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Safety and Immunogenicity of a Tetravalent Dengue Vaccine Candidate in Healthy Children and Adults in Dengue-Endemic Regions: A Randomized, Placebo-Controlled Phase 2 Study

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Background. A safe, effective tetravalent dengue vaccine is a global health priority. The safety and immunogenicity of a live attenuated, recombinant tetravalent dengue vaccine candidate (TDV) were evaluated in healthy volunteers from dengue-endemic countries.

Methods. This multicenter, double-blind, phase 2 study was conducted in Puerto Rico, Colombia, Singapore, and Thailand. During stage I, 148 volunteers aged 1.5–45 years were sequentially enrolled into 4 age-descending groups and randomized at a ratio of 2:1 to receive TDV or placebo. In stage II (group 5), 212 children aged 1.5–11 years were randomized at a ratio of 3:1 to receive TDV or placebo. Participants received a subcutaneous injection of TDV or placebo on days 0 and 90 and were followed for analysis of safety, seropositivity, and neutralizing antibodies to DENV-1–4.

Results. Injection site pain, itching, and erythema (mostly mild) were the only solicited adverse events more frequently reported with TDV than with placebo in all age groups. After 2 TDV doses, seropositivity was >95% in all 5 groups for DENV-1–3 and 72.7%–100% for DENV-4; geometric mean titers ranged from 582 to 1187 for DENV-1, from 582 to 1187 for DENV-2, from 196 to 630 for DENV-3, and from 41 to 210 for DENV-4 among the 5 groups.

Conclusions. TDV was well tolerated and immunogenic in volunteers aged 1.5–45 years, irrespective of prevaccination dengue exposure.

Keywords. live attenuated tetravalent dengue vaccine; children; adults; safety; immunogenicity.

Dengue fever is a mosquito-borne infection caused by any one of 4 dengue virus serotypes (DENV-1–4). DENV infection can result in a range of symptoms, from subclinical disease to an acute febrile illness with other manifestations, which may include headache, arthralgia, myalgia, retro-orbital pain, rash, bleeding, or leukopenia [1, 2]. A subset of patients progress to severe disease, including dengue hemorrhagic fever or dengue shock syndrome [3, 4]. Bleeding manifestations and organ involvement are more common among adults with severe dengue, while vascular leakage and shock occur more frequently and severely in children [5–7].

With an estimated 390 million infections annually, of which approximately 96 million are symptomatic [8], dengue fever is the most rapidly spreading mosquito-borne viral disease in the world [1]. Mosquito control efforts in dengue-endemic areas have been ineffective in preventing dengue outbreaks or further geographical spread of the disease [9], and no vaccines or treatments specific for dengue fever have yet been approved.

All 4 DENV serotypes generally cocirculate in areas of endemicity [7]. Primary infection with any one of the serotypes is thought to result in lifelong protection from reinfection by the same serotype but does not provide long-term protection against a secondary infection by another DENV [10–12]. Previous infection with 1 DENV serotype leads to an increased risk of dengue hemorrhagic fever or dengue shock syndrome upon secondary infection with a different serotype, which may be caused by antibody-dependent enhancement (ADE) [13].

Vaccine development has focused on tetravalent vaccines that provide protection against all 4 DENV serotypes simultaneously [10] because of concerns about ADE [14]. To date, ADE has not been observed in long-term monitoring of participants in clinical trials of tetravalent dengue vaccine candidates [14–17].

The Takeda tetravalent dengue vaccine (TDV) comprises attenuated DENV-2 (TDV-2) and 3 chimeric viruses for DENV-1,

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-3, and -4 that are generated by substituting the structural surface protein genes *prM* and *E* of TDV-2 with those of DENV-1, -3, or -4 [18, 19]. The safety and immunogenicity of TDV were previously demonstrated in 2 phase 1 clinical trials in healthy, flavivirus-naive adults aged 18–45 years [20, 21]. This study was performed to evaluate the safety and immunogenicity of TDV in healthy children and adults in 4 dengue-endemic areas: Puerto Rico, Colombia, Singapore and Thailand.

METHODS

This multicenter, double-blind, randomized, placebo-controlled, phase 2 study (ClinicalTrials.gov registration: NCT01511250) was conducted at 8 centers (3 in Puerto Rico, 1 in Colombia, and 2 each in Singapore and Thailand), at which participants were enrolled between 16 November 2011 and 2 August 2013. The study was conducted in accordance with the institutional review board regulations stated in the US Code of Federal Regulations, as well as all applicable local regulations.

Participants

Eligible participants were aged 1.5–45 years, in good general health, and negative for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus. Stage I participants had to have normal values for blood and urine laboratory tests. Participants had to give written informed consent to participate; children had to provide verbal or written assent and had to have written informed consent given by a parent or legal guardian, according to local regulations.

Key exclusion criteria included febrile illness (temperature, ≥38°C) or acute infection within 3 days of vaccination; a history of significant dermatologic disease in the last 6 months; use of systemic corticosteroid therapy (≥0.5 mg/kg/day) within the previous 6 months; use of any nonsteroidal antiinflammatory drugs, acetaminophen, or antihistamines for the 3 days immediately before each dose; and previous participation in a dengue vaccine trial.

Study Design

Study stage I was an age-descending phase in which participants aged 1.5–45 years were sequentially enrolled and randomized at a ratio of 2:1 to receive TDV or placebo in 4 age-descending groups, as shown in Figure 1. Twelve participants aged 21–45 years were enrolled into group 1, vaccinated at day 0, and monitored for safety. After 28 days, the safety data were evaluated by the data safety monitoring board (DSMB). Following DSMB approval, the additional participants in group 1 were enrolled, and, concurrently, the next group of 12 participants (group 2; ages 12–20 years) received their first dose of study treatment (Figure 1). For safety reasons, the same sequence was followed for enrollment and randomized treatment of the remainder of participants in group 2, group 3 (aged 6–11 years), and group 4 (aged 1.5–5 years).

Stage II was an expansion phase, in which a single group of children aged 1.5–11 years (group 5) were enrolled and randomized at a ratio of 3:1 to receive TDV or placebo. Stage II was initiated after DSMB approval following their review of 28-day safety data from at least 12 participants from group 4.

All participants received a second vaccination of the appropriate treatment on day 90.

Participants, investigators, and serology laboratory personnel were blinded to treatment allocation. Participants are being followed over 3 years for determination of the long-term safety, immunogenicity, and incidence of dengue; this report describes the immunogenicity and safety data collected up to day 120.

