Safety and immunogenicity of the tetravalent, live-attenuated dengue vaccine Butantan-DV in adults in Brazil: a two-step, double-blind, randomised placebo-controlled phase 2 trial

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Summary

Background The Butantan Institute has manufactured a lyophilised tetravalent live-attenuated dengue vaccine Butantan-DV, which is analogous to the US National Institutes of Health (NIH) TV003 admixture. We aimed to assess the safety and immunogenicity of Butantan-DV.

Methods We did a two-step, double-blind, randomised placebo-controlled phase 2 trial at two clinical sites in São Paulo, Brazil. We recruited healthy volunteers aged 18–59 years; pregnant women, individuals with a history of neurological, heart, lung, liver or kidney disease, diabetes, cancer, or autoimmune diseases, and individuals with HIV or hepatitis C were excluded. Step A was designed as a small bridge-study between Butantan-DV and TV003 in DENV-naive participants. In step A, we planned to randomly assign 50 dengue virus (DENV)-naive individuals to receive two doses of Butantan-DV, TV003, or placebo, given 6 months apart. In step B, we planned to randomly assign 250 participants (DENV-naive and DENV-exposed) to receive one dose of Butantan-DV or placebo. Participants were randomly assigned, by computer-generated block randomisation (block sizes of five); participants in step A were randomly assigned (2:2:1) to receive Butantan-DV, TV003, or placebo and participants in step B were randomly assigned (4:1) to receive Butantan-DV or placebo. Participants and study staff were unaware of treatment allocation. The primary safety outcome was the frequency of solicited and unsolicited local and systemic adverse reactions within 21 days of the first vaccination, analysed by intention to treat. The primary immunogenicity outcome was seroconversion rates of the DENV-1-4 serotypes measured 91 days after the first vaccination, analysed in the per-protocol population, which included all participants in step A, and all participants included in step B who completed all study visits with serology sample collection. This trial is registered with ClinicalTrials.gov, NCT01696422.

Findings Between Nov 5, 2013, and Sept 21, 2015, 300 individuals were enrolled and randomly assigned: 155 (52%) DENV-naive participants and 145 (48%) DENV-exposed participants. Of the 155 DENV-naive participants, 97 (63%) received Butantan-DV, 17 (11%) received TV003, and 41 (27%) received placebo. Of the 145 DENV-exposed participants, 113 (78%) received Butantan-DV, three (2%) received TV003, and 29 (20%) received placebo. Butantan-DV and TV003 were both immunogenic, well-tolerated, and no serious adverse reactions were observed. In step A, rash was the most frequent adverse event (16 [845] of 19 participants in the Butantan-DV group and 13 [76%] of 17 participants in the TV003 group). Viraemia was similar between the Butantan-DV and TV003 groups. Of the 85 DENV-naive participants in the Butantan-DV group who attended all visits for sample collection for seroconversion analysis and thus were included in the per-protocol analysis population, 74 (87%) achieved seroconversion to DENV-1, 78 (92%) to DENV-2, 65 (76%) to DENV-3 , and 76 (89%) to DENV-4. Of the 101 DENV-exposed participants in the Butantan-DV group who attended all visits for sample collection for seroconversion analysis, 82 (81%) achieved seroconversion to DENV-1, 79 (78%) to DENV-2, 83 (82%) to DENV-3, and 78 (77%) to DENV-4.

Interpretation Butantan-DV and TV003 were safe and induced robust, balanced neutralising antibody responses against the four DENV serotypes. Efficacy evaluation of the Butantan-DV vaccine is ongoing.

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See **Comment** page 761

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Research in context

Evidence before the study

At present, dengue virus (DENV) is endemic in more than 100 countries. The circulation of multiple serotypes is common in most affected countries. Around 2·5 billion people (two-fifths of the world population) are estimated to be at risk of contracting dengue virus and 50 million infections are estimated to occur worldwide each year. Thus, WHO defined the development of a tetravalent vaccine against infection with dengue virus as a priority. We searched PubMed and the WHO website for articles published between March 1, 2008, and March 3, 2013, using the search terms "dengue vaccine", "dengue", "dengue epidemiology", "vaccine development", and "antibody-enhancement", without language restrictions. Our search yielded 81 articles.

Several studies with live-attenuated dengue vaccines have been done in the past 60 years; however, only one vaccine (CYD-TDV) has gained licensure from regulatory agencies. A previous review has shown that age and previous exposure status of vaccine recipients to dengue virus had a significant effect on the safety and efficacy of CYD-TDV. Such differences led to the restriction of CYD-TDV use to individuals aged older than 9 years with previous exposure to DENV. Individuals without previous exposure to dengue became more susceptible to severe dengue after CYD-TDV vaccination. Subsequently, assessment of safety and immunogenicity stratified by previous exposure has become a key component of the analyses of other live dengue vaccine candidates.

In the past 12 years, the Butantan Institute, the US National Institute of Allergy and Infectious Diseases and National Institutes of Health (NIH) have collaborated to develop and manufacture live-attenuated vaccines, including the live-attenuated tetravalent dengue vaccine. Initially, attenuated dengue vaccine strains were developed by NIH scientists and were extensively evaluated in preclinical studies and phase 1 clinical trials in the USA as monovalent and tetravalent formulations. TV003 is one of the tetravalent formulations selected by Butantan Institute for further development and manufacture as a lyophilised tetravalent dengue vaccine (Butantan-DV).

Added value of this study

The present study bridges the gap between TV003 clinical evaluations done in the USA and the clinical evaluation of the Butantan-DV done in Brazil by demonstrating similarities in safety and immunogenicity. Additionally, Butantan-DV was shown to be safe and able to induce robust balanced neutralising antibody responses against the four serotypes of dengue virus (DENV-1–4) in both naive and pre-exposed volunteers after a single dose. Data also suggested an association between rash and tetravalent antibody response. No significant differences in safety or immunogenicity outcomes were observed between DENV-naive and DENV-exposed participants. Furthermore, elicitation of CD8-positive T-cell responses to non-structural dengue proteins was also demonstrated in the Butantan-DV recipients. Furthermore, we hypothesise that the Butantan-DV one-dose regimen would be easier to incorporate into expanded immunisation programmes worldwide than existing vaccines that require multiple doses.

Implications of all the available evidence

The results of this study have contributed to the advancement of the Butantan-DV to a phase 3 evaluation trial; if the safety data observed in this study and efficacy are confirmed and demonstrated in the ongoing phase 3 clinical trial, a single dose of the live-attenuated tetravalent dengue vaccine should be available in the near future for the prevention of dengue.

Introduction

The four serotypes of dengue virus (DENV-1–4) are the major causes of mosquito-borne viral disease and impose a substantial burden on global public health. At present, the largest burden of DENV is in southeast Asia and central and South America.[1,2]

Any DENV serotype can cause the full spectrum of clinical disease from asymptomatic or subclinical infection to a life-threatening syndrome. Although severe disease can be observed with primary DENV infection, most cases are observed with secondary, heterotypic DENV infection[3] and antibody-dependent enhancement is considered important in the pathophysiology.[4,5] Therefore, the ideal dengue vaccine should provide protection via the stimulation of a balanced immune response against all serotypes.

