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# Immunogenicity and Safety of a Recombinant Tetravalent Dengue Vaccine in Children and Adolescents Ages 9–16 Years in Brazil

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*Abstract.* Immunogenicity and safety of a recombinant, live-attenuated, tetravalent dengue disease vaccine (CYD-TDV) was evaluated in children/adolescents in Brazil. In this observer-blind, placebo-controlled, phase II single-center study, children/adolescents (ages 9–16 years) were randomized to receive CYD-TDV or placebo at 0, 6, and 12 months. Immunogenicity was assessed using a 50% plaque neutralization test. Overall, 150 participants were enrolled (CYD-TDV: $N = 100$; placebo: $N = 50$). Injection site pain and headache were the most common solicited injection site and systemic reactions. Unsolicited adverse events (AEs) and serious AEs were similar between groups. No serious AEs were vaccine-related. Geometric mean titers against all dengue virus serotypes increased with CYD-TDV vaccination and were 267, 544, 741, and 432 1/dil for serotypes 1–4, respectively, after dose 3, representing a mean fold increase from baseline of 5, 6, 6, and 20, respectively. CYD-TDV vaccination elicited a neutralizing antibody response against serotypes 1–4 and was well-tolerated in children/adolescents in a dengue-endemic region.

## INTRODUCTION

Dengue disease is caused by one of four dengue virus serotypes. The primary arthropod vector of the dengue virus is the urban-adapted *Aedes aegypti* mosquito.[1] Most infections are asymptomatic but may manifest as dengue fever (DF) or potentially, fatal severe dengue disease.[2] Infection with one serotype leads to lifelong immunity against that particular serotype. However, subsequent infections by different serotypes may increase the risk of developing severe dengue disease.[3]

Worldwide, dengue disease is one of the most important arthropod-transmitted diseases.[2] It has been suggested that up to one-half of the world's population (3.5 billion people) are at risk of dengue disease (Beatty M and others, unpublished data). In 2009, the World Health Organization (WHO) estimated that at least 50 million dengue infections occurred annually.[2] Between 2000 and 2010, there was an upward trend in the overall burden of dengue disease in Brazil from around 200,000 cases in 2000 to over 1 million cases in 2010.[4–6] All four serotypes have been reported in Brazil.[7] Although dengue disease is prevalent across the entire country,[6] the northeast and southeast regions are the most affected by dengue disease.[4]

No licensed vaccine or specific antiviral treatment of dengue disease exists; prevention relies on vector control measures or individual protection against mosquitoes. One dengue vaccine candidate that shows promise is recombinant, live-attenuated, tetravalent dengue disease vaccine (CYD-TDV; Sanofi-Pasteur, Lyon, France). CYD-TDV is in the late stages of clinical development and has been evaluated in clinical trials in different populations and age ranges.[8–16] It contains four recombinant viruses (CYD-1 to -4), which express the dengue premembrane and envelope proteins of one of four dengue serotypes and the non-structural and capsid proteins of the attenuated yellow fever (YF) vaccine virus YF-17D.[17,18] One of the completed studies was a phase IIb study conducted in the Ratchaburi province in Thailand that investigated the efficacy of the vaccine against virologically confirmed symptomatic dengue.[8] This study showed, for the first time, that a safe and efficacious vaccine against dengue is possible, with protection observed against serotypes 1, 3, and 4. Surprisingly, no protection was seen in this study against serotype 2, despite satisfactory neutralizing antibody titers that were in the same range after three vaccinations as for the other serotypes.

We report on a phase II study conducted in Vitória, the capital city of the Brazilian state of Espírito Santo, where dengue epidemiology is representative of the southeastern region.[7] Trends in age distribution are similar to those trends observed nationwide[19] and throughout Latin America.[20] This study was conducted to determine the immunogenicity and safety of CYD-TDV in children and adolescents in preparation for a large phase III study to determine the efficacy of CYD-TDV in children and adolescents in Latin America.

## METHODS

**Study design and participants.** This study was a phase II, randomized, observer-blind, controlled, single-center study conducted in Vitória, Espírito Santo, Brazil (National Clinical Trials Identifier NCT01187433). Children and adolescents (ages 9–16 years at enrollment) who were healthy based on medical history and physical examination at enrollment were randomized in a 2:1 ratio to receive three subcutaneous injections of CYD-TDV or three subcutaneous placebo injections (NaCl 0.9%) at 0, 6, and 12 months. Randomization was performed by telephone using an interactive voice recognition system and the permuted block method.

The four major exclusion criteria were (1) any immunodeficiency, chronic illness, or treatment that could interfere with the vaccine immunological response, (2) known systemic hypersensitivity to any components of the trial vaccine, (3) receipt of any other vaccine within 4 weeks of the first vaccination, and (4) pregnancy or breastfeeding. Female participants of childbearing potential had to use effective contraception or abstain from sexual intercourse for at least 4 weeks before the first vaccination and until at least 4 weeks after the last vaccination.

