Efficacy of a tetravalent dengue vaccine in healthy children aged 4–16 years: a randomised, placebo-controlled, phase 3 trial

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Summary

Background A substantial unmet need remains for safe and effective vaccines against dengue virus disease, particularly for individuals who are dengue-naive and those younger than 9 years. We aimed to assess the efficacy, safety, and immunogenicity of a live attenuated tetravalent dengue vaccine (TAK-003) in healthy children aged 4–16 years.

Methods We present data up to 18 months post-vaccination from an ongoing phase 3, randomised, double-blind trial of TAK-003 in endemic regions of Asia and Latin America (26 medical and research centres across Brazil, Colombia, Dominican Republic, Nicaragua, Panama, Philippines, Sri Lanka, and Thailand). Healthy children aged 4–16 years were randomly assigned 2:1 (stratified by age and region) to receive two doses of TAK-003 or two doses of placebo, 3 months apart. Investigators, participants and their parents or guardians, and sponsor representatives advising on trial conduct were masked to trial group assignments. Participants presenting with febrile illness were tested for virologically confirmed dengue (VCD) by serotype-specific RT-PCR. In timeframes beginning 30 days post-second dose, the primary endpoint (overall vaccine efficacy) was assessed in the first 11 months, and the secondary endpoints (efficacy by baseline serostatus, serotype, hospitalised dengue, and severe dengue) in the first 17 months. This study is registered with ClinicalTrials.gov, NCT02747927.

Findings 20 099 participants were randomly assigned and vaccinated between Sept 7, 2016, and Aug 18, 2017; 19 021 (94·6%) were included in the per protocol analysis, and 20 071 (99·9%) in the safety set. The primary endpoint was achieved with an overall vaccine efficacy of 80·2% (95% CI 73·3 to 85·3; 61 cases of VCD in the TAK-003 group *vs* 149 cases of VCD in the placebo group). In the secondary endpoint assessment timeframe, an overall vaccine efficacy of 73·3% (95% CI 66·5 to 78·8) was observed. Analysis of secondary endpoints showed efficacies of 76·1% (95% CI 68·5 to 81·9) in individuals who were seropositive at baseline, 66·2% (49·1 to 77·5) in individuals who were seronegative at baseline, 90·4% (82·6 to 94·7) against hospitalised dengue, and 85·9% (31·9 to 97·1) against dengue haemorrhagic fever. Efficacy varied by individual serotypes (DENV 1, 69·8% [95% CI 54·8 to 79·9]; DENV 2, 95·1% [89·9 to 97·6]; DENV 3, 48·9% [27·2 to 64·1]; DENV 4, 51·0% [–69·4 to 85·8]). Cumulative rates of serious adverse events were similar in TAK-003 (4·0%) and placebo (4·8%) recipients, and were consistent with expected medical disorders in the study population. Infection was the most frequent reason leading to serious adverse events. 20 participants (<0·1% of the safety set) were withdrawn from the trial due to 21 adverse events by the end of part two; 14 of these participants received TAK-003 and six received placebo.

Interpretation TAK-003 was well tolerated and efficacious against symptomatic dengue in children regardless of serostatus before immunisation. Vaccine efficacy varied by serotype, warranting continued follow-up to assess longer-term vaccine performance.

Funding Takeda Vaccines.

Lancet 2020; 395: 1423–33

Published **Online** March 17, 2020 https://doi.org/10.1016/S0140-6736(20)30414-1

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Introduction

Almost half of the global population live in dengue-endemic areas, with the highest burden of disease observed in the Americas and Asia.[1–4] According to one estimate, 390 million cases of dengue infection occur annually and 96 million of those manifest clinically.[1,2] Dengue—a leading cause of hospitalisation and death among children and adults in most Asian and Latin American countries—is characterised by periodic outbreaks, which have a substantial effect on human health and on global and national economies.[5] Dengue is caused by four virus serotypes (DENV 1–4), which are transmitted by mosquito vectors.[6] A tetravalent dengue vaccine (CYD-TDV; Dengvaxia, Sanofi Pasteur, Lyon, France) is approved in several endemic countries for individuals aged 9 years and older who have evidence of

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See Online for appendix

Research in context

Evidence before this study

Between database inception and Feb 10, 2020, we searched PubMed for publications in English with terms including "dengue vaccine", "dengue vaccine clinical trial", and "dengue vaccine phase 1, 2, and 3". We also searched ClinicalTrials.gov, and national and international public health agencies. All reports of phase 1–3 dengue vaccine clinical trials were included as being relevant or of interest. At the time this phase 3 study was first conceived and designed, no dengue vaccines were licensed for use in any country. Since then, CYD-TDV (Dengvaxia, Sanofi Pasteur, Lyon, France) has been registered in 18 dengue-endemic countries for individuals aged 9 years and older; this vaccine is not recommended for use in individuals who are dengue-naive and hence requires evidence of previous dengue exposure for its use. Two additional vaccine candidates are under advanced clinical development: TV003/TV005 and TDENV PIV. Several phase 1 and 2 clinical trials have shown two doses of TAK-003 to be immunogenic against all four dengue serotypes in adults and children regardless of previous dengue exposure. This phase 3 trial was designed to assess the efficacy and safety of TAK-003 in children aged 4–16 years in eight dengue-endemic countries in Asia and Latin America. A previous report described primary endpoint data for this study, with TAK-003 showing an overall vaccine efficacy of 80%. Rapid onset of protection was observed after a first dose along with encouraging data from exploratory analysis of secondary efficacy endpoints.

Added value of this study

Our data show that TAK-003 has an acceptable safety profile in healthy children aged 4–16 years, and is efficacious in the prevention of symptomatic dengue disease in both individuals who are dengue-naive and those previously exposed. Efficacy varied against individual serotypes, with an overall efficacy of 66% in individuals who were dengue-naive and 76% in those who were pre-exposed. In addition, TAK-003 reduced the number of dengue cases that were hospitalised by 90% along with an 86% reduction in dengue haemorrhagic fever. These data represent a major step forward in the development of an effective and safe dengue vaccine for use in people of all ages, irrespective of previous dengue exposure at the time of vaccination.

Implications of all the available evidence

A solution is required urgently to the major public health challenges raised by dengue disease. The efficacy and safety data presented in this report suggests potential for TAK-003 as a component of the multimodal approach to control dengue.

previous infection.[7–10] Vaccines that can provide protection in all age groups including young children, regardless of serostatus before immunisation, are still needed.

The Takeda tetravalent dengue vaccine candidate (TAK-003) was originally designed and constructed by scientists at the Division of Vector-Borne Diseases of the US Centers for Disease Control and Prevention using a live-attenuated dengue serotype 2 virus, which provides the genetic backbone for all four vaccine viruses.[11,12] The DENV 2 strain (TDV-2) is based on an attenuated laboratory-derived virus, DEN-2 PDK-53.[13] The other three vaccine strains (TDV-1, TDV-3, and TDV-4) are chimeras generated by replacing the envelope and pre-membrane genes of TDV-2 with those from wild-type DENV 1, DENV 3, and DENV 4 strains.[12,14–16]

Phase 1 and 2 studies have shown TAK-003 to be well tolerated and immunogenic against serotypes 1–4.[17–22] In a placebo-controlled study published alongside this Article, in which safety and immunogenicity were assessed up to 48 months after vaccination, antibodies persisted above baseline at 48 months and there was a lower relative risk of virologically confirmed dengue (VCD; 0·35, 95% CI 0·19–0·65).[23] We did a large, phase 3, randomised clinical trial in three parts at sites in Latin America and Asia to assess the efficacy, immunogenicity, and safety of two doses of TAK-003 in healthy children aged 4–16 years. At the end of part 1 (ie, 12 months after the second dose) of this study, we presented an interim report with assessment of the primary endpoint, showing a high overall vaccine efficacy of 80·2% (95% CI 73·3–85·3) against VCD.[24] Onset of efficacy was observed after one dose, with 81·0% (64·1–90·0) efficacy during the period between the two doses. Exploratory analyses of secondary efficacy endpoints during part 1 found vaccine efficacy was 74·9% (57·0–85·4) in individuals who were seronegative at baseline and 95·4% (88·4–98·2) against hospitalised VCD. Here, we present the main findings after completion of part 2 of the study (ie, 18 months after the second dose), in which secondary vaccine efficacy endpoints by baseline serostatus, against individual serotypes, hospitalised VCD, and severe VCD were assessed formally.

