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Vaccine, 2015; 33(39):5127-5134

Originally published at:
http://doi.org/10.1016/j.vaccine.2015.08.008

Vaccine 33 (2015) 5127–5134

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Lot-to-lot consistency of a tetravalent dengue vaccine in healthy adults in Australia: A randomised study

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ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 23 July 2015
Accepted 3 August 2015
Available online 13 August 2015

Trial registration:
ClinicalTrials.gov: NCT01134263

Keywords:
Flavivirus
Dengue
Vaccine
Immunogenicity

ABSTRACT

Background: The recombinant yellow fever-17D-dengue virus, live, attenuated, tetravalent dengue vaccine (CYD-TDV) has undergone extensive clinical trials. Here safety and consistency of immunogenicity of phase III manufacturing lots of CYD-TDV were evaluated and compared with a phase II lot and placebo in a dengue-naïve population.
Methods: Healthy 18–60 year-olds were randomly assigned in a 3:3:3:3:1 ratio to receive three subcutaneous doses of either CYD-TDV from any one of three phase III lots or a phase II lot, or placebo, respectively in a 0, 6, 12 month dosing schedule. Neutralising antibody geometric mean titres ($PRNT_{50}$ GMTs) for each of the four dengue serotypes were compared in sera collected 28 days after the third vaccination—equivalence among lots was demonstrated if the lower and upper limits of the two-sided 95% CIs of the GMT ratio were $\geq$0.5 and $\leq$2.0, respectively.
Results: 712 participants received vaccine or placebo and 614 (86%) completed the study; 17 (2.4%) participants withdrew after adverse events. Equivalence of phase III lots was demonstrated for 11 of 12 pairwise comparisons. One of three comparisons for serotype 2 was not statistically equivalent. GMTs for serotype 2 in phase III lots were close to each other (65.9, 44.1 and 58.1, respectively).
Conclusions: Phase III lots can be produced in a consistent manner with predictable immune response and acceptable safety profile similar to previously characterised phase II lots. The phase III lots may be considered as not clinically different as statistical equivalence was shown for serotypes 1, 3 and 4 across the phase III lots. For serotype 2, although equivalence was not shown between two lots, the GMTs observed in the phase III lots were consistently higher than those for the phase II lot. As such, in our view, biological equivalence for all serotypes was demonstrated.

1. Introduction

Although dengue is an endemic mosquito-borne viral disease in tropical and sub-tropical regions of the world [1], current globalisation trends with increased intercontinental air travel and seaborne trade have, in part, contributed to dengue becoming a major global

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http://dx.doi.org/10.1016/j.vaccine.2015.08.008

public health threat in recent decades [2]. Globally, in 2010, there were an estimated 96 million apparent dengue infections and a further 294 million subclinical infections [3]. Current dengue control measures rely on targeting the mosquito vectors, but many dengue endemic countries do not have routine preventative vector control measures in place. These are usually implemented during outbreaks (high transmission periods) and with methods of questionable effectiveness in reducing vector population density [4,5]. In addition, vector control measures, such as larval source reduction, are not sustainable indefinitely with limited resources usually reallocated to other competing needs once some sort of vector and disease control has been achieved, allowing vector populations to rebounded to levels sufficient for epidemic transmission [6]. These failures have, in part, contributed to the increasing occurrence of dengue epidemics and expansion of the geographical range of transmission. Although vaccination would likely represent an effective strategy for the management of dengue disease in endemic regions, there is currently no licensed vaccine to prevent dengue infection and there is no specific antiviral treatment. In addition, travellers from non-endemic countries to endemic regions are at significant risk of dengue infection [7–9], and would also benefit from an effective vaccine.

A recombinant yellow fever-17D–dengue virus, live, attenuated, tetravalent dengue vaccine (CYD-TDV) is currently under development for the control of dengue disease. CYD-TDV has undergone extensive safety and immunogenicity assessment in dengue endemic and non-endemic populations [10–17]. To fulfil the needs of phase III studies, the scale-up of CYD-TDV production has been undertaken in parallel with its clinical development [18]. Differences in the production of CYD-TDV lots used up to phase II and those used in phase III relate to the scale-up of the virus/cell culture. Other changes included the removal of human serum albumin from the excipients and inclusion of a proprietary stabiliser in the finished product [19].

This trial was conducted with the primary objective of testing the safety and consistency of the immune responses elicited by three consecutive lots of vaccine from the scaled-up phase III production process. Consistency studies are routinely undertaken for new biological products [20]. We conducted this lot-to-lot consistency study in people living in dengue-free areas of Australia in order to minimise the risk that natural infections would impact the assessment of the immune response following 3 doses of vaccine or placebo, approximately 13 months after vaccination commenced [10,21–23].

2. Methods

2.1. Study design

This was a randomised, multi-centre, placebo-controlled, observer-blind, phase III study performed at eight centres in Australia between 5 October 2010 and 12 June 2012 (NCT01134263). The study was conducted in accordance with the Declaration of Helsinki and the International Conference on the Harmonization-Good Clinical Practice. The study protocol and amendments were approved by the respective Institutional Review Boards or Independent Ethics Committees at each study site. Written informed consent was obtained from all participants. The site selection criteria included locations outside of dengue endemic regions.

