A multicenter, single-blind, randomized, phase-2/3 study to evaluate immunogenicity and safety of a single intramuscular dose of biological E’s Vi-capsular polysaccharide-CRM197 conjugate typhoid vaccine (TyphiBEVTM) in healthy infants, children, and adults in comparison with a licensed comparator

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ABSTRACT
The current scenario of typhoid fever warrants early prevention with typhoid conjugate vaccines in susceptible populations to provide lifelong protection. We conducted a multicenter, single-blind, randomized, Phase 2/3 study to assess the immunogenicity and safety of Biological E’s Typhoid Vi-CRM197 conjugate vaccine (TyphiBEVTM) compared to Vi-TT conjugate vaccine manufactured by Bharat Biotech International Limited (Typhbar-TCV; licensed comparator) in healthy infants, children, and adults from India. The study’s primary objective was to assess the non-inferiority of TyphiBEVTM in terms of the difference in the proportion of subjects seroconverted with a seroconversion threshold value of ≥2.0 µg/mL against Typhbar-TCV. A total of 622 healthy subjects (311 each in both vaccine groups) were randomized and received the single dose of the study vaccine. The TyphiBEVTM group demonstrated noninferiority compared to the Typhbar-TCV group at Day 42. The lower 2-sided 95% confidence interval limit of the group difference was −3.4%, which met the non-inferiority criteria of ≥10.0%. The geometric mean concentration (24.79 µg/mL vs. 26.58 µg/mL) and proportion of subjects who achieved ≥4-fold increase in antiVi IgG antibody concentrations (96.95% vs. 97.64%) at Day 42 were comparable between the TyphiBEVTM and Typhbar-TCV vaccine groups. No apparent difference was observed in the safety profile between both vaccine groups. All adverse events reported were mild or moderate in intensity in all age subsets. This data demonstrates that TyphiBEVTM is non-inferior to Typhbar-TCV in terms of immunogenicity, and the overall safety and reactogenicity in healthy infants, children, and adults studied from India was comparable.

Introduction
Typhoid fever is a devastating systemic infectious disease in humans. Enteric fever is the collective term used in describing the systemic infection caused by Salmonella enterica subspecies serovars Typhi and Paratyphi A, B, and C causing typhoid and paratyphoid fevers.1,2 Humans are the only host for Salmonella Typhi (S. Typhi). The bacteria are acquired through the ingestion of contaminated food and water. Once inside the body, via the intestinal mucosa, it enters the liver, spleen, bone marrow, and in some instances, even the gall bladder.3 Typhoid and paratyphoid infections primarily cause bacteremic febrile illnesses, with classic signs, such as prolonged high fever, headache, and malaise.4–6

The disease poses a significant risk to human health in low- and middle-income countries, especially due to extensively drug resistant strains of S. Typhi.3 An estimated 14.3 million cases of typhoid and paratyphoid fever and 135.9 thousand deaths due to the disease have occurred globally in 2017.2 Enteric fever is a major health problem in developing countries like India.5,6 India had reported 24, 45,611 cases of enteric fever in the year 2019.7 Large-scale community studies conducted in an urban slum setting in India have reported the incidence of enteric fever as high as 2/1000 population/year in those under 5 years and 5.1/1000 population/year in under 10 years of age.6 Children are affected disproportionately by typhoid fever. A prospective surveillance study of a community-based cohort has reported the incidence rate of typhoid per 1000 person-years as 27.3 for children under 5 years, 11.7 for those between 5 and 19 years and 1.1 for those between 19 and 40 years.8 Based on hospital-based estimates, another study from India suggested the disease incidence in children in the 1–4 age group to be between 1 and 5 per 1000 child years.9 A study from Bangladesh has also reported the incidence of typhoid fever to be highest among preschool children. The study reported 18.7 episodes/1,000 person-years typhoid fever incidence among preschool children compared to 2.1 episodes/1,000 person-years among older participants.10 Another study from sub-Saharan Africa reported the adjusted incidence rate of typhoid fever significantly higher in children aged 2–14 years than those above 15 years.11 Higher incidences of typhoid fever are also reported in children between the age group of 5–15 years from South Asian countries, including India, Indonesia, and Pakistan.12
Biological E’s Typhoid Vi-CRM197 conjugate vaccine (TyphiBEV™) is a glyco-conjugated vaccine developed based on the conjugation of the Vi polysaccharide separately with the CRM197 carrier protein. Due to the T-cell-dependent immunological properties of glycol-conjugates, this conjugate vaccine is expected to overcome the limitations of the currently available polysaccharide vaccines and offer an effective solution for immunizing not only young children ≥2 years but also in infants and toddlers <2 years of age with a long-lasting immune response against S. Typhi infection. Peda-Typh™ from Biomed India and the Vi-TT conjugate vaccine from Bharat Biotech International Limited (Tybar-TCV) were the only two typhoid vaccines available in India at the time of conduct of this study. The present study was conducted to demonstrate safety and immunogenic non-inferiority in terms of difference in the seroconversion rates after a single intramuscular (IM) dose of Biological E’s Vi-CRM197 conjugate vaccine (TyphiBEVTM) in ≥6 months to <64-year-old healthy infants, children, adolescents, and adults in comparison with the Tybar-TCV (licensed comparator) at Day 42.

