Safety and immunogenicity of one versus two doses of Takeda's tetravalent dengue vaccine in children in Asia and Latin America: interim results from a phase 2, randomised, placebo-controlled study

Xavier Sáez-Llorens, Vianney Tricou, Delia Yu, Luis Rivera, Suely Tuboi, Pedro Garbes, Astrid Borkowski, Derek Wallace

Summary

Background Dengue is the most common mosquito-borne viral disease in human beings, and vector control has not halted its spread worldwide. A dengue vaccine for individuals aged 9 years and older has been licensed, but there remains urgent medical need for a vaccine that is safe and effective against all four dengue virus serotypes (DENV-1–4) in recipients of all ages. Here, we present the preplanned interim analyses at 6 months of a tetravalent dengue vaccine candidate (TDV), which is comprised of an attenuated DENV-2 virus strain (TDV-2) and three chimeric viruses containing the premembrane and envelope protein genes of DENV-1, DENV-3, and DENV-4 genetically engineered into the attenuated TDV-2 genome backbone (TDV-1, TDV-3, and TDV-4).

Methods An ongoing phase 2, randomised, double-blind, placebo-controlled trial of a TDV is being done at three sites in dengue-endemic countries (Dominican Republic, Panama, and the Philippines) to determine its safety and immunogenicity over 48 months in healthy participants aged 2–17 years who were randomly assigned (1:2:5:1) using an interactive web response system (stratified by age) to subcutaneous TDV injection (one 0·5 mL dose containing $2·5\times10^4$ plaque-forming units [PFU] of TDV-1; $6·3\times10^3$ PFU of TDV-2; $3·2\times10^4$ PFU of TDV-3; and $4·0\times10^5$ PFU of TDV-4) in different dose schedules (two-dose regimen at 0 and 3 months, one dose at 0 months, or one dose at 0 months and a booster at 12 months) or placebo. The primary endpoint of this 6 month interim analysis was geometric mean titres (GMTs) of neutralising antibodies against DENV-1–4 in the per-protocol immunogenicity subset at 1 month, 3 months, and 6 months after the first injection. Safety was assessed as a secondary outcome as percentage of participants with serious adverse events in all participants who were injected (safety set), and solicited and unsolicited adverse events (immunogenicity subset). This trial is registered with ClinicalTrials.gov, number NCT02302066.

Findings 1800 participants were enrolled between Dec 5, 2014, and Feb 13, 2015. 1794 participants were given study injection as follows: 200 participants were given two-dose regimen at 0 and 3 months (group 1), 398 were given one dose at 0 months (group 2), 998 were given one dose at 0 months and will be given (trial ongoing) a booster at 12 months (group 3), and 198 were given placebo (group 4). These 1794 participants were included in the safety set; 562 participants were randomly assigned to the immunogenicity subset, of which 503 were included in the per-protocol set. TDV elicited neutralising antibodies against all DENV serotypes, which peaked at 1 month and remained elevated above baseline at 6 months. At 6 months, GMTs of neutralising antibodies against DENV-1 were 489 (95% CI 321–746) for group 1, 434 (306–615) for group 2, 532 (384–738) for group 3, and 62 (32–120) for group 4; GMTs of neutralising antibodies against DENV-2 were 1565 (1145–2140) for group 1, 1639 (1286–2088) for group 2, 1288 (1031–1610) for group 3, and 86 (44–169) for group 4; GMTs of neutralising antibodies against DENV-3 were 160 (104–248) for group 1, 151 (106–214) for group 2, 173 (124–240) for group 3, and 40 (23–71) for group 4; and GMTs of neutralising antibodies against DENV-4 were 117 (79–175) for group 1, 110 (80–149) for group 2, 93 (69–125) for group 3, and 24 (15–38) for group 4. No vaccine-related serious adverse events occurred; 15 (3%) of 562 participants in the immunogenicity subset reported vaccine-related unsolicited adverse events. The reactogenicity profile of TDV was acceptable, and similar to previous findings with TDV.

Interpretation TDV is safe and immunogenic in individuals aged 2–17 years, irrespective of previous dengue exposure. A second TDV dose induced enhanced immunogenicity against DENV-3 and DENV-4 in children who were seronegative before vaccination. These data supported the initiation of phase 3 evaluation of the efficacy and safety of TDV given in a two-dose schedule 3 months apart, with analyses that take into account baseline age and dengue serostatus.

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Hospital del Niño Dr José Renán Esquivel, Panama City, Panama (Prof X Sáez-Llorens MD); **Sistema Nacional de Investigación, SENACYT, Panama City, Panama** (Prof X Sáez-Llorens); **Takeda Vaccines, Singapore, Singapore** (V Tricou DPhil); **De La Salle Health Sciences Institute, Dasmariñas, Philippines** (D Yu MD); **Hospital Maternidad Nuestra Senora de La Altagracia, Santo Domingo, Dominican Republic** (L Rivera MD); **Takeda Farmacêutica, Rio de Janeiro, Brazil** (S Tuboi MD); **Takeda Vaccines, Boston, MA, USA** (P Garbes MD); **and Takeda Pharmaceuticals International, Zürich, Switzerland** (A Borkowski MD, D Wallace MBBS)

Correspondence to: Dr Vianney Tricou, Takeda Vaccines, Singapore 138567, Singapore **vianney.tricou@takeda.com**

Introduction

Dengue is the most common mosquito-borne viral disease in human beings, occurring in more than 125 countries and causing approximately 100 million symptomatic infections per year.[1] Dengue transmission by *Aedes* spp mosquitoes is ubiquitous throughout the tropics, with the highest incidence in the Americas and Asia.[1,2] Vector control efforts have not prevented the rapid

Research in context

Evidence before this study

The search for an efficacious dengue vaccine started decades ago, but challenges include the need to induce a simultaneous, durable immune response to all four DENV serotypes to avoid the theoretical concern of antibody-dependent enhancement caused by the vaccine. When this study was designed, no dengue vaccines had yet been licensed, although a chimeric yellow fever-tetravalent dengue vaccine candidate (CYD-TDV) had entered phase 3 efficacy trials. We searched PubMed on Nov 4, 2016, with the terms "dengue" AND "vaccine" AND "phase 2 OR phase II" and identified 12 primary reports of phase 2 clinical trials of dengue vaccine candidates. Additionally, in phase 3 studies done in Asia and Latin America, CYD-TDV given in a three-dose schedule (0, 6, and 12 months) provided protection against all-serotype dengue fever in 57–61% of recipients, but efficacy and safety were affected by previous exposure to dengue. Hence, CYD-TDV has now been licensed for those older than 9 years. A tetravalent dengue live attenuated virus (TDENV) was evaluated in a two-dose schedule (0 and 6 months), but the induced immunity had low persistence, so its manufacturers are currently evaluating TDENV given with a tetravalent purified inactivated vaccine via a prime-boost strategy in a phase 1 study. The other vaccine candidate that is most advanced in development includes admixtures of monovalent live attenuated tetravalent vaccines, TV003 and TV005, which have entered phase 3 evaluation. Although phase 2 data had not been published at the time of writing, small phase 1 studies indicated that it was immunogenic after one or two doses (0 and 6 months).

