# Immunogenicity and safety of booster CYD-TDV dengue vaccine after alternative primary vaccination schedules in healthy individuals aged 9–50 years: a randomised, controlled, phase 2, non-inferiority study

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## Summary

**Background** Dengue is endemic in many countries throughout the tropics and subtropics, and the disease causes substantial morbidity and health-care burdens in these regions. We previously compared antibody responses after one-dose, two-dose, or three-dose primary regimens with the only approved dengue vaccine CYD-TDV (Dengvaxia; Sanofi Pasteur, Lyon, France) in individuals aged 9 years and older with previous dengue exposure. In this study, we assessed the need for a CYD-TDV booster after these primary vaccination regimens.

**Methods** In this randomised, controlled, phase 2, non-inferiority study, healthy individuals aged 9–50 years recruited from three sites in Colombia and three sites in the Philippines (excluding those with the usual contraindications to vaccinations) were randomly assigned 1:1:1 via a permuted block method with stratification by site and by age group using an independent voice response system to receive, at 6-month intervals, three doses of CYD-TDV (three-dose group), one dose of placebo followed by two doses of CYD-TDV (two-dose group), or two doses of placebo followed by one dose of CYD-TDV (one-dose group). Participants were also randomly assigned (1:1) to receive a CYD-TDV booster at 1 year or 2 years after the last primary dose. Each CYD-TDV dose was 0·5 mL and administered subcutaneously in the deltoid region of the upper arm. The investigators and sponsor, study staff interacting with the investigators, and participants and their parents or legally acceptable representatives were masked to group assignment. Neutralising antibodies were measured by 50% plaque reduction neutralisation testing, and geometric mean titres (GMTs) were calculated. Due to a change in study protocol, only participants who were dengue seropositive at baseline in the Colombian cohort received a booster vaccination. The primary outcome was to show non-inferiority of the booster dose administered at 1 year or 2 years after the two-dose and three-dose primary regimens; non-inferiority was shown if the lower limit of the two-sided adjusted 95% CI of the between-group (day 28 post-booster dose GMT from the three-dose or two-dose group *vs* day 28 GMT post-dose three of the three-dose primary regimen [three-dose group]) geometric mean ratio (GMR) was higher than 0·5 for each serotype. Non-inferiority of the 1-year or 2-year booster was shown if all four serotypes achieved non-inferiority. Safety was assessed among all participants who received the booster. This trial is registered with ClinicalTrials.gov, NCT02628444, and is closed to accrual.

**Findings** Between May 2 and Sept 16, 2016, we recruited and enrolled 1050 individuals who received either vaccine or placebo. Of the 350, 348, and 352 individuals randomly assigned to three-dose, two-dose, and one-dose groups, respectively, 108, 115, and 115 from the Colombian cohort were dengue seropositive at baseline and received a booster; 55 and 53 in the three-dose group received a booster after 1 year and 2 years, respectively, as did 59 and 56 in the two-dose group, and 62 and 53 in the one-dose group. After the three-dose primary schedule, non-inferiority was shown for serotypes 2 (GMR 0·746; 95% CI 0·550–1·010) and 3 (1·040; 0·686–1·570) but not serotypes 1 (0·567; 0·399–0·805) and 4 (0·647; 0·434–0·963) for the 1-year booster, and again for serotypes 2 (0·871; 0·673–1·130) and 3 (1·150; 0·887–1·490) but not serotypes 1 (0·688; 0·479–0·989) and 4 (0·655; 0·471–0·911) for the 2-year booster. Similarly, after the two-dose primary schedule, non-inferiority was shown for serotypes 2 (0·809; 0·505–1·300) and 3 (1·19; 0·732–1·940) but not serotypes 1 (0·627; 0·342–1·150) and 4 (0·499; 0·331–0·754) for the 1-year booster, and for serotype 3 (0·911; 0·573–1·450) but not serotypes 1 (0·889; 0·462–1·710), 2 (0·677; 0·402–1·140), and 4 (0·702; 0·447–1·100) for the 2-year booster. Thus, non-inferiority of the 1-year or 2-year booster was not shown after the three-dose or two-dose primary vaccination regimen in dengue-seropositive participants. No safety concerns occurred with the 1-year or 2-year CYD-TDV booster.

**Interpretation** CYD-TDV booster 1 year or 2 years after the two-dose or three-dose primary vaccination regimen does not elicit a consistent, meaningful booster effect against all dengue serotypes in participants who are seropositive for dengue at baseline.

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For the Spanish translation of the abstract see Online for appendix 1

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## Introduction

Dengue, a mosquito-borne viral disease caused by four dengue virus serotypes, remains a public health concern in tropical and subtropical regions of the world. The expanding geographical distribution of the vector in the past few decades has led to new regions being at risk of dengue transmission, including parts of Europe and the USA,[1] and transmission of dengue is also increasingly affecting international travellers.[2,3] Dengue epidemiology has been characterised by increased frequency and magnitude of outbreaks, and the disease is widely considered to be the most prevalent and fastest spreading mosquito-borne viral disease.[4] Infection with dengue is thought to confer lifelong immunity against the infecting serotype but only short-lived cross-immunity against other serotypes.[5,6] Severe dengue is usually associated with secondary heterologous dengue infections because of the production of non-neutralising antibodies.[7,8]

A tetravalent dengue vaccine (CYD-TDV; Dengvaxia, Sanofi Pasteur, Lyon, France) has been licensed as a three-dose primary vaccination regimen for use in dengue-seropositive (ie, those who had previous dengue infection) individuals aged 9 years or older.[9] However, global uptake has been hampered by the restriction of its use to dengue-seropositive individuals aged 9 years or older and the need for a three-dose schedule.[10] Dengue-seronegative individuals should not receive CYD-TDV because they have an increased risk of severe dengue after vaccination.[11] Therefore, a pre-vaccination screening strategy is recommended by WHO in countries considering routine vaccination in their dengue control programme, to minimise the risk of inadvertently vaccinating dengue-seronegative individuals.[9]

We previously showed that a two-dose regimen elicited a non-inferior response to the three-dose regimen and no safety concerns were identified; therefore, a two-dose regimen could be used as an alternative to the three-dose regimen in dengue-seropositive individuals aged 9 years or older.[12] However, it is not clear whether a booster dose would be needed after the primary regimen to maintain immunity. Previous studies had suggested that a booster could be of benefit.[13–15] This follow-up study assessed the immunogenicity and safety of a booster CYD-TDV dose administered either 1 year or 2 years after two-dose or three-dose primary vaccination regimens with CYD-TDV. The main objective in this study was to show the non-inferiority of the dengue serotype-specific neutralising antibody responses elicited 28 days after a booster in individuals who received the CYD-TDV booster dose 1 year or 2 years after the two-dose or three-dose primary vaccination regimen relative to that observed 28 days after completion of the three-dose primary regimen in participants who were dengue seropositive at baseline.

