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Safety and immunogenicity of a single dose, live-attenuated 'tetravalent dengue vaccine' in healthy Indian adults; a randomized, double-blind, placebo controlled phase I/II trial

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ABSTRACT

Background: Dengue fever is the most prevalent mosquito-borne viral disease in the world, with 390 million dengue infections occurring every year. There is an unmet medical need to develop a safe, effective and affordable dengue vaccine against all four Dengue serotype viruses-DENV1, DENV-2, DENV-3 and DENV-4. Panacea Biotec Ltd (PBL) has developed a cell culture-derived, live-attenuated, lyophilized Tetravalent Dengue Vaccine (TDV). Here, in phase I/II study we assessed the safety and immunogenicity of single dose 'Dengue Tetravalent Vaccine' in healthy Indian adults.

Methods: In the study, 100 healthy adult volunteers aged 18–60 years were enrolled. The participants were allocated to TDV and placebo groups in 3:1 ratio, i.e. 75 participants to TDV group and 25 participants to the placebo group. Enrolled participants were administered a single dose of 0.5 ml of the test vaccine / placebo by subcutaneous route. Primary outcome for safety included all solicited AEs up to 21 days, unsolicited AEs up to 28 days and all AEs/serious adverse events (SAEs) till day 90 post-vaccination. For immunogenicity assessment the primary outcome was seroconversion & seropositivity rate by $PRNT_{50}$ to all four serotype till 90 days.

Results: Overall, 100 subjects were vaccinated out of which 8 subjects (5 subjects in vaccine group and 3 subjects in placebo group) dropped out from the study. The most commonly reported solicited local AE was pain and most common solicited systemic AE was headache and fever. No SAE was reported during the study. There was no statistically significant difference between TDV and placebo groups in terms of AEs. Of the 92 subjects who completed all scheduled visits in the study, 59 (81.9%) achieved seroconversion for DENV-1, 56 (77.8%) for DENV-2; 59 (81.9%) for DENV-3 and 57 (79.2%) for DENV-4 in TDV group. The seroconversion rate in the TDV group was statistically significant ($p < 0.001$) compared to placebo.

Clinical trial registration: CTRI/2017/02/007923.

Abbreviations: ADE, Antibody Dependent Enhancement; GMT, Geometric Mean Titer; PP, Per Protocol; PFU, Plaque Forming Unit; PRNT, Plaque Reduction Neutralization Test; SAE, Serious Adverse Event; TDV, Tetravalent Dengue Vaccine.

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Introduction

Dengue is a mosquito-borne viral disease which manifests a wide spectrum of clinical presentations that ranges from fever, headache, muscle pain, arthralgia, rash, nausea and vomiting [1] to more severe presentation like Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [2,3]. DHF/DSS can threaten a patient's life by increasing vascular permeability and shock. The Dengue virus is a single-stranded RNA virus of the genus Flavivirus with four serotypes[2] DENV-1, DENV-2, DENV-3, and DENV-4, all of which circulate globally and in recent years, most endemic countries are reporting circulation of all serotypes [4]. In India, Aedes aegypti is the main mosquito vector in urban areas; however, Aedes albopictus is also found in many states [5].

According to World Health Organization (WHO), the global incidence of dengue has grown dramatically in recent decades and about half of the world's population is at risk for dengue [2]. As per a cartographic model, it was estimated 390 million dengue virus infections/year (CI: 284–528), among which 96 million (CI: 67–136) manifest clinically [6]. Another study of dengue estimates 3.9 billion people are at risk of dengue infection [7]. Despite a risk of infection existing in 129 countries, [8] 70% of the actual burden of dengue is in Asia [6]. As per the estimates, 1–2 million persons are hospitalized with severe illnesses, including DHF/DSS, and 0.1–5% die [6–8]. Dengue causes about 20,000 deaths/year [9]. In India around 157,315 cases of confirmed dengue with 166 deaths due to dengue were reported in 2019 [10].

There is no known specific antiviral treatment for dengue/severe dengue, the management is symptomatic and supportive [5]. The financial, social, and individual cost of dengue is significant [11]. Given the dramatic urban growth and lack of adequate surveillance for dengue in tropical developing countries, it is likely that the true disease burden of dengue is underestimated. A unique feature of dengue is that initial infection (primary infection) by any one of the DENV serotype can offer protection (homotypic) against subsequent infection by that serotype alone. While such protection is generally presumed to be life-long, protection against heterotypic DENVs is transient. When such cross-protection wanes, a subsequent infection (secondary infection) by a different DENV serotype can actually result in severe dengue disease [17]. Epidemiological evidence suggests that secondary infections correlate with an increased risk of severe dengue disease [12]. Antibody dependent enhancement of infection (ADE) is thought to be a significant contributor to pathophysiology [20]. In this scenario, the strategic administration of a safe and efficacious Tetravalent Dengue Vaccine (TDV) which will protect against all four serotypes can be a game changer and can reduce the global dengue burden [12]. As per WHO, the first and only licensed dengue vaccine, Sanofi Pasteur's Dengvaxia® (CYD-TDV) has been shown to be efficacious and safe in clinical trials in persons who have had a dengue virus infection in the past (seropositive individuals), but carries an increased risk of hospitalization and severe dengue in those who experience their first natural infection after vaccination (seronegative individuals) [13]

