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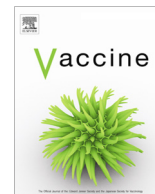
Efficacy of a novel, protein-based pneumococcal vaccine against nasopharyngeal carriage of *Streptococcus pneumoniae* in infants: A phase 2, randomized, controlled, observer-blind study

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ABSTRACT

Background: Conserved pneumococcal proteins are potential candidates for inclusion in vaccines against pneumococcal diseases. In the first part of a two-part study, an investigational vaccine (PHiD-CV/dPly/PhtD-30) containing 10 pneumococcal serotype-specific polysaccharide conjugates (10VT) combined with pneumolysin toxoid and pneumococcal histidine triad protein D (30 µg each) was well tolerated by Gambian children. Part two, presented here, assessed the efficacy of two PHiD-CV/dPly/PhtD formulations against pneumococcal nasopharyngeal carriage (NPC) prevalence in infants.
Methods: In this phase 2, randomized, controlled, observer-blind trial, healthy infants aged 8–10 weeks, recruited from a peri-urban health center, were randomized (1:1:1:1:1:1) into six groups. Four groups received PHiD-CV/dPly/PhtD (10 or 30 µg of each protein), PHiD-CV, or 13-valent pneumococcal conjugate vaccine at ages 2–3–4 months (3 + 0 infant schedule) and two groups PHiD-CV/dPly/PhtD-30 or PHiD-CV at 2–4–9 months (2 + 1 infant schedule). The primary objective was impact on non-10VT NPC at ages 5–9–12 months. Secondary objectives included confirmatory analysis of protein dose superiority and safety/reactogenicity. Impact on pneumococcal NPC acquisition, bacterial load, and *ply* and *phtD* gene sequencing were explored.
Results: 1200 infants were enrolled between June 2011 and May 2012. Prevalences of pneumococcal (60–67%) and non-10VT (55–61%) NPC were high at baseline. Across all post-vaccination time points, efficacy of PHiD-CV/dPly/PhtD-10 and PHiD-CV/dPly/PhtD-30 against non-10VT NPC (3 + 0 schedule) was 1.1% (95% CI −21.5, 19.5) and 2.1% (−20.3, 20.3), respectively; efficacy of PHiD-CV/dPly/PhtD-30 (2 + 1 schedule) was 0.5% (−22.1, 18.9) versus PHiD-CV. No differences were observed in pneumococcal NPC acquisition, clearance, or bacterial load. Both protein-based vaccines elicited immune responses to pneumococcal proteins.

Conclusions: In this high carriage prevalence setting, inclusion of pneumococcal proteins in the PHiD-CV/dPly/PhtD investigational vaccine had no impact on pneumococcal NPC in infants, regardless of protein dose or schedule. Future evaluations will assess its impact against pneumococcal disease endpoints.

1. Introduction

Remarkable reductions in the incidence of vaccine serotype invasive pneumococcal disease (IPD) and nasopharyngeal carriage (NPC) prevalence have been recorded in countries that have included a pneumococcal conjugate vaccine (PCV) in their infant immunization program [1–3]. This was observed in The Gambia following the introduction of the seven-valent PCV (PCV7) into its immunization program in August 2009 followed by replacement with the 13-valent vaccine (PCV13) in May 2011 [4,5]. Immunization with PCVs also has indirect effects, leading to decreases in the incidence of vaccine serotype carriage and disease in non-vaccinated populations [6]. However, use of PCVs is limited by the potential for serotype replacement and high manufacturing costs [3,7]. Protein antigens that are conserved across all pneumococcal serotypes have therefore been explored as an alternative and a number, used either alone or in combination with other proteins or polysaccharide conjugates, are being evaluated in clinical trials after demonstration of protection against pneumococcal disease or carriage in animal models [3,8–14].

Two protein candidates, pneumolysin (Ply) [15] and pneumococcal histidine triad protein D (PhtD) [16] (alone or in combination), have shown protection against lethal challenge, septicemia, pneumonia, and NPC prevalence in animal models [17–23] and have been selected for clinical development by GSK (Belgium). Investigational vaccines containing dPly (a pneumolysin toxoid) and PhtD (two formulations with either 10 or 30 µg of each protein) plus 10 serotype-specific polysaccharide conjugates of the pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV/dPly/PhtD-10 or PHiD-CV/dPly/PhtD-30) were well tolerated and immunogenic in healthy European adults [11] and toddlers [12]. The PHiD-CV/dPly/PhtD-30 formulation was also well tolerated and immunogenic in children aged 2–4 years in the first part of this study conducted in The Gambia [13]. In the second part, we evaluated the impact of the two protein-based pneumococcal vaccine formulations on NPC prevalence of pneumococci, and their reactogenicity, safety, and immunogenicity in infants when given in a three-dose schedule together with routine Expanded Program on Immunization (EPI) vaccines.

2. Methods

2.1. Study design and participants

A phase 2 randomized, controlled, observer-blind study was conducted at the Fajikunda Health Center situated in a peri-urban area of The Gambia. The prevalence of pneumococcal NPC in infants in Fajikunda was known to be high (76% in children aged 15–53 weeks) [24]. The study area and population have been described previously [24]. The study was conducted in accordance with Good Clinical Practice guidelines and principles of the Declaration of Helsinki. Written informed consent was obtained from a parent or legally-acceptable representative of each infant before any study procedure was performed except when deviations from the informed consent process occurred (Supplementary Text 1).

Interest in participating in the trial was determined from mothers at the time of delivery in the health center or when the infant was brought for first immunizations shortly after birth. Healthy infants aged 8–10 weeks were recruited at the health center when brought for immunizations scheduled at two months of age. Inclusion and exclusion criteria are listed in Supplementary Text 2.

Eligible participants were randomized (1:1:1:1:1:1) into one of six groups using a computer-generated, block randomization program (block size of six). The methods for pneumococcal vaccine allocation and blinding are described in Supplementary Text 2. Infants in four groups were vaccinated at 2, 3, and 4 months of age with PHiD-CV/dPly/PhtD-30, PHiD-CV/dPly/PhtD-10, PHiD-CV (*Synflorix*; GSK, Belgium), or PCV13 (*Prevenar 13*; Pfizer, USA) (3 + 0 infant schedule) (Fig. 1). Infants in the remaining two groups received either PHiD-CV/dPly/PhtD-30 or PHiD-CV at 2, 4, and 9 months of age (2 + 1 infant schedule).

Administration of other vaccines (Supplementary Text 2) was in accordance with the routine Gambian EPI schedule.

2.2. Study endpoints

The primary trial endpoint was the detection of non-10VT serotypes/groups in the nasopharynx at post-immunization visits at ages 5, 9, and 12 months, which was one, five, and eight months after the third vaccine dose in the 3 + 0 groups, and one and five months after the second dose and three months after the booster dose in the 2 + 1 groups. Non-10VT serotypes/groups included any pneumococcal isolates with unknown or determined serotype or serogroup other than those included in the 10-valent PHiD-CV (serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F). The first secondary endpoint was the immune response to Ply and PhtD given at a dose of 10 or 30 µg. A list of primary and secondary endpoints is provided in Supplementary Text 2.