The study was conducted in accordance with the Declaration of Helsinki and compliant with the International

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Conference on Harmonization guidelines for Good Clinical Practice. The study protocol was approved by the Independent Ethics Committee and Institutional Review Board of the Universidade Federal do Espírito Santo and Comissão Nacional de Ética em Pesquisa em Seres Humanos, Brazil's National Institutional Review Board and National Regulatory Agency. Written informed consent was obtained from each participant and their parent or legal guardian.

**Safety and reactogenicity.** Adverse events (AEs) monitored throughout the study included unsolicited systemic AEs within 30 minutes of each vaccination, solicited injection site reactions (pain, erythema, or swelling) up to 7 days after each vaccination, solicited systemic reactions (fever, headache, malaise, myalgia, or asthenia) up to 14 days after each vaccination, unsolicited AEs up to 28 days after each vaccination, and serious AEs (SAEs) throughout the trial. AE data after the initial 30-minute observation period were collected using a diary card. AEs were collected using a diary card with the specific solicited adverse reactions and an open field for unsolicited AEs.

AEs were graded (one, two, or three) from mild to severe according to a pre-defined intensity scale based on appropriate measurements or observations. SAEs were defined as events that were life-threatening or resulted in death, required in-patient hospitalization or prolonged existing hospitalization, resulted in persistent or significant disability or incapacity, resulted in a congenital anomaly or birth defect, or were regarded as an important medical event.

Participants were followed up at 6 months after the last injection for information on AEs occurring since the last visit. An Independent Data Monitoring Committee regularly reviewed the safety data.

**Immunogenicity.** Blood samples for the assessment of immunogenicity were taken before and 28 days after each vaccination (a total of six blood samples per participant). Serum levels of neutralizing antibodies against each of the four parental dengue strains of CYD-TDV (i.e., Dengue virus (DENV)-1 strain PUO-359, DENV-2 strain PUO-218, DENV-3 strain PaH881/88, and DENV-4 strain 1228) were determined before and 28 days after each dose using a 50% plaque reduction neutralization test ($PRNT_{50}$) at Sanofi-Pasteur's Global Clinical Immunology Department.[21–23] Briefly, serial twofold dilutions of heat-inactivated serum were mixed with a constant challenge dose of each dengue virus. The mixtures were inoculated into plate wells confluent with Vero cell monolayers. After adsorption, the cell monolayers were incubated for a few days. Plaques in each well, which indicated the presence of cells infected with dengue virus, were fixed, immunostained, and counted. Titers were expressed as the highest reciprocal serum dilution at which the mean number of plaques was reduced by 50% compared with the count obtained from control wells. Geometric mean titers (GMTs) were calculated.

A $PRNT_{50}$ assay was used to assess YF seropositivity in the first blood sample. This assay was also performed in Vero cells, and it used the YF-17D strain with crystal violet staining for enumeration. Seropositivity was defined as antibody titers ≥ 10 1/dil.

**Detection of symptomatic cases.** Detection of symptomatic dengue cases was made using passive surveillance of febrile episodes by parents or legally acceptable representatives combined with active detection of febrile episodes based on planned study visits, phone calls/home visits, and school-based detection (such as monitoring absenteeism). Febrile episodes were defined in alignment with the WHO case definition[24] (e.g., temperature ≥ 38°C on at least 2 consecutive days with a suspicion of dengue disease). In the event of a febrile episode, a blood sample was taken in the acute phase (i.e., as soon as possible but no later than 7 days after the onset of fever). The following tests were performed to virologically confirm dengue cases: quantitative dengue reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) against the dengue non-structural protein 1 (NS1) antigen. In the event of temperature ≥ 38°C on at least 2 consecutive days within 28 days after each vaccination, the occurrence and the level of dengue vaccine viruses in serum were measured by quantitative and serotype-specific RT-PCRs.

**Statistical methods.** This study was descriptive and not powered to conclude on vaccine efficacy. The sample size for this study was set at 100 in the CYD-TDV vaccine group and 50 in the placebo group using a 2:1 randomization ratio. A sample size of 100 individuals in the CYD-TDV vaccine group was selected to provide a 95% probability of observing any events that have a true incidence of 3%. The following analysis sets were performed: safety analysis set (those children who received at least one dose of study vaccine) and full analysis set (those children who received at least one dose of study vaccine and had at least one blood sample taken).

Analyses were descriptive with no testing hypothesis. For the main parameters, 95% confidence intervals (95% CIs) of point estimates were calculated using normal approximation for quantitative data and exact binomial distribution (Clopper–Pearson method) for proportions.[25] Reverse cumulative distribution curves (RCDCs) for pre- and post-vaccination antibody titers were derived to illustrate immune responses to each serotype.

## RESULTS

**Baseline characteristics.** Between August 19 and October 20, 2010, 150 children and adolescents were enrolled in the study and randomized to receive CYD-TDV ($N$ = 100) or placebo ($N$ = 50). A total of 89 participants in the CYD-TDV group and 46 participants in the placebo group received all three injections (Figure 1).

Overall, 55% of the participants were female; there were more females than males in the CYD-TDV group (60%) but fewer females than males in the placebo group (45%). The mean overall age was 12.7 years (SD = 2.1), and mean ages were similar in both vaccination groups. All participants in the study were Hispanic.