The experimental dengue vaccine used in Phase I/II clinical trial is prepared on a cell culture based technology developed at Panacea Biotec using the four recombinant live-attenuated dengue virus serotypes- DENV-1, DENV-2, DENV-3 and DENV-4 from National Institute of Health, USA. Viruses were grown on mammalian cells (Vero). The cells were infected with a single serotype with required multiplicity of infection (MOI). The production process was optimized using cell factory systems (Corning Cell BIND) with multiple harvests and the final product designated Dengue Tetravalent Vaccine, Live Attenuated (Recombinant, Lyophilized). Several NIH studies have evaluated each of candidate vaccine viruses in juvenile rhesus monkeys [22] and in the novel rodent model consisting of severe combined immunodeficiency (SCID) mice bearing intraperitoneal tumors of the human liver cell line HuH-7 [26–29]. Replication of these candidate vaccine viruses in the SCID-HuH-7 mouse model was compared with that of their wild type parent viruses. The tetravalent vaccine admixture elicited neutralizing antibody titers against the component vaccine viruses. It was safe and well tolerated in rhesus monkeys, and elicited antibody responses against the component viruses. The vaccine also has previously been shown to confer complete protection against dengue in a human challenge model [23]Based on the research and development, the final vaccine for clinical use was manufactured at Panacea Biotec's WHO complied GMP facility. The final formulation had a potency of not less than 3 $\log_{10}$ plaque-forming units for each dengue virus serotype per 0·5 ml dose.

Multiple clinical trials have been done by NIH on individual monovalent as well as tetravalent dengue vaccines in flavivirus naïve and experienced individuals. Monovalent and Tetravalent Dengue Vaccine phase I trials have been done on more than 700 and 200 participants using different formulations respectively [14] Though all the tetravalent vaccine formulations were found to be safe and immunogenic, two candidate vaccine admixtures, namely TV003 and TV005, showed best safety and immunogenicity profile [15]. The Dengue Tetravalent Vaccine described in this study is based on NIH TV003 liquid admixture vaccine.

A phase I/II study was conducted with an aim to assess the safety and immunogenicity of a single dose, subcutaneous TDV lyophilized formulation in healthy adults in India.

Materials and methods

Cells and viruses

The live attenuated Tetravalent Dengue Vaccine (TDV) has been developed using Vero cell line as cell substrate. The WHO Vero cell line [16] (10–87) has been subjected to broad range of tests to establish its suitability for vaccine production. Food and Drug Administration (FDA) is custodian of supply of WHO Vero 10–87 cell line. US-FDA has approved Panacea Biotec's request and forwarded the same to ATCC for shipping of WHO Vero cell line (10–87) to Panacea Biotec Limited. Accordingly, Panacea Biotec imported Vero cells and prepared Master Cell Bank (MCB) and Working Cell Bank (WCB) in a GMP facility. Cells were grown in SFM4 MegaVir (Hyclone) medium and cultured in incubators set at 37 °C with 5% CO_2 and passaged every 2–3 days.

Dr. Stephen S. Whitehead and team at the laboratory of Infectious diseases, National Institute of Allergy and Infectious Diseases (NIAID), located at National Institute of Health (NIH), Bethesda, MD, USA has generated attenuated Dengue vaccine viruses by employing two genetic engineering strategies. As a first strategy, DENV-1, DENV-3 and DENV-4 were attenuated by deleting 30 nucleotides (Δ30) in the 3′ UTR of Dengue genome. Secondly, chimeric viruses were generated by replacing the antigenic structural proteins (viz. prM and E) coding region of attenuated DENV-4 genome by that of another serotype. Attenuated DENV-2 was generated by this technology [24]. Site directed mutagenesis was used to introduce point mutations in attenuated DENV-1, DENV-2, DENV-3 and DENV-4 to produce a stable virus.

Panacea Biotec in-licensed the dengue vaccine viruses developed as above from the NIAID/NIH. The dengue vaccine viruses received from the NIH, which have been selected for making a tetravalent dengue vaccine were DENV-1 (rDEN-1Δ30-1545), DENV-2 (rDEN-2/4 Δ 30(ME)-1495, 7163), DENV-3 (rDEN-3 Δ 30/31-7164) and DENV-4 (rDEN-4 Δ 30-7132, 7163, 8308). Research seed lots for serotypes DENV-1, DENV-2, DENV-3 and

DENV-4 were prepared under GLP conditions to create a sufficient source of virus required for upstream, downstream and analytical method development. These viruses were also used for the development of plaque reduction neutralization test (PRNT).

