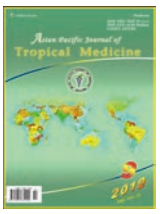

Original Article
Asian Pacific Journal of Tropical Medicine
journal homepage: www.apjtm.org

doi: 10.4103/1995-7645.267582 Impact factor:1.77

# Long-term safety follow-up of children from a randomized-controlled phase Ⅱb proof-of-concept efficacy study of the live, attenuated, tetravalent dengue vaccine (CYD-TDV) in Thailand

Kriengsak Limkittikul[1], Weerawan Hattasingh[1], Danaya Chansinghakul[2✉], Arunee Sabchareon[1], Wut Dulyachai[3], Carina Frago[4], T Anh Wartel[4], Edith Langevin[5], Sophia Gailhardou[6], Alain Bouckenooghe[4]

[1]*Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok, 10400, Thailand*
[2]*Sanofi Pasteur Clinical Sciences, 87/2 CRC Tower 23rd Floor, All Seasons Place, Wireless Road, Lumpini, Pathumwan, Bangkok, Thailand*
[3]*Ratchaburi Hospital, 77/4, Kathatorn Road, Tambon Na Muang, Amphoe Muang Ratchaburi, Thailand*
[4]*Sanofi Pasteur Clinical Sciences, 38 Beach Road #18-11 South Beach Tower, Singapore 189767*
[5]*Sanofi Pasteur Clinical Programs, Marcy L'Etoile, France*
[6]*Sanofi Pasteur Global Pharmacovigilance, Lyon, France*

ARTICLE INFO

*Article history:*
Received 19 April 2019
Revised 20 June 2019
Accepted 6 August 2019
Available online 30 September 2019

*Keywords:*
CYD-TDV
Dengue vaccine
Hospitalized dengue
Severe dengue
Virologtically confirmed dengue

ABSTRACT

**Objective:** To investigate the long-term safety of a tetravalent dengue vaccine (CYD-TDV) in children in a phase Ⅱb follow-up study in Thailand.
**Methods:** In the phase Ⅱb study, children aged 4-11 years were randomized (2:1) to receive three injections of CYD-TDV or serve as control at 6-month intervals, with 25 months' active follow-up (active phase). This study was an additional four-year passive surveillance for hospitalized virologically-confirmed dengue (VCD; hospital phase). Cases of hospitalized VCD, severe hospitalized VCD, vaccine-related serious adverse events, and deaths were reported for the total population, with *post-hoc* analyses by enrollment age (<9 and ⩾9 years).
**Results:** Of 3 997 participants receiving ⩾1 injection, 80.1% were recruited to the hospital phase [2 131 (CYD-TDV); 1 072 (control)]. Eighty-five hospitalized VCD cases were reported in the CYD-TDV group and 46 in the control group during the four-year hospital phase [relative risk (RR): 0.93, 95% confidence interval (*CI*): 0.64-1.36]. The RR over six years of follow-up was 0.77 (95% *CI*: 0.57-1.05). In those aged ⩾9 years, the cumulative RRs in the active phase, hospital phase, and entire six years were 0.28 (95% *CI*: 0.08-0.81), 0.51 (95% *CI*: 0.25-1.05), and 0.42 (95% *CI*: 0.24-0.75), respectively. In the overall population, there were ten severe hospitalized VCD cases in the CYD-TDV group and five in the control group over six years (RR: 1.00, 95% *CI*: 0.31-3.75).
**Conclusions:** Over six years of follow-up, in children aged ⩾9 years, CYD-TDV administration is associated with a reduced risk of hospitalized VCD.

## 1. Introduction

The efficacy of a tetravalent dengue vaccine (CYD-TDV) was first evaluated in the phase Ⅱb proof-of-concept study, CYD23 (NCT00842530)[1]. CYD23 was a randomized, controlled study conducted at a single center in Thailand with enrollment initiated in February 2009 and active follow-up for 25 months. The vaccine was well-tolerated and had overall efficacy of 30.2% against virologically-confirmed dengue (VCD) during the first 25 months[1]. CYD-TDV efficacy was subsequently confirmed in two pivotal phase Ⅲ studies that included over 30 000 children from Asia/Pacific and Latin America. Vaccine efficacy against

✉Corresponding author: Danaya Chansinghakul, Sanofi Pasteur Clinical Sciences, 87/2 CRC Tower 23rd Floor, All Seasons Place, Wireless Road, Lumpini, Pathumwan, Bangkok 10330, Thailand.
Tel: +66 2 2648813
E-mail: danaya.chansinghakul@sanofi.com



**How to cite this article:** Limkittikul K, Hattasingh W, Chansinghakul D, Sabchareon A, Dulyachai W, Frago C, et al. Long-term safety follow-up of children from a randomized-controlled phase Ⅱb proof-of-concept efficacy study of the live, attenuated, tetravalent dengue vaccine (CYD-TDV) in Thailand. Asian Pac J Trop Med 2019; 12(9): 396-403.

symptomatic VCD was 56.5%[2] and 64.7%[3] in the two regions, in 2-14 years old and 9-16 years old children, respectively. In addition, an integrated analysis of these three clinical trials, encompassing more than 33 000 children aged 2-16 years, demonstrated efficacies against symptomatic dengue during the first 25 months of 60.3% for all participants, 65.6% for those aged ⩾9 years, and 44.6% for those aged <9 years[4]. CYD-TDV was efficacious against all four dengue virus serotypes, albeit to varying degrees[4].

