ORIGINAL ARTICLE

Efficacy of a Tetravalent Dengue Vaccine in Children in Latin America

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ABSTRACT

BACKGROUND

In light of the increasing rate of dengue infections throughout the world despite vector-control measures, several dengue vaccine candidates are in development.

METHODS

In a phase 3 efficacy trial of a tetravalent dengue vaccine in five Latin American countries where dengue is endemic, we randomly assigned healthy children between the ages of 9 and 16 years in a 2:1 ratio to receive three injections of recombinant, live, attenuated, tetravalent dengue vaccine (CYD-TDV) or placebo at months 0, 6, and 12 under blinded conditions. The children were then followed for 25 months. The primary outcome was vaccine efficacy against symptomatic, virologically confirmed dengue (VCD), regardless of disease severity or serotype, occurring more than 28 days after the third injection.

RESULTS

A total of 20,869 healthy children received either vaccine or placebo. At baseline, 79.4% of an immunogenicity subgroup of 1944 children had seropositive status for one or more dengue serotypes. In the per-protocol population, there were 176 VCD cases (with 11,793 person-years at risk) in the vaccine group and 221 VCD cases (with 5809 person-years at risk) in the control group, for a vaccine efficacy of 60.8% (95% confidence interval [CI], 52.0 to 68.0). In the intention-to-treat population (those who received at least one injection), vaccine efficacy was 64.7% (95% CI, 58.7 to 69.8). Serotype-specific vaccine efficacy was 50.3% for serotype 1, 42.3% for serotype 2, 74.0% for serotype 3, and 77.7% for serotype 4. Among the severe VCD cases, 1 of 12 was in the vaccine group, for an intention-to-treat vaccine efficacy of 95.5%. Vaccine efficacy against hospitalization for dengue was 80.3%. The safety profile for the CYD-TDV vaccine was similar to that for placebo, with no marked difference in rates of adverse events.

CONCLUSIONS

The CYD-TDV dengue vaccine was efficacious against VCD and severe VCD and led to fewer hospitalizations for VCD in five Latin American countries where dengue is endemic. (Funded by Sanofi Pasteur; ClinicalTrials.gov number, NCT01374516.)

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Dengue is a mosquito-borne disease that is present in many parts of the world. From 2003 through 2013, the number of dengue cases that were reported to the Pan American Health Organization (PAHO) increased by a factor of five.[1-3] The disease is caused by one of four closely related virus serotypes from the genus flavivirus. Mosquitoes that transmit the virus are present in tropical and subtropical regions worldwide and in some temperate areas of the United States, Europe, Africa, and the Middle East.[4] Dengue is an increasing public health problem despite efforts to manage epidemics through vector control.[5]

Several dengue vaccine candidates are in development.[6,7] As part of the clinical development of a recombinant, live, attenuated, tetravalent dengue vaccine (CYD-TDV), twin phase 3 clinical trials were initiated in Asia and Latin America to assess the efficacy of a schedule of three doses (administered at 0, 6, and 12 months) against symptomatic, virologically confirmed dengue (VCD). The Asian trial involving children between the ages of 2 and 14 years showed an overall vaccine efficacy of 56.5% after three injections, which increased to 80.8% for efficacy against severe dengue, as defined by the independent data monitoring committee.[8] Reports of clinical trials so far, which included 25 months of active surveillance in the Asian efficacy trial, have shown no substantial safety concerns associated with this vaccine.[8-15] Here we report the first results from the randomized, blinded, placebo-controlled efficacy trial involving healthy children between the ages of 9 and 16 years in five countries in Latin America where dengue is endemic.

Methods

Study Participants and Oversight

The methods that we used in this study were similar to those used in the Asian trial.[8] The main differences were the age range and number of participants.

From June 2011 through March 2012, we enrolled healthy children between the ages of 9 and 16 years in a total of 22 centers in Colombia (9 centers), Brazil (5 centers), Mexico (5 centers), Puerto Rico (2 centers), and Honduras (1 center). We selected the five countries on the basis of the incidence of dengue.

The trial complied with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and relevant local regulations. Ethics review committees approved the protocol, amendments, and consent and assent forms and are reviewing the conduct of the ongoing trial. In accordance with local regulations, parents or guardians provided written informed consent, and participants signed informed-assent forms before enrollment. Details regarding the study conduct and analyses are provided in the protocol, available with the full text of this article at NEJM.org.