2.3. Study vaccines

The investigational pneumococcal vaccines (PHiD-CV/dPly/PhtD-10 or PHiD-CV/dPly/PhtD-30) contained dPly and PhtD at 10 or 30 µg each, combined with the 10 serotype-specific PHiD-CV polysaccharide conjugates, as described previously [13]. The antigens were adsorbed on aluminum phosphate (vaccine adjuvant; aluminum content 500 µg). The control vaccines were PHiD-CV and PCV13. All pneumococcal vaccines were provided by GSK as mono-dose vials (PHiD-CV and PHiD-CV/dPly/PhtD vaccines) or pre-filled syringes (PCV13) and administered intramuscularly into the right thigh. All other co-administered injectable vaccines were administered intramuscularly into the left thigh.

2.4. Study procedures

Nasopharyngeal swabs were obtained (as described previously [24], apart from the use of flocked nylon-tipped swabs in this study) from all participants at recruitment (age 8–10 weeks), and at ages 5, 9, and 12 months. A study-specific microbiological laboratory standard operating procedure was followed at the Medical
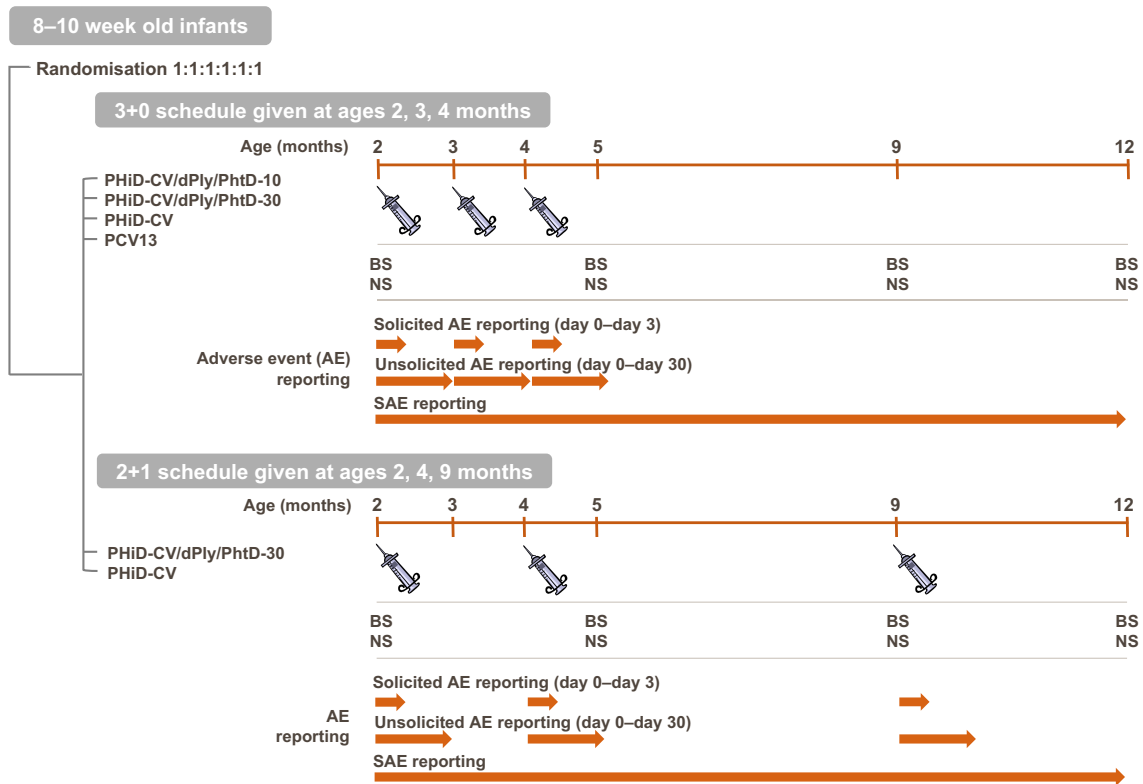
Fig. 1. Study design. BS, blood sample; NS, nasopharyngeal swab sample; SAE, serious adverse event; ↘, vaccine administration. Diphtheria-tetanus-whole cell pertussis-hepatitis B/*Haemophilus influenzae* type b and oral polio vaccines were administered at ages 2, 3, and 4 months; yellow fever, measles, and oral polio vaccines were administered at age 9 months.

Research Council (The Gambia) laboratory for identification of *S. pneumoniae*, *H. influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis,* and Group A *Streptococcus* by standard microbiological culture and biochemical tests, and serotyping of *S. pneumoniae* isolates. Results for bacteria other than *S. pneumoniae* will be presented elsewhere. The adapted broth enrichment method for *S. pneumoniae* identification, latex agglutination method for serotyping of *S. pneumoniae* isolates, and bacterial load measurement by quantitative polymerase chain reaction (qPCR) testing and by culture semi-quantitative counts are described in Supplementary Text 2. Because of technical limitations with the latex agglutination method, results were presented at the serogroup level, except when vaccine or vaccine-related serotypes were determined. Serogroup 6 and serotypes 9A/9V underwent final serotyping by Quellung reaction at Q^2 Solutions (USA). The *phtD* and *ply* gene sequencing method for a subset of nasopharyngeal swab isolates containing non-10VT serotypes/serogroups is described in Supplementary Text 2.

Blood samples for immunogenicity assessments were taken before vaccination and at ages 5, 9, and 12 months. Antibodies against Ply and PhtD were quantified using GSK in-house individual enzyme-linked immunosorbent assays (ELISAs), with technical cut-off of 12 and 17 ELISA units per mL, respectively. Results of other immunogenicity assessments will be described elsewhere.

The reactogenicity and safety assessments are described in Supplementary Text 2.

2.5. Statistical analysis

A sample size of 200 participants per group allowed detection of 35% reduction in non-10VT carriage prevalence with 82% power, assuming that non-10VT carriage prevalence in the comparator

PHiD-CV group was 40%. Vaccine efficacy against NPC prevalence was estimated as 1 minus the relative risk at all post-vaccination time points in the total vaccinated cohort, which included all vaccinated infants. The assessment of vaccine efficacy against NPC prevalence was descriptive, with no pre-defined criteria.

Percentages of infants with nasopharyngeal swabs positive for *S. pneumoniae* were calculated with non-adjusted 95% confidence intervals (CIs) at each collection time point, as well as the frequency of acquisition or clearance of serotypes/groups. Acquisition at a particular visit was determined when a nasopharyngeal swab was positive for a specific serotype/group after a negative sample for the same infant at the previous visit. Clearance was determined when a swab was negative for a specific serotype/group after a positive swab at the previous visit. Per episode analyses were conducted for acquisition and clearance, in which each new or cleared serotype/group at a visit was counted as a separate episode, allowing for the carriage of multiple serotypes/groups in an individual.