Methods

Study design and participants

We completed part 2 (18 months post-vaccination) of an ongoing phase 3, randomised, double-blind trial. Healthy children aged 4–16 years inclusive were enrolled and randomly assigned at 26 medical and research centres in eight dengue-endemic countries: Brazil (four sites); Colombia (four sites); Dominican Republic (two sites); Nicaragua (one site); Panama (four sites); the Philippines (four sites); Sri Lanka (four sites); and Thailand (three sites). The main exclusion criteria were febrile illness at the time of randomisation, impaired or altered immune function, hypersensitivity or allergy to any vaccine component, pregnancy or breastfeeding, and previous receipt of a dengue vaccine (study inclusion and exclusion criteria are described fully in the appendix p 3).

Informed assent or consent forms, and the study protocol and its amendments were reviewed and approved by institutional review boards, independent ethics committees, or health authorities. Written informed assent or consent was obtained from all participants or their parents or legal guardians before enrolment. During the study, re-consent was obtained from participants as they legally became adults. At the time of analysis at the end of part 1, adult re-consent was still in process for some participants; however, all data were included and analysed. In the subsequent analysis at the end of part 2 of the trial, any data collected after legal adult age was reached was removed if re-consent had not been obtained within a reasonable timeframe; however, this did not involve the censoring of any dengue cases. This trial is in accordance with the Declaration of Helsinki and the ICH harmonised tripartite guidelines for good clinical practice, with applicable local regulations.

Randomisation and masking

Children who met the study entry criteria were randomly assigned 2:1 to receive two doses of TAK-003 or two doses of placebo, 3 months apart. Randomisation was stratified by region and age (4–5 years, 6–11 years, and 12–16 years) using an interactive web response system and dynamic block assignment. Randomisation information was generated by personnel authorised by the trial sponsor, and stored in a secure area accessible only to authorised personnel. A subset of 4000 of the 20 099 participants was randomly selected as described for additional safety and immunogenicity assessments. Investigators, participants, and their parents or guardians, and sponsor representatives advising on trial conduct were unaware of trial group assignments. One or more designated pharmacists or vaccine administrators were unmasked at each site, but had no role in the collection or assessment of participant safety data. These individuals accessed randomisation information through a web portal. To maintain masking, medical writers and some sponsor-affiliated authors had access to group and anonymised individual-level study data. Other authors had access only to the data presented in this report. An independent data monitoring committee with responsibility for safety oversight had access to unmasked data on request.

Procedures

The study consists of three parts for each participant, with active surveillance during part 1 and part 2, and modified active surveillance during part 3 (appendix p 7). Participants or their parents or guardians were contacted at least weekly for the entire duration of the study to remind them to present for evaluation of febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) to ensure robust identification of dengue cases. Part 1 was completed once at least 120 VCD cases were confirmed and participants had 12 months follow-up post-second vaccination. Part 2, as reported here, lasted for a further 6 months for the assessment of secondary efficacy endpoints, and is being followed by an additional 3-year period (part 3) for long-term efficacy and safety evaluation. The total duration of follow-up at the end of part 2 was around 21 months after the first dose, or 18 months after second dose administration.

One 0·5 mL dose of TAK-003 contained approximately 3·6, 4·0, 4·6, and 5·1 $\log_{10}$ plaque forming units of TDV-1, TDV-2, TDV-3, and TDV-4, respectively. Placebo was a 0·5 mL injection of saline. Vaccine or placebo was administered subcutaneously into the upper arm. The lyophilised vaccine kits were kept at 2–8°C during shipping and storage, and reconstituted before administration in PBS solution.

Blood samples were taken from all participants on day 1 (pre-vaccination) and day 120 to measure amounts of dengue neutralising antibodies by microneutralisation test. Additional microneutralisation test blood samples were taken on days 30, 90, 270, and 450, and then annually from the subset participants. Microneutralisation test titres are expressed as the reciprocal of the highest dilution of test serum that shows a 50% reduction in plaque counts compared with that of virus controls. Subset safety assessments included diary-recorded local reactions for 7 days post-vaccination and systemic adverse events for 14 days post-vaccination, and unsolicited adverse events for 28 days post-vaccination. Serious adverse events and adverse events leading to withdrawal from the study were collected in all study participants for the duration of the trial.

During active surveillance, participants presenting with febrile illness or clinically suspected dengue had blood samples taken in the acute (ie, as soon as possible and preferably within 5 days of fever onset) and convalescent phases (ie, 7–14 days after the acute sample; appendix p 7). Testing included: quantitative serotype-specific RT-PCR, dengue NS1/IgM/IgG ELISA, haematocrit, liver enzyme (aspartate aminotransferase and alanine aminotransferase), and platelet counts. RT-PCR and NS1 ELISA assays were done only on the acute sample. Febrile illnesses were evaluated clinically, and additional tests could be done as per local standard of care. Additional details of study methods and procedures are provided in the appendix (p 3).

Outcomes

For efficacy objectives, VCD was defined as febrile illness or illness clinically suspected to be dengue by the investigator with confirmation by positive serotype-specific RT-PCR. Only the first VCD case in a participant was included in the overall vaccine efficacy analysis. However, vaccine efficacy analysis by serotype included the first VCD case for a specific serotype in an individual participant. The primary endpoint was vaccine efficacy of two doses of TAK-003 in preventing VCD induced by any dengue serotype occurring from 30 days