Added value of this study

Our large phase 2 cohort was drawn from two dengue endemic-regions (Asia and Latin America) and approximates the real-world population that would be vaccinated with TDV. To our knowledge, this is the first study to evaluate one versus two TDV doses given 3 months apart. We showed that the induced humoral immunogenicity remains robust 6 months after the initial dose, which is only slightly lower in vaccinees who were seronegative at baseline compared with all vaccinated participants. The second dose helped to increase the proportion of individuals responding immunologically to vaccination. The adverse event profile was consistent with that previously reported, confirming that TDV was safe and well tolerated from the age of 2 years in children and adolescents, irrespective of dengue serostatus at vaccination.

Implications of the available evidence

Although CYD-TDV has been licensed in several countries, it is not approved for children younger than 9 years. The need remains for a vaccine that is safe and effective against all four DENV serotypes in recipients of all ages, especially those younger than 9 years, irrespective of previous dengue exposure and infecting serotype. Results from our phase 2 study of TDV supported the initiation of phase 3 evaluation of a two-dose schedule in a study designed to support the use of TDV over a wide age range; and potentially, implementation of TDV within the Expanded Programme on Immunization.

global spread of dengue,[3,4] with the annual number of cases reported in WHO member states increasing from 2·2 million to 3·2 million between 2010 and 2015.[5]

Dengue is caused by infection with one of four serotypes of the dengue flavivirus (DENV-1, DENV-2, DENV-3, and DENV-4), and usually manifests subclinically, or with symptoms that include fever, headache, arthralgia, myalgia, retro-orbital pain, rash, bleeding, thrombocytopenia, or leucopenia.[2,6] A small proportion of patients can develop severe life-threatening dengue haemorrhagic fever or dengue shock syndrome.[6] No effective antivirals are currently available; treatment is limited to supportive care. Infection with one DENV serotype leads to homologous, but not long-term, heterotypic protective immunity, and subsequent infection with a different serotype is a major risk factor for severe disease;[7,8] hence a safe and effective vaccine that simultaneously protects against all four serotypes is needed.

A chimeric yellow fever virus-tetravalent dengue vaccine (CYD-TDV; Dengvaxia, Sanofi Pasteur) was approved for use in people aged 9 years and older in more than ten countries after phase 3 efficacy trials[9–11] were completed. However, continued development of additional dengue vaccines is needed to provide high levels of protection from dengue in all age groups (especially children younger than 9 years), irrespective of previous dengue exposure or regional DENV serotype distribution. Two more vaccine candidates are in phase 3 efficacy evaluation: Takeda's live attenuated tetravalent dengue vaccine, TDV,[12–15] and National Institute of Allergy and Infectious Diseases and Institute Butantan's live attenuated tetravalent vaccine candidate TV003/TV005.[16] Several other candidates are in phase 1 clinical trials.[17–19]

Takeda's TDV comprises an attenuated DENV-2 virus strain (TDV-2) and three chimeric (dengue–dengue) viruses containing the premembrane and envelope protein genes of DENV-1, DENV-3, and DENV-4 genetically engineered into the attenuated TDV-2 genome backbone (TDV-1, TDV-3, and TDV-4).[20] In phase 1 and phase 2 studies, Takeda's TDV induced neutralising antibody responses and seroconversion to all four DENV serotypes,[12–15] as well as cross-reactive T-cell-mediated responses that might be necessary for broad protection against dengue fever.[21] TDV was generally safe and well tolerated in children and adults living in dengue-endemic and non-endemic countries.

To obtain safety and immunogenicity data in a similar population to that in a large-scale phase 3 efficacy trial (NCT02747927), the safety of TDV was evaluated in a

large cohort of healthy children and adolescents living in dengue-endemic countries in Asia and Latin America. The aim was to compare the immune responses to TDV given either as a two-dose primary series (0 months and 3 months), or as one primary dose with and without a booster at 12 months. Humoral immune responses are being assessed in a subset of participants for up to 48 months (immunogenicity subset). Additionally, the cellular immune responses to TDV will be assessed in a subset of participants aged 10 years or older, the results of which are to be published separately. Because the TDV formulations evaluated in the phase 1[12–14] and phase 2[15] studies generated a strong immune response against DENV-2 and a relatively lower immune response to DENV-4, the dose of TDV-2 in this formulation was reduced by one log relative to the other serotypes to promote a more balanced immune response to all four serotypes.[22] Here, we present data from a 6 month interim analysis of this ongoing phase 2 study.

Methods

Study design and participants

This multicentre, randomised, double-blind, placebo-controlled study is ongoing at three hospitals or clinics in the Dominican Republic, Panama, and the Philippines. Healthy participants aged 2–17 years were enrolled and assessed for eligibility, then randomly assigned (1:2:5:1) to receive either one TDV dose at 0 months and one dose at 3 months (group 1), one dose at 0 months (group 2), one dose at 0 months and a booster at 12 months (group 3), or placebo (group 4). The 1:2:5:1 randomisation ratio was chosen to provide data on a one-dose schedule, with or without a booster, to support the potential use of this dosing regimen in phase 3 development because previous studies of TDV had already established the safety and immunogenicity of a two-dose schedule (0 months and 3 months, as given to group 1).

The study protocol and informed consent forms were approved by the institutional review boards at the participating centres, namely De La Salle Health Science Institute in the Philippines, Comité de Bioetica Institucional in the Dominican Republic, and Comité de Bioética en Investigación at Hospital del Niño Dr José Renán Esquivel in Panama. This study was done in accordance with the Edinburgh revision of the Declaration of Helsinki, International Conference on Harmonisation and Good Clinical Practice (ICH-GCP), and applicable national and local regulations and requirements.

Eligible participants had to be healthy, able to comply with trial procedures, and had to be available for the duration of the study. Key exclusion criteria included previous participation in a dengue vaccine trial, being given inactivated vaccine 14 days before enrolment or live vaccine 28 days before enrolment, hypersensitivity to any study vaccine component, any moderate or severe disease, fever (38ºC or higher), pregnancy or breastfeeding, being given any investigational product 30 days before first visit, or an impaired or altered immune system.

