

# Long-term Safety and Immunogenicity of a Tetravalent Dengue Vaccine Candidate in Children and Adults: A Randomized, Placebo-Controlled, Phase 2 Study

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**Background.** We report long-term safety and immunogenicity of Takeda's tetravalent dengue vaccine candidate (TAK-003) in healthy children and adults living in dengue-endemic areas in Puerto Rico, Columbia, Singapore, and Thailand.

**Methods.** In part 1 of this phase 2, randomized, placebo-controlled trial we sequentially enrolled 1.5–45 year olds (n = 148) into 4 age-descending groups, randomized 2:1 to receive 2 doses of TAK-003 or placebo 90 days apart. In part 2, 1–11 year olds (n = 212) were enrolled and randomized 3:1 to TAK-003 or placebo groups. We assessed neutralizing antibody titers for the 4 dengue serotypes (DENV) up to month 36 in part 1, and symptomatic dengue and serious adverse events (SAEs) up to month 36 in both parts.

**Results.** At month 36, seropositivity rates were 97.3%, 98.7%, 88.0% and 56.0% for DENV-1, -2, -3 and -4, respectively. Seropositivity rates varied significantly for DENV-4 according to serostatus at baseline (89.5% in seropositives versus 21.6% in seronegatives). No vaccine-related SAEs were reported.

**Conclusions.** The trial demonstrated persistence of neutralizing antibody titers against TAK-003 over 3 years in children and adults living in dengue-endemic countries, with limited contribution from natural infection. TAK-003 was well tolerated.

**Clinical Trials Registration.** NCT01511250

**Keywords.** dengue; vaccine; immunogenicity; safety; persistence.

Dengue viruses, transmitted principally by the female *Aedes aegypti* mosquito, are endemic in over 100 countries worldwide, putting an estimated 3.9 billion people at risk of dengue viral infections [1–3]. Dengue infection can be asymptomatic or can lead to severe influenza-like symptoms, including high fever (40°C) accompanied by the following clinical manifestations: arthralgia, myalgia, headache, retro-orbital pain, nausea, vomiting, and rash, which manifest 4–10 days after a bite (blood meal) from an infected mosquito. Disease is generally self-limiting, with symptoms lasting 2–7 days; however, in a small percentage of patients, the infection may progress to potentially life-threatening severe clinical manifestations, including dengue

hemorrhagic fever and dengue shock syndrome. Mortality rates from severe dengue are around 10%–20% if left untreated, but less than 1% with appropriate and timely medical interventions [4, 5]. It has been estimated that worldwide 390 million dengue infections occur annually, of which 96 million are symptomatic [6], and 500 000 cases require hospitalization [7].

Four serotypes of dengue virus have been identified (DENV-1, DENV-2, DENV-3, and DENV-4) which cocirculate in endemic areas [7]. Recovery from infection with one serotype provides immunity to that serotype for decades, but there is an increased risk of developing severe dengue with subsequent infections from heterologous serotypes, possibly due to antibody-dependent enhancement [8]. For this reason, vaccine development is focused on tetravalent formulations aimed at providing simultaneous immunity to all 4 serotypes. In clinical trials, a live attenuated tetravalent dengue vaccine (Dengvaxia [CYD-TDV]; Sanofi Pasteur) has been shown to be efficacious and safe in seropositive individuals; however, the World Health Organization recommends prevaccination screening, and that only persons with evidence of past dengue infection receive the vaccine [4, 9]. Vaccination without screening could be considered in areas with recent documentation of seroprevalence rates of at least 80% by age 9 years [9]. The development of safe, effective, and affordable dengue vaccines for use irrespective of

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serostatus remains a high priority. While primary infection in most children is asymptomatic or mild [10], as already noted, secondary infection with a different serotype can lead to severe dengue, and the highest rates of mortality due to severe disease occur in those younger than 18 years of age [7, 11].

Takeda's tetravalent dengue vaccine candidate (TAK-003) is based on a live-attenuated dengue serotype 2 virus that provides the genetic "backbone" for all 4 vaccine viruses, which were originally designed and constructed by scientists at the Division of Vector-Borne Diseases of the US Centers for Disease Control and Prevention [12]. The DENV-2 strain (TDV-2) is based on an attenuated laboratory-derived virus, DEN-2 Primary Dog Kidney (PDK)-53 [13]. The other 3 virus strains (TDV-1, TDV-3, and TDV-4) are chimeras generated by replacing the pre-membrane (prM) and envelope (E) genes of TDV-2 with those from wild-type DENV-1, DENV-3, and DENV-4 strains [14, 15]. TAK-003 has been shown to be immunogenic and well tolerated in previous clinical trials in healthy adults and children, including those seronegative for dengue serotypes prior to vaccination [16–22].

