RESEARCH PAPER

Long-term Persistence of Immunogenicity After Primary Vaccination and Response to Booster Vaccination With Typhoid Conjugate Vaccine: Results of a Phase IV Extension Study

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Received: December 06, 2021; Initial review: January 04, 2022; Accepted: March 17, 2022.

Objective: To evaluate the persistence of antibodies three years after primary vaccination with typhoid conjugate vaccine (TCV) of either Cadila Healthcare Ltd. (Cadila-TCV) or Bharat Biotech International Ltd. (Bharat-TCV) administered in a previous phase II/III study, and to study the booster dose response to Cadila-TCV. **Methods**: This was an open-label, phase IV extension study conducted in tertiary care and multispecialty hospitals in India. 112 subjects (Cadila-TCV-57, Bharat-TCV-55) who had participated in previous study were enrolled. Of these, eligible subjects received a single-dose of Cadila-TCV and were followed-up for 28 days post-booster. Primary outcome was persistence of antibodies 3 years after primary vaccination and seroconversion (≥4-fold rise in antibody titre from baseline) 28 days post-booster. Safety was based on reported adverse events (AEs) post-booster. **Results**: The baseline GMT reported in the current study was significantly higher than pre-vaccination GMT reported in the previous study. 89/112 (79.5%) subjects had antibody titer ≥10 IU/mL at baseline; eligible subjects (*n*=17) who had baseline antibody titre <10 IU/mL were administered booster dose. All the vaccinated subjects showed seroconversion post-booster. The GMTs reported at 10 days and 28 days post-booster were significantly higher as compared to GMTs reported after primary vaccination in previous study. 4 (23.5%) vaccinated subjects reported 9 AEs; all were solicited and of mild/moderate intensity. **Conclusion**: There was a significant persistence of immunogenicity after primary vaccination with both the TCVs, and robust immune response after booster vaccination with Cadila-TCV.

Keywords: *Efficacy, Protection, Safety*.

Trial Registration: CTRI/2019/07/019996 ***Published online:*** March 28, 2022; ***PII***: S097475591600415

Typhoid conjugate vaccines (TCVs) have Vi capsular polysaccharide of *S. typhi* conjugated to different carrier proteins. Four such TCVs have been approved and marketed in India. Zyvac TCV (Cadila Healthcare Ltd.) was licensed based on the prelicensure phase II/III non-inferiority clinical trial, the results of which were published previously [1]. The current phase IV trial was conducted as an extension of the previous phase II/III trial in which the same subjects were followed-up three years after their primary vaccination to evaluate the long-term persistence of anti-Vi IgG antibodies, and response to the booster dose of TCV administered to the eligible subjects.

METHODS

This prospective, open-label, multicenter, post-marketing clinical study was conducted from September, 2019 to November, 2020 at the seven centers (tertiary care or multispecialty hospitals) which also had participated in the previous study. The study was approved by the Central Licensing Authority and also by the registered local Institutional Ethics Committees for individual sites. The study was registered with Clinical Trial Registry – India before initiation of enrollment in the study.

In the previous study, a total of 240 healthy subjects aged 6 months to 45 years were enrolled, out of which 236 subjects including 117 and 119 subjects who had received TCV of Cadila Healthcare Ltd. (Cadila-TCV) and TCV of Bharat Biotech International Ltd. (Bharat-TCV), respectively completed the study with both pre-vaccination and 6 weeks post-vaccination immuno-

genicity assessments [1]. The subjects who had participated and completed the previous study were considered for enrollment in the current extension study 3 years (±3 months) after their primary vaccination.

Prior to screening, a written informed consent was obtained from the adult subjects and parents of the pediatric (<18 years) subjects; additionally, an assent was also obtained from the pediatric subjects aged ≥7 years. Adult subjects or parents of the pediatric subjects were also required to be literate enough to provide written informed consent and fill the diary cards. The subjects were excluded if they had a history of typhoid fever or vaccination against typhoid fever after previous study; any ongoing clinically significant systemic disorder, immunological disorder, coagulation disorder or thrombocytopenia; any ongoing anticoagulant, immunosuppressive or immunostimulant therapy; history of administration of blood, blood products or immunoglobulins within past 3 months, participation in another clinical trial within past 3 months, or alcohol or drug abuse within past one year.

