Safety and immunogenicity of a hexavalent DTwP-IPV-HB-PRP~T vaccine versus separate DTwP-HB-PRP~T and IPV vaccines in healthy infants in India

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ABSTRACT

Background: Multivalent vaccines containing whole-cell pertussis (wP) antigens combined with established diphtheria (D), tetanus (T), hepatitis B (HB), Haemophilus influenzae type b (Hib), and inactivated poliomyelitis (IPV) antigens allow the provision of a high-quality, affordable DTwP-IPV-HB-PRP~T vaccine.

Methods: Phase I/II, randomized, active-controlled, open-label study in healthy toddlers (Cohort I) and infants (Cohort II). Toddlers in Cohort I who had completed primary series D, T, P, HB, Hib, and polio vaccination received a booster dose of DTwP-IPV-HB-PRP~T (N = 30) or DTwP-HB-PRP~T + IPV (N = 15) vaccines at 15–18 months of age. After satisfactory review of safety data in Cohort I, infants in Cohort II received DTwP-IPV-HB-PRP~T (N = 100) or DTwP-HB-PRP~T + IPV (N = 50) at 6–8, 10–12, and 14–16 weeks of age. All infants in Cohort II had received previous oral polio and HB vaccines per country recommendations.

Results: Booster and primary series vaccinations were well tolerated with no clinically significant differences between vaccine groups. Most adverse events were mild and resolved spontaneously; there were no vaccine-related serious adverse events and no deaths. In both vaccine groups, anti-D, anti-T, anti-HB, anti-Hib, and anti-polio 1, 2, and 3 seroprotection was 100% post-booster and post-primary series. For the pertussis antigens, booster response rate was > 86% in both groups. For the primary series, vaccine response rate was slightly higher for DTwP-IPV-HB-PRP~T than DTwP-HB-PRP~T + IPV for anti-PT (80.2% and 70.8%) and anti-FHA (81.3% and 68.8%), slightly lower for anti-PRN (72.5% and 81.3%), and similar in each group for anti-FIM (95.6% and 97.9%).

Conclusions: This study demonstrated a good safety and immunogenicity profile of the hexavalent DTwP-IPV-HB-PRP~T vaccine for infant primary series vaccination at 6–8, 10–12, and 14–16 weeks of age and booster vaccination at 15–18 months of age and supported progression to the next development phase.

Introduction

Pediatric vaccines that incorporate several antigens in a single administration facilitate high vaccine coverage and compliance. Such vaccines are used routinely globally and have been critical in achieving dramatic decreases in the global incidence of childhood diseases such as diphtheria (D), tetanus (T), pertussis, hepato-
tis B (HB), *Haemophilus influenzae* type b (Hib) infection, and polio [1,2].

A pentavalent vaccine containing D, T, whole cell pertussis (wp), HB, and Hib antigens (SHAN5®) was licensed in India by Sanofi Healthcare India Private Limited (SHIPL) (formerly Shanta Biotechnics Private Ltd) in March 2014 and pre-qualified by the World Health Organization (WHO) in April of the same year. It is currently licensed in 20 countries globally with approximately 150 million doses having been administered. The inactivated poliomyelitis vaccine (IPV) SHANIVP™ was licensed in India in 2015 on the basis of the international licenses of Imovax Polio manufactured by the parent company of SHIPL (Sanofi Pasteur).

To support the objective of the Global Polio Eradication Initiative outlined in the last edition of its 2019–2023 Endgame Strategic Plan [3] and in alignment with market needs [4] SHIPL developed SHAN6™, a fully liquid, ready-to-use, wp-IPV hexavalent vaccine (DTwP-IPV-HB-PRP-T vaccine) by combining the SHAN5 and SHANIPV antigens. The D, T, wp, HB, Hib, and IPV antigens contained in SHAN5 have been extensively evaluated in prior clinical development. The D, T, and wp antigens are the same as those included in SHAN5 with the exception of thiemersal preservative [5] and the wp antigen is derived from the historical Bordetella pertussis seed strains used by Sanofi Pasteur for its established DTwP trivalent (DTC00™), DTwP-IPV quadrivalent (TETRACoq™), and pentavalent DTwP-IPV/Hib (PENTACoq™) vaccines [6–8]; the HB antigen is *Hansenula polymorpha*-derived and is the same as the HB component of Sanofi Pasteur’s Hexaxim™ (an aP-containing hexavalent vaccine other than the birth dose of OPV, HB, and BCG vaccines) [9] produced using its manufacturing facility in Argentina [10]; the Hib component is produced by SHIPL in India by the conjugation of purified capsular polysaccharide (PRP) provided by Sanofi Pasteur France and used in Hexaxim and IMOVAX® Polio and bulk is supplied by Sanofi Pasteur France, with formulation, filling, and packaging steps conducted at SHIPL. The Hib and IPV components are also used in Sanofi Pasteur’s well-established aP-containing pentavalent vaccine, Pentaxim® [11]. Good primary series safety and immunogenicity have previously been demonstrated for Hexaxim and Pentaxim in a 6, 10, 14 week schedule in India [12,13] and South Africa [14,15].