Participants or their legally acceptable representative had to sign and date a written informed consent form (and assent form, where required) before the initiation of any trial procedures, according to local regulatory requirements.

Randomisation and masking

Participants from each group were also randomly selected for inclusion in the immunogenicity subset in a final ratio of approximately 1:2:2:1. Fewer participants were randomly assigned into the immunogenicity subset from group 1 (the two-dose immunogenicity control for the groups receiving one dose, with or without a booster) and from group 4 (the control for the groups receiving TDV) to reduce the number of participants undergoing repeated blood sampling.

Participants were randomly assigned into the four groups using an interactive web response system (IWRS). Randomisation was stratified by age group based on the participants' age when informed consent was given: 2–5 years, 6–11 years, and 12–17 years. Participants were also randomly selected by IWRS for inclusion in the immunogenicity subset. The IWRS generated unique identification numbers for study vials.

Designated pharmacists or vaccine administrators who were unblinded at each site had no role in the assessment of participant safety. This interim analysis was done by a separate unblinded team at a clinical research organisation independent of the funder. This team had access to individual treatment assignments, but was not involved in subsequent trial assessments. Personnel at the study funder, clinical research organisation, and study sites who are involved in the study will remain blinded to individual participant data until unblinding after study completion.

Procedures

TDV's serotype composition was $2{\cdot}5\times10^4$ plaque-forming units (PFU) of TDV-1, $6{\cdot}3\times10^3$ PFU of TDV-2, $3{\cdot}2\times10^4$ PFU of TDV-3, and $4{\cdot}0\times10^5$ PFU of TDV-4, in a lyophilised formulation. The placebo was phosphate-buffered saline. TDV was reconstituted in water-for-injection at the time of administration, and 0·5 mL of either TDV or placebo was injected subcutaneously into the upper arm.

We collected blood samples for the measurement of neutralising antibodies from participants in the immunogenicity subset before each study injection at 0 months and 3 months, and at 1 month and 6 months, and analysed centrally.

Participants in the immunogenicity subset were given diary cards and instructed to record solicited (predefined conditions of specific interest) local reactions for 7 days; solicited systemic adverse events for 14 days; and unsolicited (spontaneously reported) adverse events for 14 days; and unsolicited adverse events for 28 days after

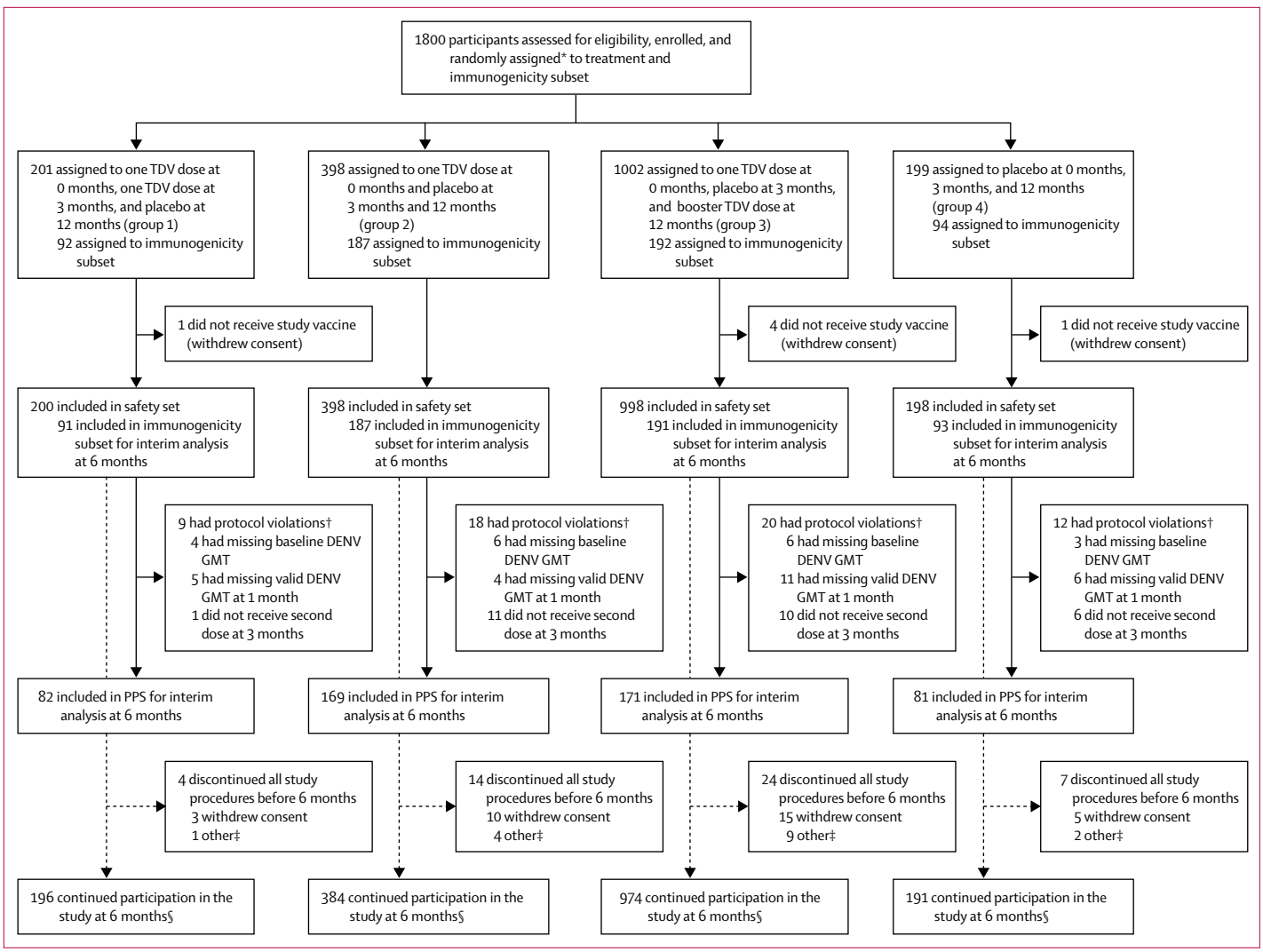


Figure 1: **Trial profile**
GMT=geometric mean titres. TDV=Takeda's tetravalent dengue vaccine. PPS=per-protocol set. *Randomisation stratified by age at time of consent. †Some participants had several protocol violations; also included two participants who used prohibited medications during the study, and one participant who had a protocol violation identified based on blind data review. ‡Included 11 lost to follow-up, two pregnancies, one adverse event, one who moved out of the study area, and one who was discontinued by investigator because of poor adherence to protocol; data not available by group to maintain blinding in ongoing study. §Included four participants who did not have the second injection, but continued study safety follow-up, and four participants who missed the visit or phone call at 6 months, but received the second injection and participated in study procedures beyond 6 months; data not available by group to maintain blinding in ongoing study.

each study injection. Safety oversight was under the direction of an independent data monitoring committee to protect the ethical and safety interests of recruited participants.

