Safety and Immunogenicity of Recombinant, Live Attenuated Tetravalent Dengue Vaccine (CYD-TDV) in Healthy Vietnamese Adults and Children

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Abstract

Background: Dengue viruses (DENV1-4) are estimated to infect 50-100 million individuals per year worldwide including an estimated 500,000 people with severe dengue who require hospitalization every year. The live, attenuated, tetravalent dengue vaccine (CYD-TDV) candidate, containing four recombinant dengue viruses (CYD1-4), is in clinical phase III.

Methods: In an observer-blind, phase II trial in Long Xuyen, Vietnam, 180 children and adults (range: 2-45 years) were randomized 2:1 to receive 3 CYD-TDV vaccinations at months (M) 0, 6 and 12 or meningococcal vaccine in the control group. Reactogenicity of CYD-TDV decreased after each vaccination, was slightly higher (SAEs), unrelated to vaccination, were reported including 2 virologically-confirmed dengue cases after the second vaccination in the control group. Reactogenicity of CYD-TDV decreased after each vaccination, was slightly higher than placebo, but not higher than either active control.

Results: At baseline 139(77%) were seropositive (titer ≥ 10 l/dil) against dengue or Japanese encephalitis; 36% were seropositive to all four dengue serotypes. After the first CYD-TDV vaccination, 53% were seropositive to all four serotypes, increasing to 72% and 92% after the second and third vaccinations. In the control group seropositivity against all four dengue serotypes was 28% at baseline and slightly increased at 36% after the third vaccination 13 months later. After the third CYD-TDV vaccination, 96% were seropositive to at least 3 serotypes, and geometric mean titers against DENV1-4 were respectively 129, 216, 169, and 146. Six serious adverse events (SAEs), unrelated to vaccination, were reported including 2 virologically-confirmed dengue cases after the second vaccination in the control group. Reactogenicity of CYD-TDV decreased after each vaccination, was slightly higher than placebo, but not higher than either active control.

Conclusions: Safety and reactogenicity of CYD-TDV were satisfactory and consistent with results from phase I and other phase II studies. Three doses of CYD-TDV induced a balanced neutralizing antibody response against the four dengue serotypes in children and adults living in a dengue endemic country.

Keywords: Dengue; Live attenuated tetravalent; CYD-TDV dengue vaccine; Recombinant vaccine; Safety; Immunogenicity; Phase II trial

Introduction

In recent decades dengue has expanded in tropical and subtropical areas around the world to become one of the most important vector-borne diseases of humans, with around 2.5 billion people living in regions where the disease is transmitted [1]. The overall annual burden of dengue as reported by World Health Organization (WHO) is an estimated 50-100 million dengue infections and 500,000 hospitalizations for severe form of the disease [1]. This burden is projected to continue to increase, notably in association with the geographic expansion of the mosquito vectors (i.e., Aedes aegypti and Aedes albopictus) [2].

It is a high public priority to develop a vaccine against dengue, specifically for highly endemic areas such as the Mekong delta, considering that no specific treatment is available, and the high cost and challenges around vector control programs to sustainably control dengue [3]. The impact of dengue on patients and their families is important in terms of economics and quality of life, the health burden is considerable for endemic regions [4].

Several candidate vaccines against dengue are in clinical or pre-clinical development, one of which is currently being evaluated in clinical efficacy trials in Asia and Latin America [5]. This vaccine – the CYD tetravalent dengue vaccine has been developed in compliance with WHO guidelines for the production and quality control of candidate tetravalent dengue vaccine [6], guidelines for the clinical evaluation of dengue vaccines in endemic areas [7], and guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses [8].

This vaccine contains four recombinant, live, attenuated viruses (CYD-1–4) developed based on the attenuated yellow fever vaccine virus, YF-17D, from which the genes encoding for pre-membrane and
envelope proteins were removed and replaced with those of one of the four wild-type dengue viruses, one per serotype [5]. Phase 1 and early phase 2 trials have shown that a three-dose regimen of CYD-TDV elicits a balanced neutralizing antibody response against all four serotypes in flavivirus-naive populations, as well as in populations previously exposed to dengue. The safety and reactivity profile observed in these studies were considered to be promising, and provided reassurance to progress to larger scale trials [5].