Among the various platforms used to manufacture dengue vaccines, live-attenuated vaccines are the most promising since they have been used to protect against other flavivirus diseases (ie, yellow fever and Japanese encephalitis), have a low production cost,[6] generally require only a single dose for protection, and induce long-lasting humoral and cellular immune responses.

At present, CYD-TDV is the only vaccine that has been licensed for dengue prophylaxis, but this vaccine requires a three-dose schedule given over 1 year. CYD-TDV (Dengvaxia; Pasteur, Lyon, France) is a live chimeric dengue vaccine that uses the 17D-yellow fever vaccine virus as its genetic backbone. However, due to mid-term safety concerns, CYD-TDV is only recommended for use in individuals aged 9–45 years with previous exposure to DENV.[7–13]

The Butantan Institute (São Paulo, Brazil) has licensed the four live-attenuated DENV components (rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30) from the Laboratory of Infectious Diseases (LID) at The National Institutes of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH; Bethesda, MD, USA),

for the development and manufacturing of a new lyophilised tetravalent live-attenuated dengue vaccine (Butantan-DV). The resulting Butantan-DV lyophilised vaccine and TV003 admixture from the NIH are analogous tetravalent formulations, which contain the same amount of attenuated virus strains per dose ($10^3 \pm 0 \cdot 5$ plaque-forming units of rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30). However, Butantan-DV, is a lyophilised ten-dose vial vaccine,[14] whereas TV003 is a single dose frozen admixture.

The live-attenuated DENV components licensed from the NIAID (NIH) were extensively evaluated in dengue-naive volunteers in phase 1 clinical trials in the USA, as monovalent and tetravalent (TV003) formulations, which were found to be safe and immunogenic.[15–20] Therefore, the aim of this phase 2 clinical trial was to assess the safety and immunogenicity of the Butantan-DV in DENV-exposed and DENV-naive individuals in Brazil.

Methods

Study design and participants

We did a two-step, double-blind, randomised placebo-controlled trial at two clinical sites in São Paulo, Brazil. Step A was designed as a small bridge-study between Butantan-DV and TV003 in DENV-naive participants. In step A, we planned to randomly assign 50 DENV-naive participants to receive two doses of Butantan-DV (n=20), TV003 formulation (n=20), or placebo (n=10) given 6 months apart. The second dose was part of an exploratory investigation to assess the ability of a second dose to boost neutralising antibody titres to each of the four DENV serotypes. In step B, we planned to randomly assign 250 DENV-exposed and DENV-naive participants to receive one dose of either Butantan-DV (n=190) or placebo (n=60).

Screening of volunteers for inclusion included a medical history, physical examination, and laboratory testing (eg, serology, neutrophil count, platelet count, measurement of liver enzymes) and previous DENV exposure (appendix p 3).

Eligible individuals were healthy men or women aged 18–59 years who would be available for the entire study period (5 years). Pregnant women, individuals with a history of neurological, heart, lung, liver or kidney disease, diabetes, cancer, or autoimmune diseases, and individuals with HIV or hepatitis C were excluded. Individuals who had been vaccinated with a live virus vaccine within the previous 28 days or with an inactivated virus vaccine within the previous 14 days before inclusion or had any immunisation scheduled for the first 42 days after the inclusion in the study were also excluded. Full inclusion and exclusion criteria are included in the appendix (pp 3–5).

All patients provided written informed consent. This study was approved by the local ethics committee of the Medical School of the University of São Paulo, the Brazilian Research National Ethics Council, the Brazilian National Technical Biosafety Commission, and the Brazilian Health Regulatory Agency. The study was done in accordance with the Declaration of Helsinki, International Conference of Harmonization guidelines, and Good Clinical Practice guidelines.

Randomisation and masking

All participants were randomly assigned according to results within 4 weeks of the serological screening for infection with dengue virus. Participants were randomly assigned using a computer-generated randomisation schedules generated by the study statistician using SAS version 9.3, with permuted blocks of five. Randomisation was stratified by DENV exposure. The randomisation schedules were prepared in advance by the study statistician and then provided to the clinical site pharmacist. DENV-naive individuals were divided into two equal groups: participants in step A were randomly assigned (2:2:1) to receive Butantan-DV, TV003, or placebo and participants in step B were randomly assigned (4:1) to receive Butantan-DV or placebo. Therefore, the final allocation ratio among DENV-naive participants was 3:1:1 (Butantan-DV: TV003: placebo). DENV-exposed participants were randomly assigned (3:1) to receive Butantan-DV or placebo. Therefore, the overall planned allocation ratio among Butantan-DV and placebo was 3:1, regardless of previous DENV exposure. Participants and study staff were unaware of treatment allocation. TV003, Butantan-DV, and placebo preparations were identical in appearance.

Vaccines

The TV003 admixture is a live-attenuated vaccine (TV003), which contains all four dengue serotypes (DENV1–4) in a point-of-care formulated tetravalent admixture. Vaccine components were produced as seed viruses and propagated in Vero cells at the LID (NIAD, NIH), according to Current Good Manufacturing Practices.[21] Each monovalent vaccine was stored at –80°C (±15°C) and shipped to the Butantan Institute. Immediately before administration, the monovalent vaccines were thawed, diluted, and combined as an admixture with a final potency of 3 $\log_{10}$ plaque-forming units for each component per 0·5 mL dose.[22]

See Online for appendix

Butantan-DV is a live-attenuated lyophilised vaccine containing all four dengue serotypes (DENV-1–4). The Butantan Institute licensed the seed viruses (rDEN1Δ30, rDEN2/4Δ30(ME), rDEN3Δ30/31, and rDEN4Δ30) from the NIAID and established new master and working virus banks. The resulting viruses were propagated in Vero cell tissue culture. At the time of formulation, vaccine bulk stocks were thawed, diluted, combined, and lyophilised as ten-dose vials. After reconstitution, the final formulation had a potency of 3 $\log_{10}$ plaque-forming units for each component per 0·5 mL dose.

The placebo was also formulated and lyophilised as a ten-dose vial (0·5 mL per dose). A phosphate-citrate

solution was used as a diluent for Butantan-DV and the placebo. Butantan-DV, placebo, and the diluent were stored at 2–8°C at the clinical sites.

Procedures

The investigational products were administered by subcutaneous injection into the non-dominant arm. After immunisation, all participants remained under observation for 1 h. Medical and laboratory (safety and immunogenicity) assessments were done every 3 days until day 21, and on days 28, 91, and 180 after each injection thereafter. Participants were instructed to measure their axillary temperature daily for 21 days after immunisation and anytime they felt febrile. Women of childbearing potential had a urine human chorionic gonadotropin test immediately before and 3 weeks after immunisation to assure that pregnant women were not exposed to the vaccine.

