Turkish Journal of Medical Sciences

Volume 47 | Number 4 Article 30

1-1-2017

A fully liquid DTaP-IPV-HB-PRP-T hexavalent vaccine for primaryand booster vaccination of healthy Turkish infants and toddlers

MEHMET CEYHAN

İNCİ YILDIRIM

HASAN TEZER

İLKER DEVRİM

EMMANUEL FEROLDI

TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2017) 47: 1247-1256

doi:10.3906/sag-1609-62

A fully liquid DTaP-IPV-HB-PRP-T hexavalent vaccine for primary and booster vaccination of healthy Turkish infants and toddlers*

Mehmet CEYHAN[1], İnci YILDIRIM[1,2], Hasan TEZER[1], İlker DEVRİM[1,3], Emmanuel FEROLDI[4,]**

[1]Faculty of Medicine, Hacettepe University, Ankara, Turkey
[2]Departments of Pediatrics and Epidemiology, Schools of Medicine and Public Health, Boston University, Boston, MA, USA
[3]Pediatric Infectious Disease Unit, Dr Behçet Uz Children's Hospital, İzmir, Turkey
[4]Sanofi Pasteur, Lyon, France

Received: 16.09.2016 • **Accepted/Published Online:** 15.04.2017 • **Final Version:** 23.08.2017

Background/aim: Immunogenicity and safety of a primary series of a fully liquid, hexavalent DTaP-IPV-HB-PRP-T vaccine given at 2, 3, and 4 months of age compared to licensed comparators and a DTaP-IPV-HB-PRP-T booster at 15–18 months were evaluated.

Materials and methods: This was a Phase III, randomized, open-label trial. Primary series (no hepatitis B [HB] at birth) of DTaP-IPV-HB-PRP-T (N = 155) (group 1) or licensed control vaccines (DTaP-IPV//PRP-T and standalone HB: N = 155) (group 2) and DTaP-IPV-HB-PRP-T booster were administered. Noninferiority was evaluated 1 month postprimary series for anti-HB seroprotection (SP). All other analyses were descriptive. Safety was assessed from parental reports.

Results: Postprimary series noninferiority of anti-HB ≥ 10 mIU/mL was demonstrated for the DTaP-IPV-HB-PRP-T vaccine (94.0%) compared to the licensed control (96.1%). Postprimary series primary SP and seroconversion (SC) rates were high and similar for both groups. Antibody persistence (prebooster) was high for each antigen and similar between groups except for HB, which was lower for DTaP-IPV-HB-PRP-T than for standalone HB. For each antigen except HB, DTaP-IPV-HB-PRP-T booster responses were high and similar in each group. Safety was good for primary and booster series and similar between groups.

Conclusion: The DTaP-IPV-HB-PRP-T vaccine is immunogenic and safe when administered in a challenging primary series schedule without HB vaccination at birth.

Key words: Hexavalent, vaccine, pediatric, Turkey

1. Introduction

In recent years the improved safety of acellular pertussis (aP) combination vaccines compared to whole cell pertussis (wP) vaccines (1,2) together with the growing importance of the inactivated poliovirus vaccine (IPV) compared to the oral poliovirus vaccine (OPV) in the context of the polio eradication endgame (3) has driven the need for their wider availability. In this context, Sanofi Pasteur has built on the wide experience with Pentaxim/Pentavac, an established pediatric pentavalent diphtheria (D), tetanus (T), aP, IPV, and *Hemophilus influenzae* type b (PRP-T) vaccine (4), to develop a fully liquid, hexavalent DTaP-IPV-hepatitis B (HB)-PRP-T vaccine (Hexaxim/Hexyon/Hexacima) that has the same D, T, aP, IPV, and PRP-T composition as Pentaxim/Pentavac. Additionally, the hexavalent vaccine incorporates a HB antigen of proven immunogenicity and safety (5–8). Such combination vaccines are increasingly pivotal to national immunization schedules globally and are increasingly important in addressing regional disparities in vaccination coverage and composition (4,9).

In previous studies, the investigational DTaP-IPV-HB-PRP-T vaccine has been shown to be safe and immunogenic when administered in a 3-dose primary series schedule at 2, 4, and 6 months (7) and 6, 10, and 14 weeks (10), with or without the administration of a standalone HB vaccine at birth (10–13). Additionally, a booster dose in the second year of life has been shown to be safe and to elicit an anamnestic response irrespective of HB vaccination at birth (11,14).

As part of an extensive clinical development plan conducted largely outside Europe (Central America

* Data presented at the 5th Asian Congress of Pediatric Infectious Diseases, 23–26 September 2010, Taipei, Taiwan

** Correspondence: emmanuel.feroldi@sanofipasteur.com

(11), South America (8,13,15,16), Thailand (12), and South Africa (10, 14), and prior to the approval of this vaccine by the European Medicines Agency in 2013 via the Centralised Procedure (17), the present study was performed in Turkey to evaluate the performance of the vaccine when used with a 2, 3, 4 month infant primary series versus control vaccines (without a HB vaccination at birth) and also to assess the immunogenicity before and after a toddler booster dose of the investigational vaccine in the second year of life. This corresponded to the infant DTaP vaccination schedule in use in Turkey at the time of the study. The 2, 3, 4 month schedule, with only 1 month between vaccinations, is more challenging than the more widely studied 2, 4, 6 month primary series schedule in terms of generating strong and lasting immune responses against HB, especially in the absence of a birth dose of a standalone HB vaccine. However, previous results following administration of DTaP-IPV-HB-PRP-T in the even more challenging 6, 10, 14 week schedule in South African infants with or without HB vaccination at birth were encouraging (10,14), and in order for a new combination vaccine to have global implications, a wide range of vaccination schedules needs to be evaluated.

2. Materials and methods

2.1. Study design and participants

Two consecutive Phase III studies were carried out at a single center in Turkey (Hacettepe University Medical Faculty, Ankara, Turkey): the first, infant primary series vaccination study, was randomized, active-controlled, and open-label (ClinicalTrials.gov: NCT00315055), and in the second study all toddler participants received the same booster vaccination (ClinicalTrials.gov: NCT00619502). The study site's independent ethics committee approved the study protocols and amendments. Both studies conformed to Good Clinical Practice, applicable International Conference on Harmonization guidelines, the ethical principles of the Declaration of Helsinki (Edinburgh revision, October 2000), and the European Directive 2001/20/EC, and were conducted in accordance with local regulations. Prior to enrolment, at least one parent or legally acceptable representative (and witness for the booster study only), as well as the study investigator, signed an informed consent form. The primary and booster studies took place between June 2006 and June 2007 and December 2007 and July 2008, respectively.