Viet Nam has had amongst the highest dengue disease burden in the Asia Pacific region, with an estimated 1 million dengue cases and more than 2000 deaths during the period 1991–2004 [9]. The Mekong Delta area of Southern Viet Nam is particularly affected, with reported incidence rates among school-age children of 16.9–40.4 per 1000 person-years [10]. All four dengue serotypes have co circulated in this area in recent years, usually with one serotype dominating at a given time [11,12]. This dominant serotype however changes periodically [9].

We report on the first clinical study of CYD-TDV in Viet Nam, conducted in an area where dengue is highly endemic. Dengue and Japanese encephalitis (JE) viruses are the primary vector-borne flaviviruses in the country, [13] and while JE vaccine is included in the national immunization program this had only been partially implemented in the South of Viet Nam at the time of the study.

Methods

Trial design and participants

This randomized, controlled, blind-observer, monocenter, phase II study took place in Long Xuyen city, An Giang province of Viet Nam. We recruited 180 individuals from four age cohorts (respectively 60, 60, 30, and 30 aged 2–5, 6–11, 12–17, and 18–45 years), and randomized them in 2:1 ratio to receive CYD-TDV or control vaccines at Months 0, 6 and 12.

Individuals aged 2–45 years and in good health based on medical history, normal physical examination and laboratory parameters were eligible for inclusion. The main exclusion criteria were: personal or family history of thymic pathology, thymectomy or myasthenia; HIV, hepatitis B or C seropositivity tested at screening; immunodeficiency or receipt of immunosuppressive treatment; systemic hypersensitivity to any vaccine component; history of life threatening reaction to the trial vaccine or to vaccine containing any of the same substances; family history of thymic pathology, thymectomy or myasthenia; HIV, and administered in blind-observer conditions to prevent safety assessment bias.

Safety and reactogenicity

Participants were kept under observation for 30 minutes after each vaccination and were followed-up for safety until 6 months after the last vaccination. Participants or parents were provided with a thermometer, a ruler and a diary card to record any adverse event (AE) following each vaccination. The following AEs were documented and graded using a pre-defined severity scale as reported elsewhere solicited injection site reactions within 7 days (i.e., pain, erythema and swelling), solicited systemic reactions within 14 days (i.e., fever, headache, malaise, myalgia, and asthena) and unsolicited AEs up to 28 days after each vaccination. All serious adverse events (SAE) were documented up to 6 months after the last vaccination.

CYD-TDV vaccine viremia

Cases of fever with body temperature ≥ 38°C for ≥ 48 hours within 28 days after vaccination were assessed for CYD-TDV viremia by quantitative RT-PCR. Serology and viremia analyses were performed at the sponsor’s global clinical immunology laboratory (dengue PRNT, RT-PCR; Swift water, PA) and at the Center for Vaccine Development (dengue ELISAs, JE PRNT; University of Mahidol, Thailand). All testing was performed under blind conditions.

Serology

In serum collected at screening, Japanese encephalitis virus neutralizing antibodies levels were assessed by a plaque reduction neutralization test with a 50% endpoint (PRNT50) as described elsewhere [13,14]. Neutralizing serum antibody responses against dengue were measured using PRNT50 before and 28 days after each vaccination. Briefly, serial two-fold dilutions of heat-inactivated serum were mixed with a constant challenge dose of the dengue vaccine’s four parental dengue wild-type viruses (target 80 PFU per well for each serotype) and inoculated into wells of a 24-well plate of confluent Vero cells. The neutralizing antibody titer was calculated and expressed as the highest reciprocal serum dilution (l/dil) reducing the mean plaque count by 50% as compared with the mean virus plaque number obtained from the control wells. The lower limit of quantization (LLOQ) of the dengue PRNT50 was 10 (l/dil) and seropositivity rate expressed the percentage of subjects with seropositive samples.