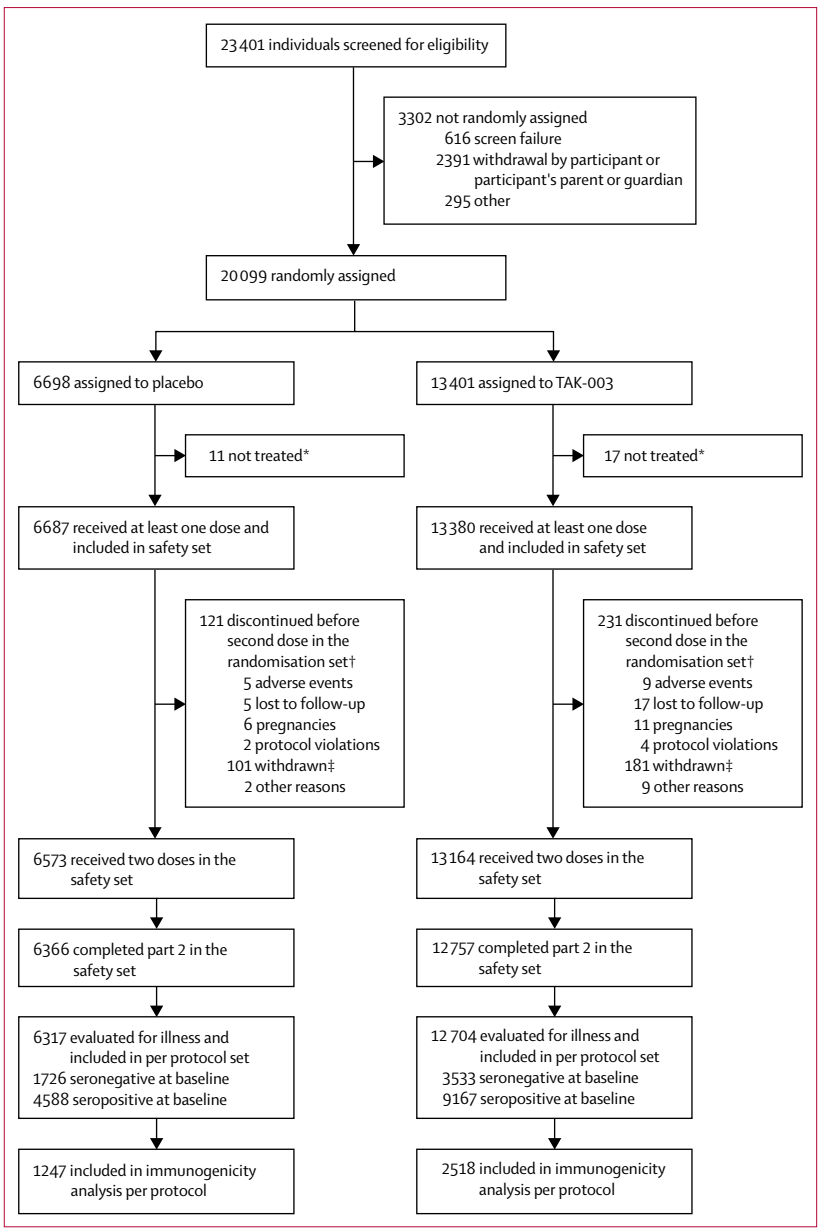

Figure 1: **Trial profile**
Participants who did not receive a vaccine dose are included in the total numbers of participants who discontinued the trial before the second dose. Three participants in the vaccine group and two in the placebo group did not receive a second dose, but continued in the study. Four participants (three assigned to the vaccine group and one assigned to the placebo group) received both vaccine and placebo due to an administrative error; these participants were consequently excluded from the vaccine and placebo groups in the safety population. One participant assigned to the vaccine group received placebo; this participant was consequently included in the placebo group in the safety population. Participants had 12 months of follow-up after second dose at the time of completing part 2 of the trial. Some data might differ from what has been previously published[24] due to the inclusion of updated datasets. *Reason not listed to preserve masking. †Includes non-vaccinated participants. ‡Withdrawn by participant or parent or guardian.

post-second vaccination until the end of part 1.[24] Secondary endpoints of vaccine efficacy against individual serotypes, by baseline serostatus, and efficacy in prevention of hospitalisation and severe dengue were assessed in the timeframe of 30 days post-second vaccination to the end of part 2. Specific criteria for hospitalisation were not defined in the study protocol; participants were hospitalised according to the judgment of individual investigators. Severity of VCD was assessed using two approaches: (1) masked review by the Dengue Case Adjudication Committee (DCAC) using predefined criteria, and (2) by a programme developed by the study statisticians to analyse data according to the WHO 1997 dengue haemorrhagic fever criteria.[25] Details of the DCAC criteria are provided in the appendix (p 5).

Statistical analysis

Efficacy endpoint analyses were done on the per protocol set data (ie, all participants without any major protocol violations; all analysis sets are defined in the appendix p 5). Vaccine efficacy is defined as 1–(λV/λC), where λV and λC denote the hazard rates for the TAK-003 and placebo groups, respectively. Hazard ratios and corresponding 95% CIs were estimated using a Cox proportional hazard model with trial group as a factor, adjusted for age, and stratified by region. The primary vaccine efficacy objective was considered to be met if the lower bound of the 95% CI for vaccine efficacy was above 25%. The sample size calculation was based on the assumption of true vaccine efficacy of 60% and a background annual dengue incidence of 1%. Randomisation of 20 100 participants in a 2:1 ratio (TAK-003:placebo) could enable identification of 120 VCD cases between 30 days post-second vaccination and the end of part 1, providing at least 90% power to rule out a vaccine effect of 25% or more (with a two-sided significance level of 0·05). Secondary vaccine efficacy endpoints were evaluated on the per protocol set using the same methods as the primary endpoint analysis with the aim to rule out vaccine efficacy of 0% in the assessment period 30 days post-second vaccination until the end of part 2. Additional analyses were done on the per protocol set, safety set, full analysis set, and safety and immunogenicity subsets. Statistical analyses were done using SAS version 9.3 software. An independent data monitoring committee was used in the study. This study is registered with ClinicalTrials.gov, NCT02747927.

Role of the funding source

The sponsor was responsible for overall study design (taking into consideration investigators' input), study site selection, and data analysis. The study investigators were responsible for data collection and day-to-day study site management. Employees and subcontractors of the sponsor had a role in study design, data collection, data analysis, data interpretation, and writing and critical review of the report. Based on a manuscript outline agreed on by the authors, a medical writer prepared a first draft manuscript (funded by the sponsor). All authors had full access to the presented data, provided critical input during manuscript preparation, and approved the final version for submission. All authors had final responsibility for the decision to submit for publication.

