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Safety and immunogenicity of a tetravalent dengue vaccine in healthy children aged 2–11 years in Malaysia: A randomized, placebo-controlled, Phase III study☆,☆☆

Amar-Singh Hss[a], Mia-Tuang Koh[b], Kah Kee Tan[c], Lee Gaik Chan[d], Lynn Zhou[e], Alain Bouckenooghe[f], Denis Crevat[g], Yanee Hutagalung[f,*]

[a] Department of Pediatrics, Hospital Raja Permaisuri Bainun, Jalan Hospital, Ipoh, Perak, Malaysia
[b] Department of Pediatrics, University of Malaya Medical Centre, Jalan Universiti, 59100 Kuala Lumpur, Malaysia
[c] Department of Pediatrics, Hospital Tuanku Jaafar, Jalan Rasah, 70300 Seramban, Negeri Sembilan, Malaysia
[d] Department of Pediatrics, Sarawak General Hospital, Jln Tun Ahmad Zaidi Adruce, 93586 Kuching, Sarawak, Malaysia
[e] Biometry China, Sanofi Pasteur, Beijing, PR China
[f] Clinical R&D, Sanofi Pasteur, C/o Sanofi-Aventis Singapore Pte Ltd., 6 Raffles Quay #18-00, Singapore 048580, Singapore
[g] Clinical R&D, Sanofi Pasteur, Marcy l'Etoile 69280, France

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ABSTRACT

Background: Dengue disease is a major public health problem across the Asia-Pacific region for which there is no licensed vaccine or treatment. We evaluated the safety and immunogenicity of Phase III lots of a candidate vaccine (CYD-TDV) in children in Malaysia.
Methods: In this observer-blind, placebo-controlled, Phase III study, children aged 2–11 years were randomized (4:1) to receive CYD-TDV or placebo at 0, 6 and 12 months. Primary endpoints included assessment of reactogenicity following each dose, adverse events (AEs) and serious AEs (SAEs) reported throughout the study, and immunogenicity expressed as geometric mean titres (GMTs) and distribution of dengue virus (DENV) neutralizing antibody titres.
Results: 250 participants enrolled in the study (CYD-TDV: $n = 199$; placebo: $n = 51$). There was a trend for reactogenicity to be higher with CYD-TDV than with placebo post-dose 1 (75.4% versus 68.6%) and post-dose 2 (71.6% versus 62.0%) and slightly lower post-dose 3 (57.9% versus 64.0%). Unsolicited AEs declined in frequency with each subsequent dose and were similar overall between groups (CYD-TDV: 53.8%; placebo: 49.0%). Most AEs were of Grade 1 intensity and were transient. SAEs were reported by 5.5% and 11.8% of participants in the CYD-TDV and placebo groups, respectively. No deaths were reported. Baseline seropositivity against each of the four DENV serotypes was similar between groups, ranging from 24.0% (DENV-4) to 36.7% (DENV-3). In the CYD-TDV group, GMTs increased post-dose 2 for all serotypes compared with baseline, ranging from 4.8 (DENV-1) to 8.1-fold (DENV-3). GMTs further increased post-dose 3 for DENV-1 and DENV-2. Compared with baseline, individual titre increases ranged from 6.1-fold (DENV-1) to 7.96-fold (DENV-3).
Conclusions: This study demonstrated a satisfactory safety profile and a balanced humoral immune response against all four DENV serotypes for CYD-TDV administered via a three-dose regimen to children in Malaysia.

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1. Introduction

Dengue disease is caused by dengue virus (DENV) serotypes (1–4) and is transmitted mainly by *Aedes aegypti* mosquitoes [1]. Dengue disease is classified as with or without warning signs, or

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* Corresponding author. Tel.: +65 6225 3836/6431 2396; fax: +65 6431 2445.
E-mail addresses: amarhss@gmail.com (A.-S. Hss), kohmt@um.edu.my (M.-T. Koh), kktalk@gmail.com (K.K. Tan), dr_chanlg@hotmail.com (L.G. Chan), Linghua.Zhou@sanofipasteur.com (L. Zhou), Alain.Bouckenooghe@sanofipasteur.com (A. Bouckenooghe), Denis.Crevat@sanofipasteur.com (D. Crevat), Yanee.Hutagalung@sanofipasteur.com (Y. Hutagalung).

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severe dengue disease [2]. Infection with one DENV serotype usually imparts immunity to that serotype [3]. No licensed vaccine or treatment for dengue disease currently exists. Prevention relies on individual protection and vector control measures, which have limited effectiveness [1].

The Asia-Pacific region is considered the global epicentre of the disease, with ~1.8 billion people at risk [4]. Dengue disease is endemic in Malaysia [5–7] and its incidence has increased dramatically, from <20/100,000 in the 1970s [7] to >150/100,000 in 2010 [8], with an increased prevalence in adults relative to children (the main target population for potential dengue disease vaccines) and several changes in serotype distributions and increases in disease severity [7–13].