## Methods

### Study design and participants

In this randomised, controlled, phase 2, non-inferiority study, we assessed the immunogenicity and safety of CYD-TDV administered, in the second stage of a two-stage study, as a booster dose 1 year or 2 years after a primary vaccination regimen. A one-dose, two-dose, or three-dose regimen was administered in the first stage of the study.

### Research in context

#### Evidence before this study

CYD-TDV is currently licensed as a three-dose vaccination regimen for use in dengue-seropositive individuals aged 9 years and older living in endemic areas. We previously showed that one-dose and two-dose CYD-TDV primary regimens elicited dengue neutralising antibody responses at 28 days after the last dose that were similar to those with the licensed three-dose regimen, and no safety concerns were identified. However, the need for a CYD-TDV booster after the primary regimen remains to be established. We searched PubMed with no date or language restrictions up to Feb 24, 2021, for studies reporting the immunogenicity and safety of a booster CYD-TDV dose using the search terms "dengue" AND "vaccine" OR "prevention" OR "immunization". We identified two previous studies (NCT02623725 and NCT02824198), which showed that a booster CYD-TDV dose tended to restore dengue neutralising antibody concentrations back towards those observed after the third primary dose, although this restoration was followed by a gradual decrease in neutralising antibody concentrations during long-term follow-up. High dengue neutralising antibody titres are associated with vaccine efficacy.

#### Added value of this study

An additional CYD-TDV dose after primary dengue vaccination boosts the immune response. However, there was no consistent meaningful booster effect against all dengue serotypes after either the two-dose or three-dose primary vaccination regimens relative to that observed after completion of the three-dose primary regimen (ie, the approved regimen) in participants who were seropositive for dengue at baseline, irrespective of whether the booster was administered 1 year or 2 years after the last dose.

#### Implications of all the available evidence

CYD-TDV booster after a two-dose or three-dose primary vaccination regimen does not provide any additional benefit in participants who are seropositive at baseline.

Full details of the study have been described previously, along with the results for the first stage of the study, which was conducted between May 2, 2016, and Dec 20, 2018 (NCT02628444).[12] Participants were recruited from three sites in Colombia (Centro de Estudios en Infectología Pediátrica, Cali; Clínica de la Costa, Barranquilla; and Hospital Pablo Tobón Uribe, Medellín) and three sites in the Philippines (Research Institute for Tropical Medicine and San Pablo City Health Office, Muntinlupa; Philippine General Hospital, Manila; and Manila Doctors Hospital, Manila). Here, we describe the results from the second stage of the study, conducted between May 2, 2017, and Dec 20, 2018.

Healthy individuals in Colombia and the Philippines aged 9–50 years were recruited for this study. Participants were excluded if they had previously been vaccinated against dengue virus with either the trial vaccine or another vaccine; planned receipt of any vaccine within 4 weeks after any trial vaccination; were pregnant, breastfeeding, or of childbearing potential; had self-reported or suspected congenital or acquired immunodeficiency; had received immunosuppressive therapy within the past 6 months; or had received long-term systemic corticosteroid therapy within the past 3 months.

This study was done in compliance with the International Conference on Harmonisation guidelines for Good Clinical Practice and the principles of the Declaration of Helsinki. The protocol and amendments were approved by applicable independent ethics committees or institutional review boards and the regulatory agencies as per local regulations. Written informed consent and assent, when applicable, were obtained from the participants or their parents or legal guardians before any study procedures were performed. The protocol is available online.

## Randomisation and masking

For the first stage of the study, on the day of enrolment, participants were randomly assigned (1:1:1), by a permuted block method with stratification by site and age group (9–11 years, 12–17 years, 18–39 years, and 40–50 years) and an independent voice response system, to one of three groups: three doses of CYD-TDV (on day 0, month 6, and month 12; three-dose group), one dose of placebo (on day 0) followed by two doses of CYD-TDV (at month 6 and month 12; two-dose group), or two doses of placebo (on day 0 and month 6) followed by one dose of CYD-TDV (at month 12; one-dose group). For the second stage of the study reported here, participants randomly assigned to each of the three study groups were also randomly assigned (1:1) to receive a CYD-TDV booster at either 1 year (one-dose, two-dose, and three-dose subgroups with booster at 1 year) or 2 years (one-dose, two-dose, and three-dose subgroups with booster at 2 years) after the last primary dose. 60 participants (ten in each of the six subgroups) at Hospital Pablo Tobón Uribe in Medellin, Colombia, consented to participate in additional immunological testing. The investigators and sponsor, study staff interacting with the investigators, and participants and their parents or legally acceptable representatives were masked to group assignment. Both vaccine and placebo were presented in 0·5 mL single-dose vials, with dose numbers defined using a random list, so they could not be used to distinguish between treatment groups. Only the individuals in charge of preparing and administering the injections had access to randomisation documents; these individuals were not involved in collecting any safety data.

## Procedures

The vaccine (CYD-TDV; Sanofi Pasteur, Normandy, France) was presented in a single-dose vial as a powder for immediate reconstitution in 0·4% NaCl solution and administered subcutaneously into the deltoid region of the upper arm. Each 0·5 mL dose of reconstituted vaccine contained a 4·5–6·0 $\log_{10}$ 50% cell-culture infectious dose of each live-attenuated, recombinant dengue virus serotype (1, 2, 3, and 4). A booster dose was administered in the same manner. As previously described,[12] participants received three injections at 6-month intervals, either of CYD-TDV (three-dose group), one injection of placebo (0·9% NaCl solution) followed by two doses of CYD-TDV (two-dose group), or two injections of placebo followed by one dose of CYD-TDV (one-dose group). After 1 year or 2 years, participants in the one-dose, two-dose, and three-dose groups received one dose of CYD-TDV booster. Blood samples during the initial primary regimen were taken before the first injection as well as before and 28 days after the second and third injections. For booster analyses, blood samples were obtained at 1 year or 2 years after the last injection but before the booster, and 28 days after the booster, for measurement of neutralising antibody titres against each of the four dengue serotypes. Dengue neutralising antibody concentrations were measured by 50% plaque reduction neutralisation assay ($PRNT_{50}$; Sanofi Pasteur Global Clinical Immunology, Swiftwater, PA, USA) with parental dengue virus strains of CYD dengue vaccine constructs, as previously described.[16] The lower limit of quantitation of the assay was 10 (1/dil). Dengue-seropositive participants were defined as those with results of 10 (1/dil) or higher for at least one serotype with the parental dengue virus strain.

