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Efficacy, safety and immunogenicity of a pneumococcal protein-based vaccine co-administered with 13-valent pneumococcal conjugate vaccine against acute otitis media in young children: A phase IIb randomized study

Laura L. Hammitt [a,*], James C. Campbell [a], Dorota Borys [b], Robert C. Weatherholtz [a], Raymond Reid [a], Novalene Goklish [a], Lawrence H. Moulton [a], Magali Traskine [b], Yue Song [c], Kristien Swinnen [b], Mathuram Santosham [a], Katherine L. O'Brien [a]

[a] Center for American Indian Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States
[b] GSK, Wavre, Belgium
[c] XPE Pharma & Science c/o GSK, Wavre, Belgium

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ABSTRACT

Background: Native American populations experience a substantial burden of pneumococcal disease despite use of highly effective pneumococcal conjugate vaccines (PCVs). Protein-based pneumococcal vaccines may extend protection beyond the serotype-specific protection elicited by PCVs.
Methods: In this phase IIb, double-blind, controlled trial, 6–12 weeks-old Native American infants randomized 1:1, received either a protein-based pneumococcal vaccine (dPly/PhtD) containing pneumolysin toxoid (dPly, 10 μg) and pneumococcal histidine triad protein D (PhtD, 10 μg) or placebo, administered along with 13-valent PCV (PCV13) at ages 2, 4, 6 and 12–15 months. Other pediatric vaccines were given per the routine immunization schedule. We assessed vaccine efficacy (VE) against acute otitis media (AOM) and acute lower respiratory tract infection (ALRI) endpoints. Immunogenicity, reactogenicity and unsolicited adverse events were assessed in a sub-cohort and serious adverse events were assessed in all children.
Results: 1803 infants were randomized (900 dPly/PhtD; 903 Control). VE against all episodes of American Academy of Pediatrics (AAP)-defined AOM was 3.8% (95% confidence interval: −11.4, 16.9). Point estimates of VE against other AOM outcomes ranged between 2.9% (−9.5, 14.0) and 5.2% (−8.0, 16.8). Point estimates of VE against ALRI outcomes ranged between −4.4% (−39.2, 21.8) and 2.0% (−18.3, 18.8). Point estimates of VE tended to be higher against first than all episodes but the confidence intervals included zero. dPly/PhtD vaccine was immunogenic and had an acceptable reactogenicity and safety profile after primary and booster vaccination in Native American infants.
Conclusions: The dPly/PhtD vaccine was immunogenic and well tolerated, however, incremental efficacy in preventing AAP-AOM over PCV13 was not demonstrated.
Clinical trials registration: NCT01545375 (www.clinicaltrials.gov)

Abbreviations: AAP, American Academy of Pediatrics; ABS, active bacterial surveillance; AE, adverse event; ALRI, acute lower respiratory tract infection; AOM, acute otitis media; ATP, according-to-protocol; CI, confidence interval; dPly, pneumolysin toxoid; ELISA, enzyme linked immunosorbent assay; EL.U, ELISA units; GMC, geometric mean concentration; HCP, health-care provider; IHS, Indian Health Service; IPD, invasive pneumococcal disease; LLOQ, lower limit of quantification; MA, medically-attended; mATP, modified according-to-protocol; PCV, pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine; PhtD, pneumococcal histidine triad protein D; Ply, pneumolysin; SAE, serious adverse event; SAS, Statistical Analysis System; TVC, total vaccinated cohort; ULOQ, upper limit of quantification; VE, vaccine efficacy.

* Corresponding author at: Johns Hopkins Bloomberg School of Public Health, 415 N. Washington St., 4th Floor, Baltimore, MD 21231, United States.
E-mail address: LHammitt@JHU.edu (L.L. Hammitt).

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1. Introduction

Routine use of licensed pneumococcal conjugate vaccines (PCVs) has led to a substantial reduction in invasive pneumococcal disease (IPD) among vaccinated and unvaccinated individuals [1]. However, currently available vaccines protect against only a fraction of the more than 90 pneumococcal serotypes. *Streptococcus pneumoniae* remains an important cause of IPD, pneumonia, and acute otitis media (AOM). In 2015, there were an estimated 3.7 million episodes of severe pneumococcal disease globally, in a setting where 129 countries had included PCV as part of the infant vaccination programs [1,2]. Prior to the use of PCVs, the rate of IPD among Native Americans was one of the highest in the world [3–6]. While use of PCVs has greatly reduced the burden of IPD, disparities persist. Age-group specific IPD incidence among Native Americans in the southwest United States remains 2–4-fold higher than the general US population; the majority of this residual pneumococcal disease is caused by serotypes not covered by currently available pneumococcal vaccines [7–9].

Protein antigens that are conserved across pneumococcal serotypes may offer an alternative to serotype-restricted vaccination strategies [10]. Two such proteins are pneumolysin (Ply) – a cytolytic protein that contributes to the host inflammatory response, facilitates colonization and plays a role in acute lung injury – and pneumococcal histidine triad protein D (PhtD), which has a role in ion homeostasis and pneumococcal virulence [11–13]. Alone or in combination Ply and PhtD have demonstrated protection against pneumococcal disease or carriage in *in vitro* and animal studies and were selected by GSK for clinical development [12,14–18]. Vaccines containing dPly (a pneumolysin toxoid), PhtD and 10 serotype-specific polysaccharide conjugates of the pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV, *Synflorix*, GSK) were found to be immunogenic and well-tolerated in children and infants; however, efficacy against pneumococcal nasopharyngeal carriage prevalence, density, acquisition or clearance was not observed [19–22]. We report results of a phase IIb randomized controlled trial that assessed the incremental efficacy (over 13-valent PCV [PCV13], *Prevenar 13*/*Prevnar 13*, Pfizer) of an investigational pneumococcal protein-based vaccine, dPly/PhtD, against AOM and acute lower respiratory tract infection (ALRI) in Native American infants. We also report immunogenicity of dPly/PhtD antigens and safety/reactogenicity of the dPly/PhtD vaccine.

2. Material and methods

We conducted this study between May 2012 and July 2016 at five sites on the Navajo Nation and White Mountain Apache tribal lands in the southwest United States. Health care is administered free of charge to tribal members through the Indian Health Service (IHS). The institutional review boards of the Johns Hopkins Bloomberg School of Public Health, the Navajo Nation, the Phoenix Area Indian Health Service, and the Tribal Council of the White Mountain Apache Tribe approved the study. The study was conducted in accordance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. Written informed consent was obtained from a parent or legally-authorized representative of each infant before enrollment. An independent data monitoring committee was charged with oversight of participant safety. This study was registered at www.clinicaltrials.gov (NCT01545375). A protocol summary is available at www.gsk-studyregister.com (study ID: 115597).