Methods

Study population and study design

Study population

Healthy subjects of either gender between ≥6 months to <64 years of age were included in the study. Subjects with a history of typhoid infection, vaccination against typhoid, contact to an individual with laboratory confirmed S. Typhi infection, any serious or chronic disease, bleeding disorder, autoimmune disease, use of immunosuppressants, allergic reaction to vaccine-related components, body temperature ≥38.0°C within 3 days prior to the day of vaccination, or unwillingness or inability to understand and follow study procedures were excluded from the study recruitment.

Of the 10 study sites chosen, the study protocol was approved by the respective Institutional Ethics Committees (IEC) of 9 study sites where the study was carried out. One of the selected sites could not take up the protocol for ethics review as the IEC was not active during the period of the study. The study was registered prospectively with Clinical Trial Registry of India (CTRI) [Regd. No. CTRI/2018/11/016419]. The study was conducted in accordance with the ethical principles defined in the Declaration of Helsinki, International Council for Harmonization Good Clinical Practices (ICH-GCP) guidelines, and applicable regulatory requirements. Written informed consent and/or assent was obtained from each subject, or from their parents/legal authorized representatives of all children involved in the study before the enrollment.

Study design

This was a multicenter, single-blind, randomized, Phase 2/3 clinical study to assess the overall safety and immunogenic non-inferiority of TyphiBEVTM vaccine compared to Tybar-TCV available in India in healthy infants, children, and adults. A total...
of 622 subjects were enrolled in the study. The total duration of the study was 42 days, with 2 visits, that is, Visit 1 (Day 0) and Visit 2 (Day 42) with +7-day time window. Post-screening, all eligible healthy subjects of either gender were block randomized into one of the 2 treatment groups viz., the TyphiBEVTM group or the Typhbar-TCV group in 1:1 ratio using Interactive Web Response System (I WRS) using Statistical Analysis System (SAS) program. A stratified block randomization (Proc Plan) was used for treatment allocation in this study.

Both vaccine groups comprised of three age subsets: ≥6 months to <2 years, ≥2 years to <18 years, and ≥18 years to <64 years. The subjects in both vaccine groups received a single .5 mL dose of study vaccine intramuscularly.

**Immunogenicity evaluations**

Immunogenicity was the primary objective of this study. Immunogenicity analysis was based on anti-Vi IgG serum antibodies measured at approximately 42 days following completion of single-dose immunization. The subject sera samples (collected pre-vaccination and 42 days after vaccination) were tested for anti-Vi IgG antibodies using the Vacciyme kit (Vaccyme Human Anti-S Typhi Vi IgG Enzyme Immunoassay Kit manufactured by the Binding Site Group UK). Reference standard for anti-Vi IgG (expressed in microgram/mL) provided by Center for Biologics Evaluation and Research (CBER)/National Institutes of Health (NIH) and National Institute for Biological Standards and Control (NIBSC) (expressed in IU/mL) were also tested on the same kit to establish the conversion factors for reporting of anti-Vi IgG concentration in Vacciyme units/mL to µg/mL and IU/mL.

The immunogenicity measurements included:

1. Assessment of seroconversion rates in both vaccine groups was measured by the number and percentage of subjects who achieved anti-Vi IgG antibody concentration ≥2.0 µg/mL in the post-vaccination blood sample (Day 42).
2. The comparison of the geometric mean concentration (GMC) of antiVi IgG antibodies as determined by enzyme-linked immunosorbent assay (ELISA) in both vaccine groups during pre (Day 0) and post vaccination (Day 42) period.
3. The proportion of subjects who achieved ≥4-fold rise in antiVi IgG antibodies at Day 42 from baseline was assessed in both vaccine groups. Geometric mean fold rise (GMFR) in antiVi IgG antibody concentrations was assessed along with their 2-sided 95% confidence interval (CI) from baseline at Day 42.
4. Immunogenicity assessment in terms of percentage of subjects equal to or above the suggested protective threshold value of ≥4.3 µg/mL (measured by ELISA) was also assessed in the present study on an exploratory basis. As per the WHO TRS No.1030, 2021, Annex 2, this threshold value was found to be associated with a high level of sustained protection lasting 4 years after vaccination.31