The development of a wp-containing hexavalent vaccine in addition to existing acellular pertussis (aP)-containing vaccines was considered important to offer a high-quality vaccine at a cost that is affordable for developing countries. Since the antigens included in SHAN6 have been approved and marketed as either established standalone or combination vaccines, robust immunogenicity was expected for each antigen. The primary objective of the present study was therefore to evaluate the safety profile of the new hexavalent vaccine in a Phase I/II stepwise trial design approach. Assessment of immunogenicity was included as secondary objectives.

Materials and methods

Study design and participants

This was a Phase I/II, randomized, active-controlled, open-label study conducted in toddlers (Cohort I) and infants (Cohort II) at 4 sites in India (Clinical Trials Registry India Number CTRI/2016/11/007434). The study protocol and one amendment were approved by the institutional ethics committee of each study site and the study was performed according to local and national regulations and was consistent with the standards established by the Declaration of Helsinki and compliant with the International Council for Harmonization guidelines for Good Clinical Practice. An informed consent form was signed by each participant’s parents or legally acceptable representatives before enrolment into each study. The study was conducted between November 2016 and October 2017.

In Cohort I, healthy toddlers aged 15–18 months who had completed a primary infant series vaccination against D, T, P, HB, Hib, and polio were eligible for inclusion. In Cohort II, healthy infants aged 6–8 weeks, born at full-term (≥37 weeks), with birth weight ≥2.5 kg, who had received a birth dose of oral poliovirus vaccine (OPV), HB vaccine, and Bacillus Calmette-Guérin (BCG) vaccine ≥4 weeks prior to the first study vaccination were eligible for inclusion. The main exclusion criteria in both groups were recent (in the 4 weeks prior to the first vaccination) or planned participation in another clinical study; known hypersensitivity to any vaccine component; any chronic illness that could interfere with study conduct or completion; receipt of blood products in the 30 days prior to inclusion or planned during the study; history of diphtheria, tetanus, pertussis, Hib, HB, or poliovirus infection; personal/maternal history of human immunodeficiency virus, HB or hepatitis C infection; known thrombocytopenia, bleeding disorder, or receipt of anticoagulants in the 3 weeks prior to inclusion; history of seizures; acute illness or febrile illness on the day of vaccination. Additionally, participants were excluded for receipt of immunosuppressive therapy for more than 2 consecutive weeks in the past 3 months (Cohort I) or for more than 2 consecutive weeks (Cohort II); any planned vaccination in the 4 weeks following study vaccination or booster dose scheduled in the second year of life (Cohort I), planned receipt of any other non-study vaccine from 8 days before to 8 days after each study vaccination or previous vaccination (other than the birth dose of OPV, HB, and BCG vaccines) or planned receipt of any D, T, P, HB, Hib, or poliomyelitis vaccine other than the study vaccines in the 4 weeks following study vaccination (Cohort II).

In Cohort I, 45 toddlers were randomized in a 2:1 ratio using permuted blocks and stratified by site to receive a single dose of either the fully liquid hexavalent vaccine (DTwP-IPV-HB-PRP-T vaccine) or separately administered DTwP-HB-PRP-T vaccine at 15–18 months of age. After independent review by a Data Safety Monitoring Board (DSMB) of the safety data from Cohort I, 30 infants in Cohort II were randomized in a 2:1 ratio to receive either the DTwP-IPV-HB-PRP-T vaccine or separately administered DTwP-IPV-HB-PRP-T vaccine at 6–8, 10–12, and 14–16 weeks of age. A further 120 infants were enrolled in Cohort II following review of the first dose safety data by the DSMB from the first 30 infants in Cohort II.

Study vaccines were administered by intramuscular injection into the anterolateral aspect of the left thigh (DTwP-IPV-HB-PRP-T or DTwP-HB-PRP-T vaccines) and right thigh when applicable (IPV vaccine).

Study vaccines

The hexavalent DTwP-IPV-HB-PRP-T vaccine (SHAN6, batch number HPCK0216, expiry date January 2018) 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-tetanus toxoid, ≥60 IU T-tetanus toxoid, ≥4 IU whole-cell B. pertussis organisms, 10 μg tDNA HB surface antigen (HBsAg), 12 μg Hib purified capsular polysaccharide conjugated to 20–40 μg tetanus toxoid carrier protein, 40, 8 and 32 D antigen units of poliovirus type 1 (Mahoney strain), type 2 (MEF-1 strain), and type 3 (Saukett strain), respectively, and 0.625 mg aluminum phosphate as adjuvant.
The pentavalent DTwP-HP-PRP–T vaccine (SHAN5, batch number PLK033B15, expiry date September 2017) was manufactured by SHPL 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 HB surface antigen (HBsAg), and 0.625 mg aluminum phosphate as adjuvant.