We performed a phase 2 study to evaluate the safety and immunogenicity of TAK-003 in healthy children and adults in 4 dengue-endemic countries. This was the first TAK-003 study in a dengue-endemic setting, and the only study performed in children under 2 years of age. Having previously reported data from the first 4 months of the study [23], we now present

the safety and immunogenicity data obtained during 3 years of follow-up after first dose administration, from study months 4 to 36.

## METHODS

This phase 2, double-blind, randomized, placebo-controlled study was conducted at 8 study sites across 4 countries (Puerto Rico, Columbia, Thailand, and Singapore) between November 2011 and April 2016. The protocol was approved by all appropriate institutional review boards/ethics committees and competent authorities, and was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines, and applicable regulatory requirements, including registration on [www.clinicaltrials.gov](http://www.clinicaltrials.gov): NCT01511250. Written informed consent (or assent where appropriate) was obtained from each participant and their legal guardian prior to enrollment in the study. We have previously reported the details of the materials and methods regarding the first 4 months of the study [23].

### Participants

The study was initially conducted in 2 parts (Figure 1), participants in both parts being eligible for inclusion if they were in good health, and tested negative for human immunodeficiency virus (HIV), hepatitis B, and hepatitis C infections. In addition, participants in part 1 were required to have normal values for

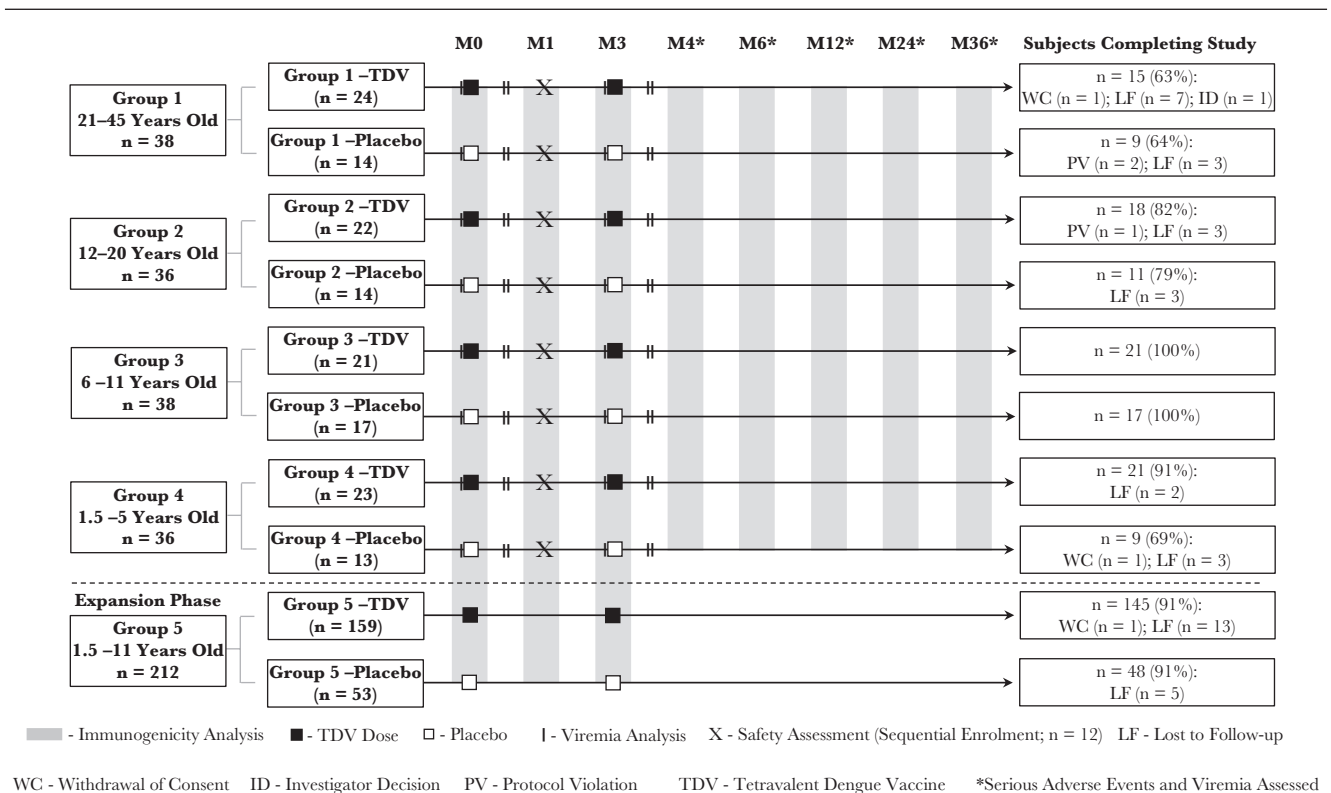


Figure 1. Study design and participant disposition (safety set).

blood and urine laboratory tests at enrollment. The main exclusion criteria included febrile illness (temperature  $\geq 38^{\circ}\text{C}$ ) or acute infection within 3 days of vaccination; significant dermatologic disease in the last 6 months; systemic corticosteroid therapy ( $\geq 0.5$  mg/kg/day) within the previous 6 months; suspected immunodeficiency; or previous participation in a dengue vaccine trial [23].

### Study Design

In part 1, 1.5 to 45-year-old participants were enrolled sequentially in 4 age-descending groups and randomized 2:1 to receive either TAK-003 or placebo (Figure 1) [23]. The sequential enrollment involved 12 participants in group 1 being enrolled, vaccinated, and monitored for safety for 28 days. These data were evaluated by the data safety monitoring board and once approved, the remaining participants were enrolled in group 1, concurrently with the first 12 participants in group 2; this system of enrollment was maintained for all 4 study groups. All participants then received a second injection 90 days after the first injection (ie, TAK-003 or placebo were administered on study days 0 and 90).