**Safety evaluations**

The evaluation of the safety and tolerability of the investigational vaccine was the secondary objective of this study. Following the vaccination, the subjects were observed for 30 minutes for any adverse reactions. During the 7-day follow-up (Day 0 to Day 6), the solicited local and systemic adverse events (AEs), were recorded in a diary by the subjects’ parents, or legally acceptable representative. The proportion of subjects with unsolicited AEs during the follow-up period until Day 42 of post vaccination in both study groups was also reported. Medically attended and serious adverse events (SAEs), if any, during the post-vaccination 42-day follow-up period, was also recorded in both vaccine groups. Vital signs, physical examination, recording of body temperature, and clinical symptomatology were also evaluated during the study.

**Statistical methods**

**Sample size determination**

As per published data, the licensed comparator vaccine is shown to offer a seroconversion rate of 98.05% in 6–23 month old healthy infants and toddlers and 97.29% in 2–45-year-old healthy subjects after single dose.32 For a formal power of 80% and for a significance level of 5%, a sample size of 282 infants and toddlers in age subset 1 (n = 141/group) and a sample size of 340 children, adolescents, and adults in age subset 2 (n = 170/group), was needed to demonstrate immunogenic noninferiority against licensed comparator. The non-inferiority margin was set at minus 10% points that was based on statistical considerations and clinical judgment. The sample size included a 10% dropout allocation at each age subset.

**Analysis sets**

The intent-to-treat analysis (ITT) population included all subjects randomized to one of the treatment groups. The per protocol (PP) population included all evaluable subjects who met all the eligibility criteria and followed the procedures defined in the protocol for whom data concerning immunogenicity endpoint measures were available. The safety population included all subjects who entered the study and received the vaccination.

**Statistical analyses**

Demographic and baseline characteristics were summarized descriptively. For continuous variables n, mean, standard deviation (SD), median, minimum, and maximum were presented. For categorical data, frequencies, and relative frequencies were computed.

The primary objective of the study was to show non-inferiority, in terms of the difference in the proportion of subjects who achieved anti-Vi IgG antibody concentration ≥2.0 µg/mL after administration of TyphiBEVTM compared with Typhbar-TCV at Day 42. A 2-sided 95% CI for the difference in the proportion of subjects between both vaccine groups, achieving seroconversion ≥2.0 µg/mL threshold, was calculated by pooled Z-test. Non-inferiority was established if the lower limit of the 2-sided 95% CI for difference in proportions was on the positive side of the minus 10% points NI margin set.
Geometric mean concentrations of anti-Vi IgG antibodies, pre (Day 0) and post-vaccination (Day 42), as determined by ELISA, the proportion of subjects who achieved ≥4-fold rise in anti-Vi IgG antibodies at Day 42, and GMFR in anti-Vi IgG antibody concentrations along with their 2-sided 95% CI at Day 42 were summarized descriptively. Summary analysis of data was also presented in terms of using NIBSC and by both standard (IU/mL) and by Vacczyme units (U/mL) for GMC evaluations.

The AEs were recorded throughout the study duration (42 + 7 days). All reported AEs during the entire study period were summarized by calculating frequencies and relative frequencies by treatment group and were listed, including severity, relationship to the vaccine (causality) and action taken. Number, percentage, and 95% CI for percentage of subjects with AEs (solicited local and systemic AEs, and unsolicited AEs) and SAEs, and cumulative incidence rates were presented overall and by body system/preferred term and by treatment group. The 2-sided 95% CIs were calculated by Clopper–Pearson method for all the occurrence rates of reported AEs and SAEs during the study. All SAEs and medically attended AEs reported during the study were analyzed for expectedness and causality. All SAEs and/or AEs leading to withdrawal were also listed by subject and treatment group. All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) v22.0.

Comparative statistics for the safety variables were calculated, the study was designed to detect differences in the incidence of local and systemic reactions between vaccination groups descriptively. Systemic tolerability was assessed through recording of body temperature and clinical symptomatology. Changes in body temperature, from study start to end of study was analyzed descriptively by treatment group and was part of study evaluation, only in case of clinically significant changes.