Outcomes

The primary safety outcome was the frequency of local and systemic, solicited and unsolicited adverse reactions within 21 days of the first vaccination in step A or after the single vaccination in step B. The primary immunogenicity outcome was the seroconversion rate for each of the four virus serotypes measured 91 days after the first vaccination in step A or after the single vaccination in step B.

Secondary safety outcomes were vaccine safety (unsolicited adverse reactions between days 21 and 180 after the first vaccination in step A or single vaccination in step B, serious adverse reactions for the duration of the study), vaccine-induced viraemia for each of the four viruses, and suspected and confirmed dengue cases up to 5 years after first vaccination in step A or after the single vaccination in step B. Secondary immunogenicity outcomes were antibody response (difference in geometric mean titre of neutralising antibodies for each of the four vaccine viruses up to 5 years after first vaccination and proportion of monovalent, bivalent, trivalent, and tetravalent antibody responses). The serological response was characterised as a 50% or greater reduction in plaque counts ($PRNT_{50}$), measured at days 28, 56, and 91 and annually thereafter following vaccination. Exploratory outcomes were assessment of cell-mediated immune responses to vaccination; seroconversion rate for each of the four vaccine viruses according to previous exposure to other flaviviruses; solicited and unsolicited, local and systemic adverse reactions within 21 days of the second vaccination in step A; unsolicited adverse reactions between day 21 and 6 months after the second vaccination of step A; quantitative and functional patterns from cells and mediators of the immune response pre-vaccination and post-vaccination after the first and second vaccinations and once a year for 5 years for step A; viraemia for each of the four vaccine viruses measured on days 3, 6, 9, 12, 15, and 21 after the second vaccination in step A; difference in seroconversion rates for each of the four vaccine serotypes after the second vaccination in step A; quantitative and functional patterns of cells and mediators of the immune response pre-vaccination and post-vaccination after the first vaccination and annually for 5 years for the first 40 step B participants with previous exposure to dengue; the proportion of monovalent, bivalent, trivalent, and tetravalent responses at days 28, 56, and 91 after the second vaccination of step A.

We used a simplified two-dilution plaque reduction neutralisation test (PRNT) to identify 50 DENV-naive participants for allocation into step A. The simplified PRNT assay was done against all four DENV serotypes. For the remaining participants, assessment of DENV exposure was done using the Panbio Dengue IgG Indirect ELISA (Abbott, Seoul, South Korea).

We used full-dilution PRNT assays to define DENV exposure immediately before immunisation according to the PRNT protocol from the NIAID-NIH. Serum neutralising antibody response was measured by the $PRNT_{50}$ assay in accordance with other live-attenuated DENV vaccine evaluations.[23,24] For all participants, an initial serum dilution of 1/5 was used for $PRNT_{50}$ assays. Seropositivity was defined by $PRNT_{50}$ cutoff titres (≥1/10) before immunisation and at any timepoint up to 91 days after vaccination (day 28, 56, or 91). For DENV-naive participants, seroconversion was defined by $PRNT_{50}$ cutoff titres(≥1/10). For participants previously exposed to DENV, seroconversion was defined as a four-fold or higher increase in pre-existing neutralising antibody titre after immunisation.

Viraemia was assessed in all DENV-naive participants in step A on days 3, 6, 9, 12, 15, and 21 after the first dose and in the first 40 DENV-exposed participants from step B. Sera samples collected were incubated in Vero cell culture. The supernatant was assessed by real-time RT-PCR, as described by Johnson and colleagues.[25] To distinguish vaccine strains from wild-type dengue viruses, all positive samples were sequenced to identify the Δ30 deletion in the 3′-untranslated region of the vaccine viruses.

We assessed T-cell responses by intracellular staining for antigen-specific cytokines using flow cytometry using the following monoclonal antibodies: anti-CD8a V500, anti-CD3 Alexa Flour 700, and anti-interferon-γ (IFNγ) fluorescein isothiocyanate (BD Biosciences, San Diego, CA, USA). For intracellular cytokine staining, peripheral blood mononuclear cells were cultured in the presence of HLA-matched peptide pools (10 μg/mL) and GolgiPlug containing brefeldin A (BD Biosciences) for 6 h and subsequently permeabilised, stained, and analysed as previously described.[26] T-cell immune responses were considered positive for all individuals with IFNγ-production from CD8-positive T cells that was higher than the 95th percentiles observed among DENV-naive individuals before vaccination.

The definition of adverse reactions following immunisation, the severity classification of the adverse event