Assessment of cell-mediated immune responses (memory B-cell responses and T-cell responses) was conducted in a subset of participants who consented to participate in additional immunological testing at day 0 (before the first injection) and before the booster at year 1 in seropositive and seronegative participants, and before the booster at year 2 in seropositive participants only, as previously described.[15,17] To assess the cell response, CD8+ T cells were stimulated by YF17D NS3 or DEN NS3 peptide pools. Post-booster responses at year 1 and year 2 were measured at days 7, 14, and 28 in seropositive participants only.

For the **protocol** see https://clinicaltrials.gov/ProvidedDocs/44/NCT02628444/Prot_000.pdf

### Outcomes

The primary endpoint for this second-stage study was to show the non-inferiority of the dengue serotype-specific neutralising antibody responses (geometric mean titres [GMTs]) elicited 28 days after the 1-year or 2-year booster in three-dose and two-dose groups relative to that observed 28 days after the last primary dose in the three-dose group (ie, in terms of geometric mean ratios [GMRs] for each dengue serotype for comparisons within the three-dose group [after 1-year booster *vs* after third primary dose, and after 2-year booster *vs* after third primary dose] and between the three-dose and two-dose groups [two-dose group after 1-year booster *vs* three-dose group after third primary dose, and two-dose group after 2-year booster *vs* three-dose group after third primary dose]). GMRs were also described for each dengue serotype for comparisons in the one-dose group after the 1-year booster versus the three-dose group after the third primary dose, and after the 2-year booster versus the three-dose group after the third primary dose. Secondary endpoints were antibody responses against each dengue serotype in terms of GMTs at 28 days after the last primary injection relative to those immediately before receiving the booster by baseline serostatus, and seroconversion rates after the booster in one-dose, two-dose, and three-dose groups. Seroconversion rates 28 days after the booster against each of the four CYD-TDV parental dengue virus strains were defined as the percentage of participants with either a pre-booster titre of less than 10 (1/dil) and a post-booster titre of 40 or higher (1/dil), or a pre-booster titre of 10 or higher (1/dil) and a 4-fold or higher increase in post-booster titre, as determined by $PRNT_{50}$.

The safety profile of CYD-TDV was assessed as a secondary endpoint among all participants who received the booster (up to 6 months after the booster) in the same manner as described after any injection during the primary vaccination regimen.[12] Solicited injection-site reactions (pain, erythema, and swelling) occurring up to 7 days after injection, solicited systemic reactions (fever, headache, malaise, myalgia, and asthenia) occurring up to 14 days after injection, unsolicited non-serious adverse events occurring up to 28 days after each injection, and serious adverse events throughout the study were assessed in terms of timing, duration, and intensity, irrespective of whether the adverse event led to study discontinuation. Unsolicited adverse events were assessed by the investigator to be related or not related to vaccination. The following adverse events of special interest were also assessed: hypersensitivity or allergic reactions occurring within 7 days after injection; serious viscerotropic or serious neurotropic disease occurring within 30 days after injection; and serious dengue virus disease, including virologically confirmed dengue, requiring admission to hospital throughout the trial.

Cell-mediated immunity was assessed as a descriptive exploratory endpoint. For CYD-specific memory B-cell responses, only the response against serotype 1 was assessed to be representative of the other serotypes. Although antibody specificity and affinity maturation outcomes were planned, these outcomes were not assessed because the current reported results were considered enough for informing about cell immunity.

### Statistical analysis

As initially planned, assuming a dropout rate of 15%, we estimated that a sample size of 1050 participants would provide 888 evaluable participants (296 per group) and an overall power of approximately 91% for a successful trial as defined per protocol for stage one of the study (reported previously[12]), and an overall power of 69–98% for stage two of the study (reported here). However, during the conduct of the study, the Independent Data Monitoring Committee for this study recommended that any subsequent planned vaccination should only be administered in participants who were dengue seropositive at baseline (dengue antibody PRNT titre ≥10), and the study protocol was amended accordingly (protocol amendment dated Dec 12, 2017). By this time, the administration of the primary vaccination regimen had already been completed. The protocol amendment was only approved by the Colombian independent ethics committees and national regulatory authority. Therefore, only participants from Colombia who were dengue seropositive at baseline received CYD-TDV booster according to the protocol amendment. Participants from the Philippines could not receive the booster injection but continued with the other study assessments as planned.

Non-inferiority analyses were performed using the paired *t* test (in the three-dose group) or two sample *t* test (between groups; three-dose group *vs* two-dose group) with booster at 1 year and booster at 2 years, with an adjusted two-sided 95% CI of the difference of the means of the $\log_{10}$ transformed titres after vaccination between the booster dose and the third dose in the three-dose group ($\alpha$=2·5% one-sided). For each serotype, non-inferiority was shown if the lower limit of the two-sided adjusted 95% CI of the between-group GMR was higher than 0·5 (ie, the $\log_{10}$ of the difference had to be higher than –0·301). Bonferroni adjustments were applied to control for multiplicity. Non-inferiority of the 1-year or 2-year booster was shown if all four serotypes achieved non-inferiority.

$PRNT_{50}$ titres and associated 95% CIs were calculated using $\log_{10}$ transformation of the titres for each of the four serotypes, assuming normal distribution of the $\log_{10}$ transformed titres, with antilog transformations applied to the results to compute the GMTs, GMRs, and 95% CIs on their original scale. The incidences of adverse events were calculated with associated 95% CIs determined with the exact binomial method (Clopper–Pearson method) by allocated group.

The per-protocol dataset, which included vaccinated participants who had no protocol deviations, was used for the analysis for the primary objective. The full

analysis dataset, which included all participants who received at least one injection of either CYD-TDV or placebo and had at least one blood sample drawn with valid post-injection serological results, was used for the analysis of the secondary endpoints. The safety analysis dataset, which included all participants who received at least one injection of CYD-TDV or placebo, was used for the description of clinical safety.

This trial is registered with ClinicalTrials.gov, NCT02628444.

### Role of the funding source

The funder of the study had a role in the study design, data collection, data analysis, data interpretation, writing of the report, and the decision to submit the paper for publication.