The first secondary (confirmatory) objective was to compare the immunogenicity of protein doses (10 or 30 μg) given in the 3 + 0 schedule. Superiority of one formulation over the other was to be demonstrated if the 95% CIs for the antibody geometric mean concentration (GMC) ratios (analysis of covariance model with adjustment for baseline concentrations; pooled variance) between the 30 μg group and 10 μg group did not include 1 for antibody responses against both Ply and PhtD.

qPCR was performed on swabs from 50% of enrolled subjects (100 infants per group; total vaccinated cohort for qPCR analysis). Immunogenicity analyses were performed on the according-to-protocol cohort for immunogenicity, defined as vaccinated infants who met all eligibility criteria, complied with all study procedures, and for whom immunogenicity data were available. Safety analyses were done on the total vaccinated cohort. Exploratory analyses

were conducted in infants with or without pneumococcal NPC at first study visit and in infants grouped according to timing of first study vaccination: August to October 2011, November 2011 to February 2012, and March to May 2012 and July 2011 (rainy season: June to October). In all exploratory analyses, 95% CIs were used to highlight potential group differences. Since there was no adjustment for multiplicity, such differences were to be interpreted with caution.

The statistical analyses were performed using SAS Drug and Development web portal version 4.3.2 and SAS version 9.3 (SAS Institute, USA).

3. Results

3.1. Demographics

Of 1200 infants enrolled between June 2011 and May 2012, 1152 (96%) completed the study. Participant flow is shown in Fig. 2. The last study visit took place on 18 March 2013. Baseline characteristics were similar across the groups (Supplementary Table S1).

3.2. Serotype/group NPC prevalence and vaccine efficacy

A nasopharyngeal swab sample was cultured for all infants at the first study visit and for at least 99.0% of infants who attended subsequent study visits in each group. The most frequent pneumococcal serotypes/groups identified in the nasopharyngeal swab samples across all time points were 15 (22.0–32.5%), 19A (20.5–31.5%), and 35 (21.5–28.0%) (Supplementary Table S2). Among PHiD-CV serotypes, serotype 19F (10.5–18.0%) followed by 23F (5.0–11.0%) had the highest incidence, while the most common

vaccine-related serotypes were 6A (13.0–22.5%) and 19A (20.5–31.5%).

In this study setting, there were no differences in prevalence of non-10VT NPC between groups receiving the pneumococcal protein-based vaccine compared to PHiD-CV control groups, regardless of dose (10 or 30 μg) or schedule (3 + 0 or 2 + 1) (Table 1). The carriage prevalence of non-10VT, any pneumococci, or PHiD-CV serotypes was in the same range among groups at each post-vaccination visit (Table 1). Similarly, assessment of the numbers of serotypes/groups acquired or cleared at the next visit did not show differences between PHiD-CV/dPly/PhtD and PHiD-CV groups for any serotype/group category (Tables 2 and 3). Acquisition of non-10VT carriage was more frequent than their clearance (Tables 2 and 3), leading to an increase in non-10VT NPC prevalence after primary vaccination (Table 1). No differences were observed across groups in pneumococcal load as measured by qPCR (Fig. 3) or semi-quantitative culture assessment (Supplementary Table S3) at any post-vaccination time point.

Exploratory analyses of infants with or without pneumococcal NPC before vaccination did not suggest any differences in prevalence, acquisition, or clearance of non-10VT or any pneumococci between the groups who received the pneumococcal protein-based vaccine and PHiD-CV groups (Supplementary Tables S4 and S5). Timing of first vaccination according to season also did not alter the vaccine's effect on carriage prevalence of non-10VT or any pneumococci following vaccination, although seasonal trends were observed (Supplementary Table S6, Fig. S1).

3.3. Immunogenicity

Before vaccination, all infants in all groups had detectable antibody concentrations against Ply and PhtD (above the cut-off values). There was a 10- to 18-fold increase in antibody
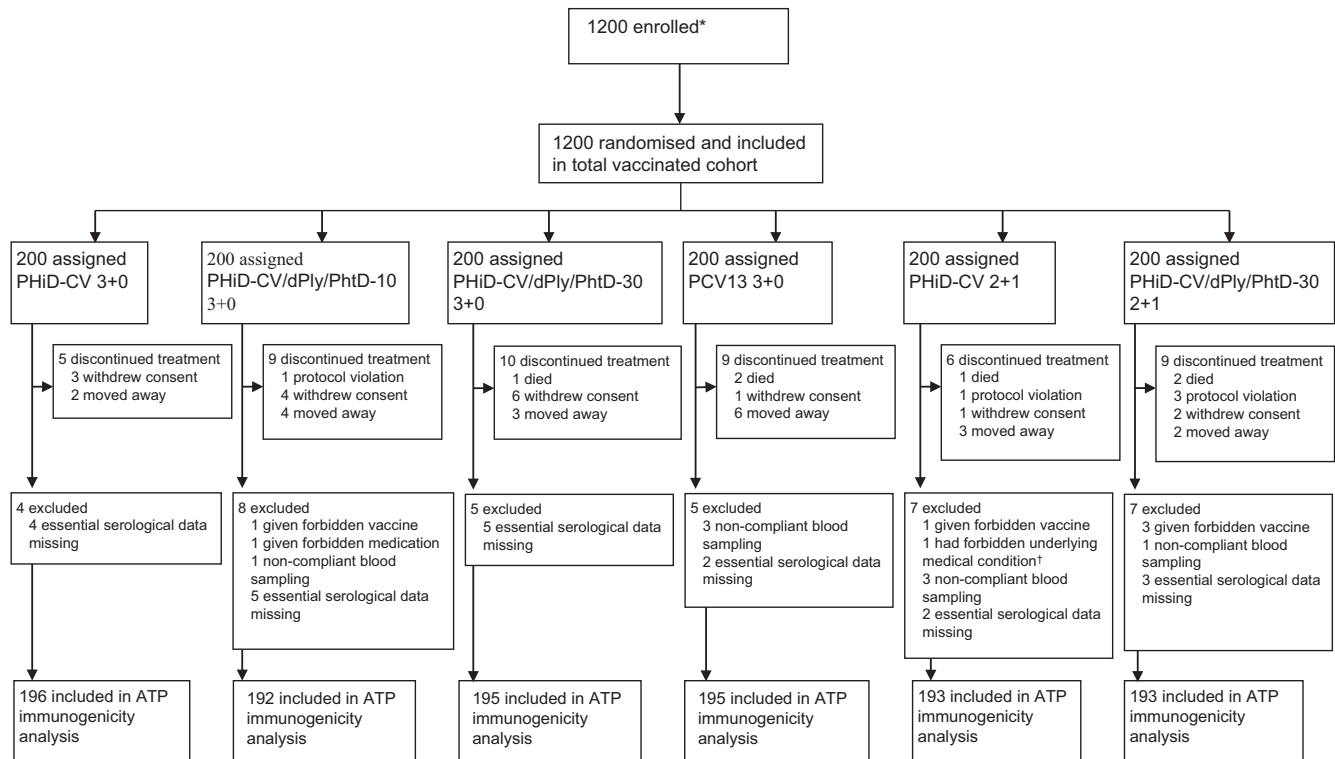

Fig. 2. Study profile. * Number who were enrolled and underwent study procedure. †Concomitant infection not related to the vaccine which might influence immune response. ATP, according-to-protocol.