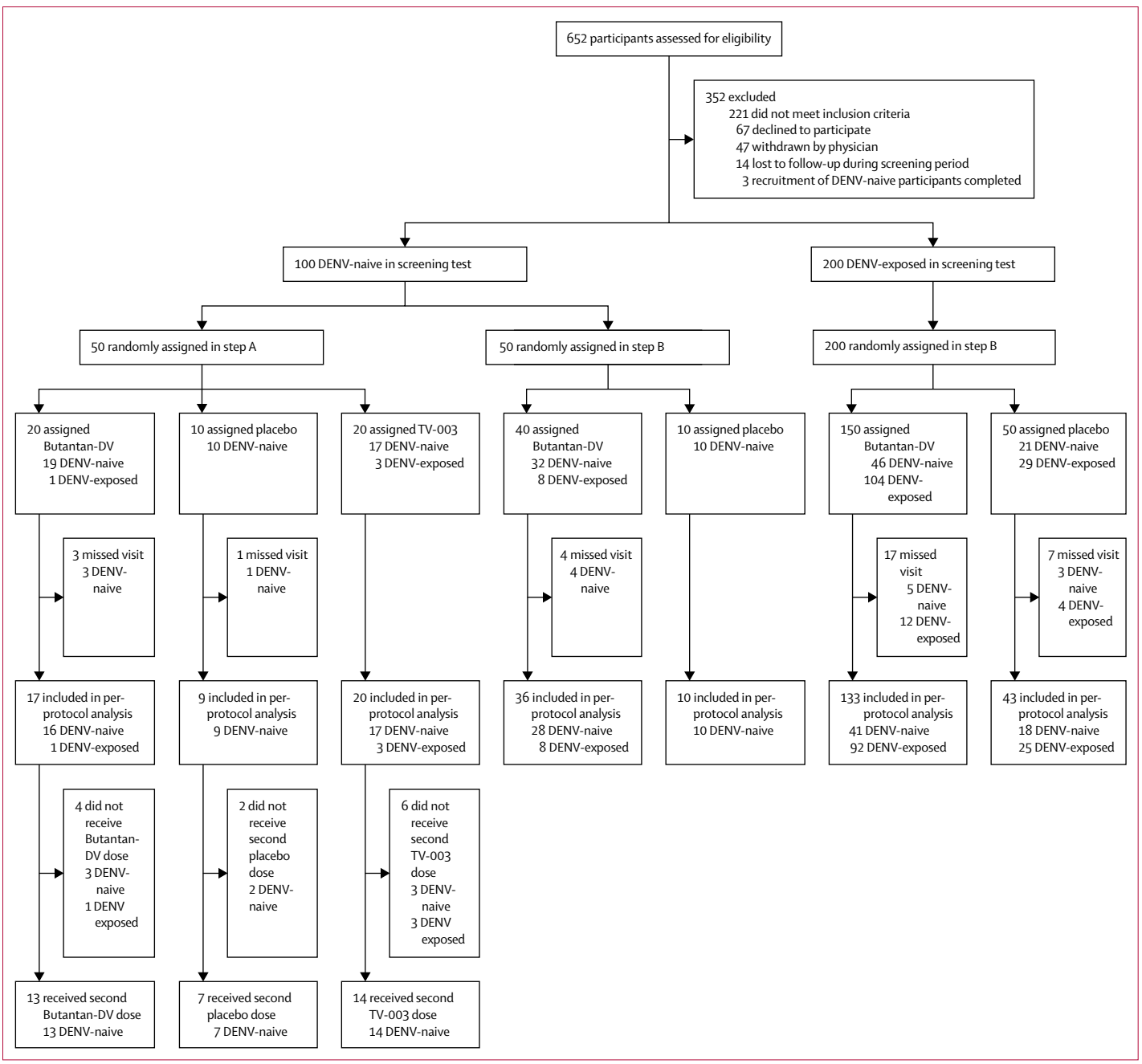

Figure: **Trial profile**
DENV=dengue virus.

(solicited clinical and laboratory; appendix p 6), and the causality relationship between intervention and adverse event were assessed according to international guidelines[27–30] (appendix p 9) and monitored by the data and safety monitoring board.

Statistical analysis

No formal sample size calculation was done. The analysis of all safety outcomes was done by intention to treat. The primary immunogenicity outcome was assessed by modified intention to treat, which included all patients who received one dose of vaccine or placebo, and per-protocol. Viraemia was assessed per-protocol, which included all participants included in step A (only DENV-naive participants) and the first 40 DENV-exposed participants in step B, which included all participants who completed all study visits with sample collection for viraemia. Seroconversion was assessed per-protocol in

	All participants			DENV-naive participants			DENV-exposed participants		
	Butantan-DV (n=210)	Placebo (n=70)	TV003 (n=20)	Butantan-DV (n=97)	Placebo (n=41)	TV003 (n=17)	Butantan-DV (n=113)	Placebo (n=29)	TV003 (n=3)
Sex									
Women	138 (66%)	50 (71%)	14 (70%)	67 (78%)	32 (78%)	13 (76%)	71 (63%)	18 (62%)	1 (33%)
Men	72 (34%)	20 (29%)	6 (30%)	9 (22%)	9 (22%)	4 (24%)	42 (37%)	11 (38%)	2 (67%)
Age (years)	41·3 (18·5–59·3)	40·6 (19·7–59·5)	40·3 (18·5–57·1)	38·3 (18·5–58·2)	40·1 (20·6–59·5)	38·0 (18·5–57·1)	41·7 (18·7–59·3)	41·7 (19·7–59·0)	42·6 (33·3–45·2)
Race									
White	139 (66%)	37 (53%)	13 (65%)	68 (70%)	20 (49%)	11 (65%)	71 (63%)	17 (59%)	2 (67%)
Black	19 (9%)	9 (13%)	1 (5%)	10 (10%)	8 (20%)	1 (6%)	9 (8%)	1 (3%)	0
Pardo	41 (20%)	18 (26%)	5 (25%)	16 (16%)	9 (22%)	4 (24%)	25 (22%)	9 (31%)	1 (33%)
Asian	10 (5%)	3 (4%)	1 (5%)	3 (3%)	3 (7%)	1 (6%)	7 (6%)	0	0
Indigenous	0	2 (3%)	0	0	1 (2%)	0	0	1 (3%)	0
Other	1 (<1%)	1 (1%)	0	0	0	0	1 (1%)	1 (3%)	0

Data are n (%), or median (range). DENV=dengue virus.

Table 1: **Demographic characteristics of all study participants (n=300)**

step A and for all participants included in step B, who completed all study visits with serology sample collection. Viraemia was also assessed by modified intention to treat for step A and the first 40 DENV-exposed participants included in step B, who completed one or more study visits following vaccination.

We used Fisher's exact and χ^2 tests to compare the frequency of adverse reactions, viraemia, seroconversion, multivalent immune response, and dengue serostatus before immunisation between the treatment groups. We used Mann-Whitney U and Kruskal-Wallis tests to compare mean peak virus titres and onset and duration of adverse reactions for each investigational product. All statistical analysis was done using Stata (version 13.0). $p<0·05$ was considered to indicate a statistically significant difference. An independent data safety monitoring board oversaw the study. This trial is registered with with ClinicalTrials.gov, NCT01696422.

Role of the funding source

The funders were involved in the study design, data collection, data analyses, data interpretation, and writing of the report. All authors had full access to all study data and had final responsibility for the decision to submit for publication.

Results

Between November 5, 2013, and September 21, 2015, 652 individuals were assessed for eligibility, of whom 300 were randomly assigned: 155 (52%) DENV-naive participants and 145 (48%) DENV-exposed participants (figure). Among the 155 DENV-naive participants, 97 (63%) received Butantan-DV, 17 (11%) received TV003, and 41 (27%) received placebo. Among the 145 DENV-exposed participants, 113 (78%) received Butantan-DV, three (2%) received TV003, and 29 (20%) received placebo. At screening, three participants who received TV003 were seronegative for DENV, but were later found to be seropositive according to the NIH-PRNT protocol. Overall, 210 (70%) participants received Butantan-DV, 20 (7%) received TV003, and 70 (23%) received placebo. Demographic characteristics of participants are presented in table 1. The follow-up period is 5 years for all participants and will be completed in September, 2020. Here, we present the results related to the period of assessment of the main study outcomes.

Solicited and unsolicited adverse reactions following one dose of Butantan-DV, TV003, or placebo in DENV-naive participants in step A are shown in the appendix (p 11). No significant differences in the frequency of solicited laboratory adverse reactions, solicited local adverse reactions, and unsolicited adverse reactions, apart from rash, were identified between the Butantan-DV, TV003, and placebo groups. No significant difference in the frequency of rash was observed between the Butantan-DV and TV003 groups (16 [84%] of 19 participants *vs* 13 [76%] of 17 participants; p=0·684).

No significant differences were identified in the onset, duration, or intensity of rash between Butantan-DV and TV003 groups (appendix p 14). 14 (88%) of 16 participants in the Butantan-DV group and 12 (92%) of 13 participants in the TV003 group had grade 1 rash (appendix p 6). All adverse reactions were self-limiting, and no medical treatment was required.