The subjects fulfilling the eligibility criteria were enrolled and their blood samples were collected to evaluate the baseline anti-Vi IgG antibody titre. The subjects whose baseline antibody titre was <10 IU/mL, which corresponds to the proposed seroprotective cut-off titre of 2 mcg/mL [2,3], were considered eligible to receive a booster dose of TCV. A 0.5 mL single-dose of Cadila-TCV was given as a booster dose within 90 days of their enrollment, irrespective of the TCV administered for primary vaccination in the previous study. The vaccine in each dose of 0.5 mL contained 25 mcg of purified Vi capsular polysaccharide of *S. typhi* conjugated to tetanus toxoid (TT) as carrier protein. The vaccine was administered intramuscularly in the deltoid region under aseptic precautions following which the subjects were closely observed for at least 30 minutes for occurrence of any immediate adverse events (AEs). Further blood samples were collected during follow-up visits at 10 (+3) days and 28 (+7) days after vaccination, for assessment of post-vaccination antibody titres. The immunogenicity assessment was performed at the central accredited laboratory. The assessment of antibody titers was performed using the commercial Vacczyme ELISA kits (Binding Site Group Ltd.). The antibody titres were also derived in IU/mL using the WHO International Standard for anti-typhoid capsular Vi polysaccharide IgG (human) (NIBSC code 16/138) [2].

The subjects were not administered the booster dose of TCV if they had received any typhoid vaccine after enrollment, received any vaccine within the past one month, history of fever or any infectious disorder of >3 days within the past one month, and fever (≥37.5°C) at the time of planned vaccination. Urine pregnancy test was performed prior to vaccination for females of child bearing potential to rule out pregnancy. Pregnant or lactating females, and females of child bearing potential not using acceptable contraceptive measures were also not administered the booster dose.

Adult subjects or parents of the pediatric subjects were dispensed diary cards to record the solicited local (pain, redness, swelling and induration) and systemic (fever, headache, nausea, vomiting, malaise, arthralgia and myalgia) AEs till 7 days after vaccination and unsolicited AEs till the completion of post-vaccination follow-up. Any abnormality reported in vitals or physical examination was also planned to be dealt as an AE. The intensity of AEs was graded as mild, moderate or severe as described earlier [1], and causality was assessed based on the World Health Organization (WHO) criteria for AEs following immunization [4].

The primary immunogenicity variables were long-term persistence of anti-Vi IgG antibodies 3 years after primary vaccination and seroconversion, which was defined as a four-fold or greater rise in antibody titer at 28 days after vaccination, as per the WHO recommendations [2,5] . The secondary immunogenicity variables were seroconversion at 10 days after vaccination and geometric mean titer (GMT) of antibodies at 10 days and 28 days after vaccination as compared to the baseline. The safety variables were local or systemic AEs, serious AEs (SAEs) reported, if any, and overall tolerability evaluation by the investigators based on the reported AEs as follows: Excellent - no AE, Good – mild AE(s), Fair - moderate AE(s) and Poor - severe or serious AE(s). The maintenance of seroconversion at the baseline, defined as four-fold or greater rise in antibody titre at baseline in the current study as compared to antibody titre reported before primary vaccination in the previous study has also been evaluated as an exploratory variable.

Since this was an extension study, no minimum sample size was defined for this study. All the 236 subjects who had participated in and completed the previous study were considered for enrollment in the current study.