2.2. Participants

Healthy adults (aged 18 to 60 years) were eligible for inclusion. Exclusion criteria included: pregnancy; breast feeding; history of flavivirus infection or vaccination; prolonged habitation in a dengue endemic area (for more than 1 year); receipt of blood products within 3 months before enrolment, chronic illnesses including alcohol and drug abuse that were at a stage that may interfere with trial conduct or completion; and congenital or acquired immunodeficiency or immunosuppressive conditions or treatments. Study investigators were advised to avoid recruiting participants planning to travel to dengue endemic areas during the study period. In the event that participants needed to travel to dengue endemic areas during the trial, they were instructed on how to protect themselves against mosquito bites.

2.3. Random assignment and blinding

Participants were randomly assigned in permuted blocks of 13 (stratified by site) to one of five groups via an interactive voice response system or interactive web response system (IVRS/IWRS) in a 3:3:3:3:1 ratio to receive either vaccine from any one of three phase III lots, or a phase II lot, or placebo, respectively. At each site a designated, unblinded vaccinator not involved in data collection or safety assessments reconstituted and administered the assigned vaccine or placebo.

2.4. Vaccines and placebo

Dengue vaccine was presented as a lyophilised powder and saline solvent for reconstitution immediately before use in 0.5 mL volumes containing $5 \pm 1 \log_{10}$ cell-culture infectious dose 50% (CCID50) of each of the four live attenuated recombinant CYD vaccine virus serotypes. Solvent was 0.5 mL NaCl 0.4% and human serum albumin 2.5% for the phase II lots, and NaCl 0.4% without human serum albumin for the phase III lots. The placebo was 0.5 mL NaCl 0.9%. The study products were administered subcutaneously in the deltoid region of the upper arm. Participants received a 3-dose series of injections scheduled at time-points 0, 6, and 12 months.

2.5. Immunogenicity assessments

Dengue neutralising antibody titres were determined on the serum samples collected before the first injection on Day 0 and 28 days (per-protocol range 28 to 32 days) after the third injection using a 50% plaque reduction neutralisation test ($PRNT_{50}$) with parental dengue virus strains of the four CYD-TDV constructs as described previously (Sanofi Pasteur GCI, Swiftwater, USA) [24,25]. The assay had a lower limit of quantification titre of 10 (1/dilution): titres below the lower limit of quantification were assigned a value of 5.

2.6. Reactogenicity and safety

All safety assessments were performed by staff blind to the study product assigned to participants. Participants were monitored for 30 min after each injection for any immediate adverse events/reactions. They were provided with digital thermometers, flexible rulers and diary cards and were instructed to record solicited injection site reactions (pain, redness and swelling) for 7 days and systemic reactions (fever determined by axillary temperature, headache, malaise, myalgia, asthenia) for 14 days, as well as unsolicited adverse events for 28 days after each study injection. All adverse events were graded on a three point scale as described elsewhere [16]. Serious adverse events with onset at any time from study enrolment to 6 months after the last injection were recorded. Investigators assigned the causal relationship to study product for each unsolicited adverse event and serious adverse event.

Adverse events of special interest were hypersensitivity/allergic reactions within 7 days of injection, viscerotropic disease or neurotropic disease within 30 days after each injection, and dengue episodes requiring hospitalization at any time during the study [26].

Women who became pregnant during the study received no further study product and were followed for the duration of the pregnancy until outcome. A pregnancy was classed as "exposed" if CYD-TDV was administered within ≤30 days before the last menstruation or thereafter.

2.7. Definitions and statistical analyses

The primary objective was to demonstrate that three phase III CYD-TDV lots induce equivalent immune responses in terms of $PRNT_{50}$ geometric mean titres (GMTs) against the four vaccine parental strains 28 days after the third vaccination. Equivalence among the three phase III lots would be demonstrated if equivalence was demonstrated for all serotypes. The GMTs for each pairwise lot-to-lot comparison were considered equivalent if the lower and upper limits of the two-sided 95% confidence intervals (CIs) of the GMT ratio were ≥0.5 and ≤2.0, respectively [27,28]. The 95% CIs were calculated using the normal approximate method for GMTs. If equivalence of the three phase III lots was shown, a secondary objective was planned to test if the phase III vaccine (pooled data for all three lots) is equivalent to the phase II lot.

In order to have over 90% power to determine GMT equivalence between the three phase III lots for all four serotypes and to provide 97.5% power for each serotype, assuming a 2-fold maximum acceptable difference between GMTs, an alpha level of 5% (two-sided hypotheses), a standard deviation of 0.54 (log_{10} titres) for each serotype, and that approximately 80% of participants would be included in the per-protocol analysis set, the sample size required for each dengue vaccine group was set at 165. With a placebo control group of 55, the total sample size required was set at 715.