Eligible infants aged 6–12 weeks were randomized 1:1 to receive dPly/PhtD vaccine or placebo (Control group) at ages 2, 4, 6 and 12–15 months, each co-administered with PCV13 (Fig. 1). Inclusion and exclusion criteria are listed in Supplementary Text. A subset of children was enrolled into the immunogenicity/reactogenicity sub-cohort. Randomization was stratified by site with a block size of six and treatment allocation at the investigator site for dPly/PhtD was performed using a central randomization system on the internet. All study participants, investigators, funding staff, and monitoring staff were masked to drug allocation. dPly/PhtD vaccine and placebo were supplied to the site in identical-looking and -labelled vials in coded kits and stored at 2–8 °C until the time of dosing. The investigational pneumococcal vaccine (dPly/PhtD) contained dPly and PhtD at 10 μg each, adsorbed on aluminum phosphate (vaccine adjuvant; aluminum content 500 μg). The placebo contained 500 μg aluminum phosphate. The placebo was visually indistinguishable from the dPly/PhtD vaccine. Study product (vaccine or placebo) was administered intramuscularly into the right thigh (primary vaccination) or deltoid (booster vaccination). Co-administered PCV13 was given intramuscularly on the

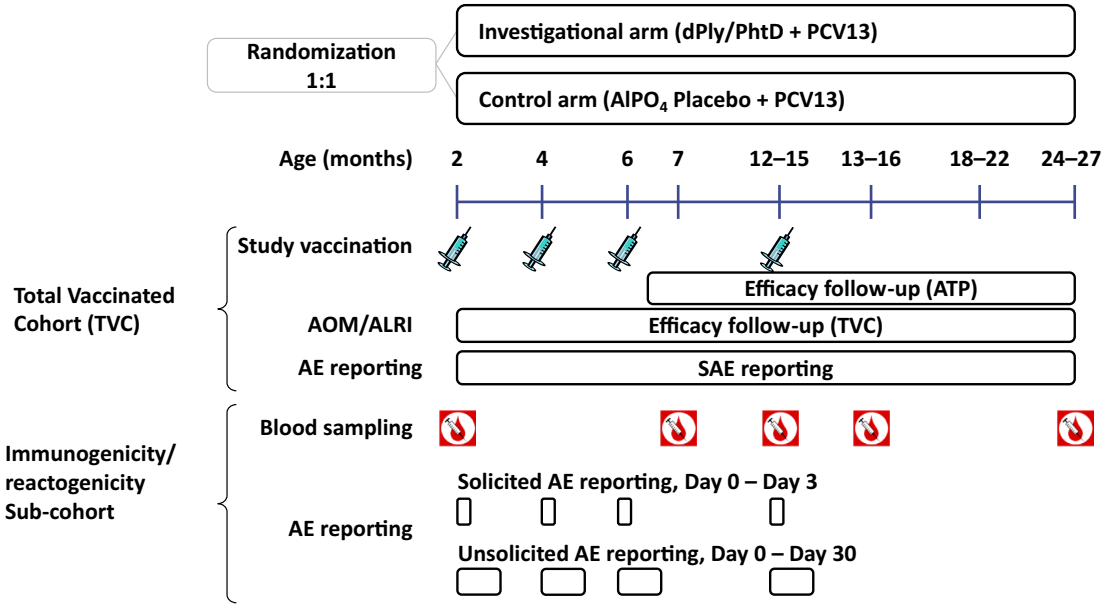

Fig. 1. Study design. dPly/PhtD, pneumolysin toxoid/pneumococcal histidine-triad protein D; PCV13, 13-valent pneumococcal conjugate vaccine; AOM, acute otitis media; ALRI, acute lower respiratory tract infection; ATP, according-to-protocol; AE, adverse event; SAE, serious adverse event.

opposite side. All other vaccines were given according to the recommended childhood immunization schedule in the United States, including influenza vaccine for children ≥6 months old which was allowed during the influenza seasons, as per Advisory Committee on Immunization Practices recommendations [23].

2.1. Study endpoints

We assessed dPly/PhtD vaccine efficacy (VE) against all episodes of clinically diagnosed AOM meeting the 2004 American Academy of Pediatrics criteria (AAP-AOM [2004]; primary objective) and other AOM and ALRI endpoints (Table 1) [24]. In addition, we report on the following secondary objectives: the safety of the dPly/PhtD vaccine in all participants in terms of serious adverse events (SAEs) and the immunogenicity and reactogenicity of the dPly/PhtD vaccine in a sub-cohort of participants. Results of other objectives (i.e., immunogenicity of co-administered pneumococcal and Hib vaccines, and bacterial nasopharyngeal carriage) will be described elsewhere.

2.2. Study procedures

Active surveillance for any medical event was conducted by study staff at study facilities and other facilities providing care in the study communities starting from administration of the first dose through study end for each participant to identify SAEs and episodes of AOM or ALRI. Immunogenicity and unsolicited adverse events (AEs) occurring within one month following administration of each dose of study vaccine were assessed in the immunogenicity/reactogenicity sub-cohort (N = 400). Solicited local and general AEs were documented on a diary card during 4 days post-vaccination by the parent/guardian of each participant in the immunogenicity/reactogenicity sub-cohort. Participants in this sub-cohort also had blood collected at five timepoints (prior to dose 1, one month post-dose 3, prior to the booster vaccination, one month and 12 months post-booster vaccination). Samples were centrifuged and serum was stored at −20 °C or colder until assayed for anti-Ply and anti-PhtD antibodies by GSK using an in-house enzyme linked immunosorbent assay (ELISA) [25].

Table 1
Case definitions for efficacy endpoints.

Case definitions	Elements of Acute Otitis Media (AOM) Case Definition						
	History of acute onset of signs and symptoms	Middle Ear Effusion (MEE)				Middle Ear Inflammation (MEI)	
		Bulging of tympanic membrane	Limited or absent mobility of tympanic membrane	Air-fluid level behind the tympanic membrane	Otorrhea	Erythema of the tympanic membrane	Distinct otalgia
AAP-AOM (2004)	Yes	At least 1 of 4 findings of MEE				At least 1 of 2 findings of MEI	
Study Modified AAP-AOM (2004)	Yes	At least 1 of 5 findings of MEE and MEI					Not necessary
HCP-AOM	All AOM cases diagnosed by a Health-Care Provider						
Recurrent HCP-AOM	At least 3 episodes of HCP-AOM in 6 months or at least 4 episodes of HCP-AOM in 12 months						
Draining AOM	AOM with otorrhea or with spontaneously perforated tympanic membrane. Collection of middle ear fluid for culture was done at the discretion of the clinical provider and was not a study-specific procedure						
ALRI	Tachypnea (respiratory rate >50 amongst children 2 to 12 months of age or >40 in children over 1 year of age) plus at least two of: cough, fever (>38.0°C by any route), increased work of breathing, auscultatory findings						
MA-ALRI	Medically-attended ALRI						
MA-HCP-ALRI	Medically-attended Health-Care Provider diagnosed ALRI						
MA-ALRI with fever or MA-HCP-ALRI with fever	MA-ALRI or MA-HCP-ALRI events accompanied by fever (>38.0°C by any route) at the time of the visit or history of fever in the 3 days preceding a given episode						

AAP, American Academy of Pediatrics; MA, medically-attended; ALRI, acute lower respiratory tract infection; HCP, health-care provider.