Concerns about excess hospitalizations for dengue among children aged 2-5 years vaccinated with CYD-TDV prompted an assessment of the effect of baseline dengue serostatus in a *post–hoc* analysis of data pooled from the three efficacy studies[5]. Among seropositive participants, the risk of severe VCD and hospitalization for VCD across all ages was approximately 70% lower in vaccinated participants *vs*. controls; however, among seronegative participants the rates of hospitalization for VCD and severe VCD were higher in the vaccinated participants versus controls[5].

Long-term monitoring for severe dengue in vaccinated participants was recommended in selected areas[6] to assess the risk of vaccine-induced sensitization/antibody-dependent enhancement leading to severe disease with subsequent wild-type dengue infection[7,8]. Expert consensus suggests a follow-up period of at least 3-5 years after the last dose was needed to confirm safety[9,10]. In this study, all participants in CYD23 trial were invited to participate in a passive, prospective, four-year, safety follow-up study, CYD57 (NCT01983553). We present here the safety data for the entire six-year follow-up period following the first injection.

## 2. Materials and methods

### 2.1. Study design and participants

The methodology of the phase Ⅱb randomized double-blind proof-of-concept study has been previously described[1]. In brief, healthy children aged 4-11 years, living in the Muang District, Ratchaburi Province of Thailand, were randomized 2:1 to receive three injections of CYD-TVD or those serving as a control (rabies vaccine or saline placebo) at Months 0, 6, and 12, with active follow-up until month 25 after first injection (active phase of the study)[1]. Those participants who received at least one study injection were eligible for inclusion in this follow-up study; initially it was planned to last for two years after completion of the active phase of the study (*i.e.*, up to four years after the first injection). However, this follow-up period was extended following Independent Data Monitoring Committee (IDMC) guidance for up to four years after the end of the active phase, *i.e.* six years after the first dose (Figure 1), and it is consistent with World Health Organization (WHO) guidelines for the evaluation of dengue vaccines in endemic areas[9,11]. Participants or their parents/guardians received yearly follow-up visits or calls.

### 2.2. Randomization and masking

The methods describing randomization, masking, and how participants were allocated to the study groups have been previously described in detail[1]. No study vaccinations were administered during this follow-up, with participants and site staff blinded to treatment allocation until the end of the entire six-year follow-up.

### 2.3. Ethics

The trial was undertaken in compliance with good clinical practice guidelines and the principles of the Declaration of Helsinki. The protocol and amendments for the initial active phase of the study were approved by the Ministry of Public Health (MoPH) and Ethics Committees of the Ministry of Public Health, Thailand; this follow-up study was approved by the Faculty of Tropical Medicine, Mahidol University and the Ethical Review Committee for Research in Human Subjects, MoPH, Thailand [protocol approved 4 September 2013 (reference No. 5/2556); amendments approved 15 May 2014]. Parents or legal guardians provided informed consent before continued participation, and written assent was received from participants aged seven years and older.

### 2.4. Long–term monitoring

Hospitalized VCD cases were identified and recorded over the duration of the four-year passive surveillance study. In the event of an acute febrile illness, participants or their parents/guardians were requested to present to the Ratchaburi Regional Hospital, where the child was examined by a pediatrician with experience of dengue, and received appropriate supportive treatment. If the child required

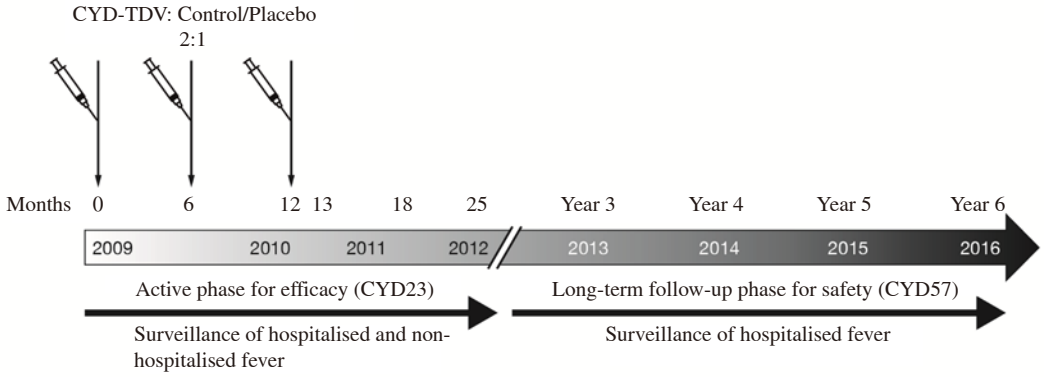


**Figure 1.** Schematic of the study design. Participants were enrolled into the original randomized-controlled phase Ⅱb study (CYD23; active phase) between Feb 5, 2009 and Feb 5, 2010 and the long term follow-up (CYD57) concluded with the last participant last follow-up on Feb 19, 2016. CYD-TDV: tetravalent dengue vaccine.

hospitalization, blood samples were taken during the acute phase of the acute febrile episode (as soon as possible, but no later than seven days after the onset of fever) and again during the convalescent phase (7-14 d after the acute sample) for virological and serological dengue tests as previously described[1]. Other health clinics in the district were also asked to refer study participants with acute febrile episodes to the Ratchaburi Regional Hospital when hospitalization was required.