The IPV vaccine (SHANIPV, batch number IPQ002A16, expiry date December 2017) was manufactured by SHPL 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 poliovirus type 1 (Mahoney strain), type 2 (MEF-1 strain), and type 3 (Saukett strain), respectively.

Reactivity and safety

Participants were observed at the study site for 30 min after each vaccination to assess immediate unsolicited adverse events (AEs). Subsequently, parent(s)/legal representative(s) used diary cards for 7 days after each vaccination to record the duration and intensity (Grade 1 [mild] to 3 [severe]) of solicited injection site reactions (tenderness, erythema, swelling) and solicited systemic reactions (fever, vomiting, crying abnormal, drowsiness, appetite lost, irritability) reactions. All solicited reactions were automatically considered to be related to the vaccination. For temperature measurement the axillary route was preferred.

Unsolicited AEs were recorded using diary cards for 28 days after each vaccination. All unsolicited injection site AEs were automatically considered to be related to the vaccination and the Investigator assessed unsolicited systemic AEs for causality and intensity (Grade 1, no interference with activity; Grade 2, some interference with activity; Grade 3, significant – prevents daily activity).

Serology

Blood samples (approximately 3–5 mL) were collected in Cohort I pre-vaccination and 28 days post-vaccination, and in Cohort II pre-first vaccination and 28 days post-third vaccination for determination of antibodies to all antigens (anti-D, anti-T, anti-pertussis, anti-Hib, anti-HB, anti-polio 1, anti-polio 2, and anti-polio 3).

Assays were performed at either Quest Diagnostics India Pvt Ltd (QDI) (Delhi, India) or Sanofi Pasteur’s Global Clinical Immunology (GCI) laboratory (Swiftwater, PA, USA).

Commercial enzyme linked immunosorbent assay (ELISA) kits were used at QDI to measure anti-D (NovaTec Immunodiagnostics GmbH, Germany), anti-T (IBL International GmbH, Germany), anti-HB (VITROS, Ortho Clinical Diagnostics, United Kingdom), and anti-Hib (Binding Site Ltd, United Kingdom) antibody concentrations. At GCI, a validated MesoScale Discovery Multiplexed Electro Chemoluminescence (DTP-ECL) immunoassay [16,17] was used to measure anti-D, anti-T, and anti-pertussis (anti-pertussis toxin [PT], anti-filamentous hemagglutinin [FHA], anti-pertactin [PRN], and anti-fimbriae 2/3 [FIM]) antibodies. Poliovirus neutralizing antibody titers were measured at GCI by micro metabolic inhibition testing (MIT) against wild type strains.

Table 1 summarizes the serological assessments for each antigen by assay (ELISA [NovaTec, IBL, VITROS, Binding Site], DTP-ECL, or MIT), laboratory (QDI or GCI), and cohort.

### Table 1

| Cohort I | Assay (LOQ) | Laboratory | Cohort II | Assay (LOQ) | Laboratory |
|----------|-------------|------------|-----------|-------------|------------|
| Diphtheria | ELISA (NovaTec) (0.01 IU/mL) | QDI | ELISA (NovaTec) (0.01 IU/mL) | QDI |
| | DTP-ECL (0.007 IU/mL) | GCI | DTP-ECL (0.007 IU/mL) | GCI |
| Tetanus | ELISA (IBL) (0.0004 IU/mL) | QDI | ELISA (IBL) (0.0004 IU/mL) | QDI |
| | DTP-ECL (0.01 IU/mL) | GCI | DTP-ECL (0.01 IU/mL) | GCI |
| Pertussis | PT DTP-ECL (4 EU/mL) | GCI | DTP-ECL (4 EU/mL) | GCI |
| | FHA DTP-ECL (3 EU/mL) | GCI | DTP-ECL (3 EU/mL) | GCI |
| | PRN DTP-ECL (4 EU/mL) | GCI | DTP-ECL (4 EU/mL) | GCI |
| | FIM DTP-ECL (4 EU/mL) | GCI | DTP-ECL (4 EU/mL) | GCI |
| | HB ELISA (VITROS) (5 mIU/mL) | QDI | ELISA (VITROS) (5 mIU/mL) | QDI |
| | Hib ELISA (Binding Site) (0.11 μg/mL) | QDI | ELISA (Binding Site) (0.11 μg/mL) | QDI |
| | Polio MIT (4 1/dil) | GCI | MIT (4 1/dil) | GCI |

See text for abbreviations.

