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Lot-to-lot consistency of a hexavalent DTwP-IPV-HB-PRP∼T vaccine and non-inferiority to separate DTwP-HB-PRP∼T and IPV antigen-matching vaccines at 6–8, 10–12, and 14–16 weeks of age co-administered with oral rotavirus vaccine in healthy infants in India: A multi-center, randomized, controlled study

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ABSTRACT

Background: Combination vaccines reduce the number of pediatric injections but must be as safe, immunogenic, and effective as each of the individual vaccines given separately. Additionally, consistency in manufacturing lots is essential for WHO prequalification. This study aimed to establish the lot-to-lot consistency of a fully liquid, hexavalent diphtheria (D)-tetanus (T)-whole-cell pertussis (wP)-inactivated poliovirus (IPV)-hepatitis B (HB)-*Haemophilus influenzae* b (PRP-T) (DTwP-IPV-HB-PRP∼T) vaccine and to demonstrate non-inferiority to licensed DTwP-HB-PRP∼T and IPV vaccines.

Methods: A Phase III, randomized, active-controlled, and open-label study was conducted at multiple centers across India. Healthy infants who had received a birth dose of oral poliovirus vaccine and hepatitis B vaccine received one of three lots of DTwP-IPV-HB-PRP∼T or separate DTwP-HB-PRP∼T and IPV vaccines at 6–8, 10–12, and 14–16 weeks of age. Oral rotavirus vaccine was co-administered at 6–8 weeks of age and 10–12/14–16 weeks of age. DTwP-IPV-HB-PRP∼T lot-to-lot consistency and non-inferiority (pooled DTwP-IPV-HB-PRP∼T) versus DTwP-HB-PRP∼T and IPV post-third dose were assessed using seroprotection rates (anti-D, anti-T, anti-HBs, anti-PRP, anti-polio 1, 2, 3) and adjusted geometric mean concentrations (anti-PT, anti-FIM). Safety was assessed by parental reports.

Results: Lot-to-lot consistency was demonstrated for DTwP-IPV-HB-PRP∼T and non-inferiority versus DTwP-HB-PRP∼T and IPV was confirmed with 95% CIs for seroprotection rate differences and adjusted geometric mean concentration ratios within pre-defined clinical margins. Pooled seroprotection rate was ≥ 99.7% for anti-D ≥ 0.01 IU/mL, anti-T ≥ 0.01 IU/mL, anti-HBs ≥ 10 mIU/mL, anti-PRP ≥ 0.15 μg/mL, and anti-polio 1, 2, and 3 ≥ 8 (1/dil) and vaccine response rate was 83.9% for anti-PT and 97.7%

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for anti-FIM. There were no safety concerns.
Conclusions: Immunogenicity of three lots of the fully liquid DTwP-IPV-HB-PRP∼T vaccine was consistent and non-inferior to licensed comparators following vaccination at 6–8, 10–12, and 14–16 weeks of age. There were no safety concerns and no evidence of any effect of co-administration with rotavirus vaccine.

Introduction

Pediatric combination vaccines are an important tool that have led to current low incidences of childhood diseases including diphtheria (D), tetanus (T), pertussis, hepatitis B (HB), *Haemophilus influenzae* type b (Hib) infection, and polio [1,2]. Such vaccines have consistently been shown to be safe and immunogenic, and add value by improving compliance to pediatric vaccination schedules [1].

Following the successful development in India of a pentavalent D, T, whole cell pertussis (wP), HB, and Hib vaccine (SHAN5™), and an inactivated poliovirus (IPV) vaccine (SHANIPV™), Sanofi Healthcare India Private Limited (SHIPL) developed a fully liquid, ready-to-use, DTwP-IPV-HB-PRP∼T vaccine (SHAN6™) which combines established SHAN5™ and SHANIPV™ antigens. Combining antigens in a fully liquid presentation reduces the time for vaccine preparation, further improving compliance to increasingly crowded vaccination schedules and helping to support the Global Polio Eradication Initiative outlined in its most recent Endgame Strategic Plan (2022–2026) [3].

The World Health Organization (WHO) guidelines for vaccine prequalification indicate that the consistency of manufacturing for vaccine lots used in clinical trials should be demonstrated. Ideally, at least three lots with the same formulation should be used, manufactured at full production scale, and the study should be designed and analyzed as an equivalence trial with pre-defined criteria to conclude comparability [4,5]. The new DTwP-IPV-HB-PRP∼T vaccine has been evaluated as safe and immunogenic in randomized, controlled, early phase clinical studies [6,7]. This study was conducted to establish that the hexavalent vaccine was safe and immunologically non-inferior to the administration of the licensed pentavalent and injectable polio vaccines. Additionally, the lot-to-lot consistency of the hexavalent vaccine was evaluated to demonstrate that different vaccine lots were consistent in terms of immunogenicity as per the WHO requirement for vaccine development and pre-qualification [4,5].

Materials AND METHODS

Study design and participants

A Phase III, randomized, active-controlled, open-label study was carried out at 13 sites in India (Clinical Trials Registry India Number CTRI/2019/01/017155). The study protocol and one amendment were approved by institutional ethics committees, and the study was performed according to local and national regulations consistent with the standards established by the Declaration of Helsinki and compliant with the International Council for Harmonization guidelines for Good Clinical Practice. Before enrolment, an informed consent form was signed by at least one parent or legally acceptable representative following an audio-visual consenting process as mandated by the Drugs Controller General of India (DCGI). Vaccinations occurred from February 2019 through December 2019.

Healthy infants aged 6–8 weeks, born at full term (≥37 weeks), with birth weight ≥ 2.5 kg, and having received a birth dose of oral poliovirus vaccine (OPV) and hepatitis B (HB) vaccine, and Bacillus Calmette-Guérin (BCG) vaccine at least 4 weeks before the first study vaccination were eligible. The main exclusion criteria were previous vaccination or planned receipt of any vaccine against D, T, P, HB, Hib, poliovirus, or rotavirus (except OPV at any time or HB vaccine at birth) in the 4 weeks following study vaccination or history of any such infection; receipt of any other vaccine in the 4 weeks preceding the first study vaccination or planned receipt within 7 days before and after each study vaccination; known hypersensitivity to any vaccine component; any chronic illness that could interfere with study conduct or completion; personal or maternal history of human immunodeficiency virus, HB surface antigen (HBsAg), or hepatitis C seropositivity or receipt of immunosuppressive therapy; previous or planned participation in another clinical study; receipt of blood products in the 30 days prior to inclusion or planned during the study; known thrombocytopenia, bleeding disorder, or receipt of anticoagulants in the 3 weeks prior to inclusion; acute illness or febrile illness on the day of vaccination; history of seizure, encephalopathy, or intussusception; natural or adopted child of anyone with direct study involvement.