Healthy infants aged 2 months, born at full term (≥37 weeks), and with birth weight ≥2.5 kg were eligible for the primary series study. The main exclusion criteria were: recent (in the 4 weeks prior to the first vaccination) or planned participation in another clinical trial or nonstudy vaccination during or in the 4 weeks prior to the study period (except Bacillus Calmette-Guerin vaccination) or any planned nonstudy vaccination in the 4 weeks after each vaccination; any prior vaccination against diphtheria, tetanus, pertussis, poliomyelitis, HB, *Hemophilus influenzae* type b (Hib) diseases, or any history of these infections; receipt of blood products since birth or history of any immune-modifying treatment; personal/maternal history of immunodeficiency, including human immunodeficiency virus, HB surface antigen (HBsAg), or hepatitis C positivity; known systemic hypersensitivity to any vaccine component; history of seizures; bleeding disorder contraindicating intramuscular (IM) injection; chronic illness that could interfere with study conduct/completion; or febrile (axillary temperature ≥37.4 °C [rectal equivalent ≥38.0 °C]) at enrolment. Children were excluded from the booster study if they had, additionally: previous booster vaccination against diphtheria, tetanus, pertussis, poliomyelitis, HB, or Hib; any vaccine-related serious adverse event (SAE) that occurred during the primary study; any known contraindication to further vaccination with a pertussis vaccination.

Participants were randomized equally in the primary study to receive the investigational DTaP-IPV-HB-PRP-T vaccine (Hexaxim/Hexyon/Hexacima) (group 1) or control vaccines, DTaP-IPV//PRP-T (Pentaxim/Pentavac) co-administered with a standalone HB vaccine (Engerix B) (group 2), at 2, 3, and 4 months of age. A permuted block randomization method was used to guarantee similar number of participants in each group at any time. All participants in the booster study received a single dose of DTaP-IPV-HB-PRP-T at 15–18 months of age. In the primary study, vaccines were administered into the right thigh (DTaP-IPV-HB-PRP-T or DTaP-IPV//PRP-T) and left thigh (HB [group 2 only]), and in the booster study DTaP-IPV-HB-PRP-T was administered into the right deltoid muscle.

2.2. Vaccines

The investigational hexavalent vaccine (batch numbers PFAGI00701B [infant primary series study] and S4009 [toddler booster study]) was manufactured by Sanofi Pasteur, France, and supplied as a fully liquid suspension for injection in single dose (0.5 mL) prefilled syringes. Each prefilled syringe contained ≥20 IU (30 limit of flocculation [Lf]) D-toxoid; ≥40 IU (10 Lf) T-toxoid; 25 μg PT; 25 μg FHA; 40, 8, and 32 D antigen units of poliovirus type 1, 2, and 3, respectively; 10 μg HBsAg; 12 μg Hib polysaccharide conjugated to 22–36 μg tetanus protein (PRP-T); and 0.6 mg aluminum hydroxide.

The control pentavalent vaccine was supplied by Sanofi Pasteur as a separate DTaP-IPV suspension (batch number Z0165) and freeze-dried PRP-T (batch number Y0660), which were reconstituted to provide a 0.5 mL dose prior

to vaccination. The pentavalent vaccine had the same D, T, aP, IPV, and PRP-T composition as the investigational vaccine. The HB standalone vaccine was manufactured by GlaxoSmithKline (commercial batch) and presented as a 0.5 mL dose containing 10 μg purified recombinant HBsAg in a prefilled syringe.

2.3. Serology

Blood samples were collected prior to the first vaccination (3 mL), 1 month after the third vaccination (5 mL: postprimary series response), prior to the booster vaccination (5 mL: antibody persistence), and 1 month after the booster vaccination (5 mL: booster response) for immunogenicity assessment of the investigational and control vaccines.

All assays were performed either at the Sponsor's Global Clinical Immunology (GCI) laboratory in the USA or at qualified contract laboratories approved by GCI. Antidiphtheria antibody concentrations (IU/mL) and antipolio 1, 2, 3 antibody titers (1/dil) were assayed by a neutralization assay (with an assay against Mahoney, MEF-1, and Saukett poliovirus strains), antitetanus (IU/mL), anti-FHA (EU/mL), anti-PT (EU/mL) concentrations by an enzyme linked immunosorbent assay (ELISA), anti-PRP-T (μg/mL) concentrations by a radioimmunoassay, and anti-HB concentrations (mIU/mL) by a commercially available chemiluminescence assay (VITROS ECi/ECiQ).

2.4. Reactogenicity and safety

Participants were monitored for immediate unsolicited adverse events (AEs) for 30 min after each vaccination. For 7 days after each vaccination, parent(s)/legal representative(s) used diary cards to record the duration and intensity (see Tables 4 and 5 for definitions of intensity)[1] of predefined (solicited) injection site (tenderness, erythema, swelling [recorded separately for DTaP-IPV//PRP-T and HB vaccination sites in group 2], and extensive swelling of the vaccinated limb[2]) and systemic (fever, vomiting, abnormal crying, drowsiness, appetite lost, irritability) reactions (also considered by definition to be related to the vaccination). For temperature measurement the axillary route was used for cultural and compliance reasons.

Unsolicited AEs were recorded using diary cards for 30 days after each vaccination: unsolicited injection site AEs were considered to be related to the vaccination and for unsolicited systemic AEs the Investigator assessed the relationship to the vaccination. Serious adverse events were collected throughout until 6 months after the last primary series or the booster vaccination. The Investigator assessed their relationship to the vaccination.

2.5. Statistical analyses

The primary statistical objective of the primary series study was to demonstrate noninferiority of the anti-HB response, based on the seroprotection threshold of 10 mIU/mL, of the investigational vaccine compared to the control vaccines 1 month postprimary series. The secondary objective of the primary series study was to describe the immune response for each antigen at 1 month postprimary series, and for the booster study objectives were to describe the prebooster (antibody persistence), and 1 month postbooster response. Safety was evaluated throughout the two studies.

Seroprotection rates (D, T, IPV, HB, PRP-T), seroconversion rates (PT, FHA), geometric mean titers (GMTs, for IPV), and geometric mean concentrations (GMCs, for D, T, PT, FHA, HB, PRP-T) with their associated 95% confidence intervals (CIs) were used to describe the immune responses, and are presented in Table 1. The 95% CIs were calculated using the normal approximation method for GMCs and GMTs and using the exact binomial distribution (Clopper–Pearson method) for percentages (18).