Study Treatment

TDV was administered subcutaneously in the upper arm at a dose of 4.7×10^5 plaque-forming units (PFU) in a total volume of 0.5 mL. The serotype composition of each dose was as follows: TDV-1, 2×10^4 PFU; TDV-2, 5×10^4 PFU; TDV-3, 1×10^5 PFU; and TDV-4, 3×10^5 PFU. This formulation was chosen on the basis of its previously demonstrated safety and immunogenicity in phase 1 studies [20, 21]. Placebo was phosphate-buffered saline.

Study Objectives, End Points, and Evaluations

The primary study objectives were to evaluate the safety, tolerability, and immunogenicity of TDV in healthy adults and children. Secondary objectives were to evaluate the incidence of vaccine virus replication and the immune response induced by TDV in a population that included those with previous exposure to dengue.

The primary safety end points were the frequency and severity of unsolicited adverse events (AEs) and of solicited AEs (via diary), including local and systemic AEs, and routine hematologic and chemistry parameters. Immunogenicity was measured by a microneutralization assay [20] to each of the 4 DENV serotypes with titers defined as the dilution resulting in a 50% reduction in plaque values [MNT50]. The primary immunogenicity endpoints were seropositivity rates, defined as group percentages with an antibody titer of ≥10, at day 120 (ie, 30 days after the second injection).

Secondary end points included geometric mean neutralizing antibody titers (GMTs) to each of the DENV serotypes, the percentage of participants who were seropositive to multiple DENV serotypes, and the incidence of vaccine viral RNA replication after each dose in stage I participants.

For immunogenicity end points, blood samples were obtained from all participants at day 0 and then on days 28, 90 (before the second dose), and 120 to determine the MNT_{50} against all 4 DENV serotypes.

Safety assessments for unsolicited AEs were conducted on days 0, 7, 14, 90, 97, 104, 111, and 120 and included physical examination, clinical laboratory evaluations (for stage I participants), and documentation of serious adverse events (SAEs). Occurrence of local injection site reactions (edema, erythema,

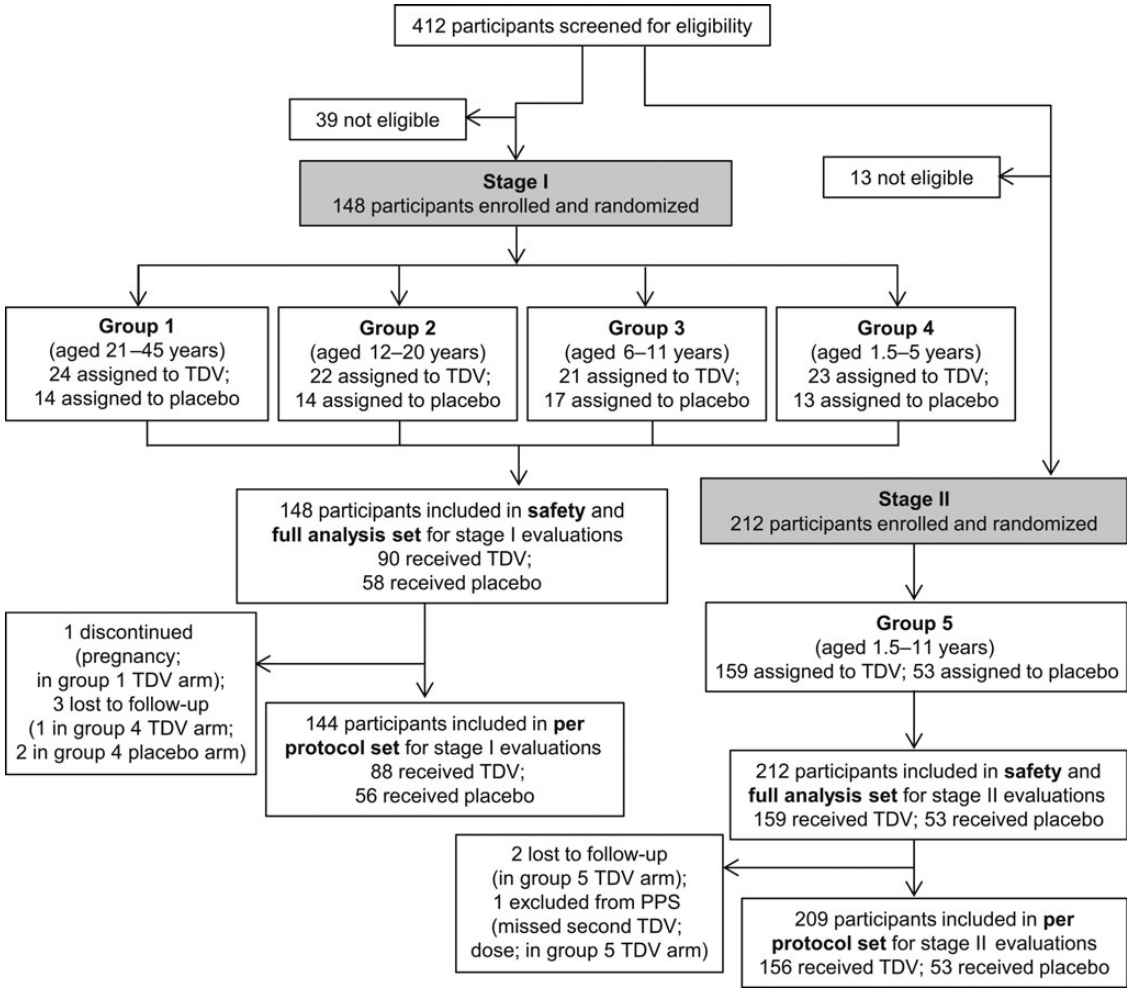

Figure 1. Study profile and participant disposition. Abbreviations: PPS, per protocol set; TDV, tetravalent dengue vaccine.

pain, and itching), were assessed by the investigator on days 0 and 90 (5, 30 and 60 minutes after injection) and at each study visit through day 120. Participants were asked to record solicited injection-site and systemic AEs on diary cards for 14 days following each vaccination.

To assess vaccine virus replication, blood samples were obtained from stage I participants at the safety assessment visits to measure viral RNA levels of each of the 4 TDV components, using a quantitative reverse-transcription polymerase chain reaction assay [20].

Sample Size, Study Populations, and Statistical Analyses

This was an exploratory study to assess the overall safety, tolerability and immunogenicity of TDV and was therefore not powered to detect any differences in potential safety and immunogenicity data between the TDV and placebo groups. The sample size of approximately 144 participants for stage I was chosen empirically, based on other similar vaccines in this stage of development. The sample size of approximately 200 participants in stage II was selected to obtain sufficient safety and immunogenicity data in the target age group (1.5–11 years) before further evaluation in larger scale phase 2 and phase 3 studies.