Infants were randomly allocated to receive one of three lots of the investigational vaccine (DTwP-IPV-HB-PRP∼T) (Lot A, B, or C) or separately administered DTwP-HB-PRP∼T and IPV vaccines at 6–8, 10–12, and 14–16 weeks of age. A permuted block method was used for the randomization to guarantee a similar ratio of participants between groups at any time. All vaccines were co-administered with oral rotavirus vaccine at 6–8 weeks of age and either 10–12 or 14–16 weeks of age.

Vaccines were administered intramuscularly into the right thigh (DTwP-IPV-HB-PRP∼T and DTwP-HB-PRP∼T vaccines), left thigh (IPV), and orally (rotavirus vaccine).

Study vaccines

The DTwP-IPV-HB-PRP∼T vaccine (SHAN6, lot numbers 2HXK001A18 [Lot A, expiry April 2020], 2HXK002A18 [Lot B, expiry May 2020], and 2HXK003A18 [Lot C, expiry May 2020]) was manufactured by SHIPL and supplied as a liquid, sterile suspension for injection in a single dose vial. Each 0.5 mL dose contained ≥ 30 IU D-toxoid, ≥60 IU T-toxoid, ≥4 IU whole-cell *B. pertussis* organisms, 10 μg rDNA HBsAg, 12 μg Hib purified capsular polysaccharide conjugated to 20–40 μg tetanus toxoid carrier protein, 40, 8 and 32 D antigen units of Vero cell-produced poliovirus type 1 (Mahoney strain), type 2 (MEF-1 strain), and type 3 (Saukett strain), respectively, and 0.625 mg aluminum phosphate adjuvant.

The DTwP-HB-PRP∼T vaccine (SHAN5, lot numbers 2PLU016B18 [expiry April 2020] and 2PLU019A18 [expiry July 2020]) was manufactured by SHIPL and supplied as a liquid, sterile suspension for injection in a 10 dose vial. Each 0.5 mL dose contained ≥ 30 IU D-toxoid, ≥60 IU T-toxoid, ≥4 IU whole-cell *B. pertussis* organisms, 10 μg Hib purified capsular polysaccharide conjugated to 20–40 μg tetanus toxoid carrier protein, 10 μg rDNA HBsAg, and 0.625 mg aluminum phosphate adjuvant.

The IPV vaccine (SHANIPV, lot numbers IPQ023A18 [expiry May 2020] and IPQ029B19 [expiry April 2021]) was manufactured by SHIPL and supplied as a liquid, sterile suspension for injection in

a 5 dose vial. Each 0.5 mL dose contained 40, 8 and 32 D antigen units of Vero cell-produced poliovirus type 1 (Mahoney strain), type 2 (MEF-1 strain), and type 3 (Saukett strain), respectively.

The commercial rotavirus vaccine (Rotarix™) (lot number A41FB629A, expiry August 2020) was manufactured by GlaxoSmithKline Biologicals and supplied as a lyophilized powder and solvent for oral suspension. After reconstitution, each 1 mL dose contained $\geq 10^{6.0}$ 50% cell culture infective doses ($CCID_{50}$) (viral infectious units) live, attenuated human rotavirus RIX4414 strain. In addition, subjects enrolled in the study received 1 to 4 $bOPV_{1\&3}$ doses as part of routine national immunization campaigns in India, in addition to the study vaccine doses, administered either before or concomitantly with the study vaccines.

Serology

All subjects provided a blood sample (approximately 5 mL) pre-first vaccination and 28 days post-third vaccination for determination of anti-D, anti-T, anti-pertussis toxin (PT), anti-filamentous hemagglutinin (FHA), anti-pertactin (PRN), and anti-fimbriae 2/3 (FIM), anti-PRP, anti-HBs, anti-polio 1, 2, and 3 antibodies. Samples from a randomized subset of half of the participants were used for determination of anti-rotavirus IgA antibodies.

Anti-D (IU/mL), anti-T (IU/mL), anti-PT (EU/mL), anti-FHA (EU/mL), anti-PRN (EU/mL), anti-FIM (EU/mL) antibody concentrations were measured by a multiplexed chemiluminescence assay using the Meso Scale Discovery platform (DTP-ECL) [7,8]. For the *B. pertussis* antigens, results were expressed in EU calibrated against the US reference pertussis antiserum (human) lot 3 and lot 4 for IgG antibodies [9,10]. Anti-HBs (mIU/mL) antibody concentrations were measured by enzyme linked immunosorbent assay (ELISA) using a commercially available kit (VITROS, Ortho Clinical Diagnostics, UK), anti-PRP (μg/mL) antibody concentrations by radioimmunoassay [11], anti-poliovirus antibody titers by micrometabolic inhibition test (MIT) against wild-type poliovirus strains [12]. Anti-rotavirus IgA antibodies were measured by enzyme immunoassay (EIA) [13].

All assays except the anti-rotavirus EIA were performed at the Sponsor's Global Clinical Immunology (GCI) laboratory (Swiftwater, PA, USA). Anti-rotavirus testing was done at the Cincinnati Children's Hospital Medical Center.

Reactogenicity and safety

Participants were monitored at the study site for immediate unsolicited adverse events (AEs) for 30 min after each vaccination. Subsequently, for 7 days parent(s)/legal representative(s) used diary cards to record the duration and intensity of pre-defined (solicited) injection site reactions (tenderness, erythema, swelling) and systemic reactions (fever, vomiting, crying abnormal, drowsiness, appetite lost, irritability). Axillary temperature was preferred. Solicited reactions were automatically considered to be vaccine-related.

Unsolicited AEs were recorded using diary cards for 28 days after each vaccination. For each unsolicited AE the investigator assigned an intensity (Grade 1 [no interference with activity], Grade 2 [some interference with activity], or Grade 3 [prevents daily activity]) and assessed the relationship to vaccination. Details of serious adverse events (SAEs) were collected throughout the study until 28 days after the last vaccination, and the investigator assessed their relationship to vaccination.