Statistical analysis: The immunogenicity analysis represents the data of full analysis set which included all the subjects with applicable immunological assessments including those with protocol deviations. The safety analysis represents the data of all the subjects who had received booster dose of TCV. The GMTs (95% CI) were computed for description of antibody titers. The GMTs between the groups were compared using unpaired *t* test

while the GMTs within the groups were compared using paired *t* test after log transformation of antibody titers. Continuous data was compared between the groups using unpaired *t* test. Categorical data was compared between the groups using Fisher exact test.

RESULTS

A total of 121 subjects were screened in this study, out of which 112 subjects (76 adults, 56 males) were enrolled while the remaining 9 subjects, who had received another dose of typhoid vaccine prior to this study, were excluded (**Fig. I**). The mean (SD) age, height, weight and body mass index of the enrolled subjects were 24.7 (12.6) years, 148.7 (22.9) cm, 51.5 (19.9) kg and 22.1 (5.2) kg/m^2, respectively. The baseline characteristics of the enrolled subjects are presented in **Table I**.

The GMT of antibodies reported at baseline in the current study (**Table I**) was significantly higher as compared to those reported before primary vaccination in the previous study (P<0.001). A total of 100 (89.3%) subjects, 50 subjects each who had received Cadila-TCV and Bharat-TCV, for primary vaccination in the previous study had maintained seroconversion at baseline in the current study (P=0.76). Based on the cut-off titer, 23 (20.5%) subjects were eligible to receive the booster dose vaccination, out of which 17 subjects were vaccinated. All the subjects followed-up on day 10 and day 28 after vaccination had shown seroconversion with a significant rise in antibody titers after vaccination as compared to baseline (P<0.001) (**Table II**). The GMT of antibodies reported at day 10 and day 28 after booster vaccination were significantly higher as compared to that reported after primary vaccination in the previous study (P<0.05) (**Table II**).

Table I Baseline Characteristics of Participants Who Had Received Typhoid Conjugate Vaccine (TCV)

Parameter	*Previous TCV Received*	
	Cadila-TCV (n=57)	*Bharat-TCV (n=55)*
Age (y)[a]	24.7 (13.1)	24.7 (12.2)
Age group		
Adult	37 (64.9)	39 (70.9)
Pediatric	20 (35.1)	16 (29.1)
Male gender	28 (49.1)	28 (50.9)
Baseline titer (EU/mL)[b]	140.8 (93.9, 211.2)	203.6 (144.3, 287.5)
Baseline titer ≥10 IU/mL	44 (77.2)	45 (81.8)

Data presented as no. (%) or [a]mean (SD) or [b]GMT (95% CI). All P>0.05.

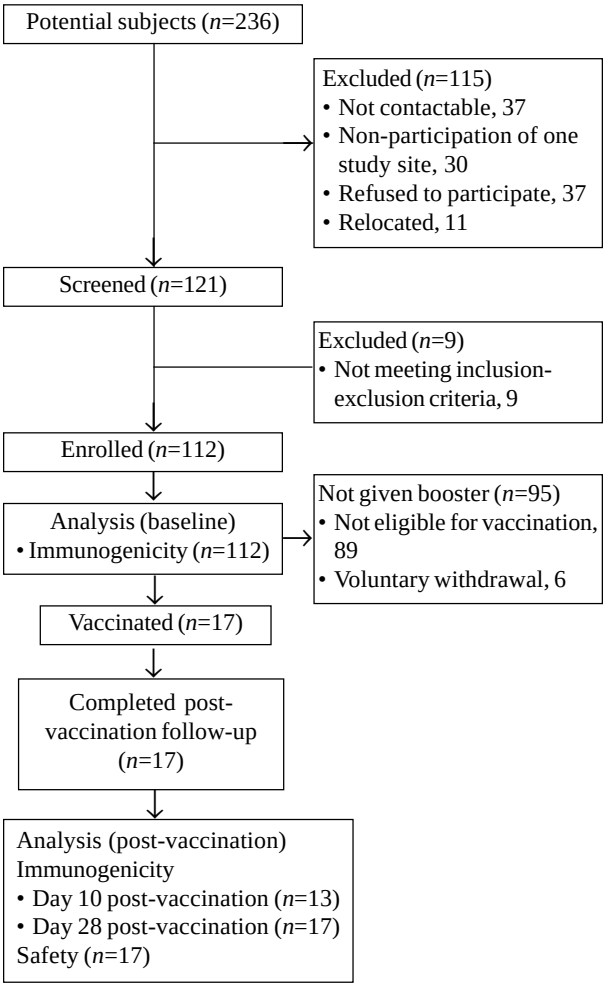

Fig. 1 Study flow chart.

