A phase I, open label, clinical study to assess the safety and immunogenicity of indigenously developed liquid (DTwP-HepB-IPV-Hib) hexavalent combination vaccine in healthy toddlers aged 16–24 months

Hitt Sharmaa, Sanjay Lalwanib, Sameer Parekhc, Pramod Pujari, Sunil Shewaleb, Sonali Palkara, Neeta Hanumanteb, Shilpa Gokhaleb, Jaganathan KS, Rakesh Kumara, Inderjit Sharmaa, and Sunil Gaironed

*Department of Clinical Research and Pharmacovigilance, Serum Institute of India Pvt. Ltd, Pune, India;  
*bDepartment of Pediatrics, Bharati Vidyapeeth (Deemed to be University) Medical college & Hospital, Pune, India;  
*cDepartment of Production, Serum Institute of India Pvt. Ltd., Pune, India;  
*dDepartment of Quality Control, Serum Institute of India Pvt. Ltd., Pune, India

**ABSTRACT**
This first in human study was designed as an open label clinical trial to assess the safety and immunogenicity of SIIPL DTwP-HepB-IPV-Hib (Hexavalent) combination vaccine in healthy toddlers, aged 16–24 months. A total of 24 healthy toddlers were administered a 0.5 ml single dose of SIIPL DTwP-HepB-IPV-Hib vaccine intramuscularly, and followed for 28 days for safety outcomes viz. immediate, solicited, unsolicited and serious adverse events. Blood samples were collected immediately prior to and 28 days after vaccination to assess the immunogenicity. Twenty four completed the study in compliance with the study protocol. None of the participants experienced any immediate or any serious adverse event. In terms of the frequency and intensity, the adverse events were comparable to DTwP-based combination vaccines. The vaccine elicited a strong booster response as demonstrated by a large increase in antibodies against all vaccine antigens. One month post booster vaccination seroresponse for diphtheria, tetanus, Hepatitis B, *Haemophilus influenza* type b and polio virus type 1 and 3 was 100%. The percentage sero-response for pertussis was 75%. Four-fold increase in antibody concentration for pertussis was achieved in 87.5% subjects. Indigenously developed DTwP-HepB-IPV-Hib vaccine by Serum Institute of India Pvt. Ltd. was found to be safe, well tolerated and showed a robust immune response in toddlers. It was concluded that this vaccine should be assessed in the next phases of clinical development in the target population.
Clinical Trial Registration – CTRI/2018/10/015875.

**INTRODUCTION**
Vaccination of infants against diphtheria, tetanus, pertussis, hepatitis B, polio and *Haemophilus influenza* type b has been recommended by the World Health Organization (WHO) for several decades and is well established in most countries around the world. Worldwide, coverage of a third dose of vaccine protecting against diphtheria, tetanus, and pertussis (DTP-3) remained at 81% in 2021, leaving almost 25 million children vulnerable to vaccine preventable diseases. Of the 25 million infants who are not fully vaccinated with DTP3, around 18 million mostly from low and middle-income countries didn’t receive an initial dose. Use of combined vaccine presentations can help to overcome reduced coverage through simplification of vaccination programmes. Although the use of oral poliovirus vaccine (OPV) in many countries has been essential toward the polio eradication effort, it carries a rare risk of vaccine associated paralytic polio and circulating vaccine derived poliovirus caused by one of the 3 Sabin vaccine-related poliovirus types. Cessation of OPV and introduction of Inactivated Poliomyelitis vaccine (IPV) in phase wise manner is essential to end polio globally. To implement this effectively, especially in developing countries consistent supply of affordable and effective vaccines is required, as herd immunity is needed to be sustained during post eradication period. IPV containing hexavalent vaccine based on whole-cell pertussis antigen (wp) represents one prospective approach to IPV access to low and middle-income countries. A pentavalent combination DTwP-HepB-Hib vaccine (Pentavac SD™) and IPV (Poliolvac™) vaccine, both manufactured by Serum Institute of India Pvt. Ltd (SIIPL) and prequalified by World Health Organization (WHO) are being used in the national immunization programs of many developing countries, including India. SIIPL has now indigenously developed a fully liquid hexavalent vaccine including Diphtheria toxoid, Tetanus toxoid, inactivated whole cell Pertussis (wp), Hepatitis B antigen, inactivated poliovirus (type 1, 2, 3) and *Haemophilus influenza* type b conjugate (adsorbed), in order to support the Global Polio Eradication strategy. SIIPL’s DTwP-HepB-IPV-Hib (Hexavalent) vaccine can be part of primary and booster vaccination of infants from 6 weeks of age. This hexavalent combination vaccine could simplify current pediatric routine immunization schedules involving multiple vaccines. As the number of injections/visits for immunization are decreased it will improve vaccine compliance and reduce costs of healthcare delivery system. This new vaccine was tested in a Phase I clinical study after successful completion of Good Laboratory Practice (GLP) compliant...
animal toxicological studies. Here, we report the results of the first in human clinical trial designed to evaluate the safety of SIIPL’s DTwp-HepB-IPV-Hib vaccine following single dose in healthy toddlers as a primary objective. The assessment of immune response was included as a secondary objective.