The sponsor of the study, Sanofi Pasteur, designed the study, performed the sample testing, and analyzed the data. The sponsor and the investigators were responsible for data interpretation and writing of the report. The investigators were responsible for data collection. The authors who were employed by Sanofi Pasteur had complete access to the study data. These authors all vouch for the completeness and accuracy of the data and the analyses. The other authors had access to the statistical analyses but not participant-level data because the blinded hospital phase of the study is ongoing. The first draft of the manuscript was written by a medical writer employed by MediCom Consult with funding from the sponsor, and all the authors provided critical input for the successive drafts and validated the submitted version.

Randomization and Blinding

We assigned the children in a 2:1 ratio to receive three doses of vaccine or placebo at 0, 6, and 12 months, using an interactive voice-response or Web-response system. Randomization was performed with the use of computer-generated permuted blocks of six, stratified according to study site and age group (9 to 11 years or 12 to 16 years). In each country, before the study-group assignments were made, children who were enrolled during the first 2 to 4 months were randomly assigned in a 1:1 ratio to a subgroup of children (representing 10% of the participants from that country) who were followed for reactogenicity and immunogenicity (for details, see the Methods section in the Supplementary Appendix, available at NEJM.org). The investigators, participants, their parents, and the sponsor were unaware of study-group assignments. The injections of vaccine or placebo were prepared and administered by staff members who were aware of study-group assignments but were not involved in study assessments.

STUDY INTERVENTIONS

The investigational vaccine consists of four recombinant dengue vaccine viruses (CYD 1 through 4), each constructed by substituting genes encoding the premembrane and envelope proteins of the yellow fever 17D vaccine virus with those from wild-type dengue viruses.[15,16] These formulations were combined into a single preparation containing 5.0 $\log_{10}$ median cell-culture infectious doses ($CCID_{50}$) per serotype and were formulated as a powder and solvent (0.4% sodium chloride) for suspension. The vaccine was stored at a temperature between 2°C and 8°C and was reconstituted immediately before administration. The placebo was a 0.9% solution of sodium chloride. Doses of vaccine or placebo were administered subcutaneously above the deltoid.

PROCEDURES

All children were scheduled for visits at months 0, 6, and 12 for vaccination and at month 13 for follow-up and blood sampling. In addition, the children were contacted by telephone or had a home visit at months 18 and 25 for follow-up (Fig. S1 in the Supplementary Appendix). Children in the reactogenicity and immunogenicity subgroup were scheduled for visits at months 1, 7, and 13 for assessment, and blood samples from these children were obtained at months 0, 7, 13, and 25 and tested for dengue serotype–specific antibodies. This assessment was performed in a central laboratory by means of a plaque-reduction neutralization test and a 50% reduction in the plaque count as the neutralizing end point ($PRNT_{50}$), with the use of standard operating procedures.[17]

Active surveillance started the day of the first injection and continued until month 25 (Fig. S1 in the Supplementary Appendix). During weekly contacts, the children or their parents or guardians were reminded to visit the trial or health care center in case of acute febrile illness (temperature, ≥38°C on ≥2 consecutive days) and were provided with a thermometer and a memory card for recording temperature. The card included instructions about how to measure and record temperature in case of fever.

We obtained two blood samples from any child who had an acute febrile illness to confirm the presence of dengue: one sample obtained within 5 days after the onset of fever (acute sample) and a second sample obtained 7 to 14 days later (convalescent sample). In the acute samples, we used both a quantitative reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay to test for VCD and an enzyme-linked immunosorbent assay to test for the presence of dengue nonstructural protein 1 antigen, in accordance with the guidelines of the World Health Organization (WHO), as described previously[8,18,19] (for details, see the Supplementary Appendix). The illness episode was classified as VCD if any test was positive.