The safety set included all randomized participants who received at least 1 dose of study vaccine or placebo. The full analysis set included all randomized participants who received at least 1 dose of study treatment and for whom a valid predosing and at least 1 valid postdosing blood sample were received. The per-protocol set included all randomized participants who received the 2 planned injections and had a day 120 evaluation.

Seropositivity rates and GMTs of DENV neutralizing antibodies were calculated with 95% confidence intervals (CIs) for each of the 4 TDV serotypes individually and for bivalent, trivalent, and tetravalent responses. These data were also presented by baseline DENV serostatus at day 0 for the combined study population. Also reported are the number and percentage of all participants with solicited and unsolicited AEs and the number and percentage of stage I participants with viremia.

RESULTS

In stage I, 148 participants aged 1.5–45 years were enrolled sequentially into 4 age-descending groups (Figure 1) according to the safety-based sequence described in Methods. Participants were randomized to receive TDV or placebo and received injections on days 0 and 90. In stage II, 212 children aged 1.5–11 years were enrolled, randomized to receive TDV or placebo, and received injections on days 0 and 90.

Six participants (1.7%) did not complete the study: 3 who received TDV and 2 who received placebo were lost to follow-up, and 1 became pregnant after the first TDV dose and was withdrawn by the investigator. One additional participant completed the study through day 120 but was excluded from the per-protocol set because he missed the second TDV dose.

Demographic characteristics and baseline seropositivity rates for DENV are summarized in Table 1. Percentages of participants seropositive to at least 1 DENV serotype at baseline increased with age (Table 1), reflecting the increased risk of exposure in these dengue-endemic regions over time.

Safety

No participants withdrew from the study because of adverse events, nor did any deaths occur. SAEs occurred in 10 participants (Table 2); none were related to vaccine, and all resolved without sequelae. SAEs that occurred during stage I of the study were cholecystitis and a severe urinary tract infection. SAEs (hospitalizations) that occurred during stage II among TDV-treated participants were mild child maltreatment syndrome in 1 child who had severe viral gastritis on a separate occasion, a severe brain contusion in a child who reported a severe fracture as a separate SAE, severe viral gastritis, moderate gastroenteritis, moderate bronchitis, moderate bronchitis and moderate influenza on a separate occasion, and mild bronchitis and moderate influenza on a separate occasion. One participant in the placebo group was hospitalized for moderate dehydration.

Unsolicited treatment-related AEs were observed in the same proportion of TDV- and placebo-treated participants overall (7.2%; Table 2). Most AEs were mild, and none were severe. The most common unsolicited AES were headache, injection site pain, and pyrexia.

Comparable proportions of participants experienced at least 1 solicited AE in the TDV and placebo groups within each age group (Table 3). The frequency and severity of solicited systemic AEs were also generally comparable between TDV and placebo groups and were mostly mild; the most common were headache, fever, and muscle pain.

No AEs detected by hematologic or chemistry tests were reported, nor were any clinically significant mean changes from baseline observed for any hematologic or chemistry laboratory parameters.

Immunogenicity

After the first TDV dose, seropositivity rates for each DENV increased from baseline in every age group (Figure 2*A*). The

Table 1. Demographic Characteristics and Baseline Seropositivity for Dengue in the Full Analysis Set of Participants

Characteristic	Group 1 21–45 y		Group 2 12–20 y		Group 3 6–11 y		Group 4 1.5–5 y		Group 5 1.5–11 y	
	TDV (n=24)	Placebo (n=14)	TDV (n = 22)	Placebo (n = 14)	TDV (n = 21)	Placebo (n = 17)	TDV (n = 23)	Placebo (n = 13)	TDV (n = 159)	Placebo (n = 53)
Male sex	14 (58.3)	6 (42.8)	10 (45.5)	7 (50.0)	15 (71.4)	9 (52.9)	16 (69.6)	6 (46.1)	71 (44.7)	34 (64.1)
Race or ethnicity										
Asian	3 (12.5)	3 (21.4)	16 (72.7)	8 (57.1)	16 (76.2)	12 (70.6)	12 (52.1)	6 (46.2)	77 (48.4)	24 (45.3)
Hispanic/Latino	20 (83.3)	10 (71.4)	6 (27.3)	6 (42.9)	4 (19.0)	5 (29.4)	11 (47.8)	7 (53.8)	3 (1.9)	2 (3.8)
White	1 (4.2)	1 (7.1)	0 (0)	0 (0)	1 (4.8)	0 (0)	0 (0)	0 (0)	79 (49.7)	27 (50.9)
Age, y	27.5 (21.0–45.0)	29.5 (21.0–40.0)	16.0 (12.0–19.0)	17.0 (12.0–20.0)	9.0 (6.0–11.0)	7.0 (6.0–11.0)	3.0 (1.5–5.0)	3.0 (2.3–5.0)	6.0 (1.6–11.0)	7.0 (2.1–11.0)
BMI[a]	28.95 (14.70–41.30)	28.15 (21.30–38.70)	20.90 (15.00–33.60)	22.70 (18.60–31.50)	17.20 (12.90–28.80)	15.90 (14.20–20.60)	15.20 (12.50–19.00)	17.10 (13.20–20.40)	16.00 (12.10–30.10)	16.50 (13.20–24.30)
Seropositivity at day 0[b]	19 (79.2)	13 (92.9)	15 (68.2)	13 (92.9)	9 (42.9)	3 (17.6)	7 (30.4)	1 (7.7)	66 (41.5)	29 (54.7)

Data are no. (%) of subjects or median value (range).

Abbreviation: TDV, tetravalent dengue vaccine.

[a] Body mass index (BMI) is calculated as the weight in kilograms divided by the height in meters squared.

[b] Seropositivity at day 0 was defined as seropositivity for any dengue virus serotype at day 0; seronegativity at day 0 defined was defined as seronegativity for all serotypes at day 0.