2.3. Sample size and statistical analysis

The primary objective was to demonstrate that the VE induced by the dPly/PhtD vaccine against clinical AOM diagnosed and verified using AAP criteria was greater than 0%, as compared to the Control group. Sample size calculations were based on the assumption that, among AOM, approximately 60% is bacterial, of which 40% is pneumococcal [26–28]. True VE against pneumococcal AOM was estimated to be similar to the observed PCV VE against vaccine-type pneumococcal AOM (approximately 50–60%) [26,29–31]. Based on 1800 enrolled participants (i.e., 1537 projected evaluable participants), the study had 97.2% power to demonstrate AOM VE >0% based on a true VE of 17%, an incidence rate in the control group of 0.6 episodes per child-year, an overdispersion of 1.4 and a one-sided test, nominal type I error of 17.8%. The interim analysis is presented in the Supplementary Text.

The primary efficacy analysis was based on (*i*) the occurrence of the primary endpoint (i.e., AAP-AOM 2004) anytime from two weeks after the administration of the third primary dose of the study vaccine up to the final study visit or the time of censoring (e.g., for participants with non-compliance with the protocol) or up to the time of withdrawal (e.g., if a participant withdrew before completing the study) and (*ii*) the modified according-to-protocol (mATP) cohort for efficacy analysis which included all participants from the ATP cohort for efficacy analysis and participants for whom non-compliance with the vaccination intervals during primary vaccination constituted the only elimination criterion from ATP cohort for efficacy analysis. The other cohorts analyzed are described in Supplementary Text.

Time-to-occurrence of primary endpoint events during the defined efficacy follow-up period was compared between groups by estimating VE and its 95% confidence interval (CI) using the Anderson & Gill model, with a robust sandwich estimator for variance matrix considered as a generalization of the Cox proportional hazard model, taking into account recurrent events [32]. VE was defined as (1 − hazard ratio) × 100%.

Antibody geometric mean concentrations (GMCs) and percentage of infants with antibody concentration above pre-specified cut-offs (12 ELISA units[EL.U]/mL for Ply and 17 EL.U/mL for PhtD) were assessed. For the purpose of GMC calculation, antibody concentrations below the lower limit of quantification (LLOQ) of the assay were given an arbitrary value of half the LLOQ and those above the upper limit of quantification (ULOQ) were assigned a value equivalent to the ULOQ.

Because acquired maternal antibodies may inhibit the infant immune response to primary immunization, we assessed as exploratory analyses the relationship between pre- and post-immunization anti-Ply and anti-PhtD antibody levels using scatter plots [33]. Further, the impact of post-primary series anti-Ply and anti-PhtD antibody levels on VE was assessed by dividing participants into "low" and "high" categories based on the median antibody concentration.

Influenza disease and vaccination may impact the incidence of AOM and ALRI, and may consequently bias estimates of VE against these diseases [34,35]. Therefore, we also assessed the effect of seasonality and influenza vaccination in a post-hoc exploratory analysis. The influenza season was defined from November 30 to May 31 and, in any given season, VE against AOM and ALRI endpoints was assessed in the total vaccinated cohort (TVC) and mATP cohort for efficacy. For each participant, VE follow-up duration for a given season was defined as shown in Supplemental Fig. 1. VE was computed within each subgroup (seasonal influenza-vaccinated or seasonal influenza-unvaccinated) to assess the efficacy of dPly/PhtD versus the Control group.

VE was estimated using the generalized Cox regression model, by using time-to-event; the point estimates were not adjusted for multiplicity.

The statistical analyses were performed using the Statistical Analysis System (SAS) in the SAS Drug Development environment.

3. Results

Of the 1803 infants in the TVC, 900 were in the Investigational group (dPly/PhtD + PCV13) and 903 in the Control group (Placebo + PCV13) (Fig. 2). 808 infants in the Investigational group and 831 in the Control group were included in the mATP cohort for efficacy. Demographic characteristics and duration of follow-up were similar in the two groups (Supplemental Tables 1 and 2). In the mATP cohort for efficacy, 485 AAP-AOM events were detected in the dPly/PhtD group and 518 in the Control group, giving an incidence of 0.43 and 0.44 episodes/child-year, respectively (Table 2); the proportion of participants experiencing 3 or more episodes of AAP-AOM was 5.1% in the dPly/PhtD group and 4.8% in the Control group. 5.7% and 5.9% of children experienced recurrent AOM diagnosed by a health-care provider (HCP) in the dPly/PhtD and Control groups, respectively (Supplemental Table 3). For both groups, the peak incidence of AOM occurred at 6–12 months of life. There were 163 medically-attended (MA)-ALRI events in the dPly/PhtD group and 165 in the Control group, giving an incidence of 0.14 episodes/child-year in each group (Table 3).

3.1. Vaccine efficacy

Incremental efficacy of dPly/PhtD in preventing AAP-AOM (2004) over PCV13 was not demonstrated (Table 2). In the mATP cohort for VE, VE against all AAP-AOM episodes was 3.8% (95% CI: −11.4, 16.9). VE against the first AAP-AOM episode was 11.3% (−3.4, 23.9). VE against all episodes of draining AOM, pneumococcal draining AOM, and non-pneumococcal draining AOM ranged from –32.2% (−298.3, 56.1) to 17.8% (−57.0, 57.0) (Supplemental Table 4). VE against all episodes of MA-ALRI, MA-ALRI with fever, and MA-HCP-ALRI with fever ranged between −4.4% (−39.2, 21.8) and 2.0% (−18.3, 18.8) (Table 3). VE against first episodes of clinical AOM or ALRI tended to be higher compared to VE against all episodes (Tables 2 and 3). This finding was more pronounced in a post-hoc analysis restricted to first episodes within the first year of life (Fig. 3; Supplemental Figs. 2 and 3; Supplemental Table 5).

Exploratory analyses of VE against various AOM and ALRI endpoints among participants with low (≤27498 EL.U/mL for Ply; ≤3739.5 EL.U/mL for PhtD) and high (>27498 EL.U/mL for Ply; >3739.5 EL.U/mL for PhtD) post-primary anti-protein antibody concentrations revealed a trend for higher efficacy against AOM endpoints with higher post-primary anti-Ply antibody levels. For ALRI endpoints, VE seemed to be higher with lower post-primary anti-Ply antibody levels but VE point estimates remained positive for both low and high antibody ranges (Supplemental Table 6). VE against AOM and ALRI endpoints appeared in the same range for low and high post-primary anti-PhtD antibody levels (Supplemental Table 7).

AOM incidence followed a seasonal pattern with peaks in winter seasons and tended to be lower in the last season of the study, reflecting the older age of the study population (Supplemental Fig. 4). No consistent impact of seasonal influenza vaccination was observed on dPly/PhtD efficacy against AOM and ALRI endpoints across the four winter seasons encompassed during the study (Supplemental Fig. 5).

