Efficacy and safety of Butantan-DV in participants aged 2–59 years through an extended follow-up: results from a double-blind, randomised, placebo-controlled, phase 3, multicentre trial in Brazil

*Mauricio L Nogueira, Monica A T Cintra, José A Moreira, Elizabeth G Patiño, Patricia Emilia Braga, Juliana C V Tenório, Lucas Bassolli de Oliveira Alves, Vanessa Infante, Daniela Haydee Ramos Silveira, Marcus Vínicius Guimarães de Lacerda, Dhelio Batista Pereira, Allex Jardim da Fonseca, Ricardo Queiroz Gurgel, Ivo Castelo-Branco Coelho, Cor Jesus Fernandes Fontes, Ernesto T A Marques, Gustavo Adolfo Sierra Romero, Mauro Martins Teixeira, André M Siqueira, Viviane Sampaio Boaventura, Fabiano Ramos, Erivaldo Elias Júnior, José Cassio de Moraes, Stephen S Whitehead, Alejandra Esteves-Jaramillo, Tulin Shekar, Jung-Jin Lee, Julieta Macey, Sabrina Gozlan Kelner, Beth-Ann G Coller, Fernanda Castro Boulos, Esper G Kallás, on behalf of the Phase 3 Butantan-DV Working Group**

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*Members listed in appendix 3 (p 6)

Faculdade de Medicina de São José do Rio Preto (FAMERP), São José do Rio Preto, Brazil (Prof M L Nogueira MD); **Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA** (Prof M L Nogueira); **Instituto Butantan, São Paulo, Brazil** (M A T Cintra MD, J A Moreira MD, E G Patiño PhD, P E Braga PhD, J C V Tenório PhD, L Bassolli de Oliveira Alves PhD, V Infante MD, D H R Silveira PharmD, F Castro Boulos MD, E G Kallás MD); **Fundação de Medicina Tropical Dr Heitor Vieira Dourado (FMT-HVD), Manaus, Brazil** (M V Guimarães de Lacerda MD); **Centro de Pesquisa em Medicina Tropical de Rôndonia, Porto Velho, Brazil** (D B Pereira MD); **Universidade Federal de Roraima, Boa Vista, Brazil** (A J da Fonseca MD); **Programa de Pós-Graduação em Ciências da Saúde, Universidade Federal de Sergipe, Aracaju, Brazil** (Prof R Q Gurgel MD); **Faculdade de Medicina da Universidade**

Summary

Background A single-dose dengue vaccine that protects individuals across a wide age range and regardless of dengue serostatus is an unmet need. We assessed the safety and efficacy of the live, attenuated, tetravalent Butantan-dengue vaccine (Butantan-DV) in adults, adolescents, and children. We previously reported the primary and secondary efficacy and safety endpoints in the initial 2 years of follow-up. Here we report the results through an extended follow-up period, with an average of 3·7 years of follow-up.

Methods In this double-blind, randomised, placebo-controlled, phase 3, multicentre trial in Brazil, healthy participants (aged 2–59 years) who had not previously received a dengue vaccine were enrolled and randomly assigned 2:1 (stratified by age 18–59 years, 7–17 years, and 2–6 years) using a central electronic randomisation system to receive 0·5 mL of Butantan-DV (containing approximately 10^3 plaque-forming units of each of the four vaccine virus strains) or placebo, administered subcutaneously. Syringes containing vaccine or placebo were prepared by an unmasked trial pharmacist who was not involved in any subsequent participant assessments; other site staff and the participants remained unaware of the group allocations. Vaccine efficacy was calculated with the accrual of virologically confirmed dengue (VCD) cases (by RT-PCR) at least 28 days after vaccination up until the cutoff (at least 2 years of follow-up from the last participant enrolled). The primary endpoint was vaccine efficacy against VCD after day 28 by any dengue virus (DENV) serotype regardless of dengue serostatus at baseline in the per-protocol population. The primary and secondary safety endpoints up until day 21 were previously reported; secondary safety endpoints include the frequency of unsolicited vaccine-related adverse events after day 22. Safety analyses were done on all participants as treated. This trial is registered with ClinicalTrials.gov (NCT02406729) and is ongoing.

Findings Of 16 363 participants assessed for eligibility, 16 235 were randomly assigned between Feb 22, 2016, and July 5, 2019, and received single-dose Butantan-DV (10 259 participants) or placebo (5976 participants). 16 162 participants (Butantan-DV n=10 215; placebo n=5947) were included in the per-protocol population and 16 235 (Butantan-DV n=10 259; placebo n=5976) in the safety population. At the data cutoff (July 13, 2021), participants had 2–5 years of follow-up (mean 3·7 years [SD 1·0], median 4·0 years [IQR 3·2–4·5]). 356 VCD cases were captured through the follow-up (128 in the vaccine group and 228 in the placebo group). Vaccine efficacy against VCD caused by any DENV serotype was 67·3% (95% CI 59·4–73·9); cases caused by DENV-3 or DENV-4 were not observed. The proportions of participants who had serious adverse events were similar between treatment groups (637 [6·2%] in the vaccine group and 395 [6·6%] in the placebo group) up until the cutoff.

Interpretation A single dose of Butantan-DV was generally well tolerated and efficacious against symptomatic VCD (caused by DENV-1 and DENV-2) for a mean of 3·7 years. These findings support the continued development of Butantan-DV to prevent dengue disease in children, adolescents, and adults regardless of dengue serostatus.

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Research in context

Evidence before this study

We searched PubMed on Feb 13, 2024, for articles published in the past 10 years using the terms "dengue" AND "vaccine" AND "clinical trial". We also searched dengue vaccine strategies that are ongoing from ClinicalTrials.gov on Feb 13, 2024. At the time that this phase 3 trial was initiated, there was one approved dengue vaccine (three-dose CYD-TDV; Dengvaxia, Sanofi Pasteur, Lyon, France). However, CYD-TDV vaccination was determined to carry a higher risk of severe disease and hospital admission among individuals without previous exposure to dengue virus (DENV). Thus, CYD-TDV is now only recommended for individuals with evidence of previous dengue exposure, typically indicated for ages 9–45 years, limiting its use. An additional dengue vaccine (two-dose TAK-003; Qdenga, Takeda, Cambridge, MA, USA) has been advanced through phase 3 and is approved in some countries, including Brazil, for use in individuals regardless of dengue serostatus. However, given the absence of protection against virologically confirmed dengue caused by DENV-3 and DENV-4 in participants without previous dengue exposure after 4·5 years, TAK-003 has been recommended to be limited to children aged 6–16 years in high-transmission settings (ie, in settings with a dengue seroprevalence of 60% or higher by age 9 years) until the efficacy–risk profile in people who are seronegative has been more precisely assessed. The single-dose TV003 and TV005 live, attenuated, tetravalent dengue vaccine candidates developed by the National Institutes of Health have undergone extensive phase 1 and phase 2 testing and have been found to be generally well tolerated and immunogenic. The vaccine virus strains that comprise TV003 and TV005 have been licensed to several developers worldwide (including Instituto Butantan, São Paulo, Brazil, and Merck Sharp & Dohme LLC, a subsidiary of Merck & Co, Rahway, NJ, USA) for further clinical development. The Butantan-Dengue Vaccine (Butantan-DV; Instituto Butantan, São Paulo, Brazil) was found to be analogous to TV003 in a phase 2 study, and since 2016 it has been under evaluation in a phase 3 trial in Brazil (NCT02406729). Initial results from the phase 3 trial were recently reported, demonstrating that a single dose of Butantan-DV prevented symptomatic, virologically confirmed dengue, regardless of baseline dengue serostatus, through 2 years of follow-up. Other vaccine strategies that were in early clinical development at the time that this phase 3 trial was initiated include a tetravalent DNA vaccine (TVDV; US Naval Medical Research Center, Silver Spring, MD, USA), a synthetic nanoparticle-based peptide vaccine (PepGNP-dengue; Emergex Vaccines, Oxfordshire, UK), another attenuated vaccine (TDENV; Walter Reed Army Institute of Research [WRAIR], Silver Spring, MD, USA and GlaxoSmithKline [GSK], Brentford, UK), and a purified formalin-inactivated virus vaccine (DPIV; WRAIR and GSK).