Increasing disease burden has spurred the development of potential dengue vaccines [14]. One live-attenuated tetravalent dengue vaccine (CYD-TDV, Sanofi Pasteur, Lyon, France), contains four recombinant viruses engineered with DENV1–4. Each CYD-TDV virus expresses dengue pre-membrane and envelope proteins of the associated serotype, and the non-structural and capsid proteins of the attenuated Yellow Fever (YF-17D) vaccine virus [15–19]. In Phase I and II studies, CYD-TDV elicited balanced neutralizing antibody responses against DENV1–4 and was well tolerated [20–28]. In a large Phase IIb study in Thai schoolchildren, CYD-TDV appeared to be efficacious against three of the four DENV serotypes and was well tolerated over 2 years of follow-up with no safety concerns [29].

The primary objectives of our study were to evaluate the safety and immunogenicity of CYD-TDV in children aged 2–11 years in Malaysia. This is the first Phase III CYD-TDV study to be reported that uses Phase III lots (manufactured by large-scale production processes) in an area where dengue disease is endemic. Additionally, one of the study sites was located in the Sarawak state of Malaysia, where Japanese Encephalitis (JE) is endemic and JE vaccination is routine.

2. Methods

2.1. Study design and participants

This multicentre, randomized, observer-blind, placebo-controlled, Phase III study was conducted between December 2010 and August 2012 at four sites in Malaysia: Kuala Lumpur, Ipoh (Perak state), Seremban (Negeri Sembilan state) and Kuching (Sarawak state). The methodology was similar to that of previous CYD-TDV Phase II studies [22,26] but used Phase III vaccine lots. Healthy children (aged 2–11 years) were assigned randomly to two groups (4 CYD-TDV: 1 placebo). All participants received three injections at 0, 6 and 12 months and were followed up for 6 months post-dose 3 to assess safety.

Participants were recruited by the study investigators, sub-investigators and research nurses. Randomization was performed by study-site personnel via an Interactive Voice Recognition System (IVRS), using a permuted block method with stratification by centre and age. A double randomization system separated treatment allocation from doses dispensed. The IVRS was used in a blind manner to ensure a balanced distribution of the number of participants with a history of JE vaccination, known JE infection or dengue disease infection in each age group (2–5 and 6–11 years) of the study groups, based on medical records or parents' recollection.

Girls of childbearing age were checked for pregnancy (urine test) and required to abstain from sexual intercourse or use contraception from four weeks before the first dose until four weeks after the last dose. Exclusion criteria included prior or current participation in another clinical study; receipt of blood or blood-derived products in the previous 3 months that could interfere with immunogenicity assessments; hypersensitivity to the vaccine components; and vaccination with any other vaccine (except for pandemic influenza) in the 4 weeks prior to enrolment. Contraindications to receiving subsequent vaccinations included significant allergic reaction (AR), serious adverse event (SAE) or ongoing adverse event (AE) related to the previous vaccination.

2.2. Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki (Seoul revision), with Good Clinical Practice (defined by the International Conference on Harmonisation), and with applicable national and local requirements. The Medical Research and Ethics Committee of the Ministry of Health of Malaysia approved the protocol. Each investigator obtained approval from an independent ethics committee or institutional review board. Participants' parents or legal representatives provided written informed consent and children aged ≥7 years signed an assent form. The trial is registered on ClinicalTrials.gov. (NCT01254422).

2.3. Study procedures

Blood samples were taken prior to vaccination, to assess flavivirus (FV) serostatus (dengue disease or JE) using plaque reduction neutralization tests ($PRNT_{50}$) [30,31], and 28 days post-dose 2 and 3 to assess immunogenicity.

2.4. Vaccine

CYD-TDV Phase III lots were manufactured using a large-scale production process. CYD-TDV vaccine details have been published [17–19]. CYD-TDV comprises a powder and solvent for suspension and must be stored at 2–8 °C. Each 0.5 ml dose of reconstituted vaccine contained approximately 5 ± 1 $\log_{10}$ cell-culture infectious dose 50% (CCID50) of each live, attenuated, recombinant CYD serotypes 1–4 (batch numbers: dose 1: S4316F02 and D0822; dose 2: S4316F08 and D1148; dose 3: S4316F14 and D1148). Placebo was 0.9% saline solution (batch numbers: dose 1: E5594F04; dose 2: E5594F08; dose 3: E5594F06). Vaccinations were by subcutaneous injection in the deltoid region of the upper arm using a 25G × 5/8 in. (0.5 mm × 16 mm) needle.

2.5. Primary endpoints

2.5.1. Safety and reactogenicity

Safety and reactogenicity data (graded in severity from 1 to 3) were collected after each dose: immediate AEs within 30 min; solicited injection-site reactions within 7 days; solicited systemic reactions within 14 days; and unsolicited AEs within 28 days of vaccination. SAEs and suspected dengue disease cases requiring hospitalization were monitored throughout the study. An independent data monitoring committee reviewed all SAEs.

2.5.2. Immunogenicity

Neutralizing antibody levels against DENV1–4 were assessed 28 days post-dose 2 and 3 using a $PRNT_{50}$ compliant with WHO guidelines [30,31] and expressed as geometric mean titres (GMTs) and seropositivity rates (percentage of participants with titres ≥10 1/dil). Distribution of individual titres at enrolment and post-dose 2 and 3 of CYD-TDV or placebo was analyzed using reverse cumulative distribution curves.

2.6. Secondary endpoints

CYD-TDV safety and immunogenicity assessments were stratified according to baseline FV-serostatus (seropositive or

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seronegative for dengue disease or JE) and by age (2–5 and 6–11 years). Pre-vaccination, post-dose 2 and post-dose 3 GMTs were also analyzed according to FV-serostatus at baseline (seropositive or seronegative for dengue disease and JE).