Outcomes

The primary endpoint was immunogenicity shown by geometric mean titres (GMT) of neutralising antibodies to each of the four DENV serotypes at 1 month, 3 months, and 6 months after the first injection using a micro-neutralisation test (MNT_{50}).[15] The secondary immunogenicity endpoint was the proportion of participants seropositive for each of the four DENV serotypes (in which seropositivity by MNT_{50} was defined as a reciprocal neutralising titre of ten or higher) at 1 month, 3 months, and 6 months. Immunogenicity endpoints were summarised for the per-protocol set, which comprised all participants from the immunogenicity subset who had no major protocol violations and for whom valid pre-dosing and post-dosing blood samples were available. The secondary safety endpoints of this interim analysis were the percentages of participants with serious adverse events in all participants who were injected (safety set), and solicited and unsolicited adverse events in the immunogenicity subset. Documentation and identification of febrile episodes potentially caused by dengue virus infection through laboratory-confirmation were included in the safety evaluation, but are not presented as

part of these interim results and will be reported in future follow-up analyses. Analysis of interim safety and immunogenicity data was done up to 6 months (day 180) after the first injection.

Statistical analyses

This trial was designed to be primarily descriptive, and was not based on testing formal null hypotheses. Therefore, the sample size was not determined based on formal statistical power calculations. The planned sample size of 1800 participants (and 600 in the immunogenicity subset) was assumed to provide a reasonable number of participants for evaluation of the persistence of immune responses after administration of a single dose (with or without a booster) versus a two-dose schedule, and to provide an adequate safety database of exposed subjects in the safety set to support a phase 3 efficacy trial.

Seropositivity rates and GMTs of dengue neutralising antibodies in the per-protocol set were calculated with 95% CIs for each of the four DENV serotypes individually at baseline and at 1 month, 3 months, and 6 months. We summarised the percentages of participants with at least bivalent, trivalent, and tetravalent seropositivity by group at each study visit. These data were also presented by baseline dengue serostatus; we defined seropositivity as a reciprocal MNT_{50} of ten or higher for one or more DENV serotype(s).

We summarised safety data from the immunogenicity subset descriptively, with solicited adverse events presented by age (<6 years and ≥6 years).

This trial is registered with ClinicalTrials.gov, number NCT02302066.

Role of the funding source

Takeda employees and subcontractors had a role in study design, data collection, data analysis, data interpretation, and writing of the report. All authors had full access to all the data in the study and had responsibility for the decision to submit for publication.

Results

Between Dec 5, 2014, and Feb 13, 2015, 1800 participants recruited in Panama, the Philippines, and the Dominican Republic from hospitals or clinics were assessed for eligibility, gave consent to participate in the study, and were randomly assigned to one of four study groups to receive TDV in different dose schedules, or placebo (figure 1). Six participants withdrew consent to participate and did not receive study vaccine; the remaining 1794 participants had the first assigned injection, and were included in the safety set to be assessed for overall safety (serious adverse events) at the 6 month interim analysis. Of these, 562 participants had also been randomly selected for inclusion in the immunogenicity subset within their study groups and were assessed for adverse events; 503 participants in the per-protocol set had their blood samples assessed for GMTs and seropositivity.

Of 1794 participants who had the first injection, 49 (3%) prematurely discontinued all study procedures before 6 months, mainly because of consent withdrawal (figure 1). 47 of 49 participants withdrew before 3 months and did not have the second injection; two participants withdrew after the second injection. Additionally, four participants who did not have the second injection continued to safety follow-up.

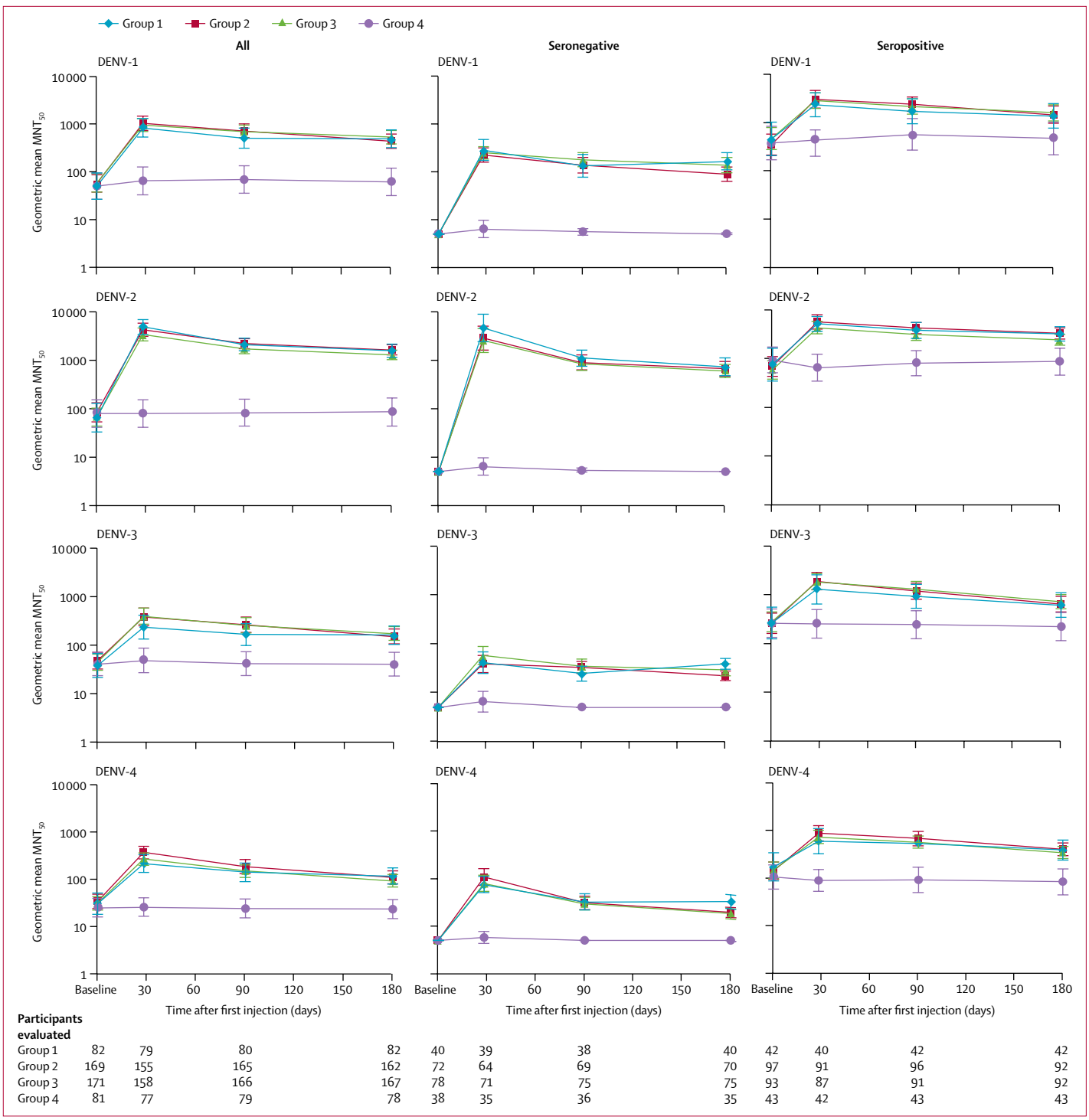
The study participants' mean age was 7·3 years (table 1). The demographic data for the per-protocol set were similar to those of the safety set, except that a smaller proportion was aged 2–5 years in the per-protocol set. The proportion of participants who were seropositive for any DENV at baseline in the per-protocol set was similar between study groups.