Added value of this study

We report the safety and efficacy of Butantan-DV through a mean of 3·7 years (SD 1·0) of follow-up from the phase 3 trial in participants aged 2–59 years in Brazil. The results were consistent with those reported after the initial 2 years of follow-up. Butantan-DV was generally well tolerated and efficacious against virologically confirmed dengue (caused either by DENV-1 or DENV-2) regardless of dengue serostatus at baseline. Cases of DENV-3 or DENV-4 were not detected during the study period and thus efficacy against DENV-3 and DENV-4 could not be assessed. We demonstrated for the first time that the vaccine was efficacious at preventing severe dengue or dengue with warning signs, regardless of hospital admission. Importantly, additional safety signals were not observed during the extended follow-up after Butantan-DV vaccination, unlike what has been reported after CYD-TDV vaccination, for which an increased risk of more severe disease among young, seronegative children aged 2–8 years was evident approximately 1 year after the third dose. In this trial, the efficacy of Butantan-DV was assessed across a wide age range (participants aged 2–59 years, and by age group) and a substantial proportion of participants enrolled (46·5%) were dengue seronegative at baseline, thereby permitting a stringent assessment.

Implications of all the available evidence

A safe and effective single-dose vaccine for the prevention of dengue disease in individuals across a wide age range and regardless of dengue serostatus is needed. A single-dose dengue vaccine regimen might be advantageous for programmatic aspects by easing logistical considerations for implementation, which might help increase vaccine uptake. The efficacy and safety findings presented in this Article support the continued development of Butantan-DV for the prevention of dengue disease in adults, adolescents, and children, with and without previous dengue exposure. The trial is ongoing, and the results from 5 years of follow-up are forthcoming. If cases of virologically confirmed dengue caused by DENV-3 or DENV-4 are not observed by the end of the trial, it will not be possible to assess efficacy against these two serotypes and other studies might be needed to evaluate the protection of the vaccine against DENV-3 and DENV-4.

Introduction

Dengue is caused by dengue virus (DENV) and is the most common arboviral disease worldwide, with the largest burden occurring in southeast Asia and central and South America.[1–4] Four serotypes of DENV (DENV-1–4) often cocirculate and can cause disease, resulting in a range of symptoms from subclinical or inapparent disease to severe disease.[2,3] Baseline dengue seropositivity can affect subsequent DENV infections by protecting from homotypic re-infection or by protecting against or enhancing heterotypic infection.[5] Thus, the goal of a dengue vaccine is to offer broad and balanced immunity against all four DENV serotypes regardless of previous DENV immunity.

Federal do Ceará, Foraleza, Brazil (Prof I Castelo-Branco Coelho MD); **Hospital Universitário Júlio Müller (UFMT), Cuiabá, Brazil** (C J F Fontes MD); **Institute Aggeu Magalhães, Fundação Oswaldo Cruz, Recife, Brazil** (E T A Marques MD); **School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA** (E T A Marques); **Núcleo de Medicina Tropical, Faculdade de Medicina, Universidade de Brasília, Brasília, Brazil** (Prof G A Sierra Romero MD); **Centre for Advanced and Innovative Therapies, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil** (Prof M M Teixeira MD); **INCT-Dengue** (Prof M M Teixeira); **Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro, Brazil** (A M Siqueira MD); **Medicina e Saúde pública de Precisão, Fundação Oswaldo Cruz–Instituto Gonçalo Moniz (IGM/FIOCRUZ), Salvador, Brazil** (V S Boaventura MD); **Hospital São Lucas da PUC do Rio Grande do Sul, Porto Alegre, Brazil** (F Ramos MD); **Faculdade de Medicina da Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Brazil** (Prof E E Júnior MD); **Departamento de Saúde Coletiva, Faculdade de Ciências Médicas Santa Casa de São Paulo, São Paulo, Brazil** (Prof J Cassio de Moraes MD); **National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA** (S S Whitehead PhD); **Merck & Co, Rahway, NJ, USA** (A Esteves-Jaramillo MD, T Shekar MS, J-J Lee PhD, S Gozlan Kelner MSc, B-A G Coller PhD); **MSD, Munro, Argentina** (J Macey MD); **Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil** (E G Kallás)

Correspondence to:
Dr Fernanda Castro Boulos, Instituto Butantan, São Paulo 05585-000, Brazil
fernanda.boulos@fundacaobutantan.org.br

See Online for appendix 3

The two currently available licensed, live, attenuated dengue vaccines require multidose schedules. CYD-TDV (Dengvaxia; Sanofi Pasteur, Lyon, France) is given in three doses and is based on an attenuated yellow fever virus genetic background. TAK-003 (Qdenga; Takeda, Cambridge, MA, USA) is a two-dose dengue vaccine based on an attenuated DENV-2 genetic background. CYD-TDV vaccination was found to increase the risk of severe disease in individuals who were dengue naive upon subsequent infection.[6,7] Therefore, CYD-TDV is only recommended for use in individuals typically aged 9–45 years, who have evidence of previous DENV infection, or in settings with seroprevalence of 80% or greater by age 9 years.[8] TAK-003 has received approval in some countries to date, including Brazil, for individuals typically aged 4 years and older, regardless of serostatus. However, TAK-003 did not provide protection against virologically confirmed dengue (VCD) caused by DENV-3 or DENV-4 among seronegative children and adolescents in endemic areas after 4·5 years of follow-up.[9] Thus, in September, 2023, the WHO Strategic Advisory Group of Experts on Immunization recommended that TAK-003 be limited to children aged 6–16 years in high-transmission settings (seroprevalence >60% by age 9 years or a mean age of peak dengue-associated hospital admission of younger than 16 years) until the efficacy–risk profile in people who are seronegative has been more precisely assessed, although prescreening for dengue serostatus is not currently required.[10] There remains an unmet need for a single-dose dengue vaccine to immunise and protect against all four serotypes across a wide age range regardless of baseline DENV serostatus.