**Materials and methods**

**Study design**

This was a Phase-I, open label clinical trial planned to evaluate the safety and immunogenicity of a single dose of DTwp-HepB-IPV-Hib vaccine administered intramuscularly in healthy toddlers, aged 16–24 months. The study was conducted during Oct 2018 to Jan 2019 at Department of Pediatrics, Pediatric Research Cell, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital, Pune, India. All the participants were enrolled after written informed consent was obtained from their parent. As per prevailing regulations, the consent process was recorded audio-visually. The study was conducted after approval from the Drugs Controller General of India and the Institutional Review Board. The study was carried out in accordance with the ICH ‘Guidance on Good Clinical Practice,’ the declaration of Helsinki and in ‘Schedule Y’ guidelines issued under Drugs and Cosmetic Act, Government of India. The study was registered on national clinical trial registry (CTR/2018/10/015875).

**Study objective**

The objective of the study was to assess safety and immunogenicity after a single booster dose administration of SIIPL DTwp-HepB-IPV-Hib vaccine in healthy toddlers aged, 16–24 months.

**Subjects and study procedures**

Eligible toddlers were between 16 and 24 months of age whose parent had signed the written informed consent, in good health and who had received three doses of diphtheria, tetanus, pertussis, hepatitis B, Hib and poliovirus vaccine as a part of routine primary vaccination procedures, during the first year of life.

Subjects were excluded from participation, if they had any acute illness or fever 7 days prior to the vaccination, history of any serious reaction to any prior vaccination or known hypersensitivity to any of the vaccine components, any clinically significant chronic disease. They were also not allowed to participate if they had received any investigational drug/vaccine within 28 days before the booster vaccination; if they had received any non-study vaccine within 28 day prior to booster vaccination, or planned administration during the active study period; if they have any immunosuppressive condition; if they had a history of infection against any pathogen against which the study vaccines were targeted or had received previous booster vaccination against diphtheria, tetanus, pertussis, hepatitis B, Hib and poliovirus.

**Dosage and administration**

The enrolled subjects received a single 0.5 mL dose of SIIPL DTwp-HepB-IPV-Hib vaccine (batch no. 2658 × 001) intramuscularly into the anterolateral aspect of the thigh. The vaccine was transported and stored at the study site between 2–8°C temperature. Each 0.5 ml dose contained Diphtheria Toxoid ≥30 IU, Tetanus Toxoid ≥40 IU, B. pertussis (whole cell) ≥4 IU, HBsAg (rDNA) 15 mcg. Hib conjugate (PRP-TT) 10 mcg, 40, 8 and 32 D antigen units of poliovirus type 1, 2 and 3 (Salk strains grown on vero cells), respectively, and Aluminium Phosphate gel 0.28 mg as an adjuvant. Pertussis vaccine (Bulk) was prepared by using four strains (134, 509, 6229 and 25,525) of inactivated B. Pertussis. Inactivation of wP antigen was done by heating in the presence of formaldehyde.