The majority of the participants were flavivirus (FV) seropositive at baseline: 81% (69% dengue seropositive and 71% YF seropositive) in the CYD-TDV group and 84% (71% dengue seropositive and 80% YF seropositive) in the placebo group. In the CYD-TDV group, the seropositivity rates at baseline (60% for serotype 1, 66% for serotype 2, 63% for serotype 3, and 48% for serotype 4; 69% for at least one serotype, 63% for at least two serotypes, 57% for at least three serotypes, and 48% for all serotypes) were similar to those rates observed in the placebo group (63% for serotype 1, 67% for serotype 2, 65% for serotype 3, and 51% for serotype 4; 71% for at least one serotype, 67% for at least two serotypes,

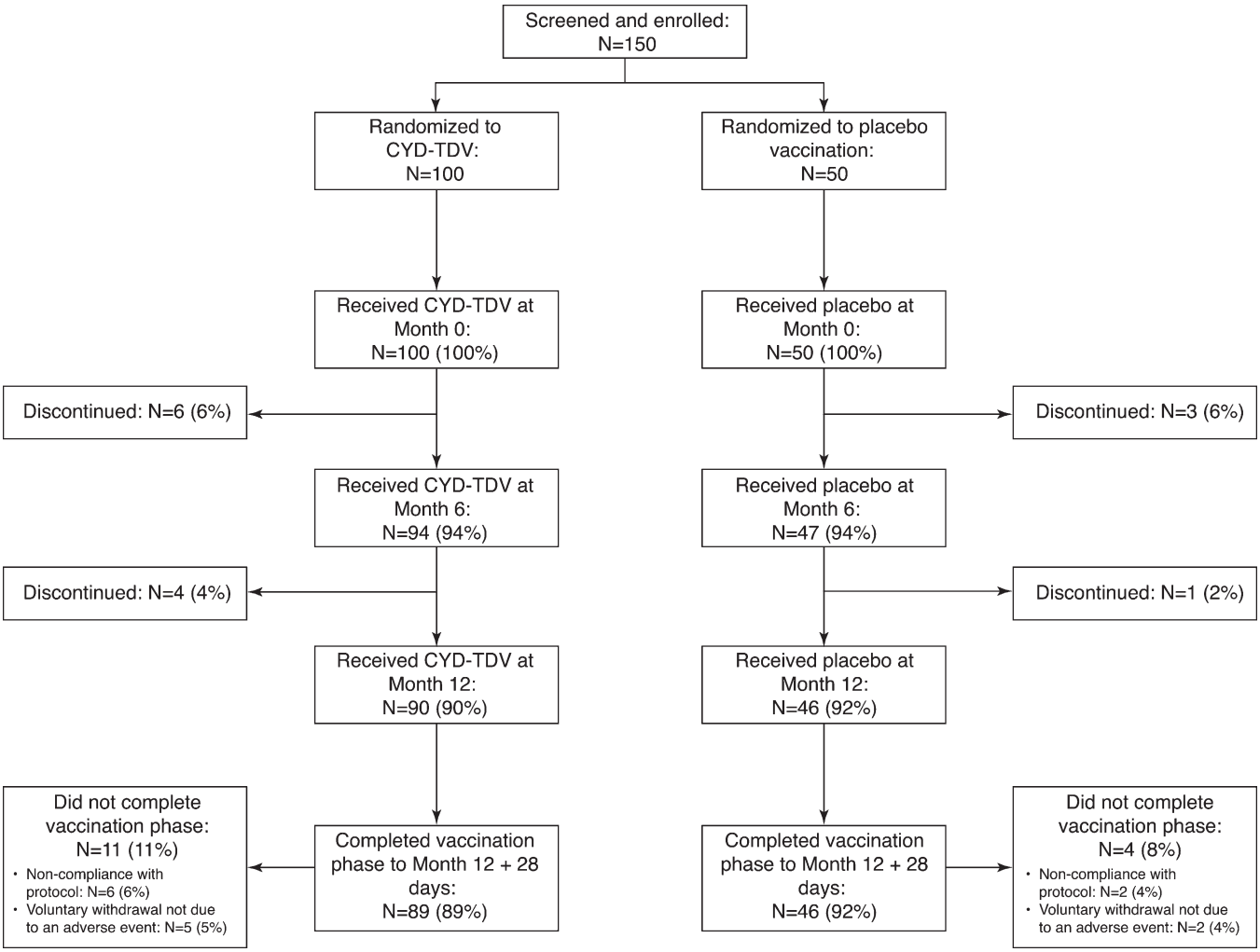


FIGURE 1. Study flow chart: progress of participants through the trial.

61% for at least three serotypes, and 47% for all serotypes). Baseline GMTs in the CYD-TDV group were 41.4, 67.0, 81.9, and 15.0 1/dil for serotypes 1, 2, 3, and 4, respectively and 47.2, 68.3, 94.7, and 17.5 1/dil for serotypes 1, 2, 3, and 4, respectively, in the placebo group.