Butantan-Dengue Vaccine (Butantan-DV) is a single-dose, live, attenuated, tetravalent dengue vaccine candidate composed of vaccine viruses representing all four DENV serotypes derived from the TV003 formulation developed by the US National Institutes of Health.[11] Three of the four vaccine viruses present in Butantan-DV and TV003 (representing DENV-1, DENV-3, and DENV-4) consist of near full-length DENV genomes (rDENV1del30, rDENV3del30/31, and rDENV4del30). The fourth component (representing DENV-2) is a chimeric vaccine virus containing the structural premembrane and envelope genes of DENV-2 on the attenuated DENV-4 genetic background (rDENV2/4del30[ME]). Phase 1 studies of TV003 and a phase 2 study of Butantan-DV found the vaccine to be generally well tolerated and immunogenic.[12–17]

In 2016, we initiated a large phase 3 trial of a single dose of Butantan-DV in participants aged 2–59 years in Brazil (NCT02406729). We previously published the efficacy and safety data throughout the first 2 years of follow-up,[18] during which the primary efficacy objective was met, with an overall vaccine efficacy against VCD (assessed by RT-PCR) caused by any serotype and regardless of baseline serostatus of 79·6% (95% CI 70·0–86·3). Analyses of secondary objectives found vaccine efficacy was 73·6% (57·6–83·7) in participants who were dengue naive, 89·2% (77·6–95·6) in those who were dengue experienced, 89·5% (78·7–95·0) against DENV-1 and 69·6% (50·8–81·5) against DENV-2. Cases caused by DENV-3 or DENV-4 were not observed in the first 2 years of the trial. Overall, the frequency of severe dengue was low during the first 2 years of follow-up, reflective of low incidence in Brazil during the study period, and thus was not previously reported. Butantan-DV was generally well tolerated with a similar safety profile as described in the phase 2 trial. Within 21 days after vaccination, solicited systemic vaccine-related adverse events were reported more frequently among Butantan-DV recipients (58·3%) versus placebo recipients (45·6%) and the incidence of rash was 22·5% in Butantan-DV recipients versus 4·2% of placebo recipients.

Here, we report findings from the phase 3 trial of Butantan-DV through the prespecified interim analysis data cutoff, at which time participants had completed a minimum of 2 years and up to 5 years of follow-up (mean 3·7 years [SD 1·0], median 4·0 years [IQR 3·2–4·5]). With additional data collected through this extended follow-up, we aimed to assess prespecified primary and secondary efficacy endpoints, including vaccine efficacy against VCD caused by any DENV serotype, by baseline dengue serostatus, by DENV serotype (DENV-1 and DENV-2), and against severe dengue or dengue with warning signs. Further, we aimed to assess vaccine efficacy by age group (2–6 years, 7–17 years, and 18–59 years) and to extend the analysis of safety.

Methods

Study design and participants

Eligible participants were randomly assigned between Feb 22, 2016, and July 5, 2019 in an ongoing double-blind, randomised, placebo-controlled, phase 3, multicentre study aimed at assessing the safety and efficacy of a single dose of Butantan-DV (NCT02406729; DEN-03-IB; appendix 3 pp 17–114). By the cutoff date (July 13, 2021), participants had completed 2–5 years of follow-up. A report of long-term efficacy and safety evaluation is planned once the full 5 years of follow-up is completed (final visit of the last participant occurred in June, 2024). Participants were enrolled and randomly assigned at 16 clinical sites across the five geographical regions (central-west, north, northeast, south, and southeast) of Brazil. The study was approved by the Brazilian Ministry of Health, National Ethics Committee, Comissão Nacional de Ética em Pesquisa (CAAE:44462915.8.1001.0068) and local ethics committees (appendix 3 p 5) and conducted in accordance with the principles of Good Clinical Practice.

Healthy male and female participants (aged 2–59 years) who met inclusion criteria and did not meet any exclusion criteria were eligible to participate. Key exclusion criteria included suspected or confirmed fever

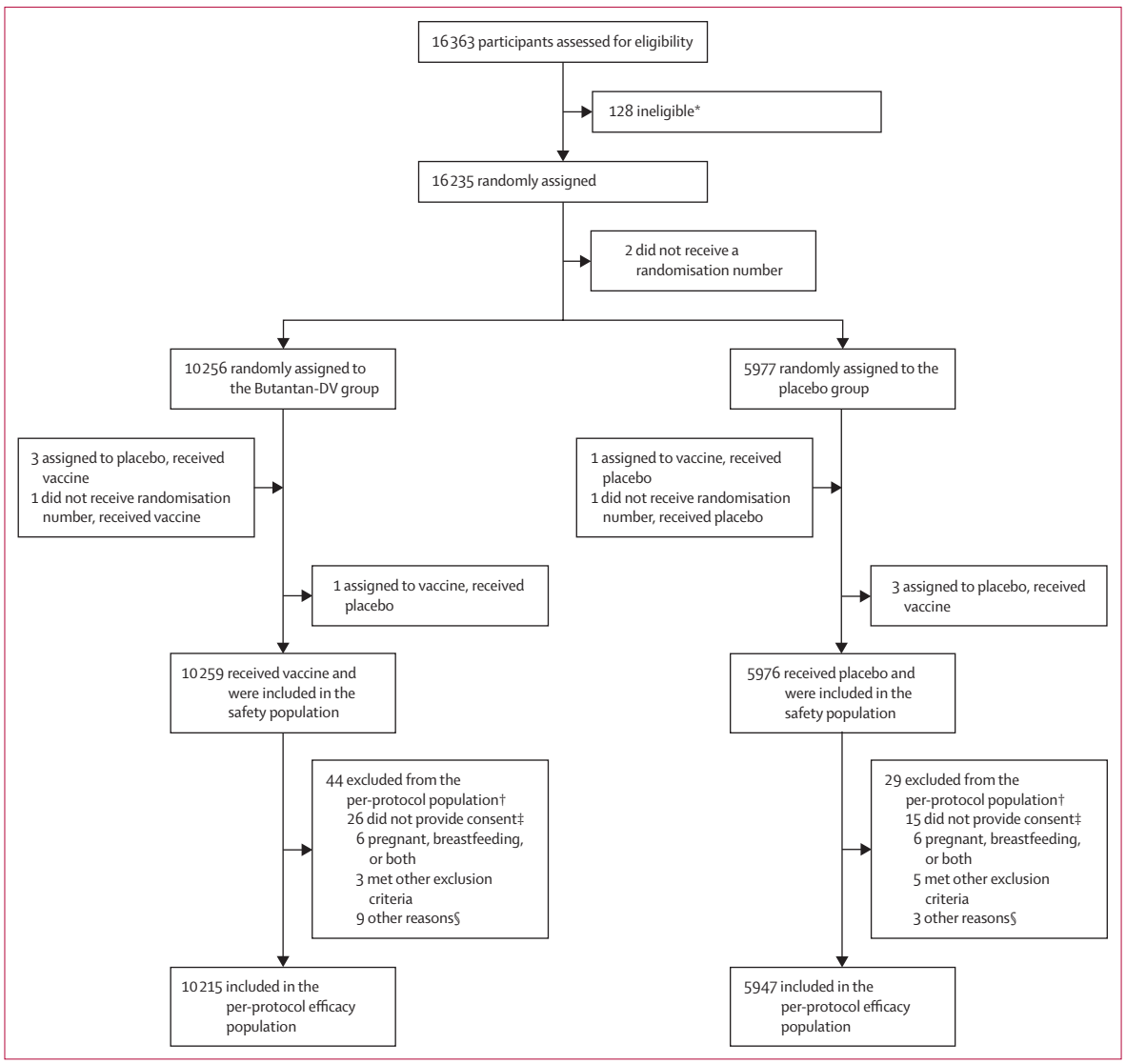


Figure 1: **Trial profile**
CONSORT diagram of participant disposition. *Reasons for exclusions after screening on the basis of eligibility criteria are provided in appendix 3 (p 11). †Participants might have more than one reason for exclusion. ‡Those who did not provide consent include participants who did not have documentation of initial consent or those who withdrew consent and were not appropriately reconsented upon return. §Other reasons consist of not receiving study vaccine according to the randomisation schedule or being administered improperly stored study vaccine. Each participant had at least 2 years of follow-up.

within 72 h before the day of vaccination, immunocompromising diseases, pregnancy or breastfeeding, or previous receipt of a dengue vaccine. A full list of inclusion and exclusion criteria can be found in the protocol (appendix 3 pp 17–114). Participants' sex determinations were made on the basis of biological sex. Before any study procedure, written informed consent was obtained from each participant or their legal representative; assent was obtained for children aged 7–17 years.