	TAK-003 dengue cases	TAK-003 cases per 100 person-years	Placebo dengue cases	Placebo cases per 100 person-years	Vaccine efficacy (95% CI)
Secondary efficacy endpoints					
Seropositive, 4–16 years	75/9167 (0·8%)	0·6	150/4589 (3·3%)	2·4	76·1% (68·5 to 81·9)
Seronegative, 4–16 years	39/3531 (1·1%)	0·8	56/1726 (3·2%)	2·4	66·2% (49·1 to 77·5)
DENV 1	38/12 700 (0·3%)	0·2	62/6316 (1·0%)	0·7	69·8% (54·8 to 79·9)
DENV 2	8/12 700 (<0·1%)	<0·1	80/6316 (1·3%)	0·9	95·1% (89·9 to 97·6)
DENV 3	63/12 700 (0·5%)	0·4	60/6316 (0·9%)	0·7	48·9% (27·2 to 64·1)
DENV 4	5/12 700 (<0·1%)	<0·1	5/6316 (<0·1%)	<0·1	51·0% (–69·4 to 85·8)
Exploratory efficacy endpoints					
Overall	114/12 700 (0·9%)	0·6	206/6316 (3·3%)	2·4	73·3% (66·5 to 78·8)
Seropositive					
4–5 years	18/957 (1·9%)	1·3	26/464 (5·6%)	4·1	67·7% (41·1 to 82·3)
6–11 years	40/4807 (0·8%)	0·6	80/2425 (3·3%)	2·4	76·2% (65·2 to 83·7)
12–16 years	17/3403 (0·5%)	0·4	44/1700 (2·6%)	1·9	81·2% (67·0 to 89·2)
Seronegative					
4–5 years	14/662 (2·1%)	1·5	9/337 (2·7%)	1·9	22·9% (–78·1 to 66·7)
6–11 years	22/2200 (1·0%)	0·7	37/1065 (3·5%)	2·5	71·2% (51·2 to 83·0)
12–16 years	3/669 (0·4%)	0·3	10/324 (3·1%)	2·3	85·7% (47·9 to 96·1)
Seropositive					
DENV 1	21/9167 (0·2%)	0·2	37/4589 (0·8%)	0·6	72·0% (52·2 to 83·6)
DENV 2	7/9167 (<0·1%)	<0·1	54/4589 (1·2%)	0·9	93·7% (86·1 to 97·1)
DENV 3	43/9167 (0·5%)	0·3	54/4589 (1·2%)	0·9	61·8% (43·0 to 74·4)
DENV 4	4/9167 (<0·1%)	<0·1	5/4589 (0·1%)	<0·1	61·2% (–44·3 to 89·6)
Seronegative					
DENV 1	17/3531 (0·5%)	0·3	25/1726 (1·4%)	1·0	67·8% (40·3 to 82·6)
DENV 2	1/3531 (<0·1%)	<0·1	26/1726 (1·5%)	1·1	98·1% (85·8 to 99·7)
DENV 3	20/3531 (0·6%)	0·4	6/1726 (0·3%)	0·3	–68·2% (–318·9 to 32·4)
DENV 4	1/3531 (<0·1%)	<0·1	0/1726 (0%)	0·0	..
Seropositive					
Asia Pacific	62/4391 (1·4%)	1·0	125/2169 (5·8%)	4·3	76·6% (68·3 to 82·7)
Latin America	13/4776 (0·3%)	0·2	25/2420 (1·0%)	0·7	73·7% (48·5 to 86·5)
Seronegative					
Asia Pacific	27/1503 (1·8%)	1·3	35/773 (4·5%)	3·4	61·8% (36·8 to 76·9)
Latin America	12/2028 (0·6%)	0·4	21/953 (2·2%)	1·6	73·4% (45·9 to 86·9)
Yellow fever vaccine received	10/2719 (0·4%)	0·3	18/1355 (1·3%)	1·0	72·4% (40·1 to 87·2)
Yellow fever vaccine not received	104/9981 (1·0%)	0·7	188/4961 (3·8%)	2·8	73·4% (66·2 to 79·1)
Japanese encephalitis vaccine received	16/3157 (0·5%)	0·4	82/1552 (5·3%)	4·0	90·8% (84·3 to 94·6)
Japanese encephalitis vaccine not received	98/9543 (1·0%)	0·7	124/4764 (2·6%)	1·9	61·5% (49·8 to 70·4)
Yellow fever and Japanese encephalitis vaccine not received	88/6825 (1·3%)	0·9	106/3409 (3·1%)	2·3	59·6% (46·4 to 69·6)

Data are n/N (%) or % (95% CI) unless otherwise specified. Per protocol set data for 30 days post-second vaccination until end of part 2 (duration 17 months). Percentages were calculated on the basis of the number of participants who underwent evaluation for VCD. In the per protocol population, 12 700 of 12 704 participants in the vaccine group and 6316 of 6317 in the placebo group were included in the evaluation of endpoints. The per protocol set was determined after exclusion of participants in a masked manner before database lock in accordance with prespecified criteria (see appendix p 5). For analyses involving the per protocol population, data from participants who discontinued were censored at the day of discontinuation. Two instances of VCD occurred in two participants during parts 1 and 2 of the study (only the first instance was included in the efficacy calculation, except when calculating efficacy by serotype). For serotype-specific vaccine efficacy calculations, only the first instance of VCD due to the individual serotype in question was included, regardless of previous instances of VCD due to other serotypes. Participants were classified as seronegative when testing seronegative for all dengue serotypes at baseline. Participants were classified as seropositive when showing a reciprocal neutralising antibody titre of 10 or more against at least one dengue serotype at baseline. VCD=virologically confirmed dengue.

Table 1: **Efficacy of TAK-003 in preventing VCD fever**

Results

We completed part 2 of this trial between March 1, 2018, and Jan 7, 2019. The trial commenced on Sept 7, 2016, and is planned to be completed by December, 2021. After screening 23 401 individuals, 20 099 participants were randomly assigned and 20 071 (99·9%) received a first injection. 6698 (33·3%) of 20 099 were assigned to the placebo group, 11 (0·2%) of whom were not treated.

	TAK-003 cases	TAK-003 cases per 100 person-years	Placebo cases	Placebo cases per 100 person-years	Vaccine efficacy (95% CI)
Secondary efficacy endpoints					
Hospitalised, 4–16 years	13/12 700 (0·1%)	<0·1	66/6316 (1·0%)	0·8	90·4% (82·6 to 94·7)
Dengue haemorrhagic fever*	2/12 700 (<0·1%)	<0·1	7/6316 (0·1%)	<0·1	85·9% (31·9 to 97·1)
Severe VCD†	2/12 700 (<0·1%)	<0·1	1/6316 (<0·1%)	<0·1	2·3% (–977·5 to 91·1)
Exploratory efficacy endpoints					
Hospitalised, 4–5 years	5/1619 (0·3%)	0·2	6/801 (0·7%)	0·5	59·1% (–33·9 to 87·5)
Hospitalised, 6–11 years	6/7009 (<0·1%)	<0·1	41/3491 (1·2%)	0·8	92·9% (83·4 to 97·0)
Hospitalised, 12–16 years	2/4072 (<0·1%)	<0·1	19/2024 (0·9%)	0·7	94·8% (77·8 to 98·8)
Dengue haemorrhagic fever*					
Seropositive	1/9167 (<0·1%)	<0·1	6/4589 (0·1%)	<0·1	91·7% (30·9 to 99·0)
Seronegative	1/3531 (<0·1%)	<0·1	1/1726 (<0·1%)	<0·1	49·4% (–709·2 to 96·8)
Severe VCD†					
Seropositive	0/9167	0	1/4589 (<0·1%)	<0·1	100% (NA)
Seronegative	2/3531 (<0·1%)	<0·1	0/1726	0	..
Hospitalised, seropositive					
4–16 years	8/9167 (<0·1%)	<0·1	45/4589 (1·0%)	0·7	91·4% (81·7 to 95·9)
4–5 years	3/957 (0·3%)	0·2	3/464 (0·6%)	0·5	51·6% (–139·7 to 90·2)
6–11 years	3/4807 (<0·1%)	<0·1	26/2425 (1·1%)	0·8	94·5% (81·9 to 98·3)
12–16 years	2/3403 (<0·1%)	<0·1	16/1700 (0·9%)	0·7	93·8% (73·0 to 98·6)
Hospitalised, seronegative					
4–16 years	5/3531 (0·1%)	0·1	21/1726 (1·2%)	0·9	88·1% (68·5 to 95·5)
4–5 years	2/662 (0·3%)	0·2	3/337 (0·9%)	0·6	65·3% (–108·0 to 94·2)
6–11 years	3/2200 (0·1%)	<0·1	15/1065 (1·4%)	1·0	90·0% (65·6 to 97·1)
12–16 years	0/669	0	3/324 (0·9%)	0·7	100% (NA)
Hospitalised, Asia Pacific	12/5894 (0·2%)	0·1	61/2942 (2·1%)	1·5	90·4% (82·2 to 94·8)
Hospitalised, Latin America	1/6806 (<0·1%)	<0·1	5/3374 (0·1%)	0·1	90·1% (15·3 to 98·8)

Data are n/N (%) unless otherwise specified. Per protocol set data for 30 days post-second vaccination until end of part 2 (duration 17 months). Percentages were calculated on the basis of the number of participants who underwent evaluation for VCD. In the per protocol population, 12 700 of 12 704 participants in the vaccine group and 6316 of 6317 in the placebo group were included in the evaluation of endpoints. The per protocol set was determined after exclusion of participants in a masked manner before database lock in accordance with prespecified criteria (see appendix p 5). For analyses involving the per protocol set, data from participants who discontinued were censored at the day of discontinuation. Participants were classified as seronegative when testing seronegative for all dengue serotypes at baseline. Participants were classified as seropositive when showing a reciprocal neutralising antibody titre of 10 or more against at least one dengue serotype at baseline. One case in the TAK-003 group met the criteria defining both dengue haemorrhagic fever and severe VCD. VCD=virologically confirmed dengue. NA=not applicable. *VCD cases meeting WHO 1997 dengue haemorrhagic fever criteria. †Determined by dengue case adjudication committee.