The noninferiority analysis for the primary objective (comparison of the HB component of the investigational vaccine to the standalone HB vaccine administered with Pentaxim/Pentavac) was carried out using the per protocol (PP) analysis set (and confirmed using the Full Analysis Set [FAS]) based on the lower bound of the two-sided 95% CI of the difference in anti-HB seroprotection rates (≥10 mIU/mL) (group 1–group 2), with noninferiority being concluded if the lower bound of this 95% CI was >−10% (the clinical delta). The 95% CI was calculated using the Wilson score method without continuity correction as quoted by Newcombe (19).

Safety was described by vaccine group after each and any vaccine administration. For each safety criterion (symptom) the percentage of participants with the given symptom was calculated with its 95% CI.

The sample size calculation was based on the noninferiority test for the primary objective, with a planned sample size of 310 participants (155 participants in each group) to allow 258 evaluable participants (assuming an attrition rate of approximately 15%). The sample size was calculated using the Farrington and Manning formula (20), with an alpha level of 2.5% (one-sided hypothesis) and to obtain an overall power of 90%.

Data from the PP population (participants with no protocol violation that could have interfered with the

[1] Not applicable for extensive swelling of the vaccinated limb, which was only recorded for the booster vaccination, and for which the circumference of the injected limb was to have been measured and recorded in the diary card and any such occurrence was to be considered Grade 3 by convention.

[2] Extensive swelling of the vaccinated limb was only assessed after the booster vaccination.

primary evaluation criteria, and analyzed according to the vaccine received) were presented for all immunogenicity assessments. The safety evaluation used the safety analysis set (SafAS) (participants who received at least one primary vaccination and all who received the booster, and analyzed according to the primary series vaccine received). Data from the FAS (those who received at least one vaccination, and analyzed according to the randomization) supported the evaluation done using the PP population.

The statistical analyses were done using SAS software Version 8.2 for the primary series study and Version 9.1 for the booster study (SAS Institute, Cary, NC, USA).

3. Results

3.1. Participants studied

In total, 310 participants were enrolled for primary infant vaccination, with 155 randomized to each group. All received at least one dose of vaccine and 152 and 150 participants in groups 1 and 2, respectively, completed the primary infant series in accordance with the protocol. Of these, 130 and 124 returned for the booster vaccination; all 254 were assessed for antibody persistence prior to the DTaP-IPV-HB-PRP-T booster vaccination, with 122 and 114 completing the booster phase in groups 1 and 2, respectively. Demographic characteristics were similar in each group, and participant disposition is summarized in Figure 1.

3.2. Immunogenicity

For the anti-HB response 1 month after the primary vaccination series, the seroprotection rate (≥10 mIU/mL) with its 95% CI was 94.0 (88.6; 97.4) in group 1 and 96.1 (91.1; 98.7) in group 2 (Table 1). The statistical difference (group 1–group 2) was –2.06 (–7.83; 3.65), thereby confirming noninferiority of group 1 to group 2, as the lower 95% CI for the difference was >–10% (Table 1).

The full immunogenicity data for each antigen, threshold, and including the GMCs and GMTs for postprimary, prebooster, and postbooster are presented in Table 2. No statistical comparisons were performed for these data. For the evaluation at 1 month postprimary series, the seroprotection rates were similar in both groups for all antigens. Prior to the booster, the majority of participants remained seroprotected against HB (≥10 mIU/L), PRP-T (≥0.15 μg/mL), D (≥0.01 IU/mL), T (≥0.01 IU/mL), polio (≥8 [1/dil]), and GMCs for anti-PT and anti-FHA were similar in each group. However, it is noted that the prebooster anti-HB ≥10 mIU/mL was significantly lower (based on nonoverlapping CIs) for group 1 (80.7% [GMC 44.2 mIU/mL)]) than for group 2 (99.0% [GMC 223 mIU/mL]). One month postbooster vaccination, the anti-HB seroprotection was similar in each group for both the 10 mIU/mL threshold (97.3% and 98.6%) and the 100 mIU/mL threshold (86.5% and 93.0%), although the GMCs were significantly lower (based on nonoverlapping 95% CIs) for group 1 (1379 mIU/mL) than for group 2 (26189 mIU/mL). For the remaining valences, strong postbooster increases in antibody GMCs and GMTs were observed, resulting in high seroprotection (PRP-T, D, T, polio 1, 2, 3) and seroconversion (PT and FHA) rates, which were similar in each group.

The anti-HB booster response was further investigated in a post hoc analysis by assessing the response in those participants with a prebooster anti-HB concentration <10 mIU/mL and those ≥10 mIU/mL (Table 3). It should be noted that this analysis was based on a small sample size and is descriptive. This analysis showed that the postbooster response at the 10 mIU/mL threshold was similar for both subgroups (100% and 95.0% of participants ≥10 mIU/mL postbooster, for a prebooster anti-HB concentration of ≥10 mIU/mL and <10 mIU/mL, respectively), i.e. was independent of the prebooster anti-HB concentration. However, at the 100 mIU/mL threshold and in terms of GMC the response was higher in participants who had prebooster anti-HB levels ≥10 mIU/mL (79.9% and 50.0% of participants ≥100 mIU/mL postbooster, for a prebooster anti-HB concentration of ≥10 mIU/mL and <10 mIU/mL, respectively, with corresponding GMCs of 2745 mIU/mL and 135 mIU/mL).

3.3. Safety and tolerability

No immediate AEs were reported, and the solicited injection site and systemic reactions for both the primary series and booster vaccination are summarized in Table 4 (injection site) and Table 5 (systemic).