For each acute febrile illness, we recorded clinical symptoms, laboratory results, and the results of imaging studies using a standardized electronic case-report form. We obtained information with respect to hospitalized patients from clinical records and updated that information in the database until discharge. An independent data monitoring committee performed a blinded review of each case on the basis of information in the database and assessed the severity of infection using predefined criteria (see the Supplementary Appendix).[8,12] The committee could request additional information from the investigators. Cases were assessed for dengue hemorrhagic fever according to criteria from the 1997 WHO guidelines with the use of a program written by the biostatistics department at Sanofi Pasteur.[20]

MONITORING COMMITTEE REVIEW

The data monitoring committee regularly reviewed dengue cases and safety data, including all serious adverse events and deaths. For each meeting, an independent external statistician who was not a committee member was charged with conducting unblinded analyses and presenting the findings in a semiblinded manner, as devised by the committee, for the purposes of signal detection (as described in the Methods section in the Supplementary Appendix). Throughout the trial, the external statisticians used an incorrect code to unblind the data. This error was detected at the end of the trial, at which time the committee reviewed the correct unblinded analyses and confirmed the safety conclusions reported here. (For details about this process, see the Methods section in the Supplementary Appendix.)

STUDY OUTCOMES

The primary outcome was vaccine efficacy against symptomatic VCD, regardless of the severity of the illness or infecting serotype, occurring between months 13 and 25 in children who had received all three injections according to protocol and

who had none of the protocol deviations in a prespecified list (per-protocol analysis) (see the Supplementary Appendix).[19,20]

Secondary outcomes included vaccine efficacy against VCD caused by each serotype that occurred at any time from month 0 to month 25 in the intention-to-treat population (i.e., participants who had received ≥1 injection) and efficacy against each serotype for episodes occurring from month 13 to month 25 in the modified per-protocol population (i.e., participants who had received all three doses, regardless of protocol deviations). Efficacy was also assessed according to age group, dengue serostatus at baseline, and country in the intention-to-treat efficacy population. In addition, we estimated vaccine efficacy against severe dengue (according to the criteria of the data monitoring committee) and against any grade of dengue hemorrhagic fever (on the basis of 1997 WHO criteria), as well as the number of hospitalizations for VCD. We also analyzed vaccine efficacy between the first dose and the second dose and between the second dose and the third dose. An exploratory Kaplan–Meier survival analysis was performed. All serious adverse events occurring at any time were documented, assessed, and reported promptly to the ethics committees and the regulatory authorities.

In the reactogenicity and immunogenicity subgroup, additional objectives were to describe vaccine immunogenicity (on the basis of $PRNT_{50}$ results) and reactogenicity, including solicited injection-site and systemic reactions and unsolicited adverse reactions after each dose. All analyses were prespecified.

STATISTICAL ANALYSIS

We estimated that enrollment of 20,875 children, with a 2:1 ratio of assignments to the vaccine group and the placebo group, would result in the identification of 57 cases of VCD and provide a power of 90% or more to show vaccine efficacy of more than 25%, assuming a true vaccine efficacy of 70% after three injections, a one-sided alpha level of 2.5%, and a lower boundary of the 95% confidence interval of more than 25%. In these calculations, we assumed a dropout rate of 20% and a disease incidence of 0.64%. The assumed incidence was based on mean dengue incidence rates in the 4 or 5 years before enrollment, according to passive surveillance data provided by the municipalities in which the trial was conducted.

We used the number of cases (i.e., children with one or more episodes of VCD) to calculate vaccine efficacy against VCD and the cumulative person-time at risk to calculate the incidence density (number of cases per 100 person-years at risk) in each group.[12] We used the above-mentioned method to calculate vaccine efficacy against severe VCD or against any grade of dengue hemorrhagic fever. We calculated the relative risk of hospitalization for VCD as the ratio of the annual incidence in the vaccine group to that in the control group, which is presented here as vaccine efficacy (1 minus the relative risk). We calculated two-sided 95% confidence intervals for vaccine efficacy and relative risk using the exact test described by Breslow and Day.[21] In the reactogenicity and immunogenicity subgroup, the probability of observing an adverse event with a true incidence of 0.23% was 95% in the vaccine group.[22]

RESULTS

STUDY POPULATION

A total of 20,869 children between the ages of 9 and 16 years were assigned to receive either vaccine (13,920) or placebo (6949). A total of 2000 of these children were assigned to the reactogenicity and immunogenicity subgroup: 1334 in the vaccine group and 666 in the control group (Fig. S2 in the Supplementary Appendix). The numbers of participants from each country were as follows: Colombia, 9743 (921 in the subgroup); Brazil, 3548 (300 in the subgroup); Mexico, 3464 (327 in the subgroup); Honduras, 2799 (300 in the subgroup); and Puerto Rico, 1315 (152 in the subgroup). More than 95% of participants in each group received all three injections, and 90% in each group were included in the per-protocol efficacy analysis.