* Analytical sensitivity.

b Refers to limit of detection.
assumed for the immunogenicity assessment and overall sample sizes were 45 participants for Cohort I and 150 participants for Cohort II.

Seroprotection was defined as anti-D antibody ≥ 0.01 IU/mL, anti-T ≥ 0.01 IU/mL, anti-HBs ≥ 10 mIU/mL, anti-Hb ≥ 0.15 μg/mL, and anti-polio 1, 2, and 3 titers ≥ 8 1/dl. For pertussis responses, booster responses (for anti-PT, anti-FHA, anti-PRN, and anti-FIM) for Cohort I were defined in participants with pre-booster concentrations < 4xLLOQ as post-booster concentrations ≥ 4x pre-booster concentrations, and in participants with pre-booster concentrations ≥ 4xLLOQ as post-vaccination titers ≥ 2x pre-booster concentrations. Vaccine responses for Cohort II participants were defined as in participants with pre-vaccination concentrations < 4xLLOQ as post-vaccination concentrations ≥ 4x pre-vaccination concentrations, and in participants with pre-vaccination concentrations ≥ 4xLLOQ as post-vaccination titers ≥ pre-vaccination concentrations. The lower limits of quantification (LLOQ) for each assay are shown in Table 1.

Data were also presented for the following thresholds: anti-D ≥ 0.1 and ≥ 1.0 IU/mL, anti-T ≥ 0.1 and ≥ 1.0 IU/mL, anti-HB ≥ 100 mIU/mL, anti-Hb ≥ 1.0 μg/mL. The percentage of participants with a ≥ 4-fold rise in anti-PT, anti-FHA, anti-PRN, and anti-FIM antibody titers are presented post-vaccination in Cohort I and post-third vaccination in Cohort II. Additionally, geometric mean concentrations (GMCs: anti-D, anti-T, anti-PT, anti-FHA, anti-PRN, anti-FIM, anti-HB, anti-Hb) geometric mean titers (GMTs: anti-polio 1, 2, and 3), and the ratio of post/pre-vaccination (Cohort I) and post-primary/pre-primary (Cohort II) are presented for all antigens.

Data are presented with their 95% confidence intervals (CIs), calculated using the exact binomial distribution (Clopper-Pearson method) [19] for proportions and the normal approximation method for GMCs and GMTs.

The safety analysis set (SafAS) population was used for all safety analyses (participants who received at least one vaccination) and the Full analysis set (FAS) was used for the immunogenicity analyses (participants who received at least one vaccination and analyzed according to the randomization).

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

Results

Participants studied

In Cohort I, a total of 45 participants were randomized to receive a single dose of either DTwP-IPV-HB-PRP-T (N = 30) or DTwP-HB-PRP-IPV (N = 15) (Fig. 1). In each group the male:female ratio (50:50 and 40:60), mean ± SD age (16.4 ± 1.0 and 16.3 ± 1.0 months), and mean ± SD weight (9.58 ± 1.16 and 9.07 ± 1.09 kg) were similar.

In Cohort II, a total of 150 participants were randomized to receive three doses of either DTwP-IPV-HB-PRP-T (N = 100) or DTwP-HB-PRP-IPV (N = 50) (Fig. 1). In each group the male:female ratio (51:49 and 50:50), mean ± SD age at first vaccination (6.3 ± 0.53 and 6.2 ± 0.51 months), and mean ± SD birth weight (2.94 ± 0.33 and 3.02 ± 0.37 kg) were similar.

All participants in Cohort I completed the study; in Cohort II, 91/100 participants (DTwP-IPV-HB-PRP-T) and 48/50 participants (DTwP-HB-PRP-IPV-T + IPV) completed the study (Fig. 1).

Safety and tolerability

There were no immediate AEs (i.e. within 30 min after any vaccination) in Cohort I or Cohort II.

In Cohort I, the overall incidence of participants with at least one solicited injection site or solicited systemic reaction was slightly higher for DTwP-IPV-HB-PRP-T (96.7% and 90.0%) than DTwP-HB-PRP-IPV (86.7% and 80.0%) (Table 2). In each group the most common solicited injection site reaction was tenderness (93.3% and 73.3%); the most common solicited systemic reactions were irritability for DTwP-IPV-HB-PRP-T (70.0%) and fever for DTwP-HB-PRP-IPV (73.3%). Grade 3 injection site reactions were only reported by 2 participants in the DTwP-IPV-HB-PRP-T group and 1 participant in the DTwP-HB-PRP-IPV group; no Grade 3 solicited systemic reactions were reported. Most solicited injection site reactions resolved spontaneously within 3 days. Unsolicited AEs occurred in 10% of participants in the DTwP-IPV-HB-PRP-T group and none in the DTwP-HB-PRP-IPV group. None was rated as Grade 3 in intensity. In the DTwP-IPV-HB-PRP-T group, 3 participants (6.7%) reported unsolicited AEs: 2 episodes of injection site nodule in 2 participants that were Grade 1 in intensity, related to the study vaccine, and which resolved within 3 weeks without medication, and a single episode of rectal abscess and staphylococcal infection in another participant that was not related to the study vaccine. The rectal abscess and staphylococcal infection resolved after hospitalization and so was classed as SAEs but not considered to be related to vaccination. No AE of special interest was reported in either cohort.