Table 2. Summary of Unsolicited Adverse Events (AEs) From the Time of Vaccine Dose 1 Receipt Through Day 30 After Vaccine Dose 2 Receipt in the Safety Set of Participants

	Group 1 21–45 y		Group 2 12–20 y		Group 3 6–11 y		Group 4 1.5–5 y		Group 5 1.5–11 y		Total	
Parameter	TDV (n = 24)	Placebo (n = 14)	TDV (n = 22)	Placebo (n = 14)	TDV (n = 21)	Placebo (n = 17)	TDV (n = 23)	Placebo (n = 13)	TDV (n = 159)	Placebo (n = 53)	TDV (n = 249)	Placebo (n = 111)
Participants with ≥1 AE[a]	14 (58.3)	9 (64.3)	15 (68.2)	8 (57.1)	16 (76.2)	12 (70.6)	17 (73.9)	12 (92.3)	111 (69.8)	40 (75.5)	173 (69.5)	81 (73.0)
Participants with ≥1 related[b] AE	2 (8.3)	1 (7.1)	4 (18.2)	3 (21.4)	1 (4.8)	1 (5.9)	1 (4.3)	2 (15.4)	10 (6.3	1 (1.9)	18 (7.2)	8 (7.2)
Participants with AE leading to any action taken	9 (37.5)	9 (64.3)	12 (54.5)	8 (57.1)	15 (71.4)	9 (52.9)	16 (69.6)	10 (76.9)	86 (54.1)	29 (54.7)	138 (55.4)	65 (58.6)
Participants with ≥1 severe AE	1 (4.2)	0 (0)	0 (0)	0 (0)	1 (4.8)	0 (0)	0 (0)	0 (0)	7 (4.4)	1 (1.9)	9 (3.6)	1 (0.9)
Participants with general disorders and administration site conditions[c]												
Injection site pain	2 (8.3)	0 (0)	2 (9.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.3)	0 (0)	6 (2.4)	0 (0)
Injection site erythema	1 (4.2)	0 (0)	2 (9.1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1.2)	0 (0)
Fatigue	0 (0)	1 (7.1)	2 (9.1)	2 (14.3)	0 (0)	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.8)	4 (3.6)
Injection site pruritus	0 (0)	0 (0)	1 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	2 (0.8)	0 (0)
Injection site swelling	0 (0)	0 (0)	1 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	0 (0)
Injection site warmth	0 (0)	0 (0)	1 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	0 (0)
Pyrexia	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)	4 (2.5)	1 (1.9)	4 (1.6)	3 (2.7)
Participants with nervous system disorders[c]												
Headache	2 (8.3)	1 (7.1)	4 (18.2)	1 (7.1)	0 (0)	1 (5.9)	1 (4.3)	0 (0)	1 (0.6)	0 (0)	8 (3.2)	3 (2.7)
Participants with eye disorders[c]												
Photophobia	1 (4.2)	0 (0)	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	1 (0.9)
Eye pain	0 (0)	1 (7.1)	0 (0)	1 (7.1)	0 (0)	0 (0)	1 (4.3)	0 (0)	1 (0.6)	0 (0)	2 (0.8)	2 (1.8)
Participants with musculoskeletal and connective tissue disorders[c]												
Joint stiffness	1 (4.2)	1 (7.1)	1 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.8)	1 (0.9)
Arthralgia	0 (0)	0 (0)	1 (4.5)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)	0 (0)	2 (0.8)	0 (0)
Myalgia	0 (0)	1 (7.1)	2 (9.1)	1 (7.1)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)	0 (0)	3 (1.2)	2 (1.8)
Participants with gastrointestinal disorders[c]												
Nausea	0 (0)	0 (0)	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)
Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)	0 (0)	0 (0)	1 (0.9)
Participants with respiratory thoracic and mediastinal disorders[c]												
Oropharyngeal pain	0 (0)	0 (0)	1 (4.5)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	1 (0.9)
Rhinitis allergic	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	1 (0.4)	0 (0)
Participants with skin and subcutaneous tissue disorders[c]												
Pruritus	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.8)	1 (5.9)	0 (0)	0 (0)	1 (0.6)	0 (0)	2 (0.8)	1 (0.9)
Rash	0 (0)	0 (0)	1 (4.5)	0 (0)	0 (0)	1 (5.9)	0 (0)	1 (7.7)	1 (0.6)	0 (0)	2 (0.8)	2 (1.8)
Participants with infections and infestations[c]												
Eczema herpeticum	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	1 (0.4)	0 (0)

Data are no. (%) of participants.

Abbreviation: TDV, tetravalent dengue vaccine.

[a] An AE was defined as any unfavorable and unintended sign, symptom, or disease temporally associated with the administration of the study treatment, whether or not it was considered to be associated with it.

[b] The AE was considered by the investigator to be related if it was temporally associated with the study treatment and no other etiology explained the event.

[c] Data are listed by the Medical Dictionary for Regulatory Activities system organ class preferred term. Unsolicited AEs were graded according to the Common Terminology Criteria for Adverse Events, version 4.03.

Table 3. Summary of Self-reported Local and Systemic Adverse Events (AEs) ≤14 Days After Either Vaccine Dose in the Safety Set of Participants