At baseline, the two study groups were similar with respect to age and sex ratio (Table 1). In the reactogenicity and immunogenicity subgroup, 79.4% of the children had a preexisting response against one or more VCD serotypes on $PNRT_{50}$ testing: 724 of 967 children (74.9%) who were 9 to 11 years of age and 819 of 977 children (83.8%) who were 12 to 16 years of age.

INCIDENCE OF VCD

A total of 10,053 febrile episodes were reported, with blood samples collected for 99.9% of the episodes, including 8965 samples (89.2%) collected

Table 1. Baseline Characteristics of the Participants in the Per-Protocol Analysis for Efficacy, the Safety Analysis, and the Intention-to-Treat Analysis for Immunogenicity.*

Characteristic	Vaccine Group	Control Group	All Participants
Per-protocol analysis for efficacy†			
No. of participants	12,574	6261	18,835
Age — yr	12.4±2.1	12.4±2.1	12.4±2.1
Sex — no. (%)			
Male	6254 (49.7)	3105 (49.6)	9359 (49.7)
Female	6320 (50.3)	3156 (50.4)	9476 (50.3)
Safety analysis‡			
No. of participants	13,915	6939	20,854
Age — yr	12.5±2.1	12.5±2.1	12.5±2.1
Sex — no. (%)			
Male	6878 (49.4)	3411 (49.2)	10,289 (49.3)
Female	7037 (50.6)	3528 (50.8)	10,565 (50.7)
Intention-to-treat analysis for immunogenicity§			
No. of participants	1301	643	1944
Age — yr	12.3±2.1	12.4±2.1	12.3±2.1
Sex — no. (%)			
Male	631 (48.5)	339 (52.7)	970 (49.9)
Female	670 (51.5)	304 (47.3)	974 (50.1)
Dengue seropositivity at baseline — % (95% CI)¶			
Any serotype	80.6 (78.3–82.7)	77.0 (73.5–80.2)	79.4 (77.5–81.2)
Serotype 1	72.8 (70.3–75.2)	70.5 (66.8–74.0)	72.0 (70.0–74.0)
Serotype 2	76.1 (73.6–78.4)	73.8 (70.2–77.1)	75.3 (73.3–77.2)
Serotype 3	76.5 (74.1–78.8)	73.6 (70.0–76.9)	75.6 (73.6–77.5)
Serotype 4	68.2 (65.6–70.8)	65.0 (61.2–68.7)	67.2 (65.0–69.3)

* Plus–minus values are means ±SD. There were no significant differences between the two groups.
† The population for the per-protocol efficacy analysis included participants who received all three injections according to protocol and who did not present with any of the criteria in a prespecified list (see the Supplementary Appendix).
‡ The population for the safety analysis included all participants who received at least one injection, and participants were evaluated according to the first dose received.
§ The population for the intention-to-treat immunogenicity analysis included all participants in the immunogenicity subgroup who received at least one injection and who had an available blood sample and a result after the specific injection.
¶ Dengue seropositivity was defined as a titer of 10 or higher on the plaque-reduction neutralization test (PRNT50). Because of serotype cross-reactivity after natural infection, the listed rates of serotype-specific seropositivity on $PRNT_{50}$ may not represent the actual percentage of participants who have been infected with each serotype.

within 5 days after the onset of fever. VCD was diagnosed in 668 episodes among 662 children (3 children in each group had two episodes). In the control group, the overall incidence of VCD was 3.8 cases per 100 person-years at risk between months 13 and 25 and 2.9 cases per 100 person-years during the entire 25-month period, with variation in incidence and serotypes among countries (Table S1 in the Supplementary Appendix).

VACCINE EFFICACY

In the per-protocol analysis, the vaccine efficacy was 60.8% (95% confidence interval [CI], 52.0 to 68.0), on the basis of 176 cases of VCD in the vaccine group and 221 in the control group that were diagnosed more than 28 days after the third dose (primary outcome) (Table 2). In the intention-to-treat analysis, which included all children who received at least one injection from month 0 to

Table 2. Vaccine Efficacy against Any Serotype of Dengue.