In Cohort II after any vaccination, the overall incidence of participants with at least one injection site reaction was similar for DTwP-IPV-HB-PRP-T (79.8%) and DTwP-HB-PRP-IPV (81.6%); for solicited systemic reactions the incidence was slightly higher for DTwP-IPV-HB-PRP-T (82.8%) than DTwP-HB-PRP-IPV (73.5%). In each group the most common injection site reaction was tenderness and the most common solicited systemic reaction was irritability (Table 1). Grade 3 injection site and systemic reactions were reported by 29.3% and 5.1% of participants in the DTwP-IPV-HB-PRP-T group and 10.2% and 4.1% of participants in the DTwP-HB-PRP-IPV group. Generally, the incidence of solicited injection site and systemic reactions either reduced or stayed similar after each subsequent vaccination. The overall incidence of unsolicited AEs was slightly higher for DTwP-HB-PRP-IPV (20.0%) than DTwP-IPV-HB-PRP-T (14.0%), with most in each group being Grade 1 in intensity, occurring later than 14 days post-vaccination, and lasting < 14 days. Of these, episodes of injection site discoloration, induration, indentation, and nodule were considered to be related to vaccination in the DTwP-IPV-HB-PRP-T group; no one solicited injection site reaction was considered to be related to vaccination in the DTwP-HB-PRP-IPV group. In 1 participant, two episodes of vaccine-related induration after the first and second dose led to discontinuation of the participant from the study. Two participants experienced SAEs, which were not related to the study vaccine: episodes of gastroenteritis, dehydration, septic shock, and hepatic ischemia in a 4 month old girl who had received 3 doses of DTwP-IPV-HB-PRP-T and which resolved within 4 days after hospitalization (the participant remained in the study), and congenital heart disease with congestive cardiac failure in a 3 month old boy who had received 2 doses of DTwP-IPV-HB-PRP-T and which resolved within 142 days after hospitalization (the participant was discontinued from the study).

There were no vaccine-related SAEs and no deaths in either cohort during the study.

Immunogenicity

Cohort I

Pre-vaccination, anti-D ≥ 0.01 IU/mL, anti-T ≥ 0.01, anti-HB ≥ 10 mIU/mL, anti-Hib ≥ 0.15 μg/mL, and anti-polio 1, 2, and 3 antibody titers ≥ 8 1/dl were all > 93% in each group, and all increased to 100.0% post-vaccination (Table 3). The results
obtained using ELISA or DTP-ECL were similar. Additionally, all other pre-defined thresholds for these antigens increased to 100.0% post-vaccination in each group. For pertussis responses (anti-PT, anti-FHA, anti-PRN, and anti-FIM) there was little difference between groups pre-vaccination and the post-vaccination vaccine response (anti-PT, anti-FHA, anti-PRN, and anti-FIM) was strong with little difference between groups (ranging from 86.7% [anti-PRN] in both groups to 96.7% [anti-FHA and anti-FIM] for DTwP-IPV-HB-PRP/C24T and 100.0% [anti-FIM] for DTwP-HB-PRP/C24T + IPV). Geometric mean concentrations and GMTs (Table 5) showed some differences between groups, with post-vaccination anti-T GMC and anti-polio 1, 2, and 3 GMTs being slightly higher for DTwP-HB-PRP-T + IPV and anti-wP, GMCs being slightly higher for DTwP-IPV-HB-PRP-T.

CoHort II
Pre-primary vaccination seroprotection rates and other pre-defined thresholds for anti-D, anti-T, anti-HB, anti-Hib, and anti-IPV (Table 4), as well as GMCs and GMTs (Table 6), were generally similar in each group.