A total of nine AEs were reported in four out of 17 (23.5%) vaccinated subjects; local pain in four participants, local swelling in two participants, and local redness, fever and headache in one participant each. All the AEs were of mild intensity except local pain in one participant, which was of moderate intensity. No severe or serious AE was reported for any subject. All AEs were solicited in nature, considered certainly related to the vaccination, and resolved completely within three days of their occurrence with/without supportive medications. Based on the reported AEs, an excellent, good and fair grade of tolerability was given for 13 (76.5%), 3 (17.6%) and 1 (5.9%) participants, respectively.

DISCUSSION

In the current study, we demonstrated that there was a significant persistence of antibodies at three years after primary vaccination with TCV, and the eligible subjects who received the booster dose mounted an antibody response which was significantly higher on day 10 and day 28 than that reported after primary vaccination. The

Table II Antibody Titers at Various Time Points for Participants Receiving a Booster Dose of Typhoid Conjugate Vaccine

Study, timepoint	*Previous TCV received*		*Overall*
	Cadila-TCV	*Bharat-TCV*	
Current phase IV study			
Baseline	*n*=8, 17.5 (7.1, 43.3)	*n*=9, 34.0 (22.9, 50.3)	*n*=17, 24.9 (15.9, 38.9)
Day 10 post-booster	*n*=7, 2734.3 (1066.1, 7013.2)	*n*=6, 1891.8 (795.3, 4500.4)	*n*=13, 2306.9 (1326.1, 4012.8)
Day 28 post-booster	*n*=8, 2107.1 (1004.5, 4420.0)	*n*=9, 1733.9 (1043.4, 2881.5)	*n*=17, 1900.5 (1288.3, 2803.7)
Previous phase II/III[a] study			
Post-primary	*n*=8, 829.5 (315.4, 2181.4)	*n*=9, 952.8 (429.5, 2114.0)	*n*=17, 892.6 (517.1, 1541.0)

Data presented as GMT (95% CI) in EU/mL. TCV: typhoid conjugate vaccine. [a]Full results available in reference 1.

booster dose administered to the selected subjects was also well tolerated.

One potential limitation of the study was that only around 50% of the subjects who had completed the previous phase II/III study were enrolled in this study. Other possible limitations could be selection of 10 IU/mL (2 µg/mL) as the cut-off titer for considering subjects eligible for booster vaccination, and non-evaluation of other immune response parameters such as IgG subclass response, functional antibodies, antibodies targeting other antigens apart from Vi capsular polysaccharide, cell mediated immune response etc.

To the best of our knowledge, there is no seroprotective titer defined for anti-Vi antibodies till date. From an earlier efficacy study of Vi-rEPA conjugate vaccine, 4.3 µg/mL was initially presumed as the seroprotective cut-off titer, which on subsequent re-examination based on the statistical modelling was estimated between 1.4-2.0 µg/mL [3], and based on the same evidence, 2 µg/mL has been used as the cut-off titer in this study. In a previous study, all the subjects who had received single-dose of Bharat-TCV and evaluated at 2 years after vaccination had maintained antibody titer ≥2 µg/mL [6]. In a further long-term follow-up study, all the subjects in a subset population aged 6-23 months at primary vaccination and who did not receive the booster dose subsequently (*n*=25) had maintained antibody titer ≥2 µg/mL at 7 years after vaccination [7]. The proportion of subjects maintaining the antibody titer ≥10 IU/mL (2 µg/mL) in the current study was relatively lower; however, it was comparable for both the TCVs.

