

## Live, Attenuated, Tetravalent Butantan–Dengue Vaccine in Children and Adults

E.G. Kallás, M.A.T. Cintra, J.A. Moreira, E.G. Patiño, P.E. Braga, J.C.V. Tenório, V. Infante, R. Palacios, M.V.G. de Lacerda, D.B. Pereira, A.J. da Fonseca, R.Q. Gurgel, I.C.-B. Coelho, C.J.F. Fontes, E.T.A. Marques, G.A.S. Romero, M.M. Teixeira, A.M. Siqueira, A.M.P. Barral, V.S. Boaventura, F. Ramos, E. Elias Júnior, J. Cassio de Moraes, D.T. Covas, J. Kalil, A.R. Precioso, S.S. Whitehead, A. Esteves-Jaramillo, T. Shekar, J.-J. Lee, J. Macey, S.G. Kelner, B.-A.G. Collier, F.C. Boulos, and M.L. Nogueira

### ABSTRACT

#### BACKGROUND

Butantan–Dengue Vaccine (Butantan-DV) is an investigational, single-dose, live, attenuated, tetravalent vaccine against dengue disease, but data on its overall efficacy are needed.

#### METHODS

In an ongoing phase 3, double-blind trial in Brazil, we randomly assigned participants to receive Butantan-DV or placebo, with stratification according to age (2 to 6 years, 7 to 17 years, and 18 to 59 years); 5 years of follow-up is planned. The objectives of the trial were to evaluate overall vaccine efficacy against symptomatic, virologically confirmed dengue of any serotype occurring more than 28 days after vaccination (the primary efficacy end point), regardless of serostatus at baseline, and to describe safety up to day 21 (the primary safety end point). Here, vaccine efficacy was assessed on the basis of 2 years of follow-up for each participant, and safety as solicited vaccine-related adverse events reported up to day 21 after injection. Key secondary objectives were to assess vaccine efficacy among participants according to dengue serostatus at baseline and according to the dengue viral serotype; efficacy according to age was also assessed.

#### RESULTS

Over a 3-year enrollment period, 16,235 participants received either Butantan-DV (10,259 participants) or placebo (5976 participants). The overall 2-year vaccine efficacy was 79.6% (95% confidence interval [CI], 70.0 to 86.3) — 73.6% (95% CI, 57.6 to 83.7) among participants with no evidence of previous dengue exposure and 89.2% (95% CI, 77.6 to 95.6) among those with a history of exposure. Vaccine efficacy was 80.1% (95% CI, 66.0 to 88.4) among participants 2 to 6 years of age, 77.8% (95% CI, 55.6 to 89.6) among those 7 to 17 years of age, and 90.0% (95% CI, 68.2 to 97.5) among those 18 to 59 years of age. Efficacy against DENV-1 was 89.5% (95% CI, 78.7 to 95.0) and against DENV-2 was 69.6% (95% CI, 50.8 to 81.5). DENV-3 and DENV-4 were not detected during the follow-up period. Solicited systemic vaccine- or placebo-related adverse events within 21 days after injection were more common with Butantan-DV than with placebo (58.3% of participants, vs. 45.6%).

#### CONCLUSIONS

A single dose of Butantan-DV prevented symptomatic DENV-1 and DENV-2, regardless of dengue serostatus at baseline, through 2 years of follow-up. (Funded by Instituto Butantan and others; DEN-03-IB ClinicalTrials.gov number, NCT02406729, and WHO ICTRP number, U1111-1168-8679.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Castro Boulos can be contacted at fernanda.boulos@fundacaobutantan.org.br or at Instituto Butantan, Avenue Vital Brasil, 1500-Butantã, São Paulo, Brazil.

N Engl J Med 2024;390:397-408.

DOI: 10.1056/NEJMoa2301790

Copyright © 2024 Massachusetts Medical Society.

**CME**  
at NEJM.org

FOUR SEROTYPES OF DENGUE VIRUS (DENV) circulate worldwide, causing an estimated 390 million infections annually.<sup>1-3</sup> The largest burden of dengue disease occurs in Southeast Asia and Central and South America.<sup>4</sup> In Brazil, DENV is hyperendemic, with varying incidence across the country.<sup>5</sup> Although most primary DENV infections are asymptomatic or subclinical,<sup>1</sup> DENV can result in severe disease,<sup>2</sup> particularly with secondary heterotypic infection.<sup>3,6,7</sup> The goal of a dengue vaccine is to offer protection against all DENV serotypes.

There are currently two licensed tetravalent, live, attenuated dengue vaccines. CYD-TDV (Dengvaxia, Sanofi Pasteur), a three-dose dengue vaccine derived from the yellow fever virus, is licensed in several countries and is typically indicated for persons 9 to 45 years of age.<sup>8,9</sup> An increased risk of severe dengue after CYD-TDV vaccination among persons with no history of dengue infection has been observed.<sup>10,11</sup> Therefore, the World Health Organization (WHO) has recommended limiting the use of CYD-TDV to persons previously exposed to dengue or, if screening is not available, to areas in which the disease is endemic (with a seroprevalence of at least 80% by 9 years of age).<sup>12</sup> Recently, TAK-003, also known as Qdenga (Takeda), a two-dose dengue vaccine derived from DENV-2,<sup>13</sup> received approval in Indonesia for persons 6 to 45 years of age,<sup>14</sup> in the European Union for persons 4 years of age or older,<sup>15</sup> and in Brazil for persons 4 to 60 years of age<sup>16</sup>; approvals were for use of the vaccine in persons regardless of their dengue serostatus. An unmet need remains for a dengue vaccine that offers protection from a single dose across a wide age range, regardless of dengue serostatus at baseline.

Butantan–Dengue Vaccine (Butantan-DV) is a single-dose, live, attenuated, tetravalent dengue vaccine candidate composed of vaccine viruses representing all four DENV serotypes analogous to the TV003 formulation developed by the National Institutes of Health.<sup>17</sup> Numerous phase 1 studies of TV003 have shown the vaccine to be immunogenic, with a generally acceptable side-effect profile.<sup>18-22</sup> In a phase 2 trial involving adults in Brazil, Butantan-DV elicited immune responses across the four serotypes and had a generally acceptable side-effect profile, both in persons with no history of dengue exposure and those who had a history of exposure to dengue.<sup>23</sup>

Here, we report the efficacy and safety findings from 2 years of follow-up in an ongoing phase 3 trial to evaluate a single dose of Butantan-DV for the prevention of symptomatic, virologically confirmed dengue infection in children, adolescents, and adults regardless of their history of dengue exposure.

## METHODS

### TRIAL DESIGN, PARTICIPANTS, AND OVERSIGHT

DEN-03-IB is an ongoing phase 3, randomized, double-blind, placebo-controlled trial, with 5 years of planned follow-up, being conducted at 16 sites in Brazil. The individual-level data according to trial-group assignment were prespecified to remain concealed through the end of the trial; thus, participant-level safety data are only available in an unblinded manner to prespecified members of the study team and to the external independent data and safety monitoring committee. Participants were eligible if they were 2 to 59 years of age and were healthy or had clinically controlled disease. Key exclusion criteria included immunocompromising conditions, pregnancy, and previous receipt of a dengue vaccine. All inclusion and exclusion criteria are listed in the protocol, available with the full text of this article at NEJM.org.