Table 2: **Efficacy of TAK-003 in preventing hospitalisations due to VCD, severe VCD, and dengue haemorrhagic fever**

13 401 (66·7) of 20 099 were assigned to the TAK-003 group, 17 (0·1%) of whom were not treated (figure 1). First injections were administered between Sept 7, 2016, and March 31, 2017. 19 741 (98·2%) participants received both injections, and 19 126 (95·2%) completed part 2 of the study in the randomisation set, which corresponded to 18 months post-second dose. Enrolment was broadly balanced across Latin America (53·5%) and Asia Pacific (46·5%) and baseline characteristics were similar across both treatment groups (appendix p 8). The mean age of participants was 9·6 years (SD 3·4) and 27·7% were seronegative for all four serotypes at baseline, as assessed by microneutralisation test. The proportion of participants who were seronegative at baseline varied by country (Panama 62·2%, Sri Lanka 38·5%, Thailand 34·4%, Brazil 28·8%, Nicaragua 22·3%, Colombia 15·4%, Philippines 12·4%, and Dominican Republic 2·8%). Other participants had a microneutralisation test titre of 10 or more to at least one dengue virus serotype at baseline and were considered to be seropositive.

During parts 1 and 2 of the study period of around 21 months, 13 881 cases of febrile illness were reported, and acute samples were collected from 13 657 (98·4%) of these cases (93·6% within 5 days of fever onset). 390 cases were VCD by serotype-specific RT-PCR, including second episodes in two participants (safety set data; appendix p 15); 98 of these cases (approximately 25%) required hospitalisation. The greatest number of VCD cases occurred in the Philippines, where 131 (74·0%) of 177 cases were due to DENV 3. In Sri Lanka, 63 (66%) of the 96 reported VCD cases were hospitalised and 57 (91%) of the 63 hospitalised cases were due to DENV 2. No VCD cases were reported in the Dominican Republic during this period of observation. Although all four serotypes were reported in Asia, almost all the reported VCD cases in Latin America were

due to DENV 1 and DENV 2, except for one DENV 4 case in Colombia. Of the 390 VCD cases during parts 1 and 2, 259 cases were in the placebo group (78 [30·1%] DENV 1, 109 [42·1%] DENV 2, 65 [25·1%] DENV 3, and seven [2·7%] DENV 4).

Data describing vaccine efficacy against VCD in the 17-month observation period starting 30 days post-second vaccination is presented in table 1. Vaccine efficacy against VCD of any serotype was 76·1% (95% CI 68·5 to 81·9) in the baseline seropositive population and 66·2% (49·1 to 77·5) in the baseline seronegative population. The overall vaccine efficacy during this time period was 73·3% (66·5 to 78·8). Secondary vaccine efficacy endpoints by serotype were met for DENV 1–3 but continued to be variable; vaccine efficacy against DENV 2 was 95·1% (89·9 to 97·6), against DENV 1 was 69·8% (54·8 to 79·7), and against DENV 3 was 48·9% (27·2 to 64·1). Vaccine efficacy remained inconclusive against DENV 4 at 51·0% (–69·4 to 85·8).

Vaccine efficacy in the prevention of hospitalisations due to VCD, dengue haemorrhagic fever as per WHO 1997 criteria, and severe VCD as per DCAC criteria are presented in table 2. Overall, 13 individuals with VCD required hospitalisation in the TAK-003 group compared with 66 individuals with VCD in the placebo group, with a vaccine efficacy of 90·4% (95% CI 82·6–94·7); overall vaccine efficacy was similar regardless of baseline serostatus (91·4% in individuals who were seropositive *vs* 88·1% in individuals who were seronegative). Vaccine efficacy against dengue haemorrhagic fever was 85·9% (31·9–97·1). Only three cases of severe VCD (all due to DENV 3) were reported with two cases occurring in the TAK-003 group and one case in the placebo group. There was little overlap in cases meeting the 1997 WHO and DCAC severity criteria; only one case in a vaccine recipient met both the criteria. There were three cases in the TAK-003 group and eight cases in the placebo group that met either criteria.

Exploratory analysis by serostatus and serotype found vaccine efficacy against DENV 1 of 72·0% (95% CI 52·2–83·6) in the seropositive populations and 67·8% (40·3–82·6) in the seronegative populations, and vaccine efficacy against DENV 2 of 93·7% (86·1–97·1) in the seropositive populations and 98·1% (85·8–99·7) in the seronegative populations; thus showing similar vaccine efficacy regardless of baseline serostatus for DENV 1 and DENV 2. Vaccine efficacy analysis by serostatus was not possible for DENV 4, with only one case reported in individuals who were seronegative at baseline.

Vaccine efficacy against DENV 3 varied by baseline serostatus. In individuals who were seropositive, vaccine efficacy was 61·8% (95% CI 43·0 to 74·4) whereas vaccine efficacy was not shown in individuals who were seronegative (–68·2%, –318·9 to 32·4). A numerical imbalance of VCD cases was seen (20 [three hospitalised] in vaccine recipients *vs* six [one hospitalised] in the placebo group), with an inconclusive relative risk of 1·63 (95% CI 0·66 to 4·05). Among the three hospitalised VCD cases in vaccinees, two were classified as severe according to the DCAC criteria. One of these two severe VCD cases in vaccinees and the only hospitalised VCD case in the placebo group also met the WHO 1997 dengue haemorrhagic fever criteria.