TDV elicited neutralising antibodies against all dengue serotypes in vaccinated participants, with the highest concentrations induced against DENV-2, and all GMTs remaining higher than at baseline at 6 months (figure 2). Notably, at the 6-month interim analysis timepoint described in this Article, the 12-month booster TDV dose had not yet been given to participants in group 3; groups 2 and 3 were, therefore, identical in terms of treatment, because individuals in both groups had been given one TDV dose at 0 months. At 6 months, GMT of neutralising antibodies against DENV-1 were 489 (95% CI 321–746)

	Group 1 (TDV at 0 months and 3 months)	Group 2 (TDV at 0 months)	Group 3 (TDV at 0 months and at 12 months)	Group 4 (placebo)	Total
Safety set					
n	200	398	998	198	1794
Age (years)	7·3 (4·01)	7·3 (4·14)	7·3 (4·06)	7·0 (3·97)	7·3 (4·06)
Age range (years)					
2–5	84 (42%)	165 (41%)	418 (42%)	84 (42%)	751 (42%)
6–11	78 (39%)	158 (40%)	393 (39%)	78 (39%)	707 (39%)
12–18	38 (19%)	75 (19%)	187 (19%)	36 (18%)	336 (19%)
Sex					
Male	100 (50%)	191 (48%)	512 (51%)	103 (52%)	906 (51%)
Female	100 (50%)	207 (52%)	486 (49%)	95 (48%)	888 (49%)
Per-protocol set					
n	82	169	171	81	503
Age (years)	8·3 (4·23)	8·0 (4·16)	8·1 (4·18)	7·7 (4·21)	8·0 (4·18)
Age range (years)					
2–5	27 (33%)	53 (31%)	60 (35%)	28 (35%)	168 (33%)
6–11	33 (40%)	74 (44%)	69 (40%)	35 (43%)	211 (42%)
12–17	22 (27%)	42 (25%)	42 (25%)	18 (22%)	124 (25%)
Sex					
Male	37 (45%)	81 (48%)	83 (49%)	43 (53%)	244 (49%)
Female	45 (55%)	88 (52%)	88 (51%)	38 (47%)	259 (51%)
Baseline seropositivity to any dengue serotype	42 (51%)	97 (57%)	93 (54%)	43 (53%)	275 (55%)

Data are n, n (%), or mean (SD). TDV=Takeda's tetravalent dengue vaccine.

Table 1: **Baseline characteristics**



Participants evaluated												
Group 1	82	79	80	82	40	39	38	40	42	40	42	42
Group 2	169	155	165	162	72	64	69	70	97	91	96	92
Group 3	171	158	166	167	78	71	75	75	93	87	91	92
Group 4	81	77	79	78	38	35	36	35	43	42	43	43

***Figure 2*: GMTs of dengue neutralising antibodies in the per-protocol immunogenicity subset**
Left panels: all participants; middle panel: participants seronegative for all DENV serotpyes at baseline; right panel: participants seropositive at baseline. GMT=geometric mean titres. MNT=microneutralisation test.

for group 1, 434 (306–615) for group 2, 532 (384–738) for group 3, and 62 (32–120) for group 4; GMT of neutralising antibodies against DENV-2 was 1565 (1145–2140) for group 1, 1639 (1286–2088) for group 2, 1288 (1031–1610) for group 3, 86 (44–169) for group 4; GMT of neutralising antibodies against DENV-3 was 160 (104–248) for group 1, 151 (106–214) for group 2, 173 (124–240) for group 3, 40 (23–71) for group 4; and GMT of neutralising

antibodies against DENV-4 was 117 (79–175) for group 1, 110 (80–149) for group 2, 93 (69–125) for group 3, 24 (15–38) for group 4. In participants who were seronegative at baseline, the two-dose schedule elicited higher DENV-3 and DENV-4 GMTs at 6 months (in group 1) than the single dose given to participants in groups 2 and 3. However, DENV-3 and DENV-4 GMTs were not different when measured at the same interval from the last TDV dose (ie, month 6 for group 1 *vs* month 3 for groups 2 and 3; figure 2). In participants who were seropositive at baseline, GMTs were similar in all TDV groups (figure 2).