Hospitalized VCD was defined as fever lasting for ≥1 d (temperature ≥37.5 ℃ measured at least twice with an interval of ≥4 h), with inpatient hospitalization and positive dengue viremia confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) or dengue non-structural protein 1 (NS1) enzyme-linked immunosorbent assay (ELISA) antigen test. These cases were reviewed by the IDMC who determined disease severity using the IDMC severity definitions (Supplementary materials) and WHO 1997 dengue hemorrhagic fever guidance[12]. The clinical signs and symptoms of VCD cases were also captured and reported. The occurrence of any vaccine-related serious adverse events (SAEs) considered by the study investigators and/or study sponsor to be related to vaccination, or any fatal SAEs (related or unrelated to study medication) were reported and reviewed by the IDMC.

Wild-type dengue viremia was assessed by RT-PCR at Sanofi Pasteur GCI, Swiftwater, PA, USA. The RT-PCR primers used were directed against the 3'-untranslated region (3'-UTR) sequence, highly conserved among dengue virus serotypes. Results were expressed as a concentration $\log_{10}$ plaque-forming unit (PFU)/mL relative to virus standards included in each RT-PCR run.

### 2.5. Statistical analysis

All statistical analyses were performed with SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Safety data were analyzed separately for the two follow-up periods, the first 25-month active surveillance after the first study injection (active phase; CYD23) and the subsequent four-year passive surveillance for hospitalized cases (hospital phase; CYD57), and also for the entire six years of follow-up, encompassing both active and hospital phases (Figure 1). All participants who received at least one study injection in the initial phase Ⅱb study were analyzed in the active phase, and all participants subsequently recruited to long-term follow-up were analyzed in the passive phase; analyses were undertaken according to the initial study product received. Hospitalized dengue incidence in each vaccination group and relative risk (RR) for the CYD-TDV group *vs*. the control group were estimated by all and each dengue serotype, and by study year. The 95% confidence intervals (95% *CI*s) for annual dengue incidence were calculated with the exact binomial distribution for percentages using Clopper-Pearson's method[13], and the 95% *CI*s for RR were calculated using the Exact method[14]. Time to hospitalized VCD from receipt of the first study injection (Day 0) was analyzed using the Kaplan-Meier method to the end of extended follow-up.

*Post-hoc* analyses by age group (<9 years and ≥9 years at enrollment for CYD23) were conducted to include an assessment of the approved age group indicated for CYD-TDV use. These were conducted for the results of each phase, active and hospital, and for the entire six-year (technically, six years+one month) follow-up period.

## 3. Results

### 3.1. Study participants

Of the 3 997 participants who received at least one study injection in the phase Ⅱb study (active phase), 3 203 (80.1%) agreed to participate in this long-term safety follow-up (hospital phase; 2 131 and 1 072 in the CYD-TDV and control groups, respectively; Figure 2). Most of those not included in this follow-up could not be reached as they had either moved to other provinces or were lost to follow-up; a few declined to participate. Baseline demographics are summarized in Table 1. All 3 203 (100%) participants were included in hospital phase Years 1 and 2, with 3 153 (98.3%) and 3 062 (95.6%) included in Years 3 and 4, respectively.

**Table 1.** Baseline demographics at enrollment in the initial study for those who continued into the safety extension study.

| Characteristics | CYD-TDV (*n*=2 131) | Control (*n*=1 072) |
|---|---|---|
| Male [*n* (%)] | 1 013 (47.5) | 493 (46.0) |
| Age at initial enrollment years | | |
| Mean±SD | 8.1±2.0 | 8.2±2.1 |
| Range | 4 to <12 | 4 to <12 |
| Aged 4-8 years [*n* (%)] | 1 338 (62.8) | 665 (62.0) |
| Aged 9-11 years [*n* (%)] | 793 (37.2) | 407 (38.0) |
| Subgroup with baseline dengue immunogenicity* (*n*†) | 165 | 85 |
| Dengue seropositive | 117 | 60 |
| 4-5 years | 13 | 8 |
| 6-8 years | 54 | 34 |
| 9-11 years | 50 | 18 |
| Dengue seronegative | 48 | 25 |
| 4-5 years | 9 | 4 |
| 6-8 years | 27 | 13 |
| 9-11 years | 12 | 8 |

*Dengue seropositive was defined as a plaque-reduction neutralization test ($PRNT_{50}$) titer of 10 or higher against at least one dengue serotype; †*n* at beginning of the hospital phase (Year 3). CYD-TDV: tetravalent dengue vaccine.

### 3.2. Risk of hospitalized VCD

There were 85 and 46 hospitalized VCD cases during the hospital phase in the CYD-TVD and control groups, respectively; of these, 67 and 28 cases were aged <9 years in the two groups, respectively, and 18 cases in both study groups were aged ≥9 years. All children hospitalized with VCD recovered fully after appropriate supportive medical care.