Post-primary series vaccination GMCs increased for anti-D, anti-PT, anti-FHA, anti-PRN, anti-HB, and anti-Hib and were generally similar for each group whereas the post-vaccination increase in anti-polio 1, 2, and 3 GMTs were higher for DTwP-IPV-HB-PRP-T than DTwP-HB-PRP-T + IPV (Table 6). Anti-T GMCs decreased slightly post-vaccination as the result of the very high pre-existing maternal antibodies. Anti-D ≥ 0.01 IU/mL, anti-T ≥ 0.01 and ≥ 0.10, anti-HB ≥ 10 mIU/mL, anti-Hib ≥ 0.15 μg/mL, and anti-polio 1, 2, and 3 ≥ 8 1/dil antibody
titers all increased to 100.0% in each group post-primary series vaccination (Table 3). Vaccine response was slightly higher for DTwP-IPV-HB-PRP–T than DTwP-HB-PRP–T + IPV for anti-PT (80.2% and 70.8%), anti-FHA (81.3% and 68.8%), slightly lower for DTwP-IPV-HB-PRP–T than DTwP-HB-PRP–T + IPV for anti-PRN (72.5% and 81.3%), and similar in each group for anti-FIM (95.6% and 97.9%). The results for anti-D and anti-T obtained using ELISA or DTP-ECL were similar.

Discussion

This study is the first to evaluate the safety and immunogenicity of the fully liquid DTwP-IPV-HB-PRP–T vaccine compared to its active control, antigen-matched vaccines (pentavalent DTwP-HB-PRP–T and standalone IPV). As such, the study was designed to administer the vaccines in a step-down manner, initially as a single dose to toddlers aged to 15–18 months (Cohort I) to establish safety before proceeding to a 3-dose primary vaccination series in infants aged 6–10, 12, and 14–16 weeks (Cohort II).

No safety concerns were observed in Cohort I and so participants in Cohort II were enrolled and received the primary series vaccination as planned. The low incidence of transient vaccine-related nodules reported following DTwP-IPV-HB-PRP–T was within the expected range for DTwP-containing vaccines [20] and was not considered to be of clinical importance, and no vaccine-related SAEs were reported. Overall, in each cohort, the hexavalent vaccine showed a similar safety profile to the comparator DTwP-HB-PRP–T and standalone IPV vaccines and the safety profile was in-line with that reported for other wP-containing hexavalent and pentavalent vaccines in India and elsewhere [5,21–23].

| Participants with at least one: | DTwP-IPV-HB-PRP–T | DTwP-HB-PRP–T + IPV |
|-------------------------------|------------------|------------------|
|                               | n/M % (95% CI)   | n/M % (95% CI)   |
| **COHORT I**                  |                  |                  |
| Immediate, unsolicited AE     | 0/30 0.0 (0.0;11.6) | 0/15 0.0 (0.0;21.8) |
| Solicited reaction            | 29/30 96.7 (82.8;99.9) | 13/15 86.7 (59.5;98.3) |
| Solicited injection site reaction | 29/30 96.7 (82.8;99.9) | 11/15 73.3 (44.9;92.2) |
| Tenderness                    | 28/30 93.3 (77.9;99.2) | 11/15 73.3 (44.9;92.2) |
| Erythema                      | 14/30 46.7 (28.3;65.7) | 3/15 20.0 (4.3;48.1) |
| Swelling                      | 18/30 60.0 (40.6;77.3) | 6/15 40.0 (16.3;67.7) |
| Solicited systemic reaction    | 27/30 90.0 (73.5;97.9) | 12/15 80.0 (51.9;95.7) |
| Fever                         | 15/30 50.0 (31.3;68.7) | 10/15 66.7 (38.4;86.2) |
| Vomiting                      | 3/30 10.0 (2.1;26.5) | 0/15 0.0 (0.0;21.8) |
| Crying abnormal               | 13/30 43.3 (25.5;62.6) | 6/15 40.0 (16.3;67.7) |
| Drowsiness                    | 6/30 20.0 (7.7;38.6) | 5/15 33.3 (11.8;61.6) |
| Appetite lost                 | 15/30 50.0 (31.3;68.7) | 5/15 33.3 (11.8;61.6) |
| Irritability                  | 21/30 70.0 (50.6;85.3) | 5/15 33.3 (11.8;61.6) |
| Unsolicited AE                | 3/30 10.0 (2.1;26.5) | 0/15 0.0 (0.0;21.8) |
| Unsolicited AR                | 2/30 6.7 (0.8;22.1) | 0/15 0.0 (0.0;21.8) |
| AE leading to study discontinuation | 1/30 3.3 (0.1;17.2) | 0/15 0.0 (0.0;21.8) |
| SAE                           | 0/30 0.0 (0.0;11.6) | 0/15 0.0 (0.0;21.8) |
| Death                         | 0/30 0.0 (0.0;11.6) | 0/15 0.0 (0.0;21.8) |
| **COHORT II**                 |                  |                  |
| Immediate, unsolicited AE     | 0/100 0.0 (0.0;3.6) | 0/50 0.0 (0.0;7.1) |
| Solicited reaction            | 87/99 87.9 (79.8;93.6) | 42/49 85.7 (72.8;94.1) |
| Solicited injection site reaction | 79/99 79.8 (70.5;87.2) | 40/49 81.6 (68.0;91.2) |
| Tenderness                    | 77/79 77.5 (68.0;85.6) | 38/49 77.6 (63.4;87.1) |
| Erythema                      | 38/99 38.4 (28.8;48.7) | 16/49 32.7 (19.9;47.5) |
| Swelling                      | 60/99 60.6 (50.3;70.3) | 28/49 57.1 (42.2;71.2) |
| Solicited systemic reaction    | 82/99 82.8 (73.5;89.7) | 36/49 73.5 (58.9;85.1) |
| Fever                         | 51/99 51.5 (41.3;61.7) | 26/49 53.1 (38.3;63.5) |
| Vomiting                      | 21/99 21.2 (13.6;30.6) | 8/49 16.3 (7.3;29.7) |
| Crying abnormal               | 23/99 23.2 (13.3;32.0) | 5/49 10.2 (3.4;22.2) |
| Drowsiness                    | 41/99 41.4 (31.6;51.8) | 15/49 30.6 (18.3;45.4) |
| Appetite lost                 | 42/99 42.4 (32.5;52.8) | 14/49 28.6 (13.4;42.2) |
| Irritability                  | 61/99 61.6 (51.3;71.2) | 28/49 57.1 (42.2;71.2) |
| Unsolicited AE                | 14/100 14.0 (7.9;22.4) | 10/50 20.0 (10.0;33.7) |
| Unsolicited AR                | 3/100 3.0 (0.6;8.5) | 0/50 0.0 (0.0;7.1) |
| AE leading to study discontinuation | 2/100 2.0 (0.2;7.0) | 0/50 0.0 (0.0;7.1) |
| SAE                           | 2/100 2.0 (0.2;7.0) | 0/50 0.0 (0.0;7.1) |
| Death                         | 0/100 0.0 (0.0;3.6) | 0/50 0.0 (0.0;7.1) |