Overall, in step A per-protocol analysis, viraemia was assessed in 18 (95%) of 19 participants in the Butantan-DV group and 15 (88%) of 17 participants in the TV003 group (table 2). No significant differences were identified in the proportion of participants with detectable vaccine viraemia, the number of serotypes detected in individual vaccine recipients, or the frequency of a particular serotype between the Butantan-DV and TV003 groups. The DENV-4 vaccine serotype was not identified in any Butantan-DV recipients.

Butantan-DV and TV003 elicited neutralising antibodies to each of the DENV serotypes (table 3). The geometric mean peak titres were not significantly different between the Butantan-DV and TV003 groups for DENV-2 (p=0·600), DENV-3 (p=0·072), or DENV-4 (p=0·275); however, for DENV-1, the mean peak titre was significantly higher in the Butantan-DV group than the TV003 group (p=0·022).

No significant differences were identified in the frequency of seroconversion for each of the dengue serotypes between the Butantan-DV and TV003 groups (all p>0·05; table 4). The frequency of seroconversion varied from 81% (13 of 16 participants) to 94% (15 of 16 participants) in the Butantan-DV group and from 82% (14 of participants 17) to 94% (16 of 17 participants) in the TV003 group. In the Butantan-DV group, the seroconversion frequency was highest for DENV-1 and DENV-2 (15 [94%] of 16 participants for both serotypes). In the TV003 group, the seroconversion frequency was highest for DENV-1 (16 [94%] of 17 participants) and DENV-4 (15 [88%] of 17 participants).

Overall, 12 (75%) of 16 participants in the Butantan-DV group and 13 (76%) of 17 participants in the TV003 group had a tetravalent neutralising humoral response after vaccination with a single dose of vaccine (appendix p 13). Additionally, three (19%) of 16 participants in the Butantan-DV group and two (12%) of 17 participants in the TV003 group had a trivalent immune response. Differences in the proportion of participants who achieved tetravalent and trivalent immune responses between the Butantan-DV and TV003 groups were not significant (p>0·999 for tetravalent immune responses; p=0·656 for trivalent responses). Ten (83%) of 12 participants in the Butantan-DV group and 11 (85%) of 13 participants in the TV003 group who achieved a tetravalent neutralising humoral response reported rash (appendix p 16).

After the second dose of vaccine in step A, one participant in the Butantan-DV group seroconverted to the four DENV serotypes and presented with DENV-3 viraemia only (appendix p 15). One participant in the TV003 group seroconverted to DENV-1 and DENV-4 serotypes and presented with viraemia for DENV-1, DENV-3, and DENV-4. Only one Butantan-DV recipient had a four-fold or higher increase in the DENV-1 neutralising antibody titre (data not shown). No increases in the antibody geometric mean titre to any dengue serotype were observed in either of these Butantan-DV and TV003 recipients after the second vaccine dose (appendix p 16).

Here, we present the combined safety and immunogenicity data after one dose of Butantan-DV from steps A and B (ie, for both DENV-naive and DENV-exposed participants), referred to as the previous exposure analysis hereafter.

The local and systemic, solicited and unsolicited, and laboratory adverse reactions following one dose of Butantan-DV are shown in table 5. Rash was more frequent in the DENV-naive (p<0·001) and DENV-exposed (p<0·001) participants in the Butantan-DV group than among placebo group participants. Furthermore, among Butantan-DV recipients, rash was also more frequent in DENV-naive participants (63 [65%] of 97) than DENV-exposed participants (51 [45%] of 113; p=0·005; table 5). However, no significant differences in day of onset, duration, or intensity of rash were identified between DENV-naive and DENV-exposed participants in the Butantan-DV group (appendix p 12). 56 (89%) of 63 DENV-naive participants and 40 (78%) of 51 DENV-exposed Butantan-DV recipients had grade 1 rash (appendix p 12). Rash episodes had a maculopapular character and were self-limiting.

Compared with participants in the placebo group, leukopenia was significantly more frequent in DENV-naive participants (p=0·001) and DENV-exposed participants (p=0·014) in the Butantan-DV group. Neutropenia was significantly more frequent in DENV-naive participants in the Butantan-DV group than participants in the placebo group (p=0·015; table 5). Arthralgia was significantly more frequent in DENV-exposed participants in the Butantan-DV group than participants in the placebo group (p=0·006; table 5). Most adverse reactions were classified as grade 1 or 2 with the exception of one case of

	Step A			Previous exposure analysis		
	Butantan-DV	TV003	p value†	DENV-naive	DENV-exposed	p value†
Per protocol‡						
Participants assessed	18	15	..	18	25	..
Viruses detected in a single participant						
0	6 (33%)	4 (27%)	0·722§	6 (33%)	15 (60%)	0·124§
1	10 (56%)	10 (67%)	..	10 (56%)	8 (32%)	..
2	2 (11%)	1 (7%)	..	2 (11%)	2 (8%)	..
Identified serotypes						
DENV-1	5 (28%)	2 (13%)	0·413	5 (28%)	7 (28%)	>0·999
DENV-2	2 (11%)	0	0·489	2 (11%)	4 (16%)	>0·999
DENV-3	7 (39%)	8 (53%)	0·494	7 (39%)	1 (4%)	0·006
DENV-4	0	2 (13%)	0·199	0	0	..
Modified intention to treat						
Participants assessed	19	17	..	19¶	29¶	..
Viruses detected in a single participant, n						
0	6 (32%)	4 (24%)	0·717§	6 (32%)	18 (62%)	0·075§
1	11 (58%)	11 (65%)	..	11 (58%)	9 (31%)	..
2	2 (11%)	2 (12%)	..	2 (11%)	2 (7%)	..
Identified serotypes						
DENV-1	5 (26%)	2 (12%)	0·408	5 (26%)	8 (28%)	>0·999
DENV-2	3 (16%)	1 (6%)	0·605	3 (16%)	4 (14%)	>0·999
DENV-3	7 (37%)	9 (53%)	0·503	7 (37%)	1 (3%)	0·004
DENV-4	0	3 (18%)	0·095	0	0	..

Data are n or n (%). *Viraemia was not detected in placebo recipients. †Fisher's exact test. ‡Participants who did not attend all study visits for sample collection for viremia were excluded from the per-protocol analysis. §None versus at least one virus. ¶19 participants were included from step A; 29 of the first 40 exposed participants in step B completed one or more study visits following vaccination and therefore were included in the modified intention-to-treat analysis.