Randomisation and masking

Participants who met the entry criteria were enrolled in a stepwise manner beginning with the oldest age group (18–59 years) and de-escalating to the younger age groups (7–17 years, and finally 2–6 years) after review of safety data by the data and safety monitoring committee (DSMC). An independent statistician, without other involvement in the trial, randomly assigned participants in a 2:1 ratio to receive a single dose of Butantan-DV or placebo using stratified randomisation (by age group, using simple randomisation within each age stratum) via a central electronic randomisation system. Syringes containing vaccine or placebo were prepared by a trial pharmacist who was not masked to vaccine allocation and was not involved in any subsequent participant assessments. The syringes containing vaccine and placebo were alike and a double-blind technique was

	Butantan-DV (n=10 259)	Placebo (n=5976)	Total (n=16 235)
Sex			
Female	5555 (54·1%)	3216 (53·8%)	8771 (54·0%)
Male	4704 (45·9%)	2760 (46·2%)	7464 (46·0%)
Age, years			
2–6	3337 (32·5%)	1679 (28·1%)	5016 (30·9%)
7–17	3376 (32·9%)	1771 (29·6%)	5147 (31·7%)
18–59	3546 (34·6%)	2526 (42·3%)	6072 (37·4%)
Median (IQR)	11 (5–31)	14 (6–36)	12 (6–33)
Race			
Pardo (multiracial Brazilian)	7017 (68·4%)	4036 (67·5%)	11 053 (68·1%)
White	2410 (23·5%)	1402 (23·5%)	3812 (23·5%)
Black	655 (6·4%)	408 (6·8%)	1063 (6·5%)
Asian	149 (1·5%)	114 (1·9%)	263 (1·6%)
Indigenous or other	28 (0·3%)	16 (0·3%)	44 (0·3%)
Ethnicity			
Not Hispanic	10 068 (98·1%)	5861 (98·1%)	15 929 (98·1%)
Hispanic	191 (1·9%)	115 (1·9%)	306 (1·9%)
Previous exposure to dengue virus (any serotype)			
Yes*	5009 (48·8%)	3041 (50·9%)	8050 (49·6%)
No	4855 (47·3%)	2700 (45·2%)	7555 (46·5%)
Unknown† or missing‡	395 (3·9%)	235 (3·9%)	630 (3·9%)

Data are n (%) unless otherwise stated. American Indian or Alaska Native are displayed as Indigenous, Black or African American are displayed as Black, multiracial is displayed as Pardo, and Hispanic or Latino is displayed as Hispanic. DENV=dengue virus. IQR=interquartile range. $VRNT_{60}$=60% virus reduction neutralisation test. *Previous exposure to dengue virus is defined as baseline DENV-1 $VRNT_{60}$ titre of 18 or higher, DENV-2 $VRNT_{60}$ titre of 15 or higher, DENV-3 $VRNT_{60}$ titre of 12 or higher, or DENV-4 $VRNT_{60}$ titre of 13 or higher. †Unknown result indicates missing test result, a sample without consent, or participants who were not tested for DENV-3. ‡Missing category indicates participants who did not provide consent for the serology testing or participants who were not tested for other reasons.

Table 1: **Baseline characteristics in the safety population**

used in which other site staff and the participants remained unaware of the group allocations. To maintain masking in this ongoing trial, medical writers and unmasked authors had full access to the data, whereas other authors involved in the follow-up of participants only had access to the data presented in this report. An external DSMC provided oversight, reviewed the safety data, and had access to unmasked data on request.

Procedures

A 0·5 mL dose of Butantan-DV aims to deliver approximately 10^3 plaque-forming units of each of the four vaccine virus strains (rDENV1del30, rDENV2/4del30[ME], rDENV3del30/31, and rDENV4del30). Butantan-DV was supplied as a lyophilised cake and placebo consisted of lyophilised customised twice-concentrated Leibovitz's L-15 medium. Vaccine and placebo were each reconstituted with sterile phosphate buffer. A single dose of Butantan-DV or placebo was administered subcutaneously on day 0.

Blood samples were collected on day 0 (before vaccination) from all enrolled participants to determine baseline dengue serostatus. Previous exposure to dengue was defined as having a 60% virus neutralisation test ($VRNT_{60}$) titre against any of the four serotypes above the lower limit of quantification in a validated VRNT, as previously described.[16,18,19] Throughout the study, participants with febrile illness or illness clinically suspected to be dengue had a blood sample collected, preferably within 9 days of symptom onset. Virological confirmation of dengue was made using a validated four-plex RT-PCR assay in a central laboratory.[20]

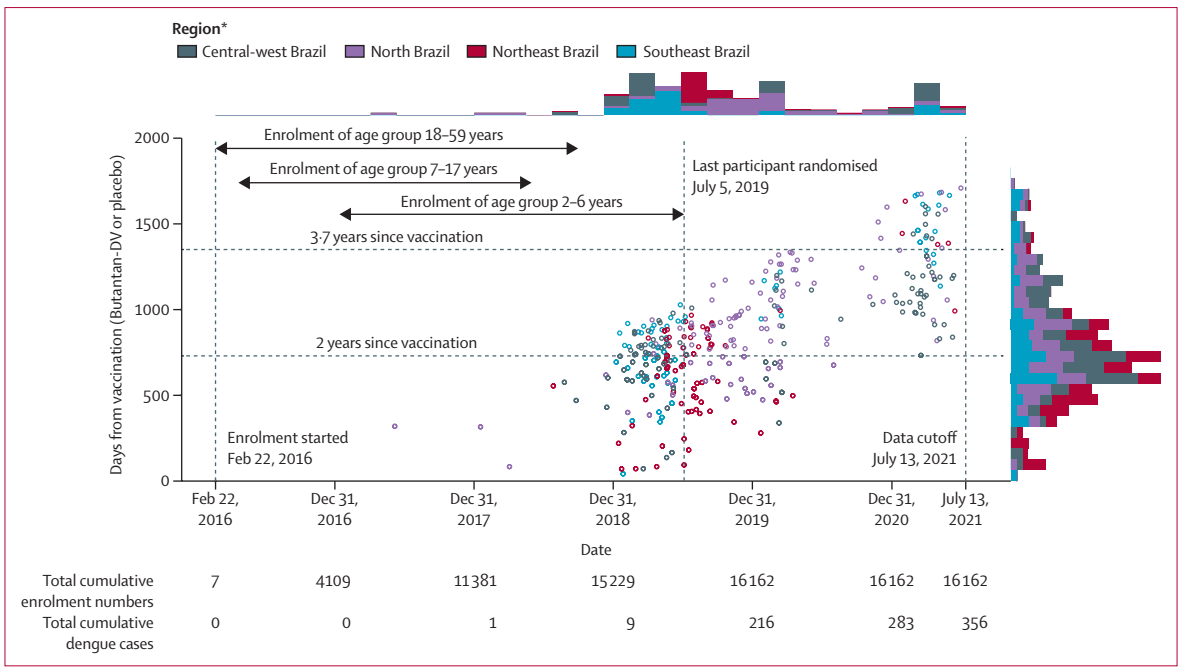
As previously described,[18] adverse events following receipt of study vaccine were recorded by participants using a patient diary for 21 days after vaccination. Unsolicited adverse events, including vaccine-related adverse events, serious adverse events (SAEs), deaths, and other reportable events were collected from all study participants throughout the duration of study participation. Solicited and unsolicited events were assessed for severity and causality by the investigator. All SAEs were reviewed by the DSMC. In this trial, all adverse events that present a reasonable causal relation to the product under investigation were considered adverse reactions and are referred to as vaccine-related adverse events in this report.