In part 2, an expansion phase of the study, 212 additional children aged 1.5–11 years were enrolled and randomized 3:1 into group 5 to receive either TAK-003 or placebo in the same schedule (Figure 1). Part 2 participants were not included in the long-term immunogenicity assessment, but they were included in this long-term safety assessment.

### Study Treatments

A 0.5-mL single dose of TAK-003 contained the following serotype composition:  $2 \times 10^4$  plaque forming units (PFU) of TDV-1;  $5 \times 10^4$  PFU of TDV-2;  $1 \times 10^5$  PFU of TDV-3; and  $3 \times 10^5$  PFU of TDV-4. The formulation was selected based on the safety and immunogenicity results from phase 1 studies [18, 19]. The placebo was 0.5 mL of sterile phosphate-buffered saline. TAK-003 or placebo were administered subcutaneously into the deltoid region of the arm.

### Assessments

The primary immunogenicity outcome was seropositivity rates to each of the 4 dengue serotypes at month 4, 30 days after the second injection, as measured by microneutralization assay [19]. Neutralizing antibody titers were defined as the dilution resulting in a 50% reduction in plaque formation ( $\text{MNT}_{50}$ ), seropositivity rates being group proportions with titers  $\geq 10$ . Secondary outcomes included geometric mean neutralizing antibody titers (GMTs) and seropositivity rates against each of the 4 TDV serotypes at months 1, 3, 4, 6, 12, 24, and 36; and the incidence of symptomatic dengue during the study, as identified by 3 consecutive days of fever ( $>38^{\circ}\text{C}$ ) and confirmed by diagnostic polymerase chain reaction (PCR) assay. In this report we present GMTs and seropositivity rates from part 1 of the study assessed over 3 years.

### Statistical Analysis

This was an exploratory study and was not powered to detect statistical differences between the TAK-003 and placebo groups, the number of participants being chosen empirically based on studies with similar vaccines in this stage of development at the time of planning. Immunogenicity assessments were performed on the per protocol set, which included all randomized participants who received the 2 planned injections and had an evaluation at month 4. These data are presented by age group with associated 95% confidence intervals (95% CI). Safety assessments were performed on the safety set, which included all randomized participants who received at least 1 dose of the study vaccine or placebo. The full analysis set included all randomized participants who received at least 1 injection of TAK-003 or placebo, and from whom valid pre- and postinjection blood samples were obtained.