Table 1
Prevalence of nasopharyngeal carriage of *Streptococcus pneumoniae* and vaccine efficacy (total vaccinated cohort).

Age at sampling	3 + 0 schedule											2 + 1 schedule				
	PHiD-CV		PHiD-CV/dPly/PhtD-10			PHiD-CV/dPly/PhtD-30			PCV13			PHiD-CV		PHiD-CV/dPly/PhtD-30		
	N	(95% CI)	N	(95% CI)	VE* (95% CI)	N	(95% CI)	VE* (95% CI)	N	(95% CI)		N	(95% CI)	N	(95% CI)	VE* (95% CI)
Any pneumococci																
2 M	200	64.5% (57.4, 71.1)	200	63.0% (55.9, 69.7)	2.3% (−25.8, 24.2)	200	59.5% (52.3, 66.4)	7.8% (−19.3, 28.7)	200	66.0% (59.0, 72.5)		200	67.0% (60.0, 73.5)	200	65.0% (58.0, 71.6)	3.0% (−24.4, 24.4)
5 M	196	84.7% (78.9, 89.4)	193	85.5% (79.7, 90.1)	−0.9% (−26.0, 19.1)	194	81.4% (75.2, 86.7)	3.8% (−20.3, 23.2)	196	79.1% (72.7, 84.6)		197	82.2% (76.2, 87.3)	197	80.7% (74.5, 86.0)	1.9% (−22.9, 21.6)
9 M	194	90.7% (85.7, 94.4)	193	86.0% (80.3, 90.6)	5.2% (−17.9, 23.8)	192	83.9% (77.9, 88.8)	7.6% (−15.1, 25.8)	193	87.0% (81.5, 91.4)		194	89.2% (83.9, 93.2)	189	86.8% (81.1, 91.3)	2.7% (−21.2, 21.9)
12 M	194	89.7% (84.5, 93.6)	189	92.1% (87.2, 95.5)	−2.6% (−27.4, 17.3)	189	87.8% (82.3, 92.1)	2.1% (−21.8, 21.3)	191	89.5% (84.3, 93.5)		193	91.2% (86.3, 94.8)	190	93.7% (89.2, 96.7)	−2.7% (−27.3, 17.1)
5, 9, 12 M visits†	196	99.5% (97.2, 100)	194	100% (98.1, 100)	−0.5% (−23.2, 18.0)	195	100% (98.1, 100)	−0.5% (−23.2, 18.0)	198	98.5% (95.6, 99.7)		198	100% (98.2, 100)	197	99.0% (96.4, 99.9)	1.0% (−21.2, 19.2)
Non-10VT serotypes/groups‡ (primary endpoint)																
2 M	200	56.0% (48.8, 63.0)	200	58.0% (50.8, 64.9)	−3.6% (−35.5, 20.8)	200	55.0% (47.8, 62.0)	1.8% (−28.9, 25.2)	200	60.5% (53.4, 67.3)		200	59.0% (51.8, 65.9)	200	54.5% (47.3, 61.5)	7.6% (−20.9, 29.5)
5 M	196	74.0% (67.2, 80.0)	193	79.8% (73.4, 85.2)	−7.9% (−36.3, 14.6)	194	70.6% (63.7, 76.9)	4.5% (−21.4, 25.0)	196	69.4% (62.4, 75.8)		197	77.2% (70.7, 82.8)	197	72.1% (65.3, 78.2)	6.6% (−18.2, 26.2)
9 M	194	82.0% (75.8, 87.1)	193	76.7% (70.1, 82.5)	6.4% (−17.8, 25.7)	192	77.6% (71.0, 83.3)	5.3% (−19.2, 24.8)	193	77.7% (71.2, 83.4)		194	82.5% (76.4, 87.5)	189	77.2% (70.6, 83.0)	6.3% (−18.0, 25.7)
12 M	194	82.0% (75.8, 87.1)	189	79.9% (73.5, 85.4)	2.5% (−22.6, 22.5)	189	81.5% (75.2, 86.7)	0.6% (−24.9, 20.9)	191	81.2% (74.9, 86.4)		193	83.9% (78.0, 88.8)	190	86.8% (81.2, 91.3)	−3.5% (−29.3, 17.2)
5, 9, 12 M visits†	196	98.5% (95.6, 99.7)	194	97.4% (94.1, 99.2)	1.1% (−21.5, 19.5)	195	96.4% (92.7, 98.5)	2.1% (−20.3, 20.3)	198	97.5% (94.2, 99.2)		198	98.0% (94.9, 99.4)	197	97.5% (94.2, 99.2)	0.5% (−22.1, 18.9)
10VT serotypes‡																
2 M	200	10.5% (6.6, 15.6)	200	7.0% (3.9, 11.5)	33.3% (−37.5, 68.6)	200	6.5% (3.5, 10.9)	38.1% (−29.5, 71.5)	200	12.5% (8.3, 17.9)		200	10.5% (6.6, 15.6)	200	13.0% (8.7, 18.5)	−23.8% (−131.4, 33.0)
5 M	196	17.3% (12.3, 23.4)	193	12.4% (8.1, 17.9)	28.3% (−24.5, 59.3)	194	16.0% (11.1, 21.9)	7.9% (−54.5, 45.2)	196	14.8% (10.1, 20.6)		197	12.2% (8.0, 17.6)	197	13.7% (9.2, 19.3)	−12.5% (−103.7, 37.5)
9 M	194	14.4% (9.8, 20.2)	193	13.0% (8.6, 18.5)	10.3% (−59.7, 49.8)	192	12.0% (7.7, 17.4)	17.0% (−49.4, 54.3)	193	15.5% (10.7, 21.4)		194	11.9% (7.7, 17.3)	189	12.7% (8.3, 18.3)	−7.1% (−98.6, 42.1)
12 M	194	8.2% (4.8, 13.0)	189	12.7% (8.3, 18.3)	−54.0% (−210.2, 21.5)	189	7.9% (4.5, 12.8)	3.8% (−107.8, 55.7)	191	11.0% (6.9, 16.3)		193	9.8% (6.0, 14.9)	190	9.5% (5.7, 14.6)	3.8% (−93.7, 52.4)
5, 9, 12 M visits†	196	32.1% (25.7, 39.2)	194	30.4% (24.0, 37.4)	5.4% (−37.1, 34.8)	195	26.7% (20.6, 33.5)	17.0% (−21.7, 43.7)	198	31.3% (24.9, 38.3)		198	28.3% (22.1, 35.1)	197	27.9% (21.8, 34.7)	1.3% (−45.8, 33.2)

CI, confidence interval. M, age in months. N, number of infants with cultured nasopharyngeal sample. VE, vaccine efficacy.

* Vaccine efficacy versus corresponding (3 + 0 or 2 + 1 schedule) PHiD-CV group. Vaccine efficacy percentages for the 2 M visit indicate group differences only because the first vaccine dose was administered at this time point.

† Nasopharyngeal carriage prevalence and vaccine efficacy across all post-immunization visits at 5, 9, and 12 months of age.

‡ 10VT corresponds to the 10 vaccine pneumococcal serotypes in PHiD-CV; non-10VT is any serotype/group excluding those in PHiD-CV.