Participants underwent randomization according to age (2 to 6 years of age, 7 to 17 years of age, and 18 to 59 years of age) and in a 2:1 ratio to receive a single dose of Butantan-DV or placebo. Enrollment occurred from February 2016 to July 2019, starting with the adult group and proceeding to the remaining age groups in descending order after interim safety analyses were performed by the data and safety monitoring committee.

The trial was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and was approved by the Brazilian Ministry of Health. Participants or their legal representatives provided written informed consent; assent was obtained for children 7 to 17 years of age. The data and safety monitoring committee provided safety oversight. The conduct of the trial was overseen by Instituto Butantan, which designed the protocol and managed the data. Investigators and site staff provided feedback on the trial design and collected the data. Additional data management and analyses were supported by Merck Sharp

 A Quick Take  
is available at  
NEJM.org

and Dohme (a subsidiary of Merck). To maintain blinding of the participants and the investigators to the data in this ongoing trial, the authors who were involved in participant follow-up had limited access to the trial data. Authors who were not involved in participant follow-up had full access to the data. The authors vouch for the completeness and accuracy of the data and for the fidelity of the trial to the protocol.

#### RANDOMIZATION AND BLINDING

Computer-generated randomization sequences for each age strata were entered into an electronic central randomization system by an independent statistician. The syringes containing vaccine or placebo were prepared by a trial pharmacist who was aware of the syringe contents and was not involved in subsequent participant assessments. Other site staff, the data management team, and the participants remained unaware of group assignments and data during the trial.

#### PROCEDURES

A blood sample was obtained before injection with vaccine or placebo to retrospectively determine the participants' dengue serostatus at baseline with the use of a validated virus reduction neutralization test with a 60% neutralization cutoff (VRNT<sub>60</sub>; details are provided in the Supplemental Methods section of the Supplementary Appendix, available at NEJM.org).<sup>21,24</sup> Previous exposure to any DENV serotype was defined as a baseline VRNT<sub>60</sub> titer against any of the four serotypes above the lower limit of quantification (i.e.,  $\geq 18$  for DENV-1,  $\geq 15$  for DENV-2,  $\geq 12$  for DENV-3, or  $\geq 13$  for DENV-4). At the same visit (day 0), a single 0.5-ml dose of either Butantan-DV or placebo was administered subcutaneously. The participants were instructed to complete a patient diary to record adverse events for 21 days after injection. A follow-up visit occurred at week 4 for all participants. Follow-up contacts to check the occurrence of adverse events and the possibility of dengue were conducted at least monthly by means of electronic communication, by telephone, or in person throughout the 2-year follow-up period.

#### VACCINE DESCRIPTION

Butantan-DV is composed of attenuated vaccine viruses for DENV-1, DENV-3, and DENV-4 (rDENV1Δ30, rDENV3Δ30/31, and rDENV4Δ30)

and a chimeric vaccine virus containing the DENV-2 genes encoding the premembrane (prM) and envelope (E) proteins on the attenuated DENV-4 background (rDENV2/4Δ30[ME]).<sup>17</sup> The vaccine was produced, formulated, and lyophilized at Instituto Butantan. The lyophilized product was reconstituted with phosphate buffer. Each 0.5-ml dose of Butantan-DV targeted delivery of  $10^3$  plaque-forming units of each vaccine virus strain. The placebo dose contained 0.5 ml of customized Leibovitz medium that had been prepared at a double concentration, lyophilized, and reconstituted with phosphate buffer.

#### EFFICACY ASSESSMENTS AND END POINTS

Vaccine efficacy was assessed on the basis of symptomatic, virologically confirmed dengue occurring more than 28 days after injection. Participants were instructed to seek out the trial team if they had a fever or other symptoms that could be present in a case of dengue. A blood sample was collected, preferably within 9 days after the onset of symptoms, to evaluate the possibility of dengue. Virologic confirmation and determination of serotype were made with the use of a fourplex reverse-transcriptase-polymerase-chain-reaction assay.<sup>25</sup> Vaccine efficacy according to previous dengue exposure was calculated for participants who had dengue serostatus at baseline (i.e., those who had preinjection VRNT<sub>60</sub> results).

Here we report the prespecified vaccine efficacy analysis from 2 years of follow-up for each participant. The primary efficacy end point was symptomatic, virologically confirmed dengue more than 28 days after injection regardless of previous exposure to dengue. Key secondary end points included virologically confirmed dengue more than 28 days after injection according to dengue serostatus at baseline and according to the dengue viral serotype. A subgroup analysis determined vaccine efficacy according to age group.

#### SAFETY ASSESSMENTS AND END POINTS

Safety assessments included the monitoring of solicited (administration-site and systemic) and unsolicited adverse events that occurred within 21 days after injection. Unsolicited adverse events, including serious adverse events, were recorded throughout the follow-up period. Participants recorded adverse events in a diary during the



21 days after injection and reported adverse event by means of periodic telephone calls; the investigators evaluated adverse events for severity and causality with respect to the vaccine or placebo. In this trial, all adverse events that presented a reasonable causal relation to the vaccine or placebo were considered to be adverse reactions and are referred to as vaccine- or placebo-related adverse events in this report.

The primary safety end point was solicited (administration-site and systemic) and unsolicited vaccine- or placebo-related adverse events through day 21 in participants 2 to 59 years of age regardless of previous exposure to dengue. Key secondary end points included solicited and unsolicited vaccine- or placebo-related adverse events through day 21 according to dengue serostatus at baseline and according to age group and the frequency of unsolicited vaccine- or placebo-related adverse events after day 22.

#### STATISTICAL ANALYSIS

To calculate the sample size for analysis of the primary end point (vaccine efficacy), we used the formula developed by Blackwelder<sup>26</sup> with Poisson approximation (see the protocol and Supplemental Methods). The primary efficacy analysis was performed in the per-protocol population, which included all eligible participants who had not used restricted medications and had provided written informed consent, undergone randomization, and received their assigned vaccine or placebo in accordance with the handling and administration conditions recommended by the manufacturer.

Vaccine efficacy was determined with the use of the following formula: vaccine efficacy =  $[1 - (1 + s)\theta] / [1 - \theta]$ , in which  $s$  is the ratio of follow-up times in the control group to that in the vaccine group and  $\theta$  is the proportion of all dengue cases that occurred in the vaccine group. Vaccine efficacy was calculated with the use of the conditional approximation described by Chan and Bohidar,<sup>27</sup> with confidence intervals calculated with the use of Blaker's method<sup>28</sup> (see Supplemental Methods). The end point of vaccine efficacy was considered to have been met if the lower bound of the two-sided 95% confidence interval was greater than 25% for DENV disease caused by any serotype (combined) for the primary end point or caused by each serotype (separately) for the secondary end points. Vaccine efficacy

was calculated on the basis of 2 years of follow-up for each participant.