Since this was a first in human clinical trial sentinel dosing was followed. The enrollment and vaccine administration were done as per the subject enrollment plan. Initially, one subject was enrolled and followed up for 3 days. Since there was no major safety concern reported, another 2 subjects were enrolled and followed for 3 days for safety assessment. Upon confirmation of safety and well-being of subjects, the study was open for recruitment of remaining 21 subjects.

**Assessment of safety**

Following vaccination, all participants were observed at the study site for 30 minutes for any immediate adverse events. Each subject’s parent was then given diary card to record the adverse events (AEs) and were asked to bring them to the site, at the next scheduled visit. Active follow up of all the vaccinated subjects was done over the 7-day period, for any solicited local and systemic AEs and further followed up until 28 days, to report any unsolicited AEs and SAEs. The diary cards were collected, reviewed and transcribed in the case report forms. In addition to these scheduled visits, the study team also contacted the parents telephonically on day 3 to know well-being of the subject, and also they were reminded to complete the diary card.

The study endpoints included incidence of immediate AEs within 30 mins of vaccination, solicited AEs within 7 days post vaccination period and incidence of unsolicited AEs and SAEs within 28 days, post vaccination. Independent oversight of this study was provided by a Data Safety Monitoring Board (DSMB), a multidisciplinary group with expertise in the field of pediatrics, vaccines, drug safety and statistics.

Since this was a Phase I clinical study, no formal sample size calculation was performed.

All statistical analyses were performed using SAS® version 9.4. All the AEs were classified according to the Medical Dictionary for Regulatory Activities (MedDRA version 21.1) System, Organ and Class (SOC). Any medication received concomitantly was coded using WHO Drug Dictionary.

**Serology**

Blood samples were taken from all subjects immediately prior to and 28 days after vaccination. Blood samples were analyzed for immunogenicity assessment at Strand Lifesciences Pvt. Ltd (India) which is NABL (National Accreditation Board for Testing and Calibration Laboratories, India) and CAP (College of American Pathologists) accredited laboratory. Anti-diphtheria and anti-tetanus antibodies were measured
using ELISA, with seroprotection defined as a titer level of ≥0.1 IU/ml. A whole-cell ELISA was used to detect antibodies to *B. pertussis*. Antibodies testing against diphtheria, tetanus and pertussis was performed using commercial CE certified kits (IBL, Germany). The commercial kit used for pertussis was coated with *B. Pertussis* antigens (containing pertussis toxin, Filamentous hemagglutinin and lipopolysaccharides) and standardized in U/ml. There is no international standard definition for seroprotection for *B. pertussis*. The quantitative threshold of 24 U/ml as per the standard curve provided in the kit literature was used to interpret the results for Anti *B. Pertussis* IgG.

Based on kit literature, pertussis sero-response was defined as titer levels >24 U/ml. The Hib ELISA specifically detects antibodies against PRP, and the seroprotection was considered as anti PRP antibody concentration ≥0.15 µg/ml. Antibodies against hepatitis B were determined by ELISA and seroprotection was defined as concentration of anti-HepB antibodies ≥10 mIU/l. The Binding site and Abbott commercial kits were used for Hib and Hepatitis B, respectively.

The testing of antibodies against inactivated polio virus type 1 and 3 by neutralization assay method was undertaken at Quest Diagnostics Infectious Disease, USA which is a CAP accredited laboratory. Seroprotection was defined anti-polio 1 and 3 titers ≥8 (1/dil). For the neutralization assay of polio type 1 and 3, Sabin strain was used. The upper limit of quantitation (ULOQ) for polio neutralization was 128 (1/dil).