Vaccine

Vaccine development process

The experimental dengue vaccine used in Phase I/II clinical trial was prepared on a cell culture based technology developed at Panacea Biotec using the four recombinant live attenuated dengue virus serotypes- DENV-1, DENV-2, DENV-3 and DENV-4 from National Institute of Health, USA. These viruses were propagated in vero cells in SFM4MegaVir medium. The cells were infected with a single serotype with required multiplicity of infection (MOI). The production process was optimized using cell factory systems (Corning Cell BIND) with multiple harvests. Further the upstream harvests were downstream processed by filtration and diafiltration, followed by analytical testing. Through these processes drug substance for each dengue serotype was prepared in GMP conditions. Drug substance for each serotype was stored at −80 °C ± 15 °C prior to thawing, formulation, and preparation of vaccine containing 3.3 $\log_{10}$ plaque-forming units (PFU) of each serotype. Final potency titers were confirmed by immunoplaque assay. Sequencing study was carried out to determine, if there is any variation in the nucleotide sequence of seed bank, drug substance and final vaccine. We found that the genetic mutation (Δ 30) is intact and the sequences at this site are similar to NIH sequences.

Vaccine composition and administration

Each 0.5 ml dose of Dengue Tetravalent Vaccine, Live Attenuated (Recombinant, Lyophilized) after reconstitution contains: (1) Active Ingredients: not less than 3.0 $\log_{10}$ PFU of each of dengue virus serotype 1 (rDEN-1Δ30), dengue virus serotype 2 (rDEN-2/4Δ30), dengue virus serotype 3 (rDEN-3Δ30/31) and dengue virus serotype 4 (rDEN-4Δ30). (2) Inactive Ingredients: Gelatin, Mannitol, Trehalose, Sucrose, Monobasic Potassium Phosphate (KH_2PO_4), Dibasic Potassium Phosphate (K_2HPO_4), Monosodium glutamate, Dulbecco's Modified Eagle's Medium & Water for injection. Reconstitution fluid: To be reconstituted with sterile water for injection. The lyophilized study drug product is to be stored at 5 ± 3 °C.

A 0.5 ml dose of the vaccine (Batch No. TDNV 01801) administered as a single dose, subcutaneously using needles suited for subcutaneous injection in the deltoid region of adults.

Study design and subjects

A double blind, placebo controlled, randomized phase I/II study was conducted at 3 sites in India i.e. KLES, Dr Prabhakar Kore Hospital & MRC, Belagavi (Karnataka), Pondicherry Institute of Medical Sciences, Pondicherry and King George's Medical University, Lucknow (Uttar Pradesh).

The study was conducted in accordance with the Declaration of Helsinki (Seoul, 2013), New Drug and Clinical Trial Rules 2019 issued by Central Drug Standard Control Organization (CDSCO) and Ethical Guidelines for Biomedical Research on Human Participants, issued by Indian Council of Medical Research (ICMR). The study protocol, amendments, informed consent process/documents, and other information which were required for the study were reviewed and approved by sites specific Ethics Committees of each study sites.

In this study, between 31 Oct 2018 and 05 March 2019, 132 adult participants were screened, of whom 100 were enrolled. Among the enrolled participants, 75 were randomly assigned to the vaccine group and 25 were randomly assigned to the placebo group (control). During the screening procedure, participants were informed about the study procedure and explained in detail about the risks associated with the vaccine trial. Written informed consent was obtained from each participant and the process was audio-video recorded. Subjects were screened on the basis of medical history, physical examination, clinical laboratory tests which included serum creatinine, alkaline phosphatase, AST, ALT, total bilirubin, complete blood count plus differential white blood cell count, PT/PTT. They were recruited based on predetermined set of inclusion and exclusion criteria.

Randomization

Eligible subjects were healthy adults of age 18–60 years. They were randomized to TDV & placebo in the ratio of 3:1. Accordingly 75 participants were assigned to TDV and 25 participants were assigned to placebo. The randomization was done by an independent biostatistician, who prepared the randomization list using R/SAS® software version 9.4. Block randomization schedule was generated with block size of four. The blinding of the investigational product was done in presence of a designated Quality Assurance Personnel with utmost care. The labelling and packaging of the TDV and placebo was done accordingly. The randomization number with the allocated treatment was sealed in separate envelopes by an independent person not involved in the study and supplied the envelopes to sponsor's Quality Assurance-Clinical Research Department and to the investigators to break the code in case of any emergency situations. The study participants were assigned a randomization number sequentially in the order of their entry in the study by the site investigators. Both TDV and placebo were identical in appearance. All study participants, site staff, persons involved in assessing outcomes and data analysis were unaware of treatment assignments.

Screening of participants was done on visit 1 and eligible participants were enrolled in the study. On visit-2 (day 0), the enrolled participants received a single dose 0.5 ml of reconstituted TDV or placebo by subcutaneous route at the deltoid region. Both TDV and placebo were lyophilized formulation (powder), stored along with diluent (sterile water for injection) at 2–8 °C at study sites. The vaccine and placebo had to be reconstituted with diluent before administration. Participants were observed at the study sites for 30 min after vaccination and were given a digital thermometer to measure body temperature, a scale to measure any injection site induration and a diary card to note any adverse event (AE). The participants were monitored on subsequent 6 visits to the study sites (visit-3 to visit-8) on day 9, 12, 28, 56, 90 and 180.