Safety analyses were performed in the as-treated population (all participants who had undergone randomization and received at least one dose of vaccine or placebo and were assessed according to the injection they actually received). Descriptive summaries, the frequency of vaccine- or placebo-related adverse events through day 21 after injection and the frequency of unsolicited vaccine- or placebo-related adverse events through 2 years of follow-up were summarized.

## RESULTS

### PARTICIPANTS

A total of 16,235 participants underwent randomization and received vaccine (10,259 participants) or placebo (5976 participants) (Fig. 1). The per protocol efficacy population included 10,215 in the vaccine group and 5947 in the placebo group. Characteristics of the participants at baseline were generally similar in the two groups (Table 1) and were generally representative of persons at risk for dengue disease in Brazil (Table S3 in the Supplementary Appendix). Approximately one third of the participants were enrolled in each of the three age groups, according to the protocol. Nearly half the participants (47.3% in the vaccine group and 45.2% in the placebo group) did not have evidence of previous exposure to any DENV serotype. Characteristics of each age group at baseline were generally consistent with the overall population. However, in both the vaccine group and placebo group, a higher percentage of participants with evidence of previous dengue exposure was observed in the older age groups (7 to 17 years of age and 18 to 59 years of age) than in the youngest age group (2 to 6 years of age) (Table S4).

### INCIDENCE OF SYMPTOMATIC VIROLOGICALLY CONFIRMED DENGUE

The cumulative incidence of symptomatic virologically confirmed dengue for any DENV serotype over time for each trial group is shown in Figure 2. During 2 years of follow-up, 135 participants had virologically confirmed dengue that occurred more than 28 days after injection: 59 cases of DENV-1 and 76 cases of DENV-2 (Table 2). Cases of DENV-3 or DENV-4 were not observed.

**EFFICACY**

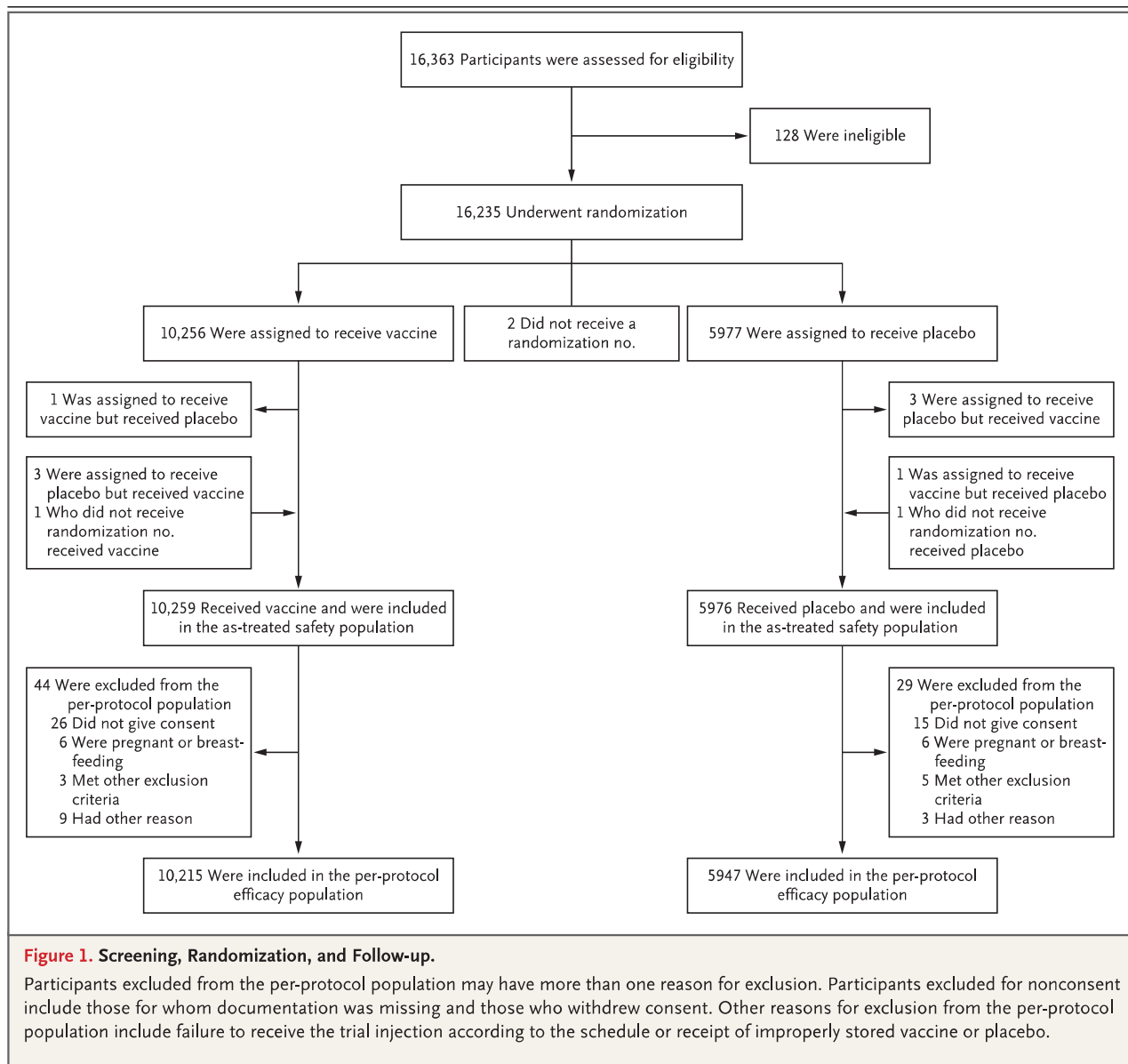
Over the 2-year follow-up, vaccine efficacy against any DENV serotype was 79.6% (95% CI, 70.0 to 86.3) (Table 2). Secondary end points of serotype-specific vaccine efficacy were 89.5% (95% CI, 78.7 to 95.0) against DENV-1 and 69.6% (95% CI, 50.8 to 81.5) against DENV-2. Regarding baseline dengue serostatus, vaccine efficacy against any serotype was 73.6% (95% CI, 57.6 to 83.7) among participants without evidence of previous dengue exposure (7516 participants) and 89.2% (95% CI, 77.6 to 95.6) among those with evidence of previous dengue exposure (8017 participants).

Vaccine efficacy through the 2-year follow-up

according to age group and according to age subgroups that were defined on the basis of dengue serostatus at baseline is shown in Table S5. Vaccine efficacy against any DENV serotype regardless of dengue serostatus at baseline, according to age group, was 80.1% among participants 2 to 6 years of age (95% CI, 66.0 to 88.4), 77.8% among those 7 to 17 years of age (95% CI, 55.6 to 89.6), and 90.0% among those 18 to 59 years of age (95% CI, 68.2 to 97.5).