The incidence of hospitalized VCD over the entire six-year study

**Table 2.** Incidence of hospitalized VCD during the entire six-year follow-up period and each phase of the trial by serotype and age group (<9 and ⩾9 years) at enrolment in the initial study.

| Serotype | <9 years | | | | | ⩾9 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CYD-TDV group | | Control group | | Relative risk (95% *CI*) | CYD-TDV group | | Control group | | Relative risk (95% *CI*) |
| | Cases | Annual incidence rate, % (95% *CI*) | Cases | Annual incidence rate, % (95% *CI*) | | Cases | Annual incidence rate, % (95% *CI*) | Cases | Annual incidence rate, % (95% *CI*) | |
| Active phase (Y1-Y2) | | | | | | | | | | |
| Any | 26/1 603 | 0.8 (0.5-1.1) | 19/796 | 1.1 (0.7-1.8) | 0.68 (0.36-1.30) | 6/1 018 | 0.3 (0.1-0.6) | 11/516 | 1.0 (0.5-1.8) | 0.28 (0.08-0.81) |
| 1 | 7/1 603 | 0.2 (0.1-0.4) | 4/796 | 0.2 (0.1-0.6) | 0.87 (0.22-4.05) | 1/1 018 | <0.1 (0.0-0.3) | 5/516 | 0.5 (0.2-1.1) | 0.10 (0.00-0.91) |
| 2 | 16/1 603 | 0.5 (0.3-0.8) | 10/796 | 0.6 (0.3-1.1) | 0.80 (0.34-1.96) | 4/1 018 | 0.2 (0.1-0.5) | 5/516 | 0.5 (0.2-1.1) | 0.41 (0.08-1.88) |
| 3 | 0/1 603 | 0.0 (0.0-0.1) | 3/796 | 0.2 (0.0-0.5) | 0.00 (0.00-1.20) | 1/1 018 | <0.1 (0.0-0.3) | 0/516 | 0.0 (0.0-0.3) | +inf (0.01-+inf) |
| 4 | 0/1 603 | 0.0 (0.0-0.1) | 1/796 | <0.1 (0.0-0.3) | 0.00 (0.00-19.37) | 0/1 018 | 0.0 (0.0-0.2) | 1/516 | <0.1 (0.0-0.5) | 0.00 (0.00-19.75) |
| Unserotyped* | 3/1 603 | <0.1 (0.0-0.3) | 1/796 | <0.1 (0.0-0.3) | 1.49 (0.12-78.21) | 0/1 018 | 0.0 (0.0-0.2) | 0/516 | 0.0 (0.0-0.3) | NC |
| Hospital phase (Y3-Y6) | | | | | | | | | | |
| Any | 67†/1 314 | 1.3 (1.0-1.6) | 28/656 | 1.1 (0.7-1.6) | 1.19 (0.76-1.93) | 18/783 | 0.6 (0.3-0.9) | 18†/402 | 1.1 (0.7-1.8) | 0.51 (0.25-1.05) |
| 1 | 17/1 314 | 0.3 (0.2-0.5) | 6/656 | 0.2 (0.1-0.5) | 1.41 (0.53-4.38) | 4/783 | 0.1 (0.0-0.3) | 5/402 | 0.3 (0.1-0.7) | 0.41 (0.08-1.91) |
| 2 | 24/1 314 | 0.5 (0.3-0.7) | 4/656 | 0.2 (0.0-0.4) | 2.99 (1.03-11.87) | 5/783 | 0.2 (0.1-0.4) | 7/402 | 0.4 (0.2-0.9) | 0.37 (0.09-1.34) |
| 3 | 15/1 314 | 0.3 (0.2-0.5) | 6/656 | 0.2 (0.1-0.5) | 1.25 (0.46-3.92) | 6/783 | 0.2 (0.1-0.4) | 4/402 | 0.3 (0.1-0.6) | 0.77 (0.18-3.71) |
| 4 | 12/1 314 | 0.2 (0.1-0.4) | 9/656 | 0.4 (0.2-0.7) | 0.67 (0.26-1.79) | 3/783 | <0.1 (0.0-0.3) | 3/402 | 0.2 (0.0-0.6) | 0.51 (0.07-3.83) |
| Unserotyped* | 0/1 314 | 0.0 (0.0-0.1) | 3/656 | 0.1 (0.0-0.3) | 0.00 (0.00-1.21) | 0/783 | 0.0 (0.0-0.1) | 0/402 | 0.0 (0.0-0.2) | NC |
| Entire study (Y1-Y6) | | | | | | | | | | |
| Any | 93†/1 411 | 1.1 (0.9-1.3) | 47/703 | 1.1 (0.8-1.5) | 0.99 (0.69-1.43) | 24/862 | 0.5 (0.3-0.7) | 29†/440 | 1.1 (0.7-1.6) | 0.42 (0.24-0.75) |
| 1 | 24/1 411 | 0.3 (0.2-0.4) | 10/703 | 0.2 (0.1-0.4) | 1.20 (0.55-2.80) | 5/862 | <0.1 (0.0-0.2) | 10/440 | 0.4 (0.2-0.7) | 0.26 (0.07-0.82) |
| 2 | 40/1 411 | 0.5 (0.3-0.6) | 14/703 | 0.3 (0.2-0.6) | 1.42 (0.76-2.83) | 9/862 | 0.2 (0.1-0.3) | 12/440 | 0.5 (0.2-0.8) | 0.38 (0.14-0.99) |
| 3 | 15/1 411 | 0.2 (0.1-0.3) | 9/703 | 0.2 (0.1-0.4) | 0.83 (0.34-2.15) | 7/862 | 0.1 (0.01-0.3) | 4/440 | 0.2 (0.0-0.4) | 0.89 (0.23-4.16) |
| 4 | 12/1 411 | 0.1 (0.1-0.2) | 10/703 | 0.2 (0.1-0.4) | 0.60 (0.24-1.54) | 3/862 | <0.1 (0.0-0.2) | 4/440 | 0.2 (0.0-0.4) | 0.38 (0.06-2.26) |
| Unserotyped* | 3/1 411 | <0.1 (0.0-0.1) | 4/703 | <0.1 (0.0-0.2) | 0.37 (0.05-2.21) | 0/862 | 0.0 (0.0-0.1) | 0/440 | 0.0 (0.0-0.1) | NC |