For the primary series, the overall incidence of solicited reactions was similar for the two vaccine groups and most were Grade 1 or 2. In each group, tenderness was the most common solicited injection site reaction (62.1% and 53.9% of participants for the DTaP-IPV-HB-PRP-T and DTaP-IPV//PRP-T vaccines, respectively) and irritability was the most common solicited systemic reaction (68.0% and 65.1% of participants for the DTaP-IPV-HB-PRP-T and DTaP-IPV//PRP-T vaccines, respectively). Overall, the incidence of fever was slightly higher in group 1 (41.2% and 28.3% of participants for the DTaP-IPV-HB-PRP-T and DTaP-IPV//PRP-T vaccines, respectively), but episodes classified as Grade 3 were similar in incidence in each group 2.0% of participants in each group); for all other solicited reactions the incidence was similar in each group both overall and for Grade 3. Unsolicited AEs within 7 days after any primary series vaccination were similar in each group (3.3% versus 5.9% in groups 1 and 2, respectively), with none in group 1 and a single episode of diarrhea in group 2 considered related to the vaccination. In both groups, unsolicited AEs occurring within 30 days of vaccine injection were reported by <19% of participants. There were no withdrawals due

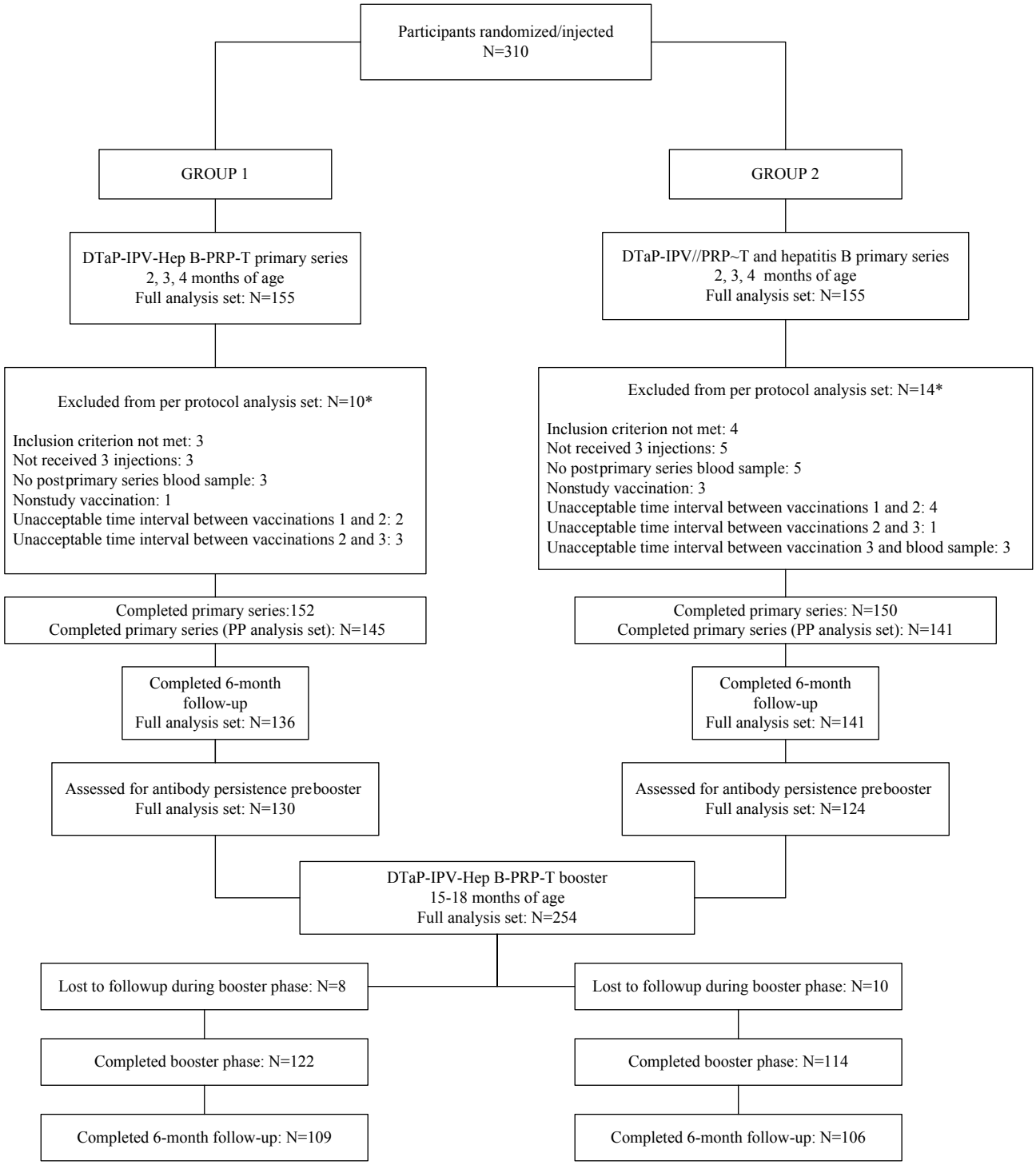

Figure 1. Disposition of study participants.
*Note that participants could have more than one reason for exclusion from the PP analysis set.

to an AE and the overall incidence of SAEs was 1.3% (2 participants) in group 1 (bronchopneumonia and upper respiratory tract infection) and 2.0% (3 participants) in group 2 (bronchiolitis, bronchopneumonia, and road traffic accident); no SAE was considered to be related to vaccination, all occurred before the 6-month follow-up period, and there were no deaths.

Table 1. Noninferiority analysis for the primary objective (anti-HB response postprimary series) (PP analysis set).

	Group 1		Group 2		Group 1 minus Group 2			
	n/M	95% CI	n/M	95% CI	% observed	2-sided 95%CI	Clinical delta[a]	Conclusion
Anti-HB ≥10 mIU/mL	126/134 (94%)	(88.6; 97.4)	123/128 (96.1%)	(91.1; 98.7)	–2.06	(–7.88; 3.65)	10	Noninferiority

n = number of participants
M = number of participants with evaluable data
Group 1= DTaP-IPV-HB-PRP-T primary series and DTaP-IPV-HB-PRP-T booster
Group 2 = DTaP-IPV//PRP-T + HB primary series and DTaP-IPV-HB-PRP-T booster
[a]Noninferiority to be concluded if lower bound of the 95% CI greater than –10

Table 2. Seroprotection rates, seroconversion rates, geometric mean concentrations, and titers 1 month postdose 3 (2, 3, 4 months of age), prebooster, and 1 month postbooster (PP analysis set).