**SAFETY**

The percentage of participants with serious adverse events occurring within 21 days after injection



**Table 1. Characteristics of the Participants at Baseline.\***

|                                                   | Vaccine<br>(N = 10,259) | Placebo<br>(N = 5976) | Total<br>(N = 16,235) |
|---------------------------------------------------|-------------------------|-----------------------|-----------------------|
| Sex — no. (%)                                     |                         |                       |                       |
| Female                                            | 5555 (54.1)             | 3216 (53.8)           | 8771 (54.0)           |
| Male                                              | 4704 (45.9)             | 2760 (46.2)           | 7464 (46.0)           |
| Age distribution — no. (%)                        |                         |                       |                       |
| 2–6 yr                                            | 3337 (32.5)             | 1679 (28.1)           | 5016 (30.9)           |
| 7–17 yr                                           | 3376 (32.9)             | 1771 (29.6)           | 5147 (31.7)           |
| 18–59 yr                                          | 3546 (34.6)             | 2526 (42.3)           | 6072 (37.4)           |
| Median age (IQR) — yr                             | 11 (5–31)               | 14 (6–36)             | 12 (6–33)             |
| Race or ethnic group — no. (%)†                   |                         |                       |                       |
| Pardo                                             | 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)              |
| Hispanic ethnic group — no. (%)‡                  |                         |                       |                       |
| No                                                | 10,068 (98.1)           | 5861 (98.1)           | 15,929 (98.1)         |
| Yes                                               | 191 (1.9)               | 115 (1.9)             | 306 (1.9)             |
| Previous exposure to DENV — no. (%)§              |                         |                       |                       |
| Yes                                               |                         |                       |                       |
| Any serotype                                      | 5009 (48.8)             | 3041 (50.9)           | 8050 (49.6)           |
| Monovalent                                        | 591 (5.8)               | 304 (5.1)             | 895 (5.5)             |
| Bivalent                                          | 340 (3.3)               | 173 (2.9)             | 513 (3.2)             |
| Trivalent                                         | 462 (4.5)               | 318 (5.3)             | 780 (4.8)             |
| Tetravalent                                       | 3616 (35.2)             | 2246 (37.6)           | 5862 (36.1)           |
| No                                                | 4855 (47.3)             | 2700 (45.2)           | 7555 (46.5)           |
| Unknown or missing data¶                          | 395 (3.9)               | 235 (3.9)             | 630 (3.9)             |
| Previous exposure to DENV, by serotype — no. (%)§ |                         |                       |                       |
| DENV-1                                            | 4295 (41.9)             | 2666 (44.6)           | 6961 (42.9)           |
| DENV-2                                            | 4487 (43.7)             | 2766 (46.3)           | 7253 (44.7)           |
| DENV-3                                            | 3801 (37.1)             | 2365 (39.6)           | 6166 (38.0)           |
| DENV-4                                            | 4538 (44.2)             | 2791 (46.7)           | 7329 (45.1)           |
| Unknown or missing data¶                          | 395 (3.9)               | 235 (3.9)             | 630 (3.9)             |

\* Each participant can be counted in one or more applicable rows. DENV denotes dengue virus and VRNT<sub>60</sub> 60% virus reduction neutralization test.

† Race and ethnic group were reported by the participants. American Indian and Alaska Native were reported as Indigenous, Black and African American were reported as Black, multiracial Brazilian was reported as Pardo, and Hispanic and Latino were reported as Hispanic.

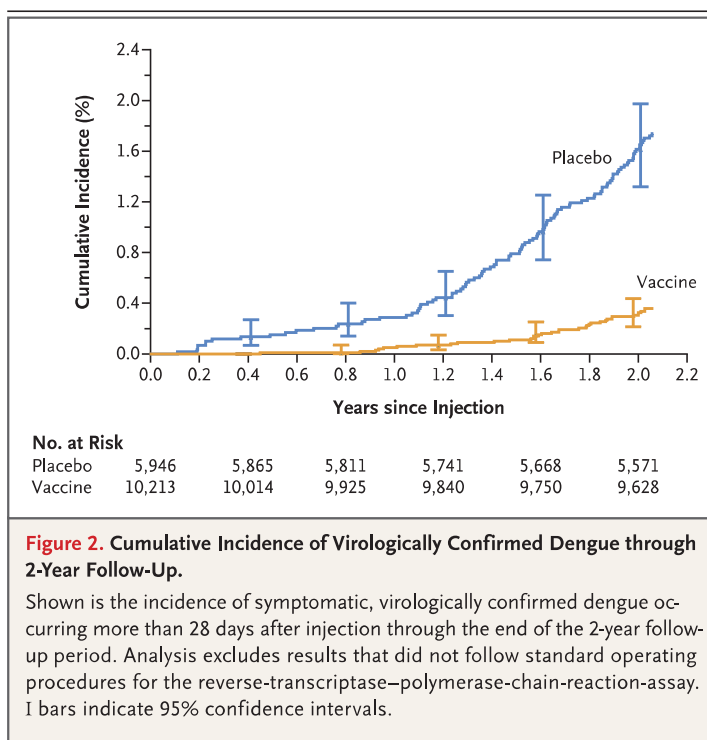
‡ Previous exposure to dengue virus is defined as a baseline DENV-1 VRNT<sub>60</sub> titer of at least 18, DENV-2 VRNT<sub>60</sub> titer of at least 15, DENV-3 VRNT<sub>60</sub> titer of at least 12, or DENV-4 VRNT<sub>60</sub> titer of at least 13.

§ Unknown results include missing test results and results from samples obtained from participants who did not provide written informed consent or who were not tested for DENV-3. Missing data denotes participants who did not undergo serologic testing because they did not provide informed consent or for other reasons.

was similar in the two groups. Serious adverse events occurred in 20 participants in the vaccine group (0.2%) and in 8 participants in the placebo group (0.1%) (Table 3 and Table S6). One death that was not related to the vaccine or placebo (as assessed by the investigator) occurred within 21 days after injection. The percentage of participants with serious adverse events in the 2-year follow-up period was 3.9% in the vaccine group and 4.0% in the placebo group (Table S7). There were 16 deaths in the 2-year follow-up period: 9 in the vaccine group and 7 in the placebo group. None of the deaths were deemed by the investigators to be caused by dengue or related to the vaccine or placebo.

Within 21 days after injection, three participants in the vaccine group and two participants in the placebo group had serious adverse events that were considered to be related to the vaccine or placebo (Table 3). Two additional participants had vaccine- or placebo-related serious adverse events occurring at 22 days or later after injection, for a total of seven vaccine- or placebo-related serious adverse events (i.e., Bell's palsy, bronchospasm, facial paralysis, Guillain-Barré syndrome, peripheral neuropathy, transverse sinus thrombosis, and viral infection) in the 2 years of follow-up. The trial-group assignments with respect to the participant-level safety data remain concealed; however, the data and safety monitoring committee reviewed all serious adverse events and did not recommend unblinding of the safety data in any case.