n, number of participants; N, number of participants in group; M, number of participants with available data; AE, adverse event; AR, adverse reaction; SAE, serious adverse event.

* Data for Cohort II are after any vaccination.
A limitation of the study is the open-label design, which could have introduced bias into the safety assessments as both the participants and investigators knew whether the investigational products that use the same antigens, this is not considered to represent marketing experience over a long period of time with several products.

In conclusion, the study demonstrated a good safety and immunogenicity profile of the hexavalent DTwP-IPV-HB-PRP~T vaccine when administered as an infant primary 3-dose vaccination series at 6–8, 10–12, and 14–16 weeks of age as well as when given to toddlers as a single booster dose and warrants further development in larger and statistically powered studies.

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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: Clinical investigators involved in these studies (SP, MM, MDR, and APD) 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. EJ, FN, and SM hold Sanofi stock. EJ, FN, and AM are employees of Sanofi Pasteur. DMP and EJ were employees of Sanofi Pasteur at the time of the study. BNP, SM, MVJ, SR, and SRJ are employees of Sanofi Healthcare India Private Ltd (SHIPL).

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Table 4
Seroprotection and seroresponse rates pre- and post-primary series vaccination (Cohort II) (FAS).

| Antigen | Assay | Threshold | DTwP-IPV-HB-PRP-T (N = 100) | DTwP-HB-PRP-T + IPV (N = 50) |
|---------|-------|-----------|-----------------------------|-------------------------------|
|         |       |           | Pre-primary                  | Post-primary                  |
|         |       |           | GMT (1/dil)                  | GMC (IU/mL)                  |
|         |       | ≥4 EU/mL  | 4.0 (3.5;5.5)                | 8.0 (7.5;9.0)                |
|         |       | >4-fold rise | NA                           | 100.0 (98.0;100.0)           |
| Diphtheria | ELISA (NovaTec) | ≥0.1 IU/mL | 45.0 (35.0;55.3) | 100.0 (96.0;100.0) |
|         |       | ≥0.1 IU/mL | 4.0 (1.1;9.8)                | 98.9 (94.0;100.0)            |
|         |       | ≥1.0 IU/mL | 0.0 (0.3;6.0)                | 62.9 (58.7;78.5)             |
|         |       | >1.0 IU/mL | 0.0 (0.3;6.0)                | 62.9 (58.7;78.5)             |
|         |       | >1.0 IU/mL | 0.0 (0.3;6.0)                | 62.9 (58.7;78.5)             |
|         |       | ≥1.0 IU/mL | 0.0 (0.3;6.0)                | 62.9 (58.7;78.5)             |
|         |       | ≥1.0 IU/mL | 0.0 (0.3;6.0)                | 62.9 (58.7;78.5)             |
| Tetanus | ELISA (IBL) | ≥0.1 IU/mL | 100.0 (96.4;100.0) | 100.0 (96.4;100.0) |
|         |       | ≥0.1 IU/mL | 100.0 (96.4;100.0) | 100.0 (96.4;100.0) |
|         |       | ≥0.1 IU/mL | 100.0 (96.4;100.0) | 100.0 (96.4;100.0) |
|         |       | ≥0.1 IU/mL | 100.0 (96.4;100.0) | 100.0 (96.4;100.0) |
|         |       | ≥0.1 IU/mL | 100.0 (96.4;100.0) | 100.0 (96.4;100.0) |
| Pertussis | PT   | ≥4 EU/mL  | 43.0 (33.1;53.3)             | 92.3 (84.8;96.9)             |
|         |       | >4-fold rise | NA                           | 75.8 (65.7;84.2)             |
|         |       | Vaccine response | NA                           | 80.2 (70.6;87.8)             |