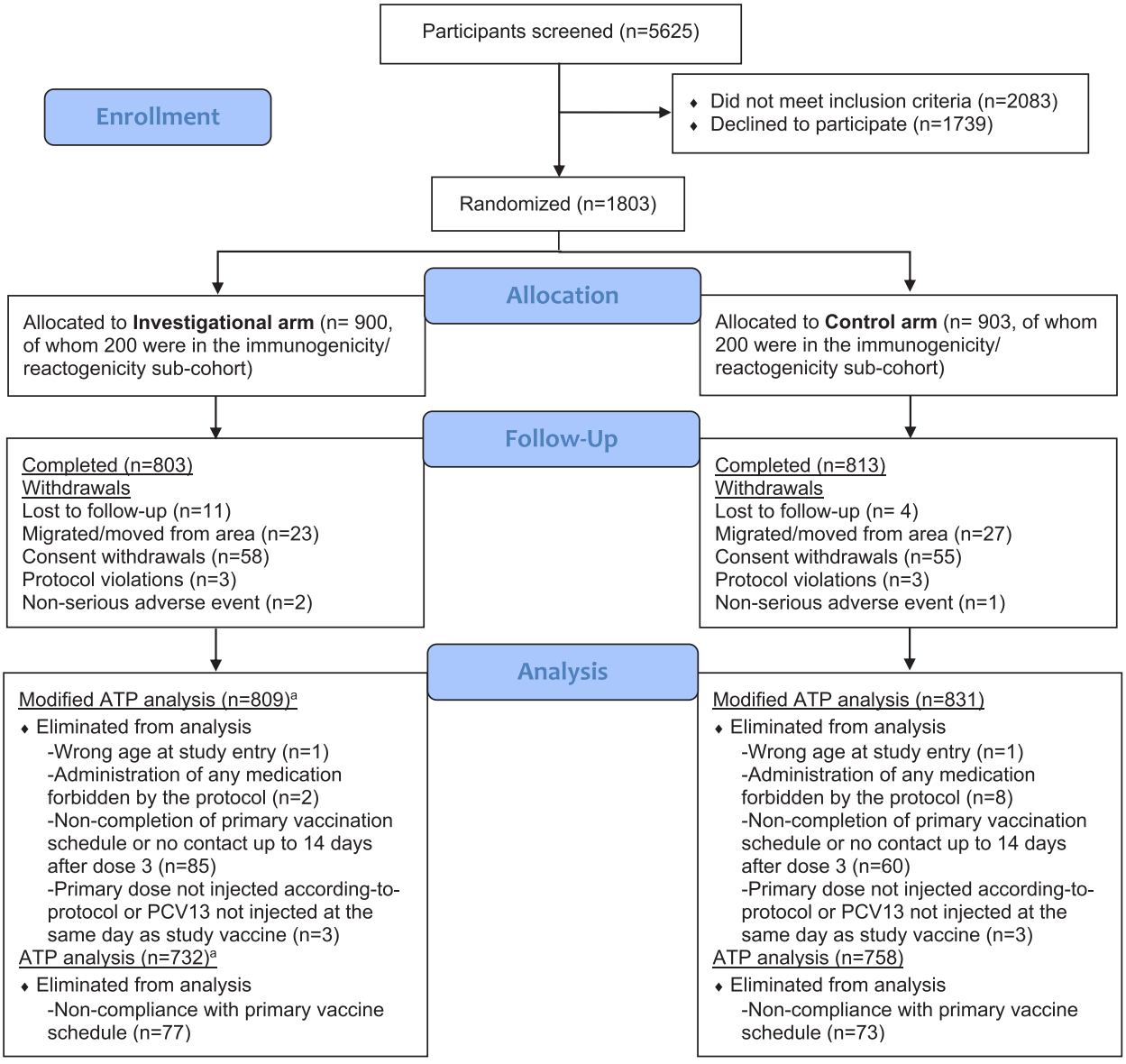

Fig. 2. Trial profile. ATP, according-to-protocol; PCV13, 13-valent pneumococcal conjugate vaccine. Note that children may have several criteria for elimination from ATP cohorts and only the primary reason is provided here. [a]One participant was censored before vaccine dose 3 and did not contribute to the efficacy analysis from two weeks after the administration of dose 3.

3.2. Reactogenicity and safety

Pain and irritability were the most frequently reported solicited local and general symptoms, respectively, after primary and booster vaccination (Table 4). Percentage of doses followed by at least one unsolicited and grade 3 unsolicited AEs were within similar ranges between groups. SAEs were reported from 229/900 (25.4%) dPly/PhtD and 232/903 (25.7%) Control children (Supplemental Table 8). Vaccination-related SAEs were reported from two children in the dPly/PhtD group (pyrexia plus diarrhea and pyrexia) and one in the Control group (febrile convulsion); no fatal SAEs were reported. IPD was reported from five children in the dPly/PhtD group and one in the Control group, all due to non-PCV13 types.

3.3. Immunogenicity

In the ATP immunogenicity cohort (N = 124/group), all children had anti-Ply and anti-PhtD antibody concentrations above the LLOQ of the assay at all timepoints. Antibody GMCs for both antigens were higher in the dPly/PhtD group than the Control group one month post-primary and post-booster vaccination and increased after primary and booster vaccination compared to baseline (Fig. 4). Twelve months post-booster vaccination, anti-Ply antibody GMCs in the dPly/PhtD group waned but remained higher compared to the Control group; while anti-PhtD antibody GMCs were within the same range for both groups. In exploratory analyses, we did not detect a clear relationship between pre- and post-primary vaccination anti-Ply and anti-PhtD antibody concentration (Supplemental Fig. 6).

4. Discussion

Currently available pneumococcal vaccines do not include antigens against all pneumococcal serotypes. Vaccines that offer serotype-independent protection, such as those targeting conserved proteins that are common to most or all pneumococcal strains, could substantially reduce global morbidity and mortality.

Table 2
Vaccine efficacy of dPly/PhtD vaccine against acute otitis media outcomes by cohort.

	Modified ATP cohort for efficacy Follow-up from 2 weeks after dose 3			ATP cohort for efficacy Follow-up from 2 weeks after dose 3			TVC Follow-up from dose 1		
	dPly/PhtD group N = 808	Control group N = 831	VE, % (95% CI)	dPly/PhtD group N = 731	Control group N = 758	VE, % (95% CI)	dPly/PhtD group N = 900	Control group N = 903	VE, % (95% CI)
All AOM episodes									
AAP-AOM	485[a]	518	3.8*	455	487	3.1	641	680	4.1
	(0.43)[b]	(0.44)	(−11.4, 16.9)	(0.44)	(0.45)	(−12.6, 16.7)	(0.40)	(0.42)	(−9.6, 16.0)
Modified AAP-AOM	648	702	5.2	607	659	4.5	853	913	4.9
	(0.57)	(0.60)	(−8.0, 16.8)	(0.58)	(0.61)	(−9.2, 16.5)	(0.54)	(0.57)	(−7.1, 15.5)
HCP-AOM	774	819	2.9	722	766	2.3	1019	1065	2.6
	(0.68)	(0.70)	(−9.5, 14.0)	(0.69)	(0.71)	(−10.8, 13.8)	(0.64)	(0.66)	(−8.7, 12.7)
First AOM episode									
AAP-AOM	309	346	11.3	289	324	9.9	382	405	6.3
	(0.37)	(0.42)	(−3.4, 23.9)	(0.38)	(0.43)	(−5.5, 23.1)	(0.33)	(0.36)	(−7.8, 18.5)
Modified AAP-AOM	380	425	11.2	356	398	9.7	459	486	5.9
	(0.50)	(0.57)	(−2.0, 22.6)	(0.52)	(0.59)	(−4.1, 21.8)	(0.44)	(0.47)	(−6.9, 17.2)
HCP-AOM	423	463	8.9	389	429	8.7	509	530	4.2
	(0.59)	(0.66)	(−3.9, 20.2)	(0.60)	(0.67)	(−4.8, 20.4)	(0.52)	(0.54)	(−8.2, 15.2)