The majority of solicited and unsolicited adverse events occurring within 21 days after injection were mild to moderate in severity (Tables S8 and S9). The percentage of participants with solicited systemic, vaccine- or placebo-related adverse events was higher among those who received vaccine (58.3%) than among those who received placebo (45.6%) (Table 3). The solicited administration-site, vaccine- or placebo-related adverse event that was most reported was administration-site pain reported by 14.9% of the participants in the vaccine group and by 11.1% of those in the placebo group. The solicited systemic, vaccine- or placebo-related adverse events that were reported most were headache (reported by 36.4% of the participants in the vaccine group and by 30.9% of those in the placebo group), fatigue (reported by 19.3% and 15.1%, respectively), and rash (reported by 22.5% and



**Figure 2. Cumulative Incidence of Virologically Confirmed Dengue through 2-Year Follow-Up.**

Shown is the incidence of symptomatic, virologically confirmed dengue occurring more than 28 days after injection through the end of the 2-year follow-up period. Analysis excludes results that did not follow standard operating procedures for the reverse-transcriptase–polymerase-chain-reaction-assay. I bars indicate 95% confidence intervals.

4.2%, respectively). The percentage of participants with adverse events occurring within 21 days after injection according to age group and dengue serostatus at baseline are shown in Table S10 and S11. The percentage of participants with solicited systemic, vaccine- or placebo-related adverse events tended to be higher as participant age increased.

## DISCUSSION

A single dose of Butantan-DV was efficacious in children, adolescents, and adults across an age range of 2 to 59 years in this ongoing phase 3 trial. Our primary efficacy criterion was met, with a vaccine efficacy of approximately 80% for protection against symptomatic, virologically confirmed dengue through 2 years of follow-up. Solicited systemic, vaccine- or placebo-related adverse events were reported more frequently by recipients of Butantan-DV than by recipients of placebo, a finding that is similar to the safety profile described in the phase 2 trial.<sup>23</sup>

Approximately half the participants in our trial did not have evidence of previous dengue exposure at baseline, which enabled robust assessments of vaccine efficacy and safety in this

**Table 2. Vaccine Efficacy at 2 Years after Injection.\***

| Confirmed Dengue          | Vaccine                         |                       |                                 | Placebo                         |                       |                                 | Cumulative<br>Vaccine Efficacy<br>(95% CI)<br>% |
|---------------------------|---------------------------------|-----------------------|---------------------------------|---------------------------------|-----------------------|---------------------------------|-------------------------------------------------|
|                           | Cases<br><i>no. (total no.)</i> | Person-Yrs<br>at Risk | Estimated Incidence<br>(95% CI) | Cases<br><i>no. (total no.)</i> | Person-Yrs<br>at Risk | Estimated Incidence<br>(95% CI) |                                                 |
| Any serotype              |                                 |                       |                                 |                                 |                       |                                 |                                                 |
| Regardless of serostatus  | 35/10,215                       | 20,452                | 0.17 (0.12 to 0.24)             | 100/5,947                       | 11,927                | 0.84 (0.68 to 1.02)             | 79.6 (70.0 to 86.3)                             |
| With previous exposure    | 8/4,994                         | 10,063                | 0.08 (0.03 to 0.16)             | 45/3,023                        | 6,092                 | 0.74 (0.54 to 0.99)             | 89.2 (77.6 to 95.6)                             |
| Without previous exposure | 26/4,826                        | 9,573                 | 0.27 (0.18 to 0.40)             | 55/2,690                        | 5,350                 | 1.03 (0.77 to 1.34)             | 73.6 (57.6 to 83.7)                             |
| DENV-1                    |                                 |                       |                                 |                                 |                       |                                 |                                                 |
| Regardless of serostatus  | 9/10,215                        | 20,463                | 0.04 (0.02 to 0.08)             | 50/5,947                        | 11,950                | 0.42 (0.31 to 0.55)             | 89.5 (78.7 to 95.0)                             |
| With previous exposure    | 1/4,994                         | 10,065                | 0.01 (0.00 to 0.06)             | 19/3,023                        | 6,101                 | 0.31 (0.19 to 0.49)             | 96.8 (81.0 to 99.8)                             |
| Without previous exposure | 8/4,826                         | 9,582                 | 0.08 (0.04 to 0.17)             | 31/2,690                        | 5,365                 | 0.58 (0.39 to 0.82)             | 85.6 (69.1 to 94.0)                             |
| DENV-2                    |                                 |                       |                                 |                                 |                       |                                 |                                                 |
| Regardless of serostatus  | 26/10,215                       | 20,458                | 0.13 (0.08 to 0.19)             | 50/5,947                        | 11,967                | 0.42 (0.31 to 0.55)             | 69.6 (50.8 to 81.5)                             |
| With previous exposure    | 7/4,994                         | 10,063                | 0.07 (0.03 to 0.14)             | 26/3,023                        | 6,107                 | 0.43 (0.28 to 0.62)             | 83.7 (63.1 to 93.5)                             |
| Without previous exposure | 18/4,826                        | 9,579                 | 0.19 (0.11 to 0.30)             | 24/2,690                        | 5,376                 | 0.45 (0.29 to 0.66)             | 57.9 (20.8 to 78.1)                             |

\* Vaccine efficacy was estimated on the basis of the exact binomial method proposed by Chan and Bohidar,<sup>27</sup> and 95% confidence intervals were estimated with the use of Blaker's exact confidence interval.<sup>28</sup> The person-years at risk was the cumulative time (in years) until the participant received a diagnosis of the first symptomatic episode of virologically confirmed dengue or until the end of the 2-year follow-up period for each participant, whichever came first. Incidence (per 100 person-years at risk) was calculated as the number of symptomatic, virologically confirmed dengue cases (the number of participants with at least one symptomatic virologically confirmed dengue episode more than 28 days after injection until the end of the follow-up period) divided by the cumulative person-years at risk. Vaccine efficacy according to previous dengue exposure was calculated for participants with dengue serostatus at baseline (those who had preinjection VRNT<sub>60</sub> results). Only DENV-1 and DENV-2 were detected during the 2-year follow-up.



population. After 2 years of follow-up, vaccine efficacy was nearly 74% in participants without evidence of previous dengue exposure. Previous evaluations of the yellow fever-based CYD-TDV dengue vaccine showed an increased risk of severe dengue after vaccination in persons without previous exposure to dengue,<sup>10</sup> a finding that highlights the need for inclusion of this population in studies of dengue vaccines. In the current trial, we enrolled a substantial percentage of participants without previous dengue exposure whom we plan to follow-up for a projected 5 years in a country where dengue is endemic, thereby allowing a careful assessment of vaccine efficacy and safety in this population.

The effect of dengue serostatus at baseline on vaccine efficacy and safety is particularly important for young children, who are less likely to have had previous dengue exposure at the time of vaccination. In previous studies of CYD-TDV, vaccine efficacy was lower among young children (<9 years of age) than among children 9 years of age or older, who were not as likely to be seronegative.<sup>11</sup> In addition, children between 2 and 5 years of age who were seronegative had an increased risk of hospitalization or severe dengue after CYD-TDV vaccination.<sup>10</sup> In the current trial, approximately one third of the participants were 2 to 6 years of age, 81% of whom had not had previous dengue exposure, which makes this age group an ideal target for vaccination to protect against first infection. After 2 years of follow-up, vaccine efficacy among children 2 to 6 years of age without previous dengue exposure was 73%, a finding which is similar to that in the older age groups. In addition, no safety concerns were identified among all three age groups regardless of dengue exposure. Longer-term follow-up of the youngest age group will be important to assess the durability of protection conferred by Butantan-DV and the severity of any breakthrough infections after vaccination.