NC, not calculable; *: RT-PCR was unable to establish dengue serotype in virologically confirmed cases; †: 1 participant infected by two serotypes in the same episode. CYD-TDV: tetravalent dengue vaccine. VCD: virologically-confirmed dengue.

**Table 3.** Incidence of hospitalized VCD during the study for any serotype, by age group (<9 and ⩾9 years) at enrolment in the initial study.

| Time period | All participants | | | | | <9 years | | | | | ⩾9 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CYD-TDV group | | Control group | | Relative risk (95% *CI*) | CYD-TDV group | | Control group | | Relative risk (95% *CI*) | CYD-TDV group | | Control group | | Relative risk (95% *CI*) |
| | Cases | Annual incidence rate, % (95% *CI*) | Cases | Annual incidence rate, % (95% *CI*) | | Cases | Annual incidence rate, % (95% *CI*) | Cases | Annual incidence rate, % (95% *CI*) | | Cases | Annual incidence rate, % (95% *CI*) | Cases | Annual incidence rate, % (95% *CI*) | |
| Active phase (Year 1-2) | | | | | | | | | | | | | | | |
| Year 1 | 8/2 666 | 0.3 (0.1-0.6) | 7/1 331 | 0.5 (0.2-1.1) | 0.57 (0.18-1.85) | 5/1 634 | 0.3 (0.1-0.7) | 5/809 | 0.6 (0.2-1.4) | 0.50 (0.11-2.15) | 3/1 032 | 0.3 (0.1-0.8) | 2/522 | 0.4 (0.0-1.4) | 0.75 (0.09-9.08) |
| Year 2 | 24/2 576 | 0.9 (0.6-1.3) | 23/1 292 | 1.6 (1.0-2.5) | 0.52 (0.28-0.97) | 21/1 572 | 1.2 (0.8-1.9) | 14/783 | 1.7 (0.9-2.8) | 0.75 (0.36-1.59) | 3/1 004 | 0.3 (0.1-0.8) | 9/509 | 1.6 (0.7-3.1) | 0.17 (0.03-0.68) |
| Active phase | 32/2 621 | 0.6 (0.4-0.8) | 30/1 312 | 1.1 (0.7-1.6) | 0.53 (0.31-0.91) | 26/1 603 | 0.8 (0.5-1.1) | 19/796 | 1.1 (0.7-1.8) | 0.68 (0.36-1.30) | 6/1 018 | 0.3 (0.1-0.6) | 11/516 | 1.0 (0.5-1.8) | 0.28 (0.08-0.81) |
| Hospital phase (Year 3-6) | | | | | | | | | | | | | | | |
| Year 3 | 22/2 131 | 1.1 (0.7-1.7) | 11/1 072 | 1.1 (0.6-2.0) | 1.01 (0.47-2.30) | 19/1 338 | 1.5 (0.9-2.4) | 6/665 | 1.0 (0.4-2.1) | 1.57 (0.60-4.81) | 3/793 | 0.4 (0.1-1.2) | 5/407 | 1.3 (0.4-3.1) | 0.31 (0.05-1.58) |
| Year 4 | 16/2 131 | 0.8 (0.4-1.2) | 17/1 072 | 1.6 (0.9-2.5) | 0.47 (0.22-1.00) | 13/1 338 | 1.0 (0.5-1.7) | 12/665 | 1.8 (0.9-3.1) | 0.54 (0.23-1.29) | 3/793 | 0.4 (0.1-1.1) | 5/407 | 1.2 (0.4-2.8) | 0.31 (0.05-1.58) |
| Year 5 | 8/2 093 | 0.4 (0.2-0.8) | 4/1 060 | 0.4 (0.1-1.0) | 1.01 (0.27-4.60) | 7/1 313 | 0.5 (0.2-1.1) | 1/658 | 0.2 (0.0-0.8) | 3.51 (0.45-158.10) | 1/780 | 0.1 (0.0-0.7) | 3/402 | 0.7 (0.2-2.2) | 0.17 (0.00-2.14) |
| Year 6 | 39/2 035 | 1.9 (1.4-2.6) | 14/1 027 | 1.4 (0.7-2.3) | 1.41 (0.75-2.80) | 28/1 268 | 2.2 (1.5-3.2) | 9/635 | 1.4 (0.7-2.7) | 1.56 (0.71-3.75) | 11/767 | 1.4 (0.7-2.6) | 5/392 | 1.3 (0.4-3.0) | 1.12 (0.36-4.13) |
| Hospital phase | 85/2 098 | 1.0 (0.8-1.3) | 46/1 058 | 1.1 (0.8-1.5) | 0.93 (0.64-1.36) | 67/1 314 | 1.3 (1.0-1.6) | 28/656 | 1.1 (0.7-1.6) | 1.19 (0.76-1.93) | 18/783 | 0.6 (0.3-0.9) | 18/402 | 1.1 (0.7-1.8) | 0.51 (0.25-1.05) |
| Entire study | 117/2 272 | 0.9 (0.7-1.0) | 76/1 142 | 1.1 (0.9-1.4) | 0.77 (0.57-1.05) | 93/1 411 | 1.1 (0.9-1.3) | 47/703 | 1.1 (0.8-1.5) | 0.99 (0.69-1.43) | 24/862 | 0.5 (0.3-0.7) | 29/440 | 1.1 (0.7-1.6) | 0.42 (0.24-0.75) |