Antibody	Criteria	Group 1			Group 2		
		Postprimary	Prebooster	Postbooster	Postprimary	Prebooster	Postbooster
Anti-HB	≥10 mIU/mL	94.0 (88.6; 97.4)	80.7 (72.1; 87.7)	97.3 (92.3; 99.4)	96.1 (91.1; 98.7)	99.0 (94.7; 100.0)	98.6 (96.0; 97.7)
	≥100 mIU/mL	64.9 (56.2; 73.0)	33.9 (25.1; 43.6)	86.5 (78.7; 92.2)	78.1 (70.0; 84.9)	76.7 (67.3; 84.5)	93.0 (88.7; 96.0)
	GMC (mIU/mL)	149 (115; 191)	44.2 (32.3; 60.7)	1379 (916; 2078)	265 (205; 342)	223 (176; 282)	26189 (19133; 35846)
Anti-PRP-T	≥0.15 µg/mL	90.7 (84.6; 95.0)	85.0 (77.0; 91.0)	100.0 (96.8; 100.0)	97.8 (93.8; 99.5)	83.3 (74.7; 90.0)	100.0 (96.5; 100.0)
	≥1 µg/mL	72.9 (64.7; 80.0)	41.6 (32.4; 51.2)	98.2 (93.8; 99.8)	76.8 (68.9; 83.6)	33.3 (24.3; 43.4)	100.0 (96.5; 100.0)
	GMC (µg/mL)	2.12 (1.62; 2.77)	0.724 (0.541; 0.968)	72.5 (55.8; 94.3)	2.37 (1.91; 2.94)	0.612 (0.443; 0.844)	86.9 (69.8; 108)
Anti-D	>0.01 IU/mL	99.3 (96.2; 100.0)	90.4 (83.0; 95.3)	100.0 (96.8; 100.0)	97.1 (92.7; 99.2)	88.3 (80.0; 94.0)	100.0 (96.3; 100.0)
	>0.1 IU/mL	34.0 (26.3; 42.4)	12.5 (6.8; 20.4)	99.1 (95.1; 100.0)	44.2 (35.8; 52.9)	14.9 (8.4; 23.7)	100.0 (96.3; 100.0)
	>1.0 IU/mL	0.0 (0.0; 2.5)	NC	90.2 (83.1; 95.0)	0.7 (0.0; 4.0)	NC	95.9 (89.9; 98.9)
	GMC (IU/mL)	0.071 (0.060; 0.084)	0.028 (0.022; 0.035)	5.09 (3.89; 6.66)	0.091 (0.075; 0.110)	0.032 (0.024; 0.041)	10.2 (7.59; 13.8)
Anti-T	>0.01 IU/mL	100.0 (97.5; 100.0)	100.0 (96.3; 100.0)	100.0 (96.7; 100.0)	100.0 (97.4; 100.00)	100.0 (96.0; 100.0)	100.0 (96.2; 100.0)
	>0.1 IU/mL	100.0 (97.5; 100.0)	83.5 (74.6; 90.3)	100.0 (96.7; 100.0)	98.6 (94.9; 99.8)	77.8 (67.8; 85.9)	100.0 (96.2; 100.0)
	>1.0 IU/mL	43.4 (35.2; 51.9)	NC	98.2 (93.5; 99.8)	32.4 (24.7; 40.8)	NC	99.0 (96.5; 99.9)
	GMC (IU/mL)	0.839 (0.731; 0.962)	0.244 (0.204; 0.292)	8.98 (7.52; 10.7)	0.709 (0.625; 0.804)	0.194 (0.158; 0.238)	13.1 (10.8; 15.8)
Antipolio type 1	≥8 (1/dil)	97.7 (91.9; 99.7)	98.9 (93.8; 100.0)	100.0 (96.5; 100.0)	97.9 (92.5; 99.7)	98.8 (93.7; 100.0)	100.0 (95.8; 100.0)
	GMT ([1/dil])	102 (74.9; 138)	110 (81.6; 148)	5477 (4401; 6814)	112 (85.4; 147)	114 (82.4; 157)	9050 (7134; 11480)
Antipolio type 2	≥8 (1/dil)	94.7 (86.9; 98.5)	100.0 (95.7; 100.0)	100.0 (96.4; 100.0)	94.0 (86.5; 98.0)	97.7 (91.9; 99.7)	100.0 (95.7; 100.0)
	GMT ([1/dil])	73.5 (52.9; 102)	114 (84.9; 153)	6099 (4916; 7566)	78.2 (58.2; 105)	131 (95.3; 179)	9170 (7170; 11727)
Antipolio type 3	≥8 (1/dil)	97.4 (90.8; 99.7)	85.2 (76.1; 91.9)	100.0 (96.4; 100.0)	100.0 (95.4; 100.0)	96.3 (90.1; 99.3)	100.0 (98.0; 100.0)
	GMT ([1/dil])	133 (93.0; 190)	47.1 (33.1; 67.1)	5542 (4156; 7392)	214 (159; 288)	101 (73.0; 141)	10152 (7806; 13205)
Anti-PT	≥4-fold rise	93.6 (88.2; 97.0)[a]	NA	96.5 (90.1; 99.3)[b]	94.2 (89.0; 97.5)[a]	NA	96.2 (89.3; 99.2)[b]
	GMC (EU/mL)	123 (109; 139)	6.08 (4.74; 7.79)	160 (137; 187)	138 (122; 155)	7.49 (597; 9.41)	237 (202; 278)
Anti-FHA	≥4-fold rise	81.9 (74.7; 87.9) [a]	NA	91.8 (83.0; 96.9)	83.1 (75.7; 89.0)[a]	NA	97.4 (90.9; 99.7)
	GMC (EU/mL)	102 (90.4; 114)	12.5 (9.59; 16.4)	222 (194; 254)	69.3 (62.0; 77.6)	8.18 (6.49; 10.3)	234 (201; 272)

Data are % (95% CI) participants with titer or concentration above threshold, GMC or GMT
Group 1 = DTaP-IPV-HB-PRP-T primary series and DTaP-IPV-HB-PRP-T booster
Group 2 = DTaP-IPV//PRP-T + HB primary series and DTaP-IPV-HB-PRP-T booster
[a]increase from preprimary series; [b]increase from prebooster; PP = per protocol; D = diphtheria; T = tetanus; PT = pertussis toxin; FHA = filamentous hemagglutinin; HB = hepatitis B; PRP-T = *Hemophilus influenzae* type b; NC = not calculated; NA = not applicable

Table 3. Postbooster antihepatitis B response in group 1 participants with a HB concentration < 10 mIU/L or ≥10 mIU/mL prebooster (PP analysis set).

Antibody	Criteria	Group 1	
		Participants with prebooster <10 mIU/mL (N = 21)	Participants with prebooster ≥10 mIU/mL (N = 88)
Anti-HB (mIU/mL)	≥10	95.0 (75.1; 99.9)	100 (95.9; 100)
	≥100	50.0 (27.2; 72.8)	97.7 (92.0; 99.7)
	GMC prebooster (IU/mL)	3.72 (2.91; 4.75)	79.9 (61.4; 104)
	GMC postbooster (IU/mL)	135 (56.9; 320)	2745 (1938; 3886)

Data are % (95% CI) participants fulfilling the given criteria or GMC
Group 1 = DTaP-IPV-HB-PRP-T primary series and DTaP-IPV-HB-PRP-T booster
PP = per protocol; HB = hepatitis B; GMC = geometric mean concentration

Table 4. Percentage of participants experiencing solicited injection site reactions occurring in the 7 days after any dose of any vaccine (SafAS).