A single dose of Butantan-DV offered protection against symptomatic, virologically confirmed dengue over the follow-up period. In previous trials, a second dose of Butantan-DV or TV003 given at 6 months or 12 months did not induce vaccine viremia or substantially boost antibody responses.<sup>19-21,23</sup> Thus, the single-dose schedule of Butantan-DV was advanced to the current phase 3 trial. The utility, if any, of a booster dose given after an extended period (>12 months) is

**Table 3. Adverse Events within 21 Days after Injection.\***

| Adverse Event Type                          | Vaccine<br>(N=10,259) | Placebo<br>(N=5976) |
|---------------------------------------------|-----------------------|---------------------|
|                                             | no. (%)               |                     |
| <b>Any adverse event</b>                    | 7137 (69.6)           | 3595 (60.2)         |
| Serious                                     | 20 (0.2)              | 8 (0.1)             |
| Unsolicited                                 | 3360 (32.8)           | 1917 (32.1)         |
| Systemic                                    | 6204 (60.5)           | 2864 (47.9)         |
| <b>Event related to vaccine or placebo†</b> | 6527 (63.6)           | 3109 (52.0)         |
| Serious                                     | 3 (<0.1)              | 2 (<0.1)            |
| Unsolicited                                 | 1391 (13.6)           | 720 (12.0)          |
| Solicited                                   | 6395 (62.3)           | 2998 (50.2)         |
| Administration-site adverse event‡          | 2012 (19.6)           | 879 (14.7)          |
| Pain                                        | 1527 (14.9)           | 665 (11.1)          |
| Pruritus                                    | 585 (5.7)             | 239 (4)             |
| Erythema                                    | 318 (3.1)             | 92 (1.5)            |
| Induration                                  | 195 (1.9)             | 94 (1.6)            |
| Swelling                                    | 120 (1.2)             | 63 (1.1)            |
| Systemic adverse event                      | 5980 (58.3)           | 2725 (45.6)         |
| Headache                                    | 3734 (36.4)           | 1846 (30.9)         |
| Asthenia                                    | 1984 (19.3)           | 905 (15.1)          |
| Exanthema                                   | 2312 (22.5)           | 250 (4.2)           |
| Myalgia                                     | 1789 (17.4)           | 757 (12.7)          |
| Pruritus                                    | 1938 (18.9)           | 526 (8.8)           |
| Retro-orbital eye pain                      | 1618 (15.8)           | 641 (10.7)          |
| Nausea                                      | 1244 (12.1)           | 631 (10.6)          |
| Arthralgia                                  | 1152 (11.2)           | 487 (8.1)           |
| Photophobia                                 | 993 (9.7)             | 486 (8.1)           |
| Pyrexia§                                    | 1047 (10.2)           | 398 (6.7)           |
| Chills                                      | 871 (8.5)             | 328 (5.5)           |
| Vomiting                                    | 574 (5.6)             | 291 (4.9)           |

\* For specific administration-site and systemic adverse events, every participant was counted once for each applicable row and column.

† All adverse events that presented a reasonable causal relation to the trial vaccine or placebo were considered to be vaccine- or placebo-related adverse events.

‡ All administration-site reactions after injection were considered to be adverse events with causal relation to the vaccine or placebo.

§ Pyrexia (fever) was solicited on the form for reporting form for data relative to suspected dengue from the time of injection through day 21 after injection.

not currently known. There are several advantages to a single-dose dengue vaccine. Protection from disease after one dose of vaccine may be especially important for travelers or for an outbreak response when rapid protection is needed. A one-dose schedule eliminates the time

between doses in which a person may have partial or incomplete immunity. Furthermore, a one-dose vaccine may ease logistic and economic considerations, simplify vaccine scheduling, and increase uptake.

The safety profile of Butantan-DV reported here is consistent with that shown in a previous phase 2 trial of Butantan-DV<sup>23</sup> and numerous phase 1 studies of the analogous vaccine TV003.<sup>18-22</sup> The incidence of rash among Butantan-DV vaccinees in this trial was numerically lower (23%) than that observed in the phase 2 trial of Butantan-DV (45 to 65%).<sup>23</sup> The reasons for the differences between the trials in the incidence of observed rash are not known, and demographic characteristics of the participants or their dengue serostatus at baseline could affect the results. The phase 2 trial was conducted in an area where most of the population had not had previous exposure to dengue and rash was observed more frequently among participants who did not have previous exposure to DENV (65%) than among those who had a history of exposure (45%).<sup>23</sup> In addition, observer perception may play a role. In this trial, rash was reported by the participant or parent; in the phase 2 trial, rash was reported by a trial physician.

Our trial had several limitations. Cases of DENV-3 or DENV-4 were not observed during the follow-up period, thereby preventing the evaluation of vaccine efficacy against these serotypes. The absence of DENV-3 and DENV-4 corresponds with the lower circulation of these DENV serotypes in Brazil during the trial period.<sup>29-32</sup> Although numerous DENV serotypes cocirculate and infect a wide range of populations, DENV-1 and DENV-2 may be more commonly associated with disease and (in the case of DENV-2) severe clinical outcomes<sup>33,34</sup> and are the serotypes against which the vaccine trial showed protection. The effect of preexisting immunity from other flaviviruses (i.e., the viruses that cause Zika and yellow fever) on subsequent DENV infection or Butantan-DV vaccination needs to be explored. The large-scale Zika outbreak in Brazil may have driven lower incidence rates of dengue in 2016 through 2018,<sup>35,36</sup> shortly after the start of this trial. The incidence of dengue with warning

signs and severe dengue during the 2-year follow-up period was low in both the vaccine and placebo groups, a finding that is reflective of the overall lower rates of virologically confirmed dengue, precluding meaningful analyses and potentially resulting in the unblinding of data in this ongoing trial. Serologic tests for dengue inherently capture cross-reactive antibodies<sup>37-39</sup>; thus, understanding the effects of type-specific immunity at baseline on vaccine efficacy is not possible at this time. We report the prespecified outcomes for the initial 2 years of the trial; ongoing assessments through 5 years of follow-up, as recommended by the WHO,<sup>40</sup> aim to provide longer-term insights regarding safety and efficacy.

In this phase 3 trial, a single administration of Butantan-DV was shown to have a favorable safety profile and be efficacious in preventing symptomatic, virologically confirmed dengue caused by DENV-1 and DENV-2, irrespective of previous dengue exposure, throughout a 2-year follow-up. These data support the continued development of Butantan-DV for the prevention of dengue disease in adults and children.

