Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial

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Summary

Background Roughly half the world's population live in dengue-endemic countries, but no vaccine is licensed. We investigated the efficacy of a recombinant, live, attenuated tetravalent dengue vaccine.

Methods In this observer-masked, randomised, controlled, monocentre, phase 2b, proof-of-concept trial, healthy Thai schoolchildren aged 4–11 years were randomly assigned (2:1) to receive three injections of dengue vaccine or control (rabies vaccine or placebo) at months 0, 6, and 12. Randomisation was by computer-generated permuted blocks of six and participants were assigned with an interactive response system. Participants were actively followed up until month 25. All acute febrile illnesses were investigated. Dengue viraemia was confirmed by serotype-specific RT-PCR and non-structural protein 1 ELISA. The primary objective was to assess protective efficacy against virologically confirmed, symptomatic dengue, irrespective of severity or serotype, occurring 1 month or longer after the third injection (per-protocol analysis). This trial is registered at ClinicalTrials.gov, NCT00842530.

Findings 4002 participants were assigned to vaccine (n=2669) or control (n=1333). 3673 were included in the primary analysis (2452 vaccine, 1221 control). 134 cases of virologically confirmed dengue occurred during the study. Efficacy was 30·2% (95% CI −13·4 to 56·6), and differed by serotype. Dengue vaccine was well tolerated, with no safety signals after 2 years of follow-up after the first dose.

Interpretation These data show for the first time that a safe vaccine against dengue is possible. Ongoing large-scale phase 3 studies in various epidemiological settings will provide pivotal data for the CYD dengue vaccine candidate.

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Introduction

Dengue has become one of the most important and widespread arthropod-borne viral diseases of human beings, with about half the world's population now at risk.[1] WHO estimates that 50–100 million dengue infections occur each year in more than 100 countries, and that half a million people develop severe dengue necessitating hospital admission, although the true figures could be higher.[1–3] Symptomatic infection is classified as either dengue or severe dengue, depending on whether the patient recovers after the initial 3–7-day febrile phase, or develops complications as a result of a systemic vascular leakage syndrome.[1,4] There is no specific treatment and, in absence of a vaccine, prevention relies on individual protection against mosquitoes and vector control strategies that, in view of the continuing expansion of dengue, have shown their limits as standalone measures.

The major challenges facing vaccine research and development include the existence of four pathogenic dengue virus serotypes (DENV1–4) that compete and interact at the immunological level, as well as more practical challenges, such as the lack of suitable animal models or a correlate of protection.[5–7] In more than half a century of research, various vaccine approaches have been attempted and several candidate vaccines are in early clinical or preclinical development.[8]

One candidate vaccine, CYD-TDV, is a recombinant, live, attenuated, tetravalent dengue vaccine based on the yellow fever 17D vaccine strain and produced in Vero cells.[7] Phase 1 and 2 trials have been undertaken in southeast Asia and Latin America in cohorts of adults and children who were either immunologically naive against dengue and other flaviviruses before vaccination or who had some degree of pre-existing flaviviral immunity due to vaccination against yellow fever or Japanese encephalitis or natural exposure in endemic areas.[7,9–13] These studies have shown that a three-dose regimen given over 12 months is well tolerated and elicits balanced neutralising antibody responses against the four serotypes in diverse epidemiological settings. We present the primary report of the first clinical trial of the protective efficacy of this investigational dengue vaccine. The trial was designed and undertaken in an endemic area according to WHO recommendations.[14] The primary objective was to assess protective efficacy after three injections against virologically confirmed symptomatic dengue, irrespective of severity or serotype. Passive surveillance for admissions to hospital owing to fever due to dengue is ongoing.

Methods

Study design and participants

We undertook an observer-blind, randomised, controlled, monocentre phase 2b trial of the efficacy of CYD-TDV against virologically confirmed symptomatic dengue. The study was done in the Muang district, Ratchaburi province, Thailand, which is about 100 km west of Bangkok. An epidemiological study to prepare this site for a dengue vaccine efficacy trial was done in 2006–09 and showed that the site was suitable for the trial in terms of disease incidence, the co-circulation of all four dengue virus serotypes (predominantly DENV1, then 2), adequate infrastructure for surveillance, and high community awareness of the disease.[15–17] The study was done at Ratchaburi Regional Hospital (RRH), the province's principal medical care facility, and involved 35 schools in the district. We enrolled schoolchildren aged 4–11 years who were in good health based on medical history and physical examination, and whose parent or legal guardian had no plans to leave the study area. We excluded children with acute febrile illness at enrolment, those with congenital or acquired immunodeficiency or other disorders listed in the protocol, and those receiving immunosuppressive therapy or other treatments or vaccines prohibited by the protocol (appendix).

See Online for appendix

The ethical review committee for research in human subjects, Ministry of Public Health, Thailand, approved the protocol, amendments, consent, and assent forms. We obtained written informed consent from parents or legal guardians of all participants, and written assent from all participants aged 7 years or older. The trial was undertaken in accordance with the principles of the Declaration of Helsinki, and in compliance with good clinical practice guidelines.