		Primary series vaccination				
			Group 2		Booster vaccination	
	Grade[a]	Group 1 (N = 153)	DTaP-IPV//PRP-T (N = 152)	Engerix B (N = 152)	Group 1 (N = 130)	Group 2 (N = 122)
Any injection site reaction	Any	69.9 (62.0; 77.1)	NC	NC	50.4 (41.2; 59.6)	65.8 (56.2; 74.5)
	Grade 3	13.7 (8.7; 20.2)	NC	NC	5.0 (1.8; 10.5)	7.2 (3.2; 13.7)
Tenderness	Any	62.1 (53.9; 69.8)	53.9 (45.7; 62.1)	49.3 (41.1; 57.6)	46.3 (37.2; 55.6)	60.4 (50.6; 69.5)
	Grade 3	11.8 (7.1; 18.0)	7.9 (4.1; 13.4)	9.2 (5.1; 15.0)	3.3 (0.9; 8.2)	1.8 (0.2; 6.4)
Redness	Any	34.0 (26.5; 42.1)	25.0 (18.3; 32.7)	15.8 (10.4; 22.6)	28.9 (21.0; 37.9)	45.0 (35.6; 54.8)
	Grade 3	0.7 (0.0; 3.6)	0.0 (0.0; 2.4)	0.7 (0.0; 3.6)	2.5 (0.5; 7.1)	3.6 (1.0; 9.0)
Swelling	Any	23.5 (17.1; 3.6)	20.4 (14.3; 27.7)	17.1 (11.5; 24.0)	21.5 (14.5; 29.9)	32.4 (23.9; 42.0)
	Grade 3	2.6 (0.7; 6.6)	0.0 (0.0; 2.4)	0.0 (0.0; 2.4)	1.7 (0.2; 5.8)	2.7 (0.6; 7.7)

Data are % of participants (95% CI)
SaFAS = safety analysis set; NC = not calculated
Group 1 = DTaP-IPV-HB-PRP-T primary series and DTaP-IPV-HB-PRP-T booster
Group 2 = DTaP-IPV//PRP-T + HB primary series and DTaP-IPV-HB-PRP-T booster
[a]Grade 1, 2, and 3 pain were defined as 'minor reaction when injection site is touched', 'cries or protests when injection site is touched', and 'cries when injected limb is moved or the movement of the injected limb is reduced'. For erythema and swelling, a diameter of <2.5 cm was assessed as Grade 1, from 2.5 to <5 cm as Grade 2, and ≥5 cm as Grade 3

For the DTaP-IPV-HB-PRP-T booster vaccination, the incidence of solicited injection site and systemic reactions was similar for the two primary series vaccine groups, and most were classified as Grade 1 or 2. In particular, there were no episodes of extensive swelling of the vaccinated limb (and so this solicited reaction for the booster phase is not presented in Table 3). The incidence of unsolicited AEs within 7 days of the booster vaccination was low (9.1% and 9.9% of participants in groups 1 and 2, respectively, with the most commonly reported being upper respiratory tract infection in group 1 [3.3%] and nasopharyngitis in group 2 [4.5%]); two participants in group 1 (1.7% [injection site hemorrhage]) and none in group 2 reported an AE considered to be related to the vaccination. In the 30 days

Table 5. Percentage of participants experiencing solicited systemic reactions occurring in the 7 days after any dose of any vaccine (SafAS).

	Grade[a]	Primary series vaccination		Booster vaccination	
		Group 1 (N = 153)	Group 2 (N = 152)	Group 1 (N = 130)	Group 2 (N = 122)
Any systemic reaction	Any	81.7 (74.6; 87.5)	76.3 (68.7; 82.8)	50.4 (41.2; 59.6)	65.8 (56.2; 74.5)
	Grade 3	36.6 (29.0; 44.8)	24.3 (17.8; 32.0)	12.4 (7.1; 19.6)	12.6 (7.1; 20.3)
Fever	Any	41.2 (33.3; 49.4)	28.3 (21.3; 36.2)	24.0 (16.7; 32.6)	32.4 (23.9; 42.0)
	Grade 3	2.0 (0.4; 5.6)	2.0 (0.4; 5.7)	0.8 (0.0; 4.5)	0.0 (0.0; 3.3)
Vomiting	Any	50.3 (42.1; 58.5)	45.4 (37.3; 53.7)	10.7 (5.8; 17.7)	9.9 (5.1; 17.0)
	Grade 3	17.6 (12.0; 24.6)	12.5 (7.7; 18.8)	1.7 (0.2; 5.8)	1.8 (0.2; 6.4)
Abnormal crying	Any	55.6 (47.3; 63.6)	40.8 (32.9; 49.0)	24.0 (16.7; 32.6)	31.5 (23.0; 41.0)
	Grade 3	17.0 (11.4; 23.9)	10.5 (6.1; 16.5)	2.5 (0.5; 7.1)	3.6 (1.0; 9.0)
Drowsiness	Any	44.4 (36.4; 52.7)	46.7 (38.6; 55.0)	19.8 (13.1; 28.1)	22.5 (15.1; 13.4)
	Grade 3	4.6 (1.9; 9.2)	7.2 (3.7; 12.6)	1.7 (0.2; 5.8)	2.7 (0.6; 7.7)
Appetite lost	Any	47.7 (39.6; 55.9)	46.7 (38.6; 55.0)	33.1 (24.8; 42.2)	38.7 (29.6; 48.5)
	Grade 3	10.5 (6.1; 16.4)	7.9 (4.1; 13.4)	7.4 (3.5; 13.7)	7.2 (3.2; 13.7)
Irritability	Any	68.0 (60.5; 75.3)	65.1 (57.0; 72.7)	42.1 (33.2; 51.5)	55.0 (45.2; 64.4)
	Grade 3	20.3 (14.2; 27.5)	14.5 (9.3; 21.1)	4.1 (1.4; 9.4)	6.3 (2.6; 12.6)