Statistical analyses were performed with SAS® software. $PRNT_{50}$ titres and their 95% CIs were calculated for each of the four serotypes assuming normal distribution of the log_{10} transformed $PRNT_{50}$ titres.

The primary immunogenicity analyses were performed on the per-protocol analysis set, and confirmed on the full analysis set. The per-protocol set included participants who met all protocol-specified inclusion criteria and did not meet any protocol-specified exclusion criteria or definitive contraindications, received the correct doses of vaccine by the correct route and site of administration within the specified times, provided a post-injection serum sample within the specified time and were dengue seronegative at baseline ($PRNT_{50}$ titres below the lower limit of quantification for each serotype). The full analysis set consisted of all participants who received at least one dose of CYD-TDV or placebo. The safety analysis set consisted of all vaccinated participants, analysed according to the treatment received at the first dose. The incidences of adverse events were calculated along with the 95% CIs by allocated group.

Because this is the first study involving adults aged older than 45 years, we undertook post-hoc exploratory comparisons of the immunogenicity and safety data for adults aged 46 to 60 years to that of younger adults aged 18 to 45 years.

3. Results

3.1. Study populations

Overall, 715 participants were enrolled and randomly assigned to the study groups (Fig. 1). Of these, 712 received at least one study injection, of whom 614 (86.2%) completed the study; 547 (76.8%) were included in the per-protocol set. Withdrawals were evenly distributed across study groups and were mainly voluntary (5.9% of participants [42/712]). Six withdrawals occurred after serious adverse events and 11 after other adverse events. The demographic characteristics for enrolled participants are summarised in Table 1.

3.2. Antibody responses

The baseline seropositivity ($PRNT_{50}$ titre ≥ 10) rates against each of the dengue serotypes were broadly similar across the CYD-TDV study groups (Table 1—FAS). Across the phase III lots, after completion of the third vaccination the seropositivity rates ranged from 59% to 71% for serotype 1, 78% to 86% for serotype 2, 91% to 95% for serotype 3, and 91% to 92% for serotype 4. For the phase II lot, post-vaccination seropositivity rates were 56%, 74%, 98% and 93% for each serotype, respectively. There were no discernable increases in seropositivity rates with placebo for serotypes 1 to 3, but a small increase from 0% (95% CI: 0–6.3) to 14.6% (95% CI: 6.1–27.8) was observed for serotype 4.

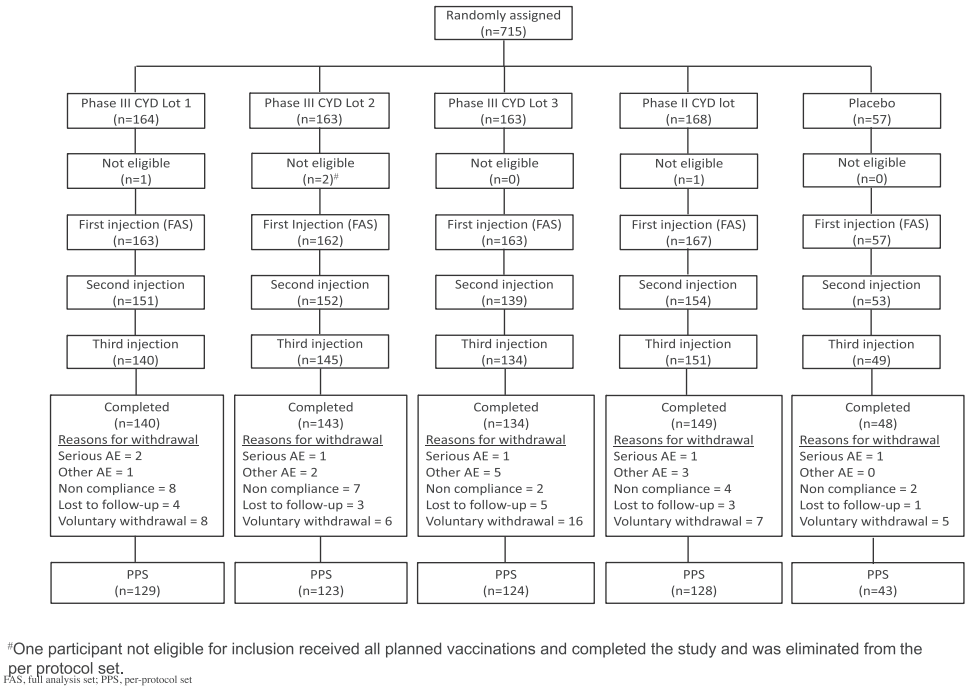
Compared to the baseline blood samples GMTs for the four serotypes were greater across all CYD-TDV groups after the third vaccination (Fig. 2—FAS). There was no discernable change to GMTs with placebo compared to baseline: GMTs ranged 5.0 to 6.2 at baseline and 5.6 to 6.6 after the third vaccination.