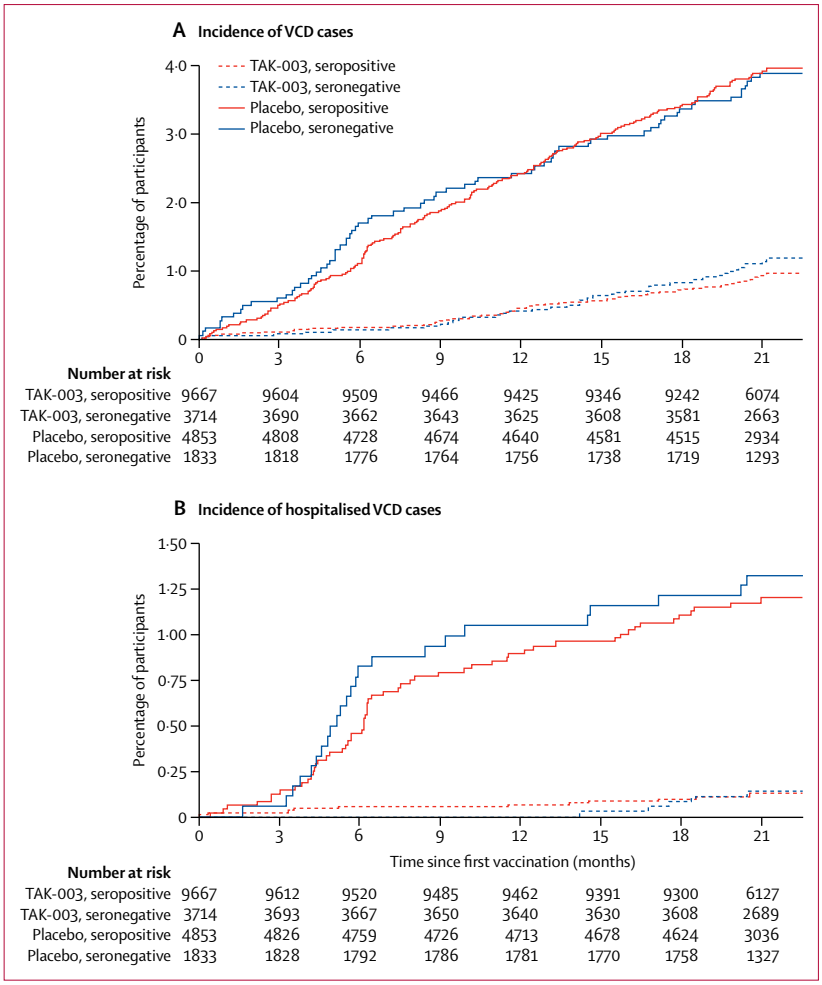

Figure 2: **Cumulative incidence of VCD and hospitalised cases of VCD by baseline serostatus (safety set data)**
VCD=virologically confirmed dengue.

The cumulative incidence of VCD and hospitalised cases of VCD by baseline serostatus over a 21-month period following first dose are presented in figures 2, 3. Additional exploratory analyses of vaccine efficacy are presented in table 1 and the appendix (pp 13–15). In general, vaccine efficacy in the subgroups could be largely explained by the variation of efficacy by serotype and the relative distribution of serotypes in the analysis subpopulations (appendix pp 12, 15).

The clinical features of all VCD cases after first dose in the safety set by serostatus are presented in the appendix (p 16). Bleeding, plasma leakage, and low platelet counts were reported in a small proportion of VCD cases, in both the vaccine and placebo groups. Bleeding was observed in

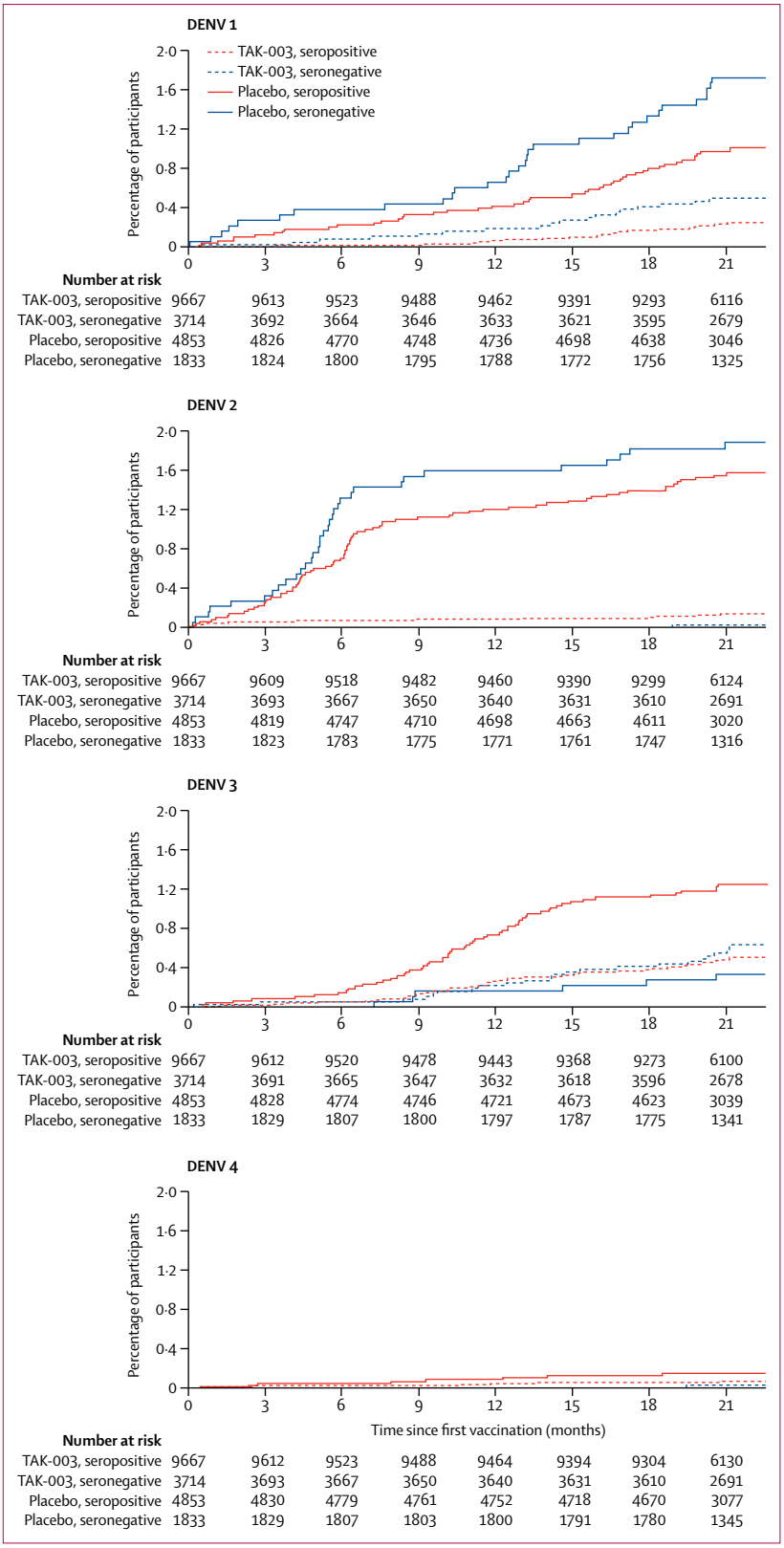

Figure 3: **Cumulative incidence of virologically confirmed dengue for each serotype by baseline serostatus (safety set data)**

four (5%) of 89 participants in the TAK-003 seropositive group versus 12 (6%) of 188 in the placebo seropositive group; and two (5%) of 42 in TAK-003 versus five (7%) of 71 in placebo seronegative groups. Plasma leakage was observed in one (1%) of 89 in TAK-003 versus 13 (7%) of 188 in placebo seropositive groups; and two (5%) of 42 in TAK-003 versus three (4%) of 71 in placebo seronegative groups. Platelet counts of 100×10^9 per L or less were observed in five (6%) of 89 in TAK-003 versus 45 (24%) of 188 in placebo seropositive groups; and three (7%) of 42 in TAK-003 versus ten (14%) of 71 in the placebo seronegative groups. Further analysis of DENV 3 cases in individuals who were seronegative at baseline found bleeding in one (5%) of 22 TAK-003 group cases versus one (17%) of six placebo group cases; plasma leakage in two (9%) of 22 TAK-003 group cases versus one (17%) of six placebo group cases; and platelet counts of 100×10^9 per L or less in two (9%) of 22 TAK-003 group cases versus one (17%) of six placebo group cases.