Analysis	Vaccine Group			Control Group			Vaccine Efficacy (95% CI)
	Cases/ Events*	Person-Yr at Risk†	Incidence Density (95% CI)‡	Cases/ Events*	Person-Yr at Risk†	Incidence Density (95% CI)‡	
	no.		*no./100 person-yr*	*no.*		*no./100 person-yr*	%
Per-protocol analysis	176/176	11,793	1.5 (1.3–1.7)	221/221	5,809	3.8 (3.3–4.3)	60.8 (52.0–68.0)
Intention-to-treat analysis	277/280§	26,883	1.0 (0.9–1.2)	385/388§	13,204	2.9 (2.6–3.2)	64.7 (58.7–69.8)

* A case was defined as a first episode of virologically confirmed dengue (VCD) by means of enzyme-linked immunosorbent assay for dengue nonstructural protein 1 antigen, dengue screening on quantitative reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay, or serotype-specific RT-PCR assay. Among the VCD cases, 90% had positive results for both dengue RNA and nonstructural protein 1 antigen, 6% for dengue RNA only, and 2% for nonstructural protein 1 antigen only.

† Data for person-years at risk are the cumulative time in years until VCD was diagnosed or until the end of the active follow-up period, whichever came first. This value is the sum of individual units of time for which the participants contributed to the analyses.

‡ Incidence density was calculated as the number of cases divided by the cumulative person-years at risk.

§ Six participants (3 in each group) who had two episodes of VCD had the following serotypes: 2 participants, unknown serotype and serotype 2; 1 participant, serotypes 1 and 2; 1 participant, serotypes 1 and 3; 1 participant, serotypes 3 and 1; and 1 participant, two unknown serotypes. A total of 14 participants (6 in the vaccine group and 8 in the control group) had two serotypes detected during the same febrile episode, with all episodes except two occurring after the third injection; 7 participants had serotypes 1 and 2 (1 after the first injection), 5 participants had serotypes 1 and 3 (1 after the second injection), and 2 participants had serotypes 2 and 3.

month 25, the vaccine efficacy was 64.7% (95% CI, 58.7 to 69.8) (Table 2). In addition, efficacy was observed between the first dose and the second dose and between the second dose and the third dose (Table S2 in the Supplementary Appendix) and throughout the 25-month period (Fig. 1).

Efficacy was highest for serotype 4 and lowest for serotype 2. For all four serotypes, the lower boundary of the 95% confidence interval for vaccine efficacy was more than 0 in both the per-protocol and intention-to-treat analyses (Table 3). Efficacy was similar in the two age groups and was 83.7% (95% CI, 62.2 to 93.7) among children who had antibodies against dengue at baseline, as compared with 43.2% (95% CI, −61.5 to 80.0) among those who did not (Table S2 in the Supplementary Appendix). Vaccine efficacy varied according to country (Table S1 in the Supplementary Appendix).

There were 17 hospitalizations for VCD after at least one injection in the vaccine group, as compared with 43 hospitalizations in the control group, for a vaccine efficacy of 80.3% (95% CI, 64.7 to 89.5). Among the children who were hospitalized, all four serotypes were detected, and fewer children in the vaccine group than in the control group had any type of hemorrhage, visceral manifestations, or plasma leakage with clinical signs (Table S3 in the Supplementary Appendix). The median length of hospitalization was 6 days in the vaccine group and 4 days in the control group, a difference that was not significant (Table S3 in the Supplementary Appendix).

There were 12 cases of severe dengue: 1 in the vaccine group (serotype 1) and 11 in the control group (3 cases of serotype 1, 4 cases of serotype 2, 3 cases of serotype 3, and 1 case of serotype 4). Efficacy against severe dengue was 95.5% (95% CI, 68.8 to 99.9) after the first injection and 91.7% (95% CI, 31.4 to 99.8) after the third injection. Eleven of the patients with severe VCD had dengue hemorrhagic fever (according to the WHO definition): 1 patient in the vaccine group (grade 2) and 10 patients in the control group (2 patients with grade 1 and 8 patients with grade 2). Efficacy against dengue hemorrhagic fever was 95.0% (95% CI, 64.9 to 99.9) after the first injection and 90.0% (95% CI, 10.7 to 99.8) after the third injection. The single episode of dengue hemorrhagic fever in the vaccine group was classified as severe on the basis of laboratory results only; the child was not hospitalized.