An independent data monitoring committee (IDMC) oversaw the study. Safety data after the first vaccination of a first cohort of 150 children were reviewed by the IDMC and their recommendation to proceed with the trial was submitted to the ethical review committee for approval. The committee approved continuation and at this point required that the study design be changed from active (rabies vaccine) to placebo control. Placebo was therefore used for all subsequent control vaccinations.

Randomisation and masking

At vaccination centres set up at RRH and in 20 of the participating schools, children were randomly assigned (2:1) to receive three doses of dengue vaccine or a control product at months 0, 6, and 12. Investigators used an interactive web-based response system (IWRS) to sequentially allocate a unique inclusion number to each enrolled child. Subsequent steps occurred in a separate room away from the masked-observer investigator. Using the IWRS, designated unmasked trial personnel (who were not involved in data collection or trial assessments) were informed of a product code assigned to the participant's inclusion number, and administered the product labelled with that code. The randomisation list that assigned a product code to each inclusion number was generated under the sponsor's responsibility by block randomisation with block sizes of six and stratification by vaccination centre.

Procedures

The recombinant, live, attenuated, tetravalent dengue vaccine (CYD-TDV) has been described elsewhere.[7] It was presented as a powder and saline solvent (0·4% NaCl, containing human serum albumin) for reconstitution immediately before use. Reconstituted vaccine contained $5\pm1\log_{10}$ median cell culture infectious dose of each of the four CYD vaccine viruses per 0·5 mL dose. The control product was inactivated rabies vaccine (Verorab, Sanofi Pasteur, Lyon France) for the first injection of the first 50 children randomly assigned to the control group, and 0·9% NaCl saline placebo for all other injections. All products were supplied by the sponsor and were injected subcutaneously in the upper arm.

We actively followed up all children to detect acute febrile illness based on daily surveillance of school registers during school terms for absenteeism, followed by phone calls or home visits to absentees, and on phone calls twice per week, mobile phone text-messages, or home visits throughout school holidays. In case of febrile illness at any time (defined as illness with two temperature readings of 37·5°C or higher at least 4 h apart), parents were asked to take their child to RRH for diagnosis and treatment. The surveillance system also captured spontaneous consultations at RRH. Consecutive febrile episodes separated by a symptom-free interval of 14 days or longer were regarded as separate episodes. Paired serum samples were collected at presentation (ie, acute sample, collected no later than 7 days after fever onset) and 7–14 days later (convalescent sample) and sent to the sponsor's Global Clinical Immunology (GCI) laboratory (Swiftwater, PA, USA) and to the Centre for Vaccine Development (Mahidol University, Bangkok, Thailand). We screened acute samples for presence of flavivirus using an initial PCR assay. Positive samples were tested for wild-type dengue virus with a serotype-specific quantitative RT-PCR, derived from a published method.[18] In parallel, all acute samples were tested for the presence of dengue NS1 antigen with a commercial ELISA kit (Platelia, Bio-Rad Laboratories, Marnes-La-Coquette, France).[19] An episode of virologically confirmed dengue was defined as a positive result in either one of the serotype-specific PCRs, or by NS1-antigen ELISA. A case was defined as a first episode of virologically confirmed dengue. Active surveillance was maintained until each participant had been followed up for at least 13 months after the third vaccination.

Severe dengue was defined as an episode that either fulfilled the 1997 WHO criteria for dengue haemorrhagic fever (DHF),[20] or was classified as severe by the IDMC on the basis of the WHO criteria and an additional list of symptoms such as visceral manifestations (appendix).

We documented and assessed all serious adverse events (SAEs) as defined in International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidance until the sixth month after the last injection, and thereafter any fatal SAE or vaccine-related SAE. The IDMC regularly reviewed all SAEs. We also assessed vaccine reactogenicity in the first 1050 enrolled children using conventional methods as described elsewhere for previous trials with this vaccine.[11,12]

We assessed dengue immune responses in the first 300 enrolled children at RRH in sera collected at enrolment, and before and 28 days after each injection. Sera were sent to GCI for measurement of neutralising antibody titres against each of the four the CYD parental dengue viruses with the plaque-reduction neutralisation test ($PRNT_{50}$), as described.[21,22] The assay's quantitation limit was 10 (1/dil). Samples lower than this value were assigned the titre 5 and regarded as seronegative.

Statistical analysis

WHO guidelines for the clinical evaluation of dengue vaccine in endemic areas recommend that the primary analysis of vaccine efficacy (VE) consider fully vaccinated volunteers.[14] Therefore our primary objective was to establish VE against cases (ie, participants with an episode) of symptomatic, virologically confirmed dengue occurring more than 28 days after the third vaccination according to