Statistical analyses

The primary statistical objectives were (i) to demonstrate equivalence in terms of immunogenicity of three lots of DTwP-IPV-HB-PRP∼T (ii) to demonstrate non-inferiority of DTwP-IPV-HB-PRP∼T to DTwP-HB-PRP∼T and IPV vaccines. Secondary objectives were to describe the immune responses for DTwP-IPV-HB-PRP∼T, DTwP-HB-PRP∼T and IPV, and rotavirus vaccines, and to describe the safety profile in each group.

Antibody thresholds and criteria used to define seroprotection (SP) and vaccine response (VR) rates are presented in Table 1, Table 2, and Table 3. In additional to SP and VR rates, geometric mean titers (GMTs) (IPV) and geometric mean concentrations (GMCs) (D, T, HB, PRP, PT, FIM, PRN, FHA) are also presented. Rotavirus GMCs and seroconversion rate (≥4-fold rise in anti-rotavirus IgA) are presented. Data are shown with 95% confidence intervals (CIs), calculated using the exact binomial distribution (Clopper-Pearson) [14] for proportions and the normal approximation method for GMCs/GMTs.

An equivalence testing approach was used to test lot-to-lot consistency using SP rates for anti-D, anti-T, anti-HBs, anti-PRP, and anti-polio 1, 2, 3 based on the use of the two-sided 95% CI of the difference between two pairs of batches. The 95% CI was calculated using Wilson score method without continuity correction [14] and equivalence was demonstrated if the 95% CI of the paired difference lay entirely with −10% to 10%. For the pertussis antigens, three paired equivalence tests on adjusted GMCs (aGMCs) were performed to test lot-to-lot consistency for anti-PT and anti-FIM responses based on the use of the two-sided 95% CI of the ratio between two pairs of batches. The 95% CI of the ratio between aGMCs was calculated using normal approximation of the log10 of the concentration, and equivalence between lots was demonstrated if the 2-sided 95% CI fell entirely within 0.5–2.0. Of note, adjusted GMCs were computed to adjust for baseline disparities and consider the correlation between pre- and post-concentration, through an analysis of covariance model using the pre-vaccination log–transformed concentration as a covariate for adjustment to account for the associated variability. Overall equivalence between lots was concluded if equivalence for both SP rates and aGMCs was demonstrated.

Following demonstration of lot-to-lot consistency for DTwP-IPV-HB-PRP∼T, the three lots were pooled for non-inferiority comparison to DTwP-HB-PRP∼T and IPV based on the 95% CI of the difference (DTwP-IPV-HB-PRP∼T *minus* DTwP-HB-PRP∼T and IPV). For SP rates non-inferiority was concluded if the lower limit of the 95 %CI of the difference was greater than −10%, and for aGMCs non-inferiority was concluded if the lower limit of the 95% CI for the ratio was > 0.5. Overall non-inferiority was concluded if non-inferiority was demonstrated for both SP rates and aGMCs.

For pertussis, equivalence and non-inferiority were also assessed using non-adjusted GMCs for anti-PT and anti-FIM endpoints as a post hoc analysis.

A total of 1280 participants was planned (320 participants in each group), providing an overall power of 90.2% for the equivalence analysis and 99.9% for the non-inferiority analysis, assuming an attrition rate of 15%. A subset of 640 participants (160 per group) was randomized for the assessment of rotavirus vaccine immunogenicity.

The immunogenicity analyses, including the equivalence and non-inferiority testing, used the per protocol (PP) population (participants with no protocol violation that could have interfered with the evaluation criteria, and analyzed according to the vaccine received). Data from the full analysis set (FAS) (those who received at least one vaccination, and analyzed according to the randomization) supported the evaluation done using the PP population. The safety evaluation used the safety analysis set (SafAS) (participants who received at least one vaccination).

Statistical analyses were done under the responsibility of Sanofi Pasteur's statistical group using SAS software, Version 9.4 (SAS Institute, Cary, NC, USA).

Table 1
Equivalence of seroprotection rate (anti-D, anti-T, anti-HBs, anti-polio 1, 2, 3, and anti-PRP) and geometric mean concentration (anti-PT and anti-FIM) between three lots of DTwP-IPV-HB-PRP∼T at 28 days after 3-dose vaccination series at 6–8, 10–14, and 16–18 weeks of age (Per Protocol population).

		DTwP-IPV-HB-PRP∼T*			Difference or ratio (95% CI)†		
		Lot A (N = 284)	Lot B (N = 286)	Lot C (N = 294)	Lot A-Lot B	Lot A-Lot C	Lot B-Lot C
Anti-D	≥ 0.01 IU/mL	100.0 (98.7;100.0)	100.0 (98.7;100.0)	100.0 (98.8;100.0)	0.00 (-1.33;1.33)	0.00 (-1.33;1.29)	0.00 (-1.33;1.29)
Anti-T	≥ 0.01 IU/mL	100.0 (98.7;100.0)	100.0 (98.7;100.0)	100.0 (98.8;100.0)	0.00 (-1.33;1.33)	0.00 (-1.33;1.29)	0.00 (-1.33;1.29)
Anti-HBs	≥ 10 mIU/mL	99.6 (98.0;100.0)	99.7 (98.1;100.0)	99.7 (98.1;100.0)	−0.00 (-1.65;1.63)	−0.00 (-1.66;1.57)	−0.00 (-1.66;1.57)
Anti-polio 1	≥ 8 (1/dil)	100.0 (98.7;100.0)	100.0 (98.7;100.0)	100.0 (98.8;100.0)	0.00 (-1.33;1.33)	0.00 (-1.33;1.29)	0.00 (-1.33;1.29)
Anti-polio 2	≥ 8 (1/dil)	99.6 (98.0;100.0)	100.0 (98.7;100.0)	99.3 (97.6;99.9)	−0.35 (-1.97;1.00)	0.33 (-1.37;2.12)	0.68 (-0.734;2.45
Anti-polio 3	≥ 8 (1/dil)	100.0 (98.7;100.0)	100.0 (98.7;100.0)	100.0 (98.7;100.0)	0.00 (-1.34;1.33)	0.00 (-1.34;1.29)	0.00 (-1.33;1.29)
Anti-PRP	≥ 0.15 µg/mL	100.0 (98.7;100.0)	100.0 (98.7;100.0)	100.0 (98.8;100.0)	0.00 (-1.33;1.33)	0.00 (-1.33;1.29)	0.00 (-1.33;1.29)
Anti-PT	aGMC (EU/mL)	81.7 (67.8;98.5)	88.7 (73.7;107)	89.9 (74.8;108)	0.921 (0.708;1.20)	0.910 (0.700;1.18)	0.988 (0.760;1.28)
Anti-FIM	aGMC (EU/mL)	1222 (1054;1416)	1259 (1086;1459)	1294 (1119;1497)	0.970 (0.787;1.20)	0.944 (0.767;1.16)	0.973 (0.791;1.20)