2.7. Statistical methods

2.7.1. Sample size and study populations

With a planned sample size of 250 participants (CYD-TDV: $n = 200$; placebo: $n = 50$) and assuming a drop-out rate of 15%, the probability of observing a common AE after three vaccinations was 100% and 82% if the incidence of the AE was 5% and 1%, respectively. Of the 200 CYD-TDV recipients, 60–140 were expected to be FV-seropositive at baseline. Based on a variability of 0.7 $\log_{10}$ for the $PRNT_{50}$ assay, the power to detect differences of ≥ 0.4 in $\log_{10}$ GMTs between FV-seropositive and -seronegative participants and between age groups was >90%. The populations analyzed included a Safety Analysis Set (SAS, participants receiving at least one dose of study vaccine) and a Full Analysis Set (FAS, participants receiving at least one dose of study vaccine, with a valid post-vaccination serology result). Further analyses were conducted stratified by FV-serostatus and by age group.

2.7.2. Statistical analyses

Analyses were descriptive with no hypothesis tested. For the main parameters, 95% confidence intervals of point estimates were calculated using a normal approximation for quantitative data and exact binomial distribution (Clopper–Pearson method) for proportions [32,33]. Analyses were conducted with SAS software, version 9.1 or above (SAS Institute, Cary, NC, USA).

3. Results

The first participant was enrolled on 2 December 2010 and the last participant's 6 month follow-up visit was 14 August 2012. We enrolled 250 participants in the study (CYD-TDV: $n = 199$; placebo: $n = 51$), of whom 196 (98.5%) and 50 (98%) in the CYD-TDV and placebo group, respectively, completed the vaccination phase and were included in the FAS ($n = 246$, Fig. 1).

In the SAS ($n = 250$), 55.8% of participants in the CYD-TDV group and 60.8% in the placebo group were FV-seropositive (seropositive for DENV and/or JE). In the CYD-TDV and placebo groups, 49.7% and 52.0% of participants were aged 2–5 years, respectively. In the CYD-TDV group, 57.3% of children aged 2–5 years were FV-seropositive and 42.7% were FV-seronegative and in children aged 6–11 years, 54.0% were FV-seropositive and 46.0% were FV-seronegative.

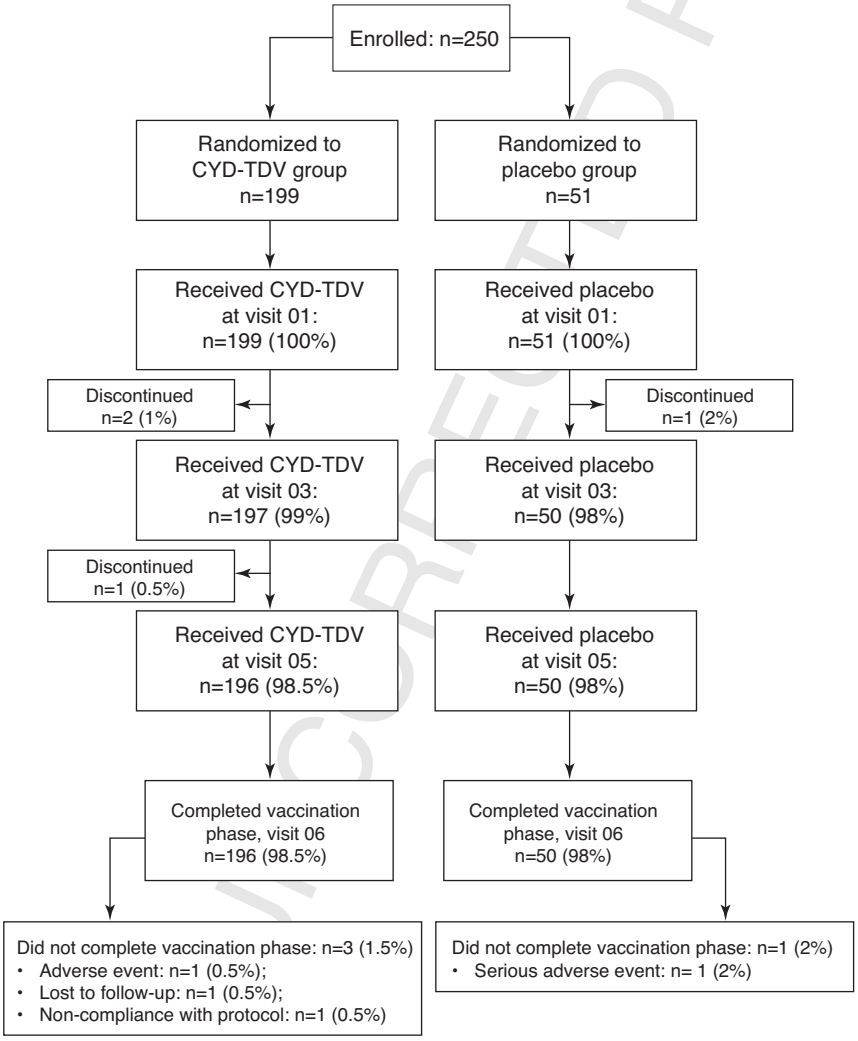

Fig. 1. Study flow chart: progress of participants through the study. Reasons for withdrawal: In the CYD-TDV group, one participant withdrew due to an AE (allergic conjunctivitis), one was lost to follow-up after the first vaccination, and one did not comply with the protocol by refusal of a blood sample before injection 3. In the placebo group, one participant experienced a SAE (right VII nerve paralysis) after the first vaccination, which was assessed by the investigator to be related to treatment.