An exploratory analysis of the antibody response across all lots in younger participants aged 18–45 years ($n = 447$) obtained the following GMTs compared to older participants aged 46–60 years ($n = 265$), respectively: 18.8 (16.4–21.7) vs. 17.7 (95% CI, 15.2–20.7) for serotype 1; 47.2 (95% CI, 39.4–56.4) vs. 54.2 (95% CI 43.4–67.7)

Table 1
Participants' baseline characteristics by randomly assigned groups (full analysis sets).

	CYD-TDV				Placebo ($n = 57$)
	Phase III lots			Phase II lot ($n = 167$)	
	Lot 1 ($n = 163$)	Lot 2 ($n = 162$)	Lot 3 ($n = 163$)		
Sex (n, %)					
Male	60 (36.8)	75 (46.3)	79 (48.5)	83 (49.7)	24 (42.1)
Age (years, mean ± SD)	39.4 ± 13.3	39.4 ± 12.9	38.7 ± 14.1	38.4 ± 13.2	39.7 ± 13.5
Dengue status (n, %)[‡]					
Seropositive	7 (4.3)	11 (6.8)	8 (4.9)	17 (10.2)	5 (8.8)
Serotype 1	0	4 (2.5)	2 (1.2)	6 (3.6)	4 (7.0)
Serotype 2	2 (1.2)	2 (1.2)	1 (0.6)	4 (2.4)	3 (5.3)
Serotype 3	5 (3.1)	6 (3.7)	3 (1.8)	10 (6.1)	4 (7.0)
Serotype 4	2 (1.2)	2 (1.2)	6 (3.7)	6 (3.6)	0

[‡] Dengue neutralising antibody titres were determined using a 50% plaque reduction neutralisation test ($PRNT_{50}$) with parental dengue virus strains of CYD-TDV constructs (Sanofi Pasteur GCI, Swiftwater, USA) [24,25]. Baseline dengue seropositivity was defined by dengue neutralisation antibodies above the lower limit of quantification (10 [1/dilution]) for at least one serotype.



#One participant not eligible for inclusion received all planned vaccinations and completed the study and was eliminated from the per protocol set.

FAS, full analysis set; PPS, per-protocol set

Fig. 1. Flow of participants through the trial.