## Results

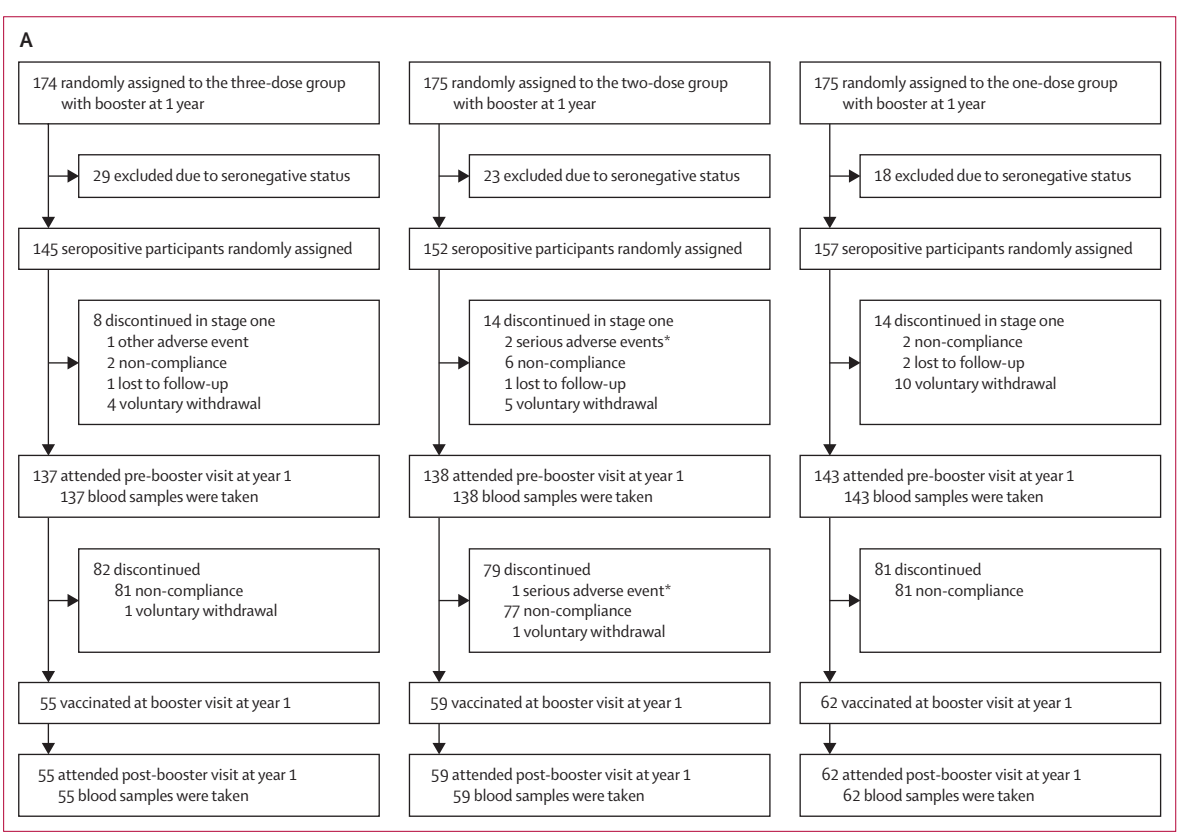
Figure 1 shows the trial profile. The large numbers of participants who discontinued before receipt of a booster at year 1 or year 2 for non-compliance reasons were due to the change in protocol to exclude those who were dengue seronegative at baseline. No participants from the Philippines received CYD-TDV booster. In Colombia, no participant who received CYD-TDV booster 1 year after the last primary dose discontinued; three participants who received CYD-TDV booster at 2 years after the primary dose discontinued. The baseline characteristics of participants who were seropositive for dengue at baseline and included in the present study are summarised in table 1.

After the three-dose primary schedule, non-inferiority of the immune response elicited by the 1-year CYD-TDV booster was shown for serotypes 2 and 3 only (ie, the lower limits of the corrected 95% CIs for paired ratios were above 0·5; 0·550 for serotype 2 and 0·686 for serotype 3; table 2). Therefore, overall non-inferiority of the 1-year booster was not achieved.

In the three-dose group, non-inferiority of the immune response elicited by the 2-year CYD-TDV booster was also only shown for serotypes 2 and 3 (ie, lower limits of the corrected 95% CIs were 0·673 for serotype 2 and 0·887 for serotype 3; table 2). Therefore, overall non-inferiority of the 2-year booster was also not achieved.

After the two-dose primary schedule, non-inferiority of the immune response elicited by the 1-year booster was shown for serotypes 2 and 3 only (ie, the lower limits of the corrected 95% CIs were 0·505 for serotype 2 and 0·732 for serotype 3; table 3). Therefore, overall non-inferiority for all the four serotypes of the 1-year booster was not achieved.

After the two-dose primary schedule, non-inferiority of the immune response elicited by the 2-year booster was shown only for serotype 3 (ie, the lower limit of the