dPly/PhtD Group = dPly/PhtD vaccine co-administered with PCV13 at 2, 4, 6 and 12–15 months of age.
Control Group = Placebo co-administered with PCV13 at 2, 4, 6 and 12–15 months of age.
dPly/PhtD, pneumolysin toxoid/pneumococcal histidine-triad protein D; ATP, according-to-protocol; TVC, total vaccinated cohort; CI, confidence interval; VE, vaccine efficacy; N, number of participants; AAP, American Academy of Pediatrics; AOM, acute otitis media; HCP, health-care provider; PCV13, 13-valent pneumococcal conjugate vaccine.
[a] Number of episodes.
[b] Incidence (episodes/child-year).
*Result for primary objective.

Table 3
Vaccine efficacy against acute lower respiratory tract infection outcomes by cohort.

	Modified ATP cohort for efficacy Follow-up from 2 weeks after dose 3			ATP cohort for efficacy Follow-up from 2 weeks after dose 3			TVC Follow-up from dose 1		
	dPly/PhtD group N = 808	Control group N = 831	VE, % (95% CI)	dPly/PhtD group N = 731	Control group N = 758	VE, % (95% CI)	dPly/PhtD group N = 900	Control group N = 903	VE, % (95% CI)
All ALRI episodes									
MA-ALRI	163[a]	165	−1.5	153	152	−4.4	236	236	−1.7
	(0.14)[b]	(0.14)	(−32.6, 22.4)	(0.15)	(0.14)	(−37.7, 20.9)	(0.15)	(0.15)	(−28.1, 19.3)
MA-ALRI with fever	125	123	−4.4	116	113	−6.4	169	162	−6.1
	(0.11)	(0.11)	(−39.2, 21.8)	(0.11)	(0.11)	(−43.8, 21.3)	(0.11)	(0.10)	(−36.7, 17.6)
MA-HCP-ALRI with fever	289	303	2.0	266	279	1.2	401	422	3.4
	(0.25)	(0.26)	(−18.3, 18.8)	(0.26)	(0.26)	(−20.3, 18.8)	(0.25)	(0.26)	(−14.4, 18.3)
First ALRI episode									
MA-ALRI	121	135	9.3	113	126	8.3	163	175	5.7
	(0.12)	(0.13)	(−15.9, 29.0)	(0.12)	(0.13)	(−18.2, 28.9)	(0.12)	(0.12)	(16.7, 23.9)
MA-ALRI with fever	99	106	4.9	91	98	4.6	127	134	4.0
	(0.09)	(0.10)	(−25.1, 27.7)	(0.09)	(0.10)	(−26.9, 28.3)	(0.09)	(0.09)	(−22.4, 24.7)
MA-HCP- ALRI with fever	207	226	8.2	188	211	10.0	267	286	6.4
	(0.22)	(0.24)	(−10.9, 24.0)	(0.22)	(0.24)	(−9.5, 26.1)	(0.21)	(0.22)	(−10.6, 20.8)

dPly/PhtD Group = dPly/PhtD vaccine co-administered with PCV13 at 2, 4, 6 and 12–15 months of age.
Control Group = Placebo co-administered with PCV13 at 2, 4, 6 and 12–15 months of age.
dPly/PhtD, pneumolysin toxoid/pneumococcal histidine-triad protein D; ATP, according-to-protocol; TVC, total vaccinated cohort; N, number of participants; CI, confidence interval; VE, vaccine efficacy; MA, medically-attended; ALRI, acute lower respiratory tract infection; HCP, health-care provider; PCV13, 13-valent pneumococcal conjugate vaccine.
[a] Number of episodes.
[b] Incidence (episodes/child-year).

In this study, dPly/PhtD vaccine was immunogenic and had an acceptable reactogenicity and safety profile after primary and booster vaccination when co-administered with PCV13; however, we did not observe significant efficacy against any of the pre-defined AOM or ALRI endpoints.

In pre-clinical studies, Ply and PhtD, administered either separately or in combination, provided protection against pneumococcal carriage and disease, including pneumonia in macaques [12,16,17,36]. Several other studies have investigated the impact of protein-based or whole cell vaccines against *colonization* endpoints in humans, but to our knowledge, this was the first study designed to evaluate an effect of pneumococcal proteins against *disease* in humans as a primary endpoint. Among infants in The Gambia study, the inclusion of pneumococcal proteins plus 10 serotype-specific polysaccharide conjugates (PHiD-CV/dPly/PhtD), administered at 2, 3, 4 months or 2, 4, 9 months of age did not reduce the prevalence or density of pneumococcal carriage beyond the effect of PHiD-CV [20]. The reasons for the differences in outcomes between animal models and human clinical trials are unclear. It has been suggested that the absence of an effect on carriage in infants in The Gambia may be a consequence of the acquisition of carriage early in life, along with other risk factors for high