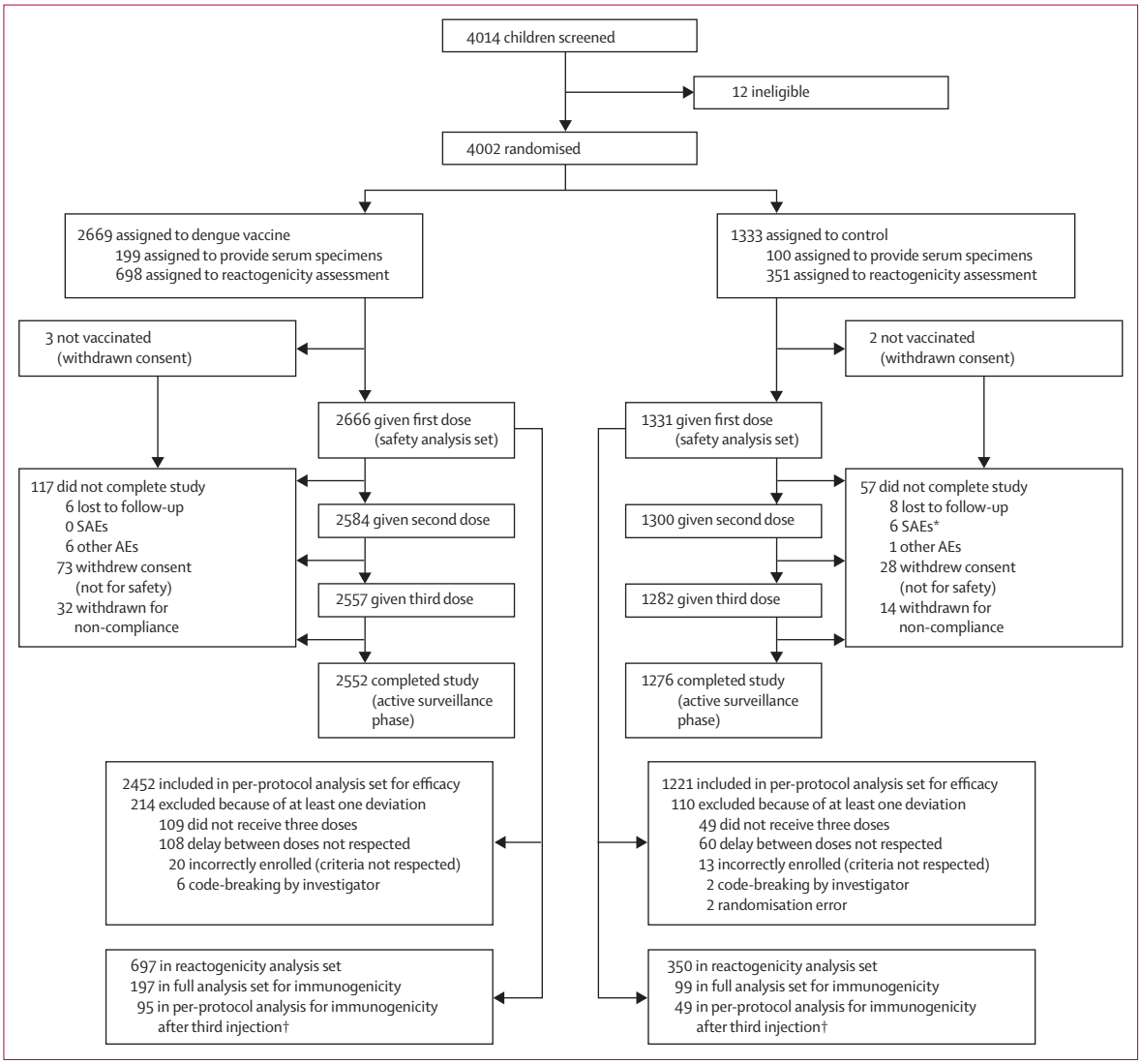

Figure: **Trial profile**

SAE=serious adverse event. AE=adverse event. *Six in the control group discontinued because of SAEs: four deaths, 1 acute idiopathic thrombocytopenic purpura, one acute febrile illness. †For the first cohort of 150 enrolled children, the ethics committee's approval to proceed with the trial was not received in time to perform the second injection within the time window allowed in the protocol (injections occurred at month 9), resulting in the exclusion of these children from the per-protocol analyses of efficacy and immunogenicity.

the equation: $VE = 100 \times (1 - ID_{CYD} / ID_{Control})$, where ID is the incidence density calculated as the number of cases divided by the total disease-free, person-time at risk in each group. With an assumed disease incidence of 1·3%, a true VE of 70%, a minimum follow-up of 1 year after the third vaccination, and a subject attrition rate of 7·5% per year, 4002 participants assigned with a 2:1 ratio to dengue vaccine or control were needed to show, with more than 80% power, and 95% confidence, that VE was not null. Analyses were based on the two-sided 95% CI of VE, calculated using the Exact method.[23] A 95% CI that excludes zero shows a significant difference at a two-sided α level of 0·05. The primary analysis was done on the per-protocol population—ie, in those who satisfied the enrolment criteria, who had correctly received all three doses of the assigned vaccine at months 0, 6 (±15 days), and 12 (±30 days), and for whom group allocation had not been unmasked.

As secondary and observational objectives, we established efficacy against cases occurring more than 28 days after the second injection, irrespective of protocol compliance, and against all cases occurring after at least one injection (intention-to-treat analysis).

We also planned to assess efficacy against severe dengue, and against cases that were either virologically confirmed or serologically suspected. There were too few severe cases to make the first of these analyses meaningful. The analysis of virologically confirmed or serologically suspected cases will be reported separately. Other secondary and observational analyses that will be reported separately were the relation between neutralising antibody titres and the occurrence of dengue, and levels of viraemia.