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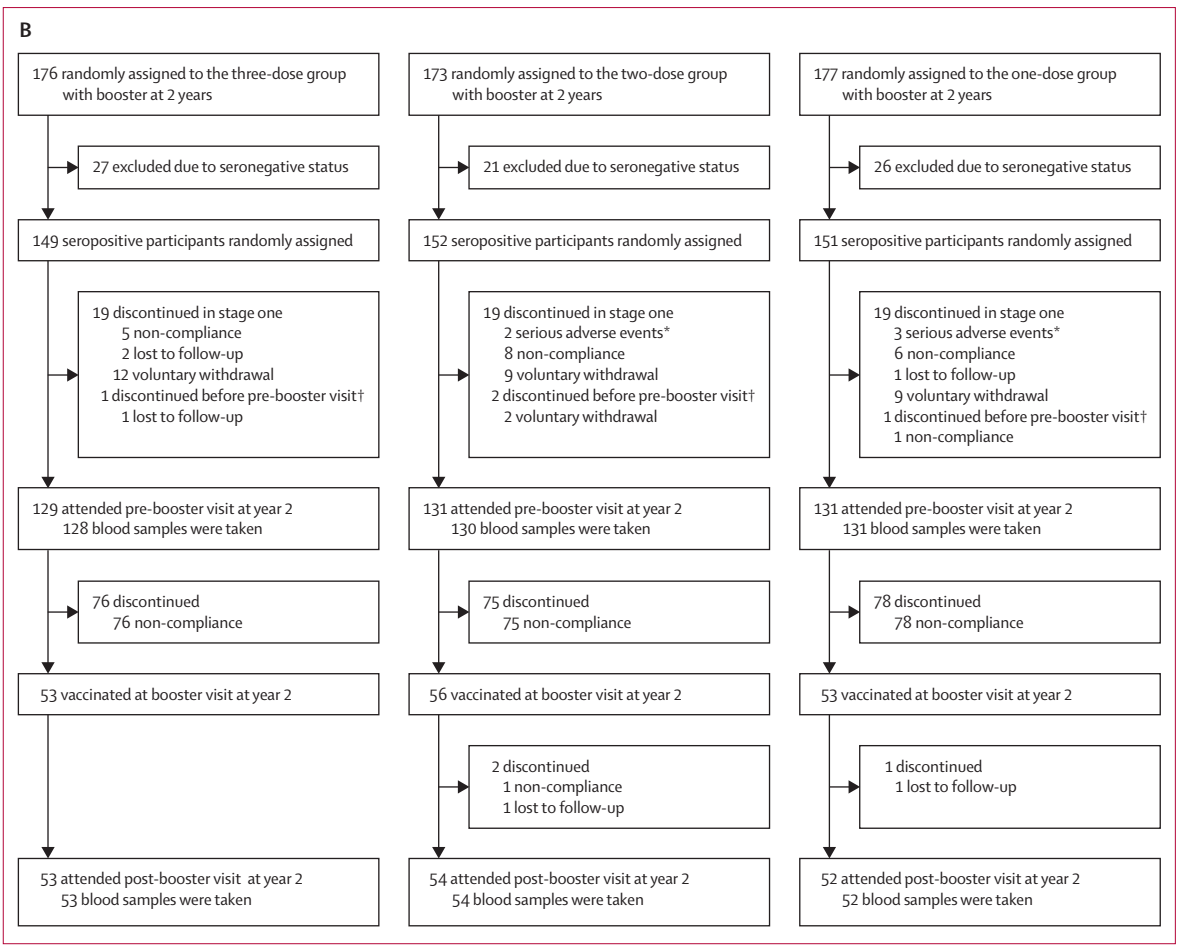


***Figure 1:*** **Trial profile**
Participants received a booster 1 year (A) and 2 years (B) after the last primary dose. *Serious adverse events were assessed and deemed not related to the study vaccination by the investigator or the sponsor. †Participant from Colombia discontinued before receipt of a booster.

| | Three-dose group after 1-year booster (n=53) | Two-dose group after 1-year booster (n=58) | One-dose group after 1-year booster (n=60) | Three-dose group after 2-year booster (n=48) | Two-dose group after 2-year booster (n=50) | One-dose group after 2-year booster (n=49) |
|---|---|---|---|---|---|---|
| Sex | | | | | | |
| Male | 28 (53%) | 28 (48%) | 25 (42%) | 17 (35%) | 20 (40%) | 20 (41%) |
| Female | 25 (47%) | 30 (52%) | 35 (58%) | 31 (65%) | 30 (60%) | 29 (59%) |
| Age, years | 33·4 (13·6) | 33·8 (14·2) | 34·0 (13·6) | 34·7 (12·9) | 31·8 (14·9) | 33·9 (14·0) |

Data are n (%) or mean (SD).

***Table 1:*** **Baseline demographics in participants who were seropositive at baseline (per-protocol analysis set)**

corrected 95% CI was 0·573; table 3). Therefore, overall non-inferiority of the 2-year booster was also not achieved.

There was no booster effect 1 year after the one-dose primary schedule compared with that observed 28 days after completion of the single primary dose for serotypes 1, 2, and 4 (appendix 2 p 2). For serotype 3, the booster injection tended to restore GMTs towards similar concentrations observed 28 days after the single primary dose. No booster effect was shown 2 years after the one-dose primary regimen (one-dose group after third primary dose *vs* one-dose group after 2-year booster) compared with that observed 28 days after completion of the single primary dose for all serotypes.

See Online for appendix 2

The antibody GMTs against each dengue serotype during the study in participants who were seropositive at baseline and received two or three doses of the vaccine are summarised in figures 2 and 3; antibody GMTs remained above those observed at baseline in the groups assessed. After administration of the 1-year and 2-year boosters, GMTs were boosted towards similar

concentrations to those observed after the last dose of the CYD-TDV primary regimen in most cases. GMTs 28 days after 1-year and 2-year booster in the one-dose group were similar to those recorded from three-dose and two-dose groups for each serotype, except for serotype 1, for which the post-booster GMT was numerically higher in the one-dose group than in the three-dose or two-dose groups.

Seroconversion rates 28 days after booster injection in participants who were dengue seropositive at baseline were null or low for each dengue serotype in all six subgroups, ranging from 0% for serotypes 1 and 2 in the three-dose group after 2-year booster to 13·0% for serotype 4 in the two-dose group after 2-year booster. However, seroconversion rates tended to be higher in the one-dose group after 1-year booster than in the three-dose group after 1-year booster or the two-dose group after 1-year booster. Rates also tended to be higher in the two-dose group after 2-year booster and one-dose group after 2-year booster than in the three-dose group after 2-year booster. These data are not shown.

Irrespective of the vaccine regimens used, an increase in the CYD-specific memory B cells was observed 1 year after the last primary vaccination dose. A clear difference between the seronegative and seropositive population was shown, with the seropositive population having higher concentrations of pre-vaccination CYD-specific memory B cells due to previous dengue infections. Seronegative participants presented a 5-fold increase from baseline (at the start of the study) to 1 year after the last study injection, compared with a lower 1·4-fold increase to pre-booster at 1 year or 2 years in seropositive participants. A 1·6-fold increase in the CYD-specific memory B-cell response was elicited 28 days after the 1-year or 2-year booster. Although no significant difference was observed in the concentration of CYD-specific memory B cells after one, two, or three doses of CYD-TDV, during the acute phase after the booster (after 1 year and 2 years), an expansion of plasmablasts, peaking at day 7 after booster, was observed in all vaccine regimen groups. Responses decreased back to baseline by day 14 after the booster. The primary vaccination induced an interferon-γ (IFN-γ) response and, to a lower extent, CD107a expression, driven by $CD8^+$ T cells against the backbone protein NS3 from yellow fever virus. Some differences in T-cell responses were evident between seronegative and seropositive participants similar to those seen with memory B-cell responses. However, in all seronegative and seropositive participants, IFN-γ, CD107a, CD154, interleukin-2, or C-C motif chemokine 4 were not expressed by $CD4^+$ T cells in response to either YF-17D NS3 or DEN NS3 peptide pools stimulation.