**Safety and reactogenicity.** There were six SAEs reported by five participants in the CYD-TDV group (5%) and three SAEs reported by three participants in the placebo group (6%). None were considered by the investigator to be related to the study medication, and all participants recovered. The SAEs in the CYD-TDV group were food poisoning, abdominal wall abscess, appendicitis, cellulitis not in the injection site (two occurrences), and pregnancy end by intrauterine death that occurred 112 days after the second dose. In the placebo group, SAEs were abdominal pain, appendicitis, and multiple injuries.

The majority of solicited systemic and injection site reactions in both treatment groups were grade 1 in intensity, began 0–3 days after injection, and resolved within 3 days of occurrence. The proportions of individuals with solicited systemic reactions tended to decrease after each injection in the CYD-TDV vaccine group (Figure 2). Headache was the most frequently reported solicited systemic reaction in both treatment groups, and it was reported by 61% of participants who received CYD-TDV and 51% of participants who received placebo (Table 1). The incidence of solicited injection site reactions in the CYD-TDV group compared with the placebo group varied after each injection (Figure 2). Overall, the proportion of individuals reporting solicited injection site reactions tended to decrease after each injection in the CYD-TDV group (Figure 2). Pain was the most frequently reported injection site reaction in both treatment groups, and it was reported by 40–41% of participants in the CYD-TDV and placebo groups (Table 1).

No unsolicited AEs occurred within 30 minutes of vaccination during the study. No marked differences in the overall incidences of unsolicited AEs were observed between the CYD-TDV and placebo groups (Figure 2). The most frequently reported unsolicited AEs were upper respiratory tract infection in the CYD-TDV group (18%) and nasopharyngitis in the placebo group (12%).

An analysis of the safety profile by baseline flavivirus serostatus suggested that solicited injection site reactions tended to be more frequent in FV seropositive participants than seronegative participants but showed no marked differences for the other categories of AEs (Figure 2). Results were similar when this analysis was performed based on dengue

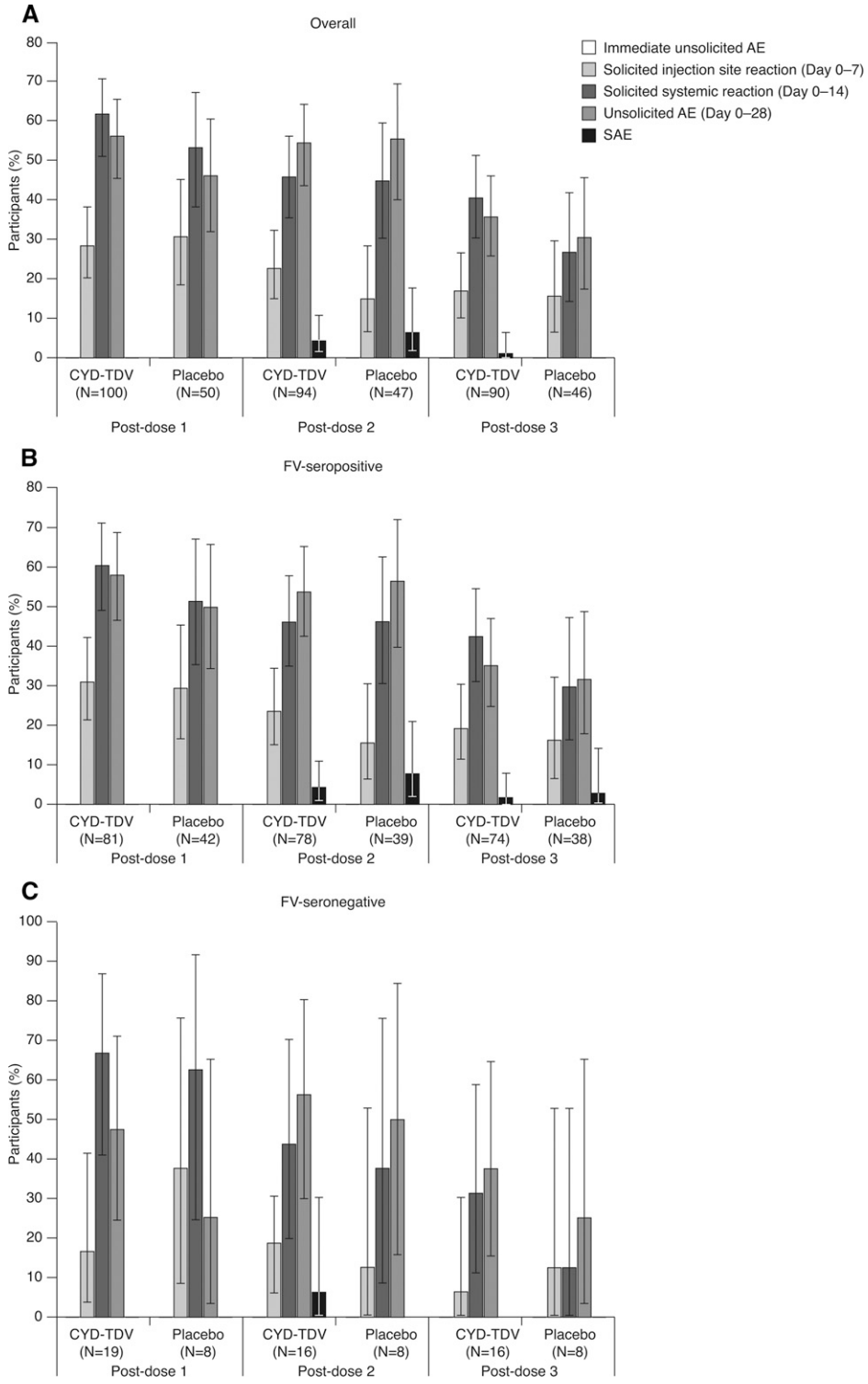


FIGURE 2. Proportion of participants with different categories of AEs after each vaccine dose by vaccine group and FV seropositivity status at baseline.

serostatus and YF serostatus at baseline rather than FV serostatus (data not shown).