	Group 1 21–45 y		Group 2 12–20 y		Group 3 6–11 y		Group 4 1.5–5 y		Group 5 1.5–11 y		Total	
Solicited AE	TDV (n = 24)	Placebo (n = 14)	TDV (n = 22)	Placebo (n = 14)	TDV (n = 21)	Placebo (n = 17)	TDV (n = 23)	Placebo (n = 13)	TDV (n = 159)	Placebo (n = 53)	TDV (n = 249)	Placebo (n = 111)
Systemic												
Fever												
Any grade	4 (16.6)	6 (42.9)	4 (18.1)	1 (7.1)	1 (4.8)	1 (5.9)	4 (17.4)	4 (30.8)	33 (20.8)	10 (18.9)	46 (18.5)	22 (19.8)
Only grade 3	3 (12.5)	3 (21.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)	1 (0.6)	2 (3.8)	4 (1.6)	6 (5.4)
Headache												
Any grade	6 (25.0)	4 (28.6)	9 (40.9)	8 (57.1)	4 (19.0)	5 (29.4)	3 (13.0)	4 (30.8)	46 (28.9)	12 (22.6)	68 (27.3)	33 (29.7)
Only grade 3	1 (4.2)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (2.5)	2 (3.8)	5 (2.0)	3 (2.7)
Muscle pain												
Any grade	4 (16.6)	3 (21.4)	4 (18.1)	5 (35.7)	1 (4.8)	3 (17.6)	5 (21.7)	2 (15.4	30 (18.9)	6 (11.3)	44 (17.7)	19 (17.1)
Only grade 3	1 (4.2)	1 (7.1)	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)	1 (7.7)	3 (1.9)	1 (1.9)	4 (1.6)	4 (3.6)
Joint pain												
Any grade	1 (4.2)	3 (21.4)	2 (9.0)	2 (14.3)	0 (0)	0 (0)	1 (4.3)	1 (7.7)	10 (6.3)	2 (3.8)	14 (5.6)	8 (7.2)
Only grade 3	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)	0 (0)	0 (0)	2 (1.8)
Eye pain												
Any grade	3 (12.5)	3 (21.4)	1 (4.5)	3 (21.4)	0 (0)	0 (0)	2 (8.7)	1 (7.7)	14 (8.8)	3 (5.7)	20 (8.0)	10 (9.0)
Only grade 3	1 (4.2)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	1 (0.9)
Light sensitivity												
Any grade	3 (12.5)	1 (7.1)	0 (0)	4 (28.6)	1 (4.8)	0 (0)	0 (0)	0 (0)	11 (6.9)	3 (5.7)	15 (6.0)	8 (7.2)
Only grade 3	1 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	0 (0)
Tiredness												
Any grade	4 (16.6)	4 (28.6)	9 (40.9)	6 (42.9)	2 (9.5)	5 (29.4)	3 (13.0)	3 (23.1)	29 (18.2)	10 (18.9)	47 (18.9)	28 (11.2)
Only grade 3	2 (8.3)	2 (14.3)	0 (0)	0 (0)	1 (4.8)	0 (0)	0 (0)	1 (7.7)	3 (1.9)	1 (1.9)	6 (2.4)	4 (3.6)
Rash												
Any grade	1 (4.2)	0 (0)	2 (9.0)	2 (14.3)	2 (9.5)	1 (5.9)	2 (8.7)	2 (15.4)	14 (8.8)	2 (3.8)	21 (8.4)	7 (6.3)
Only grade 3	0 (0)	0 (0)	0 (0)	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	1 (0.4)	1 (0.9)
Nausea												
Any grade	4 (16.6)	1 (7.1)	3 (13.6)	4 (28.6)	1 (4.8)	0 (0)	3 (13.0)	3 (23.1)	22 (13.8)	4 (7.5)	33 (13.2)	12 (10.8)
Only grade 3	1 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (2.5)	0 (0)	5 (2.0)	0 (0)
Vomiting												
Any grade	2 (8.3)	1 (7.1)	0 (0)	2 (14.3)	1 (4.8)	1 (5.9)	4 (17.4)	5 (38.5)	18 (11.3)	3 (5.7)	25 (10.0)	12 (10.8)
Only grade 3	1 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.3)	0 (0)	3 (1.2)	0 (0)

Table 3 continued.

Solicited AE	Group 1 21–45 y		Group 2 12–20 y		Group 3 6–11 y		Group 4 1.5–5 y		Group 5 1.5–11 y		Total	
	TDV (n = 24)	Placebo (n = 14)	TDV (n = 22)	Placebo (n = 14)	TDV (n = 21)	Placebo (n = 17)	TDV (n = 23)	Placebo (n = 13)	TDV (n = 159)	Placebo (n = 53)	TDV (n = 249)	Placebo (n = 111)
Local												
Injection site pain												
Any grade	7 (29.2)	0 (0)	14 (63.6)	3 (21.4)	9 (42.9)	0 (0)	7 (30.4)	2 (15.4)	56 (35.2)	6 (11.3)	93 (37.3)	11 (9.9)
Only grade 3	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.8)	0 (0)	0 (0)	0 (0)	4 (2.5)	0 (0)	5 (2.0)	0 (0)
Itching												
Any grade	3 (12.5)	1 (7.1)	7 (31.8)	1 (7.1)	2 (9.5)	1 (5.9)	2 (8.7)	0 (0)	26 (16.4)	4 (7.5)	40 (16.1)	7 (6.3)
Only grade 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	1 (0.4)	0 (0)
Erythema												
Any grade	6 (25.0)	0 (0)	4 (18.1)	0 (0)	2 (9.5)	0 (0)	3 (13.0)	0 (0)	24 (15.1)	1 (1.9)	39 (15.6)	1 (0.9)
Only grade 3	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.8)	0 (0)	0 (0)	0 (0)	1 (0.6)	1 (1.9)	2 (0.8)	1 (0.9)
Edema												
Any grade	0 (0)	0 (0)	1 (4.5)	0 (0)	3 (14.3)	0 (0)	1 (4.3)	0 (0)	13 (8.2)	0 (0)	18 (7.2)	0 (0)
Only grade 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)	0 (0)	1 (0.4)	0 (0)

Data are no. (%) of participants who self-reported the specified AE in their diary. Diaries were reviewed by the investigator and, based on their review and discussion with the participant, clinically significant events were recorded as AEs. This table summarizes all self-reported reactions from the diaries. Erythema and edema dimensions were recorded in the diary and used to grade severity according to the *Food and Drug Administration Guidance for Industry: Toxicology Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*. Temperature was recorded in the diary and used to grade fever according to the Common Terminology Criteria for Adverse Events, version 4.03.

Abbreviation: TDV, tetravalent dengue vaccine.

increase from baseline was greatest in the younger age groups (groups 3, 4, and 5), in which seropositivity rates for DENV-1–4 increased to 64%–100% after a single dose (compared with 13%–38% at baseline).

At day 120, 30 days after the second TDV dose, the seropositivity rate for DENV-1–3 was >95% in each of the 5 groups. The DENV-4 seropositivity rate was slightly lower in groups 1–4 (87.5% overall) but was 94.3% in group 5. In general, the second dose had little meaningful impact on seropositivity in adolescents and adults (groups 1 and 2). In children (groups 3, 4, and 5), the second dose either did not affect or modestly increased the numbers of participants who were seropositive for DENV-1, DENV-2, or DENV-3, but it demonstrably increased rates of seropositivity to DENV-4 (Figure 2*A*).

Figure 2*B* shows monovalent seropositivity rates over time according to baseline serostatus of the combined study population. Among participants who were seronegative to all DENV at baseline, >94% were seropositive for DENV-1, DENV-2, or DENV-3 on day 28 after the first dose. The DENV-4 seropositivity rate was 58.6% after the first dose, increasing to 87.7% by day 120 (30 days after the second dose). Among participants who were seropositive to at least 1 DENV serotype at baseline, seropositivity rates to DENV-1–4 were 91.3%–99.1% after 1 dose and 96.5%–100% after 2 doses (Figure 2*B*).