* Data are % (95% CI) (anti-D, anti-T, anti-HBs, anti-polio 1, 2, 3, and anti-PRP) or aGMC (95% CI) (anti-PT and anti-FIM).
† Difference for anti-D, anti-T, anti-HBs, anti-polio 1, 2, 3 and anti-PRP; ratio for anti-PT and anti-FIM.
For anti-D, anti-T, anti-HB, anti-polio 1, 2, 3, and anti-PRP, equivalence concluded as the limits of 2-sided 95% CI of difference lay between batches are between the interval (-10%, 10%) For anti-PT and anti-FIM, equivalence concluded as the limits of 2-sided 95% CI of difference between batches are between the interval (0.5, 2.0).

Table 2
Non-inferiority of seroprotection rate (anti-D, anti-T, anti-HBs, anti-polio 1, 2, 3, and anti-PRP) and geometric mean concentration (anti-PT and anti-FIM) of DTwP-IPV-HB-PRP∼T versus DTwP-HB-PRP∼T and IPV at 28 days after 3-dose vaccination series at 6–8, 10–14, and 16–18 weeks of age (Per Protocol population).

				DTwP-IPV-HB-PRP∼T* versus DTwP-HB-PRP∼T and IPV	
		DTwP-IPV-HB-PRP∼T*† (N = 864)	DTwP-HB-PRP∼T and IPV† (N = 285)	Difference or ratio (95% CI)‡	Conclusion**
Anti-D	≥ 0.01 IU/mL	100.0 (99.6;100.0)	100.0 (98.7;100.0)	0.00 (-0.443;1.33)	Non-inferiority
Anti-T	≥ 0.01 IU/mL	100.0 (99.6;100.0)	100.0 (98.7;100.0)	0.00 (-0.443;1.33)	Non-inferiority
Anti-HBs	≥ 10 mIU/mL	99.7 (99.0;99.9)	100.0 (98.7;100.0)	−0.35 (-1.02;1.01)	Non-inferiority
Anti-polio 1	≥ 8 (1/dil)	100.0 (99.6;100.0)	100.0 (98.7;100.0)	0.00 (-0.443;1.33)	Non-inferiority
Anti-polio 2	≥ 8 (1/dil)	99.7 (99.0;99.9)	100.0 (98.7;100.0)	−0.35 (-1.02;1.00)	Non-inferiority
Anti-polio 3	≥ 8 (1/dil)	100.0 (99.6;100.0)	100.0 (98.7;100.0)	0.00 (-0.443;1.33)	Non-inferiority
Anti-PRP	≥ 0.15 µg/mL	100.0 (99.6;100.0)	100.0 (98.7;100.0)	0.00 (-0.443;1.33)	Non-inferiority
Anti-PT	aGMC (EU/mL)	86.2 (77.3;96.1)	64.6 (53.4;78.1)	1.33 (1.07;1.66)	Non-inferiority
Anti-FIM	aGMC (EU/mL)	1251 (1148;1365)	1260 (1083;1465)	0.994 (0.835;1.18)	Non-inferiority

* Pooled data from 3 lots of DTwP-IPV-HB-PRP∼T vaccine.
† Data are % (95% CI) (anti-D, anti-T, anti-HBs, anti-polio 1, 2, 3, and anti-PRP) or aGMC (95% CI) (anti-PT and anti-FIM).
‡ Difference for anti-D, anti-T, anti-HBs, anti-polio 1, 2, 3 and anti-PRP; ratio for anti-PT and anti-FIM.
** For anti-D, anti-T, anti-HBs, anti-polio 1, 2, 3, and anti-PRP, non-inferiority concluded as the lower limit of 2-sided 95% CI of difference between groups is greater than −10%; for anti-PT and anti-FIM, non-inferiority concluded as the lower limit of 2-sided 95% CI of ratio between 2 groups is >0.5.

Results

Participants studied

A total of 1280 participants received at least one vaccination, with 959 randomized to DTwP-IPV-HB-PRP∼T (318, 321, and 320 participants for Lots A, B, and C, respectively) and 321 randomized to DTwP-HB-PRP∼T and IPV. Overall, 890 participants (DTwP-IPV-HB-PRP∼T: 296, 295, and 299 participants for Lots A, B, and C) and 297 participants (DTwP-HB-PRP∼T and IPV) completed the study. Participant disposition, including reasons for withdrawal and reasons for exclusion from the PP population, is presented in Fig. 1. Demographic characteristics were similar in each group with a comparable proportion of male and female participants (overall 47.6% female).

Immunogenicity

The three DTwP-IPV-HB-PRP∼T lots were equivalent as the 95% CIs for the SP rate differences (for anti-D, anti-T, anti-HBs, anti-polio 1, 2, 3, and anti-PRP) and aGMC ratios (for anti-PT and anti-FIM) were within the pre-defined clinical margins (Table 1). Therefore, immunogenicity data were pooled for the three DTwP-IPV-HB-PRP∼T lots and the 95% CIs for the SP rate differences and aGMC ratios between DTwP-IPV-HB-PRP∼T and DTwP-HB-PRP∼T and IPV confirmed overall non-inferiority (Table 2). The post hoc evaluation using non-adjusted GMCs confirmed the equivalence and non-inferiority demonstrated using the aGMC data.