During the post-vaccination visits, the participants were clinically evaluated for any AEs and blood samples were collected to monitor clinical lab parameters, viremia, serum plaque reduction neutralization test ($PRNT_{50}$). Urine pregnancy testing was done for eligible females on specific visits. For safety and immunogenicity assessment, the data were collected till day 180 post-vaccination. However, participants will be followed up for 3 years, post-vaccination to assess the occurrence of symptomatic dengue cases. (Fig. 1)

Serological analysis

Analysis of Immunogenicity & viremia was performed at Bioanalytical Research Department (BARD), Panacea Biotec Ltd, Lalru, Punjab, India. The primary immunogenicity outcomes were determination of $PRNT_{50}$ titers to all four dengue serotypes at day 0, 28, 56, 90 in post-vaccination sera. The seroconversion & seropositivity rate by $PRNT_{50}$ to all four dengue serotypes were also determined.

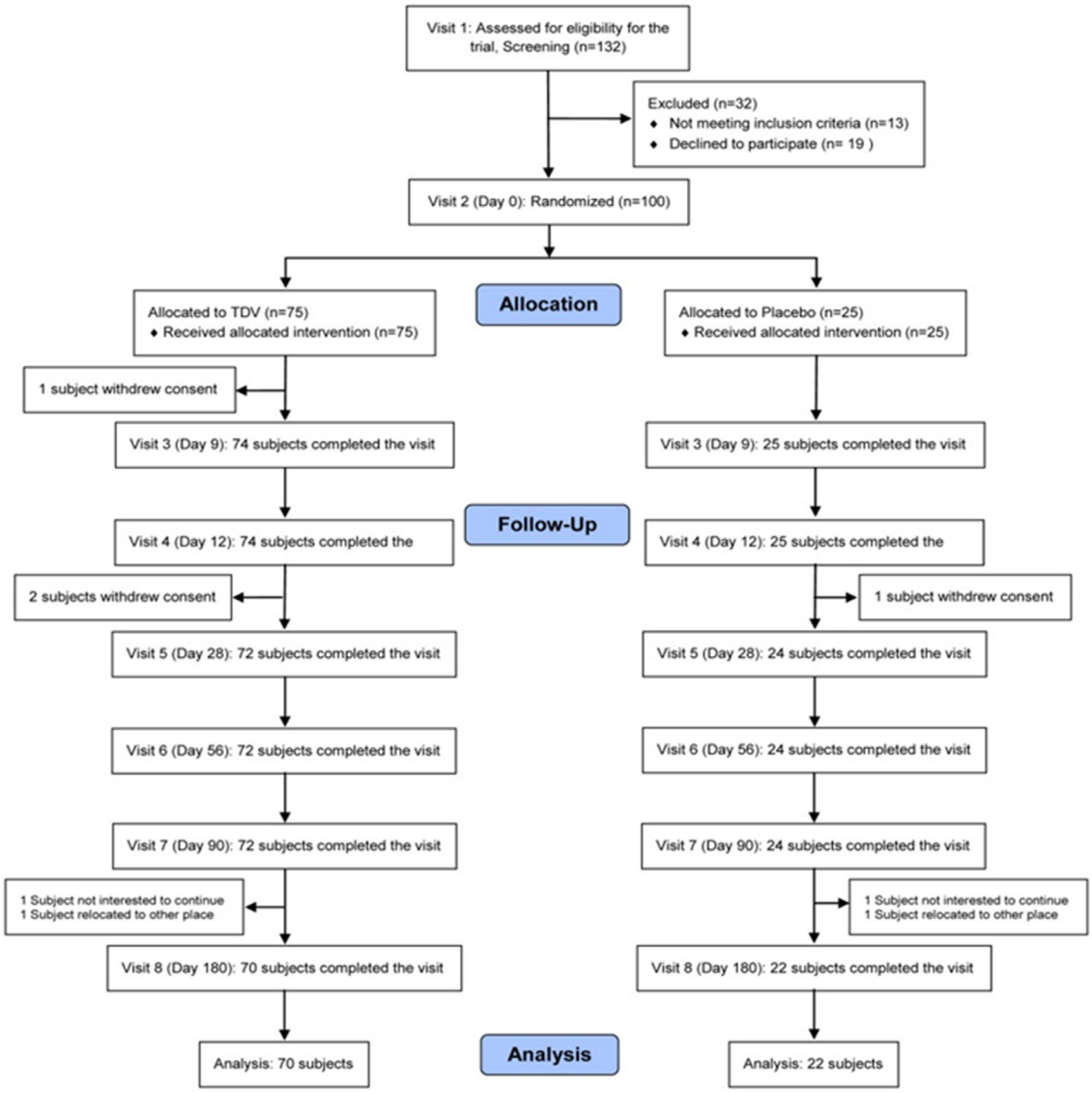

Fig. 1. Subject allocation and participation in the completion of the trial. Flow chart explaining subject participation (n = number of subject) and exclusions during different phases of the study.