## RESULTS

Of the 148 participants randomized in part 1, 121 (81.8%) completed the study to month 36. Across groups, 2 participants withdrew their consent, 21 were lost to follow-up, 1 was withdrawn by the investigator after becoming pregnant, and 3 were withdrawn due to protocol violations (Figure 1). In part 2, 193 of the 212 randomized participants (91%) completed the 3-year follow-up. Baseline demographic characteristics are described in Table 1. The mean ages of participants were similar across TAK-003 and placebo recipients within each age-descending group. As would be expected with the increased risk of exposure over time for individuals living in dengue-endemic areas, the youngest age groups had the lowest rates of baseline seropositivity, with higher rates seen in older age groups.

### Safety

In our previous report of the frequency and severity of solicited and unsolicited adverse events up to month 4, we found that only the frequency of local reactions, mainly mild in severity, was higher after TAK-003 compared with placebo [23]. In this 3-year follow-up we found no clinically meaningful differences in safety outcomes, that is serious adverse events (SAEs), between the TAK-003 and placebo groups, or among the different age groups.

Over the course of the entire study (month 0 to 36), 29 participants (24 TAK-003 recipients and 5 placebo recipients) in groups 1–5 reported SAEs (Table 2), but none of the SAEs were considered by the investigators or sponsor to be vaccine related. The most frequently reported SAEs were infections and infestations. Those occurring between months 4 and 36 in the TAK-003 group included: spontaneous abortion; cholecystitis; hand, foot, and mouth disease; dengue fever; urinary tract infection; rotaviral diarrhea; tonsillar hypertrophy; incision site hematoma; and viral infection. Participants in the placebo group reported cellulitis and an abscess, dengue fever, and influenza. No deaths were reported during the study.

**Table 1. Study Population Demographics at Baseline**

Age Group	Treatment Group	Mean Age $\pm$ SD, y	Male, %	Seropositive, %
Group 1, 21–45 y	TAK-003 (n = 24)	29 $\pm$ 7.5	58	79
	Placebo (n = 14)	30 $\pm$ 7.9	43	86
Group 2, 12–20 y	TAK-003 (n = 22)	16 $\pm$ 2.3	45	68
	Placebo (n = 14)	17 $\pm$ 2.8	50	93
Group 3, 6–11 y	TAK-003 (n = 21)	8.4 $\pm$ 1.6	71	43
	Placebo (n = 17)	7.8 $\pm$ 1.6	53	18
Group 4, 1.5–5 y	TAK-003 (n = 23)	3.3 $\pm$ 0.9	70	30
	Placebo (n = 13)	3.5 $\pm$ 0.9	46	8
Group 5, 1.5–11 y	TAK-003 (n = 159)	6.6 $\pm$ 2.7	45	42
	Placebo (n = 53)	6.8 $\pm$ 2.9	64	55

Abbreviation: TAK-003, Takeda tetravalent dengue vaccine candidate.

There were 8 pregnancies reported during the study, 4 each in the TAK-003 and placebo groups. Pregnant TAK-003 vaccinees were 21 to 35 years of age; one 35 year old with a conception date estimated to be 21 days after her first dose of TAK-003 suffered a spontaneous abortion at approximately 4 weeks of gestation and withdrew from the study. Estimated conception dates for the other 3 pregnancies were 114, 227, and 968 days after the second dose of TAK-003, and all 3 had uncomplicated deliveries of healthy babies. The 4 pregnant women in the placebo group were 20 to 40 years old, who estimated they conceived 11 to 92 days after placebo injection. One had an elective abortion at 4 weeks; the other 3 had uncomplicated deliveries of healthy babies.

#### Incidence of Symptomatic Dengue

The observed incidence of PCR-confirmed dengue fever was low during the entire study period (month 0 to 36). Of 23 suspected cases who were tested, 6 had PCR-confirmed dengue infection (Table 2); 4 participants in the TAK-003 groups (4/249; 1.6%) and 2 participants in the placebo groups (2/111; 1.8%); 4

of these 6 dengue cases required hospitalization, 2 each in the TAK-003 and placebo groups. Two cases were in baseline seronegative participants; both required hospitalization, 1 each in the TAK-003 and placebo groups.