Serotype-specific incidence was to be calculated as an observational objective. In an exploratory analysis, defined in the statistical analysis plan, the serotype-specific relative risk was estimated, which is presented here as serotype-specific VE (ie, 1–relative risk). In an exploratory analysis, defined post-hoc, the serotype distribution in the two groups was tested for heterogeneity using Fisher's exact test and χ^2 test. Analyses for safety and immunogenicity endpoints were descriptive, using 95% CI, Student *t* test for normally distributed continuous variables, and χ^2 for the homogeneity between two categorical variables. $p<0·05$ was regarded as significant.

The sample sizes of the immunogenicity and reactogenicity subsets were arbitrarily defined as no hypotheses were tested.

This trial is registered at ClinicalTrials.gov, NCT00842530.

	Dengue vaccine (n=2669)	Control (n=1333)
Per-protocol analysis set for efficacy		
n	2452	1221
Age (years)	8·18 (2·04)	8·23 (2·06)
Boys	1187 (48%)	583 (48%)
Full analysis set for immunogenicity		
n	197	99
Age (years)	8·26 (1·74)	8·12 (1·74)
Boys	84 (43%)	46 (46%)
Body-mass index (kg/m²)	16·4 (3·4)	16·8 (3·7)
Anti-DENV or anti-JEV prevalence*	179 (91%)	91 (92%)
Anti-JEV prevalence*	157 (80%)	77 (78%)
Anti-DENV prevalence (≥1 serotype)*	138 (70%)	68 (69%)

Data are n, mean (SD), or n (%). DENV=dengue virus. JEV=Japanese encephalitis virus. *Anti-DENV and anti-JEV seroprevalence defined as the percentage of participants with a plaque-reduction neutralisation test ($PRNT_{50}$) titre of 10 or higher.

Table 1: **Baseline characteristics of participants**

Role of the funding source

The sponsor contributed to all study stages, including trial design, sample testing, statistical analysis, and the writing of this report. AS, DW, CS, KL, PC, VJ, WD, KP, TAW, AM, MS, AB, SV, NGT, and JL had complete access to the data. AS, DW, and JL had primary responsibility for the decision to submit for publication.

Results

Between Feb 5, 2009, and Feb 5, 2010, 4002 children were enrolled, of whom 3839 (96%) received three injections and 3673 (92%) were included in the per-protocol analysis set for efficacy (figure). Age and sex ratios were similar in both groups (table 1). At baseline, more than 90% of children sampled (immunogenicity subset) were seropositive against DENV or Japanese encephalitis virus (table 1). Active surveillance detected 2266 febrile episodes, 2263 (99·9%) of which were tested by both RT-PCR and NS1-antigen ELISA in acute blood samples. For 2256 (>99%) episodes, these samples were collected within 7 days of fever onset.

During the study, 134 children had virologically confirmed dengue, four of whom (all in the control group) had two episodes. Of these 134 cases, 77 occurred more than 28 days after the third injection in the per-protocol population and were included in the primary analysis: 45 occurred during 2522 person-years at risk in the vaccine group, whereas 32 cases occurred during 1251 person-years at risk in the control group. The corresponding vaccine efficacy was 30·2% (95% CI –13·4 to 56·6; table 2). Efficacy after at least one injection (intention-to-treat analysis) was 34·9% (95% CI 6·7–54·3; see appendix for Kaplan-Meier curves of virologically confirmed dengue, occurring after the first injection).

Non-primary analyses assessed serotype-specific relative risk and further post-hoc analyses revealed heterogeneity in the distribution of serotypes between groups, suggesting that efficacy differed between serotypes (table 2). Against DENV1, 3, and 4, efficacy after at least one injection was 61·2%, 81·9%, and 90·0%, respectively, and statistically superior to 0. Against DENV2, which accounted for 79 (59%) of 134 episodes, VE was

	Dengue vaccine		Control		Efficacy	
	Person-years at risk	Cases or episodes*	Person-years at risk	Cases or episodes*	% (95% CI)	Heterogeneity p value†
>28 days after three injections (per-protocol analysis)						
Cases	2522	45	1251	32	30·2% (−13·4 to 56·6)	0·0340
Serotype 1 episodes	2536	9	1251	10	55·6% (−21·6 to 84·0)	..
Serotype 2 episodes	2510	31	1250	17	9·2% (−75·0 to 51·3)	0·0309
Serotype 3 episodes	2541	1	1257	2	75·3% (−375·0 to 99·6)	..
Serotype 4 episodes	2542	0	1263	4	100·0% (24·8 to 100·0)	..
NS1-antigen positive only episodes	2542	4	1265	0	ND	..
>28 days after two injections						
Cases	3824	61	1905	47	35·3% (3·3 to 56·5)	0·0057
Serotype 1 episodes	3855	10	1921	16	68·8% (27·0 to 87·4)	..
Serotype 2 episodes	3824	44	1918	22	−0·3% (−75·8 to 41·1)	0·0009
Serotype 3 episodes	3860	2	1924	6	83·4% (7·1 to 98·4)	..
Serotype 4 episodes	3864	1	1934	4	87·5% (−26·5 to 99·7)	..
NS1-antigen positive only episodes	3863	4	1936	1	−100·5% (−9771·8 to 80·2)	..
After at least one injection (intention-to-treat analysis)						
Cases	5292	76	2630	58	34·9% (6·7 to 54·3)	0·0027
Serotype 1 episodes	5343	14	2666	18	61·2% (17·4 to 82·1)	..
Serotype 2 episodes	5312	52	2662	27	3·5% (−59·8 to 40·5)	0·0007
Serotype 3 episodes	5348	4	2667	11	81·9% (38·8 to 95·8)	..
Serotype 4 episodes	5353	1	2679	5	90·0% (10·6 to 99·8)	..
NS1-antigen positive only episodes	5351	5	2681	1	−150·5% (−11748·3 to 72·0)	..