Secondary outcome of immunogenicity was assessed by detection of neutralizing antibodies, on day 180 post vaccination, as measured by $PRNT_{50}$; Determination of the seroconversion rate and seropositivity rate by $PRNT_{50}$ test to DEN-1, DEN-2, DEN-3, and DEN-4 viruses on study Day 180 post vaccination; Determination of the monovalent, bivalent, trivalent and tetravalent seropositivity and seroconversion rate on study day 180 post vaccination.

Serum neutralizing antibody levels to DEN-1, DEN-2, DEN-3 and DEN-4 was measured by plaque reduction neutralization test ($PRNT_{50}$) as per PRNT protocol from the NIAID-NIH. The reciprocal of the lowest calculated dilution which reduced virus by 50% ($PRNT_{50}$) was reported as the neutralizing titre. For all the subjects, an initial serum dilution of 1/5 was used for $PRNT_{50}$ assays. Seropositivity was defined as $PRNT_{50}$ cut off titers ($\geq$1/10) before immunization and at any time point up to 180 days after vaccination (day 28, 56, 90 or 180).Seroconversion was defined as $PRNT_{50}$ cut off ($\geq$1/10) for DENV-naïve subjects and four-fold or higher increase in $PRNT_{50}$ titre after immunization of dengue exposed subjects. The viremia detection in participants vaccinated with a single dose of TDV was done on post-vaccination serum samples of days 9 and 12 by amplification and direct titration [19] of dengue virus on vero cell monolayer using immunoplaque assay.

Safety assessment

The primary safety outcomes were assessment of all solicited AEs (Adverse Events) up to 21 days, unsolicited AEs up to 28 days and all AEs/serious adverse events (SAEs) till day 90 post-vaccination with respect to causality, severity and frequency.

Secondary outcomes – All solicited (local as well as systemic) and unsolicited AEs between the two treatment groups were recorded. Incidence, intensity and relationship to vaccination were analyzed for each AE as per the study protocol. All AEs were displayed in tabular format, with line listings of individual adverse events. Separate analysis was done for solicited and unsolicited events, local and systemic solicited events and all AEs. Assessment of AEs and SAEs occurring up to 180 days post vaccination, were analyzed with respect to causality, severity and frequency. Assessment was done to detect viremia (for each of the four vaccine virus) on Days 9 and 12 post vaccination.

Statistical analysis

There was no formal sample size calculation done. However, a sample size of 100 adult subjects was taken which was expected to give reliable estimate for safety and immunogenicity of TDV. The analysis of all safety and demographic outcomes was done by intention to treat. The primary immunogenicity outcome was assessed both by modified intention to treat, which included all subjects who received one dose of vaccine or placebo and have at least one post baseline immunogenicity assessment, as well as per protocol (PP) set which included all subjects who completed all study visits with valid sample for immunogenicity assessments and did not qualify for any major protocol deviation.

Statistical analysis was done in R/SAS® 9.4 software by an independent biostatistician. All continuous variables were summarized by number of observation, mean, standard deviation, median, minimum and maximum. Categorical variables were presented as frequencies and percentages. All p-values reported was based on two-sided tests, and p-values < 0.05 has been considered as statistically significant.

A two sample *t*-test as well as analysis of covariance model adjusted with baseline values was applied to compare the investigational vaccine and placebo arm for mean peak neutralizing antibody titers ($PRNT_{50}$) GMTs. chi-square/fishers exact test was used for comparing seropositivity and seroconversion rate as well as multivalent response. Data and Safety Monitoring Board (DSMB) reviewed the 180 days post-vaccination safety data and immunogenicity data till day 90.

Results

In this study 132 healthy adult volunteers were screened, of whom 100 eligible participants were enrolled in the study as per the inclusion criteria between 31 Oct 2018 and 05 March 2019. The participants were allocated to the TDV and placebo group in 3:1 ratio, i.e. 75 (75%) participants to TDV group and 25 (25%) participants to placebo group. During course of the study 8 participants (5 in TVD group & 3 in placebo group) withdrew from the study. No participant withdrew from the study due to adverse event. 92 participants (70 in TDV group & 22 in placebo group) who completed all scheduled visits were considered for immunogenicity analysis whereas safety was analyzed for 100 participants who were administered the study vaccine.

Demographic characteristics of participants are presented in Table 1.

Immunogenicity

For the study subjects, Geometric Mean Titers (GMTs) of neutralizing antibodies increased against the all four dengue serotypes following vaccination with TDV (**Supplementary Table S2**). Type specific mean peak neutralizing antibody response was elicited by single dose of TDV (Table 2). The neutralizing antibody peak response was obtained at Day 90 for DENV-1 & Day 28 for DENV-2, DENV-3 and DENV-4 (**Supplementary Figure S1-S4**). All p values comparing mean peak neutralizing titer from vaccine to placebo was highly significant.