SAFETY, REACTOGENICITY, AND IMMUNOGENICITY

Serious adverse events within 28 days after an injection were reported in 121 children: 81 of 13,915 children (0.6%) in the vaccine group and 40 of 6939 (0.6%) in the control group (Table 4). No deaths occurred during this period. During the entire study period, the rates of serious adverse events were similar in the two groups, with the most common events being infection and injury

not considered to be related to vaccination (Table S4 in the Supplementary Appendix). Twelve deaths (six in each group), which were all considered to be unrelated to the vaccine, were reported. In the vaccine group, four deaths were due to accidents, one was due to respiratory failure 9 months after the third injection, and one was due to systemic vasculitis with renal failure 10 months after the third injection.

Four serious adverse events were deemed to be vaccine-related by investigators: three in the vaccine group (a moderate asthma attack 16 hours after the first injection, allergic urticaria 4 hours after the second injection, and acute peripheral polyneuropathy associated with viral meningitis 3 days after the first injection, without detectable vaccine virus in samples) and one in the control group (transient visual disturbance 1 day after the first injection). A fifth serious adverse event in the vaccine group (unspecified seizures 18 hours after the first injection, without detectable vaccine virus in samples) was judged to be vaccine-related by the sponsor. All five children recovered fully without sequelae. There were no cases of viscerotropic or neurotropic diseases (adverse events of special interest, since this vaccine is based on the backbone of the yellow fever vaccine virus) or of severe anaphylactic reactions related to the vaccine. In the reactogenicity and immunogenicity subgroup, the rate and profile of solicited reactions and unsolicited adverse events were similar in the vaccine and placebo groups (Table 4; and Tables S5, S6, and S7 in the Supplementary Appendix).

Geometric mean titers of antibodies against each serotype increased after vaccination in the vaccine group but not in the control group (Table S8 in the Supplementary Appendix). Among children with seronegative status at baseline, geometric mean antibody titers increased after the second and third doses of vaccine, but the increases were lower than those in children with seropositive status at baseline.

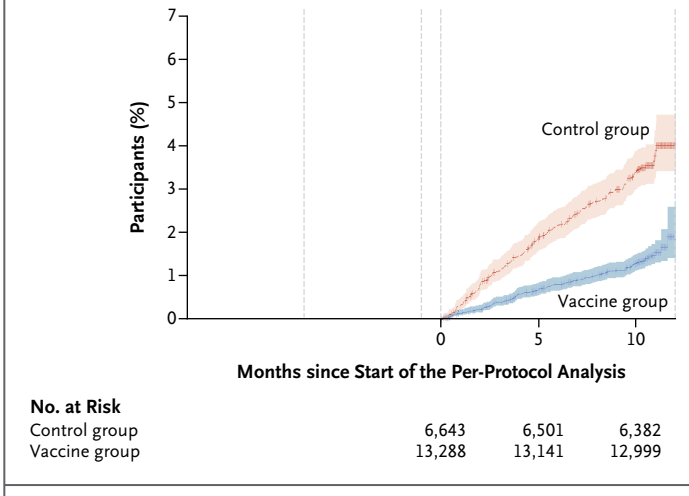

Figure 1. Incidence of Virologically Confirmed Dengue (VCD).
Shown are the cumulative incidences of the first symptomatic VCD cases caused by any serotype and occurring more than 28 days after the third dose in the modified per-protocol population (Panel A) and at any time during the active follow-up period in the intention-to-treat population (Panel B). The dashed vertical lines indicate the timing of injections (i.e., at months 0, 6, and 12), the start of the follow-up period for the modified per-protocol analysis (month 13), and the end of the active surveillance phase (month 25). The shaded areas around the curves show the 95% confidence intervals.