There were no clinically important differences between groups pre-first vaccination or post-third vaccination for antibodies to the D, T, pertussis, HB, IPV (anti-polio 1, 2, and 3), or PRP antigens in terms of the thresholds evaluated or for GMCs and GMTs, although differences were noted post-third vaccination for anti-polio GMTs (all types) (higher for DTwP-IPV-HB-PRP∼T) and anti-FHA GMC (higher for DTwP-HB-PRP∼T and IPV) (Table 3). Post-third vaccination, anti-D ≥ 0.01 IU/mL, anti-T ≥ 0.01 IU/mL, anti-HBs ≥ 10 mIU/mL, anti-PRP ≥ 0.15 µg/mL, and anti-polio 1, 2, and 3 ≥ 8 (1/dil.) were demonstrated for ≥ 99.7% of participants in the pooled DTwP-IPV-HB-PRP∼T and for 100.0% of participants in the DTwP-HB-PRP∼T and IPV groups. For pertussis antigens, VR rate was similar in each group for anti-PT (83.9% and 79.3%), anti-FIM (97.7% and 96.5%), anti-PRN (87.4% and 88.1%), and anti-FHA (74.7% and 80.7%). The increase in anti-rotavirus IgA antibodies was similar following co-administration with DTwP-IPV-HB-PRP∼T or concomitant DTwP-HB-PRP∼T and IPV (Table 3).

Safety and tolerability

There were no clinically important differences in the safety profile of the three DTwP-IPV-HB-PRP∼T lots (79.4%, 81.0%, and 81.9% of participants reporting at least one solicited reaction and 12.9%, 15.9%, and 16.3% of participants reporting at least one unsolicited

Table 3
Seroprotection rates, vaccine response rates, geometric mean concentrations, and geometric mean titers for DTwP-IPV-HB-PRP∼T and DTwP-HB-PRP∼T and IPV pre-first vaccination and post-third vaccination at 6–8, 10–14, and 16–18 weeks of age (Per Protocol population).

		DTwP-IPV-HB-PRP∼T* (N = 864[†])		DTwP-HB-PRP∼T and IPV (N = 285[†])	
		Pre-first vaccination	Post-third vaccination	Pre-first vaccination	Post-third vaccination
Anti-D	≥ 0.01 IU/mL	65.0 (61.8;68.2)	100.0 (99.6;100.0)	64.6 (58.7;70.1)	100.0 (98.7;100.0)
	≥ 0.1 IU/mL	14.6 (12.3;17.1)	99.5 (98.8;99.9)	15.4 (11.4;20.2)	99.6 (98.1;100.0)
	≥ 1.0 IU/mL	3.0 (2.0;4.4)	81.9 (79.2;84.5)	1.1 (0.2;3.0)	81.8 (76.8;86.1)
	GMC	0.020 (0.018;0.023)	2.55 (2.38;2.74)	0.020 (0.017;0.025)	2.47 (2.17;2.82)
	GMCR	NA	125 (108;144)	NA	121 (93.9;156)
Anti-T	≥ 0.01 IU/mL	100.0 (99.6;100.0)	100.0 (99.6;100.0)	100.0 (98.7;100.0)	100.0 (98.7;100.0)
	≥ 0.1 IU/mL	99.9 (99.4;100.0)	100.0 (99.6;100.0)	100.0 (98.7;100.0)	100.0 (98.7;100.0)
	≥ 1.0 IU/mL	89.6 (87.4;91.5)	87.4 (85.0;89.5)	89.1 (84.9;92.5)	88.8 (84.5;92.2)
	GMC	2.87 (2.70;3.05)	2.99 (2.79;3.20)	2.75 (2.49;3.03)	2.96 (2.62;3.35)
	GMCR	NA	1.04 (0.954;1.14)	NA	1.08 (0.926;1.26)
Anti-HBs	≥ 10 mIU/mL	17.1 (14.6;19.8)	99.7 (99.0;99.9)	22.3 (17.5;27.7)	100.0 (98.7;100.0)
	≥ 100 mIU/mL	7.5 (5.8;9.5)	97.0 (95.6;98.0)	8.0 (5.1;11.9)	97.2 (94.5;98.8)
	GMC	4.96 (4.47;5.50)	1422 (1307;1547)	5.74 (4.75;6.95)	1312 (1157;1487)
	GMCR	NA	279 (243;321)	NA	220 (173;280)
Anti-polio 1	≥ 8 (1/dil)	82.8 (80.1;85.2)	100.0 (99.6;100.0)	77.9 (72.6;82.6)	100.0 (98.7;100.0)
	GMT	40.8 (36.2;46.0)	2685 (2454;2936)	43.3 (34.6;54.2)	1546 (1289;1855)
	GMTR	NA	65.9 (57.1;76.0)	NA	35.7 (27.0;47.4)
Anti-polio 2	≥ 8 (1/dil)	71.8 (68.6;74.7)	99.7 (99.0;99.9)	71.6 (66.0;76.7)	100.0 (98.7;100.0)
	GMT	15.2 (14.0;16.6)	748 (678;825)	14.9 (12.8;17.3)	457 (391;534)
	GMTR	NA	49.2 (42.7;56.7)	NA	30.7 (24.4;38.6)
Anti-polio 3	≥ 8 (1/dil)	65.7 (62.4;68.9)	100.0 (99.6;100.0)	60.6 (54.6;66.3)	100.0 (98.7;100.0)
	GMT	22.6 (19.9;25.6)	3054 (2806;3325)	18.9 (15.2;23.6)	1419 (1186;1697)
	GMTR	NA	136 (116;159)	NA	75.3 (56.3;101)
Anti-PRP	≥ 0.15 µg/mL	38.9 (35.7;42.3)	100.0 (99.6;100.0)	36.3 (30.7;42.2)	100.0 (98.7;100.0)
	≥ 1 µg/mL	8.7 (6.9;10.8)	98.6 (97.6;99.3)	7.4 (4.6;11.1)	98.9 (97.0;99.8)
	GMC	0.108 (0.097;0.120)	18.1 (16.2;20.3)	0.099 (0.083;0.117)	17.8 (14.6;21.8)
	GMCR	NA	168 (146;194)	NA	180 (140;232)
Anti-PT	≥ 2 EU/mL	65.7 (62.5;68.9)	95.7 (94.1;97.0)	67.4 (61.6;72.8)	94.0 (90.6;96.5)
	VR[‡]	NA	83.9 (81.3;86.3)	NA	79.3 (74.1;83.9)
	≥ 4-fold rise	NA	75.2 (72.2;78.1)	NA	70.2 (64.5;75.4)
	GMC (EU/mL)	3.81 (3.51;4.14)	86.7 (77.3;97.4)	4.00 (3.46;4.63)	63.4 (51.0;78.7)
	GMCR	NA	22.8 (19.3;26.9)	NA	15.9 (11.7;21.5)
Anti-FIM	≥ 2 EU/mL	73.6 (70.5;76.5)	99.7 (99.0;99.9)	77.9 (72.6;82.6)	99.3 (97.5;99.9)
	VR[‡]	NA	97.7 (96.4;98.6)	NA	96.5 (93.6;98.3)
	≥ 4-fold rise	NA	93.4 (91.5;95.0)	NA	92.6 (89.0;95.4)
	GMC (EU/mL)	6.75 (6.08;7.50)	1258 (1154;1372)	7.68 (6.41;9.20)	1238 (1050;1461)
	GMCR	NA	186 (161;216)	NA	161 (123;212)
Anti-PRN	≥ 2 EU/mL	29.2 (26.2;32.3)	97.9 (96.7;98.8)	28.8 (23.6;34.4)	98.6 (96.4;99.6)
	VR[‡]	NA	87.4 (85.0;89.5)	NA	88.1 (83.7;91.6)
	≥ 4-fold rise	NA	85.4 (82.9;87.7)	NA	85.6 (81.0;89.5)
	GMC (EU/mL)	1.76 (1.64;1.88)	44.1 (40.3;48.3)	1.76 (1.56;1.99)	47.0 (40.3;54.8)
	GMCR	NA	25.1 (22.5;28.1)	NA	26.7 (21.8;32.7)
Anti-FHA	≥ 2 EU/mL	92.8 (90.9;94.5)	99.9 (99.4;100.0)	93.0 (89.4;95.7)	100.0 (98.7;100.0)
	VR[‡]	NA	74.7 (71.6;77.5)	NA	80.7 (75.6;85.1)
	≥ 4-fold rise	NA	46.8 (43.4;50.2)	NA	55.4 (49.5;61.3)
	GMC (EU/mL)	11.3 (10.4;12.2)	40.2 (37.3;43.4)	11.2 (9.69;12.9)	58.1 (51.0;66.2)
	GMCR	NA	3.58 (3.20;3.99)	NA	5.20 (4.27;6.35)
Anti-rotavirus[†]	≥ 4-fold rise	NA	56.6 (51.8;61.3)	NA	59.1 (50.2;67.6)
	GMC	8.06 (7.16;9.06)	61.4 (51.3;73.5)	7.37 (6.04;9.00)	57.6 (41.5;80.0)
	GMCR	NA	7.68 (6.31;9.35)	NA	7.95 (5.68;11.1)