Table 2: **Viraemia* after the first dose of immunisation in step A and in all Butantan-DV recipients stratified by previous exposure**

	Step A					Previous exposure analysis				
	Butantan-DV		TV003		p value*	DENV-naive		DENV-exposed		p value*
	n	GMT (95% CI)	n	GMT (95% CI)		n	GMT (95% CI)	n	GMT (95% CI)	
Per protocol†	16	..	17	..	..	85	..	101	..	..
DENV-1	15	412·86 (182·80–932·45)	16	114·54 (53·31–246·08)	0·022	74	280·76 (184·31–427·67)	97	841·76 (599·42–1182·09)	<0·0001
DENV-2	15	297·01 (99·98–882·32)	14	445·39 (135·78–1461·02)	0·600	78	167·52 (115·84–242·24)	100	732·34 (539·04–994·96)	<0·0001
DENV-3	13	404·20 (115·74–1411·57)	14	93·96 (24·99–353·33)	0·072	65	114·58 (71·91–182·57)	95	299·84 (214·20–419·73)	<0·0001
DENV-4	14	154·29 (78·88–301·77)	15	267·38 (143·74–497·38)	0·275	76	151·09 (104·56–218·31)	85	238·04 (169·00–335·27)	0·077
Modified intention to treat	19	..	17	..	..	97	..	113	..	..
DENV-1	16	439·34 (203·65–947·78)	16	114·54 (53·31–246·08)	0·016	81	281·10 (187·94–420·43)	104	833·01 (595·91–1164·46)	<0·0001
DENV-2	16	314·61 (113·50–872·09)	14	445·39 (135·78–1461·02)	0·677	87	167·45 (118·48–236·67)	108	720·21 (535·73–968·22)	<0·0001
DENV-3	14	451·01 (139·68–1456·25)	14	93·96 (24·99–353·33)	0·053	72	113·45 (73·55–174·99)	104	305·59 (222·15–420·37)	<0·0001
DENV-4	15	194·86 (87·82–432·37)	15	267·38 (143·74–497·38)	0·455	83	164·00 (114·35–235·22)	93	224·49 (162·26–310·59)	0·188

GMT was calculated using $PRNT_{50}$. GMT=geometric mean titre. *Mann-Whitney test. †Participants who did not attend attend all study visit with sample collection for GMT were excluded from the per-protocol analysis.

Table 3: **GMTs of the four dengue serotypes after the first dose of immunisation in step A and in all Butantan-DV recipients stratified by previous exposure analysis**

	Step A				Previous exposure analysis			
	Butantan-DV	TV003	Placebo	p value*	DENV-naive	DENV-exposed	Placebo	p value*
Per protocol†	16	17	9	..	85	101	62	..
DENV-1	15 (94%)	16 (94%)	2 (22%)	<0·0001‡	74 (87%)	82 (81%)	7 (11%)	<0·0001§
DENV-2	15 (94%)	14 (82%)	0	<0·0001¶	78 (92%)	79 (78%)	10 (16%)	<0·0001‖
DENV-3	13 (81%)	14 (82%)	0	<0·0001**	65 (76%)	83 (82%)	7 (11%)	<0·0001††
DENV-4	14 (88%)	15 (88%)	0	<0·0001‡‡	76 (89%)	78 (77%)	5 (8%)	<0·0001§§
Modified intention to treat	19	17	10	..	97	113	70	..
DENV-1	16 (84%)	16 (94%)	2 (20%)	<0·0001¶¶	81 (84%)	88 (78%)	8 (11%)	<0·0001‖‖
DENV-2	16 (84%)	14 (82%)	0	<0·0001***	87 (90%)	86 (76%)	10 (14%)	<0·0001†††
DENV-3	14 (74%)	14 (82%)	0	<0·0001‡‡‡	72 (74%)	87 (77%)	8 (11%)	<0·0001§§§
DENV-4	15 (79%)	15 (88%)	0	<0·0001¶¶¶	83 (86%)	86 (76%)	5 (7%)	<0·0001‖‖‖

Data are n, or n (%). *χ^2 test. Seroconversion was defined by $PRNT_{50}$ cutoff (≥1/10) for DENV-naive participants or a four-fold or higher increase in neutralising antibody titre after immunisation of DENV-exposed participants. †Participants who did not attend all visits for sample collection for seroconversion analysis were excluded from the per-protocol analysis. ‡p>0·999 for Butantan-DV versus TV003. §p=0·278 for DENV-naive versus DENV-exposed. ¶p=0·601 for Butantan-DV versus TV003. ‖p=0·011 for DENV-naive versus DENV-exposed. **p>0·999 for Butantan-DV versus TV003. ††p=0·336 for DENV-naive versus DENV-exposed. ‡‡p>0·999 for Butantan-DV versus TV003. §§p=0·028 for DENV-naive versus DENV-exposed. ¶¶ p=0·605 for Butantan-DV versus TV003.‖‖p=0·305 for DENV-naive versus DENV-exposed. ***p>0·999 for Butantan-DV versus TV003. †††p=0·010 for DENV-naive versus DENV-exposed. ‡‡‡p=0·695 for Butantan-DV versus TV003. §§§p=0·641 for DENV-naive versus DENV-exposed. ¶¶¶p=0·662 for Butantan-DV versus TV003. ‖‖‖p=0·085 for DENV-naive versus DENV-exposed.

Table 4: **Frequency of seroconversion after the first dose of immunisation in step A and in all Butantan-DV recipients stratified by previous exposure**

grade 3 neutropenia and grade 3 myalgia, in two DENV-naive participants in the Butantan-DV group, and one episode of grade 3 neutropenia in a DENV-exposed participants in the Butantan-DV group. One participant had grade 3 neutropenia for 7 days and the other participant had a final diagnosis of benign ethnic neutropenia. No significant differences in the frequency of unsolicited adverse reactions were identified between DENV-naive and DENV-exposed participants in the Butantan-DV group and participants in the placebo group. All unsolicited and solicited laboratory, local, and systemic adverse reactions were self-limiting and no medical treatment was required, including the episodes of grade 3 neutropenia and myalgia.

Viraemia was assessed in 46 DENV-naive participants in step A (19 participants in the Butantan-DV group, 17 participants in the TV003 group, and ten participants in the placebo group) and in the first 40 DENV-exposed participants in step B (29 participants in the Butantan-DV group and 11 participants in the placebo group; modified intention-to-treat analysis; table 2). No significant differences were identified in the number of viruses detected between DENV-naive (18 of 19 assessed) and DENV-exposed (25 of 29 assessed) Butantan-DV recipients (p=0·124). Viraemia was demonstrated for DENV-1, DENV-2, and DENV-3, but not for DENV-4. DENV-3 viraemia was significantly more frequent in DENV-naive participants than DENV-exposed participants in the Butantan-DV group (p=0·006).

The neutralising antibody geometric mean titre was significantly higher in DENV-exposed participants than DENV-naive participants in the Butantan-DV group for DENV-1 (p<0·0001), DENV-2 (p<0·0001), and DENV-3 (p<0·0001), but not for DENV-4 (p=0·077; table 3).