**Results**

**Study population**

This study was carried out at 9 sites in India. Of the 636 subjects screened, 622 subjects (311 in TyphiBEV™ group and 311 in Typhar-TCV group) were randomized into the study and received the single dose of the study vaccine. Both vaccine groups were further divided into three age subsets: age subset 1 consisting of healthy infants and toddlers aged ≥6 months to <2 years (n = 141), age subset 2 consisting of healthy children and adolescents aged ≥2 years to <18 years (n = 85) and age subset 3 consisting of old healthy adults aged ≥18 years to <64 years (n = 85) in each treatment group.

Of the 622 subjects, 595 (95.66%) subjects completed the study (296 in TyphiBEV™ group and 299 in Typhar-TCV group respectively), and 27 (4.34%) subjects did not complete the study (15 in TyphiBEV™ group and 12 in Typhar-TCV group) due to the following reasons: lost to follow-up (13 subjects), migration from the study area (7 subjects), subjects’ choice/withdrawal of consent (4 subjects), noncompliance to study protocol (2 subjects), and death due to SAE (dengue fever) in 1 subject (from Typhar-TCV group) which was considered unrelated the study vaccine. (Figure 1).

**Immunogenicity findings**

Immunogenicity assessments were primarily based on the PP population that consisted of 591/622 (95%) subjects. Overall, 295/311 (94.86%) subjects in the TyphiBEV™ group and 296/311 (95.18%) subjects in the Typhar-TCV group were included in the immunogenicity analysis. The proportion of subjects with anti-Vi IgG antibody concentrations above the seroconversion threshold ≥2.0 μg/mL (primary) were 98.98% (292/295 subjects) and 99.32% (294/296 subjects) in the TyphiBEV™ and Typhar-TCV groups, respectively. In the present study, the difference in seroconversion rates between the treatment groups at Day 42 was −0.34%, with its lower limit of 95% CI −1.82% and upper limit of 1.14% (Table 2).
Table 1. Demographics and baseline characteristics-ITT population.

| Parameter/Statistics/Category | ≥6 months to <2 years (N=282) | ≥2 years to <18 years (N=170) | ≥18 years to <64 years (N=170) | Overall (N=622) |
|------------------------------|---------------------------------|---------------------------------|---------------------------------|-----------------|
|                              | TyphiBEV™ (N₁=141) | Typhbar-TCV (N₁=141) | TyphiBEV™ (N₁=85) | Typhbar-TCV (N₁=85) | TyphiBEV™ (N₁=85) | Typhbar-TCV (N₁=85) | TyphiBEV™ (N₁=311) | Typhbar-TCV (N₁=311) |
| Gender, N₁ (%)               | Male 81 (57.45%) | 66 (46.81%) | 48 (60.00%) | 43 (50.59%) | 40 (47.06%) | 172 (55.31%) | 157 (50.48%) |
|                              | Female 60 (42.55%) | 75 (53.19%) | 37 (40.00%) | 42 (49.41%) | 45 (52.94%) | 139 (44.69%) | 154 (49.52%) |
| Age (Years)                  | Mean 1.25 | 1.25 | 8.09 | 7.11 | 31.37 | 33.34 | 11.35 | 11.62 |
|                              | SD 0.38 | 0.41 | 4.33 | 3.77 | 7.41 | 8.71 | 13.39 | 14.44 |
|                              | Median 1.21 | 1.27 | 7.40 | 6.54 | 30.52 | 31.75 | 3.04 | 3.06 |
|                              | Range (Min:Max) (0.59:1.98) | (0.52:1.99) | (2.06:17.36) | (2.01:15.96) | (19.00:49.56) | (18.30:53.85) | (0.59:49.56) | (0.52:53.85) |
| Height (Cms)                 | Mean 75.9 | 74.7 | 121.2 | 115.4 | 162.5 | 160.7 | 112.0 | 109.3 |
|                              | SD 7.0 | 7.1 | 15.2 | 12.30 | 10.10 | 9.76 | 39.54 | 38.59 |
|                              | Median 75.4 | 75.0 | 121.0 | 113.1 | 162.5 | 160.0 | 93.0 | 90.1 |
|                              | Range (Min:Max) (60.0:182.9) | (64.0:182.9) | (70.0:176.0) | (124.9:182.0) | (139.7:187.9) | (60.0:182.9) | (50.0:187.9) |
| Weight (Kgs)                 | Mean 9.5 | 9.5 | 25.7 | 22.1 | 63.2 | 60.0 | 28.6 | 26.7 |
|                              | SD 1.37 | 1.72 | 15.61 | 11.60 | 13.71 | 12.38 | 24.77 | 22.88 |
|                              | Median 9.5 | 9.5 | 20.3 | 19.5 | 60.0 | 59.2 | 12.5 | 13.0 |
|                              | Range (Min:Max) (6.5:14.0) | (6.2:14.0) | (8.5:97.0) | (7.5:70.0) | (34.7:95.6) | (35.0:98.0) | (6.5:97.0) | (6.2:98.0) |