Data are % (95% CI) participants with titer or concentration above threshold, VR, 4-fold rise from pre-first vaccination, GMT, or GMC.
*Pooled data from 3 lots of DTwP-IPV-HB-PRP∼T vaccine.
[†]For anti-rotavirus N = 440 (DTwP-IPV-HB-PRP∼T) and N = 132 (DTwP-HB-PRP∼T and IPV).
[‡]VR rate defined for anti-PT, anti-FIM, anti-PRN, and anti-FHA as: if pre-first vaccination concentration is < 4xlower limit of quantification (LLOQ) then post-third vaccination concentration ≥ 4x LLOQ; if pre-first vaccination concentration ≥ 4x LLOQ then post-third vaccination concentration ≥ pre-vaccination concentration.
NA, not applicable; GMCR, geometric mean concentration ratio; GMTR, geometric mean titer ratio; VR, vaccine response rate.

AE for Lot A, B, and C, respectively) and so only the pooled data are presented (Table 4).

There were no immediate AEs (within 30 min after vaccination). The overall incidence of participants with at least one injection site reaction was similar for DTwP-IPV-HB-PRP∼T (80.8%) and concomitant DTwP-HB-PRP∼T and IPV (81.3%), and in each group the most common injection site reaction was tenderness (64.9% and 69.4% of participants) (Table 4). Grade 3 solicited injection site reactions were reported by 15.0% (DTwP-IPV-HB-PRP∼T) and 12.2% (DTwP-HB-PRP∼T and IPV) of participants. For DTwP-HB-PRP∼T and IPV, respectively, 65.3% and 30.3% of participants reported tenderness, 17.5% and 6.9% reported erythema, and 34.7% and 11.9% reported swelling almost exclusively at the DTwP-HB-PRP∼T injection site. For solicited systemic reactions, the overall incidence was similar in each group (69.0% and 62.8% of participants), with 5.0% and 4.4% of participants reporting a Grade 3 reaction, the most common in each group being fever (43.8% and 37.8% of participants) (Table 4). Generally, the incidence of solicited injection site and systemic reactions either reduced or similar after each subsequent vaccination.

The overall incidence of participants reporting at least one unsolicited AE was similar for DTwP-IPV-HB-PRP∼T (15.0%) and DTwP-HB-PRP∼T and IPV (12.1%). Overall, the incidence after each vaccination was similar, most were Grade 1 in intensity with no