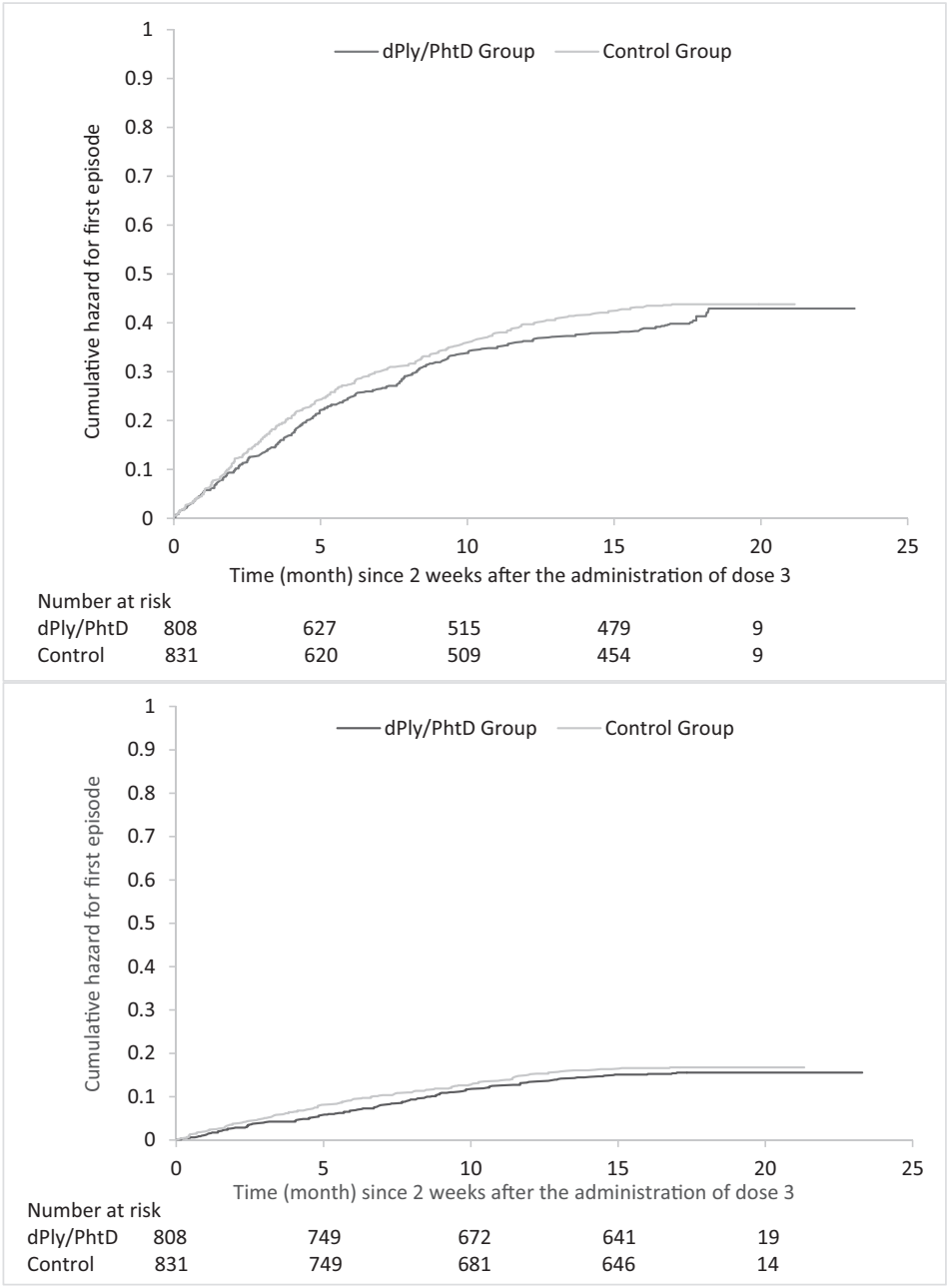

Fig. 3. Cumulative hazard curves for the first occurrence of AAP-AOM (top panel) and MA-ALRI (bottom panel), from 2 weeks after the administration of dose 3 (modified ATP cohort for efficacy). dPly/PhtD = dPly/PhtD vaccine co-administered with PCV13 at 2, 4, 6 and 12–15 months of age. Control = Placebo co-administered with PCV13 at 2, 4, 6 and 12–15 months of age. AAP, American Academy of Pediatrics; AOM, acute otitis media; MA, medically-attended; ALRI, acute lower respiratory tract infection; ATP, according-to-protocol; dPly/PhtD, pneumolysin toxoid/pneumococcal histidine-triad protein D; PCV13, 13-valent pneumococcal conjugate vaccine.

density carriage; however, populations with a high force of infection and a variety of risk factors are precisely where new vaccines are needed most.

In spite of the lack of demonstrated efficacy we observed a robust serum antibody response to the protein antigens. Antibody levels to the Ply component were substantially higher in the dPly/PhtD group compared to the Control group at all timepoints. Anti-Ply antibody concentrations were frequently beyond the assay ULOQ following the booster dose. Because values above the assay ULOQ were assigned as the ULOQ value, the anti-Ply antibody GMCs for dPly/PhtD recipients are underestimated. We were unable to quantify the functional response of anti-Ply antibodies (i.e., the inhibition of Ply hemolysis activity) because of issues with assay stability. For anti-PhtD, an increase in antibody GMCs one month post-dose 3 was observed compared to baseline; whereas antibody GMCs in the Control group declined. Levels of anti-PhtD antibody were similar at 12 months of age and, although there was an increase in antibody GMCs one month-post booster in dPly/PhtD recipients compared to the Control group, levels were similar again by 24-months of age. The pattern of anti-protein antibody responses was similar to findings in infants in The Gambia and in Europe, although the GMCs were substantially higher in the Native American infants at baseline (pre-vaccination) and post-primary vaccination compared to European infants who received dPly/PhtD containing vaccine at 2, 3, 4 and 12–15 months and Gambian infants who were vaccinated at 2, 3, 4 months [20,22]. The high baseline anti-protein antibody levels in this study presumably result from exposure at a young age or from maternal

Table 4
Percentages of doses (with 95% confidence interval) followed by solicited or unsolicited adverse events (AEs) – immunogenicity/reactogenicity sub-cohort (total vaccinated cohort).

	3-dose primary vaccination phase		Booster dose	
	dPly/PhtD group	Control group	dPly/PhtD group	Control group
Solicited AEs at dPly/PhtD vaccine or $AlPO_4$ Placebo injection site (days 0–3)				
	N = 551	**N = 553**	**N = 156**	**N = 153**
Pain	69.3 (65.3, 73.2)	66.2 (62.1, 70.1)	64.1 (56.0, 71.6)	54.2 (46.0, 62.3)
Grade 3	14.3 (11.5, 17.5)	17.9 (14.8, 21.4)	15.4 (10.1, 22.0)	11.8 (7.1, 18.0)
Redness	31.6 (27.7, 35.6)	31.1 (27.3, 35.1)	37.8 (30.2, 45.9)	39.2 (31.4, 47.4)
>30 mm	0.2 (0.0, 1.0)	0.0 (0.0, 0.7)	0.0 (0.0, 2.3)	0.0 (0.0, 2.4)
Swelling	22.7 (19.3, 26.4)	16.1 (13.1, 19.4)	25.0 (18.4, 32.6)	24.2 (17.6, 31.8)
>30 mm	0.2 (0.0, 1.0)	0.0 (0.0, 0.7)	0.0 (0.0, 2.3)	0.0 (0.0, 2.4)
General solicited AEs (days 0–3)				
	N = 551	**N = 554**	**N = 156**	**N = 154**
Drowsiness	45.0 (40.8, 49.3)	46.9 (42.7, 51.2)	41.7 (33.8, 49.8)	42.2 (34.3, 50.4)
Grade 3	6.5 (4.6, 8.9)	3.4 (2.1, 5.3)	8.3 (4.5, 13.8)	7.8 (4.1, 13.2)
Irritability	64.4 (60.3, 68.4)	62.3 (58.1, 66.3)	62.2 (54.1, 69.8)	55.8 (47.6, 63.8)
Grade 3	9.4 (7.1, 12.2)	10.8 (8.4, 13.7)	17.3 (11.7, 24.2)	10.4 (6.1, 16.3)
Loss of appetite	24.9 (21.3, 28.7)	23.6 (20.2, 27.4)	27.6 (20.7, 35.3)	26.6 (19.8, 34.3)
Grade 3	2.5 (1.4, 4.2)	1.8 (0.9, 3.3)	3.8 (1.4, 8.2)	4.5 (1.8, 9.1)
Fever[a]	7.6 (5.5, 10.2)	10.6 (8.2, 13.5)	5.1 (2.2, 9.9)	9.7 (5.6, 15.6)
>40.0 °C	0.0 (0.0, 0.7)	0.2 (0.0, 1.0)	0.0 (0.0, 2.3)	0.0 (0.0, 2.4)
Unsolicited AEs[b] (days 0–30)				
	N* = 572	**N* = 579**	**N* = 178**	**N* = 174**
Any	29.4 (25.7, 33.3)	28.0 (24.4, 31.8)	29.8 (23.2, 37.1)	27.0 (20.6, 34.3)
Grade 3	3.1 (1.9, 4.9)	3. (1.9, 4.9)	5.6 (2.7, 10.1)	2.9 (0.9, 6.6)