ITT=intent-to-treat, Max=maximum, Min=minimum, N=sample size, N1=subject count, SD=standard deviation, Typhbar-TCV=Bharat Biotech’s Typhoid Vi-TT conjugate vaccine, TyphiBEV™=Biological E’s Typhoid Vi-CRM197 conjugate vaccine.
Table 2. Summary of seroconversion (≥2 µg/ml) rates for Anti-Vi IgG antibody concentration by three age subsets and treatment groups-PP population.

| Parameter | ≥6 months to <2 years (N=259) | ≥2 years to <18 years (N=164) | ≥18 years to <64 years (N=168) | Overall (N=591) |
|-----------|--------------------------------|--------------------------------|--------------------------------|-----------------|
|           | Anti-Vi IgG antibody concentration | TyphiBEVTM (N=129) | Tybar-TCV (N=130) | Proportion difference between groups (95% CI) | TyphiBEVTM (N=82) | Tybar-TCV (N=82) | Proportion difference between groups (95% CI) | TyphiBEVTM (N=84) | Tybar-TCV (N=84) | Proportion difference between groups (95% CI) | TyphiBEVTM (N=295) | Tybar-TCV (N=296) | Proportion difference between groups (95% CI) |
| Pre-      | Seroconverted (≥2 µg/mL) | 7 (5.43%) | 4 (4.88%) | 2 | 3 | 14 | 6 |
|           | 95% CI | 2.21, 10.86 | 0.02, 4.21 | 0.30, 8.53 | 0.74, 10.08 | 0.74, 10.08 | 2.62, 7.83 | 0.75, 4.36 |
| Not       | Seroconverted (≥2 µg/mL) | 122 (94.57%) | 78 (95.12%) | 80 | 81 | 81 | 281 | 290 |
|           | 95% CI | 97.56, 0.74 | 0.30, 8.53 | 0.74, 10.08 | 0.74, 10.08 | 0.74, 10.08 | 0.74, 10.08 |
| Post-     | Seroconverted (≥2 µg/mL) | 128 (99.22%) | 130 (100.00%) | 82 | 81 | 82 | 292 | 294 |
|           | 95% CI | 95.76, 99.98 | 97.20, 99.9 | 95.60, 99.71 | 95.4, 99.97 | 95.06, 99.79 | 95.58, 99.92 |
| Not       | Seroconverted (≥2 µg/mL) | 1 (0.78%) | 0 (0.00%) | 0 | 1 (1.22%) | 2 | 3 | 2 (0.68%) |

Percentages were calculated using respective column header count as denominator. 95% CI was calculated by Clopper–Pearson Method. Two-sided 95% confidence intervals for difference in proportion of subjects between groups was calculated using pooled Z test. CI=confidence interval, IgG=immunoglobulin G, N=sample Size, N1=subject count, PP=per protocol, SD=standard deviation, Tybar-TCV=Bharat Biotech’s Typbar-Typhioid Vi-TT conjugate vaccine, TyphiBEVTM =Biological E’s Typhoid Vi-CRM197 conjugate vaccine.
**Table 3.** Summary of geometric mean concentrations for three age subsets by treatment groups—PP population.

| Anti-Vi IgG antibody concentration | ≥6 months to <2 years (N<sub>1</sub>=259) | ≥2 years to <18 years (N<sub>1</sub>=164) | ≥18 years to <64 years (N<sub>1</sub>=168) | Overall (N=591) |
|-----------------------------------|----------------------------------------|--------------------------------------|------------------------------------------|-----------------|
| TyphiBBV<sup>TM</sup> (N=129)     | TyphiBBV<sup>TM</sup> (N=130)          | TyphiBBV<sup>TM</sup> (N=82)         | TyphiBBV<sup>TM</sup> (N=84)            | TyphiBBV<sup>TM</sup> (N=295) |
| GMC Ratio (T/R)                   | 129                                    | 130                                  | 82                                      | 84              |
| 95% CI of GMC                     | (0.09, 1.12)                           | (0.07, 0.09)                         | (0.09, 1.13)                            | (0.10, 1.12)    |
| Post vaccination Parameters       |                                        |                                      |                                         |                 |
| GMC                               | 20.66                                 | 29.66                                | 32.69                                   | 29.92           |
| 95% CI of GMC                     | (18.02, 23.69)                         | (26.38, 32.72)                       | (27.34, 39.37)                          | (15.12, 22.24)  |