Passive detection and diagnosis of dengue fever

In the event of a febrile episode with a body temperature of ≥ 38°C for ≥ 48 hours and suspected dengue, participants/parents were instructed to return to the study center for laboratory diagnosis using quantitative dengue RT-PCR, and by commercial ELISA kits: NS1Ag ELISA (Plateia”, Biorad Laboratories, Marnes-La-Coquette, France),

The study site fulfilled criteria set out in WHO guidelines for clinical evaluation of dengue vaccines in endemic areas [7].

Vaccines

The CYD vaccine is composed of 5 ± 1 log10 cell–culture infectious dose 50% (CCID50) of each live, attenuated, dengue serotype 1,2,3,4 virus using NaCl 0.4% containing human serum albumin 2.5% as solvent. Control vaccines given at Months 0, 6 and 12 were, respectively: licensed meningococcal polysaccharide vaccine A+C (Mencare-A/C/Y/W-135™, Sanofi Pasteur, France), saline placebo (the solvent used to reconstitute the CYD vaccine), and licensed typhoid Vi polysaccharide vaccine (Typhim Vi™, Sanofi Pasteur, France). Vaccines were prepared and administered in blind-observer conditions to prevent safety assessment bias.
and Dengue IgM and IgG ELISA (EL1500M and EL1500G respectively, Focus Diagnostics Inc, CA, USA).

**Statistical method**

All the main analyses were descriptive. For the main parameters, 95% Confidence Intervals of point estimates were calculated using the normal approximation for quantitative data and exact binomial distribution with Clopper-Pearson method for proportions. The sample size was arbitrarily set to 120 subjects in the dengue group and 60 subjects in the control group. Antibody titers were summarized by group and age cohort before and after each vaccination in terms of geometric mean titer (GMT), number and percentage with titer ≥ 10 l/dil against each dengue serotype (seropositivity) and against at least one, two, three, or the four dengue serotypes. Analyses included all participants present at the first visit and having received at least one vaccination (i.e., safety analysis set [SAS] and full analysis set [FAS] with available results from blood taken after vaccination).

### Results

#### Demographics and flavivirus immune status

Between March and July 2009, we enrolled 180 participants (Figure 1) and vaccinated them with a first injection of CYD-TDV (N=120) or control vaccine (N=60); 172(96%) completed the study up to 6 month after the last vaccination. There were 6 early withdrawals in the dengue vaccine group and 2 in the control vaccine group. There were no discontinuations for safety reasons and none were lost to follow-up.

There were slightly more males enrolled in the control group (58%) than in the dengue vaccine group (48%). The mean age, height, weight, and body mass index (BMI), were comparable between groups (Table 1). At baseline, a high proportion of 77% of participants (76% in the dengue vaccine group and 80% in the control vaccine group) were seropositive against dengue or JE, and this proportion increased with age (Table 1). Almost 40% of participants had a baseline neutralizing antibody response against at least one dengue serotype: 39% (47/120) in the dengue vaccine group, 38% (23/60) in the control group. Seropositivity rates against dengue serotypes 1,2,3, and 4 were, respectively: 25%, 18%, 53%, and 23% among 2–5 year-olds, 50%, 53%, 55%, and 43% among 6–11 year-olds, 60%, 65%, 70%, and 56% among 12–17 year-olds, and 95%, 95%, 95%, and 85.0% among 18–45 year-olds. Baseline seropositivity rates against the JE virus was similar in the dengue group (37% [95% CI: 28.3;46.3]) and the control group (42% [95% CI: 29.1;55.1]).

#### Safety and reactogenicity

Table 2 shows the safety overview after any vaccination, by age cohort. Overall and by age group, solicited reactions were less frequently observed in the dengue vaccine group than in the control group. This was due to the solicited injection site reactions, reported for 33% [95%CI: 24.2;41.7] for the dengue vaccine group than and 80% [95% CI: 67.7;89.2] in the control group. The reporting rates of solicited systemic reactions were similar between groups (Table 2). After dengue vaccination, reporting rates of solicited reactions and unsolicited adverse events appeared lower in children (i.e., 2–5 yo and 6–11 yo) than in adolescents (i.e. 12–17 yo) or adults (18–45 yo). The reporting rates of unsolicited adverse events were similar between groups (Table 2). No adverse event led to study discontinuation, and there were no vaccine-related serious adverse events (SAE) (Table 2).