The adverse events reported after any injection during the primary regimen have been summarised previously.[12] The frequencies of solicited injection-site and systemic reactions after a booster at 1 year and 2 years are summarised by group in the appendix 2 (pp 3–4); the corresponding safety overviews by study group after CYD-TDV booster are also summarised in the appendix 2 (pp 5–8). Pain was the main local reaction reported in all groups after 1-year and 2-year booster doses, whereas headache, malaise, myalgia, and asthenia were the most frequently reported systemic reactions, reported at broadly similar rates across the groups (appendix 2 pp 3–4). Solicited reactions were reported least frequently in the three-dose group after 1-year or

| | Three-dose group after booster* | | Three-dose group after third primary dose† | | Paired ratio (three-dose group after booster*/three-dose group after third primary dose†) | |
|---|---|---|---|---|---|---|
| | N | GMT (95% CI) | N | GMT (95% CI) | N | GMR (95% CI) |
| **Booster dose at 1 year after the last primary dose** | | | | | | |
| Serotype 1 | 53 | 483 (281–832) | 53 | 853 (526–1384) | 53 | 0·567 (0·399–0·805) |
| Serotype 2 | 53 | 884 (602–1300) | 53 | 1186 (809–1738) | 53 | 0·746 (0·550–1·010) |
| Serotype 3 | 53 | 722 (458–1140) | 53 | 696 (483–1002) | 53 | 1·040 (0·686–1·570) |
| Serotype 4 | 53 | 383 (269–545) | 53 | 592 (400–876) | 53 | 0·647 (0·434–0·963) |
| **Booster dose at 2 years after the last primary dose** | | | | | | |
| Serotype 1 | 48 | 700 (401–1220) | 48 | 1017 (592–1746) | 48 | 0·688 (0·479–0·989) |
| Serotype 2 | 48 | 730 (497–1071) | 48 | 838 (554–1269) | 48 | 0·871 (0·673–1·130) |
| Serotype 3 | 48 | 559 (395–792) | 48 | 486 (333–708) | 48 | 1·150 (0·887–1·490) |
| Serotype 4 | 48 | 364 (260–510) | 48 | 556 (400–774) | 48 | 0·655 (0·471–0·911) |

The per-protocol analysis dataset included participants who were seropositive for dengue at baseline. For each serotype, non-inferiority was shown if the lower limit of the two-sided 95% CI for the GMR was higher than 0·5. Non-inferiority of the 1-year or 2-year booster was shown if all four serotypes achieved non-inferiority. GMT=geometric mean titre. GMR=geometric mean ratio. *28 days after CYD-TDV booster dose 1 year or 2 years after last vaccination. †28 days after third primary CYD-TDV dose.

***Table 2:*** **Dengue neutralising antibody titres elicited by the booster dose at 1 year and 2 years after the three-dose primary schedule versus the third CYD-TDV primary dose, for each serotype (per-protocol analysis dataset)**

| | Two-dose group after booster* | | Three-dose group after third primary dose† | | Paired ratio (two-dose group after booster*/ three-dose group after third primary dose†) |
|---|---|---|---|---|---|
| | N | GMT (95% CI) | N | GMT (95% CI) | GMR 95% CI |
| **Booster dose at 1 year after the last primary dose** | | | | | |
| Serotype 1 | 58 | 549 (331–911) | 112 | 875 (614–1248) | 0·627 (0·342–1·150) |
| Serotype 2 | 58 | 828 (569–1203) | 112 | 1023 (771–1356) | 0·809 (0·505–1·300) |
| Serotype 3 | 58 | 676 (436–1049) | 112 | 568 (433–745) | 1·190 (0·732–1·940) |
| Serotype 4 | 58 | 270 (200–364) | 112 | 540 (418–697) | 0·499 (0·331–0·754) |
| **Booster dose at 2 years after the last primary dose** | | | | | |
| Serotype 1 | 50 | 778 (429–1414) | 112 | 875 (614–1248) | 0·889 (0·462–1·710) |
| Serotype 2 | 50 | 692 (430–1116) | 112 | 1023 (771–1356) | 0·677 (0·402–1·140) |
| Serotype 3 | 50 | 517 (365–733) | 112 | 568 (433–745) | 0·911 (0·573–1·450) |
| Serotype 4 | 50 | 379 (261–551) | 112 | 540 (418–697) | 0·702 (0·447–1·100) |

The per-protocol analysis dataset included participants who were seropositive for dengue at baseline. For each serotype, non-inferiority was shown if the lower limit of the two-sided 95% CI for the GMR was higher than 0·5. Overall, non-inferiority was shown if all four serotypes achieved non-inferiority at both timepoints. GMT=geometric mean titre. GMR=geometric mean ratio. *28 days after CYD-TDV booster dose 1 year or 2 years after last vaccination. †28 days after third primary CYD-TDV dose.

***Table 3:*** **Dengue neutralising antibody titres elicited by the booster dose at 1 year and 2 years after the two-dose primary schedule versus the third CYD-TDV primary dose, for each serotype (per-protocol analysis set)**