Data are % of participants (95% CI)
SaFAS = safety analysis set
Group 1 = DTaP-IPV-HB-PRP-T primary series and DTaP-IPV-HB-PRP-T booster
Group 2 = DTaP-IPV//PRP-T + HB primary series and DTaP-IPV-HB-PRP-T booster
Grade 1, 2, and 3 fever were defined as temperature (axillary equivalent) ≥37.4 °C–≤37.9 °C, >38.0 °C–≤38.9 °C, and >39.9 °C, respectively. Other systemic symptoms were defined as: vomiting (Grade 1, 1 episode/day; Grade 2, 2 to 5 episodes/day; Grade 3, ≥6 episodes /day or requiring parenteral hydration), abnormal crying (Grade 1, <1 h; Grade 2, 1–3 h; Grade 3, >3 h), drowsiness (Grade 1, unusually sleepy; Grade 2, not interested in surroundings or did not wake up for a meal; Grade 3, sleepy most of the time or difficult to wake up), appetite lost (Grade 1, eating less than normal; Grade 2, missed 1 to 2 meals; Grade 3, missed ≥3 meals), and irritability (Grade 1, easily consolable; Grade 2, requiring increased attention; Grade 3, inconsolable)

postbooster, 15.7% and 18.0% of participants in groups 1 and 2, respectively, reported an unsolicited AE: there were no SAEs during this time. During the 6-month follow-up period 6 participants (2.4%) reported an SAE, none of which were considered to be related to vaccination. There were no deaths or withdrawals due to an AE during the booster phase.

4. Discussion

With the exception of the HB antigen, all antigens included in the fully liquid hexavalent vaccine have been extensively studied both as part of the pentavalent vaccine (Pentaxim/Pentavac) (4) and also in a clinical trial designed specifically to assess the noninferiority of all antigens to Pentaxim/Pentavac and a standalone HB vaccine (13). This previous study showed noninferiority of immune responses for all antigens, including HB (with no HB vaccination at birth). Compared to the present study, the HB response has been higher postprimary vaccination series for the less challenging 2, 4, 6 month schedule (13) or postbooster when a HB dose had been administered at birth (14). However, the differences in study design in terms of vaccination schedule and the administration of a birth dose of HB compound comparisons between studies and more importantly numerous studies have consistently shown noninferiority of immune responses against HB to both standalone HB vaccine and to HB antigens included in multivalent comparator vaccines, in a range of schedules, both with and without HB vaccination at birth. These studies have been published separately and reviewed (5).

The present study, therefore, was not powered to assess all antigens statistically, since such comparisons have been done previously in appropriately powered studies both for the investigational DTaP-IPV-HB-PRP-T vaccine (5–7) and for the D, T, aP, IPV, and PRP-T antigens that are the same as those contained in the established control DTaP-IPV//PRP-T vaccine (4); instead, the present study was designed to assess the noninferiority of only the HB antigen compared to a standalone HB vaccine (administered with Pentaxim/Pentavac) at 1 month after a 3-dose primary series in terms of SP rate. The response to the remaining antigens is presented descriptively. As expected from other studies, noninferiority was demonstrated for HB after the primary vaccination series, and the responses for all other antigens were strong and similar between the two groups.

In this study a DTaP-IPV-HB-PRP-T booster was administered to both primary vaccination groups (i.e. the same booster for participants who had received either DTaP-IPV-HB-PRP-T or Pentaxim/Pentavac and a standalone HB as the primary series) to establish the suitability of DTaP-IPV-HB-PRP-T given as a booster in either scenario. The anti-HB antibody persistence prebooster was lower for DTaP-IPV-HB-PRP-T than for the standalone HB vaccine (SP rate ≥10 mIU/mL [GMC] of 80.7% [44.2 mIU/mL] and 99.0% [223 mIU/mL] for groups 1 and 2, respectively). However, as has been seen with other challenging vaccination schedules (14), the anti-HB seroprotection rate (10 mIU/mL threshold) after a booster dose of the hexavalent vaccine is similar even when comparing groups with different prebooster seroprotection rates (97.3% and 98.6% for groups 1 and 2, respectively). In terms of SP ≥10 mIU/mL and GMC, however, the postbooster response was lower for DTaP-IPV-HB-PRP-T (86.5% and 44.2 mIU/mL) than for the standalone HB vaccine (93.0 and 223 mIU/mL), although this should be considered in the context of the high postbooster SP rates (≥10 mIU/mL) in both groups at this time point, which suggests that the difference in GMC is unlikely to be of clinical significance. To further investigate the postbooster anti-HB response, a post hoc analysis was performed for those participants in group 1 with an anti-HB prebooster seroprotection rate <10 mIU/mL compared to those with a prebooster seroprotection rate of ≥10 mIU/mL; while descriptive and exploratory, and performed using only a small sample size, this analysis shows that—based on the 10 mIU/mL threshold—the postbooster response is strong (≥95.0%) irrespective of whether the prebooster anti-HB seroprotection rate was above or below 10 mIU/mL. These data echo those from another study of the DTaP-IPV-HB-PRP-T vaccine administered in a 6, 10, 14 week primary series schedule and including a comparison to a standalone HB vaccine (14). This adds further support to that hypothesis that if adequately primed then T and B cell memory would be expected even in the event of antibody waning to subprotective levels (21,22), meaning that a strong and adequate response would be expected even in such individuals when exposed to wild-type virus. The WHO also supports this hypothesis, stating 'the loss of detectable anti-HBs in participants who had responded to satisfactorily to a primary series does not necessarily indicate a lack of protection' (23,24).

The safety profile was good for both the primary vaccination series (DTaP-IPV-HB-PRP-T or control vaccines) and the DTaP-IPV-HB-PRP-T booster (in both groups) and there were no clinically important differences between groups. This is as would be expected based on the extensive experience with the DTaP-IPV-HB-PRP-T vaccine: despite the possibility for cultural differences in AE reporting using diary cards (e.g., the anal route for temperature measurement is not always acceptable to parents), all clinical studies of DTaP-IPV-HB-PRP-T, conducted over four continents, have consistently shown no clinically important safety findings (5).

To conclude, the new hexavalent DTaP-IPV-HB-PRP-T vaccine was shown to be immunogenic and safe as a 2, 3, 4 month primary series without HB vaccination at birth, comparable to Pentaxim + Engerix B, and a DTaP-IPV-HB-PRP-T booster was shown to be safe and immunogenic following a primary series of either DTaP-IPV-HB-PRP-T or Pentaxim + Engerix B.

Acknowledgments, funding source, and disclaimers

We would like to acknowledge all study staff for their contributions to the study conduct and all study participants and their parents/legal guardians.

We would also like to thank Drs Michel Scemama and Eduardo Santos-Lima for their significant contributions to the realization of this trial while employed as Clinical Trial Leaders at Sanofi Pasteur, Dr Sandrine Lentsch-Graf (Hexaxim Project Leader) for her valuable input, Mrs Siham B'Chir for the statistical analyses, and Dr Xavier DaCosta for his critical review of the manuscript (XDC, SL-G, and SB'C are employees of Sanofi Pasteur).