DISCUSSION

In this trial, we found that the CYD-TDV vaccine had an efficacy of 60.8% against symptomatic VCD after a three-dose vaccination schedule among children between the ages of 9 and 16 years (the primary outcome). We also found serotype-specific efficacy against all four serotypes, including serotype 2. Furthermore, efficacy of 80.3% against hospitalization for dengue and 95.5% against severe dengue were observed over the 25-month period. We identified no safety concerns or evidence of more severe disease in breakthrough cases in the vaccine group over the 25-month surveillance period. Higher efficacy was observed in children with a seropositive status at baseline

Table 3. Serotype-Specific Vaccine Efficacy.

Variable	Vaccine Group			Control Group			Vaccine Efficacy (95% CI)
	Cases	Person-Yr at Risk	Incidence Density (95% CI)	Cases	Person-Yr at Risk	Incidence Density (95% CI)	
	no.		*no./100 person-yr*	*no.*		*no./100 person-yr*	%
Modified per-protocol analysis*							
Serotype 1	66	12,478	0.5 (0.4–0.7)	66	6,196	1.1 (0.8–1.4)	50.3 (29.1–65.2)
Serotype 2	58	12,495	0.5 (0.4–0.6)	50	6,219	0.8 (0.6–1.1)	42.3 (14.0–61.1)
Serotype 3	43	12,514	0.3 (0.2–0.5)	82	6,213	1.3 (1.1–1.6)	74.0 (61.9–82.4)
Serotype 4	18	12,522	0.1 (0.1–0.2)	40	6,206	0.6 (0.5–0.9)	77.7 (60.2–88.0)
Unknown	6	12,540	<0.1 (0.0–0.1)	3	6,268	<0.1 (0.0–0.1)	0.0 (−517.8–78.6)
Intention-to-treat analysis							
Serotype 1	99	27,016	0.4 (0.3–0.4)	109	13,434	0.8 (0.7–1.0)	54.8 (40.2–65.9)
Serotype 2	84	27,035	0.3 (0.2–0.4)	84	13,461	0.6 (0.5–0.8)	50.2 (31.8–63.6)
Serotype 3	55	27,060	0.2 (0.2. 0.3)	106	13,459	0.8 (0.6–1.0)	74.2 (63.9–81.7)
Serotype 4	32	27,063	0.1 (0.1–0.2)	83	13,442	0.6 (0.5–0.8)	80.9 (70.9–87.7)
Unknown	15	27,079	<0.1 (0.0–0.1)	14	13,514	0.1 (0.1–0.2)	46.5 (−19.6–75.9)

* The modified per-protocol analysis was performed at least 28 days after the third injection in all participants who had received three doses, regardless of protocol deviations.

than in those with a seronegative status (83.7% vs. 43.2%). Differences in efficacy according to country are probably explained by differences in baseline antibody levels and in serotype circulation.[23]

The efficacy results reported here are consistent with those of the similarly designed Asian trial.[8] In the two studies, efficacy was higher against serotypes 3 and 4 than against serotypes 1 and 2. In Asia, efficacy against serotype 2 was 35% after the third injection, which was not significant in comparison with placebo, whereas in our study, the point estimate was 42.3 and was significant. In the two trials, point estimates of efficacy were similar in per-protocol and intention-to-treat analyses (60.8% and 64.7%, respectively, in our study, as compared with 56.5% and 54.8%, respectively, in Asia).

Different efficacy estimates between children with seropositive status and those with seronegative status at baseline were also observed in the two studies. As previously reported, post-vaccination geometric mean antibody titers differed significantly according to baseline serostatus, a factor that may have contributed to the difference in efficacy.[14] Efficacy in the small subgroup of children who had seronegative status at baseline was 43.2%, which was not significant in comparison with placebo but was similar to that in the Asian study (35.5%). Moreover, the vaccine's safety profile showed no clinically significant difference according to serostatus during the observation period, although the power to detect severe disease among children with seronegative status was limited. This consistency between the twin efficacy studies is important, given the epidemiologic differences between and within the regions.

In our study, the estimated efficacy between injections suggests that some protection may be provided by the first injection. However, the second and third vaccinations increased antibody responses in the children without previous exposure to dengue, which might also have increased the quality of the antibody response (e.g., avidity) and the duration of protection. Planned investigations of the mechanisms of protection afforded by CYD-TDV in regions where the disease is endemic may improve our understanding of the contribution of each dose to protection.