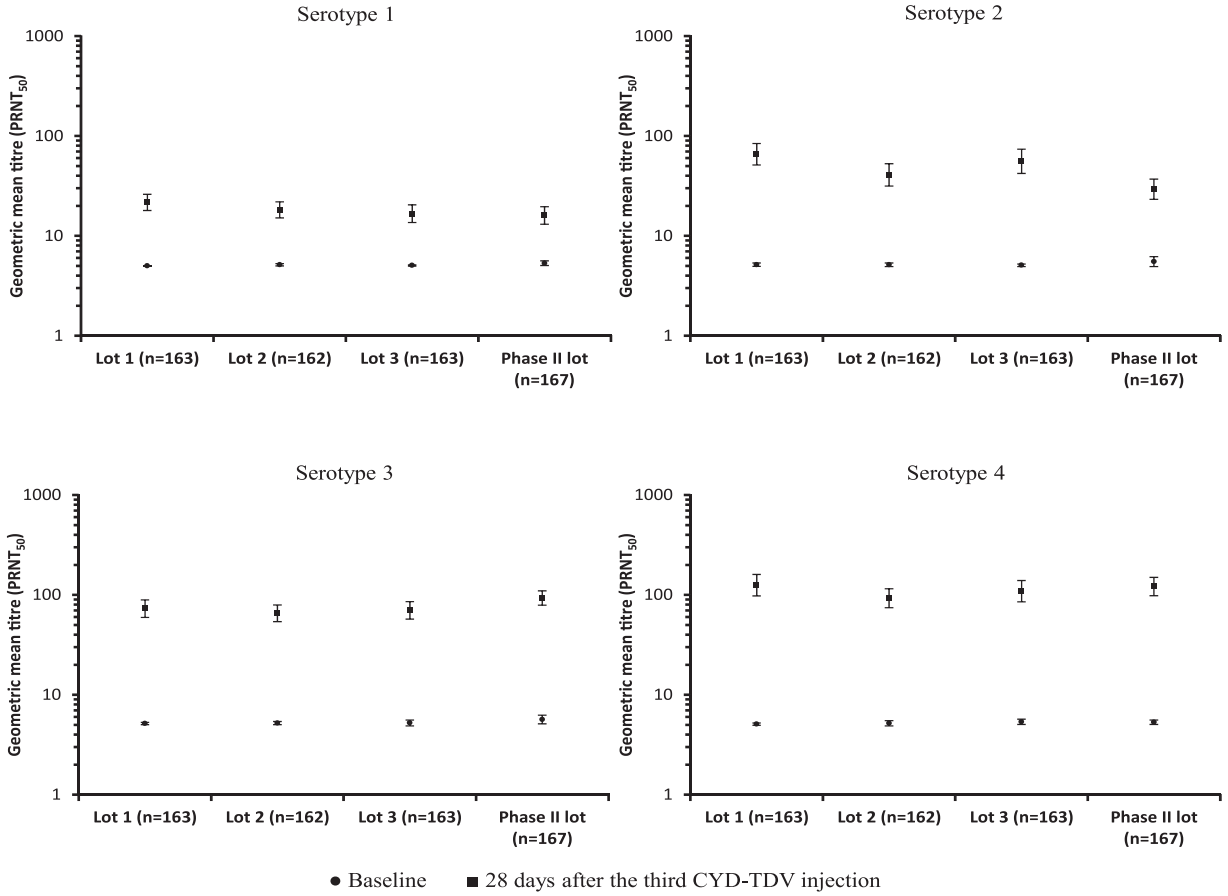


Fig. 2. Geometric mean titres (GMTs) and 95% CIs for each dengue serotype at baseline and 28 days following the third injection according to vaccine lot. Data shown for the full analysis set.

Table 2
GMT of antibodies against parental dengue virus serotypes among the CYD-TDV vaccine lots, placebo, and the phase III lot-to-lot comparisons 28 days after the third injection (per-protocol set)[†,‡].

	Serotype 1	Serotype 2	Serotype 3	Serotype 4
GMT (95% CI)				
Phase III				
Lot 1 (n = 129)	20.6 (16.9; 25.1)	65.9 (50.6; 85.7)	74.2 (60.1; 91.7)	131.8 (101.4; 171.3)
Lot 2 (n = 123)	18.1 (14.8; 22.2)	44.1 (33.3; 58.3)	65.0 (53.2; 79.3)	94.6 (75.3; 118.7)
Lot 3 (n = 124)	17.1 (13.9; 21.2)	58.1 (43.2; 78.2)	71.6 (58.2; 88.2)	108.5 (84.2; 139.7)
Phase II				
Lot (n = 128)	15.1 (12.4; 18.4)	25.7 (20.6; 32.0)	83.6 (71.1;98.4)	115.4 (92.8;143.5)[a]
Placebo (n = 43)	5 (NC)	5 (NC)	5 (NC)	6.09 (5.1; 7.4)
Lot–Lot comparisons GMT ratio (95% CI)				
Phase III				
Lot 1/Lot 2	1.14 (0.86; 1.51)	1.49 (1.02; 2.19)	1.14 (0.86; 1.53)	1.39 (0.99; 1.97)
Lot 2/Lot 3	1.06 (0.79; 1.42)	0.76 (0.50; 1.14)	0.91 (0.68; 1.21)	0.87 (0.62; 1.22)
Lot 3/Lot 1	0.83 (0.63; 1.11)	0.88 (0.60; 1.31)	0.96 (0.72; 1.30)	0.82 (0.57; 1.18)

NC, not calculated. The lower limit of quantitation (LLOQ) of the assay was 10 (1/dil). For all calculations, any titre reported as <LLOQ was converted to a value of 0.5 LLOQ.

[a] n = 127.

[‡] Data are presented as GMT (95% CIs) and GMT ratios between paired lots (95% CIs). Lot-to-lot equivalence was demonstrated if the lower and upper limits of the two-sided 95% CIs of the GMT ratio were ≥0.5 and ≤2.0, respectively.

[†] The per-protocol set included participants who met all protocol-specified inclusion criteria and did not meet any protocol-specified exclusion criteria or definitive contraindications, received the correct doses of vaccine within the specified times, provided a post-injection serum sample and were dengue seronegative at baseline (i.e. $PRNT_{50}$ titres below the lower limit of quantification for all 4 serotypes).

for serotype 2; 63.6 (95% CI, 55.1–73.5) vs. 83.3 (71.2–97.5) for serotype 3; and 91.2 (95% CI, 75.2–111) vs. 144 (95% CI, 123–168) for serotype 4.

3.3. Equivalence analyses

Statistical equivalence of the GMTs for the 3 phase III lots was demonstrated for 11 of 12 pairwise comparisons in the per-protocol analysis (Table 2). For one of three comparisons for serotype 2, the GMT ratio between a pair of lots (lots 1 and 2) was 1.49 (95% CI: 1.02; 2.19); i.e. the upper 95% CI exceeded the pre-set threshold of 2.0. The same result was observed in the full analysis set (data not shown).

Because the strict protocol-specified definition of phase III lot equivalence had not been met, comparison of phase III lots (pooled data) to the phase II lot could not proceed according to protocol. However, the pooled GMTs for the phase III vaccine lots for serotypes 1, 3 and 4 after the third injection (18.6, 70.2 and 110.9, respectively) were similar to those with the phase II lot (15.1, 83.6 and 115.4, respectively). For serotype 2, the GMTs were greater in each individual phase III lot (65.9, 44.1 and 58.1) compared to the phase II lot (25.7).

3.4. Reactogenicity and safety

The frequencies of adverse events following each of the three injections are shown in Table 3 and Fig. 3. All the lots had a similar reactogenicity and safety profile. No deaths were reported. Forty-four serious adverse events were reported by 34 participants; 5.0% [33/655] of CYD-TDV recipients and 1.8% [1/57] of placebo

Table 3
Frequency of solicited injection site and systemic reactions after any injection by group (safety analysis set)[†].