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Table 1
Demographic and baseline characteristics of participants: Safety Analysis Set (all participants and categorized according to flavivirus serostatus at baseline and stratified by age [2–5 and 6–11 years]).

	CYD-TDV					Placebo
	All	FV-seropositive at baseline	FV-seronegative at baseline	Age 2–5 years	Age 6–11 years	
N	199	111	88	99	100	51
Sex, *n* (%)						
Male	96 (48.2)	51 (45.9)	45 (51.1)	51 (51.5)	45 (45.0)	32 (62.7)
Female	103 (51.8)	60 (54.1)	43 (48.9)	48 (48.5)	55 (55.0)	19 (37.3)
Age (years)						
Mean (±SD)	6.4 (2.8)	6.5 (2.9)	6.2 (2.7)	4.0 (1.2)	8.7 (1.7)	6.5 (3.0)
Range	2.0–11.9	2.1–11.9	2.0–11.6	2.0–5.9	6.0–11.9	2.1–11.8
Height (cm)						
Mean (±SD)	113.7 (18.1)	114.4 (19.1)	112.8 (16.9)	99.5 (10.6)	127.8 (11.9)	115.7 (19.4)
Range	78.0–157.0	78.0–153.0	81.0–157.0	78.0–121.0	104.0–157.0	84.0–158.0
Weight (kg)						
Mean (±SD)	21.9 (10.6)	22.9 (11.5)	20.7 (9.2)	15.6 (4.7)	28.2 (11.0)	22.7 (10.8)
Range	8.9–77.0	9.0–77.0	8.9–61.5	8.9–37.6	15.4–77.0	11.0–54.9
Body mass index (kg/m^2)						
Mean (±SD)	16.2 (3.6)	16.6 (3.6)	15.7 (3.5)	15.6 (2.9)	16.8 (4.0)	16.2 (3.8)
Range	11.7–34.8	12.1–34.7	11.7–34.8	11.7–34.8	12.1–34.7	12.0–32.5

FV, flavivirus; *n*, number of participants; SD, standard deviation.

Baseline demographic characteristics were similar between groups and in the FV-serostatus subsets (Table 1). However, baseline GMTs against DENV1–4 were slightly higher in those aged 6–11 years than in those aged 2–5 years (Table 2). The majority of participants were of Asian origin ($n = 248$) and two participants were of Asian/Caucasian origin.

3.1. Safety and reactogenicity

The proportion of participants reporting solicited reactions (total and injection site and systemic reactions) was similar in the CYD-TDV (89.4%) and placebo (94.1%) groups (Table 3). However, there was a trend for slightly higher reactogenicity with CYD-TDV compared with placebo post-dose 1 and 2 (75.4% versus 68.6% and 71.6% versus 62.0%, respectively) and slightly lower reactogenicity in the CYD-TDV group compared with placebo post-dose 3 (57.9% versus 64.0%, Fig. 2).

The number of solicited systemic reactions decreased after each injection in both groups. Solicited injection site reactions in the CYD-TDV group tended to be more frequent post-dose 2 compared with post-dose 1 and less frequent post-dose 3. In the placebo group, solicited injection site reactions were more frequent post-dose 2 and 3 than post-dose 1 (Fig. 2).

Most solicited injection site reactions and systemic reactions were Grade 1 and lasted <3 days. The most frequently reported injection site reactions were pain (CYD-TDV: 69.3%; placebo: 56.9%), erythema (46.7% and 49.0%) and swelling (38.7% and 35.3%). In both study groups, malaise (CYD-TDV: 54.3%; placebo: 41.2%) and headache (52.3% and 39.2%) were the most frequently reported solicited systemic reactions, followed by asthenia, myalgia and fever. Fever was the most commonly reported Grade 3 reaction in both groups (CYD-TDV: 6.6%; placebo: 3.9%), and approximately half of these reported concomitantly with intercurrent infections (Supplementary table S1).

Table 2
Geometric mean titres (GMTs) for dengue serotype-specific plaque reduction neutralization tests ($PRNT_{50}$) antibodies before vaccination and 28 days after two and three injections of CYD-TDV or placebo: Full Analysis Set for all participants and stratified according to flavivirus serostatus at baseline and age (2–5 years and 6–11 years).