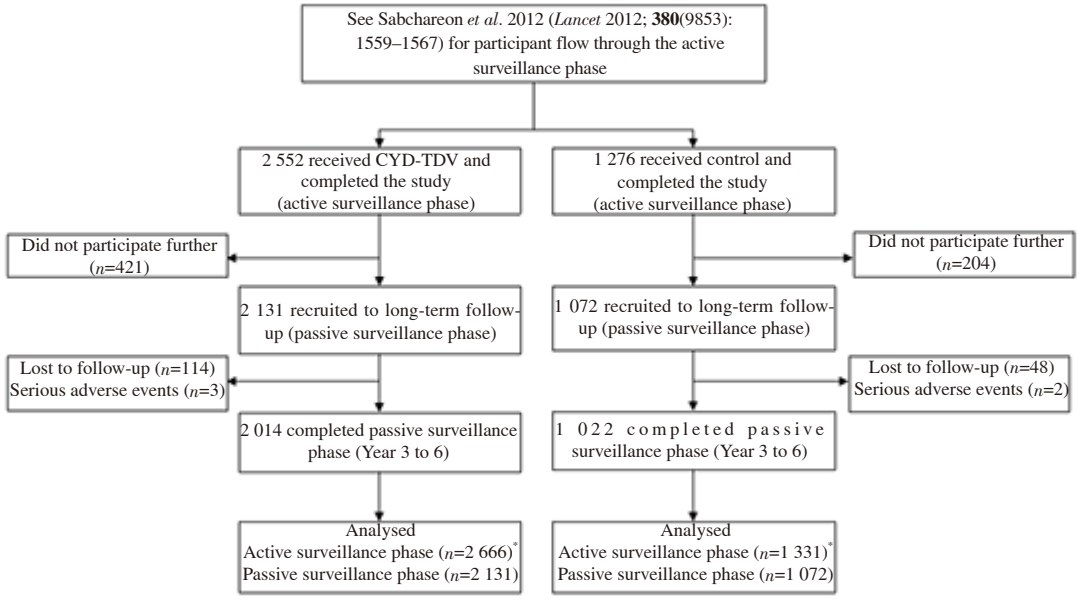


**Figure 2.** Trial profile (*safety analysis set). CYD-TDV: tetravalent dengue vaccine.

period is summarized by dengue virus serotype in Supplementary Table S1, and by the two age groups in Table 2. Serotype 2 was the predominant cause of hospitalized VCD during the active phase, however, during the hospital phase the cases of VCD due to each serotype were more evenly distributed in both groups (Table S1). The risk of hospitalized VCD was lower in the CYD-TDV group than control group; the RR of hospitalized VCD due to any serotype in the CYD-TDV group compared with control was lowest in the active phase (RR: 0.53, 95% *CI*: 0.31-0.91), followed by the entire six-year follow-up period (RR: 0.77, 95% *CI*: 0.57-1.05), and was less marked during the hospital phase (RR: 0.93, 95% *CI*: 0.64-1.36) (Table S1).