Symptom	Severity	CYD-TDV						Placebo (n = 57)		
		Phase III lots (n = 488)			Phase II lot(n = 167)					
		n/M	%	95% CI	n/M	%	95% CI		%	95% CI
Injection site reaction	Any	231/481	48.0	43.5; 52.6	84/164	51.2	43.3; 59.1	11/57	19.3	10.0; 31.9
Pain	Any	220/481	45.7	41.2; 50.3	78/164	47.6	39.7; 55.5	11/57	19.3	10.0; 31.9
	Grade 3	3/481	0.6	0.1; 1.8	0/164	0	0; 2.2	0/57	0	0.0; 6.3
Erythema	Any	62/481	12.9	10.0; 16.2	24/164	14.6	9.6; 21.0	0/57	0	0.0; 6.3
	Grade 3	0/481	0	0; 0.8	0/164	0	0; 2.2	0/57	0	0.0; 6.3
Swelling	Any	14/481	2.9	1.6; 4.8	5/164	3	1.0; 7.0	1/57	1.8	0.0; 9.4
	Grade 3	0/481	0	0; 0.8	0/164	0	0; 2.2	0/57	0	0.0; 6.3
Systemic reaction	Any	366/481	76.1	72.0; 79.8	125/164	76.2	69.0; 82.5	36/57	63.2	49.3; 75.6
Asthenia	Any	157/481	32.6	28.5; 37.0	69/164	42.1	34.4; 50.0	20/57	35.1	22.9; 48.9
	Grade 3	23/581	4.8	3.1; 7.1	9/164	5.5	2.5; 10.2	2/57	3.5	0.4; 12.1
Fever[a]	Any	21/480	4.4	2.7; 6.6	5/164	3.0	1.0; 7.0	1/57	1.8	0.0; 9.4
	Grade 3	1/480	0.2	0; 1.2	0/164	0	0; 2.2	0/57	0	0.0; 6.3
Headache	Any	304/481	63.2	58.7; 67.5	104/164	63.4	55.5; 70.8	28/57	49.1	35.6; 62.7
	Grade 3	47/481	9.8	7.3; 12.8	14/164	8.5	4.7; 13.9	4/57	7.0	1.9; 17.0
Malaise	Any	244/481	50.7	46.2; 55.3	90/164	54.9	46.9; 62.6	26/57	45.6	32.4; 59.3
	Grade 3	46/481	9.6	7.1; 12.5	13/164	7.9	4.3; 13.2	3/57	5.3	1.1; 14.6
Myalgia	Any	210/481	43.7	39.2; 48.2	84/164	51.2	43.3; 59.1	21/57	36.8	24.4; 50.7
	Grade 3	25/481	5.2	3.4; 7.6	13/164	7.9	4.3; 13.2	3/57	5.3	1.1; 14.6

n, number of participants in specified category; M, number of participants available.

[a] Defined by a temperature of ≥38.0 °C.

[†] The safety analysis set consisted of all vaccinated participants, analysed according to the treatment received at the first dose.