Cl=confidence interval, GMC=geometric mean concentration, N<sub>1</sub>=subject count, N=sample size, PP=per protocol, T/R=Test/Reference, Typhi-TCCB=Biotech’s Typhi-TT conjugate vaccine, TyphiBBV<sup>TM</sup>=Biological E’s Typhoid Vi-CRM197 conjugate vaccine.
In the age subsets of ≥6 months to <2 years, ≥2 years to <18 years, and ≥18 years to <64 years, the difference (95% CI) in seroconversion rates between the treatment groups at Day 42 was −0.78%, 1.22%, and −1.19%, respectively, with a lower and upper confidence limit at (2.29%, 0.74%) for ≥6 months to <2 years, (1.16%, 3.60%) for ≥2 years to <18 years, and (5.19%, 2.81%) for ≥18 years to <64 years. Hence, the TyphiBEV™ demonstrated non-inferiority compared to TypbarTCV as the lower limit of 95% CI of the difference in seroconversion rate was above the predefined non-inferiority limit of −10%. In all three age subgroup analyses, the proportion of subjects seroconverted was similar in the TyphiBEV™ and Typbar-TCV groups.

Overall, the GMC at Day 42 was 24.79 µg/mL in the TyphiBEV™ group and 26.58 µg/mL in the Typbar-TCV groups. The geometric mean ratio between the TyphiBEV™ group and the Tybar-TCV group was .93. The difference in GMCs between the treatment groups was considered not clinically meaningful. The GMCs at Day 42 in the TyphiBEV™ vs TypbarTCV groups were 20.66 vs 29.66 µg/mL, 27.24 vs 32.69 µg/mL, and 29.92 vs 18.34 µg/mL, respectively, in the age subset of ≥6 months to <2 years, ≥2 years to <18 years, and ≥18 years to <64 years, respectively (Table 3).

The proportion of subjects who achieved ≥4-fold increase in anti-Vi IgG antibody concentrations at Day 42 from baseline were 96.95% (286/295 subjects) with a GMFR of 223.38 in the TyphiBEV™ group against 97.64% (289/296 subjects) with a GMFR of 260.81 in the Typbar-TCV group (Table 4).

In the additional exploratory immunogenicity analysis, a threshold value of 4.3 µg/mL anti-Vi antibody measured by ELISA was used as mentioned in Annex-2 of WHO TRS No.1030, 2021, which was found to be associated with a high level of sustained protection lasting 4 years after vaccination.28 On using this threshold of ≥4.3 µg/mL of anti-Vi IgG antibody concentrations, it was observed that the seroconversion rate was 95.59% (282/295 subjects) and 96.28% (285/296 subjects) in the TyphiBEV™ group and Typbar-TCV group, respectively. The seroconversion rate corresponding to

### Table 4. Fold increase of IgG antibody concentrations from baseline to Day 42 by vaccine group-PP population.

| Parameter | Day 42 (Post Vaccination) |
|-----------|---------------------------|
|           | TyphiBEV™ (n=295) | Typbar-TCV (n=296) |
| ≥4 Fold Rise | 96.95% | 97.64% |
| GMFR | 223.38 | 260.81 |

GMFR=geometric mean fold rise, n=subject count, IgG=Immunoglobulin G, N=sample size, PP=per protocol, Typbar-TCV=Bharat Biotech’s Typhoid Vi-TT conjugate vaccine, TyphiBEV™ =Biological E’s Typhoid Vi-CRM197 conjugate vaccine.

### Local Adverse Events

|                      | Severe | Moderate | Mild |
|----------------------|--------|----------|------|
| TyphiBEV™ Injection Site Erythema | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Injection Site Induration | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Injection Site Pain | 0.00%  | 0.00%    | 0.64%|
| TyphiBEV™ Injection Site Pruritus | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Injection Site Swelling | 0.00%  | 0.00%    | 0.00%|

### Systemic Adverse Events

|                      | Severe | Moderate | Mild |
|----------------------|--------|----------|------|
| TyphiBEV™ Vomiting    | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Fatigue     | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Malaise     | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Pyrexia     | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Arthralgia  | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Myalgia     | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Headache    | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Cough       | 0.00%  | 0.00%    | 0.00%|
| TyphiBEV™ Rash        | 0.00%  | 0.00%    | 0.00%|

Figure 2. Local and systemic adverse events reported. TyphiBEV™ =Biological E’s Typhoid Vi-CRM197 conjugate vaccine, Typbar-TCV=Bharat Biotech’s Typhoid Vi-TT conjugate vaccine.
sustained protection threshold in the TyphiBEV™ group vs Tybar-TCV group was 96.90% vs 99.23%, 95.12% vs 97.56%, and 94.05% vs 90.48%, respectively, in the age subset of ≥6 months to <2 years, ≥2 years to <18 years, and ≥18 years to <64 years.