Supported by Instituto Butantan, Merck Sharp and Dohme (a subsidiary of Merck), the Ministry of Health of Brazil, the Brazilian National Bank for Economic and Social Development, the São Paulo Research Foundation, and Fundação Butantan.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank the participants and their parents, the investigators, trial site staff, contract research organization team (IQVIA Brazil), and the sponsor personnel for their contributions to the implementation and conduct of the trial; former and current Instituto Butantan employees who were crucial to bringing about the trial; former Instituto Butantan President-Directors Isaias Raw, M.D., Ph.D., and Rui Curi, Ph.D., for their scientific contributions to the program; Anna Durbin, M.D., for her decades of work in clinically evaluating the dengue vaccine candidates developed by the Laboratory of Infectious Diseases at the National Institute of Allergy and Infectious Diseases for the guidance she provided when the vaccine manufacturing capabilities and clinical development plan for Butantan-DV were being established at Instituto Butantan, and for accepting the role as a member of the data and safety monitoring board; Merck Sharp and Dohme (a subsidiary of Merck) for statistical analyses and additional data management support; and Elizabeth Anderson, Ph.D., and Kara Cox, M.S., for medical writing assistance with the first draft of the manuscript, and Karyn Davis, B.A., for editorial assistance with an earlier draft of the manuscript (all of Merck Sharp and Dohme).

We dedicate this trial to the memory of Prof. Isaias Raw.

## APPENDIX

The authors' full names and academic degrees are as follows: Esper G. Kallás, M.D., Ph.D., Monica A.T. Cintra, M.D., Ph.D., José A. Moreira, M.D., Ph.D., Elizabeth G. Patiño, Ph.D., Patricia Emilia Braga, Ph.D., Juliana C.V. Tenório, Ph.D., Vanessa Infante, M.D., Ricardo Palacios, M.D., Ph.D., Marcus Vinicius Guimarães de Lacerda, M.D., Ph.D., Dhelio Batista Pereira, M.D., Ph.D., Allex Jardim da Fonseca, M.D., Ph.D., Ricardo Queiroz Gurgel, Ph.D., Ivo Castelo-Branco Coelho, M.D., Ph.D., Cor Jesus Fernandes Fontes, M.D., Ph.D., Ernesto T.A. Marques, M.D., Ph.D., Gustavo Adolfo Sierra Romero, M.D., Ph.D., Mauro Martins Teixeira, M.D., Ph.D., André M. Siqueira, M.D., Ph.D., Aldina Maria Prado Barral, M.D., Ph.D., Viviane Sampaio Boaventura, M.D., Ph.D., Fabiano Ramos, M.D., Erivaldo Elias Júnior, M.D., Ph.D., José Cassio de Moraes, M.D., Ph.D., Dimas T. Covas, M.D., Ph.D., Jorge Kalil, M.D., Ph.D., Alexander Roberto Precioso, M.D., Ph.D., Stephen S. Whitehead, Ph.D., Alejandra Esteves-Jaramillo, M.D., M.P.H., Tulin Shekar, M.S., Jung-Jin Lee, Ph.D., Julieta Macey, M.D., Sabrina Gozlan Kelner, M.Sc., Beth-Ann G. Collier, Ph.D., Fernanda Castro Boulos, M.D., and Mauricio L. Nogueira, M.D., Ph.D.

The authors' affiliations are as follows: Instituto Butantan (E.G.K., M.A.T.C., J.A.M., E.G.P., P.E.B., J.C.V.T., V.I., R.P., D.T.C., J.K., A.R.P., F.C.B.), Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (E.G.K.), Departamento de Saúde Coletiva, Faculdade de Ciências Médicas Santa Casa de São Paulo (J.C.M.), and Departamento de Clínica Médica, Faculdade de Medicina, Universidade de São Paulo (J.K.), São Paulo, Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus (M.V.G.L.), Centro de Pesquisa em Medicina Tropical de Rondonia, Porto Velho (D.B.P.), Universidade Federal de Roraima, Boa Vista (A.J.F.), Programa de Pós-graduação em Ciências da Saúde, Universidade Federal de Sergipe, Aracaju (R.Q.G.), Faculdade de Medicina da Universidade Federal do Ceará, Fortaleza (I.C.-B.C.), Hospital Universitário Júlio Müller, Cuiabá (C.J.F.F.), Instituto Aggeu Magalhães, Fundação Oswaldo Cruz, Recife (E.T.A.M.), Núcleo de Medicina Tropical, Faculdade de Medicina, Universidade de Brasília, Brasília (G.A.S.R.), the Center for Advanced and Innovative Therapies, Universidade Federal de Minas Gerais, Belo Horizonte (M.M.T.), Instituto Nacional de Infectologia Evandro Chagas, Rio de Janeiro (A.M.S.), Medicina e Saúde Pública de Precisão, Fundação Oswaldo Cruz—Instituto Gonçalo Moniz (IGM/FIOCRUZ), Salvador (A.M.P.B., V.S.B.), Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (F.R.), Faculdade de Medicina da Universidade Federal de Mato Grosso do Sul, Campo Grande (E.E.J.), Faculdade de Medicina de Ribeirão Preto, Universidade of São Paulo, Ribeirão Preto (D.T.C.), and Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto (M.L.N.) — all in Brazil; the School of Public Health, University of Pittsburgh, Pittsburgh (E.T.A.M.); the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD (S.S.W.); Merck, Rahway, NJ (A.E.-J., T.S., J.-J.L., S.G.K., B.-A.G.C.); Merck Sharp and Dohme, Munro, Argentina (J.M.); and the Department of Pathology, University of Texas Medical Branch, Galveston (M.L.N.).