Proportions of TDV recipients who became seropositive for multiple DENV serotypes are shown in Figure 3. The majority of individuals (59%–86%) in each study group had tetravalent responses after a single TDV dose (Figure 3*A*). Again, the increases were greatest in the younger age groups (groups 3–5), in which more participants were seronegative at enrollment. A second TDV dose increased the proportion who were seropositive for all 4 DENV serotypes at day 120 in the youngest age groups but had little effect in adolescents and adults (groups 1 and 2).

Seropositivity to multiple DENV was analyzed by DENV serological status at enrollment (Figure 3*B*). After a single TDV dose, the proportion of participants who were seropositive to all 4 DENV serotypes increased from 0% to 59% among those who had been seronegative at enrollment and from 63% to 89% among the initially seropositive participants. The proportions who were seropositive to all 4 DENV serotypes after 2 TDV doses increased to 88% and 97% of those who had been initially seronegative and seropositive, respectively.

A substantial increase in GMT was observed at day 28 for DENV-1–3 among TDV recipients in all study groups after the first dose (Figure 4*A*), with lower comparative increases observed for DENV-4. The increase in GMT with the first dose was statistically significant for all 4 serotypes, regardless of serostatus at enrollment (Figure 4*B*).

GMT increases after the second dose were more variable. DENV-2 GMTs did not increase meaningfully in any of the 5 groups after the second TDV dose (compare day 90 with day 120; Figure 4*A*). However, DENV-1, -3 and -4 GMTs showed

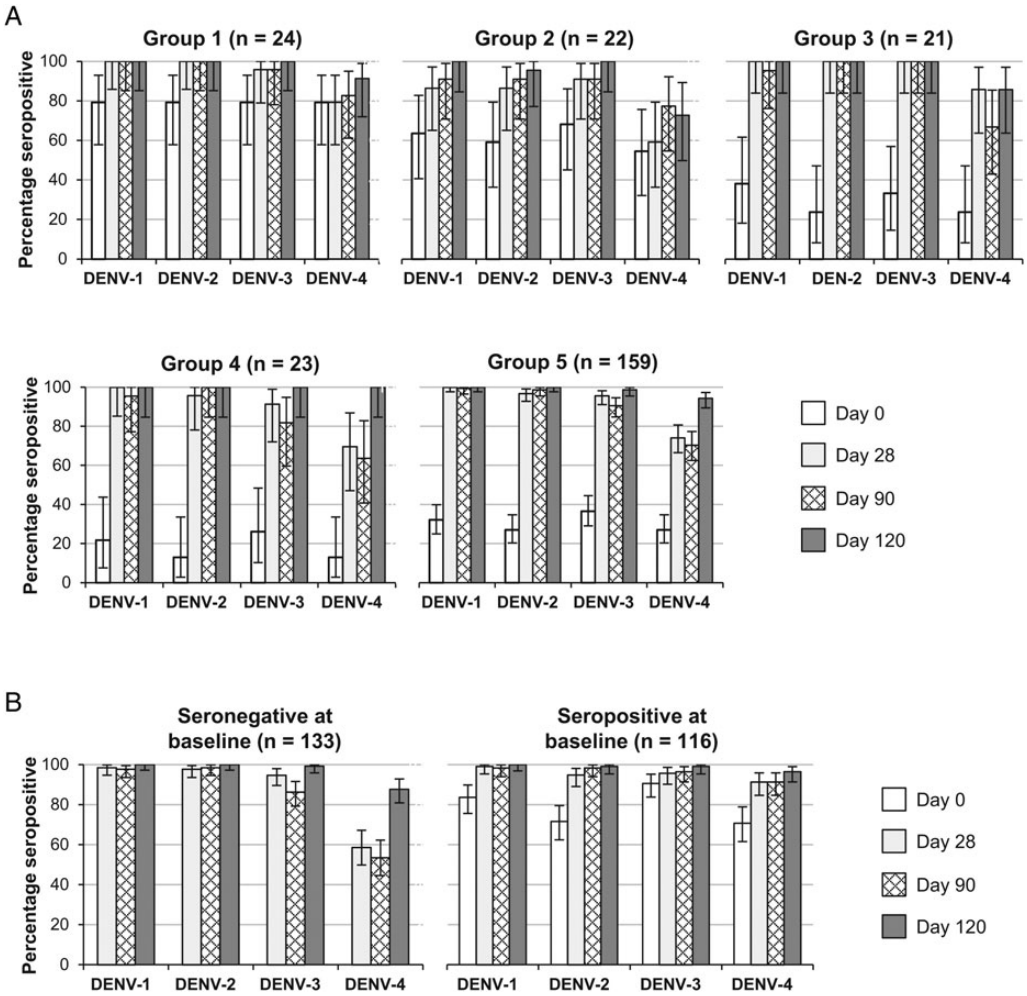

Figure 2. Monovalent seropositivity rates in tetravalent dengue vaccine recipients over time in the full analysis set. *A*, Seropositivity rates in each study group at days 0, 28, 90, and 120. *B*, Seropositivity rates according to baseline dengue virus (DENV) serostatus at days 0, 28, 90, and 120. Seropositivity at baseline was defined as seropositivity for any DENV serotype at day 0. Data are percentages of participants, with 95% confidence intervals.

upward trends in children (groups 3, 4, and 5) after the second dose. When GMTs were analyzed by baseline DENV serostatus, the second dose had no meaningful effect on any GMTs in seropositive participants (Figure 4*B*). In contrast, DENV-1, -3 and -4 GMTs increased significantly after the second dose in participants who were seronegative at baseline.

In placebo-treated participants, the GMTs for each of the 4 DENV serotypes remained essentially unchanged from day 0 levels after both the first and second doses (data not shown).

Immunogenicity data for the per-protocol set were essentially the same as for the full analysis set (data not shown).

Replication of TDV

After the first dose, TDV viral RNA was only observed for TDV-2 in the vaccinated participants. At day 7, 18 TDV-treated participants (20% overall) showed evidence of TDV viral replication; 2 (8%) were in group 1, 3 (13%) were in group 2, 4 (19%) were in group 3, and 9 (39%) were in group 4. At day 14, TDV-2 RNA was only observed in group 2 (4 recipients [18%]), group 3 (6 [28%]), and group 4 (4 [17%]; 15% of groups 1–4 overall). By day 90, viremia was not detected in any TDV recipient.

At day 97, after the second dose, 1 participant in group 2 showed evidence of TDV-2 RNA, and at day 104, 1 participant in group 1 was positive for TDV-2 RNA (1.1% overall in each case).