The testing was performed using Vacczyme kit from Binding Site Group Ltd., and initial number was reported in Vacczyme units (VU/mL). These values were then converted to µg/mL based on CBER standard and into IU/mL based on NIBSC standard.

**Safety findings**

Safety analysis was performed on the safety cohort, which included 622 subjects (311 subject each in the TyphiBEV™ and Tybar-TCV groups) who received the vaccination. Overall, 113 AEs (35 in the TyphiBEV™ group and 78 in the Tybar-TCV group) were reported in the study by 26/311 (8.36%) subjects in the TyphiBEV™ group and 47/311 (15.11%) subjects in the Tybar-TCV group (Supplementary Table S1). The incidence rate of AEs was lower in the TyphiBEV™ group compared with the Tybar-TCV group in all age subsets; 9.93% vs 15.60% in the subset of ≥6 months to <2 years, 10.59% vs 15.29% in the subset of ≥2 years to < 18 years, and 3.53% vs 14.12% in the subset of ≥18 years to < 64 years.

The majority of AEs were mild or moderate in severity. The most commonly reported AEs (>2% of subjects in any treatment group) in the TyphiBEV™ groups were injection site pain (14/311 [4.50%] subjects) vs 29/311 [9.32%] subjects), pyrexia (7/311 [2.25%] subjects vs 13/311 [4.18%] subjects), injection site erythema (2/311 [0.64%] subjects vs 10/311 [3.22%] subjects), and injection site swelling (1/311 [0.32%] subjects vs 8/311 [2.57%] subjects) (Supplementary Table S2). The incidence of local AEs was 5.14% (16/311 subjects) in the TyphiBEV™ group and 11.90% (37/311 subjects) in the Tybar-TCV group. The most commonly reported local AEs (>2% of subjects in any treatment group) in the TyphiBEV™ and Tybar-TCV groups were injection site pain (14/311 [4.50%] subjects vs 29/311 [9.32%] subjects), injection site erythema (2/311 [0.64%] subjects vs 10/311 [3.22%] subjects), and injection site swelling (1/311 [0.32%] subjects vs 8/311 [2.57%] subjects) (Figure 2).

The incidence of systemic AEs was 3.86% (12/311 subjects) in the TyphiBEV™ group and 5.47% (17/311 subjects) in the Tybar-TCV group. The most commonly reported systemic AE (>2% of subjects in any treatment group) in the TyphiBEV™ and Tybar-TCV groups was pyrexia (7/311 [2.25%] subjects vs 13/311 [4.18%] subjects) (Figure 2). There were no AEs reported in the first 30-min post-vaccination period. One subject from the Tybar-TCV group reported an SAE of dengue fever of Grade 3 severity on Day 29. On Day 38, the subject died due to dengue shock syndrome with multiple organ dysfunction syndrome. The investigator considered the event unrelated to the study medication.

The incidence of medically attended AEs was 2.25% (7/311 subjects) in the TyphiBEV™ group and 4.50% (14/311 subjects) in the Tybar-TCV group. The most commonly reported medically attended AE (>2% of subjects in any treatment group) in the TyphiBEV™ and Tybar-TCV groups was pyrexia (7/311 [2.25%] subjects vs 9/311 [2.89%] subjects). All the medically attended AEs resolved without sequel: the incidence of AEs within 7 days post-vaccination period was lower in the TyphiBEV™ (26/311 [8.36%] subjects) group compared with the Tybar-TCV (46/311 [14.79%] subjects) group. Three unsolicited AEs were reported in the study by three subjects from the Tybar-TCV group. All three AEs (dengue fever [SAE led to the death of subject], Varicella zoster [considered to be mild in intensity by the investigator], and wheezing) were considered unrelated to the study medication by the investigator. No subject was withdrawn from the study due to any adverse event. No marked clinically significant changes over time were observed in the vital signs recorded.