Participants were followed up electronically, by telephone, or in person to check the occurrence of unsolicited adverse events and dengue cases. During the initial 2 years, follow-up occurred once per month. In the remaining 3 years of the trial, follow-up was done every 3 months. Additionally, sites had the ability to increase contact frequency during times of localised dengue outbreaks.

Outcomes

The primary efficacy endpoint was the efficacy of a single dose of Butantan-DV in reducing the incidence of symptomatic VCD (by any DENV serotype) at least 28 days after vaccination regardless of previous exposure to dengue. Secondary efficacy endpoints were vaccine efficacy in individuals with and without previous dengue exposure (for participants with known baseline dengue serostatus), for individual DENV serotypes, and against dengue with warning signs or severe dengue regardless of hospital admission. Cases of severe dengue or dengue with warning signs were determined according to the Brazilian Ministry of Health,[21] which adopts the definitions proposed by the 2009 WHO classification (appendix 3 pp 17–114). A prespecified subgroup analysis determined vaccine efficacy by age group.

Primary and secondary safety outcomes were previously reported through the initial 2 years of follow-up.[18] The primary safety endpoint was frequency of solicited (administration site and systemic) and unsolicited vaccine-related adverse events through 21 days after vaccination in participants aged 2–59 years,



	Feb 22, 2016	Dec 31, 2016	Dec 31, 2017	Dec 31, 2018	Dec 31, 2019	Dec 31, 2020	July 13, 2021
Total cumulative enrolment numbers	7	4109	11381	15229	16162	16162	16162
Total cumulative dengue cases	0	0	1	9	216	283	356

Figure 2: **Temporospatial distribution of symptomatic virologically confirmed dengue**
Symptomatic virologically confirmed dengue cases (open circles) over the study period by geographical region are presented considering days from vaccination (vaccine or placebo) on the y-axis and calendar days on the x-axis. Bar graphs display frequency of virologically confirmed dengue cases in each timeframe. Key dates in the study are represented, including the enrolment start date, enrolment periods for each age group, date of random assignment of the last participant, and the data cutoff date (based on the time that the last participant completed 2 years of follow-up). Cumulative enrolment and cumulative dengue cases at the end of each calendar year are shown below the graph. Dengue cases were not observed in the south region during follow-up. *Cases were not observed in the south.

regardless of their baseline dengue serostatus. Secondary safety endpoints included the frequency of solicited and unsolicited vaccine-related adverse events through day 21 after vaccination in individuals with and without previous dengue exposure (for participants with known baseline dengue serostatus) or by age group, and the frequency of unsolicited vaccine-related adverse events after day 22.

Statistical analysis

The sample size for the main trial endpoint (efficacy) was determined using Blackwelder's formula[22] with Poisson approximation. The sample size calculation was based on the assumptions of an expected vaccine efficacy of 80% and a background annual DENV incidence of 0·337% (on the basis of the median incidence of dengue in Brazil between 2008 and 2013) and considered a 90% evaluability rate (appendix 3 p 3). Thus, it was estimated that the planned sample size of 16 944 participants randomly assigned in a 2:1 ratio (Butantan-DV to placebo) would provide 90% power to detect a lower limit of the two-sided 95% CI for vaccine efficacy higher than 25%.

Efficacy analyses were done on the per-protocol population (ie, all participants without major protocol deviations; appendix 3 p 131). Supportive efficacy analyses for the primary and secondary efficacy endpoints were done on the intention-to-treat population, defined as all randomly assigned participants who received the investigational product according to their planned treatment group assignment. Vaccine efficacy was determined using the exact binomial method described by Chan and Bohidar,[23] and 95% CIs were estimated using Blaker's exact CI method[24] (appendix 3 p 4). Participants were censored at the first positive RT-PCR test for VCD or after study discontinuation. The vaccine efficacy objective was considered met if the lower bound of the 95% CI for vaccine efficacy was higher than 25% for DENV disease caused by any serotype (primary objective) or by each individual serotype (secondary objective) regardless of baseline dengue serostatus. Vaccine efficacy was calculated on the basis of the accrual of VCD cases up until the cutoff date (July 13, 2021). A post-hoc sensitivity analysis was done to estimate vaccine efficacy among participants in the per-protocol population who were enrolled at the targeted 2:1 treatment allocation ratio; in this analysis, participants aged 18–59 years who were enrolled after an irregularity in the electronic randomisation system was observed (after Jan 31, 2017) were excluded. Safety analyses were done on all randomly assigned participants according to the treatment they received. Statistical analyses were done using SAS software (version 9.4).

Role of the funding source

Instituto Butantan sponsored the study, designed the trial (with input from the investigators and site staff), and