The study showed that all the subjects remained seropositive to each DENV serotype, throughout the study (till 180 days post-vaccination). The seroconversion rate obtained in TDV group for DENV-1 as 59(81.9%), for DENV-2 as 56(77.8%), for DENV-3 as 59 (81.9%) and for DENV-4 as 57(79.2%) whereas in placebo group seroconversion rates obtained for DENV-1 as 8(33.3%), for DENV-2 as 3(12.5%), for DENV-3 as 6(25.0%); and for DENV-4 as 5 (20.8%). The higher seroconversion rate in TDV group as compared to placebo group was statistically significant ($p < 0.0001$) for all four dengue serotypes. Maximum seroconversion was noted in DENV-1 and DENV-3 followed by DENV-4 and then DENV-2. (Table 3). In terms of valency of DENV response, ~45% of the subjects achieved tetravalent antibody response and ~ 37% of the subject achieved trivalent antibody response. Cumulatively ~ 81% of the subjects achieved trivalent response or more (**Supplementary Table S1**)

Safety Assessments:

During the follow up visits no clinically significant change in vital signs were recorded and there was no clinically significant effect on hematological and biochemical evaluation of the analyzed subject's blood samples during screening, day 9 and day 28. The number and percentage of participants who reported any AE (solicited/unsolicited, local/systemic) during the trial were 51(68.0%) and 16(64.0%) in TDV and placebo groups respectively. 27(36.0%) participants in TDV group and 11(44.0%) participants in placebo group reported solicited local AEs and 39(52.0%) participants in TDV group and 12(48.0%) participants in placebo group reported solicited systemic AEs. The proportion of participants with solicited AEs overall and by severity are presented in Fig. 2. The most common local solicited AE was pain followed by tenderness in both the TDV and placebo groups. The most common systemic solicited AE reported in the TDV group was headache followed by fever and myalgia, whereas in the placebo group it was fever followed by myalgia. There was no statistically significant difference between

Table 1
Demographic characteristics of study participants (n = 100).

Parameter	Statistics	Vaccine (N = 75)	Placebo (N = 25)	Overall (N = 100)
Gender	Male	48 (64.0%)	13 (52.0%)	61 (61.0 %)
	Female	27 (36.0%)	12 (48.0%)	39 (39.0%)
Age at the time of vaccination(in years)	N	75	25	100
	Mean	34.61	29.80	33.41
	SD	9.72	7.22	9.36
	Median	34.0	29.0	31.0
	Minimum	18.0	20.0	18.0
	Maximum	57.0	53.0	57.0
Weight(kg)	N	75	25	100
	Mean	64.15	58.79	62.81
	SD	11.24	7.44	10.65
	Median	65.0	58.3	62.5
	Minimum	35.0	43.0	35.0
	Maximum	90.0	70.0	90.0

Note: Respective header count was used as denominator for percentage calculation.

Table 2
Mean peak neutralizing antibody response to each of DENV serotypes post vaccination.

Modified intention to treat				
Serotype	**Vaccine (N = 72)**	**Placebo (N = 24)**	**p value**	**Overall (N = 96)**
DENV-1	1001.42 (29.60:5097.70)	442.05 (29.70:3919.20)	0.0037	816.26 (29.60:5097.70)
DENV-2	1630.54 (303.70:5116.50)	934.72 (96.50:4277.80)	0.0004	1418.79 (96.50:5116.50)
DENV-3	645.26 (48.80:4961.70)	206.65 (24.60:1163.10)	0.0017	485.41 (24.60:4961.70)
DENV-4	562.39 (63.70:4828.10)	265.41 (17.60:4236.20)	0.0023	466.13 (17.60:4828.10)
Per protocol				
Serotype	**Vaccine (N=70)**	**Placebo (N=22)**	**p value**	**Overall (N= 92)**
DENV-1	1019.51 (29.60:5097.70)	446.22 (29.70:3919.20)	0.0073	836.72 (29.60:5097.70)
DENV-2	1624.83 (303.70:5116.50)	928.84 (96.50:4277.80)	0.0009	1421.45 (96.50:5116.50)
DENV-3	650.46 (77.10:4961.70)	183.07 (24.60:1163.10)	0.0030	480.35 (24.60:4961.70)
DENV-4	546.96 (63.70:4828.10)	235.54 (17.60:4236.20)	0.0059	447.16 (17.60:4828.10)

p-value from the Regression model adjusted with baseline values as covariate.

Table 3
Seroconversion rate of four DENV serotypes in vaccine and placebo group.

Modified intention to treat			
Serotype	**Vaccine (N = 72)** n (%)	**Placebo (N = 24)** n (%)	**p value**
DENV-1	59 (81.9%)	8 (33.3%)	<0.0001
DENV-2	56 (77.8%)	3 (12.5%)	<0.0001
DENV-3	59 (81.9%)	6 (25.0%)	<0.0001
DENV-4	57 (79.2%)	5 (20.8%)	<0.0001
Per protocol			
Serotype	**Vaccine (N = 70)**	**Placebo (N = 22)**	**p value**
DENV-1	58 (82.9%)	8 (36.4%)	<0.0001
DENV-2	55 (78.6%)	3 (13.6%)	<0.0001
DENV-3	57 (81.4%)	6 (27.3%)	<0.0001
DENV-4	55 (78.6%)	5 (22.7%)	<0.0001

Percentages were calculated using respective header count as denominator. p-value was calculated using Chi-Square/Fisher Exact test.