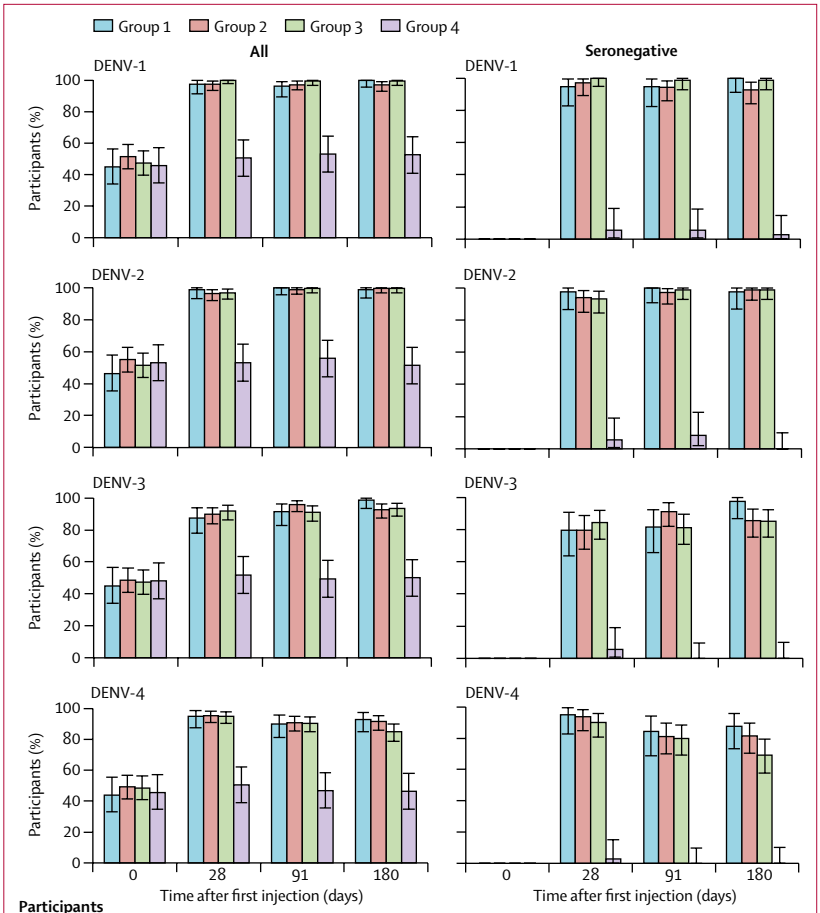
The proportion of participants who were seropositive for individual DENV in TDV-vaccinated participants increased to 87–100% in all study groups by month 1, and remained high for each DENV at month 6 (85–100%; figure 3). In participants who were seronegative at baseline, the two-dose schedule led to higher seropositivity rates to DENV-3 (98%) and DENV-4 (88%) at month 6 than the one-dose schedule (86% and 85% for DENV-3 and 81% and 69% for DENV-4). Unlike GMTs, this observation was not an artefact of a different interval since the last dose of TDV, and is also present when seropositivity at the same interval from the last TDV dose is considered (figure 3; group 1 *vs* groups 2 and 3 at months 3 and 6).

At baseline, 225 (45%) of the 503 participants in the per-protocol set had tetravalent seropositivity to DENV. By month 1, more than 80% of TDV-vaccinated participants in each study group were seropositive for all four serotypes, and 96% or more were seropositive for at least three serotypes (table 2). Multivalent seropositivity rates were maintained at month 6; the 6-month rates being slightly higher after two TDV doses (table 2). Of participants who were seronegative at baseline, a higher proportion of those who had two doses were seropositive for four seroptypes (85% for group 1) than in those who had one dose (70% for group 2 and 65% for group 3 at month 6, and 74% for group 2 and 68% for group 3 at month 3).

Overall, 1402 participants had one TDV dose, and 194 received two doses. None of the 40 serious adverse events reported by 32 participants (2% of the safety set) was related to the study vaccine or procedures. Two serious adverse events led to early study discontinuation (allergic reaction to food colourant and immune thrombocytopenia purpura), but the participants continued safety follow-up. One death unrelated to the study vaccine or procedures occurred after the 6 month analysis cutoff (due to pneumonia, pulmonary tuberculosis, and septic shock). Two pregnancies led to discontinuation of study injections, but the participants subsequently gave birth normally and their babies were healthy.

No major differences in unsolicited adverse event rates were seen between TDV and placebo, either after the first dose versus the second dose, or related to seropositivity at baseline, and most were unrelated to the study vaccination (table 3). Of 186 participants who reported any unsolicited adverse events, 161 (87%) reported mild adverse events. 15 (3%) of 562 participants in the immunogenicity subset reported vaccine-related unsolicited adverse events. Of participants who were seropositive at baseline, five (2%) of 260 reported vaccination-related unsolicited adverse events after the first TDV injection (*vs* one [2%] of 47 participants who were given placebo) and none of 45 participants reported vaccination-related unsolicited adverse events after the second TDV injection (*vs* two [1%] of 252 who were given placebo). In participants who were seronegative at baseline, vaccine-related unsolicited adverse events were reported by four (2%) of 202 participants after the first TDV injections (*vs* none of 43 who were given placebo) and one (2%) of 42 participants after the second TDV injection (*vs* two [1%] of 203 who were given placebo).

The most frequently reported injection-site reaction in participants was pain, with rates differing according to age and intervention (appendix p 1). In children younger than 6 years, proportions of participants who reported pain were similar after TDV or placebo after the first injection;



Participants evaluated	All				Seronegative			
Group 1	82	79	80	82	40	39	38	40
Group 2	169	155	165	162	72	64	69	70
Group 3	171	158	166	167	78	71	75	75
Group 4	81	77	79	78	38	35	36	35

Figure 3: **Seropositivity rates for each dengue serotype in the per-protocol immunogenicity subset**
Left panels: all participants; right panels: participants seronegative for all DENV at baseline.