#### Immunogenicity

For the study population as a whole, GMTs of neutralizing antibodies against the 4 dengue serotypes increased in all age groups following vaccination with TAK-003. Persisting antibody titers were observed in the peripheral blood of participants for up to 3 years after the administration of the first vaccine dose (Figure 2). Note, data from month 3 and 24 timepoints are not presented in Figure 2 and Figure 3 due to space restrictions and clarity; the overall trends of the antibody response profiles are not altered by the absence of these data. No substantial changes from baseline GMT levels were observed in any of the placebo groups. For participants who were seropositive for any of the DENV serotypes at baseline, GMTs at month 0 tended to increase with age, with the highest titers observed within the oldest age group.

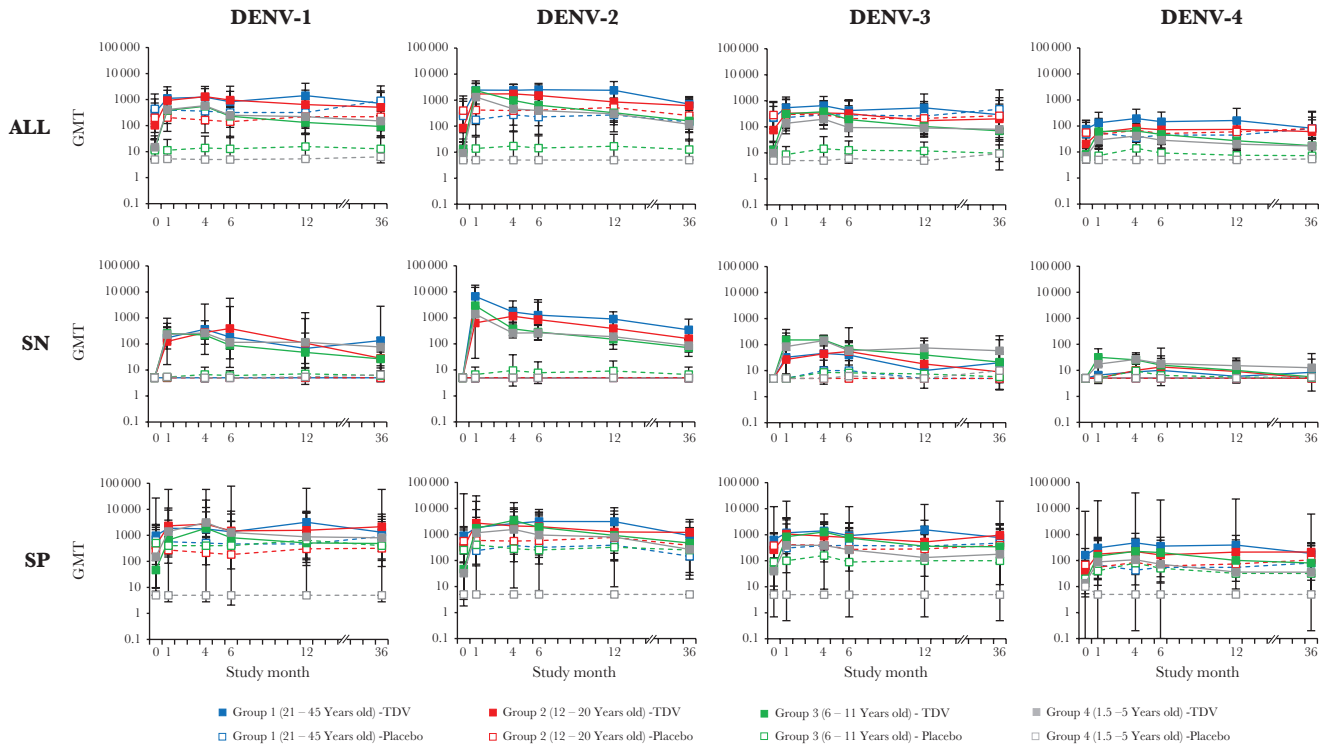
**Table 2. Occurrences of SAEs and PCR-Confirmed Dengue Fever in the Different Groups Over the 3 Years of the Study**