dPly/PhtD Group = dPly/PhtD vaccine co-administered with PCV13 at 2, 4, 6 and 12–15 months of age.
Control Group = Placebo co-administered with PCV13 at 2, 4, 6 and 12–15 months of age.
dPly/PhtD, pneumolysin toxoid/pneumococcal histidine-triad protein D; N/N*, number of documented/administered doses; grade 3, crying when limb was moved/limb spontaneously painful (pain), preventing normal everyday activity (drowsiness, unsolicited AEs), crying inconsolably/preventing normal everyday activity (irritability), not eating at all (loss of appetite); PCV13, 13-valent pneumococcal conjugate vaccine.
[a] ≥38.0 °C (regardless of the route of measurement; axillary temperature presented here).
[b] The most frequently reported unsolicited AEs were upper respiratory tract infection, viral infection, pyrexia, cough and rhinorrhea post-primary vaccination, and pyrexia and upper respiratory tract infection post-booster.

antibody transfer *in utero*, both of which are plausible given the high burden of pneumococcal carriage and disease in this population [8,37,38]. In a post-hoc analysis, we did not find any evidence to suggest that pre-vaccination antibody interfered with dPly/PhtD immunogenicity.

It is important to acknowledge that this study measured serum but not mucosal antibodies. In a prospective study of 100 healthy infants 6–24 months of age, higher mucosal antibody levels to PhtD and dPly correlated with reduced risk of development of pneumococcal AOM in children, although no significant difference was found in mucosal antibody levels to PhtD and dPly between children with and without pneumococcal carriage [39]. Results were not stratified by episode number so it is unclear if these findings were similar between first and subsequent episodes of AOM. While the study presented herein did not measure mucosal antibodies – a step that should be considered in future studies of pneumococcal protein vaccines – the serum antibody levels intimate that mucosal levels were also likely high among dPly/PhtD vaccine recipients.

There are several possible reasons that efficacy was not observed, despite very good serum antibody responses to the dPly/PhtD vaccine. Pre-clinical studies did not examine the effect of GSK's dPly or PhtD antigens on AOM. Other studies have shown potential protection by pneumococcal proteins, including PhtD and Ply, against AOM in a murine model; however, these findings might not translate to humans [40]. Additionally, differences in the production method might lead to antigenic differences that could affect efficacy. The study was powered to detect an incremental VE of 17% or greater against the syndrome of AOM; this may have been too high a bar given that the dPly/PhtD vaccine was being co-administered with PCV13. The reported VE of licensed PCVs against clinical AOM assessed in clinical trials ranged up to a 15% reduction, but was non-statistically significant [41]; we used a slightly higher estimated VE given the serotype-independent nature of the dPly/PhtD vaccine. At the time the study was designed, the available data suggested that around a quarter of AOM was pneumococcal; however, the burden of pneumococcal AOM, particularly amongst cases of recurrent AOM, has continued to decline in the PCV era and more recent data suggest an increasing role of other pathogens, particularly *H. influenzae* [42,43]. Accurately diagnosing AOM can be challenging and overdiagnosis of AOM is common; inclusion of events that were not truly AOM would reduce the ability to detect efficacy. We attempted to

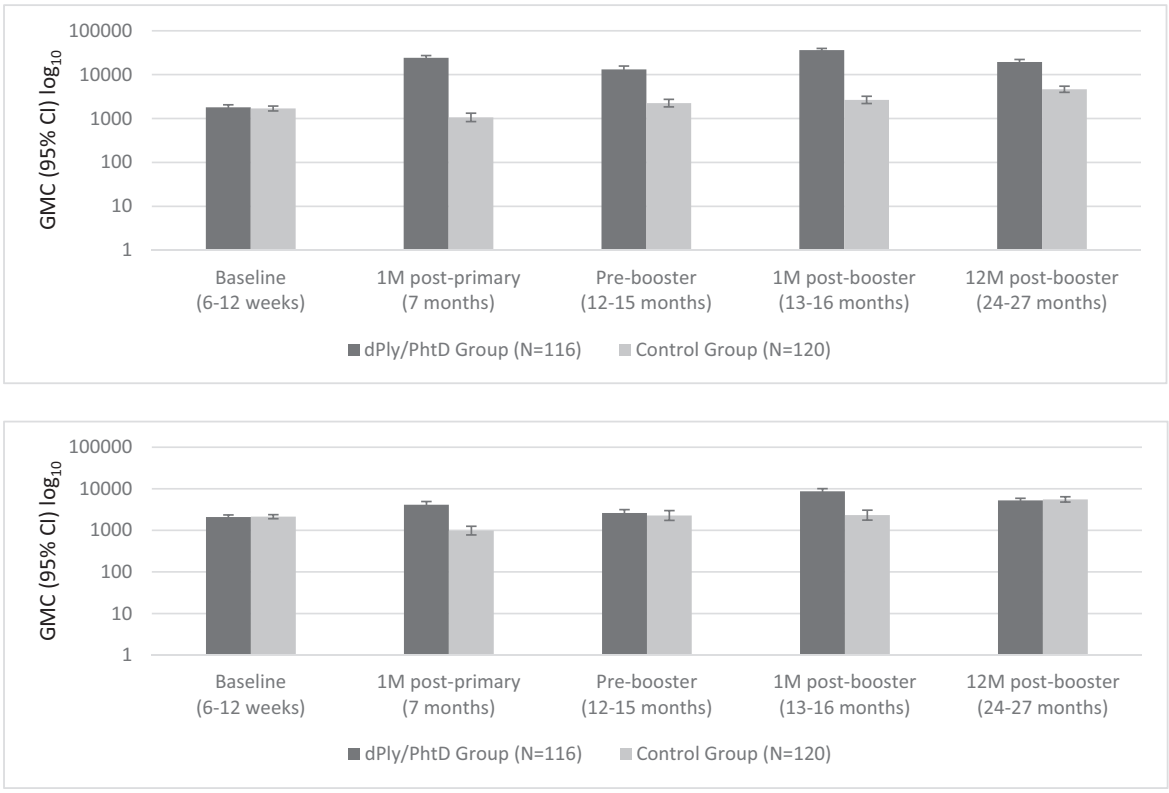

Fig. 4. Antibody geometric mean concentrations (GMCs) against pneumococcal Ply (top panel) and PhtD (bottom panel) proteins (ATP cohort for immunogenicity). dPly/PhtD Group = dPly/PhtD vaccine co-administered with PCV13 at 2, 4, 6 and 12–15 months of age. Control Group = Placebo co-administered with PCV13 at 2, 4, 6 and 12–15 months of age. dPly/PhtD, pneumolysin toxoid/pneumococcal histidine-triad protein D; ATP, according-to-protocol; CI, confidence interval; M, month; N, maximum number of participants with available results; PCV13, 13-valent pneumococcal conjugate vaccine.