Table 2
Acquisition of pneumococcal serotypes or serogroups between two consecutive visits (per episode analysis; total vaccinated cohort).

| | 3 + 0 schedule | | | | | | | | | 2 + 1 schedule | | | | |
| | PHiD-CV | | PHiD-CV/dPly/PhtD-10 | | | PHiD-CV/dPly/PhtD-30 | | | PCV13 | | PHiD-CV | | PHiD-CV/dPly/PhtD-30 | | |
Consecutive sampling ages	N	(95% CI)	N	(95% CI)	VE* (95% CI)	N	(95% CI)	VE* (95% CI)	N	(95% CI)	N	(95% CI)	N	(95% CI)	VE* (95% CI)
Any pneumococci															
2 M, 5 M	196	89.8% (77.5, 104.1)	193	89.6% (77.2, 104.0)	0.2% (−23.1, 19.1)	194	84.5% (72.5, 98.5)	5.9% (−16.5, 23.9)	196	87.8% (75.6, 101.9)	197	89.3% (77.1, 103.6)	197	85.8% (73.8, 99.7)	4.0% (−18.6, 22.2)
5 M, 9 M	194	90.7% (78.3, 105.2)	192	79.7% (68.0, 93.4)	12.2% (−9.1, 29.3)	191	78.5% (66.9, 92.2)	13.4% (−7.6, 30.4)	191	90.1% (77.6, 104.6)	193	94.8% (82.0, 109.6)	189	85.7% (73.5, 100)	9.6% (−11.7, 26.8)
9 M, 12 M	193	79.3% (67.7, 92.9)	189	80.4% (68.6, 94.3)	−1.4% (−27.0, 18.9)	189	76.2% (64.7, 89.7)	3.9% (−20.7, 23.5)	190	79.5% (67.8, 93.2)	192	73.4% (62.3, 86.6)	188	83.5% (71.4, 97.7)	−13.7% (−42.8, 9.4)
Non-10VT serotypes/groups[†]															
2 M, 5 M	196	74.0% (62.9, 87.1)	193	77.2% (65.8, 90.6)	−4.4% (−31.2, 17.0)	194	67.0% (56.4, 79.6)	9.4% (−14.8, 28.5)	196	72.4% (61.5, 85.4)	197	79.7% (68.2, 93.2)	197	72.1% (61.1, 85.0)	9.6% (−13.5, 27.9)
5 M, 9 M	194	76.3% (64.9, 89.6)	192	66.1% (55.6, 78.7)	13.3% (−9.9, 31.6)	191	69.1% (58.3, 82.0)	9.4% (−14.5, 28.4)	191	76.4% (65.0, 89.9)	193	81.9% (70.0, 95.7)	189	73.5% (62.3, 86.8)	10.2% (−12.8, 28.5)
9 M, 12 M	193	71.0% (60.0, 83.9)	189	70.4% (59.4, 83.4)	0.9% (−25.8, 21.9)	189	67.7% (57.0, 80.5)	4.6% (−21.4, 25.0)	190	69.5% (58.6, 82.4)	192	64.1% (53.7, 76.4)	188	74.5% (63.1, 87.9)	−16.2% (−48.1, 8.8)
10VT serotypes[†]															
2 M, 5 M	196	13.8% (9.4, 20.1)	193	9.8% (6.3, 15.4)	28.5% (−28.5, 60.3)	194	13.9% (9.5, 20.3)	−1.0% (−72.2, 40.7)	196	12.8% (8.6, 18.9)	197	8.1% (5.0, 13.3)	197	11.2% (7.4, 17.0)	−37.5% (−161.8, 27.8)
5 M, 9 M	194	10.8% (7.1, 16.6)	192	9.9% (6.3, 15.5)	8.6% (−70.0, 50.8)	191	7.9% (4.7, 13.0)	27.4% (−40.7, 62.6)	191	12.0% (8.0, 18.1)	193	10.9% (7.1, 16.7)	189	10.1% (6.4, 15.8)	7.6% (−71.8, 50.3)
9 M, 12 M	193	6.7% (3.9, 11.6)	189	9.0% (5.6, 14.5)	−33.5% (−174.9, 35.1)	189	6.9% (4.0, 11.8)	−2.1% (−120.3, 52.7)	190	8.9% (5.6, 14.4)	192	6.8% (3.9, 11.7)	188	6.9% (4.0, 11.9)	−2.1% (−120.3, 52.7)

CI, confidence interval. M, age in months. N, number of infants with cultured nasopharyngeal sample. VE, vaccine efficacy.

[*] Vaccine efficacy versus corresponding (3 + 0 or 2 + 1 schedule) PHiD-CV group.

[†] 10VT corresponds to the 10 vaccine pneumococcal serotypes in PHiD-CV; non-10VT is any serotype/group excluding those in PHiD-CV.

Table 3
Clearance of pneumococcal serotypes or serogroups between two consecutive visits (per episode analysis; total vaccinated cohort).

Consecutive sampling ages	3 + 0 schedule PHiD-CV N	(95% CI)	PHiD-CV/dPly/PhtD-10 N	(95% CI)	VE* (95% CI)	PHiD-CV/dPly/PhtD-30 N	(95% CI)	VE* (95% CI)	PCV13 N	(95% CI)	2 + 1 schedule PHiD-CV N	(95% CI)	PHiD-CV/dPly/PhtD-30 N	(95% CI)	VE* (95% CI)
Any pneumococci															
2 M, 5 M	196	50.5% (41.5, 61.5)	193	51.8% (42.6, 63.0)	−2.6% (−35.4, 22.3)	194	47.9% (39.1, 58.7)	5.1% (−26.0, 28.5)	196	59.7% (49.8, 71.6)	197	54.8% (45.4, 66.2)	197	55.3% (45.9, 66.8)	−0.9% (−31.7, 22.7)
5 M, 9 M	194	85.1% (73.0, 99.1)	192	85.9% (73.8, 100.1)	−1.0% (−25.4, 18.6)	191	85.3% (73.2, 99.5)	−0.3% (−24.6, 19.2)	191	88.5% (76.1, 102.9)	193	94.8% (82.0, 109.6)	189	85.2% (73.0, 99.4)	10.2% (−11.0, 27.3)
9 M, 12 M	193	91.2% (78.7, 105.7)	189	84.1% (72.0, 98.3)	7.7% (−14.3, 25.6)	189	73.5% (62.3, 86.8)	19.4% (−0.7, 35.4)	190	86.8% (74.6, 101.2)	192	80.7% (69.0, 94.5)	188	81.4% (69.5, 95.4)	−0.8% (−26.0, 19.4)
Non-10VT serotypes/groups[†]															
2 M, 5 M	196	41.3% (33.2, 51.4)	193	47.2% (38.4, 57.9)	−14.1% (−53.9, 15.4)	194	41.8% (33.6, 51.9)	−1.0% (−37.5, 25.7)	196	46.9% (38.3, 57.6)	197	47.2% (38.5, 57.8)	197	41.6% (33.5, 51.7)	11.8% (−18.7, 34.5)
5 M, 9 M	194	69.6% (58.8, 82.4)	192	75.0% (63.7, 88.3)	−7.8% (−36.3, 14.8)	191	69.1% (58.3, 82.0)	0.7% (−26.2, 21.9)	191	74.9% (63.6, 88.2)	193	82.4% (70.5, 96.2)	189	72.0% (60.8, 85.1)	12.7% (−9.8, 30.5)
9 M, 12 M	193	74.1% (62.9, 87.3)	189	71.4% (60.3, 84.6)	3.6% (−22.0, 23.8)	189	60.3% (50.2, 72.5)	18.6% (−4.1, 36.4)	190	72.1% (61.0, 85.2)	192	70.8% (59.9, 83.8)	188	69.7% (58.7, 82.7)	1.6% (−25.0, 22.6)
10VT serotypes[†]															
2 M, 5 M	196	7.1% (4.2, 12.1)	193	4.7% (2.4, 9.0)	34.7% (−50.8, 71.7)	194	4.6% (2.4, 8.9)	35.1% (−50.1, 71.9)	196	10.7% (7.0, 16.4)	197	7.1% (4.2, 12.0)	197	10.7% (7.0, 16.3)	−50.0% (−195.0, 23.7)
5 M, 9 M	194	13.4% (9.1, 19.7)	192	9.4% (5.9, 14.9)	30.0% (−27.6, 61.6)	191	12.0% (8.0, 18.1)	10.1% (−57.5, 48.7)	191	11.0% (7.2, 16.9)	193	10.9% (7.1, 16.7)	189	10.6% (6.8, 16.4)	2.7% (−79.4, 47.3)
9 M, 12 M	193	12.4% (8.3, 18.6)	189	10.1% (6.4, 15.8)	19.2% (−47.6, 55.7)	189	11.1% (7.2, 17.0)	10.6% (−60.5, 50.3)	190	13.2% (8.9, 19.5)	192	8.3% (5.1, 13.6)	188	10.1% (6.4, 15.8)	−21.3% (−135.8, 37.6)