The frequency of seroconversion for each DENV serotype after one dose of Butantan-DV in DENV-naive and DENV-exposed participants is shown in table 4. According to the per-protocol analysis, in the Butantan-DV group, the frequency of seroconversion was significantly higher for DENV-2 (p=0·011) and DENV-4 (p=0·028) in DENV-naive participants than DENV-exposed participants. However, no significant differences were identified in the frequency of seroconversion between DENV-naive and DENV-exposed participants for DENV-1 (p=0·278) or DENV-3 (p=0·336).

Overall, 54 (64%) of 85 DENV-naive and 56 (55%) of 101 DENV-exposed participants in the Butantan-DV group had a tetravalent antibody response, and 21 (25%) of 85 DENV-naive participants and 26 (26%) of 101 DENV-exposed participants in the Butantan-DV group had a trivalent antibody response following a single dose of Butantan-DV (appendix p 13). No significant differences were identified in the frequency of tetravalent (p=0·296) and trivalent (p>0·999) immune responses between DENV-naive and DENV-exposed participants in the Butantan-DV group.

The results suggest an association between multivalent immune response and rash; 36 (67%) of 54 DENV-naive participants and 31 (55%) of 56 DENV-exposed participants in the Butantan-DV group who achieved a tetravalent immune response presented with rash (appendix p 14). No significant differences in the frequency of association of tetravalent immune response and rash were identified between DENV-naive and DENV-exposed participants in the Butantan-DV group (p=0·246).

We assessed T-cell responses in 55 participants (32 DENV-naive participants and 23 DENV-exposed participants). Measurement of IFNγ production by CD8-positive T cells after stimulation with a DENV peptide pool indicated significant responses after vaccination in DENV-naive and DENV-exposed Butantan-DV recipients.

	Butantan-DV		Placebo (n=70)	p value*
	DENV-naive (n=97)	DENV-exposed (n=113)		
Solicited laboratory adverse reactions				
Increased alanine aminotransferase concentrations	11 (11%)	15 (13%)	2 (3%)	0·064
Haemoglobin†	10 (10%)	8 (7%)	5 (7%)	0·649
Leukopenia	12 (12%)	9 (8%)	0	0·011‡
Neutropenia	11 (11%)	8 (7%)	1 (1%)	0·049§
Lymphopenia	3 (3%)	5 (4%)	1 (1%)	0·534
Thrombocytopenia	1 (1%)	3 (3%)	0	0·312
Lymphocytosis	2 (2%)	1 (1%)	0	0·429
Leukocytosis	1 (1%)	1 (1%)	1 (1%)	0·940
Monocytopenia	1 (1%)	0	0	0·388
Neutrophilia	0	0	0	..
Solicited local adverse reactions				
Pain	5 (5%)	8 (7%)	6 (9%)	0·678
Erythema	8 (8%)	6 (5%)	0	0·053
Swelling	0	2 (2%)	0	0·226
Local pruritus	0	0	0	..
Induration	0	0	0	..
Solicited systemic adverse reactions				
Rash	63 (65%)	51 (45%)	5 (7%)	<0·0001¶
Headache	48 (49%)	48 (42%)	22 (31%)	0·066
Myalgia	19 (20%)	28 (25%)	8 (11%)	0·087
Retro-orbital pain	12 (12%)	11 (10%)	2 (3%)	0·096
Arthralgia	6 (6%)	15 (13%)	1 (1%)	0·011‖
Nausea	4 (4%)	7 (6%)	5 (7%)	0·681
Fatigue	5 (5%)	8 (7%)	1 (1%)	0·233
Pyrexia	4 (4%)	6 (5%)	4 (6%)	0·880
Photophobia	1 (1%)	3 (3%)	0	0·312
Vomiting	0	3 (3%)	0	0·106
Chills	3 (3%)	0	0	0·057
Unsolicited adverse reactions**				
Systemic pruritus	11 (11%)	18 (16%)	4 (6%)	0·113
Dizziness	4 (4%)	5 (4%)	1 (1%)	0·533
Oropharyngeal pain	4 (4%)	4 (4%)	0	0·245
Diarrhoea	3 (3%)	3 (3%)	2 (3%)	0·982
Back pain	4 (4%)	3 (3%)	0	0·240

Data are n (%). *χ^2 test. †Change from baseline value. ‡p=0·358 for DENV-naive versus DENV-exposed; p=0·001 for DENV-naive versus placebo; and p=0·014 for DENV-exposed versus placebo. §p=0·338 for DENV-naive versus DENV-exposed; p=0·015 for DENV-naive versus placebo; and p=0·156 for DENV-exposed versus placebo. ¶p=0·005 for DENV-naive versus DENV-exposed; p<0·0001 for DENV-naive versus placebo; and p<0·0001 for DENV-exposed versus placebo. ‖p=0·108 for DENV-naive versus DENV-exposed; p=0·241 for DENV-naive versus placebo; and p=0·006 for DENV-exposed versus placebo.**Adverse reactions with overall incidence of 2% or higher.

Table 5: **Solicited and unsolicited adverse reactions following the first dose of Butantan-DV or placebo**

Responses observed in 15 seronegative volunteers (ten Butantan-DV recipients and five placebo recipients), who received two vaccine doses, are shown in the appendix (p 10). At 91 days after the first vaccine dose, significant CD8-positive T cell responses were detected above the cutoff value of 0·06% IFNγ-positive cells in nine of ten Butantan-DV recipients, whereas no significant changes in IFNγ-producing CD8-positive

T cells were observed among the five placebo recipients. No significant changes in antigen-specific IFNγ production were observed in any of the participants after the second vaccine dose when compared with repsonses observed after the first dose. One placebo recipient developed a positive response between the first and second doses; this participant was confirmed to have been infected with wild-type DENV-1 during this interval. The immunological responses of the 32 DENV-naive (23 participants in the Butantan-DV group; nine participants in the placebo group) and 23 DENV-exposed (14 participants in the Butantan-DV group; nine participants in the placebo group) participants after one dose of vaccine or placebo are shown in the appendix (p 10). At day 91, 35 (94%) of 37 vaccinees (DENV-naive and DENV-exposed) had antigen-specific IFNγ responses compared with two (13%) of the 15 placebo recipients assessed at this timepoint. At day 12, immune responses were slightly higher among DENV-exposed participants than DENV-naive participants, indicating that the pre-existing DENV immunity can lead to an early CD8-positive T-cell response in DENV-exposed participants, but this difference was not statistically significant at day 91.

Discussion

In this study, vaccination with Butantan-DV was found to be safe, well tolerated, and immunogenic in both DENV-naive and DENV-exposed participants after one dose of vaccine. The results also show that despite a different formulation, Butantan-DV and TV003 are analogous vaccines.