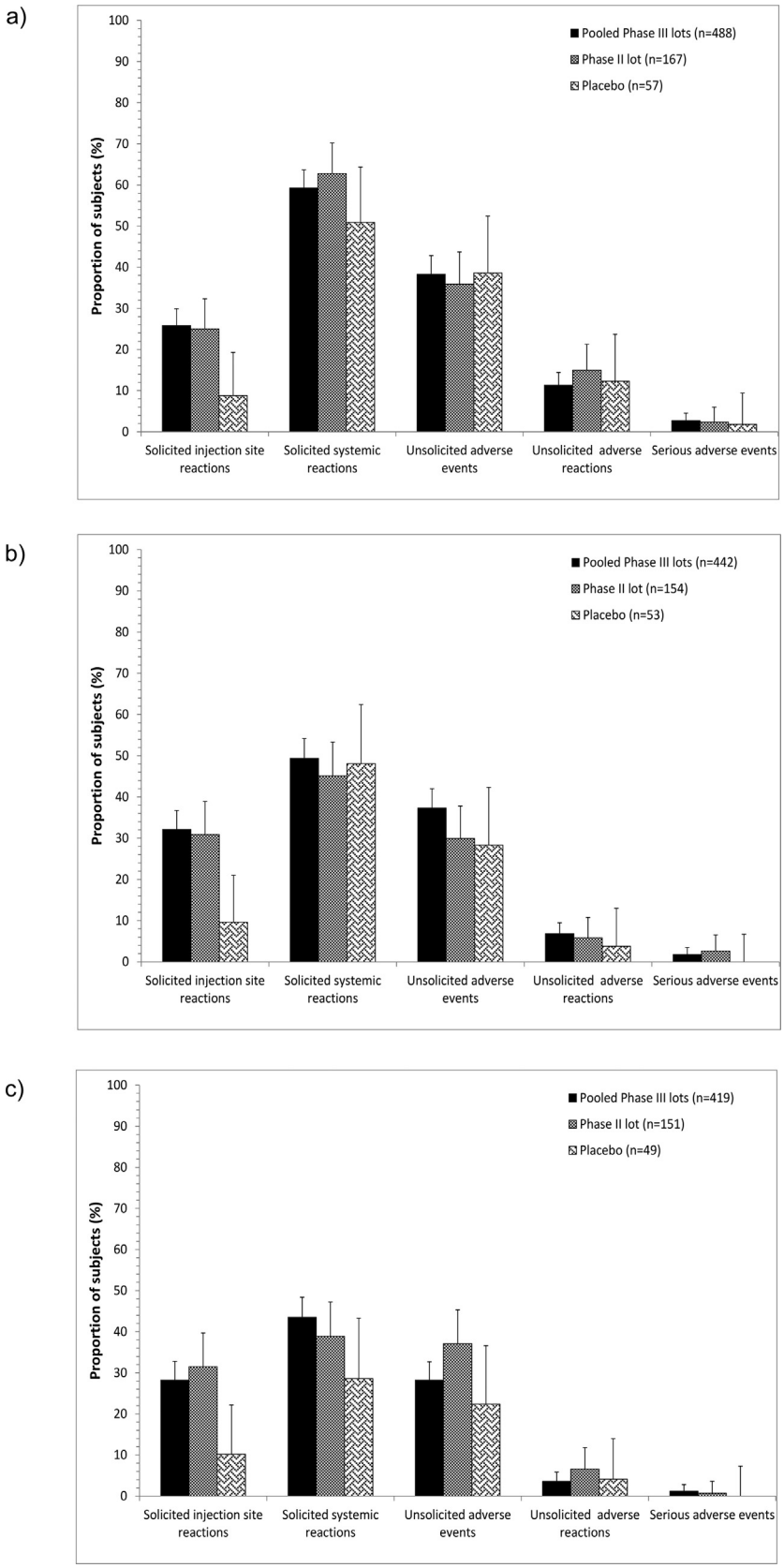


Fig. 3. Safety overview after the first (a), second (b) and third (c) injections (safety analysis set). Data are shown with upper 95% confidence limit.

recipients. Six participants (five CYD-TDV recipients and one placebo recipient) discontinued participation in the study following serious adverse events, of which two were considered related to CYD-TDV vaccine: headache 10 days after the first vaccination in a 49-year-old woman with hypertension, and polymyalgia rheumatica in the month after the second vaccination in a 58-year-old man with underlying chronic osteoarthritis.

Eleven participants (all CYD-TDV recipients) had non-serious adverse events leading to discontinuation—four of these (erythematous rash, migraine, upper respiratory tract infection and periorbital infection) were assessed as related to the study product.

Immediate unsolicited adverse events occurred at a rate of 0.6% (4/655) after any injection with CYD-TDV. These events (facial erythema, pruritus and dysgeusia, respectively, in three participants with the phase III vaccine, and dizziness in one participant with the phase II vaccine) were of Grade 1 intensity and all resolved spontaneously within one day. There were no immediate unsolicited adverse events in the placebo group.

Systemic and injection site unsolicited adverse events considered vaccine-related were reported by 18% (90/488), 23% (39/167) and 18% (10/57) of participants in the pooled phase III, phase II, and placebo groups, respectively.

Older participants aged 46–60 years had a similar safety profile compared with younger participants aged 18–45 years (data not shown).

Among the adverse events of special interest sought, there were no cases of serious or severe allergic reactions, no serious neurotropic or viscerotropic events, and no hospitalized dengue disease reported. While allergic reactions within seven days after study injection (1 pruritus, 7 rash, 1 facial swelling) were reported by: 8/488 (2%) participants receiving any phase III vaccine and 1/167 (1%) receiving the phase II vaccine, and none in the placebo group.

Four pregnancies were reported; all in CYD-TDV groups. One pregnancy was 'exposed to vaccine', i.e. the vaccine was administered 18 days before the last menstrual period. The woman chose to have an elective abortion for unrelated personal reasons. The other three pregnancies were considered unexposed to the vaccine and in each case a healthy infant was delivered at or near full-term.

4. Discussion

This study was designed to establish the lot-to-lot consistency of neutralising antibody responses produced by three phase III lots of CYD-TDV. The secondary aims were: to demonstrate equivalence of immune response of the phase III lots (pooled data) and a phase II lot, and to describe the safety of CYD-TDV in an adult population living in dengue-free locations. As this is the largest CYD-TDV clinical trial in adults to date, and the first to use phase III vaccine lots and include adults older than 45 years, we could specifically assess the immunogenicity and safety of CYD-TDV for the first time in this older adult age group.