**Immunogenicity.** Neutralizing antibody responses as measured by PRNT increased against all four serotypes in response to CYD-TDV vaccination (Table 2). A slight increase was also seen in the placebo group between baseline titers and those titers measured 13 months later. GMTs increased in all volunteers after the first CYD-TDV dose and in most cases, after the second vaccination. GMTs were generally slightly lower 28 days after the third CYD-TDV vaccination than after the second vaccination (Table 2). In the CYD-TDV group, GMTs 28 days after the third vaccination were 267, 544, 741, and 432 l/dil, representing a mean fold increase from baseline of 5, 6, 6, and 20, for serotypes 1, 2, 3,

TABLE 1
Solicited AEs by vaccine group (safety analysis set)

| | CYD-TDV group ($N$ = 100) | | | Placebo group ($N$ = 50) | | |
|---|---|---|---|---|---|---|
| | $n/N$ | Percent | 95% CI | $n/N$* | Percent | 95% CI |
| Injection site reactions (days 0–7) | | | | | | |
| Pain | | | | | | |
| Any | 40/100 | 40.0 | 30.3–50.3 | 20/49 | 40.8 | 27.0–55.8 |
| Grade 3 | 0/100 | 0.0 | 0.0–3.6 | 2/49 | 4.1 | 0.5–14.0 |
| Erythema | | | | | | |
| Any | 4/100 | 4.0 | 1.1–9.9 | 1/49 | 2.0 | 0.1–10.9 |
| Grade 3 | 0/100 | 0.0 | 0.0–3.6 | 0/49 | 0.0 | 0.0–7.3 |
| Swelling | | | | | | |
| Any | 5/100 | 5.0 | 1.6–11.3 | 2/49 | 4.1 | 0.5–14.0 |
| Grade 3 | 0/100 | 0.0 | 0.0–3.6 | 0/49 | 0.0 | 0.0–7.3 |
| Systemic reactions (days 0–14) | | | | | | |
| Fever | | | | | | |
| Any | 30/100 | 30.0 | 21.2–40.0 | 9/49 | 18.4 | 8.8–32.0 |
| Grade 3 | 8/100 | 8.0 | 3.5–15.2 | 3/49 | 6.1 | 1.3–16.9 |
| Headache | | | | | | |
| Any | 61/100 | 61.0 | 50.7–70.6 | 25/49 | 51.0 | 36.3–65.6 |
| Grade 3 | 15/100 | 15.0 | 8.6–23.5 | 10/49 | 20.4 | 10.2–34.3 |
| Malaise | | | | | | |
| Any | 40/100 | 40.0 | 30.3–50.3 | 16/49 | 32.7 | 19.9–47.5 |
| Grade 3 | 11/100 | 11.0 | 5.6–18.8 | 3/49 | 6.1 | 1.3–16.9 |
| Myalgia | | | | | | |
| Any | 42/100 | 42.0 | 32.2–52.3 | 21/49 | 42.9 | 28.8–57.8 |
| Grade 3 | 6/100 | 6.0 | 2.2–12.6 | 4/49 | 8.2 | 2.3–19.6 |
| Asthenia | | | | | | |
| Any | 35/100 | 35.0 | 25.7–45.2 | 10/49 | 20.4 | 10.2–34.3 |
| Grade 3 | 8/100 | 8.0 | 3.5–15.2 | 2/49 | 4.1 | 0.5–14.0 |

*One participant in the placebo group did not have available data.

and 4, respectively. In the placebo group, GMTs 28 days after the third dose were 79.2, 132, 140, and 33.4 l/dil for dengue serotypes 1, 2, 3, and 4, respectively (Table 2).

At post-dose 3, participants in the CYD-TDV group who were FV seropositive at baseline had higher GMTs (381, 835, 1,031, and 485 for serotypes 1, 2, 3, and 4, respectively; $N$ = 73) than those participants who were FV seronegative at baseline (53.4, 76.7, 163, and 255 1/dil for serotypes 1, 2, 3, and 4, respectively; $N$ = 16) (Figure 3). A similar pattern was observed when this analysis was performed based on dengue serostatus and YF serostatus at baseline rather than FV serostatus (data not shown). In the placebo group, nominal increases in GMTs were observed relative to baseline at post-dose 3 for all four serotypes.