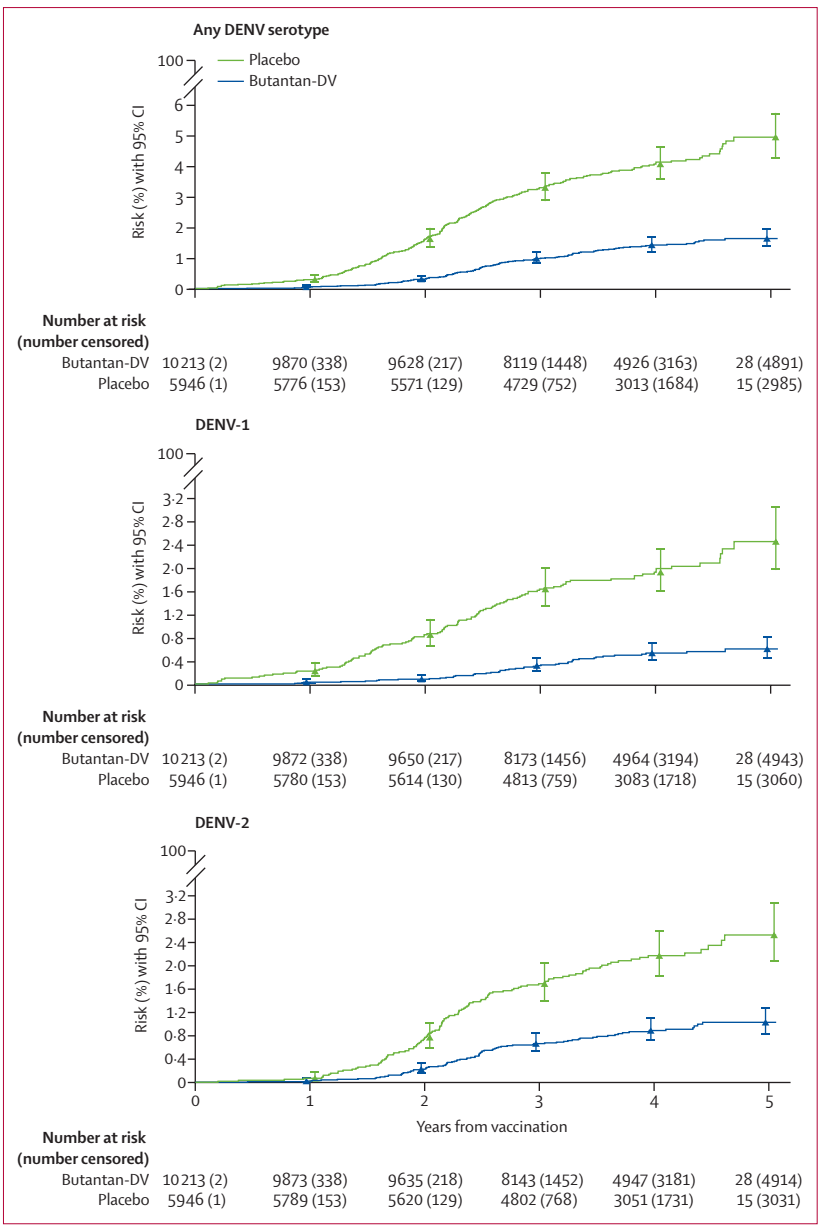


Figure 3: **Risk of symptomatic virologically confirmed dengue through the cutoff date, overall and by serotype**

Cases were defined as symptomatic episodes of virologically confirmed dengue occurring at least 28 days after vaccination and until the data cutoff (July 13, 2021). Participants with more than one dengue episode were counted as a single case. The numbers of participants in the per-protocol population under follow-up at various timepoints are shown beneath the graphs, with the numbers of participants censored in parentheses. Analyses excluded results that did not follow the standard operating procedure for the RT-PCR. The error bars represent 95% CIs.

managed the data. Additional support for the statistical analyses, $VRNT_{60}$ testing, data management, and medical writing assistance was provided by Merck Sharp & Dohme LLC, a subsidiary of Merck & Co, Rahway, NJ, USA.

Results

Between Feb 22, 2016, and July 5, 2019, 16 363 participants were assessed for eligibility, of whom 16 235 were randomly assigned and received Butantan-DV (10 259 [63·2%]) or placebo (5976 [36·8%]; figure 1; appendix 3 p 11). Because of an observed irregularity in the electronic randomisation system, the incurred treatment allocation ratio was lower than expected for participants aged 18–59 years (table 1). Participants had completed at least 2 years of follow-up by July 13, 2021, at the time of the data cutoff, when 112 (0·7%) participants had completed the study (69 in the vaccine group and 43 in the placebo group; figure 2). 16 162 (99·6%) participants were included in the per-protocol efficacy population (10 215 in the Butantan-DV group and 5947 in the placebo group). As previously reported,[18] the baseline characteristics were generally similar between groups and approximately half of participants did not have evidence of previous dengue exposure before vaccination (table 1).

From day 0 through a mean of 3·7 years of follow-up, the proportions of participants with a fever episode, as reported in the fever and suspected dengue case form, were generally similar between intervention groups (7210 [70·6%] of 10 215 in the vaccine group and 4077 [68·6%] of 5947 in the placebo group), with the majority of fever episodes occurring more than 28 days after vaccination (appendix 3 p 12). RT-PCR results were available for 23 080 (94·6%) of 24 393 fever episodes reported more than 28 days after vaccination in the vaccine group and for 12 516 (94·1%) of 13 297 fever episodes in the placebo group. A total of 356 VCD cases were captured through the cutoff date (128 in the vaccine group and 228 in the placebo group). We assessed the temporospatial distribution of VCD by geographical region through the cutoff date (figure 2). The incidence of VCD cases among participants in the trial varied by year and geographical region. During the follow-up period, 157 cases were caused by DENV-1 and 201 cases by DENV-2; one participant had a co-infection of DENV-1 and DENV-2, and one participant had an infection of DENV-1 followed by a subsequent infection of DENV-2, 88 days apart. Both of these participants were counted as a single case for the total VCD. Cases caused by DENV-3 or DENV-4 were not observed during follow-up.

We calculated the risk of VCD over a mean follow-up of 3·7 years in the per-protocol population (figure 3). The risk of VCD was higher in the placebo group than the Butantan-DV group, regardless of DENV serotype. At the data cutoff, the vaccine efficacy against symptomatic VCD in participants aged 2–59 years, regardless of baseline dengue serostatus, was 67·3% (95% CI 59·4–73·9) against any serotype (figure 4). Vaccine efficacy was 75·8% (65·8–83·1) against DENV-1 and 59·7% (46·5–69·8) against DENV-2. Thus, the primary and secondary efficacy objectives met the prespecified statistical criterion for success, with the lower bounds of the 95% CIs greater than 25%. Primary and secondary vaccine efficacy estimates for the intention-to-treat population were generally similar to those in the per-protocol population

(appendix 3 p 13). In general, vaccine efficacy against symptomatic VCD tended to be higher among individuals with evidence of previous DENV exposure (figure 4). Vaccine efficacy against VCD by any DENV serotype was 64·6% (49·4–75·5) in children aged 2–6 years, 70·6% (57·8–79·8) in those aged 7–17 years, and 72·8% (57·5–82·8) in adults aged 18–59 years. The incidence of VCD caused by DENV-1 or DENV-2 by age group was low during follow-up, resulting in wide CIs.

We also evaluated the secondary endpoint of vaccine efficacy against severe dengue or dengue with warning signs. There were 19 cases (three in the vaccine group and 16 in the placebo group) of severe dengue or dengue with warning signs, regardless of hospital admission, during follow-up, resulting in a vaccine efficacy of 89·0% (62·5–97·3).

During follow-up, the proportion of participants who had SAEs was similar between the treatment groups (637 [6·2%] of 10 259 in the vaccine group and 395 [6·6%] of 5976 in the placebo group; table 2). We have previously reported seven cases of treatment-related SAEs during the first 2 years of follow-up.[18] Additional unsolicited vaccine-related adverse events or vaccine-related SAEs were not reported after the initial 2 years through the data cutoff date (July 13, 2021). The cumulative frequency of death reported in the study through the data cutoff was similar between treatment groups (0·4% [37 deaths] in the vaccine group and 0·5% [30 deaths] in the placebo group). None of the deaths were deemed to be related to the study treatment. The most common adverse event resulting in death was COVID-19 (n=12; appendix 3 p 14).