Age Group	Treatment Group	SAEs, No. (%)	Cases of Dengue Fever	
			Suspected, No. <sup>a</sup>	PCR-Confirmed, No., d <sup>b</sup>
Group 1, 21–45 y	TAK-003 (n = 24)	4 (16.7)	1	0
	Placebo (n = 14)	0	0	0
Group 2, 12–20 y	TAK-003 (n = 22)	1 (4.5)	1	1, 372 d
	Placebo (n = 14)	0	0	0
Group 3, 6–11 y	TAK-003 (n = 21)	4 (19.0)	2	1, 250 d
	Placebo (n = 17)	1 (5.8)	0	0
Group 4, 1.5–5 y	TAK-003 (n = 23)	1 (4.3)	3	0
	Placebo (n = 13)	2 (15.4)	1	1, 290 d
Group 5, 1.5–11 y	TAK-003 (n = 159)	14 (8.8)	10	2, 883 and 884 d
	Placebo (n = 53)	2 (3.8)	5	1, 51 d
All age groups	TAK-003 (n = 249)	24 (9.6)	17 (6.8%)	4 (1.6%)
	Placebo (n = 111)	5 (4.5)	6 (5.4%)	2 (1.8%)

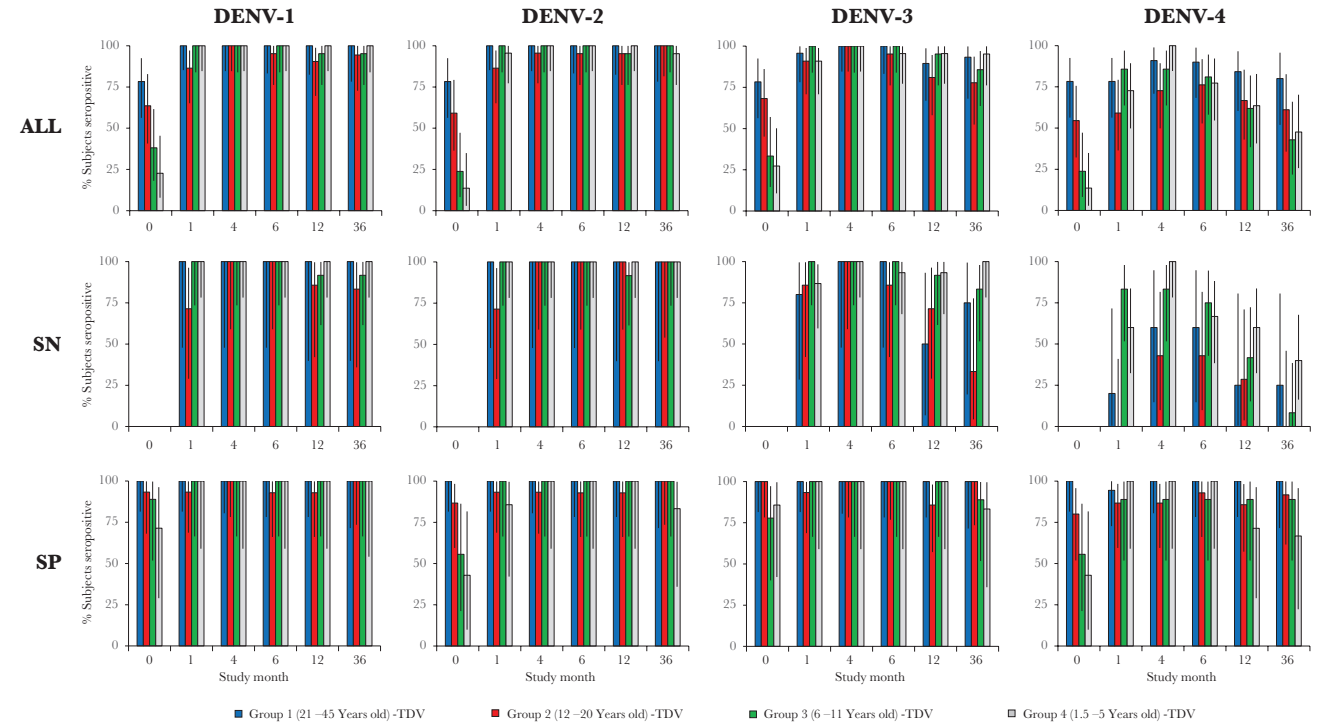
Abbreviation: PCR, polymerase chain reaction; SAE, serious adverse event; TAK-003, Takeda tetravalent dengue vaccine candidate.

<sup>a</sup>Participants who had a blood draw to test for suspected dengue infection.

<sup>b</sup>Day after second injection on which dengue infection was confirmed.