ND=not determined. *A case was defined as a first episode of virologically confirmed dengue by either serotype-specific PCR, or NS1-antigen ELISA. Serotype-specific efficacy was calculated including all episodes of that serotype; four children with two virologically confirmed dengue episodes during the study were therefore included once in each of the two serotype-specific analyses concerned. †Fisher's exact test was used to test heterogeneity of serotype distribution between groups among the four serotypes and χ^2 was used to test the distribution between groups of serotype 2 versus the other three serotypes; NS1-antigen positive only cases (ie, RT-PCR negative cases) were excluded from heterogeneity testing.

Table 2: **Serotype-specific and overall efficacy of CYD tetravalent dengue vaccine against virologically confirmed dengue disease**

	Dengue vaccine group			Control group					
				First cohort*			Second cohort*		
	n	%	95% CI	n	%	95% CI	n	%	95% CI
Safety analysis set									
n	2666	..	..	50	..	..	1281	..	..
SAEs at any time	315	11·8	10·6–13·1	8	16·0	7·2–29·1	168	13·1	11·3–15·1
Vaccine-related SAEs at any time	0	0·0	0·0–0·1	0	0·0	0·0–7·1	1	0·1	0·0–0·4
SAEs within 28 days after any injection	50	1·9	1·4–2·5	2	4·0	0·5–13·7	35	2·7	1·9–3·8
Vaccine-related SAEs within 28 days after any injection	0	0·0	0·0–0·1	0	0·0	0·0–7·1	1	0·1	0·0–0·4
Reactogenicity analysis set									
n	697	..	..	50	..	..	300	..	..
AE within 30 min of injection	0	0·0	0·0–3·6	0	0·0	0·0–7·1	0	0·0	0·0–1·2
Solicited injection site reaction within 7 days of injection	426	61·6	57·8–65·2	29	58·0	43·2–71·8	189	63·2	57·5–68·7
Solicited systemic reaction within 14 days of injection	538	77·7	74·5–80·8	38	76·0	61·8–65·9	223	74·3	69·0–79·2
Unsolicited AE within 28 days of injection	317	45·5	41·7–49·3	20	40·0	26·4–54·8	142	47·3	41·6–53·2
Vaccine-related unsolicited AE within 28 days of injection	10	1·4	0·7–2·6	0	0·0	0·0–7·1	1	0·3	0·0–1·8
AE leading to study discontinuation	0	0·0	0·0–0·5	0	0·0	0·0–7·1	0	0·0	0·0–1·2

Data are n, %, or 95% CI of participants with AEs at least once during the study. Safety analysis set=all vaccinated participants, analysed according to the vaccine received. AE=adverse event. SAE=serious adverse event. Injection site reaction=pain, erythema, or swelling. *The first cohort received one injection of rabies vaccine followed by two placebo injections; the second cohort received three placebo injections.

Table 3: **Summary of all reported SAEs and of AEs reported in the reactogenicity subset after at least one injection**

low and not statistically superior to 0. In data from the control group, the incidence rate of virologically confirmed dengue was 1·73% in 2010 and 2·33% in 2011.

584 SAEs occurred during the study: 366 were reported for 315 (12%) of 2666 children in the vaccine group, and 218 were reported for 176 (13%) of 1331 children in the

	All episodes			Dengue virus serotype 2		
	Dengue vaccine group	Control group	p value*	Dengue vaccine group	Control group	p value*
Number of episodes	76	62	..	52	27	..
Duration of clinical syndrome (days)						
Mean	5·39 (2·34)	5·84 (2·68)	0·30	5·23 (2·39)	5·89 (2·21)	0·24
Median	5·0 (3·5–7·0)	5·5 (4·0–8·0)	..	5 (3–7)	6 (4–8)	..
Range	1–12	1–15	..	1–12	2–10	..
Duration of fever (days)						
Mean	4·13 (1·98)	4·40 (1·97)	0·42	4·08 (1·96)	4·22 (1·58)	0·74
Median	4·0 (2·5–5·0)	5 (3–6)	..	4·0 (2·5–5·0)	5 (3–5)	..
Range	1–10	1–9	..	1–10	1–7	..
Episodes leading to hospital admission	32 (42%)	30 (48·4)	0·46	20 (38·5)	15 (55·6)	0·14
Duration of hospital stay (days)						
Mean	4·91 (1·33)	5·17 (1·97)	0·55	5·05 (1·50)	4·80 (1·78)	..
Median	5 (4–6)	5 (4–6)	..	5 (4–6)	5 (3–6)	0·66
Range	3–8	2–11	..	3–8	2–9	..
Episodes with any haemorrhagic signs	28 (37%)	23 (37%)	0·98	16 (31%)	12 (44%)	0·22
Spontaneous bleeding	10 (13%)	10 (16%)	..	4 (8%)	5 (19%)	..
Bleeding with blood transfusion	0	0	..	0	0	..
Plasma leakage	4 (5%)	3 (5%)	..	2 (4%)	1 (4%)	..
Thrombocytopenia ≤50×10^9/L	6 (8%)	8 (13%)	..	4 (8%)	6 (22%)	..
Thrombocytopenia ≤100×10^9/L	16 (21%)	16 (26%)	..	10 (19%)	8 (30%)	..
Shock	0	2 (3%)	..	0	0	..
Organ impairment	1 (1%)	1 (2%)	..	1 (2%)	0	..