Discussion

Here we report the safety and efficacy of Butantan-DV, a live, attenuated tetravalent dengue vaccine, throughout a mean of 3·7 years of follow-up in participants aged 2–59 years enrolled in an ongoing phase 3 clinical trial in Brazil. Encouragingly, the results were similar to what was previously reported after the initial 2 years of follow-up.[18] A single dose of Butantan-DV was generally well tolerated and additional safety signals were not observed. Butantan-DV demonstrated efficacy against symptomatic VCD (caused by DENV-1 and DENV-2), regardless of baseline serostatus, in each age group. Further, Butantan-DV was efficacious against dengue with

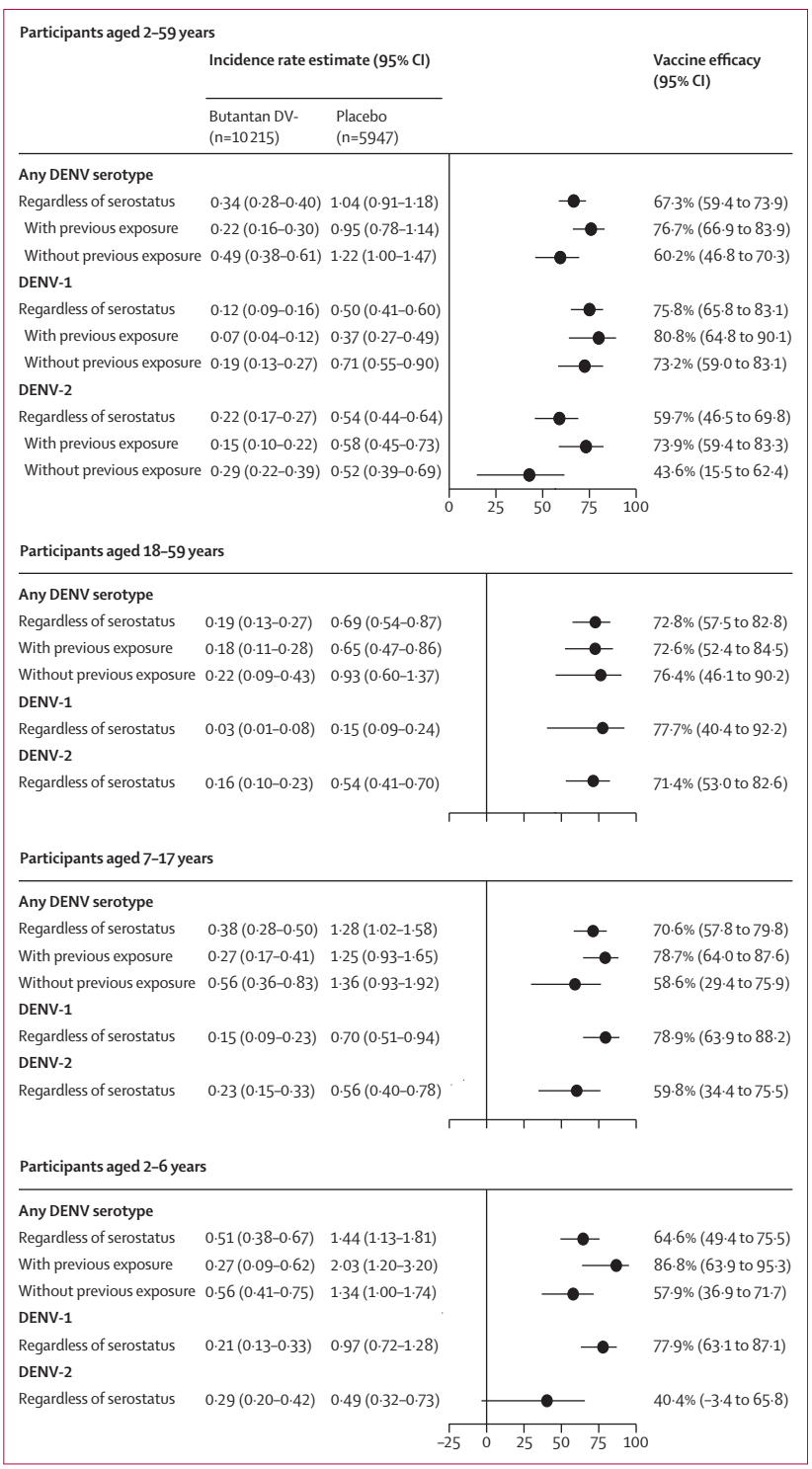


Figure 4: **Vaccine efficacy against symptomatic virologically confirmed dengue, overall and according to baseline serostatus, serotype, and age subgroup, through an average of 3·7 years of follow-up**

Forest plots indicate vaccine efficacy with 95% CIs against symptomatic virologically confirmed dengue after day 28 in the per-protocol population through the cutoff date (July 13, 2021). Incidence rate was cases per 100 person-years at risk. The vaccine efficacy objective was considered met if the lower bound of the 95% CI for vaccine efficacy was higher than 25% for virologically confirmed dengue caused by any serotype (primary objective) or by each individual serotype (secondary objective) in participants aged 2–59 years, regardless of baseline dengue serostatus. Participants with several dengue episodes were counted as a single case. Participants with more than one dengue episode were counted as a single case for the total virologically confirmed dengue incidence and individually by dengue-specific serotype for heterotypic virologically confirmed dengue episodes. Only DENV-1 and DENV-2 were detected in this study.

	Butantan-DV (n=10 259)	Placebo (n=5976)	Total (n=16 235)
Participants with one or more adverse events	7288 (71·0%)	3725 (62·3%)	11 013 (67·8%)
Participants with one or more unsolicited adverse events	3751 (36·6%)	2170 (36·3%)	5921 (36·5%)
Participants with serious adverse events*	637 (6·2%)	395 (6·6%)	1032 (6·4%)
Participants who died	37 (0·4%)	30 (0·5%)	67 (0·4%)

*Three serious adverse events were associated with infants of the participants, not the participants themselves; all occurred at least 22 days after vaccination and none were considered to be related to study vaccine by the investigator.

Table 2: **Adverse event summary from day 0 after vaccination to the cutoff date (July 13, 2021)**

warning signs or severe dengue, regardless of hospital admission.

Trial enrolment occurred over a 3-year period, and thus participants had between 2 years and 5 years of follow-up at the time of the data cutoff. This timeframe allowed assessment of longer-term safety and efficacy over several dengue seasons. The temporospatial distribution of VCD cases reflected the patterns of DENV circulation in Brazil during the period in which the trial was done.[25–27] Following a large epidemic of Zika virus in Brazil, which peaked in 2016, the incidence of DENV was relatively low across the country.[28] However, a resurgence of DENV to pre-Zika outbreak levels was seen in Brazil by 2019 once the Zika virus outbreak had subsided,[28] when trial enrolment of the youngest age group (2–6 years) was still underway. In 2020, the COVID-19 pandemic swept across the country and a decline in VCD incidence was seen again.[26] The effect of social distancing on reducing DENV circulation is not well understood and other factors, such as a decline in arbovirus surveillance or limited capacity as resources were redirected to pandemic response, might have contributed to fewer recorded VCD cases in 2020.[29,30]

Future work is needed to investigate the effects of previous exposure to other flaviviruses (such as Zika and yellow fever) on DENV-specific immune responses upon subsequent vaccination or infection. Being a randomised controlled trial, the incidence of previous Zika virus exposure is probably balanced between the groups in this study. The reduction of DENV incidence following the Zika virus epidemic is hypothesised to be caused by cross-protective antibodies afforded by widespread Zika virus infection.[31] However, it is not fully understood how primary Zika virus infection affects dengue clinical outcomes or for how long Zika virus-induced cross-protective antibodies last. Previous studies[32–34] have found that after an initial 2 years of cross-protection, previous Zika virus infection exacerbated dengue pathogenesis. Exploratory analyses to investigate the effects of baseline Zika virus serostatus on vaccine efficacy are planned after study completion.