**Figure 2.** Serotype-specific geometric mean antibody titers (GMTs) in all participants, participants seronegative (SN) at baseline, and participants seropositive (SP) at baseline (per protocol set). Abbreviations: DENV, dengue virus; TDV, tetravalent dengue vaccine.



**Figure 3.** Serotype-specific seropositivity rates in all participants, baseline seronegative (SN) participants, and baseline seropositive (SP) participants (per protocol set). Abbreviations: DENV, dengue virus; TDV, tetravalent dengue vaccine.



At month 4, following vaccination with 2 doses of TAK-003, the antibody titers in the overall population were 853, 1177, 358, and 81 against DENV-1, -2, -3, and -4 serotypes, respectively. The degree of response varied by age groups with a trend to higher GMTs in older age groups (Figure 2). Across all serotypes, the GMTs were higher in baseline-seropositive than baseline-seronegative participants. In baseline seronegatives, the GMTs against DENV-1 and -2 were higher in the adults and adolescent age groups, while for DENV-3 and -4 the GMTs were higher in the 2 younger age groups. At this timepoint (month 4), the GMT against DENV-4 was comparatively low (GMT of 19) in the baseline seronegative vaccinees.

At month 4, high seropositivity rates were observed for all 4 serotypes in all age groups (Figure 3). After 2 doses of TAK-003, seropositivity rates were 100%, 98.9%, 100%, and 87.4% for DENV-1, -2, -3, and -4 in comparison to 50.9%, 49.1%, 54.5%, and 41.8% in the placebo groups, respectively. In vaccinees, seropositivity rates were high for DENV-1, -2, and -3 irrespective of baseline serostatus but there was a minor difference for DENV-4 (79.5% in baseline seronegatives versus 93.8% in baseline seropositives). In baseline seronegative vaccinees, the seropositivity rates against DENV-4 varied by age groups (60%, 42.9%, 83.3%, and 100% in 21–45, 12–20, 6–11, and 1.5–5 years age groups, respectively).

By month 36, 3 years after first vaccination, GMTs remained higher than those observed in the placebo groups for all 4 serotypes; at this timepoint GMTs were 240, 274, 125, and 32 in TAK-003 vaccinees, and 47, 34, 43, and 19 in placebo recipients for serotypes DENV-1, -2, -3, and -4, respectively. In baseline seropositive TAK-003 vaccinees, 36-month GMTs for DENV-1, -2, -3, and -4 serotypes were 1127, 701, 543, and 123, respectively, compared with 399, 209, 287, and 71 in baseline seropositive placebo recipients. Despite generally lower GMTs in the baseline seronegative vaccinees, antibody titers against DENV-1, -2, and -3 (49, 104, and 28, respectively) still persisted above both baseline and placebo group levels (6.2, 6.0, and 7.0, respectively) at the end of the 3-year follow-up period (Figure 2). However, GMTs against DENV-4 were similar in the baseline seronegative vaccinees (8.0) and placebo recipients (5.3) at this timepoint. In participants seronegative at baseline, GMTs were higher in the adult vaccinee group for DENV-1 and -2 and in the youngest group for DENV-3 and -4, while in the baseline seropositive groups, GMTs were higher for all the serotypes in the adult and the adolescent vaccinee groups compared to the other 2 younger age groups.

The seropositivity rates at month 36 persisted to different extents for the 4 serotypes: 97.3%, 98.7%, 88.0%, and 56.0% of the vaccinees were seropositive for DENV-1, -2, -3, and -4, respectively, compared with 48.9%, 44.4%, 46.7%, and 42.2% in the placebo recipients. Overall seropositivity rates were higher for DENV-1, -2, and -3 serotypes, than for DENV-4 in vaccinees. While in baseline seropositive participants there were higher

rates of seropositivity for DENV-4, in those seronegative at baseline this declined at 12 months (Figure 3) and was 21.6% at 36 months (versus 89.5% in baseline seropositives). For DENV-3, there was also a trend for seropositivity rates to decline in the adult and adolescent seronegative vaccinee groups, with 2 of 4 and 1 of 6 participants seropositive at 24 months, while 3 of 4 and 2 of 6 were seropositive at 36 months, respectively.

In terms of multivalent seropositivity, 87% of participants in the full analysis set were tetravalent seropositive at month 4 compared with 56% at month 36. These rates were 79% and 22% in baseline seronegatives versus 94% and 89% in baseline seropositives, respectively.