Cumulative rates of serious adverse events (parts 1 and 2) were similar between groups at 4·0% in the vaccine group and 4·8% in the placebo group (table 3). No additional cases of related serious adverse events during part 2 of the study were observed. One TAK-003 and four placebo recipients had serious adverse events during part 1, which were considered by the investigators to be related to receiving masked investigational product (two had hypersensitivity, two were diagnosed with dengue, and one with dengue haemorrhagic fever). Additional serious adverse event data are provided in the appendix (pp 9–11). 20 participants (<0·1% of safety set) were withdrawn from the trial due to 21 adverse events by the end of part 2; 14 of these participants received TAK-003 and six received placebo. The six deaths that occurred in parts 1 and 2 of the study (table 3; aseptic meningitis, arteriovenous malformation of the cerebral vessels, anaplastic ependymoma, gunshot wound, suffocation, and road traffic accident) were all considered to be unrelated to the investigational product or study procedures by the investigators, sponsor, and data monitoring committee.

Among those who presented with a febrile illness within 30 days of vaccination, vaccine viraemia was detected in 34 (7%) of 479 participants after the first dose (31 of 34 with serotype 2), and in one (<1%; serotype 2) of 503 participants after the second dose (30 [88·2%] of 34 cases had onset of fever within 7–13 days of first dose administration). This included 15 participants with replication-competent vaccine virus (all serotype 2), with four isolates showing a single reversion at the 5′NCR attenuation locus. None of these four febrile illnesses were clinically diagnosed as dengue or had evidence of bleeding, positive tourniquet test, low platelet count, or plasma leakage. Two of these cases presented with rash, which resolved within 7 days.

Seropositivity rates (defined as group proportions with reciprocal neutralising titres ≥10) against each serotype over time are presented in the appendix (p 17). On day 120

(ie, 1 month after second dose), tetravalent seropositivity rates in individuals who were seropositive at baseline were 99·8% versus 85·2% in TAK-003 versus placebo recipients, and in individuals who were seronegative at baseline, 99·5% versus 3·5%.

Geometric mean titres of neutralising antibodies against each serotype are presented in table 4. Generally, geometric mean titres were of similar magnitude to those observed in previous studies of this vaccine.[20] On day 120 (1 month after second dose), TAK-003 induced geometric mean titres of 2115 (DENV 1), 4897 (DENV 2), 1761 (DENV 3), and 1129 (DENV 4) in individuals who were seropositive at baseline, and 184 (DENV 1), 1730 (DENV 2), 228 (DENV 3), and 144 (DENV 4) in individuals who were seronegative at baseline. On day 270, TAK-003 induced geometric mean titres of 1446 (DENV 1), 3691 (DENV 2), 1088 (DENV 3), and 778 (DENV 4) in individuals who were seropositive at baseline, and 87 (DENV 1), 929 (DENV 2), 72 (DENV 3), and 64 (DENV 4) in individuals who were seronegative at baseline. Antibody persistence was observed on day 450 with TAK-003-induced geometric mean titres of 1243 (DENV 1), 2993 (DENV 2), 799 (DENV 3), and 817 (DENV 4) in individuals who were seropositive at baseline, and 77 (DENV 1), 656 (DENV 2), 53 (DENV 3), and 64 (DENV 4) in individuals who were seronegative at baseline.

Discussion

We previously reported the initial vaccine efficacy analyses from part 1 of this 3-part study.[24] The 6 months of additional follow-up in part 2 have allowed us a more precise look into the efficacy measures, particularly the final analyses of secondary objectives. It also enabled observation of how the vaccine performed beyond the first year after completion of the vaccine course, when potential cross-protection from the dominant serotype 2 component might have declined. Broadly, the results from part 2 were similar to those of part 1.[24]

The trial met all of the secondary objectives for which there were sufficient VCD cases to enable assessment. It confirmed the earlier exploratory findings of overall efficacy regardless of baseline serostatus (66·2% in individuals who were seronegative *vs* 76·1% in individuals who were seropositive), a high overall efficacy against hospitalised dengue (90·4%), and variable efficacy by serotype (48·9–95·1%). The highest vaccine efficacy was seen against DENV 2, which provides the genetic backbone of TAK-003, and it was likely to be contributed by anti-NS1 antibodies as well as cell-mediated responses.[26,27] Overall vaccine efficacy at the end of part 2 was 73·3%, which is lower than the 80·2% reported at the end of part 1. This finding can be explained largely by two factors: (1) a relatively higher proportion of DENV 1 (30·1% *vs* 20·1% in the placebo group and 33·3% *vs* 26·2% in the TAK-003 group) and lower proportion of DENV 2 (38·3% *vs* 43·0% in the placebo group and 7·0% *vs* 4·9% in the TAK-003 group) VCD cases being included in the secondary versus primary endpoint analyses timeframes; and (2), some decline in vaccine efficacy primarily against DENV 3 (62·6% vaccine efficacy at the end of part 1 *vs* 48·9% at the end of part 2).

A comprehensive subanalysis was planned to fully understand the underlying determinants of efficacy

	TAK-003 (n=13 380)	Placebo (n=6687)
Serious adverse events	538 (4·0%)	324 (4·8%)
Non-investigational product-related* serious adverse events	537 (4·0%)	320 (4·8%)
Investigational product-related* serious adverse events	1 (<0·1%)	4 (<0·1%)
Serious adverse events leading to investigational product withdrawal or trial discontinuation	18 (0·1%)	8 (0·1%)
Deaths	5 (<0·1%)	1 (<0·1%)

Data are number of participants with events (%). Participants with at least one adverse event after any injection (vaccine or placebo administration). *As assessed by investigator.

Table 3: **Overview of safety set data until end of part 2**

	TAK-003 seropositive (n=1816)	Placebo seropositive (n=902)	TAK-003 seronegative (n=702)	Placebo seronegative (n=345)
DENV 1				
Day 1	410 (365–461)	445 (377–524)	5 (5–5)	5 (5–5)
Day 30	2404 (2204–2622)	430 (361–512)	118 (106–131)	6 (5–6)
Day 90	1945 (1791–2112)	410 (349–481)	91 (82–102)	6 (5–6)
Day 120	2115 (1957–2286)	451 (381–534)	184 (169–201)	6 (6–7)
Day 270	1446 (1328–1573)	415 (350–492)	87 (79–97)	6 (6–7)
Day 450	1243 (1139–1357)	451 (380–536)	77 (68–86)	7 (6–8)
DENV 2				
Day 1	745 (674–825)	802 (697–924)	5 (5–5)	5 (5–5)
Day 30	6696 (6300–7116)	744 (635–870)	6277 (5648–6977)	7 (6–7)
Day 90	4826 (4571–5095)	724 (624–839)	1682 (1543–1833)	7 (6–8)
Day 120	4897 (4646–5163)	766 (654–896)	1730 (1614–1855)	8 (7–9)
Day 270	3691 (3496–3898)	776 (665–906)	929 (855–1009)	9 (7–10)
Day 450	2993 (2831–3165)	747 (637–875)	656 (601–717)	8 (7–10)
DENV 3				
Day 1	357 (321–398)	356 (305–415)	5 (5–5)	5 (5–5)
Day 30	2254 (2093–2428)	349 (298–409)	194 (173–218)	6 (5–6)
Day 90	1563 (1453–1682)	321 (277–374)	94 (85–104)	6 (5–6)
Day 120	1761 (1646–1884)	353 (301–414)	228 (212–246)	6 (5–7)
Day 270	1088 (1008–1174)	307 (261–360)	72 (66–78)	6 (6–7)
Day 450	799 (737–865)	282 (240–331)	53 (49–59)	6 (6–7)
DENV 4				
Day 1	218 (198–241)	234 (203–270)	5 (5–5)	5 (5–5)
Day 30	1306 (1224–1393)	222 (191–258)	111 (98–125)	5 (5–6)
Day 90	1002 (940–1069)	215 (187–248)	63 (57–70)	6 (5–6)
Day 120	1129 (1066–1196)	241 (208–280)	144 (134–155)	6 (5–6)
Day 270	778 (730–830)	229 (197–266)	64 (59–70)	6 (6–7)
Day 450	817 (765–873)	293 (253–341)	64 (58–71)	6 (6–7)