	CYD-TDV group GMT 1/dil (95% CI)					Placebo group GMT 1/dil (95% CI)
	All ($n = 196$)	FV-seropositive at baseline ($n = 109$)	FV-seronegative at baseline ($n = 87$)	Age 2–5 years ($n = 96$)	Age 6–11 years ($n = 100$)	All ($n = 50$)
DENV-1						
Pre-vacc	15.3 (11.5; 20.4)	37.5 (24.0; 58.6)	5.0 (NC)	10.4 (7.51; 14.5)	22.2 (14.1; 34.8)	18.6 (9.69; 35.8)
Post-dose 2	119 (90.7; 155)	248 (171; 361)	47.1 (35.3; 62.9)	95.9 (69.0; 133)	146 (95.4; 222)	21.0 (10.6; 41.9)
Post-dose 3	151 (121; 188)	247 (178; 343)	81.6 (66.3; 101)	117 (91.2; 151)	192 (136; 272)	18.9 (9.94; 35.8)
DENV-2						
Pre-vacc	15.9 (11.8; 21.3)	39.9 (25.2; 63.1)	5.0 (NC)	11.1 (7.79; 15.7)	22.5 (14.2; 35.6)	18.6 (10.0; 34.5)
Post-dose 2	160 (127; 203)	306 (221; 424)	71.4 (55.4; 92.1)	138 (106; 181)	185 (126; 272)	17.9 (9.91; 32.2)
Post-dose 3	180 (146; 221)	292 (217; 395)	97.5 (77.5; 123)	158 (125; 201)	203 (144; 285)	16.3 (9.59; 27.7)
DENV-3						
Pre-vacc	15.6 (12.3; 19.9)	38.8 (27.3; 55.4)	5.0 (NC)	12.1 (8.97; 16.3)	20.0 (13.7; 29.2)	15.9 (9.57; 26.5)
Post-dose 2	196 (163; 235)	296 (230; 381)	116 (92.6; 146)	167 (134; 207)	228 (170; 306)	15.9 (9.27; 27.4)
Post-dose 3	193 (161; 231)	287 (219; 376)	117 (97.4; 141)	168 (136; 208)	220 (164; 295)	16.3 (9.81; 27.0)
DENV-4						
Pre-vacc	9.92 (8.17; 12.0)	17.1 (12.5; 23.5)	5.0 (NC)	8.28 (6.44: 10.6)	11.8 (8.79: 15.8)	12.3 (7.96; 19.0)
Post-dose 2	110 (88.9; 136)	152 (115; 201)	73.3 (53.7; 100)	101 (75.8: 134)	119 (87.0: 164)	13.3 (7.94; 22.1)
Post-dose 3	114 (97.0; 134)	155 (125; 191)	78.0 (62.0; 98.1)	105 (86.0: 129)	123 (95.7: 159)	10.9 (7.34; 16.2)

CI, confidence interval; FV, flavivirus; GMT, geometric mean titre; n, number of participants; NC, not calculated.

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Table 3
Overview of safety data up to 28 days after any injection: Safety Analysis Set.

Participants experiencing at least one of:	CYD-TDV group ($n = 199$)			Placebo group ($n = 51$)		
	n	%	95% CI	*n*	%	95% CI
Solicited reaction	178/199	89.4	(84.3; 93.3)	48/51	94.1	(83.8; 98.8)
Solicited injection site reaction	163/199	81.9	(75.8; 87.0)	40/51	78.4	(64.7; 88.7)
Solicited systemic reaction	141/199	70.9	(64.0; 77.1)	37/51	72.5	(58.3; 84.1)
Unsolicited AE	107/199	53.8	(46.6; 60.8)	25/51	49.0	(34.8; 63.4)
Unsolicited non-serious AE	105/199	52.8	(45.6; 59.9)	20/51	39.2	(25.8; 53.9)
Unsolicited AR	22/199	11.1	(7.1; 16.3)	5/51	9.8	(3.3; 21.4)
Unsolicited non-serious AR	22/199	11.1	(7.1; 16.3)	4/51	7.8	(2.2; 18.9)
AE leading to study discontinuation[a]	1/199	0.5	(0.0; 2.8)	1/51	2.0	(0.0; 10.4)
SAE[b]	11/199	5.5	(2.8; 9.7)	6/51	11.8	(4.4; 23.9)

AE, adverse event; AR, allergic reaction; CI, confidence interval; n, number of participants; SAE, serious adverse event.
[a] Identified in the termination form as SAE or other AE.
[b] Includes SAEs collected up to after the third injection analysis.

The proportion of participants experiencing unsolicited AEs was similar between groups overall (CYD-TDV: 53.8%; placebo: 49.0%) and post-dose 1 and 2 (30.7% and 27.5%; and 26.9% and 24.0%, respectively, Table 3 and Fig. 2), and slightly higher with CYD-TDV than with placebo post-dose 3 (CYD-TDV: 22.4%; placebo: 14.0%). Infections and infestations were most frequently reported, with upper respiratory tract infections predominant (CYD-TDV: 13.8%; placebo: 9.8%). The proportion of participants experiencing unsolicited ARs was similar between groups (CYD-TDV: 11.1%; placebo: 9.8%). Injection site induration was the most frequent AR. The proportions of both unsolicited AEs (Fig. 2) and unsolicited ARs were generally lower after each injection for both groups (unsolicited ARs: CYD-TDV: 7.5%, 2%, 2.6%; placebo: 7.8%, 2%, 0%). Five participants experienced Grade 1 rash episodes post-dose 1 (CYD-TDV: $n = 3$; placebo: $n = 1$), or post-dose 2 (CYD-TDV: $n = 1$) lasting ≤ 6 days.

Most unsolicited AEs and ARs were non-serious and did not lead to study discontinuation (Table 3). SAEs were reported by 5.5% of participants in the CYD-TDV group and 11.8% in the placebo group (Table 3). All SAEs were assessed as unrelated to study vaccine, except for an SAE of VII nerve paralysis in the placebo group. This participant did not receive any further vaccinations and recovered 4 months later. No deaths were reported.