|         |       | ≥3 EU/mL  | 88.0 (80.0;93.6)             | 100.0 (96.0;100.0)           |
|         |       | >4-fold rise | NA                           | 51.6 (40.9;62.3)             |
|         |       | Vaccine response | NA                           | 81.3 (71.8;88.7)             |
|         |       | ≥4 EU/mL  | 13.0 (7.1;21.2)              | 94.5 (87.9;88.2)             |
|         |       | >4-fold rise | NA                           | 82.4 (73.0;99.6)             |
|         |       | Vaccine response | NA                           | 72.5 (62.2;81.4)             |
|         |       | ≥4 EU/mL  | 54.0 (43.7;64.0)             | 98.9 (94.0;100.0)            |
|         |       | >4-fold rise | NA                           | 87.9 (79.4;93.8)             |
|         |       | Vaccine response | NA                           | 95.6 (89.1;98.8)             |
|         |       | ≥10 mIU/mL | 12.0 (6.4;20.0)              | 100.0 (96.0;100.0)           |
|         |       | >10 mIU/mL | 5.0 (1.6;11.3)               | 95.6 (89.1;98.8)             |
|         |       | >10 mIU/mL | 12.0 (6.4;20.0)              | 100.0 (96.0;100.0)           |
|         |       | >10 mIU/mL | 5.0 (1.6;11.3)               | 95.6 (89.1;98.8)             |
|         |       | >10 mIU/mL | 5.0 (1.6;11.3)               | 95.6 (89.1;98.8)             |
|         |       | >10 mIU/mL | 5.0 (1.6;11.3)               | 95.6 (89.1;98.8)             |
|         |       | ≥0.01 IU/mL | 100.0 (96.0;100.0)            | 100.0 (96.0;100.0)           |
|         |       | ≥0.01 IU/mL | 100.0 (96.0;100.0)            | 100.0 (96.0;100.0)           |
|         |       | ≥0.01 IU/mL | 100.0 (96.0;100.0)            | 100.0 (96.0;100.0)           |
|         |       | ≥0.01 IU/mL | 100.0 (96.0;100.0)            | 100.0 (96.0;100.0)           |
|         |       | ≥0.01 IU/mL | 100.0 (96.0;100.0)            | 100.0 (96.0;100.0)           |

Data are % (95% CI) participants with titer or concentration above threshold.
NA = not applicable; NC = not calculated.

Table 5
Geometric mean concentrations (GMCs) and geometric mean titers (GMTs) pre- and post-vaccination (Cohort I) (FAS).

| Antigen | Assay | Threshold | DTwP-IPV-HB-PRP-T (N = 30) | DTwP-HB-PRP-T + IPV (N = 15) |
|---------|-------|-----------|-----------------------------|-------------------------------|
|         |       |           | Pre-vaccination              | Post-vaccination              |
|         |       |           | GMT (1/dil)                  | GMC (IU/mL)                  |
|         |       | ≥1.0 IU/mL | 1.06 (0.73;1.51)             | 17.7 (14.3;21.9)             |
|         |       | ≥1.0 IU/mL | 1.06 (0.73;1.51)             | 17.7 (14.3;21.9)             |
|         |       | ≥1.0 IU/mL | 1.06 (0.73;1.51)             | 17.7 (14.3;21.9)             |
|         |       | ≥1.0 IU/mL | 1.06 (0.73;1.51)             | 17.7 (14.3;21.9)             |
|         |       | ≥1.0 IU/mL | 1.06 (0.73;1.51)             | 17.7 (14.3;21.9)             |
|         |       | ≥1.0 IU/mL | 1.06 (0.73;1.51)             | 17.7 (14.3;21.9)             |

Data are geometric mean titer (GMT) or geometric mean concentration (GMC) (95% CI).
NA = not applicable; NC = not calculated.
Data are geometric mean titer (GMT) or geometric mean concentration (GMC) (95% CI). NA = not applicable; NC = not calculated.

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