See Online for appendix

	Group 1 (TDV at 0 months and 3 months)	Group 2 (TDV at 0 months)	Group 3 (TDV at 0 months and at 12 months)	Group 4 (placebo)
Seropositive or seronegative for dengue at baseline				
Baseline				
n	82	169	171	81
Seropositivity to 4 DENV serotypes	34 (41%), 31–53	80 (47%), 40–55	76 (44%), 37–52	35 (43%), 32–55
Seropositivity to ≥3 DENV serotypes	35 (43%), 32–54	83 (49%), 41–57	81 (47%), 40–55	39 (48%), 37–60
Seropositivity to ≥2 DENV serotypes	37 (45%), 34–57	85 (50%), 43–58	83 (49%), 41–56	39 (48%), 37–60
Month 1				
n	79	155	158	77
Seropositivity to 4 DENV serotypes	64 (81%), 71–89	131 (85%), 78–90	138 (87%), 81–92	37 (48%), 37–60
Seropositivity to ≥3 DENV serotypes	78 (99%), 93–100	150 (97%), 93–99	152 (96%), 92–99	39 (51%), 39–62
Seropositivity to ≥2 DENV serotypes	78 (99%), 93–100	152 (98%), 94–100	158 (100%), 98–100	41 (53%), 42–65
Month 3				
n	80	165	166	79
Seropositivity to 4 DENV serotypes	66 (83%), 72–90	145 (88%), 82–92	141 (85%), 79–90	37 (47%), 36–58
Seropositivity to ≥3 DENV serotypes	77 (96%), 89–99	159 (96%), 92–99	158 (95%), 91–98	39 (49%), 38–61
Seropositivity to ≥2 DENV serotypes	79 (99%), 93–100	163 (99%), 96–100	166 (100%), 98–100	40 (51%), 39–62
Month 6				
n	82	162	167	78
Seropositivity to 4 DENV serotypes	75 (91%), 83–97	139 (86%), 80–91	139 (83%), 77–89	36 (46%), 35–58
Seropositivity to ≥3 DENV serotypes	81 (99%), 93–100	156 (96%), 92–99	159 (95%), 91–98	39 (50%), 39–62
Seropositivity to ≥2 DENV serotypes	82 (100%), 96–100	159 (98%), 95–100	165 (99%), 96–100	40 (51%), 40–63
Seronegative for all DENV at baseline				
Month 1				
n	39	64	71	35
Seropositivity to 4 DENV serotypes	28 (72%), 55–85	44 (69%), 56–80	53 (75%), 63–84	1 (3%), 0–15
Seropositivity to ≥3 DENV serotypes	38 (97%), 87–100	62 (97%), 89–100	66 (93%), 84–98	1 (3%), 0–15
Seropositivity to ≥2 DENV serotypes	38 (97%), 87–100	63 (98%), 92–100	71 (100%), 95–100	2 (6%), 1–19
Month 3				
n	38	69	75	36
Seropositivity to 4 DENV serotypes	26 (68%), 51–83	51 (74%), 62–84	51 (68%), 56–78	0, 0–10
Seropositivity to ≥3 DENV serotypes	36 (95%), 82–99	64 (93%), 84–98	68 (91%), 82–96	0, 0–10
Seropositivity to ≥2 DENV serotypes	37 (97%), 86–100	67 (97%), 90–100	75 (100%), 95–100	0, 0–10
Month 6				
n	40	70	75	35
Seropositivity to 4 DENV serotypes	34 (85%), 70–94	49 (70%), 58–80	49 (65%), 54–76	0, 0–10
Seropositivity to ≥3 DENV serotypes	39 (98%), 87–100	65 (93%), 84–98	67 (89%), 80–95	0, 0–10
Seropositivity to ≥2 DENV serotypes	40 (100%), 91–100	67 (96%), 88–99	73 (97%), 91–100	0, 0–10

Data are n or n (%), 95% CI. TDV=Takeda's tetravalent dengue vaccine.

***Table 2*: Seropositivity to multiple dengue serotypes by study group and baseline serostatus in the per-protocol immunogenicity subset**

pain was reported less frequently after the second injection (appendix p 1). More participants older than 6 years reported pain after TDV injection than placebo, and the reporting rates were similar after the first TDV injection (87 [28%] of 308 participants *vs* five [9%] of 57 participants who were given placebo) and second TDV injections (19 [32%] of 60 participants *vs* 42 [15%] of 289 participants who were given placebo; appendix p 1). Pain intensity was mostly mild or moderate, with very few participants (all older than 6 years) reporting severe pain. Erythema and swelling were reported only after the first injection and by very few participants (five [<1%] of 527 participants for erythema and three [<1%] for swelling), all at mild or moderate severity (appendix p 1). Local reactions generally occurred in the 3 days after study injection and lasted for 1–2 days.

In children younger than 6 years, the most common systemic adverse events reported 14 days after either injection were loss of appetite after the first TDV injection, and fever of 38·0°C or more and irritability or fussiness after the second injection (appendix pp 2–3). After the second dose, fever and irritability were reported more frequently in the TDV groups than the placebo groups (four [16%] of 25 participants who had TDV *vs* ten [8%] of

131 participants who had placebo for fever; four [15%] of 26 participants given TDV *vs* six [5%] of 131 participants given placebo for irritability), and the proportions of participants reporting these adverse events were higher than after the first injection. Solicited systemic adverse events generally occurred in the 7 days post-vaccination and lasted 2–5 days. In participants aged 6 years or older, headache was the most commonly reported systemic adverse event in TDV and placebo groups (appendix pp 2–3). Myalgia was the second most common adverse event, reported at similar rates after first or second injection, and more commonly after TDV than placebo (46 [15%] of 307 participants *vs* four [7%] of 57 participants given placebo after the first injection; ten [17%] of 59 participants *vs* 16 [6%] of 287 participants given placebo after the second injection). Few systemic adverse events were described as severe (the highest incidence was for severe headache reported by five [1·4%] of 364 participants who were 6 years or older after the first injection), and the proportion of participants reporting severe adverse events did not differ noticeably between TDV and placebo groups. Systemic adverse events generally occurred in the first week after either injection and lasted for roughly 2–3 days.

No clinically important mean changes from baseline were observed in heart rate, diastolic blood pressure, systolic blood pressure, or body temperature during any study visits in any group.

Discussion

This study is a 6 month interim evaluation of the safety and immunogenicity of TDV given in one-dose or two-dose schedules to a large paediatric cohort. The study participants were seropositive or seronegative to dengue and lived in dengue-endemic countries in Asia and Latin America. TDV was well tolerated, safe, and elicited neutralising antibodies against all DENV in recipients aged 2–17 years, irrespective of previous dengue exposure. Two doses elicited higher proportions of participants who were seropositive for DENV-3 and DENV-4 than one dose in participants who were seronegative before vaccination. At month 6, 85% of participants were seropositive for all four serotypes after two doses, compared with 68% of participants after one dose.

The safety profile was consistent with that observed in earlier phase 1 and 2 studies.[23,24] No serious adverse events were related to TDV. Both TDV regimens were well tolerated in terms of solicited local reactions and systemic adverse events, with no major differences related to baseline serostatus observed. In children younger than 6 years, fever and irritability were the only systemic adverse events observed more frequently with TDV than placebo. The number of adverse events reported were similar or lower than those reported for other live attenuated vaccines including varicella, measles-mumps-rubella, and measles-mumps-rubella-varicella (MMRV).[25–27] In recipients older than 6 years, only headache and myalgia were more frequently reported with TDV than placebo.