3.1.1. *Safety and reactogenicity by baseline FV-serostatus and age*

An analysis by subset showed that safety and reactogenicity data were not markedly affected by FV-serostatus or age (Fig. 2). The decrease in the incidence of systemic reactions post-dose 3 appeared more marked in the FV-seropositive group than the FV-seronegative group.

3.2. *Immunogenicity*

Baseline seropositivity against DENV1–4 was similar in both treatment groups (CYD-TDV: 31.1%, 27.6%, 36.7% and 24.0%; placebo: 32.0%, 30.0%, 36.7% and 30.0%, for DENV1–4, respectively). A large proportion of participants were seropositive at baseline for at least one DENV serotype (CYD-TDV: 44.9%; placebo: 48%). A small proportion of participants were seropositive at baseline for JE only (CYD-TDV: 10.7%; placebo: 13.7%). In the CYD-TDV group, pre-vaccination GMTs against DENV1–4 ranged from 9.92 1/dil (DENV-4) to 15.9 1/dil (DENV-2). Baseline seropositivity rates against DENV1–4 differed between age groups in both treatment groups, being higher in children aged 6–11 years compared with those aged 2–5 years (Supplementary table S2). Similarly in the CYD-TDV group, GMTs were higher in children aged 6–11 years compared with children aged 2–5 years (Table 2). When stratified by FV serostatus, GMTs were higher in those aged 6–11 years than in those aged 2–5 years in participants who were FV-seropositive at baseline (Supplementary table S3).

Robust immune responses were reported after vaccination with CYD-TDV regardless of FV-serostatus (Table 2). The geometric mean fold rise of individual antibody titres ranged from 4.8-fold (DENV-1) to 8.1-fold (DENV-3) post-dose 2 and from 6.1-fold (DENV-1) to 8.0-fold (DENV-3) post-dose 3. Post-dose 2 and 3 GMTs were higher in the FV-seropositive group. Post-dose 3 GMTs showed a greater increase in the FV-seronegative group than in the FV-seropositive group. Pre-vaccination and post-dose 3 GMTs were higher in participants who were seropositive for dengue disease at baseline than in participants who were seronegative, regardless of JE serostatus. Post-dose 3 GMTs were slightly higher in JE seropositive participants versus JE seronegative participants. However, this difference was not significant. The highest post-dose 3 GMTs were observed in participants who were seropositive for both dengue disease and JE at baseline. Robust immune responses were also reported regardless of age, although post-dose 2 and 3 GMTs were higher in 6–11 year-olds compared with 2–5 year-olds (Table 2).

The post dose 2 and 3 antibody reverse cumulative distribution curves demonstrated good similarity of responses (curves close together and parallel, Fig. 3).In FV-seronegative children, the dose 3 vaccination increased the overall neutralizing titres with a left shift of the curves for serotypes 1 and 2.

4. Discussion

CYD-TDV is in advanced stages of clinical development and large Phase III efficacy studies are underway in Latin America (NCT01374516) and Asia (NCT01373281); no other dengue disease candidate vaccine has reached this stage of development to date. As the first paediatric trial to use Phase III CYD-TDV lots manufactured using large-scale processes, the present study is a key component of the global clinical trial programme for CYD-TDV [17–19]. Results showed that CYD-TDV had a satisfactory safety profile and generated a balanced humoral immune response against DENV1–4 in this paediatric population.

All four DENV serotypes circulate in Malaysia and their relative geographic distributions vary over time [11]. Baseline seropositivity against DENV1–4 was similar in both groups, which was expected, given that dengue disease is endemic in Malaysia.

The safety profile of CYD-TDV was satisfactory for the total population and in the subsets analyzed. Reactogenicity decreased with subsequent doses of CYD-TDV, consistent with observations from previous phase I and II studies [11]. Injection site erythema and swelling were reported frequently, but at Grade 1 intensity and at similar rates in both treatment groups. Reactogenicity was similar in the subsets of FV-serostatus and age ranges and in the whole