Because the participants in this study lived in dengue-endemic areas, it was likely they would be naturally exposed to dengue viruses during the 3-year follow-up period, which may have impacted antibody persistence. The inclusion of the placebo group allowed some estimation of impact of this natural exposure during follow-up. Only 4.3% (DENV-2) and 8.7% (DENV-1, -3, and -4) of placebo recipients who were seronegative at baseline had become seropositive to individual serotypes at 36 months showing there was limited natural exposure, suggesting it would have had little influence on antibody persistence.

## DISCUSSION

This study demonstrates long-term persistence of immune response 3 years after the first vaccination with TAK-003 in a population of 1.5–45 year olds. Three years after vaccination, the seropositivity rates in the overall population against DENV-1, -2, and -3 were high (88% to 97%), but modest (56%) against DENV-4. This was driven by lower initial immune response in baseline seronegatives against DENV-4 (GMT of 19 at month 4) that subsequently declined to a GMT of 8.0 and a seropositivity rate of 21.6% at 3 years. However, immune persistence against DENV-4 remained high (90%) in baseline seropositives at that timepoint. Three years after vaccination, GMTs tended to be higher for all serotypes in the 2 older age groups in baseline seropositives, but this varied by serotype in baseline seronegatives. An inference about age effect in seronegatives is also limited by the low numbers of such participants in the 2 older age groups.

The finding of relatively low immunogenicity to DENV-4 with the earlier vaccine formulations in neutralization assays, as well as possible interference between the serotypes (ie, TDV-2—the strongest replicator—inhibiting the replication of the other serotypes) was noted in the development program [18, 19, 23]. This has led to reformulation and testing of newer TAK-003 formulations (all differing in the potency of individual serotypes and the balance between the serotypes). The final clinical formulation containing a reduced quantity (1 log unit) of TDV-2 relative to other serotypes was found to provide a more balanced immune response to the

other serotypes, particularly DENV-4 [21, 22]. These studies also supported the optimal dose regimen required for individuals seronegative at baseline, that is 2 doses administered 3 months apart.

No important safety risks were found during the initial study period [23] or in this 3-year follow-up period. No vaccine-related SAEs occurred at any time during the study.

Symptomatic dengue in the study population was infrequent and the low numbers of cases preclude any conclusions about a vaccine effect. Importantly, there was no indication of disease enhancement in the longer term. Recent papers by Biswal et al report data from a large-scale phase 3 trial conducted to assess the efficacy of the final TAK-003 formulation in over 20 000 children and adolescents (4–16 years old) living in dengue-endemic areas [16, 17]. In the secondary endpoint assessment timeframe (18 months post vaccination), TAK-003 demonstrated an overall vaccine efficacy of 73.3% (95% CI, 66.5–78.8); efficacy against dengue leading to hospitalization was 90.4% (95% CI, 82.6–94.7) [16]. In TAK-003 recipients seronegative at baseline (n = 702), serotype-specific GMTs of 184, 1730, 228, and 144 were observed at the 4-month timepoint for DENV-1, -2, -3, and -4 [16]; these data compare favorably to the corresponding (4-month) GMTs in seronegative vaccinees observed during the present study (group 2 [age 12–20 years] GMTs, 276, 1159, 44, and 10; group 3 [age 6–11 years] GMTs, 220, 381, 151, and 25, respectively). Persistence of antibody response to the final formulation and its clinical relevance will be assessed in the long-term part of this phase 3 trial (NCT02747927), which has a 4.5-year follow-up period after second dose administration.

In summary, 3 years after the administration of the first of 2 doses of TAK-003, persistence of antibody titers against TAK-003 was demonstrated in 1.5 to 45-year-old participants in dengue-endemic countries. The vaccine was well tolerated in this population without any important safety risks identified over the 3 years. The clinical relevance of antibody persistence will be further assessed in the ongoing efficacy trial.

## Notes

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**Author contributions.** Principal investigators were C. S., E. B.-S., and I. E. R. Data analyses and interpretation was carried out by J. K., M. R., A. B., A. P., and D. W. All authors made a significant intellectual contribution to the development of this manuscript from conception until approval of the final version for submission.

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**Potential conflicts of interest.** At the time of the study, J. K., M. R., A. B., A. P., and D. W. were permanent employees of Takeda. All other authors report no potential conflicts of interest. 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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