TABLE 2
GMTs against each of the four dengue virus serotypes at baseline and 28 days after each vaccine dose by vaccine group (full analysis set)

| | CYD-TDV group ($N$ = 99) GMT | | Placebo group ($N$ = 49) GMT | |
|---|---|---|---|---|
| Time point | 1/dil | 95% CI | 1/dil | 95% CI |
| Serotype 1 | | | | |
| Baseline | 41.4 | 27.7–62.1 | 47.2 | 26.9–82.7 |
| Post-dose 1 | 256 | 151–433 | 50.7 | 29.5–87.3 |
| Post-dose 2 | 436 | 287–662 | 130 | 59.7–283 |
| Post-dose 3 | 267 | 181–394 | 79.2 | 42.4–148 |
| Serotype 2 | | | | |
| Baseline | 67 | 44.0–102 | 68.3 | 36.3–129 |
| Post-dose 1 | 352 | 210–592 | 62.8 | 33.9–116 |
| Post-dose 2 | 647 | 449–932 | 137 | 61.4–306 |
| Post-dose 3 | 544 | 378–782 | 132 | 67.0–259 |
| Serotype 3 | | | | |
| Baseline | 81.9 | 49.3–136 | 94.7 | 43.6–206 |
| Post-dose 1 | 690 | 423–1,125 | 110 | 50.3–242 |
| Post-dose 2 | 1,031 | 704–1,512 | 227 | 92.8–556 |
| Post-dose 3 | 741 | 516–1,062 | 140 | 66.0–298 |
| Serotype 4 | | | | |
| Baseline | 15 | 11.6–19.4 | 17.5 | 11.9–25.8 |
| Post-dose 1 | 383 | 262–559 | 19.2 | 13.3–27.7 |
| Post-dose 2 | 346 | 281–425 | 31.2 | 18.1–53.7 |
| Post-dose 3 | 432 | 335–556 | 33.4 | 21.5–51.9 |

$N$ = number of participants with a valid serology result for the particular dengue serotype, including results reported as less than the lower limit of quantification or greater than the upper limit of quantification.

An increase in seropositivity rates after each CYD-TDV injection was observed, which was more pronounced post-dose 1 for serotypes 3 and 4. Post-dose 3 seropositivity rates were between 97% and 100%. For these two serotypes, there were no appreciable changes in seropositivity rates after any injection in the placebo group (post-dose 3 seropositivity rates ranged from 70% to 76%). Similar seropositivity rates were observed in FV seropositive and FV seronegative participants after the third dose of CYD-TDV for serotypes 3 and 4. A lower response was also observed for serotypes 1 and 2 in FV seronegative participants. Seropositivity rates for at least one, two, or three serotypes at baseline were similar among all participants who received CYD-TDV or placebo. After three doses of CYD-TDV, all participants (100%) were seropositive for at least one or two serotypes, 98.9% of participants were seropositive for at least three serotypes, and 96.6% of participants were seropositive for all four serotypes. Table 3 shows the number of responders before and after vaccination against at least one, two, three, or all four serotypes according to the flavivirus serostatus. There were no appreciable changes in seropositivity rates among those patients who received placebo after any injection.

**Detection of symptomatic cases.** The incidence of virologically confirmed dengue disease cases was lower in the CYD-TDV group compared with the placebo group; 31 of 100 (31.0%) participants in the CYD-TDV group and 15 of 50 (30.0%) participants in the placebo group were reported with suspected cases of dengue disease during the study. Of these cases, 3 of 14 (21.4%) participants in the placebo group with available blood samples had virologically confirmed cases of dengue disease compared with 0 of 30 participants with available blood samples in the CYD-TDV group. All three cases of virologically confirmed dengue disease (one case was both NS1- and RT-PCR serotype 1-positive, and the other two cases were NS1-positive but with no serotype identified by RT-PCR) occurred before the second placebo injection and were seronegative post-injection 1. CYD-TDV viremia was not detected in the study.

## DISCUSSION

This study was an important component of the global clinical trial program for CYD-TDV, which is in the final stages of clinical development.[26,27] It is the only study to date to provide data on safety and immunogenicity of CYD-TDV in a Brazilian cohort of children and adolescents, which is currently the age group most affected by severe dengue disease in Brazil.[19,20] (Teixeira MG and others, unpublished data.). This study served as preparation for the large multicenter phase III study recently launched throughout Latin America in children and adolescents (NCT01374516) as well as a similar preparatory study recently completed in other Latin American countries.[16]

The present study confirmed the findings from previous phase II studies, which showed that vaccination with a