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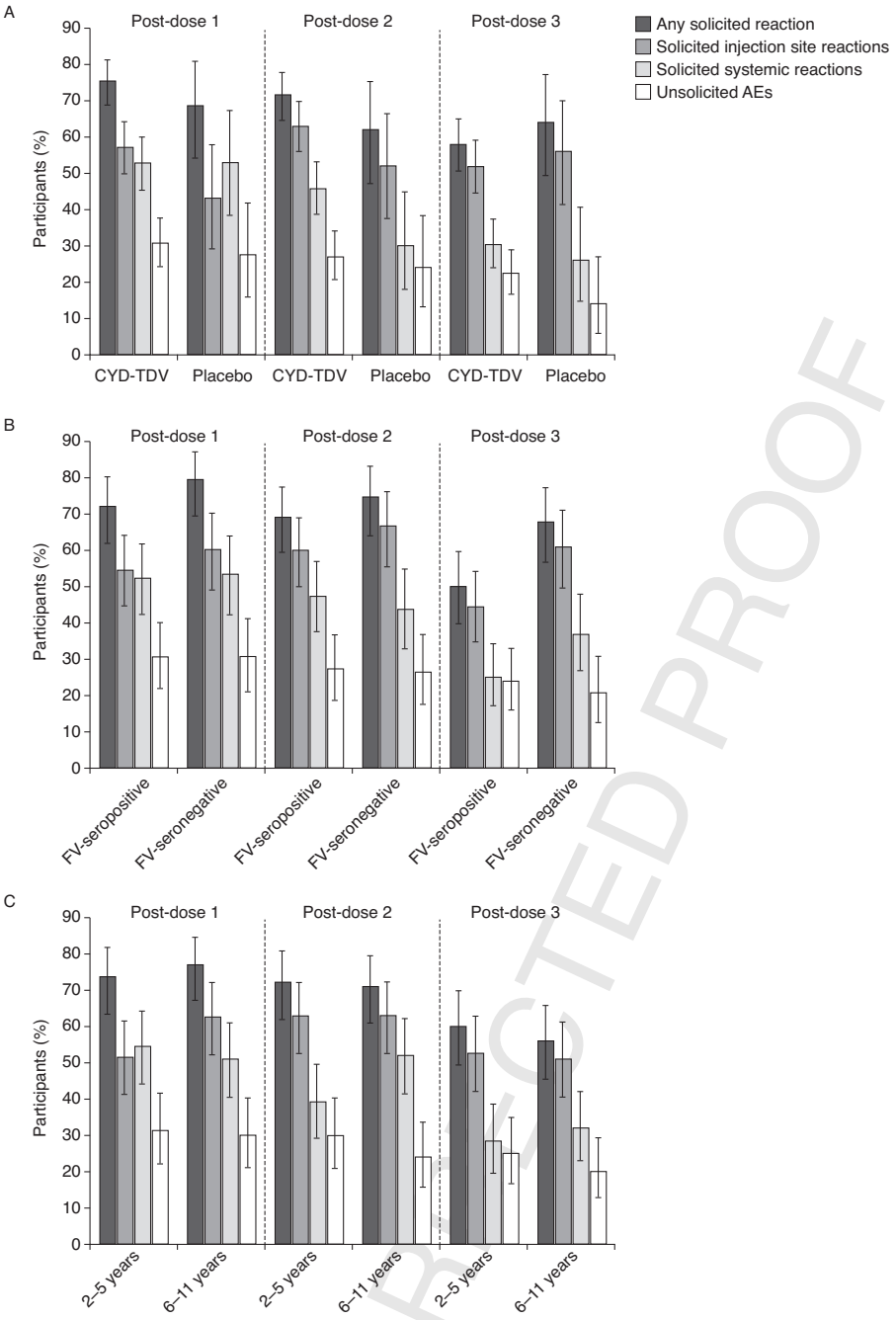

Fig. 2. Proportion of participants with different categories of adverse events and adverse reactions after each vaccination: Safety Analysis Set for (A) all participants and (B) in the CYD-TDV Group by FV-serostatus at baseline and (C) in children stratified by age (2–5 and 6–11 years). AE, adverse event; FV, flavivirus.

population, without increased reactogenicity in 2–5 year-olds, or baseline FV-seropositive children compared with 6–11-year-olds or FV-seronegative children. The favourable safety and reactogenicity results observed are similar to those from previous Phase I and II studies [20–29]. Our data from Malaysia are consistent with those reported from 2 to 11-year olds in Peru [22] and Singapore [26], from 4 to 11-year olds in Thailand [29] and from 9 to 16-year olds in Latin America [28].

The three-dose regimen of CYD-TDV elicited a good immune response in terms of GMTs, regardless of FV-serostatus or age group. GMTs increased both post-dose 2 and 3 in the overall population. The robust and balanced antibody responses against DENV1–4 were similar to those reported in other studies of CYD-TDV in children [22,26,28]. However, the results of the Phase IIb proof-of-concept efficacy study by Sabchereon et al. challenged the hypothesis that such a robust, balanced antibody response profile, as assessed by $PRNT_{50}$, translates to similar levels of protection against all viruses of each DENV serotype [29]. The higher GMTs reported after vaccination in the FV-seropositive group compared with the FV-seronegative group were also observed in children vaccinated in Latin America [28]. The third dose had little impact in the FV-seropositive group but was more marked in the FV-seronegative group. Therefore, in the context of a mixed population with both FV-seropositive and FV-seronegative children, a three-dose regimen is beneficial to induce a balanced immune response. Baseline serostatus for dengue disease but not for JE had an impact on post-dose 3 GMTs. However, it should be noted that the sample size of this subset was limited-only 67 participants in the CYD-TDV