**Discussion**

The present study was a multicentric, single-blind, randomized, controlled, Phase 2/3 study to evaluate immunogenicity and safety of single IM dose of TyphiBEV™ in infants and toddlers ≥6 months to <2 years, in children and adolescents ≥2 years to <18 years, and in adults ≥18 years to 64 years of age, in comparison with a licensed comparator TybarTCV. Studies have reported the Vi conjugate vaccine to be superior to unconjugated vaccines in offering better and longer protection, involving memory cells in addition to the production of Vi antibodies. The Vi conjugate vaccine is reported to be safe and immunogenic in infants and children. The results of present study are in line with these findings. Animal studies have shown that glycoconjugate vaccine ViCRM197 stimulates the production of Vi-specific serum IgG1 titers that persisted for more than 60 days post vaccination. The intestinal washes of the animals studied also had Vi-specific IgG antibodies. The study also reported the ViCRM197 to stimulating T cell response that augmented the Vi-specific B cells to produce antibodies. In the present study, a single-dose Vi-CRM197 conjugate vaccine induced a strong anti-Vi immune response as demonstrated with the overall proportion of subjects seroconverted (99%) in the age group of ≥6 to <64 years. This finding was in line with the findings of another study that reported a single dose of Vi-CRM197 conjugate vaccine to induce a strong anti-Vi immune response in adults, children, and infants aged 9–11 months with a seroconversion rate of 100%. A Phase 1 study comparing EuTCV, a Vi-CRM197 conjugate vaccine to Tybar-TCV and Vi Polysaccharide vaccine Typhim Vi™ in Filipino adults has also reported anti-Vi IgG antibody titer to be higher in ViCRM197 vaccine than Tybar-TCV and Typhim Vi. The proportion of subjects seroconverted in EuTCV and Tybar-TCV was 100%, compared to 84% in Typhim Vi. In line with the study, the proportion of subjects seroconverted was comparable across all 3 age subsets in both vaccine groups in the present study. There was also no significant difference in terms of proportion of subjects achieving ≥4-fold increase in anti-Vi IgG antibody concentrations at Day 42 from baseline between treatment groups. The seroconversion rates corresponding to the sustained protection threshold were also comparable in the TyphiBEV™ group vs Tybar-TCV group in all age subsets in the present study. The primary objective of demonstrating
immunogenic non-inferiority of TyphiBEV™ compared with the TypbarTCV was also achieved with the lower limit of 95% CI (−1.82%, 1.14%) for the difference in proportion (−0.34%) of subjects seroconverted (≥2.0 μg/mL) was above the minus 10% points.

In contrast to the previous study, which reported comparable rates of adverse events in Vi-CRM197 vaccine and other vaccines, the overall incidence of AEs (including local, systemic, medically attended, and unsolicited AEs) reported during the present study was lower in the TyphiBEV™ group (8.36%; 26/311 subjects) compared with Typbar-TCV group (15.11%; 47/311 subjects). The incidence of AEs was similar in all three age subsets studied in the TyphiBEV™ group. The most commonly reported AEs in both vaccine groups were injection site pain, pyrexia, injection site erythema, and injection site swelling. The majority of AEs in both vaccine groups were mild in their intensity. The present data clearly shows that the TyphiBEV™ vaccine is well tolerated in all age groups tested, and its’ safety profile is consistent with that of licensed Typbar-TCV used as a comparator in the present study.

Study limitations: The present study is not double-blinded. In this study, we presented immunogenicity and safety data only until Day 42 as the follow-up of a Phase 3 study is ongoing, assessing immunological persistence of anti-Vi IgG antibodies at 12, 24, and 36 months. The Phase 3 study also will assess the immune response to a booster dose of conjugate typhoid vaccine in both treatment groups administered at 36 months in subjects (6 months to 45-year-old) who participated in the present study.

In conclusion, this study demonstrated the primary immunogenicity objective of noninferiority of TyphiBEV™ in terms of the difference in the proportion of subjects seroconverted (protective threshold value ≥2.0 μg/mL) against TypbarTCV. In addition, the non-inferior immunogenicity was also shown by the exploratory analysis using WHO referred correlate of protection threshold value of ≥4.3 μg/mL. The safety and tolerability profile of the TyphiBEV™ was comparable to the TypbarTCV in healthy infants, children, adolescents, and adults from India.

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Authors’ contribution
All authors met the ICMJE criteria for authorship and were responsible for conception and design of the research, acquisition of data, and its analysis. All the authors were involved in revising the manuscript critically for important intellectual content and approved the final manuscript.
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