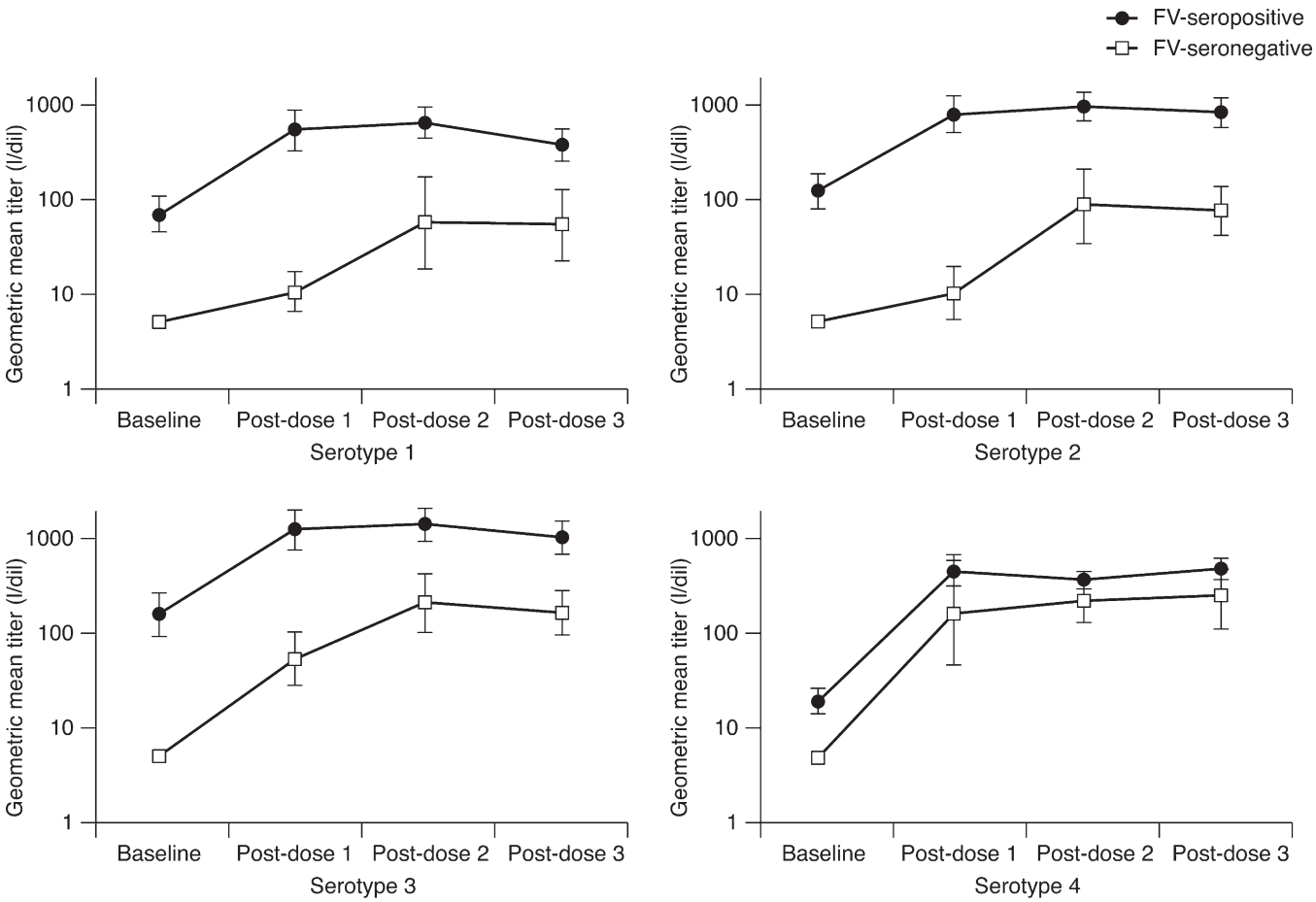


FIGURE 3. Dengue serotype-specific GMTs of antibodies at baseline and 28 days after each dose of CYD-TDV according to whether participants were flavivirus seropositive ($N = 80$) or seronegative ($N = 19$) at baseline.

TABLE 3

Percentage of responders (titer ≥ 10) against at least one, two, three, and all four dengue virus serotypes at baseline and 28 days after each dose of CYD-TDV by baseline flavivirus serostatus (full analysis set)

| | Percentage of response in flavivirus seropositive subjects at baseline | | Percentage of response in flavivirus seronegative subjects at baseline | |
|---|---|---|---|---|
| Time point | Percent | *n*/*N* | Percent | *n*/*N* |
| At least one serotype | | | | |
| Baseline | 85.0 | 68/80 | 0.0 | 0/19 |
| Post-dose 1 | 97.5 | 78/80 | 94.7 | 18/19 |
| Post-dose 2 | 100.0 | 78/78 | 100.0 | 16/16 |
| Post-dose 3 | 100.0 | 73/73 | 100.0 | 16/16 |
| At least two serotypes | | | | |
| Baseline | 77.5 | 62/80 | 0.0 | 0/19 |
| Post-dose 1 | 97.4 | 76/78 | 78.9 | 15/19 |
| Post-dose 2 | 100.0 | 78/78 | 100.0 | 16/16 |
| Post-dose 3 | 100.0 | 73/73 | 100.0 | 16/16 |
| At least three serotypes | | | | |
| Baseline | 70.0 | 56/80 | 0.0 | 0/19 |
| Post-dose 1 | 82.5 | 74/80 | 36.8 | 7/19 |
| Post-dose 2 | 100.0 | 78/78 | 93.8 | 15/16 |
| Post-dose 3 | 100.0 | 73/73 | 93.8 | 15/16 |
| All four serotypes | | | | |
| Baseline | 58.8 | 47/80 | 0.0 | 0/19 |
| Post-dose 1 | 87.5 | 70/80 | 10.5 | 2/19 |
| Post-dose 2 | 100.0 | 78/78 | 75.0 | 12/16 |
| Post-dose 3 | 98.6 | 72/73 | 87.5 | 14/16 |

three-dose CYD-TDV regimen was well-tolerated and also elicited neutralizing antibody responses against all four dengue virus serotypes in both FV seropositive and FV seronegative participants.[9,12–15,28] A higher immune response to vaccination was observed in FV seropositive participants compared with patients who were FV seronegative, which was described in prior studies.[12,13,16] This finding suggests that pre-existing FV seropositivity may increase the vaccine-induced antibody response to CYD-TDV.