minimize incorrect AOM diagnoses by training providers on the diagnosis of AOM through in-person sessions at the start of the study and online refresher modules during the study, and by using a standardized template for capture of AOM outcomes that included all relevant elements of the history and exam [44]. During the course of the study, the AAP published updated criteria for diagnosis of AOM, which were intended to more clearly differentiate AOM from otitis media with effusion. Because the study was ongoing, no changes to the study objectives were made apart from collection of new data to allow assessment of efficacy according to the updated definition (AAP-AOM [2013]) [45]. VE against episodes of AAP-AOM was similar using the 2004 and 2013 definitions; however, this analysis was limited because some details of the AOM events could not be collected retrospectively. Reassuringly, the incidence rate of AOM in this study (0.43–0.44 episodes/child-year) was similar to that documented by tympanocentesis among children aged 6–36 months in Rochester, New York with AAP-AOM [2013] in the PCV era (0.38 episodes/child-year during the first year of life and 0.48 episodes/child-year during the second year of life) [42]. Nevertheless, AOM may still have been mis- or overdiagnosed, which could have resulted in the study being underpowered and biased toward the null hypothesis. In addition, culture of middle ear fluid to assess the etiology of draining AOM was done at the discretion of the clinical provider and not as a study procedure.

Efficacy tended to be higher against first AOM and ALRI episodes compared to subsequent episodes. This finding, along with the observed antibody profiles over the course of the study, prompted us to conduct a post-hoc analysis in the mATP cohort of VE against first AOM and first MA-ALRI events at age <12 months. There was a suggestion of incremental VE of dPly/PhtD vaccine beyond that of PCV13, which was more pronounced for MA-ALRI. Compared to the Control group, dPly/PhtD vaccine recipients had higher antibody GMCs to PhtD at 7 months of age, but this difference was no longer evident by 1 year of age. PhtD plays a role in adherence of *S. pneumoniae* to human nasopharyngeal epithelial cells [46]. It is possible that the trend toward greater protection among dPly/PhtD vaccine recipients against first AOM or ALRI episodes in the first year of life relates to the notable difference in anti-PhtD levels, and that this clinical benefit was no longer evident when levels became more similar to those of the Control group. However, the lack of consistent and statistically significant findings limits our ability to make conclusions. Another possible reason that efficacy may have been greater against first episodes relates to the role of biofilms. Although not assessed in this study, biofilms are typically associated with recurrent infections and allow bacteria to resist host immune responses [47]. Dispersed biofilm bacteria have increased virulence; animal models that evaluate the impact of anti-protein antibodies on disease using broth-grown bacteria, as opposed to dispersed biofilm bacteria, may not accurately reflect pathogenesis in humans.

Because influenza vaccination and season might impact the incidences of AOM and ALRI [34,35], we also conducted an exploratory analysis to understand their potential impact on dPly/PhtD efficacy. This post-hoc analysis did not show a consistent impact of influenza vaccination on the VE of dPly/PhtD against AOM and ALRI. The small number of children in the subsets and hence the low number of AOM and ALRI cases recorded are a limitation for this analysis.

The frequency of solicited and unsolicited AEs was similar in dPly/PhtD and Control participants after primary and booster vaccination. IPD events, which were identified through the surveil-

lance for SAEs since they were not disease outcome events in the protocol, were more common in the dPly/PhtD group (n = 5) compared to the Control group (n = 1), although this was not a statistically significant difference. As part of a long-standing population-based Active Bacterial Surveillance (ABS) system for IPD at the study sites, two additional cases of IPD in participants from the Control group were identified: one in a participant who completed the study before IPD occurred at 31 months of age, and one in a participant that had withdrawn from the study prior to the occurrence of IPD at 25 months of age. Because of these circumstances the events were not included in the clinical study database although they were reported to ABS. The study was not powered to detect a difference in IPD outcomes and the work-up for possible invasive bacterial infection was done at the discretion of the child's provider, rather than systematically as part of the study.

In conclusion, this study showed that vaccination with dPly and PhtD antigens was well-tolerated and immunogenic but did not show additional protection to children against AOM or ALRI. The interactions between immunity, carriage, and progression to disease are complex and poorly understood. Future studies should aim to improve our understanding of the effect of anti-protein antibodies on pathogenesis of pneumococcal disease.

5. Trademark statement

Synflorix is a trademark of the GSK group of companies. *Prevenar 13/Prevnar 13* is a trademark of Pfizer, Inc.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [**Potential Conflict of interest:** L.L. Hammitt, J.C. Campbell, R.C. Weatherholtz, R. Reid, M. Santosham and K.L. O'Brien report institutional research grants from the GSK group of companies, during the conduct of the study. L.L. Hammitt, R.C. Weatherholtz and K.L. O'Brien also report institutional grants from Pfizer, Novavax and Merck outside the submitted work. M. Santosham also reports an institutional research grant for Rota Council from the GSK group of companies. K.L. O'Brien also reports to be an external scientific advisor for Pfizer, Sanofi Pasteur, Merck and the GSK group of companies. L.H. Moulton reports personal fees from a Merck Scientific Advisory Board outside the submitted work. N. Goklish has no conflict of interest to disclose. M. Traskine, K. Swinnen and D. Borys are employees of the GSK group of companies, and K. Swinnen and D. Borys hold shares of the GSK group of companies. Y. Song worked for XPE Pharma & Science as a consultant for the GSK group of companies.]

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Authors' contributions

All authors have substantially contributed to the submitted work. L. H. codesigned and performed the study, collected, analyzed, and interpreted the data, and wrote the manuscript. J. C., R. W., and M. S. codesigned and performed the study, and collected, analyzed, and interpreted the data. D. B., K. S., and K. O'B. codesigned the study and analyzed and interpreted the data. R. R. performed the study and collected, analyzed, and interpreted the data. N. G. performed the study and collected the data. M. T., Y. S., and L. M. analyzed and interpreted the data. All authors reviewed and edited the manuscript, and approved its submission.

All authors attest they meet the ICMJE criteria for authorship.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.09.076.

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