## REFERENCES

- Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature* 2013;496:504-7.
- Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization, 2009.
- Simmons CP, Farrar JJ, van Vinh Chau N, Wills B. Dengue. *N Engl J Med* 2012; 366:1423-32.
- Stanaway JD, Shepard DS, Undurraga EA, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. *Lancet Infect Dis* 2016; 16:712-23.
- Júnior JBS, Massad E, Lobao-Neto A, Kastner R, Oliver L, Gallagher E. Epidemiology and costs of dengue in Brazil: a systematic literature review. *Int J Infect Dis* 2022;122:521-8.
- Halstead SB. Pathogenesis of dengue: challenges to molecular biology. *Science* 1988;239:476-81.
- Katzelnick LC, Gresh L, Halloran ME, et al. Antibody-dependent enhancement of severe dengue disease in humans. *Science* 2017;358:929-32.
- Capeding MR, Tran NH, Hadinegoro SR, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 2014;384:1358-65.
- Villar L, Dayan GH, Arredondo-García JL, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med* 2015;372:113-23.
- Sridhar S, Luedtke A, Langevin E, et al. Effect of dengue serostatus on dengue vaccine safety and efficacy. *N Engl J Med* 2018;379:327-40.
- Hadinegoro SR, Arredondo-García JL, Capeding MR, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N Engl J Med* 2015; 373:1195-206.
- Dengue vaccine: WHO position paper, September 2018 — recommendations. *Vaccine* 2019;37:4848-9.
- Biswal S, Reynales H, Saez-Llorens X, et al. Efficacy of a tetravalent dengue vaccine in healthy children and adolescents. *N Engl J Med* 2019;381:2009-19.
- Takeda. Takeda's Qdenga (dengue tetravalent vaccine [live, attenuated]) approved in Indonesia for use regardless of prior dengue exposure. August 22, 2022 (<https://www.takeda.com/newsroom/newsreleases/2022/takedas-qdenga-dengue-tetravalent-vaccine-live-attenuated-approved-in-indonesia-for-use-regardless-of-prior-dengue-exposure>).
- Takeda. Takeda's Qdenga (dengue tetravalent vaccine [live, attenuated]) approved for use in European Union. December 8, 2022 (<https://www.takeda.com/newsroom/newsreleases/2022/takedas-qdenga-dengue-tetravalent-vaccine-live-attenuated-approved-for-use-in-european-union>).
- Agência Nacional de Vigilância Sanitária (Anvisa). Anvisa aprova nova vacina contra a dengue. March 2, 2023 (<https://www.gov.br/anvisa/pt-br/assuntos/noticias-anvisa/2023/anvisa-aprova-nova-vacina-para-a-dengue>).
- Blaney JE Jr, Durbin AP, Murphy BR, Whitehead SS. Development of a live attenuated dengue virus vaccine using reverse genetics. *Viral Immunol* 2006;19: 10-32.
- Durbin AP, Kirkpatrick BD, Pierce KK, et al. A single dose of any of four different live attenuated tetravalent dengue vaccines is safe and immunogenic in flavivirus-naïve adults: a randomized, double-blind clinical trial. *J Infect Dis* 2013;207: 957-65.
- Durbin AP, Kirkpatrick BD, Pierce KK, et al. A 12-month-interval dosing study in adults indicates that a single dose of the national institute of allergy and infectious diseases tetravalent dengue vaccine induces a robust neutralizing antibody response. *J Infect Dis* 2016;214:832-5.
- Kirkpatrick BD, Durbin AP, Pierce KK, et al. Robust and balanced immune responses to all 4 dengue virus serotypes following administration of a single dose of a live attenuated tetravalent dengue vaccine to healthy, flavivirus-naïve adults. *J Infect Dis* 2015;212:702-10.
- Russell KL, Rupp RE, Morales-Ramirez JO, et al. A phase I randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and immunogenicity of a live-attenuated quadrivalent dengue vaccine in flavivirus-naïve and flavivirus-experienced healthy adults. *Hum Vaccin Immunother* 2022;18:2046960.

22. Whitehead SS, Durbin AP, Pierce KK, et al. In a randomized trial, the live attenuated tetravalent dengue vaccine TV003 is well-tolerated and highly immunogenic in subjects with flavivirus exposure prior to vaccination. *PLoS Negl Trop Dis* 2017; 11(5):e0005584.
23. Kallas EG, Precioso AR, Palacios R, et al. 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. *Lancet Infect Dis* 2020;20:839-50.
24. Whiteman MC, Bogardus L, Giaccone DG, et al. Virus reduction neutralization test: a single-cell imaging high-throughput virus neutralization assay for dengue. *Am J Trop Med Hyg* 2018;99:1430-9.
25. Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. *J Clin Microbiol* 2005; 43:4977-83.
26. Blackwelder WC. Sample size and power for prospective analysis of relative risk. *Stat Med* 1993;12:691-8.
27. Chan ISF, Bohidar NR. Exact power and sample size for vaccine efficacy studies. *Commun Stat Theory Methods* 1998;27: 1305-22.
28. Blaker H. Confidence curves and improved exact confidence intervals for discrete distributions. *Can J Stat* 2008; 28:783-98.
29. Brasil, Ministério da Saúde, Secretaria de Vigilância em Saúde. Óbito por arboviroses no Brasil, 2008 a 2019. *Boletim Epidemiol* 2020;51(33):1-28 (<http://plataforma.saude.gov.br/anomalias-congenitas/boletim-epidemiologico-SVS-33-2020.pdf>).
30. Brasil, Ministério da Saúde, Secretaria de Vigilância em Saúde. Monitoramento dos casos de arboviroses urbanas transmitidas pelo *Aedes Aegypti* (dengue, chikungunya e zika), semanas epidemiológicas 1 a 50, 2020. *Boletim Epidemiol* 2020; 51(51):1-33 ([https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/epidemiologicos/edicoes/2020/boletim\\_epidemiologico\\_svs\\_51.pdf](https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/epidemiologicos/edicoes/2020/boletim_epidemiologico_svs_51.pdf)).
31. Brasil, Ministério da Saúde, Secretaria de Vigilância em Saúde. Monitoramento dos casos de arboviroses urbanas causados por vírus transmitidos pelo mosquito *Aedes* (dengue, chikungunya e zika), semanas epidemiológicas 1 a 48, 2021. *Boletim Epidemiol* 2021;52(45):1-13 ([https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/epidemiologicos/edicoes/2021/boletim\\_epidemiologico\\_svs\\_45.pdf](https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/epidemiologicos/edicoes/2021/boletim_epidemiologico_svs_45.pdf)).
32. Oneda RM, Basso SR, Frasson LR, Mottecy NM, Saraiva L, Bassani C. Epidemiological profile of dengue in Brazil between the years 2014 and 2019. *Rev Assoc Med Bras* (1992) 2021;67:731-5.
33. Yuan K, Chen Y, Zhong M, Lin Y, Liu L. Risk and predictive factors for severe dengue infection: a systematic review and meta-analysis. *PLoS One* 2022;17(4): e0267186.
34. Sangkaew S, Ming D, Boonyasiri A, et al. Risk predictors of progression to severe disease during the febrile phase of dengue: a systematic review and meta-analysis. *Lancet Infect Dis* 2021;21:1014-26.
35. Brito AF, Machado LC, Oidtman RJ, et al. Lying in wait: the resurgence of dengue virus after the Zika epidemic in Brazil. *Nat Commun* 2021;12:2619.
36. Mugabe VA, Borja LS, Cardoso CW, et al. Changes in the dynamics of dengue incidence in South and Central America are possibly due to cross-population immunity after Zika virus epidemics. *Trop Med Int Health* 2021;26:272-80.
37. Hammont WM, Sather GE. Problems of typing dengue viruses. *Mil Med* 1964; 129:130-5.
38. Russell PK, Nisalak A. Dengue virus identification by the plaque reduction neutralization test. *J Immunol* 1967;99: 291-6.
39. Thomas SJ, Nisalak A, Anderson KB, et al. Dengue plaque reduction neutralization test (PRNT) in primary and secondary dengue virus infections: how alterations in assay conditions impact performance. *Am J Trop Med Hyg* 2009; 81:825-33.
40. Vannice KS, Wilder-Smith A, Barrett ADT, et al. Clinical development and regulatory points for consideration for second-generation live attenuated dengue vaccines. *Vaccine* 2018;36:3411-7.

Copyright © 2024 Massachusetts Medical Society.