TDV and placebo groups in terms of AEs ($p < 0.05$). All unsolicited AEs resolved without sequelae except AEs related to eosinophilia (5 events) and hypertension (2 events). No participant was withdrawn from the study due to AE/SAE (Table 4).

Discussion

In the present phase I/II trial, a single dose of live attenuated Tetravalent Dengue Vaccine of Panacea Biotec Ltd., was shown to be safe and well tolerated in healthy adults. The vaccine induced robust, balanced neutralizing antibody responses against the four dengue virus serotypes. Other live attenuated recombinant tetravalent dengue vaccine in development include Takeda vaccine TAK-003, which is a live virus vaccine utilizing chimerization with DENV-2 PDK-53 as the backbone. The DENV-2/-1, -2/-3, and -2/-4 chimeras are created by replacing the DENV-2 prM and E genes

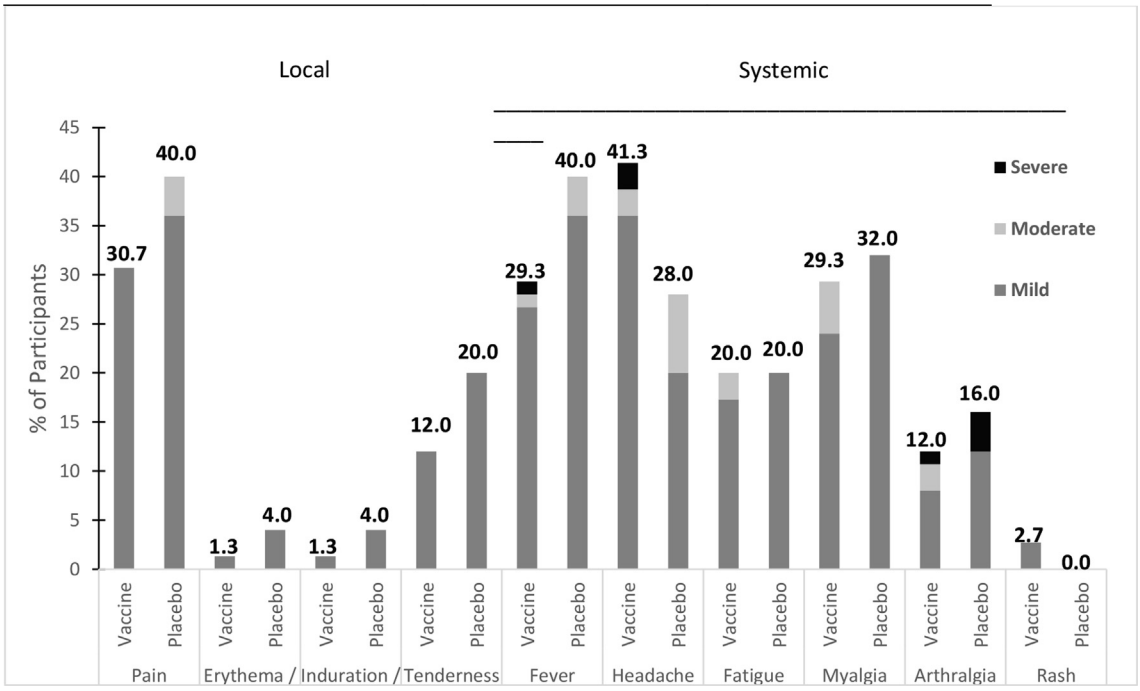

Fig. 2. Proportion of participants with Solicited Adverse Events after vaccination with TDV or Placebo by Severity. Solicited adverse events collected postvaccination [Days 1–5 for local events and Days 1–21 for systemic events] are shown with severity grades [(TDV (N = 75), Placebo (N = 25)]. The height of the stacked bar represents the total percentage of participants reporting the adverse event. The severity grades (mild, moderate, severe) within the bar indicate the proportion of total attributed to each respective category.

Table 4
Adverse events (*AEs) reported during the trial, based on ITT.