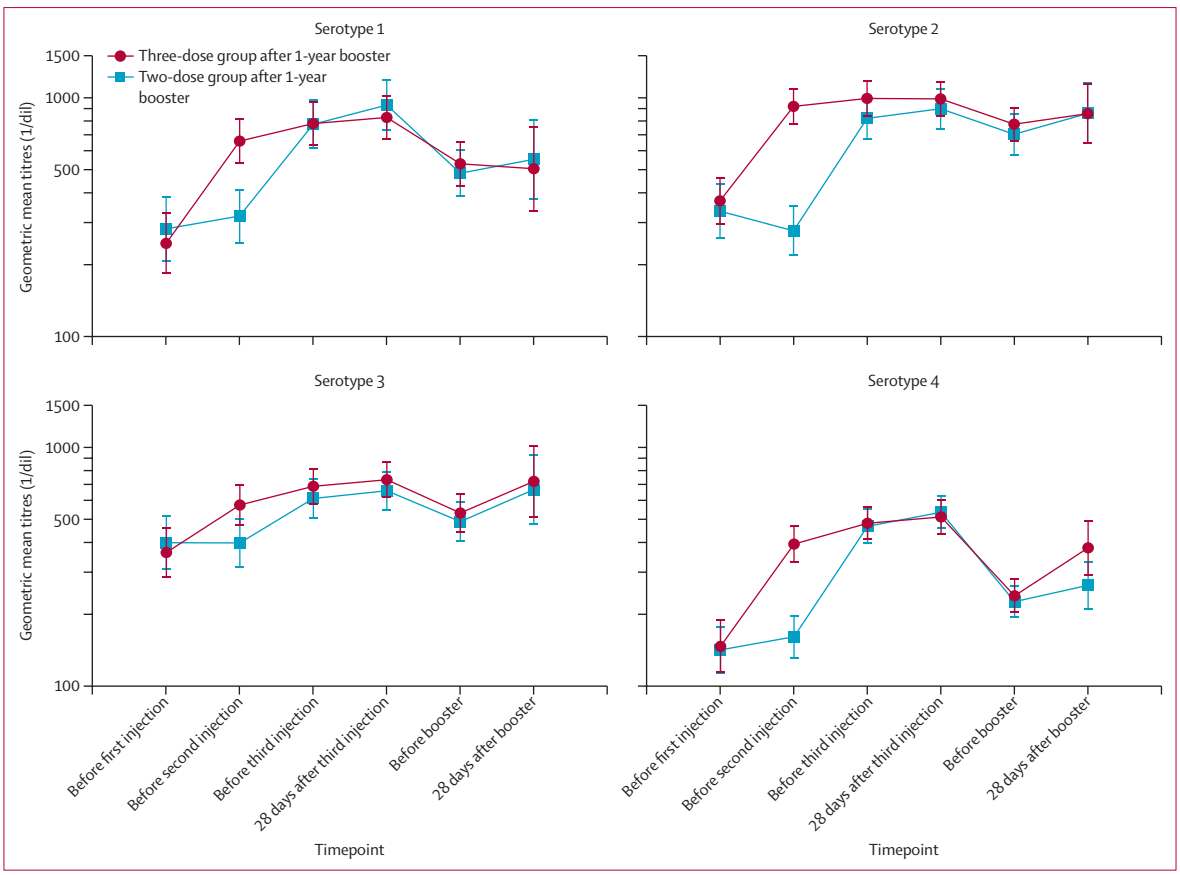


***Figure 2:*** **Dengue antibody titres at each timepoint, per serotype, in participants who were dengue seropositive at baseline who had received CYD-TDV booster 1 year after the last primary dose (full analysis dataset)**

Datapoints are geometric mean titres, with whiskers indicating 95% CIs. Data were obtained from trial participants in both Colombia and the Philippines for the primary vaccination series (from before first injection to 28 days after third injection). For the before booster analyses, data included participants due to receive a booster at 1 year or 2 years after primary vaccination from Colombia and those due to receive a booster at 1 year after primary vaccination from the Philippines. Only participants in Colombia received the booster injection at year 1.

2-year booster doses (appendix 2 pp 5–8). No immediate unsolicited adverse events or reactions were reported, and no adverse events leading to study discontinuation or serious adverse events of special interest were reported. One participant in the three-dose group after a booster dose at 2 years reported a serious adverse event (urinary tract infection) within 28 days after booster injection, but this event was considered not related to the study vaccination. One death (myocardial infarction) that was unrelated to the study vaccination was reported in the two-dose group after a booster dose at 1 year.

## Discussion

In this randomised, double-blind trial, the protocol-specified non-inferiority of the dengue serotype-specific neutralising antibody responses against all four dengue serotypes after the 1-year or 2-year booster was not shown relative to that observed after completion of the three-dose primary regimen in participants who were dengue seropositive at baseline, irrespective of study group. The 1-year booster did not achieve non-inferiority relative to that after completion of the three-dose primary regimen when administered after the two-dose or three-dose primary vaccination regimens, with non-inferiority only shown for serotypes 2 and 3 in both cases. Similar results were observed with the 2-year booster, with non-inferiority only shown for serotypes 2 and 3 when administered after the three-dose regimen and for serotype 3 only when administered after the two-dose regimen.

Therefore, our results suggest that CYD-TDV booster at 1 year or 2 years after a two-dose or three-dose primary vaccination regimen, despite boosting the immune response, does not elicit a consistent, meaningful booster effect in dengue-seropositive individuals for any of the serotypes except for serotype 3. The rationale for our analysis design was that antibody concentrations after dose three wane over time, and we wanted to assess the effect of a booster; antibody concentrations after a third primary dose of vaccine had been associated with efficacy in two independent phase 3 clinical trials.[18,19]