## Results

### Subjects and demography

A total of 24 subjects were enrolled in the study and received the study vaccination. All subjects completed the study in compliance with the study protocol. The mean age of study participants was 19.2 months (SD ± 1.84), their mean length was 78.9 cm (SD ± 3.30) and mean weight was 10.1 kg (SD ± 0.98). Out of 24 enrolled subjects, 17 (70.8%) were males and remaining were females. All the subjects received birth dose of OPV and three doses of DTwP-HepB-Hib vaccine of different manufacturers, and OPV as a part of primary immunization using 6, 10, 14 week schedule. Eight (33.3%) and 10 (41.7%) subjects received 1 and 2 fractional dose/s of IPV intradurally.

### Safety and reactogenicity

There were no any immediate AEs observed within 30 min of vaccination. The majority of subjects (up to 95.8%) reported at least one or more adverse event during the conduct of the study. Overall 72 AEs were reported. Out of these reported AEs, 67 were solicited and 5 were unsolicited AEs. Pain at injection site (70.8%), irritability (75%) and fever (58.3%) were most commonly reported solicited AEs (Table 1). The grade 3 pain at injection site was reported in 2 subjects and grade 3 loss of appetite was reported in one subject. All other local and systemic solicited AEs were mild or moderate in intensity. In 3 (12.5%) subjects, 5 unsolicited AEs of mild or moderate grade were reported. None of the unsolicited event was related to the vaccination. No AEs led to any discontinuation and no death or any serious adverse event was reported during the study.

The DSMB members reviewed the post-vaccination Day 7 and day 28 safety data of all the subjects and concluded that the study had met the primary endpoints of safety. They recommended that the vaccine should be further assessed in the next phases of clinical development.

### Table 1. Post vaccination incidence of solicited adverse events.

| Adverse event                     | n  | %   | 95% CI          |
|-----------------------------------|----|-----|-----------------|
| **Injection site pain**           |    |     |                 |
| Any                               | 17 | 70.83 | [48.91, 87.38] |
| Grade 3                           | 2  | 8.3  | [1.03, 27.00]   |
| **Injection site erythema**       |    |     |                 |
| Any                               | 4  | 16.7 | [4.74, 37.38]   |
| Grade 3                           | 0  | 0    | NE              |
| **Injection site swelling**       |    |     |                 |
| Any                               | 6  | 25   | [9.77, 46.71]   |
| Grade 3                           | 0  | 0    | NE              |
| **Fever**                         |    |     |                 |
| Any                               | 14 | 58.3 | [36.64, 77.89]  |
| Grade 3                           | 0  | 0    | NE              |
| **Irritability**                  |    |     |                 |
| Any                               | 18 | 75.0 | [53.29, 90.23]  |
| Grade 3                           | 0  | 0    | NE              |
| **Abnormal Crying**               |    |     |                 |
| Any                               | 2  | 8.3  | [1.03, 27.00]   |
| Grade 3                           | 0  | 0    | NE              |
| **Decreased appetite**            |    |     |                 |
| Any                               | 5  | 20.8 | [7.13, 42.15]   |
| Grade 3                           | 1  | 4.2  | [0.11, 21.12]   |
| **Drowsiness**                    |    |     |                 |
| Any                               | 1  | 4.2  | [0.11, 21.12]   |
| Grade 3                           | 0  | 0    | NE              |

e = Number of events, n = Number of subjects with adverse events, NE = Non Estimable, % = Percentages of subjects, CI = Confidence interval.
**Immunogenicity**