In children aged ⩾9 years, the risk of hospitalized VCD was reduced in the CYD-TDV group during the active phase (RR: 0.28,

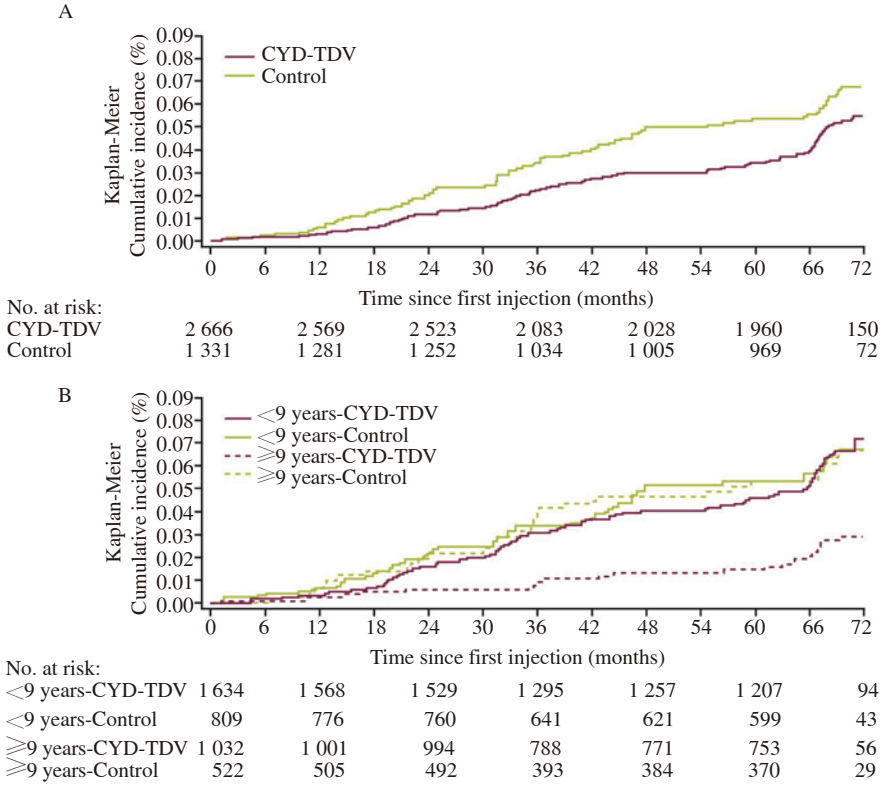


**Figure 3.** Kaplan-Meir curve for hospitalized VCD due to any serotypes from first injection to the end of follow-up, for (A) all participants and (B) participants aged <9 and ⩾9 years at enrollment. CYD-TDV: tetravalent dengue vaccine. VCD: virologically-confirmed dengue.

95% *CI*: 0.08-0.81) and during the entire six-year follow-up (RR: 0.42, 95% *CI*: 0.24-0.75), but only a trend towards a lower risk during the hospital phase (RR: 0.51, 95% *CI*: 0.25-1.05) (Table 2). No such decreases in RR over time were observed with CYD-TDV compared with controls in children aged <9 years (Table 2), or aged 4-5 years (Table S2).

While the number of cases of hospitalized VCD fluctuated over each year of the four-year hospital phase, no difference was observed in the incidences of hospitalized VCD between the two study groups for each year of the hospital phase across all participants and by age group (Table 3). In the overall population, there were ten severe hospitalized VCD cases in the CYD-TDV group and five in the control group over six years (RR: 1.00, 95% *CI*: 0.31-3.75).

The onset of a lower risk of hospitalization for VCD occurred from about 12 months after the first injection, and was maintained for the remainder of the follow-up (Figure 3A). The greatest benefits of CYD-TDV were observed in those aged ≥9 years (Figure 3B); no benefit was observed with CYD-TDV in those aged <9 years.

Dengue viremia levels among hospitalized VCD cases during the hospital phase of the study were similar between the CYD-TDV group and the control group [mean±SD (3.64±1.17) $\log_{10}$ pfu/mL and (3.38±1.21) $\log_{10}$ pfu/mL, respectively].

### 3.3. Risk of severe hospitalized VCD

There was no difference in the risk of severe hospitalized VCD between the two study groups during the hospital phase or the entire study, or by age group (Table 4). During the four-year hospital phase, there were eight and three cases of severe hospitalized VCD in the CYD-TDV and control group, respectively.

Two cases of severe hospitalized VCD in the CYD-TDV group were of grade Ⅲ severity (WHO 1997 dengue hemorrhagic fever criteria grading); these occurred during the first year of the hospital phase in those aged <9 years. In addition, four cases and one case were grade I severity in the CYD-TDV and control groups, respectively, and one and two cases were grade I. One case of severe hospitalized VCD in the CYD-TDV group had dengue encephalitis. All participants hospitalized with severe VCD recovered fully after appropriate supportive medical care.

**Table 4.** Incidence of severe hospitalized VCD-safety analysis set.

| Participants | CYD-TDV group | | Control group | | |
|---|---|---|---|---|---|
| | Cases | Annual incidence rate, % (95% *CI*) | Cases | Annual incidence rate, % (95% *CI*) | Relative risk( 95% *CI*) |
| All participants | | | | | |
| Hospital phase | 8/2 098 | <0.1 (0.0-0.2) | 3/1 058 | <0.1 (0.0-0.2) | 1.35 (0.32-7.87) |
| Entire study | 10/2 272 | <0.1 (0.0-0.1) | 5/1 142 | <0.1 (0.0-0.2) | 1.01 (0.31-3.75) |
| <9 years | | | | | |
| Hospital phase | 6/1 314 | 0.1 (0.0-0.3) | 2/656 | <0.1 (0.0-0.3) | 1.50 (0.27-15.16) |
| Entire study | 7/1 411 | <0.1 (0.0-0.2) | 3/703 | <0.1 (0.0-0.2) | 1.16 (0.27-6.96) |
| ≥9 years | | | | | |
| Hospital phase | 2/783 | <0.1 (0.0-0.2) | 1/402 | <0.1 (0.0-0.4) | 1.03 (0.05-60.56) |
| Entire study | 3/862 | <0.1 (0.0-0.2) | 2/440 | <0.1 (0.0-0.3) | 0.77 (0.09-9.17) |

CYD-TDV: tetravalent dengue vaccine. VCD: virologically-confirmed dengue.