Data are geometric mean titres (95% CI). Per protocol set for immunogenicity data. Data rounded to nearest whole number. Baseline seronegative is defined as seronegative to all serotypes. Baseline seropositive is defined as having reciprocal neutralising antibody titres of 10 or more to one or more serotype. n refers to number of participants in the analysis set (number of participants evaluated at each timepoint might vary).

Table 4: **Geometric mean titres by dengue serotype**

beyond secondary endpoints. Vaccine efficacy in these subanalyses was dependent on the relative distribution of serotypes identified in the particular subpopulation. For example, high efficacy against hospitalised dengue was influenced by a high proportion of DENV 2, and the low efficacy observed in the 4–5-year age group was influenced by a high proportion of DENV 3 along with a low proportion of DENV 2. Similarly, the higher efficacy estimates in participants previously immunised with Japanese encephalitis vaccine was influenced by the relatively high proportion of DENV 1 and DENV 2 cases in Sri Lanka and Thailand, where vaccination against Japanese encephalitis is routine. Further analysis to understand vaccine efficacy better in these subpopulations is planned.

A key finding during the analysis of part 1 was the absence of efficacy against DENV 3 in individuals who were seronegative at baseline, and this observation continued in part 2. This finding was only made possible by important features of the trial design that enabled such a detailed assessment of vaccine efficacy subgroups. These included the large sample size, the broad geographical distribution of trial sites, baseline sampling in all participants to enable full assessment of serostatus, an age range that ensured inclusion of sufficient participants who were seronegative at baseline, and the continuation of long-term active surveillance. Without these features of the trial design, the efficacy observed against DENV 3 overall and in individuals who were seronegative generally would have masked this important finding. It is important to continue monitoring this trend, particularly to see if the imbalance of febrile illness translates into an increase in hospitalisation. Currently, there are too few cases of hospitalised dengue due to DENV 3 in participants who were seronegative (n=4) for any conclusions to be drawn. Nearly all DENV 3 cases were reported from the Philippines where the proportion of participants who were seronegative was low (n=480 [12·4%]). During the long-term follow-up period, additional cases of DENV 3 outside the Philippines and in older children who are seronegative might help to provide a clearer picture.

The trial was not able to conclude on vaccine efficacy against two secondary endpoints, VCD due to DENV 4, and severe dengue as defined by the DCAC criteria. The trial design anticipated difficulties in identifying all serotypes by including 26 sites across eight countries to provide epidemiological heterogeneity and an additional 6 months of surveillance for secondary efficacy endpoints. Despite these measures, there were too few DENV 4 cases to enable a conclusion. Encouragingly, the point estimate of efficacy against DENV 4 was positive and similar to the efficacy estimates for DENV 1 and DENV 3. Comparison of RT-PCR and NS1 antigen ELISA results in the acute samples ruled out the possibility of missing cases with PCR (data not shown).

Only three cases of dengue met the DCAC criteria of severity, which required cases to be associated with either functional impairment or substantial intervention to manage complications (details of the DCAC criteria are provided in the appendix p 5). These three cases were all due to DENV 3 (one case in the placebo group and two cases in the TAK-003 group) and were associated with low blood pressure, hypotensive shock, or respiratory distress. One of the limitations of this study is that it is not large enough to enable the identification of dengue severe enough to be associated with functional impairment. In addition to DCAC-defined severe dengue, nine cases of dengue that met the WHO 1997 criteria[25] of dengue haemorrhagic fever were identified. These were due to DENV 1 (n=2), DENV 2 (n=4), and DENV 3 (n=3) and occurred frequently enough to enable the demonstration of vaccine efficacy against dengue haemorrhagic fever (85·9%, 95% CI 31·9–97·1). There was little overlap between these definitions with only one case (in the TAK-003 group) meeting both the dengue haemorrhagic fever and the DCAC definitions. The low incidence of severe cases (dengue haemorrhagic fever-defined or DCAC-defined) and the observation that the efficacy of TAK-003 varied by serotype suggests the need for cautious interpretation of these data.

We have previously reported that TAK-003 was well tolerated, and that no important safety risks were identified in part 1.[24] There was no change to this conclusion after cumulative analysis of adverse events in part 2.

With the protocol-defined primary and secondary efficacy objectives assessed up to 18 months after last vaccine dose, it is reasonable to consider the potential utility of this vaccine candidate. Dengue is a growing public health threat for more than half of the global population,[4,28,29] and there are considerable challenges in developing an effective vaccine.[30] The only licensed vaccine is not indicated in those younger than 9 years and requires the identification of individuals who are seropositive.[7–10] Other public health measures have traditionally failed to eliminate the risk of dengue.[31,32] In this context, TAK-003 has shown high overall efficacy against both symptomatic and hospitalised dengue irrespective of baseline serostatus, with rapid onset of protection after one dose. However, efficacy differs by serotype and more data are needed to understand the safety and efficacy profile of this candidate against DENV 3 and DENV 4 in individuals who are seronegative. These nuances will require careful balancing by regulators and public health officials in determining the potential utility of this vaccine as a component of a multimodal approach to reducing the global burden of dengue.

Contributors

CB-T, LMV, HV, MTA, VS, EJR-A, DY, VPW, EDM, ADF, DG, PK, FE, EL-M, LB, and XS-L were study investigators. ShB, AB, PG, ST, MR, and DW were responsible for study design. ST, YH, IE, and PG were responsible for medical monitoring and data review. ShB, AB, MR, IL, SvB, and DW were responsible for data analysis and interpretation. ShB was responsible for publication management.

Declaration of interests
ShB, ST, YH, PG, IE, MR, SvB, IL, AB, and DW are permanent employees of the Takeda group of companies. DW has a patent WO2017/179017 pending. XS-L, PK, and LB have served as advisory board members for Takeda. All other authors declare no competing interests.

Data sharing
Access to the study protocol, statistical analysis plan, and de-identified patient data will be provided to suitably qualified researchers at the discretion of an independent committee using a data sharing platform on written request when appropriate marketing authorisation has been received.

Acknowledgments
We thank the trial participants and their parents or guardians for agreeing to take part in the trial, the data monitoring committee, the dengue case adjudication committee members, the Takeda expanded study team, the study team at Pharmaceutical Product Development, and all the trial staff in each of the countries. The authors would also like to acknowledge the contribution of the late Dan Stinchcomb, and employees of Inviragen (Fort Collins, CO, USA) in the initial developmental work of TAK-003 (Inviragen was subsequently acquired by Takeda). The authors are grateful to Jamie Stirling (OLC Bioscience, London, UK) and Keith Veitch (keithveitch communications, Amsterdam, Netherlands) for editorial assistance in the preparation of this manuscript.

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