In the present study, one or two doses of CYD-TDV resulted in high GMTs, which did not increase substantially after the third dose. The present study is consistent with results from other studies in the global development program in pre-dominantly FV seropositive populations.[9,13,16,28] An increase in GMTs after the third dose was observed among FV seronegative participants in a similar study conducted in four countries in Latin America[16] and other studies conducted in FV seronegative populations.[14,29] In this study, such an increase in titers after the third dose was not observed; however, because the sample size is small and the assay variability is considerable, a fold difference of less than two is not significant enough to characterize it as higher or lower.

The GMTs reported in this study tended to be higher than the GMTs reported in similar studies conducted in the Philippines, Thailand, and Singapore, possibly because of higher baseline FV seropositivity rates and GMTs in the present

study than among adolescents in the Asian studies.[8,9,15] In Vitória, the background circulating FV is dengue, because YF is not endemic in this region.[30] Despite a low YF vaccination coverage rate reported among 10- to 14-year-old children from 2000 to 2009 (19.4%), a high percentage of samples in the present study were positive for YF at baseline. This observation may be related, in part, to cross-neutralizing or cross-reacting antibodies between dengue and YF. Similar findings have been reported in a prior study conducted in areas of Mexico, Honduras, and Puerto Rico that are non-endemic for YF in persons not known to be exposed to YF.[16] Various FV cross-reactive responses have been shown to occur after exposure to heterologous flaviviruses,[31] and antibodies are shown to neutralize all four dengue virus serotypes after a secondary dengue virus infection, regardless of the presumed serotypes responsible for primary and secondary infection,[32,33] This cross-reactivity may explain the presence of baseline serotype 4 neutralizing antibodies, despite this serotype not being in circulation at the time of the study. The biological relevance of measuring DENV neutralization in a system modeled on *in vivo* target cells (e.g., assays in FcγR cells), therefore, deserves additional study.

It was expected that a balanced and robust PRNT response to CYD-TDV against all four serotypes, which was shown in this study and other studies,[9–15,26,27] would translate into protection. However, in a phase IIb proof-of-concept efficacy trial conducted in Thailand, CYD-TDV did not provide adequate protection against infection with serotype 2, despite the presence of high antibody levels.[8] It is, thus, clear that the development of robust neutralizing antibodies may not be the only factor involved in the protective response to CYD-TDV vaccination. The possibility also exists that a minimum detectable titer of 1:10 as the threshold for seropositivity may not be indicative of true immunity. The situation is complicated further by the presence of four virus serotypes that cause dengue disease, which exhibit regional and national variations in serotype distribution, genotypic variation, and variable immune responses to vaccination. The prevalence of Japanese Encephalitis Virus (JEV) and other flaviviruses and the use of JEV vaccination are also confounding factors. Clearly, these multiple variables confirm the need for large-scale clinical trials and countenance against drawing conclusions from any one of the many studies in the global program.

This study provides reassuring information on the tolerability of CYD-TDV in children and adolescents, the age groups most affected by severe dengue disease in Brazil. The present data are also in agreement with the data from other studies in the global development program,[8,9,14,16] which also show a trend to a decrease in reactogenicity with successive doses of CYD-TDV.[9,11,14,28] There were no remarkable differences in reactogenicity according to FV seropositivity status at baseline. There were no deaths or SAEs that were considered to be related to CYD-TDV or placebo, which underscores the favorable safety profile of the vaccine in this population.

A total of 3 of 14 participants in the placebo group with suspected dengue disease had virologically confirmed dengue disease compared with 0 of 31 participants with suspected dengue disease in the CYD-TDV group. These observations suggest that CYD-TDV may have afforded some protection, a possibility that is strengthened given the higher numbers receiving CYD-TDV compared with placebo (2:1 randomization). All three cases occurred between doses 1 and 2 and coincided with the dengue peak season in Brazil (Teixeira MG and others, unpublished data). However, firm conclusions cannot be drawn, because the study was not powered to determine the efficacy of CYD-TDV for the prevention of dengue disease. In addition, because this study was a single-center study, it should be noted that the findings may not be applicable to Brazil as a whole.

In conclusion, this phase II study provides the first data relating to the use of CYD-TDV in a Brazilian cohort. The study confirmed that CYD-TDV vaccination elicited a neutralizing antibody response against all four dengue virus serotypes and was well-tolerated in children and adolescents ages 9–16 years in a region of Brazil that is endemic for dengue disease. The findings support observations from other phase I and II trials and the continued development of CYD-TDV. Additional information on the efficacy and safety of CYD-TDV will be provided by ongoing phase III studies in Latin America (NCT01374516) and Asia (NCT01373281).

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