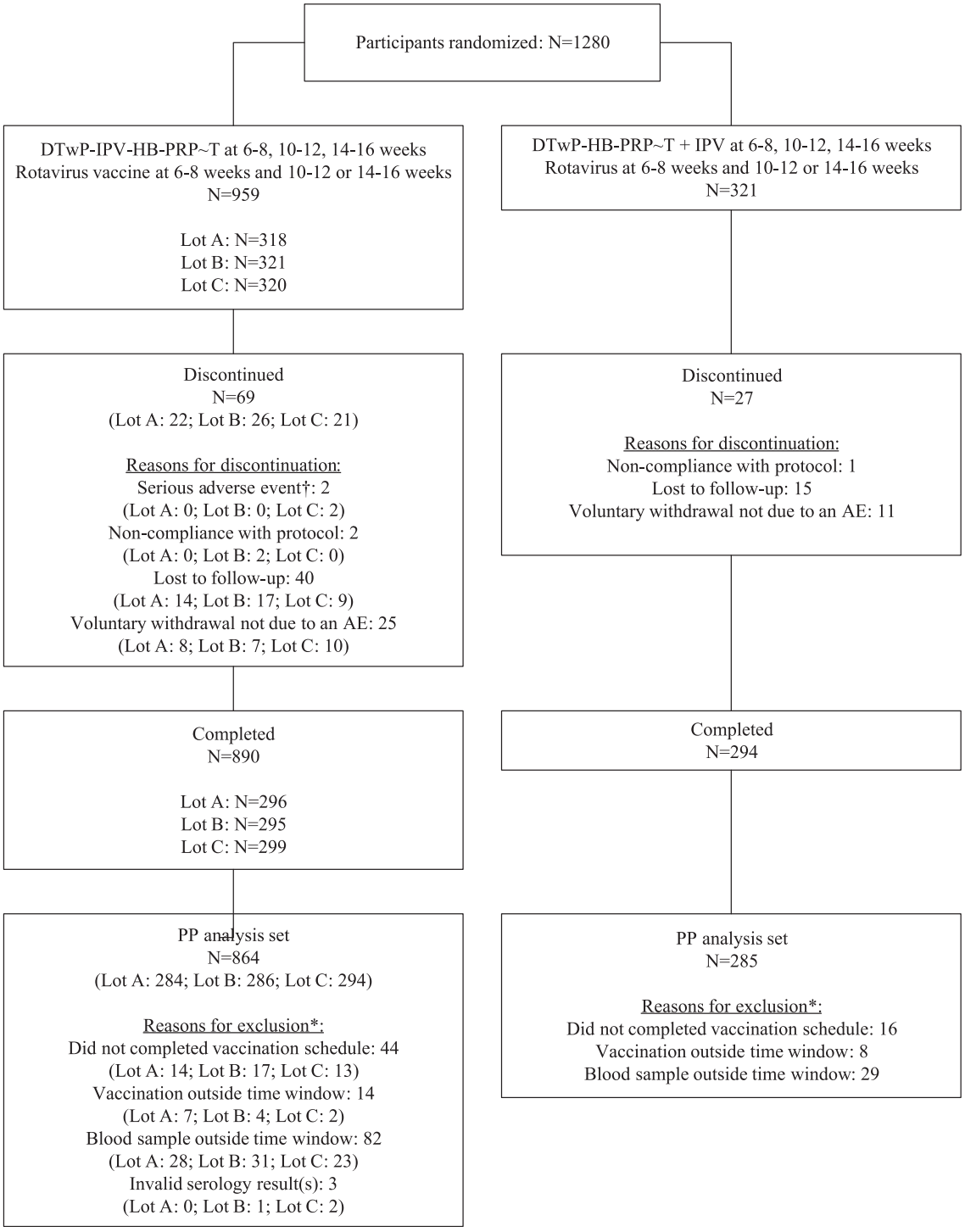

Fig. 1. Disposition of study participants *A participant could have more than one reason for exclusion from the PP analysis set †One fatal SAE (SIDS) and one SAE of tracheobronchitis that led to hospitalization.

Grade 3 unsolicited AEs, and none was considered to be clinically important. One participant in the DTwP-IPV-HB-PRP~T group (Lot C) and one participant in the DTwP-HB-PRP~T and IPV group reported an unsolicited AE considered to be related to the vaccination; both were episodes of injection site induration following the first vaccination and both participants received the subsequent vaccinations and completed the study as planned.

Serious adverse events that were not considered to be vaccine-related were reported by 9 participants in the DTwP-IPV-HB-PRP~T (4 SAEs in 4 participants for Lot A [lower respiratory tract infection, sepsis, upper respiratory tract infection], 2 SAEs of lower respiratory tract infection in 2 participants for Lot B, and 4 SAEs in 3 participants for Lot C [sudden infant death syndrome (SIDS), lower respiratory tract infection, tracheobronchitis (which led to hospitalization and subsequent participant discontinuation from the study), and gastroesophageal reflux]) and by no participant in the DTwP-HB-PRP~T and IPV group. The SIDS occurred 28 days after the second dose of DTwP-IPV-HB-PRP~T (Lot C) and there was no evidence of any causal relationship with vaccination.

Table 4
Immediate, solicited, unsolicited, and serious adverse events during the study (Safety Analysis Set).

	DTwP-IPV-HB-PRP∼T*(N = 959)			DTwP-HB-PRP∼T and IPV(N = 321)		
Participants with at least one:	n/M	%	(95% CI)	n/M	%	(95% CI)
Immediate unsolicited AE	0/959	0.0	(0.0;0.4)	0/321	0.0	(0.0;1.1)
Solicited reaction	772/956	80.8	(78.1;83.2)	260/320	81.3	(76.5;85.4)
Grade 3†	160/956	16.7	(14.4;19.3)	40/320	12.5	(9.1;16.6)
Solicited injection site reaction	665/956	68.5	(65.5;71.5)	235/320	73.4	(68.2;78.2)
Grade 3†	143/956	15.0	(12.8;17.4)	39/320	12.2	(8.8;16.3)
Tenderness	620/956	64.9	(61.7;67.9)	222/320	69.4	(64.0;74.4)
Erythema	235/956	24.6	(21.9;27.4)	62/320	19.4	(15.2;24.1)
Swelling	352/956	36.8	(33.8;40.0)	122/320	38.1	(32.4;43.7)
Solicited systemic reaction	660/956	69.0	(66.0;72.0)	201/320	62.8	(57.3;68.1)
Grade 3†	48/956	5.0	(3.7;6.6)	14/320	4.4	(2.4;7.2)
Fever	419/956	43.8	(40.7;47.0)	121/320	37.8	(32.5;43.4)
Vomiting	116/956	12.1	(10.1;14.4)	28/320	8.8	(5.9;12.4)
Crying abnormal	292/956	30.5	(27.6;33.6)	79/320	24.7	(20.1;29.8)
Drowsiness	186/956	19.5	(17.0;22.1)	54/320	16.9	(12.9;21.4)
Appetite lost	228/956	23.8	(21.2;26.7)	70/320	21.9	(17.5;26.8)
Irritability	374/956	39.1	(36.0;42.3)	116/320	36.3	(31.0;41.8)
Unsolicited AE	144/959	15.0	(12.8;17.4)	39/321	12.1	(8.8;16.2)
Unsolicited AR	1/959	0.1	(0.0;0.6)	1/321	0.3	(0.0;1.7)
AE leading to study discontinuation	2/959	0.2	(0.0;0.8)	0/321	0.0	(0.0;1.1)
SAE	9/959	0.9	(0.4;1.8)	0/321	0.0	(0.0;1.1)
Death	1/959	0.1	(0.0;0.6)	0/321	0.0	(0.0;1.1)

*Pooled data from 3 lots of DTwP-IPV-HB-PRP∼T vaccine.
†Grade 3 solicited injections site and systemic reactions were defined as follows: tenderness, cries when injected limb is moved or the movement of the injected limb is reduced; erythema and swelling, a diameter of ≥ 5 cm; fever, temperature > 39.5 °C; vomiting, ≥6 episodes/day; crying abnormal crying, >3 h; drowsiness, sleeping most of the time or difficult to wake up; appetite lost, refused ≥ 3 meals or refused most meals; irritability, inconsolable.
n, number of participants; M, number of participants with available data; AE, adverse event; AR, adverse reaction; SAE, serious adverse event.