Data are n (%), mean (SD), median (IQR), or range. Episodes=all symptomatic, virologically confirmed dengue occurring during the study. *t test was used to compare means, χ^2 test was used to compare proportions between groups.

Table 4: **Clinical characteristics of virologically confirmed dengue episodes**

	Dengue vaccine (n=2669)			Control (n=1333)	
	12-year-old girl	6-year-old girl	6-year-old boy	12-year-old girl	10-year-old boy
Onset	182 days after dose 2	328 days after dose 3	344 days after dose 3	48 days after dose 3	170 days after dose 3
Presented with	Headache, anorexia, vomiting, abdominal pain, confusion	Rash, cough, anorexia, vomiting, abdominal pain	Headache, anorexia, abdominal pain	Headache, rash, abdominal pain	Headache, rash, vomiting, anorexia, abdominal pain
Additional clinical information	No clinical shock, CSF normal, CT brain normal, tonsillitis	No clinical shock, no other visceral manifestations	No clinical shock, no other visceral manifestations	Clinical shock (BP 80/50 mm Hg) after defervescence, hepatomegaly	No clinical shock, no other visceral manifestations
Duration of hospital stay (days)	7	6	3	11	10
Tourniquet test	Negative	Positive	Not done	Negative	Positive
Spontaneous bleeding	No	No	Haematemesis	Mucosal bleeding	Haematemesis
Minimum platelet count (×10^9/L)	48	6	25	18	51
Plasma leakage	No	>20% increase in haematocrit	>20% increase in haematocrit	Clinical pleural effusion, confirmed by chest radiograph, >20% increase in haematocrit	>20% increase in haematocrit
Dengue WT qRT-PCR					
Serotype	DENV2	DENV1	PCR negative	DENV1	DENV2
Virus titre (log_{10} GEq/mL)	9·89	5·46	NA	9·76	9·83
NS1 antigen ELISA	10·5	8·60	8·54	10·3	5·85
Diagnosis	Dengue fever with encephalopathy	DHF grade 1	DHF grade 2	DHF grade 3	DHF grade 2

BP=blood pressure. NA=not applicable. DHF=dengue haemorrhagic fever.

Table 5: **Summary of dengue episodes classified as severe according to independent data monitoring committee or WHO 1997 classifications[20]**

control group (table 3). No vaccine-related SAEs were reported in the dengue group, but one occurred in the placebo control group. SAEs were medical disorders consistent with the age group and occurred at similar rates in each group when we considered the study period as a whole, as well as SAEs occurring within 28 days of an injection. In the reactogenicity analysis set, the proportion of each group with adverse events (AEs) was similar (table 3). Unsolicited AEs within 28 days of any vaccination were reported for 317 (45%) of 697 children in the vaccine group and 142 (47%) of 300 receiving placebo control. Vaccine-related unsolicited AEs within 28 days of any vaccination were reported for ten (1%) of 697 children in the vaccine group and one (<1%) of 300 receiving placebo control. Four children in the control group died during the study (drowning, traffic accident, T-cell lymphoma, head injury).

The clinical characteristics of dengue episodes were similar in the two groups when we considered all episodes, irrespective of serotype (table 4). These characteristics were also similar in the two groups when we considered serotype 2 episodes only (ie, the serotype against which the vaccine did not show efficacy). Five dengue episodes were classified as severe dengue according to IDMC or WHO 1997 definitions. Three occurred in the vaccine group, two in the control group (table 5). The incidence density of severe dengue after at least one injection was 0·058% (3 cases/5149 person-years) in the vaccine group, and 0·078% (2 cases/2578 person-years) in the control group. One episode of grade 3 DHF occurred in the control group; other episodes were grade 2 DHF or milder. All five children recovered without sequelae within 12 days.

In the immunogenicity subset, geometric mean titres (GMTs) increased after the first dengue vaccination compared with baseline (n=197) and were higher after the second and third injections compared with after injection 1 (table 6). 4 weeks after the third injection, GMTs were in the range 146 against DENV1 to 405 against DENV3, decreasing to values in the range 76·5–153 at 1 year after the third injection. In the control group (n=99), GMTs were in the range of 20–50 at each timepoint and were highest against DENV2 and DENV3.