DISCUSSION

This phase 2 study involved the first use of TDV in children and adolescents and its first use in dengue-endemic regions. The vaccine was well tolerated in all age groups, and substantial increases in all immunogenicity end points were observed for all 4 DENV serotypes after 1 or 2 doses. No participant was naturally infected with DENV during the study period. The study's strengths include its wide geographic spread and participants' previous exposure to different DENV serotypes, the large age

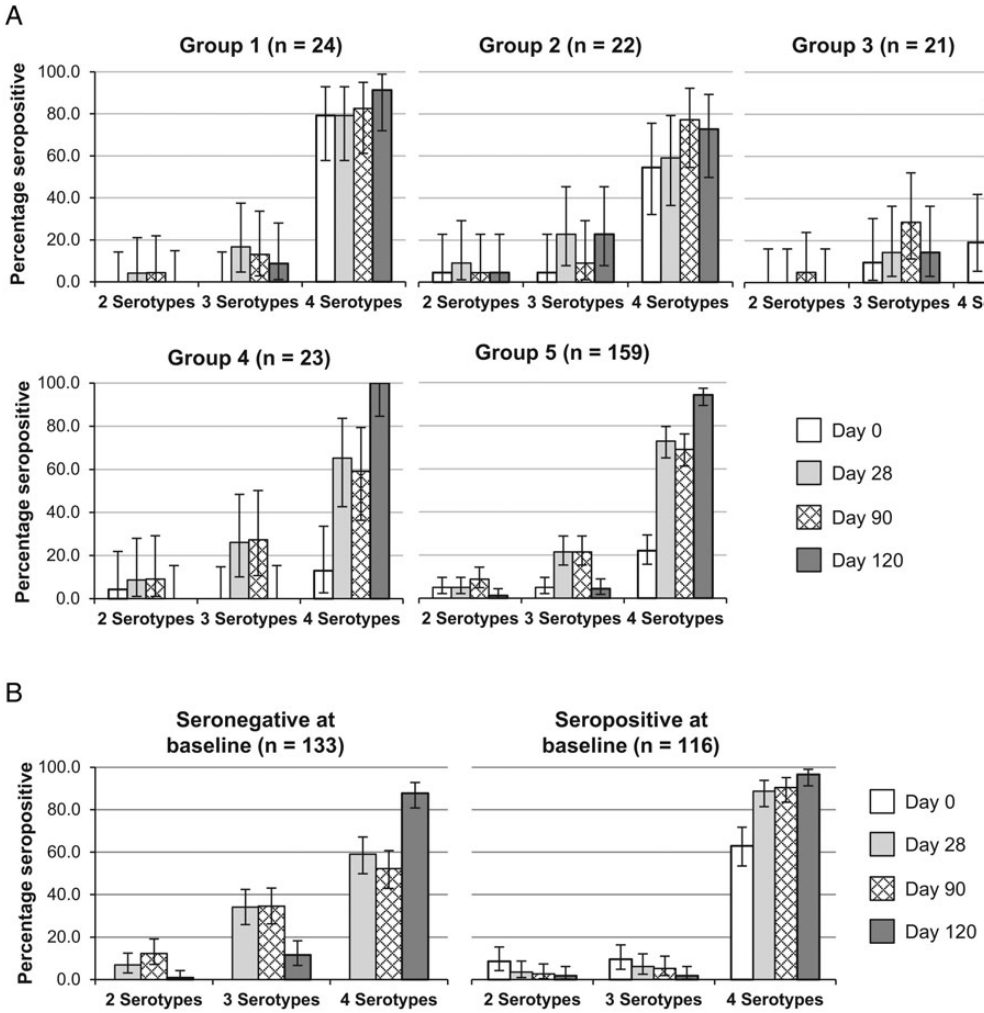

Figure 3. Multivalent seropositivity rates in tetravalent dengue vaccine (TDV) recipients over time in the full analysis set. *A*, Seropositivity rates for 2, 3, or 4 dengue virus (DENV) serotypes in each study group at days 0, 28, 90, and 120. *B*, Seropositivity rates for 2, 3, or 4 DENV serotypes according to baseline DENV serostatus at days 0, 28, 90, and 120. Seropositivity at baseline was defined as seropositivity for any DENV serotype at day 0. Data are percentages of participants, with 95% confidence intervals.

range of the participants, and the use of a placebo. Although the relatively short follow-up reported here might be regarded as a study weakness, ongoing assessments of long-term immunogenicity and safety are being performed over 3 years.

No clinically relevant safety or tolerability issues were observed in any age group, which is consistent with previous findings from phase 1 studies of TDV in healthy flavivirus-naive adults [20, 21]. Mild injection-site pain was the most frequently reported solicited AE (by 46% of TDV-treated participants aged 12–45 years and by 35% of children aged 1.5–11 years). The incidence of unsolicited AEs was much lower. The most common were headache (3.2% of TDV recipients and 2.7% of placebo-treated participants) and injection site pain (2.4% of TDV recipients and 0% of placebo-treated participants).

The study population included participants who were seropositive for up to 4 DENV serotypes at enrollment. As expected, baseline seropositivity to ≥1 serotype was more prevalent among adults (around 84%) than children (40%) in these dengue-endemic countries. No increase was observed in safety signals or in vaccine virus replication in participants who were seropositive at baseline. Hence, preexisting antibodies did not increase reactogenicity or the magnitude or duration of TDV viral replication following TDV administration. In fact, the proportion of participants in whom TDV replication was observed was lower in adults with DENV preexposure (8%) than in the youngest children (38%).

TDV administration induced robust immune responses in the participants in these dengue-endemic countries. Seropositivity increases were observed after TDV vaccination in all age groups. Among adults, the first TDV dose improved seropositivity to DENV-1, DENV-2, and DENV-3 in the small group of individuals who were seronegative upon enrollment, while the second dose increased the seropositivity rate for DENV-4. Among the younger age groups, the vaccine was very immunogenic: 72%