The single-center phase 2b study in Thailand provided the first useful insights into the performance of the vaccine. In particular, it provided the first evidence that efficacy varied according

Table 4. Safety Analysis and Subgroup Analysis of Reactogenicity Events Reported within 28 Days after Any Injection.*

Event	Vaccine Group		Control Group	
	no./total no.	*% (95% CI)*	*no./total no.*	*% (95% CI)*
Safety analysis				
Serious adverse event	81/13,915	0.6 (0.5–0.7)	40/6939	0.6 (0.4–0.8)
Death	0/13,915	NA	0/6939	NA
Reactogenicity subgroup analysis				
Unsolicited nonserious adverse event	595/1333	44.6 (41.9–47.4)	292/664	44.0 (40.2–47.8)
Immediate unsolicited nonserious adverse event	3/1333	0.2 (0.0–0.7)	1/664	0.2 (0.0–0.8)
Unsolicited nonserious adverse reaction	16/1333	1.2 (0.7–1.9)	5/664	0.8 (0.2–1.7)
Injection site				
Solicited reaction	675/1328	50.8 (48.1–53.6)	279/658	42.4 (38.6–46.3)
Unsolicited nonserious reaction	9/1333	0.7 (0.3–1.3)	3/664	0.5 (0.1–1.3)
Systemic				
Solicited reaction	909/1328	68.4 (65.9–70.9)	458/659	69.5 (65.8–73.0)
Unsolicited nonserious adverse reaction	7/1333	0.5 (0.2–1.1)	2/664	0.3 (0.0–1.1)
Unsolicited nonserious adverse event	592/1333	44.4 (41.7–47.1)	290/664	43.7 (39.9–47.5)

* Listed are events that occurred at least once in any participant. Safety data were analyzed according to the first dose of vaccine. NA denotes not applicable.

to serotype despite similar antibody levels. This highlights the need for large, multicenter phase 3 trials to test investigational dengue vaccines in heterogeneous epidemiologic settings and to obtain confirmatory data for serotype-specific efficacy.

Reductions in the rates of hospitalization and severe dengue caused by any serotype were observed during the active phase of both phase 3 trials. This finding is pertinent from a public health viewpoint, given the debilitating burden of dengue on hospitals during endemic transmission seasons and epidemic outbreaks.[24-27]

Safety and reactogenicity profiles from 25 months of active surveillance were consistent with previous reports that identified no major concerns.[8,10-15,28,29] No pattern of serious adverse events was identified.

Vaccine immunogenicity was consistent with previous data from the region.[10,14,15,30] Serotype-specific geometric mean antibody titers in the vaccine group did not reflect the serotype-specific efficacy observed. Although this finding highlights the challenge of linking functional antibody responses with efficacy, it does not preclude the identification of a correlate of protection through further analysis of patient-level data.[31]

One limitation of our study is that dengue serostatus was assessed in a subgroup of 10% of the children, of whom only approximately 20% were seronegative. Thus, the efficacy and safety estimates for children with seronegative status were based on approximately 2% of the study population. Furthermore, this subgroup was enrolled during the first few months of overall enrollment. The $PRNT_{50}$ assay is known to have cross-reactivity among serotypes, which makes it difficult to determine dengue serotype-specific seropositivity in participants with multiple infection episodes. Similarly, we could not determine the effect on vaccine efficacy of preexisting immunity to yellow fever because of cross-reactivity with dengue on $PRNT_{50}$ assay for yellow fever, which was performed as outlined in the protocol (data not shown). Another limitation is

that the safety profile is described for a 25-month observation period, although safety follow-up for an additional 4 years is ongoing.

The efficacy that was observed both in our study and in the Asian trial reflects country-specific epidemiologic features, in terms of circulating viruses, incidence, and prior exposure, factors. Post-licensure studies and robust surveillance systems will be necessary to evaluate vaccine efficacy and the effect on the clinical and epidemiologic features of dengue disease.

Overall, the results of our study and the Asian study provide a consistent picture of the efficacy and safety of this dengue vaccine after 25 months of active surveillance in 10 countries among different populations (including a variety of ages and ethnic backgrounds) over different seasons with different circulating serotypes and levels of endemicity.

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APPENDIX

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