Dr Andrew Lane (Lane Medical Writing) provided professional medical writing assistance in the preparation and development of the manuscript in accordance with the European Medical Writers Association guidelines and Good Publication Practice.

This study and professional medical writing support were funded by Sanofi Pasteur.

No clinical investigator involved in these studies received any direct payment from Sanofi Pasteur in this regard, but may have received expenses for conference attendance for the presentation of data from these studies. EF is an employee of Sanofi Pasteur.

References

1. Edwards KM and Decker MD. Pertussis vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editor. Vaccines. 6th ed. Philadelphia, PA, USA: Saunders Co.; 2013. pp. 447-492.
2. WHO. Pertussis vaccines: WHO position paper, September 2015. Wkly Epidemiol Rec. 2015; 90: 433-458.
3. WHO. Polio vaccines: WHO position paper, January 2014. Wkly Epidemiol Rec. 2014; 89: 73-92.
4. Plotkin SA, Liese J, Madhi SA, Ortiz E. A DTaP-IPV//PRP~T vaccine (Pentaxim): a review of 16 years' clinical experience. Expert Rev Vaccines 2011; 10: 981-1005.
5. McCormack PL. DTaP-IPV-Hep B-Hib vaccine (Hexaxim®): a review of its use in primary and booster vaccination. Paediatr Drugs 2013; 15: 59-70.
6. Nunes MC, Madhi SA. Review of a new fully liquid, hexavalent vaccine: Hexaxim. Expert Opin Biol Th 2013; 13: 575-593.
7. Santos-Lima E, B'Chir S, Lane A. Combined immunogenicity data for a new DTaP-IPV-Hep B-PRP-T vaccine (Hexaxim) following primary series administration at 2, 4, 6 months of age in Latin America. Vaccine 2013; 31: 1255-1258.
8. Tregnaghi MW, Voelker R, Santos-Lima E, Zambrano B. Immunogenicity and safety of a novel yeast Hansenula polymorpha-derived recombinant Hepatitis B candidate vaccine in healthy adolescents and adults aged 10-45 years. Vaccine 2010; 28: 3595-3601.
9. Decker MD, Edwards KM, Bogaerts HH. Combination vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editor. Vaccines. 6th ed. Philadelphia, PA, USA: Saunders Co.; 2013. pp. 981-1007.
10. Madhi SA, Cutland C, Jones S, Groome M, Ortiz E. Immunogenicity and safety of an acellular pertussis, diphtheria, tetanus, inactivated poliovirus, Hib-conjugate combined vaccine (Pentaxim) and monovalent hepatitis B vaccine at 6, 10 and 14 weeks of age in infants in South Africa. S Afr Med J 2011; 101: 126-131.
11. Aquino AG, Brito MG, Doniz CE, Herrera JF, Macias M, Zambrano B, Plennevaux E, Santos-Lima E. A fully liquid DTaP-IPV-Hep B-PRP-T hexavalent vaccine for primary and booster vaccination of healthy Mexican children. Vaccine 2012; 30: 6492-6500.
12. Kosalaraksa P, Thisyakorn U, Benjaponpitak S, Chokephaibulkit K, Santos-Lima E. Immunogenicity and safety study of a new DTaP-IPV-Hep B-PRP-T combined vaccine compared to a licensed DTaP-IPV-Hep B//PRP-T comparator, both concomitantly administered with a 7-valent pneumococcal conjugate vaccine at 2, 4, and 6 months of age in Thai infants. Int J Infect Dis. 2011; 15: e249-256.
13. Tregnaghi MW, Zambrano B, Santos-Lima E. Immunogenicity and safety of an investigational hexavalent diphtheria-tetanus-acellular pertussis-inactivated poliovirus-hepatitis B-Haemophilus influenzae B conjugate combined vaccine in healthy 2-, 4-, and 6-month-old Argentinean infants. Pediatr Infect Dis J 2011; 30: e88-96.
14. Madhi SA, Koen A, Cutland C, Groome M, Santos-Lima E. Antibody persistence and booster vaccination of a fully liquid hexavalent vaccine coadministered with measles/mumps/rubella and varicella vaccines at 15-18 months of age in healthy South African infants. Pediatr Infect Dis J 2013; 32: 889-897.
15. Tregnaghi M, Zambrano B, Santos-Lima E. Antibody persistence after a primary series of a new DTaP-IPV-Hep B-PRP-T combined vaccine or separate DTaP-IPV//PRP-T and hepatitis B vaccines at 2, 4, and 6 months of age and the effect of a subsequent DTaP-IPV//PRP-T booster vaccination at 18 months of age in healthy Argentinean infants. Pediatr Infect Dis J 2012; 31: e24-30.
16. Macias M, Lanata CF, Zambrano B, Gil AI, Amemiya I, Mispireta M, Ecker L, Santos-Lima E. Safety and immunogenicity of an investigational fully liquid hexavalent DTaP-IPV-Hep B-PRP-T vaccine at two, four and six months of age compared with licensed vaccines in Latin America. Pediatr Infect Dis J 2012; 31: e126-132.
17. European Medicines Agency. EMEA procedural advice on medicinal products intended exclusively for markets outside the community under Article 58 of regulation EC no. 726/2004 in the context of co-operation with the World Health Organization (WHO) April 2009. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2010/02/WC500074039.pdf. Accessed 18 April 2017.
18. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med 1998; 17: 857-872.
19. Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med 1998; 17: 873-890.
20. Farrington CP, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. Stat Med 1990; 9: 1447-1454.
21. Bauer T, Jilg W. Hepatitis B surface antigen-specific T and B cell memory in individuals who had lost protective antibodies after hepatitis B vaccination. Vaccine 2006; 24: 572-577.
22. Zanetti AR, Romano L, Giambi C, Pavan A, Carnelli V, Baitelli G, Malchiodi G, Valerio E, Barale A, Marchisio MA et al. Hepatitis B immune memory in children primed with hexavalent vaccines and given monovalent booster vaccines: an open-label, randomised, controlled, multicentre study. Lancet Infect Dis 2010; 10: 755-761.
23. WHO. Hepatitis B vaccines. Wkly Epidemiol Rec. 2009; 84: 405-419.
24. WHO. Recommendations to assure the quality, safety and efficacy of recombinant hepatitis B vaccines 2010. Available from: http://www.who.int/biologicals/HEP_B_Recomm_after_ECBS_endorsment_final.pdf. Accessed 18 April 2017.