Long-term persistence of serum anti-Vi antibodies has already been demonstrated upto 7 years after primary vaccination with TCVs in the earlier clinical studies [6-8]. In a study conducted with Bharat-TCV, the proportion of subjects maintaining seroconversion at 2, 3 and 5 years after primary vaccination was 59.5%, 73.5% and 73.2% in 6-23 months age group and 74.1%, 76.2% and 69.2% in 2-45 years age group [9]. In a subset of subjects from the former age group, 44% had maintained seroconversion at 7 years after vaccination [7]. Likewise, in a study of another TCV, the seroconversion was maintained in 83% subjects in the immunogenicity subset at 12 months after primary vaccination [8]. Although the data of current study cannot be directly compared with that of previously published studies, the baseline GMTs and the proportion of subjects maintaining seroconversion at 3 years after primary vaccination reported in our study was found comparable for both the TCVs.

Although the number of subjects receiving the booster vaccination was small, the robust booster response seen with TCVs can be explained by generation of immunological memory owing to conjugation of Vi polysaccharide with TT carrier protein which renders the antigen T-cell dependent leading to production of plasma cells and memory B cells [10]. Administration of the same vaccine for booster dose may be a possible explanation of a higher booster response observed in the subjects who had received Cadila-TCV for primary vaccination. The safety data of the booster vaccination reported in this study is also consistent with that reported with the marketed TCVs [11-13], even though our study had limited numbers.

Overall, this study provides useful insights on the long-term immunological persistence and response to booster dose of TCV. Further long term studies are warranted to confirm waning of antibody titres and to evaluate efficacy/effectiveness of TCVs over the years of follow-up. Pursuant to limited availability of long-term follow-up data, there is currently no recommendation provided for booster dose of TCVs [2,14,15]. The data derived from the current study and that generated from long-term follow-up studies of other TCVs will help policymakers to take appropriate decision on the requirement and timing of booster dose of TCV.

Ethics clearances: Institute's Ethics Committee, Indo-US Superspeciality Hospital, Hyderabad; No. Nil dated June 22, 2019. Institute's Ethics Committee, Panchsheel Hospital Pvt. Ltd., New Delhi; No. Nil dated July 02, 2019. Institute's Ethics Committee, Sparsh Hospitals and Critical Care Pvt. Ltd, Bhubaneswar; No. Nil dated July 15, 2019. Institute's Ethics Committee, Gandhi Medical College, Secunderabad; No. IEC/GMC/2019/05/05 dated September 11, 2019. Institute's Ethics Committee, GCS Medical College, Hospital and Research Center, Ahmedabad, No. GCSMC/EC/TRIAL/APPROVE/2019/2291 dated September 28, 2019. Institute's Ethics Committee, Institute of Child Health, Kolkata, No. ICH/IEC/81/2019 dated October 10, 2019. Institute's Ethics Committee, SMS Medical College and Attached Hospitals, Jaipur; No. 625/MC/EC/2019 dated November 1, 2019.

Contributors: AKK,KGU,SS,MRK,KSP,VKG,SKJ: study conduct, medical care of the study participants and data acquisition; PD,RM: study concept and design, overall study coordination, data analysis and interpretation; KM: study concept and design, and manufacture of the vaccine. All authors had full access to clinical trial data. PD, RM: prepared the manuscript and other authors provided their feedback for revising it for the intellectual content. All authors have approved the final version of this manuscript. All authors agree with the interpretation of data and its representation in the manuscript.

Funding: The monetary and material support including the vaccine supplies was provided by M/s. Cadila Healthcare Ltd., Ahmedabad.

Competing interests: AKK,KGU,SS,MRK,KSP,VKG,SKJ: were the clinical trial investigators and they received honorarium from the sponsor for the conduct of the study. PD, RM, KM are employees of M/s. Cadila Healthcare Ltd.

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