Injection site reactions after dengue vaccination were of grade 1 or 2 severity and resolved spontaneously, while a few grade 3 erythema and swelling reactions were recorded among 6–11 year-olds in the control group (Table 3). Most solicited injection site reactions after dengue vaccination were of grade 1 severity, and appeared within 3 days (data not shown). A few grade 3 systemic reactions (fever among 2–5 year-olds, and headache and asthenia among 18–45 year-olds) were reported after dengue vaccination (Table 3).
When considering reactogenicity specifically after each vaccination, solicited injection site reactions reported were more frequently after Meningococcal A+C vaccination (75% [95%CI: 62.1; 85.3]) than after the first dose of CYD-TDV (20% [95%CI: 13.3; 28.3]), and were more frequent after Typhoid fever vaccination (43% [95%CI: 30.2; 56.8]) than after the third dose of CYD-TDV (13% [95%CI: 7.6; 20.8]). The solicited reactions rate of the control vaccine was comparable to the dengue vaccine group for the second injection respectively 15.5% [95%CI: 7.2; 27.4] and 18.1% [95%CI: 11.6; 26.3]. For the third vaccination, the solicited injection site reactions rate reported in the control group with

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Subjects experiencing at least one/n, % (95%CI)

- Solicited reaction: any adverse event (AE) pre-listed in the protocol that is automatically considered to be vaccine-related, and as such termed an 'adverse reaction' (AR)
- Solicited injection site reaction: any AR associated with the site of injection that is pre-listed in the protocol (pain, erythema, swelling)
- Solicited systemic reaction: any AR not associated with the site of injection that is pre-listed in the protocol (fever, headache, malaise, myalgia, asthenia)
- Unsolicited AE: any observed AE that is not a solicited injection site or solicited systemic reaction
- Unsolicited AR: any unsolicited AE that is considered to be vaccine-related
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SAE: any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, or is an important medical event

Table 2: Safety overview after any vaccination by age cohort.

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Subjects experiencing at least one / n, %

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| Pain                     | Any      | 5, 13% | 12, 30% | 10, 50% | 7, 35% | 34, 29% | 13, 65% | 19, 95% | 9, 90% | 7, 70% | 48, 80% |
| Erythema                 | Any      | 1, 3%  | 5, 13%  | 2, 10%  | 0      | 8, 7%  | 3, 15%  | 4, 20%  | 3, 30%  | 3, 30%  | 13, 22% |
| Swelling                 | Any      | 0      | 2, 5%   | 1, 5%   | 2, 10% | 5, 4%  | 4, 20%  | 4, 20%  | 0      | 1, 10% | 9, 15%  |
| Fever                    | Any      | 12, 30%| 10, 25% | 9, 45%  | 2, 10% | 33, 28%| 6, 30%  | 5, 25%  | 2, 20% | 3, 30% | 16, 27% |
| Headache                 | Any      | 1, 3%  | 1, 1%   | 1, 2%   | 10, 50%| 9, 45%  | 42, 35% | 5, 25%  | 7, 35%  | 5, 50%  | 1, 10%  | 18, 30% |
| Malaise                  | Any      | 7, 18% | 9, 23%  | 11, 55% | 8, 40% | 35, 29%| 2, 10%  | 4, 20%  | 3, 30%  | 5, 50%  | 14, 23% |
| Myalgia                  | Any      | 3, 8%  | 8, 21%  | 8, 40%  | 5, 25% | 24, 20%| 3, 15%  | 3, 15%  | 5, 50%  | 3, 30%  | 14, 23% |
| Asthenia                 | Any      | 3, 8%  | 6, 15%  | 5, 25%  | 2, 10% | 16, 13%| 2, 10%  | 0      | 0      | 1, 10%  | 3, 5%   |