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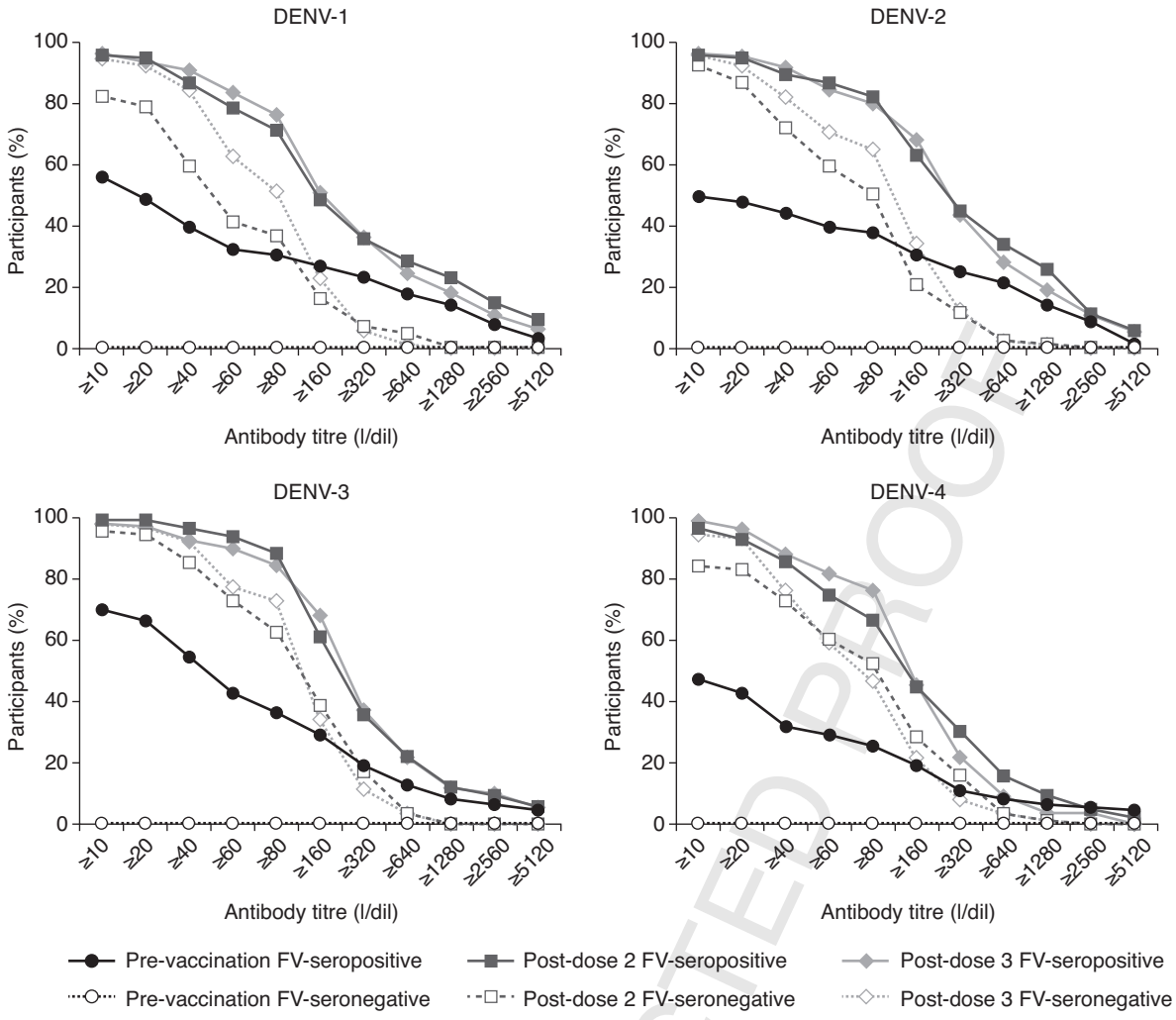

Fig. 3. Reverse cumulative distribution of serotype-specific $PRNT_{50}$ antibody titres curves for DENV serotypes 1–4 by baseline FV-serostatus, pre-vaccination and after two and three doses of CYD-TDV (Full Analysis Set). DENV, dengue virus; FV, flavivirus; PRNT, plaque reduction neutralization test.

group and 17 participants in the placebo group were seropositive for JE antibodies ($PRNT_{50}$ titre $\geq$ 10 l/dil). The immune response in the current study was satisfactory in both age groups, although higher GMTs were reported in those aged 6–11-years olds than 2–5-year olds post-dose 2 and 3. This observation was probably the result of the difference in baseline DENV serostatus between the two age groups (difference in pre-vaccination GMTs for DENV1–4) as the main driver for vaccine immunogenicity. The link between baseline FV-serostatus and age range on the immune response is presumed to be the result of a longer period of exposure to natural dengue infection in the 6–11 years group compared with the younger cohort.

Although the highest attack rate for dengue infection is commonly observed in young adults aged 20–24 years old [34], the large proportion of children who were seropositive at baseline for at least one DENV serotype indicates that dengue disease is endemic and infects children in Malaysia at a young age. However, no suspected cases of dengue disease requiring hospitalization were reported by the investigators or participants' parents in this study and antibody titres in the placebo group did not increase, suggesting the absence or very limited circulation of wild-type DENV strains in this population during the study period. Therefore, the results of vaccine immunogenicity were probably not biased by natural dengue infections. However, the placebo group was small and conclusions based on these results need to be kept in context.

This study was not designed to assess CYD-TDV efficacy and did not address long-term vaccine safety or immune persistence, with follow-up limited to 6 months. However, large Phase III efficacy studies are underway and long-term follow-up is ongoing in these studies, as well as in other Phase I [20] and Phase II studies [29]. Furthermore, the sample size of this study was too small to accurately analyze any potential priming effect of previous exposure to individual FV subtypes (e.g. JE).

5. Conclusion

In conclusion, this Phase III study demonstrated a satisfactory safety profile and a balanced humoral immune response against DENV1–4 for CYD-TDV administered via a three-dose regimen to children in Malaysia living in an area where dengue disease is endemic.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.10.013.

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