	Dengue vaccine group (n=197)			Control group (n=99)		
	m	GMT (95% CI)	Seropositive* (n, %)	m	GMT (95% CI)	Seropositive* (n, %)
Baseline						
Serotype 1	197	42·8 (30·7–59·6)	108 (55%)	99	26·6 (17·6–40·2)	48 (48%)
Serotype 2	197	56·8 (40·3–80·1)	115 (58%)	98	43·7 (27·8–68·7)	57 (58%)
Serotype 3	197	31·5 (24·2–41·0)	119 (60%)	99	28·7 (19·3–42·6)	55 (56%)
Serotype 4	197	28·1 (21·7–36·4)	111 (56%)	99	23·2 (15·6–34·6)	45 (45%)
28 days after first injection						
Serotype 1	197	94·4 (66·4–134·3)	144 (73%)	98	27·7 (18·1–42·3)	47 (48%)
Serotype 2	197	195 (143–266)	172 (87%)	99	42·9 (27·2–67·6)	56 (57%)
Serotype 3	197	111·9 (85·8–145·9)	169 (86%)	99	27·0 (18·3–39·8)	52 (53%)
Serotype 4	197	138 (106–178)	169 (86%)	99	24·2 (16·4–35·8)	46 (46%)
28 days after second injection						
Serotype 1	94	120·7 (79·4–183·5)	84 (89%)	49	21·9 (12·4–38·6)	22 (45%)
Serotype 2	94	326 (230–462)	93 (99%)	49	43·5 (23·3–81·1)	28 (57%)
Serotype 3	94	195 (144–263)	93 (99%)	49	24·6 (14·4–42·1)	27 (55%)
Serotype 4	94	159 (121–210)	91 (97%)	49	21·2 (12·3–36·4)	22 (45%)
28 days after third injection						
Serotype 1	95	146·1 (98·5–216·7)	90 (95%)	49	23·9 (14·0–40·9)	27 (55%)
Serotype 2	95	310 (224–431)	94 (99%)	49	52·2 (26·8–101·7)	29 (59%)
Serotype 3	95	405 (307–534)	95 (100%)	49	48·9 (25·5–93·9)	29 (59%)
Serotype 4	95	155 (123–196)	93 (98%)	49	19·4 (11·6–32·2)	21 (43%)
1 year after third injection						
Serotype 1	95	76·5 (48·2–121·5)	73 (77%)	48	20·7 (12·0–35·8)	22 (46%)
Serotype 2	95	122·5 (78·8–190·4)	81 (85%)	48	38·5 (20·6–72·1)	27 (56%)
Serotype 3	95	94·8 (65·2–137·9)	85 (89%)	48	25·6 (14·5–45·3)	26 (54%)
Serotype 4	95	153 (110–212)	89 (94%)	48	37·5 (20·1–69·2)	26 (54%)

m=number of participants per protocol at that point in the study and for whom data are available for that endpoint. $PRNT_{50}$=plaque-reduction neutralisation test. GMT=geometric mean titre. *Titre 10 or higher.

Table 6: **Geometric mean $PRNT_{50}$ antibody titre against vaccine parental dengue strains at baseline and after each injection (per-protocol immunogenicity analysis)**

Discussion

This phase 2b, proof-of-concept study was designed with the primary objective of establishing efficacy against virologically confirmed dengue of any serotype after three injections, under the assumption that the number of cases would not allow a meaningful estimation of serotype-specific efficacy. Our primary estimate of efficacy was lower than projected and was not significant. However, the number of observed cases was substantially higher than expected, allowing us to consider efficacy per serotype. Efficacy estimates against DENV1, 3, and 4 were in a range consistent with our assumed overall efficacy of 70% after three injections, and these estimates were significant after at least one vaccination, but not after the third possibly because of the lower number of cases. Conversely, efficacy was not shown against DENV2. This lack of efficacy against DENV2, and the fact that DENV2 was the prevalent serotype during the study, diminished the overall vaccine efficacy in this setting (panel).

More than 91% of enrollees completed the study per protocol, and the active surveillance system implemented for the study successfully collected acute blood samples for virological confirmation from more than 2200 febrile episodes within 7 days of fever onset. This high compliance probably reflects the importance of site preparation activities, and beyond that the importance of dengue for the study team and the population of Ratchaburi.[15–17]

The vaccine's safety and reactogenicity profile was good and consistent with previous clinical trials using the recombinant CYD dengue vaccine technology.[7,9–12] The absence of vaccine-related SAEs or any other safety signal after 2 years of active follow-up of more than 2600 vaccinated children is particularly noteworthy. Theoretical safety concerns associated with the potential increase in the rate or severity of dengue disease by an incomplete immune response against the four serotypes of dengue

***Panel:* Research in context**

Systematic review

A search of PubMed with the search terms "dengue", "vaccine", and "efficacy" did not show any previous clinical study of efficacy of a dengue vaccine. No date or language restrictions were applied.