CI, confidence interval. M, age in months. N, number of infants with cultured nasopharyngeal sample. VE, vaccine efficacy.
* Vaccine efficacy versus corresponding (3 + 0 or 2 + 1 schedule) PHiD-CV group; negative vaccine efficacy indicates more clearance in PHiD-CV/dPly/PhtD group than in PHiD-CV group.
[†] 10VT corresponds to the 10 vaccine pneumococcal serotypes in PHiD-CV; non-10VT is any serotype/group excluding those in PHiD-CV.
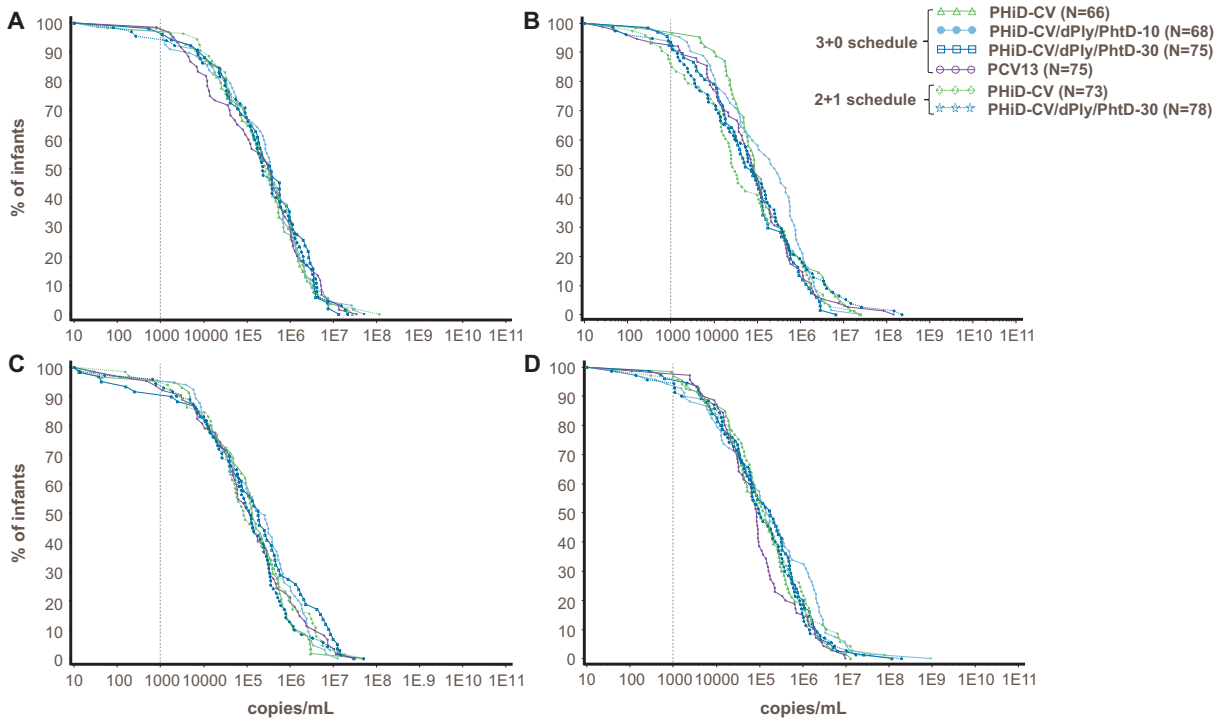
Fig. 3. Reverse cumulative distribution curves for pneumococcal bacterial load in nasopharyngeal swabs at 2 (A), 5 (B), 9 (C), and 12 (D) months of age at sampling (total vaccinated cohort for quantitative PCR analysis). N, maximum number of infants with available results at each time point; copies/mL, gene copies per mL. The dotted line indicates the lower limit of detection (cut-off: 1000 copies/mL).
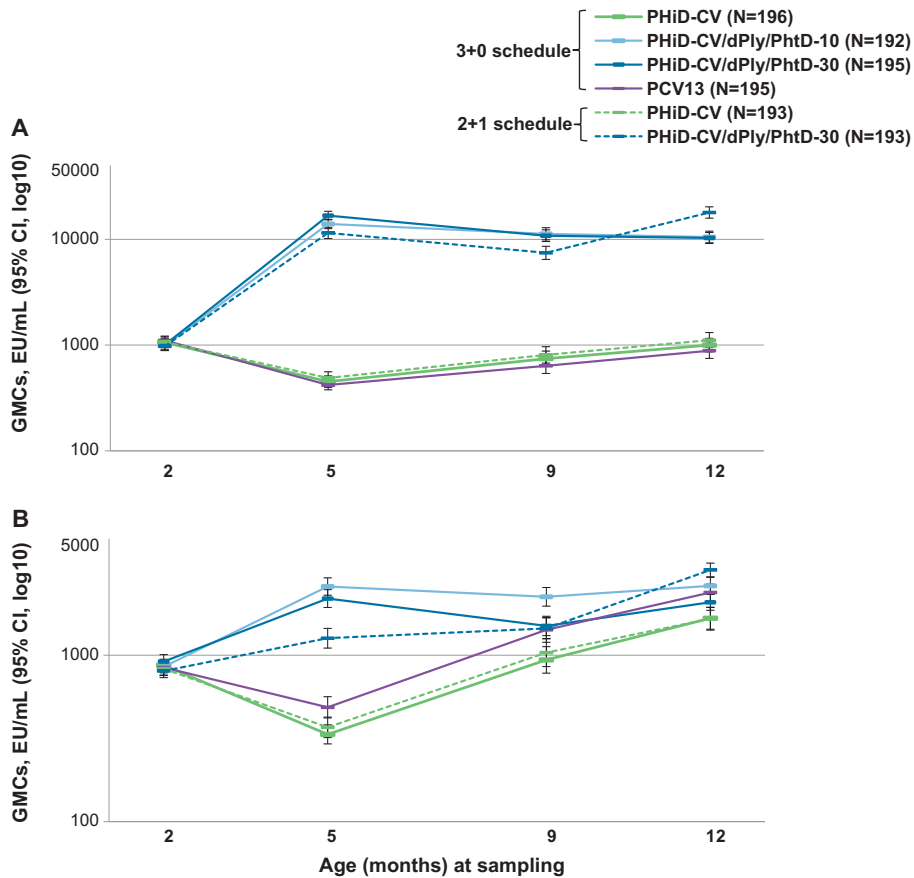