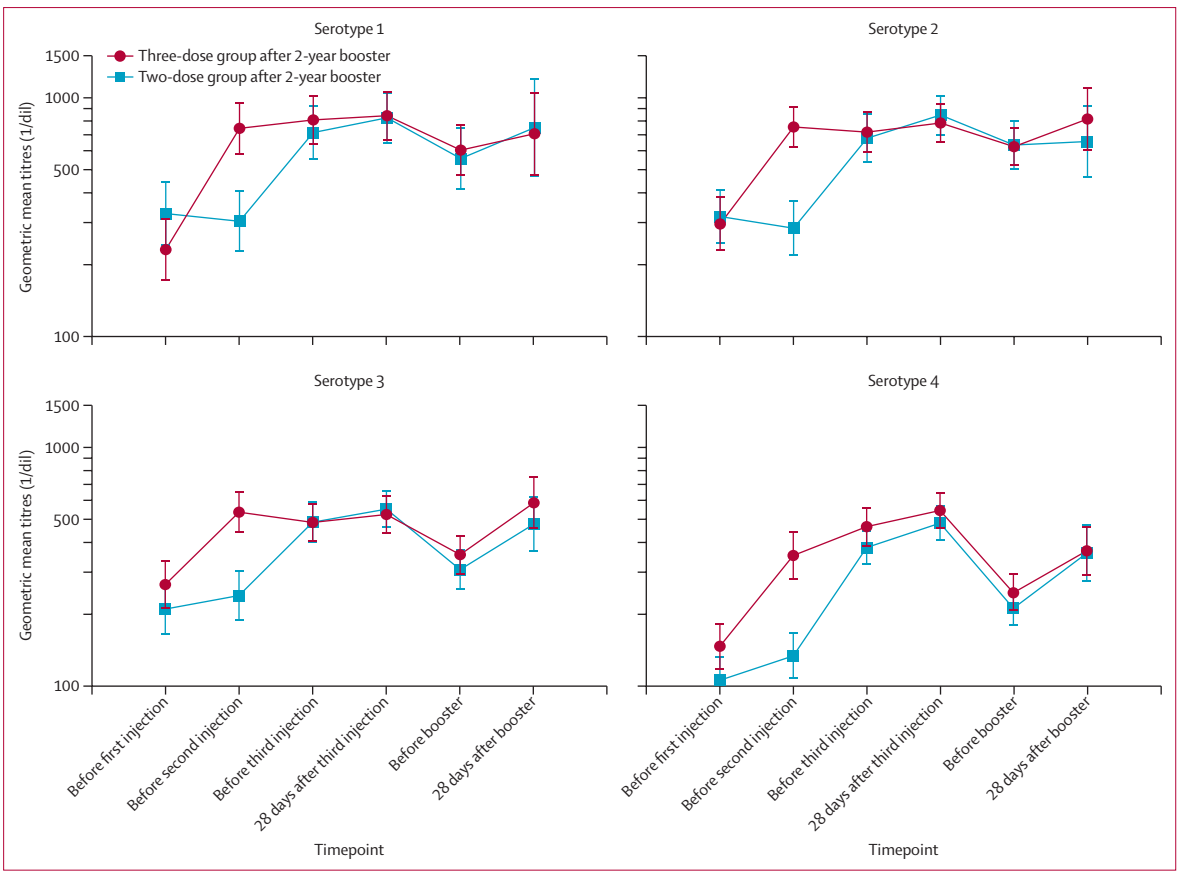


***Figure 3:*** **Dengue antibody titres at each timepoint, per serotype, in participants who were seropositive at baseline who had received CYD-TDV booster 2 years after the last primary dose (full analysis dataset)**

Datapoints are geometric mean titres, with whiskers indicating 95% CIs. Data were obtained from trial participants in both Colombia and the Philippines for the primary vaccination series (from before first injection to 28 days after third injection). For the before booster analyses, data included participants due to receive a booster at 2 years after primary vaccination from Colombia and the Philippines. Only participants from Colombia received the booster injection at year 2.

Previous studies that have assessed the CYD-TDV booster 4 years or more after completion of the three-dose primary regimen in Singapore and Latin America showed that the booster dose tended to restore neutralising antibody concentrations to concentrations shown after the third dose of the primary regimen, which then declined thereafter.[13–15] In our study, no marked difference between the 1-year and 2-year booster responses were observed when administered after a two-dose or three-dose primary regimen.

Dengue neutralising antibody concentrations after the two-dose and three-dose primary vaccination regimens persisted above concentrations observed at baseline throughout the 1-year and 2-year periods before booster dose administration in our study. The persistence of dengue neutralising antibody concentrations above baseline in dengue-seropositive participants over time has been reported previously.[20] A phase 1 study conducted in the Philippines, which assessed the persistence of antibodies in predominantly dengue-seropositive participants, showed that the antibody concentrations remained above baseline for up to 5 years after CYD-TDV administration; however, exposure to wild-type dengue was suggested to have contributed to antibody persistence.[21] Additionally, a 2020 study assessing dengue seroprevalence in healthy children and adults in Colombia between 2013 and 2015 showed that 211 (16%) of 1318 individuals had evidence of recent or current (within the last 90 days) dengue secondary infection.[22]

The absence of meaningful booster response might be explained by a number of reasons, including the reduced sample size (the effects of a booster dose were assessed only in participants who were seropositive at baseline and participants from the Philippines were excluded from receiving a booster) and previous exposure to wild-type dengue. The memory B-cell response measured before and shortly after the booster vaccination given 1 year after the primary immunisation regimen seems to support this theory, although the lower than planned number of participants available for assessment of cell-mediated immune responses limits the robustness of these observations. Nonetheless, in the seropositive population, a 1·4-fold increase in the percentage of CYD-specific antibody-secreting cells was

observed from baseline (before the primary schedules) to 1 year or 2 years later just before the booster (a 5-fold increase was observed in those who were seronegative 1 year later). A marked but moderate increase was subsequently measured 28 days after the booster. This small increase might be due to pre-existing CYD-specific memory B cells in this population. Similar findings were observed in two other studies in populations living in endemic areas,[13,14] with a booster dose given after a longer period of time, 5–6 years after primary vaccination.

The 1-year and 2-year CYD-TDV boosters were well tolerated after the two-dose or three-dose primary vaccination regimens, and no safety concerns were reported during the entire study in any of the groups assessed. Overall, fewer participants reported adverse events or adverse reactions after booster injections than after any injections in the primary vaccination regimen.

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Our study has some important limitations. The study could not be conducted as originally planned given that only those who were dengue seropositive at baseline were eligible to receive a booster injection and no participant from the Philippines received booster vaccination. Thus, only 40% of participants enrolled in the study received a booster dose, all from Colombia. This reduction in evaluable participants might have affected randomisation and reduced the power of the study to establish non-inferiority. Furthermore, natural exposure to circulating dengue serotypes, as well as other flaviviruses (eg, yellow fever virus and Zika virus), with the potential for antibody cross-reactivity interactions, might have affected the evolution of GMT concentrations over time and booster responses in our study. Colombia was affected by Zika outbreaks in 2016, resulting in high Zika seroprevalence when study participants were enrolled and received study injections,[23] and participants who received booster injections were exclusively from the Colombian cohort.

In conclusion, there was no marked difference in booster responses after primary vaccination with the two-dose or three-dose regimens in our study. Also, no marked difference was shown in the immune response after booster versus 28 days after the third dose of the three-dose primary vaccination regimen. However, with the caveat of the aforementioned limitations, our study did not show a booster effect against all four serotypes 1 year or 2 years after either primary vaccination regimen in dengue-seropositive individuals before vaccination (ie, the indicated population). Thus, CYD-TDV booster does not appear to provide additional benefit in participants who are seropositive at baseline after a two-dose or three-dose primary vaccination regimen.

#### Contributors

DLC-M, BZ, GD, ZC, MB, and FN contributed to the concept or design of the study. EL-M, MRC, AACB, MCM, IR, MLAG, JCR, and MAV were involved in data acquisition and DLC-M, JP, GD, ZC, HW, MB, and AR were involved in the analysis or interpretation of the data. ZC and HW accessed and verified all the data. All authors critically revised the manuscript and approved the final version for publication.

#### Declaration of interests

DLC-M, JP, BZ, GD, ZC, HW, MB, AR, JCR, MAV, and FN are Sanofi Pasteur employees and might hold shares or stock options, or both, in the company. EL-M, MRC, AACB, MCM, IR, and MLAG received funds from Sanofi Pasteur through their institutions to support their work in the CYD65 trial.

#### Data sharing

Qualified researchers can request access to patient-level data and related study documents, including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Patient-level data will be anonymised and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found online.

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