Equivalence between the phase III vaccine lots was statistically demonstrated for 11 of the 12 comparisons, but not for the 12th pairwise comparison between lots 1 and 2 for serotype 2; thereby failing to satisfy the study's strict per protocol equivalence definition. However, equivalence between the phase III and II vaccine lots was demonstrated for vaccine dengue serotypes 1, 3, and 4, but not for serotype 2. Two factors that adversely affected the power of the study may account for this outcome. First, 76.8% of participants were eligible for inclusion in the per-protocol analysis while it had been assumed that 80% would be included. In addition, at the planning stages of the study, the intrinsic variability of the neutralising antibody assay was assumed to be 2, but was later determined to be 3 [25].

A post-hoc power analysis of the study observations revealed that those two factors mentioned above reduced the power of the study to determine equivalence to 62%. As lot-to-lot consistency was not fully statistically demonstrated for all 12 comparisons, the secondary objective of demonstrating equivalence between phase III and phase II vaccine lots could not be formally undertaken as described in the study protocol. Nevertheless, considering that only one lot-to-lot comparison (Lot 1 versus Lot 2 for serotype 2) failed the equivalence test by a narrow margin, we judge that biological equivalence was nonetheless established. The GMTs for serotype 2 were consistently higher in each individual phase III lot compared to the phase II lot. Therefore, although consistency for serotype 2 was narrowly missed between two phase III lots, the phase III lots may be considered as not clinically different.

There were no safety concerns with CYD-TDV in this study. The safety and reactogenicity profile of the vaccine lots was acceptable and consistent with previous CYD-TDV clinical trials [10,11,13,14,19]. Both the phase III and phase II vaccines in this study were equally well tolerated, with reporting patterns for the different categories of adverse events and reactions similar between groups. There was a trend of reduced reactogenicity with second and third dose of CYD-TV compared to the first dose (Fig. 2). Most of the local and systemic reactions were of mild intensity, and of short duration. CYD-TDV had a similar safety and tolerability profile in participants older than 45 years compared with younger participants (see Supplementary Fig. S1).

The immunogenicity profiles of the phase III and phase II vaccine lots tested in this study were consistent with those observed in two phase II studies in non-endemic areas, one conducted in healthy adults in the USA [13] using the same study product and vaccination schedule as the current study, and the other in dengue-naïve children, adolescents, and adults in Mexico City using a slightly different vaccination schedule [12].

There is no recognised $PRNT_{50}$ seroprotection threshold for any dengue serotype yet. Two of the three phase III vaccines lots in our study were also used in two phase III studies conducted in South East Asia (10,275 children aged 2 to 14 years) [29] and Latin America (20,869 children aged 9 to 16 years) [30]. One year of follow-up, beginning 28 days after the third dose of vaccine, showed that CYD-TDV had 56.5% and 60.8% efficacy (with narrow 95% confidence intervals) against virologically-confirmed dengue in the two phase III studies, respectively. Additional analyses of the phase III efficacy trials may provide information on potential $PRNT_{50}$ seroprotection threshold values.

In summary, these results support the change in manufacturing process required for the scaled up production of the CYD-TDV. There were no safety concerns observed in this study with the CYD-TDV, and the vaccination schedule was well tolerated.

Contributors

Dv-dV, AB, MB, DW, YH, and LC were involved in the design and operational management of the study. JT, LGH, MQ, JM, MDN and PCR were principal investigators in this study. All authors contributed to the interpretation of the data and contributed to this publication and approved the final manuscript for submission. All authors had access to the study data and are responsible for the veracity and completeness of the data reported.

Role of funding source

This study was sponsored by Sanofi Pasteur. The sponsor participated in the trial design and managed all operational aspects of the study, including monitoring data collection, statistical analyses, and writing of the report.

Conflict of interest statement

Dv-dV, MB, AB, YH, and LC are employees of Sanofi Pasteur. DW was an employee of Sanofi Pasteur when the study was undertaken. All other author's institutions have received funding from Sanofi Pasteur to conduct this clinical study. JT has received lecture fees from Sanofi Pasteur, GSK, CSL, Gilead/MSD. LGH has received funding from Baxter, Biota, CSL, Gilead, GSK, Merck, Novartis, Pfizer, Roche, Romark and Sanofi Pasteur for the conduct of research, and, from some of the listed companies, for travel to present at conferences or consultancy work. All funding received by LGH was directed to research accounts at The Children's Hospital at Westmead. MQ, JM and MDN report no relevant conflict of interest. PCR has received funding from Sanofi Pasteur for the conduct of research through the Telethon Kids Institute and for travel to present at conferences.

Acknowledgements

Editorial assistance with the preparation of the manuscript was provided by Richard Glover, in Science Communications, Springer Healthcare. Funding for this assistance was provided by Sanofi Pasteur.

We thank Maureen McKeirnan (Brisbane South Clinical Research Centre, Australia) for her participation in this study. We also thank Sutee Yoksan from CVD Mahidol, Thailand, for his dengue assay contributions, Linghua Zhou for her comments on the manuscript, Edith Langevin for assistance with the statistical analyses, and Charlene Stevens for coordinating study activities. We acknowledge the valuable contributions of each participant who volunteered to participate in the study and the staff who contributed to the conduct of the study at each site.

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.2015.08.008

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