Seroprotection rates and Geometric Mean Concentration (GMC) before and after the booster vaccination for all antigens are shown in Table 2. Before the booster dose, majority of the subjects (>83%) had seroprotective antibody concentrations against tetanus, hepatitis B, Hib, Polio type 1 and 3. One-month post booster vaccination, seroprotection for Diphtheria, Tetanus, Hepatitis B, *Haemophilus influenzae* type ‘b’ and polio virus type 1 and 3 was 100%. No subject had pre-vaccination anti-D ≥ 1.0 IU/ml. Pre-vaccination anti-T ≥ 1.0 was observed in 45.8% subjects. Post-vaccination, anti-D ≥ 1.0 IU/ml was reported in 54.2% subjects and anti-tetanus ≥1.0 IU/ml was reported in all the subjects. Anti PRP antibody concentration ≥1 µg/ml was observed in 83.3% subjects pre-vaccination which increased to 100%, post-vaccination. Pre-vaccination anti Hep B ≥ 100 mIU/ml was 50% that increased to 100%, post-vaccination. The percentage sero-response for pertussis based on kit literature (>24 U/ml) after booster vaccination was 75%. Four-fold or more increase in antibody concentration for pertussis was achieved in 87.5% subjects.

**Discussion**

Inclusion of the three IPV antigens in a wP-based pentavalent combination vaccine though technically complex, has a potential cost advantage, considering it will be the ultimate combination vaccine in routine immunization programs in developing countries.4,7

The present Phase I study evaluated safety and immunogenicity of a fully liquid DTwP-HepB-IPV-Hib vaccine manufactured by SIIPL, in toddlers aged 16–24 months. The safety and reactogenicity following single booster dose was assessed by collecting solicited local injection site AEs and systemic signs and symptoms over the protocol defined time period. wP-based combination vaccines and IPV vaccine, manufactured by SIIPL have been evaluated in toddlers, in previous studies.5 Also, another wP-based hexavalent vaccine has been assessed in toddlers in the published literature. The safety collection and assessment method in trials with was similar and comparable to the current study.8,9 No immediate or serious adverse events were reported in the study. Pain at injection site was the most common solicited local AE whereas, fever and irritability were most common solicited systemic AEs reported during the study. No safety concerns were reported, following single booster administration of the hexavalent vaccine. The overall incidence of the AEs observed in this study is in line with the published data for other wP-based combination vaccines.8,10,11

The vaccine elicited a strong immune response for D, T, wP, Hep B and Hib antigens and demonstrated a substantial increase in antibodies against all these antigens. The seroprotection/seroresponse rates were comparable to other whole cell hexavalent combination vaccines and Pentavalent + IPV vaccines.8,9,11 The GMCs of all antibodies exceeded the seroprotection/seroresponse thresholds by very large margins and are consistent with long-term protection reported in previous studies.12,13 Similar to other clinical trials of wP-based DT combination vaccines8,12 a diagnostic commercial ELISA kit was used to measure anti *B. pertussis* antibodies and the quantitative threshold of 24 U/ml was used to interpret the results, as per the kit literature. These kits are coated with mixed pertussis antigens and not suited for precise evaluation of specific pertussis antigens especially Pertussis toxin (PT) which is considered the main virulence factor for pertussis. Measurement of the anti-PT antibody response is crucial which will be considered in the further course of clinical development of this vaccine.

A limitation in the study is that the antibodies against polio type 2 using neutralization assay could not be assessed as the bio-analytical laboratory performing the neutralization assay was acting in accordance with WHO’s containment strategy to destroy type 2 poliovirus in their facilities.14 However, this is not considered a major limitation, as this is a first in human study to assess safety primarily, and immune response against type 2 is being assessed in the late stage pivotal clinical studies.
Also, as the ULOQ for polio neutralization was 128 (1/dil), an actual rise induced by the vaccine could not be assessed in this Phase I study, as reported in literature.9,11

In conclusion, the SIPII’s DTwP-HepB-IPV-Hib vaccine was found to be well-tolerated and immunogenic when administered to toddlers, as a single booster dose. This phase-I study supports the evaluation of safety and immunogenicity of DTwP-HepB-IPV-Hib vaccine in the next phases of clinical development.

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Disclosure statement

The authors HS, SP, PP, SS, JK, RK, IS and SG are employees of Serum Institute of India Pvt. Ltd.

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Author contributions

All authors attest they meet the ICMJE criteria for authorship.

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