	TDV	Placebo	Total
After first injection			
Study groups	1–3	4	1–4
n	469	93	562
Any adverse events	98 (21%)	19 (20%)	117 (21%)
Mild	83 (18%)	14 (15%)	97 (17%)
Moderate	13 (3%)	5 (5%)	18 (3%)
Severe	..	..	2 (<1%)
Hypersensitivity	..	..	1 (<1%)
Varicella	..	..	1 (<1%)
Related adverse events	9 (2%)	1 (1%)	10 (2%)
Mild	..	..	9 (2%)
Abdominal Pain	..	..	1 (<1%)
Vomiting	..	..	1 (<1%)
Pyrexia	..	..	2 (<1%)
Gastroenteritis	..	..	2 (<1%)
Viral Infection	..	..	1 (<1%)
Rash	..	..	2 (<1%)
Moderate	..	..	1 (<1%)
Gastroenteritis	..	..	1 (<1%)
After second injection			
Study groups	1	2–4	1–4
n	90	444	534
Any adverse events	12 (13%)	57 (13%)	69 (13%)
Mild	10 (11%)	54 (12%)	64 (12%)
Moderate	2 (2%)	2 (1%)	4 (1%)
Severe	..	..	1 (<1%)
Pneumonia	..	..	1 (<1%)
Related adverse events	1 (1%)	4 (1%)	5 (1%)
Mild	..	..	4 (1%)
Vomiting	..	..	1 (<1%)
Gastroenteritis	..	..	1 (<1%)
Nasopharyngitis	..	..	1 (<1%)
Viral infection	..	..	1 (<1%)
Moderate	..	..	2 (<1%)
Injection site haemorrhage	..	..	1 (<1%)
Pyrexia	..	..	1 (<1%)

Data are n (%) of participants reporting adverse events. Numbers of participants after the second injection are those who had a second TDV or placebo injection. Some data have been removed because they could potentially unblind the participants' treatment allocation. TDV=Takeda's tetravalent dengue vaccine.

Table 3: **Severity and vaccine relatedness of unsolicited adverse events reported within 28 days of injection in the immunogenicity subset**

Consistent with results from previous studies,[12–15] GMTs and seropositivity to all four DENVs peaked 1 month after the first TDV dose, and remained elevated at the interim 6 month evaluation timepoint, including in participants who were seronegative at baseline. The neutralising antibody profiles were similar overall, with the exception of DENV-4 titres and seropositivity, which were higher than at the same timepoints in previous studies, particularly in participants who were seronegative at baseline. In previous studies, DENV-4 GMTs after the

subcutaneous injection of high-dose formulation were between 8 and 17 at month 1, and between 6 and 12 at month 3,[12–15] compared with 75–108 at month 1 and 30–33 at month 3 in this study. Similarly, the proportions of participants who were seropositive were between 24% and 59% at month 1 and 18% and 53% at month 3 in previous studies,[12–15] compared with 90–95% at month 1 and 80–84% at month 3 in this study. Hence, reducing the relative potency of the TDV-2 component of the tetravalent formulation might have enhanced immune responses to TDV-4.

The GMTs and seropositivity rates in the baseline-seropositive participants were not affected by the number of doses. However, in participants who were seronegative at baseline, the DENV-3 and DENV-4 GMTs at month 6 were higher after two doses than after one dose, with no difference when measured at the same interval (ie, 3 months) from the last TDV dose. The proportion of participants who were seropositive for DENV-3 increased from around 80% after the first TDV dose to 98% after the second dose (figure 3), but there was no similar increase in seropositivity rates for DENV-4, which were already high (>90%) after the first dose. These results suggest that most individuals who had not mounted an immune response to DENV-3 after the first dose responded after the second dose. Multiple doses of live attenuated vaccines are often given to improve the proportion of responders or vaccine take. For example, greater protection against varicella was observed after a compressed two-dose schedule of the MMRV vaccine than after a single dose of monovalent varicella vaccine. The two-dose schedule was also associated with increased anti-varicella zoster virus antibody concentrations and seropositivity rates at all timepoints.[25]

Although correlates of protection for dengue vaccines have not been identified, the high, sustained levels of immunogenicity induced by TDV against DENV-1–4, even in seronegative vaccinees, are encouraging. A multivariate regression analysis of plaque reduction neutralisation test ($PRNT_{50}$) immunogenicity data from several phase 2 studies of CYD-TDV showed that induced GMTs were higher in participants who were seropositive at baseline for dengue, yellow fever, and Japanese encephalitis.[28] The efficacy of CYD-TDV also varied according to immune status at vaccination—ie, lower in individuals who were initially seronegative.[9,11,24] In dengue-seropositive individuals, the level and quality of the immune response to CYD-TDV were boosted, possibly because the vaccine mimics an attenuated and subclinical secondary infection with a heterotypic DENV serotype.[29] This finding prompted our analysis of TDV's immunogenicity according to baseline dengue serostatus. Although TDV also induced reduced GMTs in baseline-seronegative participants, seropositivity in this vulnerable population after a single TDV dose was high. Seropositivity after vaccination might be an important measure of vaccine performance, because it provides evidence of a measurable response to vaccination. In the absence of a correlate of protection, it is not possible to say what magnitude of response is required for protection. However, a vaccine that generates humoral and cellular immunity and shows measurable seropositivity to all dengue serotypes in most individuals, even those without previous exposure to dengue, suggests that such a vaccine is suitable for assessment in a large-scale vaccine efficacy trial. The dosing schedule chosen for this trial should generate multivalent responses in the highest proportion of individuals who were initially seronegative.

Our study has some limitations, such as the short period of this interim analysis (although the participants are being followed up for 48 months), and the fact that dengue vaccine efficacy cannot necessarily be predicted from humoral immunogenicity, as shown by phase 3 studies of CYD-TDV. However, TDV also induces cell-mediated immune responses to dengue non-structural proteins, as a result of its dengue backbone,[21] which might further contribute to protection against dengue disease.[24] Phase 3 efficacy studies will be needed to confirm this possibility. Another dengue vaccine candidate, TV003/TV005, has also been shown to induce neutralising antibody and T-cell responses that persist 6 months after vaccination, but its developers acknowledge that only efficacy studies will show whether these responses will protect against natural DENV infection.[16] One strength of our study is that it was done in large cohorts recruited from dengue endemic-regions that approximate the real-world population that would be vaccinated with TDV, and we included regions in which different dengue serotypes are prevalent.

In conclusion, this study confirms previous observations that TDV is well tolerated, safe, and immunogenic in children and adolescents aged 2–17 years, irrespective of previous dengue exposure. A second TDV dose induces enhanced immunogenicity against DENV-3 and DENV-4 in children who are seronegative at the time of vaccination, suggesting that a two-dose schedule of TDV induces a more robust humoral immune response than one dose. These data support phase 3 evaluation of the efficacy and safety of TDV given in a two-dose schedule 3 months apart, with analyses that take into account baseline age and dengue serostatus.

Contributors

All authors contributed to the manuscript drafting, and reviewed and approved the final version. XS-L, DY, and LR were the principal investigators of the trial. VT did the data analysis and interpretation, and coordinated the manuscript drafting. ST and PG designed and monitored the trial. DW oversaw the trial design, data analysis interpretation, and writing of the manuscript. AB supervised the clinical development of the tetravalent dengue vaccine candidate, designed the trial, and interpreted the data.

Declaration of interests

VT, ST, PG, AB, and DW are employed by Takeda Vaccines. XS-L, DY, and LR received funds to their organisations from Takeda Vaccines to support their work in this trial.

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