Fig. 4. Antibody geometric mean concentrations with 95% confidence intervals against Ply (A) and PhtD (B) by age at sampling (according-to-protocol cohort for immunogenicity). CI, confidence interval; GMC, geometric mean concentration; N, maximum number of infants with available results at each time point; EL.U/mL, ELISA units per mL.

concentrations against Ply from pre-vaccination to last post-vaccination time point in PHiD-CV/dPly/PhtD groups compared with no, or slight, increase in control PHiD-CV or PCV13 groups (Fig. 4). The magnitude of increased antibody responses to PhtD was smaller (a 2.3–4.0-fold increase in PHiD-CV/dPly/PhtD groups versus a 1.9–2.8-fold increase in control PHiD-CV or PCV13 groups) (Fig. 4). In the control groups, Ply and PhtD antibody GMCs dropped by 42–62% between pre-vaccination and age of 5 months, while antibody GMCs increased in the PHiD-CV/dPly/PhtD groups. For the 3 + 0 schedule, the confirmatory objective on dose superiority was not met as the 95% CI for the GMC ratio for antibody responses against both Ply and PhtD after the third vaccine dose did not exclude 1 (Supplementary Table S7). For antibody responses against Ply, the adjusted GMC was higher for the 30 µg than the 10 µg dose while for anti-PhtD responses, the trend was the reverse.

No differences in IgG antibody response against Ply or PhtD were observed in subsets of infants with or without pneumococcal NPC before vaccination (Supplementary Fig. S2).

3.4. Gene sequencing

Among 350 selected NPC isolates containing non-10VT serotypes/groups, 347 were positive for *ply* and 342 were positive for *phtD* genes. Sequencing results are provided in Supplementary Text 3 and Tables S8–11. Briefly, 18 Ply protein variants were obtained, with variant 1 (100% identity with Ply used for PHiD-CV/dPly/PhtD vaccine) being the most common and present in 62% of samples positive for *ply*. High sequence identity (at least 98.5%) was observed, corresponding to a maximum of seven amino acid substitutions among all Ply protein variants. For PhtD, 84 protein sequences were detected, with no clear major variant. All PhtD variants except four (but including the variant used for PHiD-CV/dPly/PhtD vaccines) showed 91.8–99.9% protein identity. Comparison of protein-based vaccine and control PHiD-CV groups showed PHiD-CV/dPly/PhtD vaccination had no impact on the prevalence of Ply variant 1. There was also no evidence of an impact on average Ply or PhtD sequence identity relative to the amino acid sequences of the proteins in the vaccine.

3.5. Reactogenicity and safety

Incidences of solicited and unsolicited adverse events (AEs) were comparable among groups (Supplementary Tables S12 and S13). Reports of pain at the PHiD-CV/dPly/PhtD vaccine injection site tended to be less frequent than reported for co-administered vaccines (Supplementary Fig. S3). There were few reports of related or grade 3 unsolicited AEs (Supplementary Table S13). A total of 21 serious AEs (SAEs) were reported for 19 infants, none of which was assessed by the investigator as causally related to vaccination. Six SAEs were fatal (Supplementary Text 4), three in the investigational arms and three in the control groups, with diagnoses of aspiration pneumonia for one case in the PHiD-CV/dPly/PhtD-30 (3 + 0 schedule) group, acute gastroenteritis and sudden infant death syndrome for one case each in the PHiD-CV/dPly/PhtD-30 (2 + 1) group, gastroenteritis for one case in the PHiD-CV (2 + 1) group, and bronchopneumonia and sepsis for one case each in the PCV13 group.

4. Discussion

We evaluated the impact of two formulations of an investigational vaccine containing two pneumococcal proteins combined with polysaccharide conjugates of the 10-valent PHiD-CV (PHiD-CV/dPly/PhtD) on NPC prevalence and their immunogenicity and safety in infants. The study was carried out in a community in The Gambia where the prevalence of pneumococcal carriage among infants was known to be very high [24]. In this trial setting, we did not show that addition of the pneumococcal proteins dPly and PhtD reduces the prevalence of non-10VT carriage beyond that provided by PHiD-CV, the primary trial endpoint. We found that the prevalence of pneumococcal carriage, acquisition or clearance of carriage, and pneumococcal load as detected by qPCR and culture were similar across study groups. The trial was well powered to detect a statistical difference of 25% between each of the PHiD-CV/dPly/PhtD groups and the PHiD-CV control groups, given the incidence of non-10VT carriage observed in the study. Protein dose superiority (10 or 30 µg) in terms of immunogenicity for both proteins was not demonstrated. Gene sequencing results did not suggest that the lack of impact on carriage was due to an absence of *ply* or *phtD* genes in *S. pneumoniae* strains colonizing infants in The Gambia or divergence in Ply or PhtD protein sequences. Also, exploratory analyses showed baseline carriage status of infants and the time of year when infants presented for first vaccination had no clear effects on efficacy against NPC prevalence of non-10VT or any pneumococci.

Despite the fact that all infants had background antibodies to both proteins before vaccination, robust antibody responses to the Ply component were seen, resulting in substantially higher antibody levels in dPly-containing vaccine recipients compared to control and this difference was sustained up to the age of 12 months. For PhtD, the magnitude of the antibody response versus control was greatest at 5 months, with diminishing differences between groups at 9 and 12 months. The fold increase in antibody concentration from pre- to post-vaccination was lower for PhtD than for Ply in all groups. Anti-Ply GMCs were higher in infants who received the 30 µg vaccine formulation than in those who received the 10 µg formulation, whereas the reverse trend was observed for anti-PhtD concentrations. This differs from findings in European infants, where superiority with the 30 µg dose was demonstrated for both proteins one month after three-dose PHiD-CV/dPly/PhtD priming [25]. In the control groups, antibody GMCs against Ply and PhtD decreased between pre-vaccination and 5 months of age, which is attributed to waning maternal antibodies [26,27].