When considering reactogenicity specifically after each vaccination, solicited injection site reactions reported were more frequently after Meningococcal A+C vaccination (75% [95%CI: 62.1; 85.3]) than after the first dose of CYD-TDV (20% [95%CI: 13.3; 28.3]), and were more frequent after Typhoid fever vaccination (43% [95%CI: 30.2; 56.8]) than after the third dose of CYD-TDV (13% [95%CI: 7.6; 20.8]). The solicited reactions rate of the control vaccine was comparable to the dengue vaccine group for the second injection respectively 15.5% [95%CI: 7.2; 27.4] and 18.1% [95%CI: 11.6; 26.3]. For the third vaccination, the solicited injection site reactions rate reported in the control group with
Typhoid fever vaccine was higher (43.1% [95% CI:30.2; 56.8]) than in the dengue vaccine group (13.2% [95%CI: 7.6; 20.8]) (data not shown).

Solicited systemic reactions rates diminished after each successive dose of the dengue vaccine, and were similar in the control and dengue vaccine groups; respectively 48% (95%CI: 35.2; 61.6) and 42% (95%CI: 32.7; 51.0) after the first dose, 17% (95%CI: 8.6; 29.4) and 27.6% (95%CI: 19.7; 36.7) after the second dose, and 24% (95%CI: 13.9; 37.2) and 15% (95%CI: 8.9; 22.8) after the third. Overall, after any vaccination, headache (35%) was the most frequently reported solicited systemic reaction in the dengue group followed by malaise (29%), fever (28%), myalgia (20%) and asthenia (13%) while in the control group, the following reactions were reported: headache (30%), fever (27%), malaise (23%), myalgia (23%) and asthenia (5%). A summary of safety results according to age group is shown in table 3.

### Serious adverse events

There were six serious adverse events (SAEs) due to hospitalization, none of which were related to vaccination. Three occurred in the dengue group (3/120, 2.5%), and three occurred in the control group (3/60, 5%). The three SAEs in the dengue group were acute gastritis, acute Pharyngitis, and dengue hemorrhagic fever (DHF) grade 1 meeting the 1997 WHO case definition [15]. The three SAEs in the control group were confirmed grade I DHF due to serotype 1, one virologically confirmed grade II DHF also due to serotype 1, and one serologically probable grade I DHF. In the dengue vaccine group, there was one hospitalized grade I DHF in an 18-year-old male subject that occurred 93 days after the first vaccination. Virological tests for dengue were negative, although anti-dengue IgM was detected in acute and convalescent samples. He was seropositive for both dengue and JE viruses at baseline and on Day 28 after the first vaccination, his neutralizing antibody levels against serotypes 1–4 were 106, 191, 86, and 169 respectively. Of the 7 suspected dengue cases, dengue infection was therefore virologically confirmed in 2 cases in the control group (N=60), and none in the dengue vaccine group (N=120).

### Immunogenicity

Antibody responses increased after each of the three doses of dengue vaccines, CYD-TDV against each serotype for all four serotypes in all age cohorts (Figure 2). On Day 28 after the complete 3-dose CYD-TDV schedule, GMTs against serotypes 1–4 were respectively 129, 216, 169, and 146, 92% of vaccines were seropositive to all 4 dengue serotypes, compared with 36.2% in the control group (Table 4). Antibody responses after vaccination appeared to increase with increasing age with GMTs for each serotype after the third CYD-TDV dose in the range 64.7–143 among 2–5 years-old, 93.9–185 among 6–11 years-old, 135–334 among 12–17 years-old, and 375–825 among adults (Figure 2).

Antibody responses to CYD-TDV were higher among those who were flavivirus seropositive at baseline compared with those who were flavivirus seronegative (Figure 3). In the flavivirus positive subgroup, GMTs increased to values in the range 187–316 after 3 doses of CYD-TDV, at which point 97.7% were seropositive against all four serotypes. In those who were flavivirus negative, GMTs increased to values in the range 31.2–75.5, at which point 75% were seropositive against all four serotypes.

### Discussion

This is the first clinical trial of a dengue vaccine candidate in...


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9. World Health Organization. Regional Office for South-East Asia and for the Western Pacific, Health in Asia and the Pacific 2008, New Delhi, India. WHO Regional Office for South-East Asia: 539.