Rash was the most common solicited systemic adverse reaction observed in both the DENV-naive TV003 and Butantan-DV groups. The frequency and characteristics of rash were similar between the TV003 and Butantan-DV groups. Viraemia, as an indicator of vaccine infectivity, was also not associated with any increase of vaccines reactogenicity. The safety profiles of Butantan-DV and TV003 in this study are consistent with the safety findings of phase 1 clinical studies done in the USA with the monovalent vaccines and the TV003 admixture.[15,17,22,23]

Butantan-DV and TV003 admixture provided a similar robust balanced neutralising antibody response to all DENV serotypes after a single dose. Overall, no significant differences were identified between the Butantan-DV and TV003 groups with regard to post-vaccination neutralising antibody geometric mean titres, frequency of seroconversion to the four dengue viruses, tetravalent immune response, and the frequency of association of tetravalent immune response and rash. The association between rash and tetravalent immune response described in this study was also found in the phase 1 clinical studies done in the USA with the TV003 admixture.[15] The similarities in safety and immunogenicity between Butantan-DV and TV003 were also demonstrated after the second dose in Step A whereby the second dose was not associated with antibody boost, an increase in the seroconversion frequency to any of the DENV serotypes, rash, or detectable viraemia. Such findings strongly support data published by Kirkpatrick and colleagues[15] in which TV003 admixture elicited complete protection against DENV-2 challenge in a controlled human infection model.

Overall, previous dengue exposure was not associated with a higher frequency of adverse reactions in Butantan-DV vaccine recipients. Rash is observed in live-attenuated vaccines and was common in the Butantan-DV group: rash was the most frequent solicited systemic adverse event among DENV-naive and DENV-exposed participants. However, all cases were considered mild and rash is unlikely to affect vaccine uptake.

DENV exposure before vaccination was associated with significantly higher neutralising antibody titres, with the exception of DENV-4. Higher neutralising antibody titres in flavivirus-exposed participants before vaccination with TV003 were found by Whithead and colleagues.[23]

The proportion of patients who achieved seroconversion varied between 77% and 92% among DENV-naive participants, and between 77% and 82% among DENV-exposed participants. The proportion of patients who achieved seroconversion for DENV-2 and DENV-4 was significantly higher in DENV-naive participants than DENV-exposed participants in the Butantan-DV group. Variation in the frequency of seroconversion for each dengue virus after vaccination in endemic settings, such as Brazil, should be expected and reflects the variable serotypic patterns of the exposed participants. However, after vaccination, antibody immune responses to previously unencountered serotypes and seroconversion were observed (represented by an increase in antibody titre), which is not expected to occur when a previous immune response already exists to a specific serotype.[31]

Overall, 64% of DENV-naïve participants and 55% DENV-exposed participants in the Butantan-DV group achieved a tetravalent antibody response. No significant differences were identified in the frequency of association of tetravalent immune response and rash between DENV-naive and DENV-exposed participants in the Butantan-DV group.

Protective immunity to DENV is thought to be comprised of neutralising antibody and activated CD8-poistive and CD4-positive T cells.[18,26] A previous study showed that CD8+ T cells predominantly target non-structural proteins of DENV in natural infection and after immunisation with the live-attenuated DENV vaccine TV003.[16] Similarly, CD4+ T cells target predominantly the capsid and NS3 and NS5 proteins.[18] Our results showed a robust expansion of antigen-specific IFNγ-producing cells after a single vaccination, both in DENV-naive (91% responders) and DENV-exposed (100% responders) participants vaccinated with Butantan-DV. Expansion of CD8-positive T cells has also been demonstrated after immunisation with the NIH candidate vaccines,

including the monovalent formulations and TV003 admixture.[16,26] When each of the four components of the TV003 admixture are administered as monovalent formulations, they induce serotype-specific cell-mediated immune responses. However, when administered as a tetravalent admixture (TV003), the CD8+ T cell response was directed to the highly conserved epitopes among all four DENV serotypes.[17]

Studies have shown that pre-existing immunity to one DENV serotype is the greatest risk factor for more severe disease on secondary, heterotypic DENV infection,[3] which is more frequent in hyperendemic areas, where diverse patterns of DENV co-circulation and serotype replacement are observed. Therefore, an ideal dengue vaccine would induce a robust and broader immune response to the four dengue serotypes.

Several significant differences in the composition of Butantan-DV and TV003 and in the type of immune response they induce in DENV-naive and DENV-exposed individuals distinguish them from other live-attenuated DENV vaccines. First, Butantan-DV and TV003 contain three of four full-length DENV with all wild-type structural and non-structural proteins: only DENV-2 is a chimeric virus that does not contain DENV-2 non-structural proteins. Second, since CD8-positive T-cell epitopes are predominantly localised to the non-structural proteins,[32] Butantan-DV and TV003 elicit a broad cellular immune response to three DENV serotypes. Third, the balanced infectivity and neutralising antibody response results from a single vaccine dose regime.

Therefore, it is expected that Butantan-DV should confer a safer and broader protective immune response than vaccines with protection solely based on DENV structural protein expression[33] and vaccines containing non-structural proteins from only one DENV serotype.[34] In conclusion, one dose of Butantan-DV has been found to be safe and immunogenic in DENV-naive and DENV-exposed adults. These findings support the evaluation of the Butantan-DV in phase 3 efficacy trials.

Contributors

EGKa and LMAC were the principal investigators of the trial. ARP, RP, BT, and JLM designed the study and supervised the clinical development of Butantan-DV. TV and MdGS contributed to the writing and revision of the final version of the manuscript. GM coordinated the monitoring activities of the trial. AdS and HME were the monitors of the trial. JdPS was in charge of the clinical database. IR supervised the manufacture of Butantan-DV. JK supervised the entire project. PEB did the statistical analysis. CLSS and MdCSTT coordinated and did the immunogenicity and viraemia analysis of Butantan-DV. LF, RG, RA, AM, AMES, JCOAF, SCLF, and NKS conducted the study at the clinical sites. SW provided the TV003 admixure. DW and AS designed the dengue virus peptide pools for cellular immune evaluation and oversaw the cellular immune response analysis experiment. CGTS and PRC did the cellular immune assays and analysed the results.

Declaration of interests

AP, RP, PEB, TV, NMFG, AdS, HME, JdPS, and MdGS are employees of the Butantan Institute. IR, BT, JLM, JK, and GM are former employees of the Butantan Institute. SSW and APD acted as consultants for the manufacturing and clinical development of Butantan-DV. All other authors declare no competing interests.

Data sharing

The individual, identifiable data and the data dictionary will not be shared. Some de-identified safety data will be provided to a centralised database consortium sponsored by the The National Institutes of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH), which is only available to the NIAID–NIH, the International Institute of Vaccines, and vaccine licensees with access to the database; thus, they will not be publicly available. The study protocol, statistical analysis plan, and informed consent forms may be shared with licensees of the TV003 and TV005 candidate vaccines from the NIAID–NIH with access to the centralised database consortium. This data will be shared upon request after the members of the consortium sign the data access agreement. Furthermore, the data access will be limited to members of the consortium only, who can share the information with their respective regulatory agencies.

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