Discussion

Equivalence was demonstrated for three lots of the final DTwP-IPV-HB-PRP∼T vaccine formulation, showing the manufacturing process to be reproducible. Subsequent non-inferiority of the pooled DTwP-IPV-HB-PRP∼T lots to the established DTwP-HB-PRP∼T and IPV vaccines confirmed the strong immunogenicity of each antigen administered concurrently in the hexavalent vaccine. For the *Bordetella pertussis* antigens, there are no established correlates of protection, and anti-PT and anti-FIM are the main drivers of pathogenicity and *Bordetella pertussis* agglutination [15–19]. Furthermore, anti-PT antibodies are considered important for protection against clinical symptoms, and WHO recommendations include the documentation of anti-PT responses [20,21]. For these reasons, anti-PT and anti-FIM were selected as the primary endpoints for the evaluation of the pertussis response using a robust qualified assay [8], and were included in the main statistical analyses. A descriptive comparison of all immune response thresholds, GMCs, and GMTs confirmed the robust response. Despite isolated differences, such as higher anti-polio GMTs in the DTwP-IPV-HB-PRP∼T group and higher anti-FHA GMC in the DTwP-HB-PRP∼T and IPV group, no differences between the two groups were considered to be clinically important and such isolated differences were expected for this type of vaccine [22–24]. These data are comparable to previous data for the new hexavalent vaccine [6,7] as well as other vaccines of this type [25–32] suggesting no interaction due to the co-administration with rotavirus vaccine. In particular, the high anti-polio antibody titers for the DTwP-IPV-HB-PRP∼T vaccine, which were almost twice as high as for standalone IPV and expected due to the adjuvant effect of aluminum contained in the vaccine [33–36] and consistent with previous studies, ensure alignment with the polio endgame strategy and withdrawal of OPV [3,37]. The literature for poliovirus vaccines is extensive, and documents various vaccination regimens that can be used in association with DTwP-containing vaccines, eg, bivalent OPV1&3-only, IPV-only, OPV followed by IPV, IPV followed by OPV, or mixed IPV/OPV [24,38]. These data demonstrate that SHAN6 can be administered following any regimen that uses IPV-containing vaccines and OPV. Additionally, the immune response to routine rotavirus vaccination was similar in each group and in the expected range [39,40].

The use of the ECL assay for the evaluation of the pertussis response in our study provides data that are precise, accurate, and reproducible [8]. Commercially available assays developed for routine pertussis diagnostic purposes that have been used in the evaluation of pertussis immunogenicity in many other clinical studies of wP-containing vaccines [28–32,41–43] have unknown specificity and are less well-suited to the robust evaluation that is required in clinical trials. Additionally, the pertussis component of the DTwP-IPV-HB-PRP∼T vaccine is similar to that contained in an established range of vaccines (DTCoq, Tetracoq, Pentacoq) that have been used extensively and have shown efficacy and field effectiveness against pertussis [44–46].

There were no safety concerns associated with administration of the DTwP-IPV-HB-PRP∼T vaccine in this study, consistent with other wP-containing multivalent vaccines in India and elsewhere [30,31,42,47,48]. There were very few unsolicited vaccine-related AEs, no vaccine-related SAEs, and no episodes of convulsions, hyporesponsive state, or encephalopathy. There was an imbalance in SAEs that were not related to vaccination, which could have been due to the imbalanced sample size between the DTwP-IPV-HB-PRP∼T and DTwP-HB-PRP∼T and IPV groups or possibly due to unconscious bias in the collection of safety data in the investigational vaccine group in this open-label study. No differences between groups were considered to be of clinical importance, and overall reactogenicity was similar in each group. One participant died of SIDS before the third dose of DTwP-IPV-HB-PRP∼T was due, and this was not considered to be related to vaccine

administration. The DTwP-IPV-HB-PRP∼T safety profile was comparable to previous studies with this vaccine [6,7] and its wP-antigen matching predecessor [48–50].

Limitations of the study included the open-label design, which could have introduced bias in the reporting of safety, and the lack of a control group without rotavirus vaccination although comparison to historical data, described earlier, was not indicative of any interaction.

In conclusion, three lots of the fully liquid DTwP-IPV-HB-PRP∼T vaccine showed consistency of manufacturing by showing consistent immunogenicity which was comparable to licensed vaccines following vaccination of infants at 6–8, 10–12, and 14–16 weeks of age in India. There was no evidence of any effect of co-administration with rotavirus vaccine, and there were no safety concerns. Hexavalent vaccines containing IPV are expected to increase compliance to vaccination schedules, favor access to multi-dosing IPV regimens, and help to support switching to IPV use globally.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper: Clinical investigators involved in these studies received fees from Sanofi Pasteur through their institutions for the conduct of these clinical studies, but did not receive any direct payment from Sanofi Pasteur in this regard. They may have received expenses for conference attendance for the presentation of data from these studies. AM, FN, and KV are employees of Sanofi Pasteur and may hold shares and/or stock options in the company; BNP, SM, SR, and VJM are employees of Sanofi Healthcare India Private Ltd (SHIPL); RSS reports grants from Shantha Biotechnics Private Limited; RS holds shares in Sanofi India Private Ltd and reports investigator fees from Maulana Azad Medical College; AK, DK, IVP, MD, MM (Maurya), MM (Mitra), SP (Palkar), SP (Prasanth), RZK, SD, VE, and VNT have nothing to declare.

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