To our knowledge, this was the first study designed and powered to evaluate the effect of pneumococcal proteins against bacterial carriage in infants as the primary endpoint. There are few reports from other studies that have investigated the impact of protein-based vaccines against pneumococcal NPC prevalence in humans. A study of a trivalent recombinant protein pneumococcal vaccine containing PhtD, PlyD1 (a genetically detoxified Ply), and pneumococcal choline-binding protein A (PcpA) conducted in Bangladesh examined impact on carriage but not as a primary or secondary outcome measure [10]. Initial reports from this study suggested there was no significant difference in carriage prevalence of pneumococci between vaccinated and control groups [28]. First results are also available from an experimental pneumococcal challenge study of 96 adults in the UK with a protein-based vaccine containing three T-cell antigens, SP0148, SP1912, and SP2108 [14]. At sampling points up to 14 days after inoculation, pneumococcal NPC prevalence was reduced by 18–36% versus placebo but the group differences were not statistically significant.

In pre-clinical studies, Ply and PhtD, administered either separately or in combination, showed protection against lethal challenge and pneumococcal disease [17–23,29] and were therefore considered for vaccine development to provide broader protection against pneumococcal disease. They also showed protection against pneumococcal NPC prevalence [22,23,30]. In studies conducted by our group, protection against NPC was observed in mice after immunization with dPly and PhtD only when the proteins were administered via an intranasal route and using *Escherichia coli*

labile toxin as adjuvant [22,23]; there was no protection when the proteins were administered systemically (unpublished data), which is consistent with the results of the present clinical study. In contrast, another study reported protection against pneumococcal NPC in mice administered with anti-Ply and anti-PhtD antibodies intravenously [30]. Therefore, although pre-clinical assessments suggest that immunization with dPly and PhtD antigens may play a role in protection against NPC, this effect may depend on the route of administration of the antigens.

It is possible that the characteristics of the induced immune response leading to an impact on carriage in animals differ from those induced in the clinical study. As well as the difference in immunization route, this may be due to differences in the adjuvant and/or initial immune status of mice versus Gambian infants. In the pre-clinical studies conducted by our group, *E. coli* labile toxin was used as adjuvant [22,23], while aluminum phosphate was used in the clinical study. Immune responses may vary with different adjuvants, particularly stronger induction of T-cell responses, which is thought to be involved in protection against NPC [31], but this was not assessed in this study.

Moreover, the clinical study was conducted in a setting where the prevalence of pneumococcal NPC among infants is high (pneumococcal and non-10VT NPC prevalence at baseline were 60–67% and 55–61%, respectively) and therefore most, if not all, infants would have been immunologically primed by prior or ongoing carriage events. Thus intramuscular immunization of Gambian infants might have boosted a mucosal immune response primed by natural NP exposure. This was contrary to the initial unexposed and therefore immunologically naïve status of animals in the pre-clinical studies.

The trial was well conducted with a very high completion rate and detailed evaluation of the impact of the vaccine including measurement of bacterial acquisition, clearance, and load. The methodology developed will be of interest to other groups contemplating pneumococcal vaccine trials with NPC as an endpoint. However, our study had some limitations. Intervals between sampling time points were relatively long and consequently the sensitivity of tests for acquisition or clearance of carriage was probably not optimal. Another limitation was that the study was not powered to detect a smaller reduction (below 25%) in non-10VT carriage due to the initial assumption of higher vaccine efficacy against this endpoint. Reduction of carriage by less than 25% might not preclude efficacy on disease since a dynamic model of pneumococcal infection predicted that 20% effectiveness with PCV7 against vaccine serotype carriage would have a major impact on IPD caused by these serotypes [32]. No observed effect on carriage prevalence also does not preclude an effect on host-to-host transmission as other mechanisms such as reduction of local inflammation might be involved [33], but this was not evaluated in this study. We also did not investigate T-cell responses to the investigational vaccine, which might have enabled us to better understand the nature of the immune response to the pneumococcal proteins.

In conclusion, our study showed that inclusion of pneumococcal proteins in the PHiD-CV/dPly/PhtD investigational vaccine had no impact on pneumococcal NPC prevalence in infants, regardless of protein dose or schedule. However, the quantitative relationship between the pneumococcal protein-containing vaccines' impact on NPC prevalence and its impact on pneumococcal disease in humans is yet to be defined, and no effect on carriage does not preclude protection against disease. Another infant trial (ClinicalTrials.gov, NCT01545375) is evaluating protection against acute otitis media in infants receiving dPly and PhtD antigens. If the protein antigens are proven effective in disease protection, their use in combination with conjugates could add value by extending protection to a larger set of pneumococcal serotypes while maintaining the well established effect of the existing PCV.

Authors' contributions

AO was involved in planning, data collection, site coordination of study, review of the reported study, and trained and supervised clinicians and staff on study procedures, clinically evaluated and investigated the patients, maintained quality assurance over clinical procedures, and drafted the report. MOCO was involved in planning, data collection, review, project oversight on site and was involved in the conception of the study design, trained and supervised clinicians and staff on study procedures, clinically evaluated and investigated the patients, maintained quality assurance over clinical procedures, and supervised the laboratory work. MAn was involved in planning/design/review of the reported study, interpretation of the results and was involved in planning, designing, and reviewing the reported study, the analysis plan, and interpretation of the results. EOO clinically evaluated and investigated the patients and maintained quality assurance over clinical procedures. EOO also participated in the training of some of the staff on study procedures. YS trained and supervised clinicians and staff on study procedures and clinically evaluated and investigated patients and maintained quality assurance over clinical procedures. EFN was involved in the generation, quality control, and interpretation of laboratory results, and laboratory work supervision. OTI and OO were involved in clinical evaluation and investigation of the study participants, trained study team staff on procedures and maintained quality assurance over clinical procedures. PKO, FC, AB, SJ, and ID supervised the laboratory work. AW was involved in center coordination, data collection, and quality checks. BK was involved in project oversight, laboratory work supervision and interpretation of results. BMG contributed to the study design, development of the study protocol, analysis plan, interpretation of the results, and writing the final report. MAl was involved in planning, designing, and reviewing the reported study and interpretation of the results. MT was involved in planning, designing, and reviewing the reported study and statistical analysis of the data. ND was involved in the generation of study data and in the interpretation of the results. SS contributed to the study design, development of the study protocol, development of the analysis plan, interpretation of the results, and laboratory work supervision. KS was involved in the generation of study data and in the interpretation of the results. VV contributed to study design, review of the analysis plan, interpretation of the results, and laboratory work supervision. KD was involved in interpretation of the results, coordination, and reporting of the study. DB was involved in planning, designing, and reviewing the reported study, analysis plan, interpretation of the results, safety (interaction with and reporting to the Data and Safety Monitoring Board/Independent Data Monitoring Committee), project oversight, and writing the final report.

All authors had full access to all data in the study, contributed to the writing of this report, and had final responsibility for the decision to submit for publication.

Conflict of interest

MOCO, MAn, EOO, YS, EFN, PKO, FC, AW, OTI, OO, AB, SJ, ID, and MAl have no conflicts of interest to disclose. AO received support

Trademark statement

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.03.071.

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