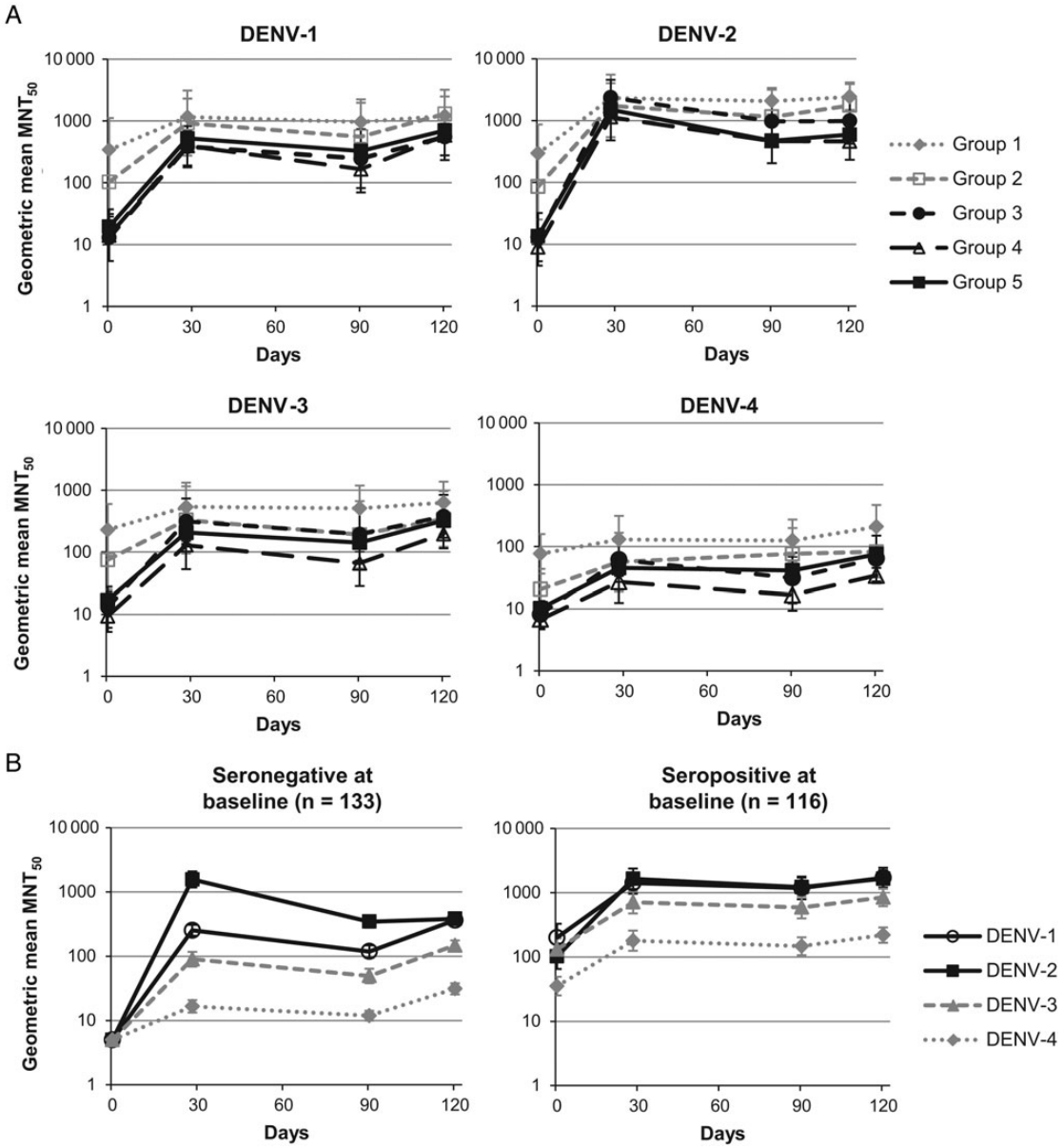

Figure 4. Titers for each dengue virus (DENV) serotype over time in tetravalent dengue vaccine (TDV) recipients in the full analysis set. Findings are stratified by DENV serotype in groups 1–5 (*A*) and by baseline DENV serostatus for DENV-1–4 (*B*). Seropositivity at baseline was defined as seropositivity for any DENV at day 0. Data are geometric mean 50% microneutralization titers (MNT_{50}), with 95% confidence intervals.

of children aged 1.5–11 years in group 5 became seropositive to all 4 serotypes after a single dose; this proportion increased to 93% after 2 doses (compared with 30% in the placebo group).

In general, after 1 or 2 doses of TDV, GMTs of neutralizing antibodies were highest for DENV-1 or DENV-2, followed by DENV-3 and DENV-4 (Figure 4). A single TDV dose induced increases in GMTs for all DENV serotypes in all age groups; a second dose increased DENV-1, DENV-3, and DENV-4 GMTs, particularly in children. The second dose had little impact on GMTs in initially seropositive participants, but it slightly increased (by <3-fold) neutralizing antibody responses to DENV-1, DENV-3, and DENV-4 in seronegative participants. These increases in GMTs can reflect an increase in the number of seropositive subjects and/or an increase in titer for subjects who were already seropositive. To further explore these observations, an ongoing clinical study of TDV is comparing immune responses after 1 or 2 doses in children in dengue-endemic countries.

The GMTs and seropositivity rates for multiple DENV serotypes observed after a single dose of TDV in this study are encouraging, particularly in participants who were seronegative at baseline. As expected, TDV induced higher levels of neutralizing antibodies to all 4 DENV serotypes in participants who were

initially seropositive, consistent with findings that preimmunity to flaviviruses (including DENV) has a priming effect on both the humoral and cellular response to subsequent dengue vaccination [22, 23]: the relative increase was smaller, but the GMTs induced to all 4 DENV serotypes were higher.

Transient viral RNA indicative of replication of TDV-2 (the only type detected) was observed after the first dose and mainly in younger seronegative children. Although TDV-2 replicated in some recipients, it did not cause any detectable clinical signs: no association was observed between the presence of viral RNA and unsolicited or solicited local or systemic AEs. As in the phase 1 studies, very little TDV viral replication was observed after the second dose. These data suggest that the immune response induced by a single dose of TDV is sufficient to inhibit TDV viral replication on exposure to live, attenuated DENV. TDV also induced the highest GMTs to DENV-2, followed by DENV-1, in both seronegative and seropositive participants. Although correlates of protection have not yet been determined for dengue vaccines [24], another tetravalent live attenuated dengue vaccine (CYD-TDV) that has a yellow fever backbone induced relatively equivalent levels of neutralizing antibodies to the 4 DENV serotypes after 3 doses [25], but it provided lower efficacy [26, 27] against DENV-1 and -2 than against DENV-3 and -4.

In conclusion, TDV is generally well tolerated in people aged 1.5–45 years who live in dengue-endemic countries. The vaccine candidate is immunogenic for all DENV serotypes in all age groups after 1 or 2 doses, irrespective of prevaccination dengue serostatus.

Notes

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Potential conflicts of interest. M. K. S. and D. W. are employed by Takeda Vaccines. M. R. is employed by Takeda Development Center Americas. G. S. G. and D. T. S. were employed by Inviragen at the time the study was conducted. D. T. S. is an advisor to Takeda Vaccines. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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