As with the initial 2-year report,[18] Butantan-DV demonstrated efficacy against symptomatic VCD (caused by DENV-1 and DENV-2), regardless of baseline serostatus and cases of symptomatic VCD caused by DENV-3 or DENV-4 were not observed by the time of data cutoff in this study. Although vaccine efficacy estimates were numerically lower in this study than in the initial report at 2 years, this finding was not formally tested and the 95% CIs for those estimates overlap. Moreover, the prespecified statistical criterion for success was met for the primary and secondary safety objectives in this study. Furthermore, vaccine efficacy estimates remained positive through the extended follow-up, even among young children, the majority of whom (81%) did not have evidence of previous DENV exposure. Data obtained through the full 5 years of follow-up will assess the durability of protection.

The DENV-2 vaccine component is the only chimeric vaccine virus in Butantan-DV.[11] Despite this, Butantan-DV afforded continued protection against symptomatic VCD caused by DENV-2 through an average of 3·7 years, even among participants without evidence of previous DENV exposure. DENV-2 vaccine efficacy point estimates were numerically lower than vaccine efficacy against VCD caused by DENV-1; however, this finding was not formally tested in prespecified analyses and the 95% CIs of the vaccine efficacy estimates against DENV-2 were overlapping with those against DENV-1. Whether this observed trend was caused by the chimeric nature of the DENV-2 vaccine component or other factors, such as the circulating strains in Brazil at the time of the study, is unknown. Data through the full 5 years of follow-up and future work to investigate the immune responses after Butantan-DV vaccination could provide further insights on these findings.

The number of severe dengue cases during the first 2 years of follow-up was low overall, probably reflective of the overall lower rates of VCD, and thus was not previously reported because of the potential to unblind the study randomisation.[18] By using cases accrued during the extended follow-up period, we demonstrated that Butantan-DV was efficacious against severe dengue and dengue with warning signs, regardless of hospital admission. Data through the full 5 years of follow-up will provide longer-term insights regarding efficacy against severe disease.

Consistent with our previous report detailing safety after the initial 2 years of follow-up,[18] Butantan-DV was found to be generally well tolerated through an average of 3·7 years of follow-up. Additional safety signals were not observed during the extended follow-up, unlike what has been reported after CYD-TDV vaccination, for which an increased risk of more severe disease, particularly among young, seronegative children, was evident approximately 1 year after the third dose.[6] Overall, the safety profile was consistent with previous studies, including phase 1 and 2 studies of Butantan-DV[17] and TV003.[12–15] Importantly, no additional serious vaccine-related adverse events were observed during the extended follow-up. An increase in the number of participants having an adverse event resulting in death since the intitial 2 years of follow-up can be attributed to the

unprecedented COVID-19 pandemic that spread across Brazil in 2020, resulting in substantial loss of life.[35]

This study has some limitations. Methodological limitations include possible selection bias in the per-protocol estimates, confounding in the as-treated estimates, and selection bias caused by missing outcome data. An observed irregularity in the electronic randomisation system incurred an overall treatment allocation ratio lower than 2:1 in the 18–59 years age group. However, results from a post-hoc sensitivity analysis suggest that the lower than expected treatment allocation ratio did not affect the primary findings (appendix 3 p 16). Although the follow-up period spanned several dengue seasons, there were relatively few cases of severe dengue or dengue with warning signs (n=19), and cases caused by DENV-3 or DENV-4 were not observed by the time of the data cutoff. However, the incidence of dengue and the absence of cases caused by DENV-3 and DENV-4 reflect the patterns of circulation of DENV in Brazil during the period of the trial.[28] If cases of VCD caused by DENV-3 or DENV-4 are not observed by the end of the trial, it will not be possible to assess efficacy against these two serotypes and other studies might be needed to evaluate the protection of the vaccine against DENV-3 and DENV-4. There are, however, encouraging data suggesting that the vaccine would be effective against DENV-3 and DENV-4.[36] Previous studies of TV003 found that the vaccine produces vaccine viraemia for all four serotypes[15,16] and elicited type-specific immunity.[37] Further, TV005 afforded protection against DENV-3 challenge in a controlled human-infection study.[38] This phase 3 trial is ongoing amid a re-emergence of DENV-3 in Brazil,[39] and the results from 5 years of follow-up are forthcoming. As previously stated, the effects of previous Zika virus immunity are not known, and exploratory analyses are planned. Because of the staggered enrolment design, which took approximately 3 years to complete, there is a variation in the duration of follow-up time (between 2 years and 5 years), with the youngest participants having the shortest follow-up time. Still, important safety signals were not observed, and vaccine efficacy estimates remained positive throughout the extended follow-up period regardless of age group. Although dengue incidence varies across Brazil,[40] which was captured by enrolling participants across several sites in the five geographical regions, this trial was done in a single country and there might be underlying differences in host genetics, flavivirus exposure histories, and circulating DENV strains between Brazil and other countries. Future studies might be needed to extrapolate these findings outside of Brazil.

In conclusion, a single dose of Butantan-DV was generally well tolerated and efficacious against DENV-1 and DENV-2 symptomatic VCD, regardless of baseline serostatus, throughout an average of 3·7 years of follow-up. These findings support the continued development of Butantan-DV for the prevention of dengue disease in adults, adolescents, and children with and without previous dengue exposure. Data through the full 5 years of follow-up will provide further insights on the safety and efficacy of the vaccine.

Contributors

EGK, MLN, and SSW conceptualised the study. EGP and PEB designed the methods. EGK, DBP, JCdM, EEJ, IC-BC, MLN, MVGdL, ETAM, VSB, FR, CJFF, AMS, GASR, RQG, MMT, and AJdF collected the data. DHRS, FCB, JAM, JCVT, LBdOA, MATC, VI, AE-J, B-AGC, JM, and SGK curated the data. EGP, PEB, J-JL, and TS did the formal analysis. MLN, FCB, JAM, JCVT, LBdOA, EGP, VI, AE-J, B-AGC, JM, and SGK verified the data. All authors critically reviewed the manuscript and approved the manuscript for submission. To maintain masking in this ongoing trial, authors involved in the follow-up of patients had limited access to the full study data.

Declaration of interests

AE-J, TS, J-JL, and SGK are employees of Merck Sharp & Dohme (MSD) LLC, a subsidiary of Merck & Co (Rahway, NJ, USA), and may own stock or hold stock options in Merck & Co. JM is an employee of MSD, Argentina and may own stock or hold stock options in Merck & Co. B-AGC was an employee of MSD and may own or have owned stock or hold or have held options in Merck & Co at the time the study was conducted. Instituto Butantan is a non-profit public health institution of the state of São Paulo, Brazil. EGK, MATC, JAM, EGP, PEB, JCVT, LBdOA, VI, DHRS, and FCB are employees of Instituto Butantan. EGK was the primary site Principal Investigator and left to direct the Instituto Butantan, effective Jan 16, 2023. FCB reports owning stock from Novartis. SSW reports that the vaccine technology was licensed by Butantan Foundation Institute from his employer (the National Institutes of Health). VSB and FR report receipt of a research grant to their institution from Instituto Butantan intended to fund the clinical trial. All other authors declare no competing interests.

Data sharing

Data will be made available after Agência Nacional de Vigilância Sanitária approval to qualified scientific researchers with a specific purpose outlined in a proposal approved by Instituto Butantan. The complete de-identified patient dataset will be shared after the researcher enters into a standard data-sharing agreement and the proposal is approved. Researchers must commit to transparency in publication. Requests for data must be sent to ensaiosclinicos@butantan.gov.br.

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