Interpretation

Although the primary estimate of efficacy in our study was lower than projected and was not significant, the study's secondary findings have major implications for the continuing development of dengue vaccines. This candidate vaccine was immunogenic for all four serotypes and protected against three of the four serotypes (1, 3, and 4) at levels consistent with the initial hypothesis. Against the fourth serotype, however, no protection was seen in this setting despite satisfactory immunogenicity, and this factor affected the primary outcome. These findings challenge the vaccine development hypothesis that by inducing balanced levels of neutralising antibodies as measured by plaque-reduction neutralisation test, tetravalent vaccination would provide similar levels of protection against the four serotypes.[7,21] Crucially, the antibody-dependent enhancement hypothesis that has hampered vaccine development to date was not borne out by this study.[5,7] There was no sign of enhanced disease in breakthrough cases after vaccination with this tetravalent YF17D-based recombinant dengue vaccine during the 2 years of study follow-up.

have hampered vaccine development.[5,7] In this context, the absence of any sign of disease enhancement after 2 years of follow-up after the first injection, in the presence of non-protective immune responses against the circulating DENV2 viruses, is an important and reassuring finding. Indeed, the duration of fever and the rate of admission to hospital were no higher in cases in the vaccine group compared with the control group, and there was no excess in severe cases or in the classic clinical signs of dengue such as bleeding, plasma leakage, or thrombocytopenia.

The observed lack of efficacy against DENV2 despite satisfactory immunogenicity is surprising and will need further investigation. It leads us to question the robustness of the assumption that to protect against dengue, vaccination must induce balanced immunogenicity against all four serotypes as assessed by $PRNT_{50}$, regarded as the most relevant assay to measure antibodies that protect epithelial cells from dengue virus infection.[21] In vivo, however, Fc receptor-bearing cells are crucially important in supporting dengue infection. Testing the biological relevance of measuring DENV neutralisation in a system modelled on in-vivo target cells therefore deserves further study.

An antigenic mismatch between the CYD2 vaccine virus and the DENV2 virus or viruses that caused disease in our cohort is possible. The Asian 1 genotype of DENV2 circulating in southeast Asia consists of several lineages, one of which has mutations in domain 2 of the E protein (at E83, E226, and E228) that could have implications for viral fitness.[24] The aminoacid residues at these positions in the donor wild-type virus for the CYD2 vaccine (PUO-218) correspond to those of the Asian/American genotype.[24,25] Antibody responses after vaccination against DENV2 were higher than those against DENV1 and DENV3, and were similar to responses noted in earlier studies in the region.[10,12] A previous study in non-human primates showed that sera raised against CYD-TDV neutralise in an SN_{50} assay a broad range of isolates across all the DENV serotypes, genotypes, geographical origins, and isolation years,[26] and this could still be the case for the DENV2 viruses circulating in our study. Whether immune responses to DENV non-structural proteins (which are not encoded in the CYD vaccine viruses) contribute to the overall protective response to DENV2 also needs further clarification. Also, the DENV2 antibody titres might not have been high enough to protect against this serotype or the particular lineage of viruses circulating in Ratchaburi during the study period. The monocentre design of this phase 2b study in a single area of Thailand and the predominance of this one serotype therefore constitutes its principal limitation.

The worldwide dynamics of dengue viruses are complex. The prevailing serotype and the distribution of viruses differ from region to region and evolve with time as a result of serotype and lineage replacement events.[4,15,24,27–29] Intrinsic and extrinsic host factors can also play a part. Under different epidemiological circumstances, the vaccine as currently formulated might provide significant benefit. Further evaluation of vaccine efficacy in different epidemiological settings, against a wide range of dengue viruses of differing virulence and viral fitness, circulating in both epidemic and endemic contexts, is important. Ongoing phase 3 efficacy studies in more than 30 000 volunteers in ten countries in Latin America and Asia will provide pivotal data for this candidate vaccine's efficacy (NCT01374516 and NCT01373281).

In conclusion, although the assumed high efficacy against all four serotypes of dengue virus was not shown, our study constitutes the first ever demonstration that a safe dengue vaccine is possible. In the context of WHO goals to reduce dengue mortality by at least 50% and the morbidity rate by at least 25% by 2020,[30] this study represents a major milestone. These findings have important implications for dengue vaccine development and support the continued evaluation of this dengue vaccine based on the recombinant CYD dengue vaccine technology.

Contributors

All authors participated in the design or implementation of the study, or the analysis and interpretation of findings. AS, TAW, AB, and JL were involved in all phases of the study. TAW, AB, NGT, MS, and JL contributed to the study design. AS was the principal investigator. AS, CS, KL, PC, SS, VJ, WD, and KP contributed to the implementation of the study and supervision at the site. DW was the sponsor's clinical study leader and responsible medical officer throughout the study. TAW was responsible for the safety follow-up during the study. AM designed and did the statistical analysis. AS, CS, DW, SV, NGT, and JL interpreted the efficacy results. AS, DW, CS, KL, PC, VJ, WD, KP, TAW, AM, MS, AB, SV, NGT, and JL had access to the study data, contributed to data interpretation, and reviewed and revised drafts of this report; all approved the final version. The report was written by Grenville Marsh (Sanofi Pasteur).

Conflicts of interest

DW, TAW, AM, MS, AB, SV, NGT, and JL are employees of Sanofi Pasteur and own shares or stock options in the company. AS, CS, KL, PC, SS, VJ, WD, and KP declare that they have no conflicts of interest.

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