Category	Vaccine Group (N = 75) Participants, n (%)	Placebo Group(N = 25) Participants, n (%)
Total AEs reported	51 (68.0%)	16 (64.0%)
Solicited AEs	43 (57.3%)	15 (60.0%)
Unsolicited AEs	27 (36.0%)	6 (24.0%)
Solicited local AEs	27 (36.0%)	11 (44.0%)
Pain	23 (30.7%)	10 (40.0%)
Erythema / Redness	1 (1.3%)	1 (4.0%)
Induration / Swelling	1 (1.3%)	1 (4.0%)
Tenderness	9 (12.0%)	5 (20.0%)
Solicited systemic AEs	39 (52.0%)	12 (48.0%)
Fever	22 (29.3%)	10 (40.0%)
Headache	31 (41.3%)	7 (28.0%)
Fatigue	15 (20.0%)	5 (20.0%)
Myalgia	22 (29.3%)	8 (32.0%)
Arthralgia	9 (12.0%)	4 (16.0%)
Rash	2 (2.7%)	0 (0.0%)
AEs leading to trial discontinuation	0 (0.0%)	0 (0.0%)
Any SAE Reported	0 (0.0%)	0 (0.0%)
Deaths	0 (0.0%)	0 (0.0%)

with the respective genes from the other DENV serotypes, the Butantan-DV and NIH vaccines which contain DENV-1, -3, and -4 backbones and a DENV-2/-4 chimera which has been made using the DENV-4 backbone with DENV-2 prM and E replacing the DENV-4 prM and E protein [26].

In this study, the most common solicited local AE was pain and most common solicited systemic AE was headache and fever. In fact, none of the adverse events recorded in the TDV group was found to be statistically significant compared to placebo. Also no SAEs were reported during the follow up period. There was no loss to follow up, reported due to AE or SAE.

In a recent published study with an analogous vaccine namely Butantan-DV (Step-A study), rash was the most frequent adverse event recorded. Similarly, in NIH study at USA [21] rash was the only AE which occurred significantly more in vaccine group as compared to placebo. However, in the current Phase I/II study, none of the adverse events was found to be statistically significant compared to placebo.

In the current study, vaccine viremia was not detected in any participant, presumably due to the binding of pre-existing dengue antibody to the circulating virus in the dengue exposed participants. In the Butantan-DV study low viremia was demonstrated for DENV1, DENV2 and DENV3 and negligible for DENV4 [18] (p. 9). However in the NIH study, three-quarters of flavivirus naive subjects experienced viremia following the first vaccination with TV003. Subjects who were flavivirus-exposed prior to vaccination with TV003, 76% of subjects became viremic. [20] (p.12).

Further in terms of immunogenicity, the study vaccine demonstrated balanced, robust neutralizing antibody response to all dengue serotypes after a single dose. An increase in neutralizing antibody GMT response was observed across all four serotypes in the TDV group similar to that observed in Butantan and NIH vaccine groups in Butantan-DV study [18]. In terms of seroconversion, the seroconversion rate varied between 77% and 82% across all four serotypes in TDV group which was significantly higher than placebo. Overall cumulatively $\sim$ 95% of the participants achieved multivalent antibody response in the present study. This response was similar to that of Butantan-DV vaccine group and NIH vaccine group in Butantan-DV vaccine studies [18].

The present study demonstrates safety, tolerability and robust immunogenicity of the single dose of Panacea Biotec's Dengue Tetravalent Vaccine. Clinical trial sites that participated in the study were amongst the dengue endemic regions in India. Further it is important to note that in spite of higher pre-vaccination antibody titers (neutralizing antibody titers were more than 1:10 prior to vaccination) in the dengue exposed population, a significantly high number of participants were able to achieve seroconversion (>4 fold rise).

The limitations of the current study was that adequate data could not be generated for dengue naïve subjects and T cell immune response could not be assessed in the study participants. Panacea Biotec's TDV vaccine is similar to NIH TV003. NIH has done extensive clinical studies on TV003 including assessment of CD4 positive and CD8 positive T cell immune response [25,30]. Our vaccine has undergone Phase I/II trial to look up mainly safety and preliminary immunogenicity in terms of neutralizing antibody titers. We further, plan to incorporate dengue naïve subjects and assessment of T cell immune response in subsequent Phase III studies.

Conclusion

To the best of our knowledge, this is the first study that has investigated the safety and immunogenicity of Panacea Biotec's Tetravalent Dengue Vaccine (TDV) in Indian subjects. The study demonstrated that the single dose of this vaccine was safe, well tolerated and induced robust immune response in study participants. The vaccine promises to be a suitable candidate requiring evaluation in phase III clinical trial.

Author Contributions

The study was conducted by Panacea Biotec Ltd. LM was the Principal Investigator who designed the study and supervised the clinical development of TDV. SB was the Medical Monitor for the study. MKJ was the study monitor. MKJ and SB coordinated the monitoring activities of the trial. AB was responsible for medical writing who contributed to the literature search, writing and revision of the final version of the manuscript. AG was responsible for Audit & Quality Assurance for the study. GG supervised the Research and Development of TDV. MP and AJP were the Site Investigators for the study. RK was the Co-Investigator for the study & also supervised the study conduct at the site, in addition to editing the manuscript. AKM, RPK & NRA were the Co-Investigators for the study. LM, MP, AKM, RK, MKJ, AB, AG and SB have reviewed the results and verified the clinical trial data for the study. All authors reviewed and approved the final version of the manuscript.

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Informed Consent Statement

The work presented in this study has been carried out in an ethical way. Informed consent was obtained from all the study subjects who took part in the trial.

Data Availability Statement

The data that support the findings of this study are available from the authors on reasonable request pending approval from all relevant institutions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvacx.2022.100142.

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