**Table 5.** Clinical signs and symptoms for hospitalized VCD cases during the hospital phase for all participants and by age group (<9 and ≥9 years) at enrolment in the initial study.

| Signs and symptoms | All participants | | | <9 years | | | ≥9 years | | |
|---|---|---|---|---|---|---|---|---|---|
| | CYD-TDV group (*n*=85) | Control group (*n*=46) | Relative risk (95% *CI*) | CYD-TDV group (*n*=67) | Control group (*n*=28) | Relative risk (95% *CI*) | CYD-TDV group (*n*=18) | Control group (*n*=18) | Risk ratio (95% *CI*) |
| Mean duration of clinical symptoms (d) | 7.7±2.6 | 7.5±2.3 | | 8.0±2.7 | 7.3±2.3 | | 6.7±2.3 | 7.9±2.4 | |
| Mean duration of fever (d) | 5.8±1.7 | 5.7±2.0 | | 5.9±1.7 | 5.4±2.0 | | 5.2±1.5 | 6.2±2.0 | |
| Mean duration of hospital stay (d) | 4.5±1.7 | 4.3±1.7 | | 4.7±1.6 | 4.0±1.3 | | 4.1±1.9 | 4.9±2.2 | |
| Hemorrhage [*n* (%)] | 35 (41.2) | 20 (43.5) | 0.95 (0.53-1.73) | 31 (46.3) | 11 (39.3) | 1.18 (0.58-2.60) | 4 (22.2) | 9 (50.0) | 0.44 (0.10-1.59) |
| Spontaneous bleeding | 22 (25.9) | 13 (28.3) | 0.92 (0.44-1.98) | 19 (28.4) | 6 (21.4) | 1.32 (0.51-4.05) | 3 (16.7) | 7 (38.9) | 0.43 (0.07-1.88) |
| Blood transfusions | 2 (2.4) | 1 (2.2) | 1.08 (0.06-63.86) | 1 (1.5) | 0 (0) | NA | 1 (5.6) | 1 (5.6) | 1.00 (0.01-78.50) |
| Visceral manifestations [*n* (%)] | 2 (2.4) | 0 (0) | NA | 0 (0) | 0 (0) | NA | 2 (11.1) | 0 (0) | NA |
| Hepatic failure | 0 (0) | 0 (0) | NA | 0 (0) | 0 (0) | NA | 0 (0) | 0 (0) | NA |
| CNS manifestations | 2 (2.4) | 0 (0) | NA | 0 (0) | 0 (0) | NA | 2 (11.1) | 0 (0) | NA |
| Other manifestations | 0 (0) | 0 (0) | NA | 0 (0) | 0 (0) | NA | 0 (0) | 0 (0) | NA |
| Plasma leakage [*n* (%)] | 14 (16.5) | 5 (10.9) | 1.52 (0.52-5.38) | 11 (16.4) | 2 (7.1) | 2.30 (0.50-21.34) | 3 (16.7) | 3 (16.7) | 1.00 |
| Clinical signs of plasma leakage | 2 (2.4) | 2 (4.3) | 0.54 (0.04-7.47) | 2 (3.0) | 1 (3.6) | 0.84 (0.04-49.31) | 0 (0.0) | 1 (5.6) | 0.00 (0.00-39.00) |
| Hematocrit increase ≥20% | 14 (16.5) | 4 (8.7) | 1.85 (0.59-7.90) | 11 (16.4) | 1 (3.6) | 4.60 (0.67-197.87) | 3 (16.7) | 3 (16.7) | 1.00 (0.13-7.47) |
| Thrombocytopenia ($\times 10^9$ /L) | | | | | | | | | |
| Platelet count ≤$50\times10^9$ /L [*n* (%)] | 24 (28.2) | 11 (23.9) | 1.18 (0.56-2.67) | 20 (29.9) | 2 (7.1) | 4.18 (1.02-36.88) | 4 (22.2) | 9 (50.0) | 0.44 (0.10-1.59) |
| Platelet count ≤$100\times10^9$ /L [*n* (%)] | 57 (67.1) | 24 (52.2) | 1.285 (0.78-2.17) | 48 (71.6) | 11 (39.3) | 1.824 (0.93-3.89) | 9 (50.0) | 13 (72.2) | 0.692 (0.26-1.75) |
| Lowest platelet count $10^9$ /L [median (IQR)] | 70.0 (68.0) | 98.5 (102.0) | | 70.0 (73.0) | 137.0 (103.0) | | 97.0 (105.0) | 52.0 (72.0) | |
| Shock*[*n* (%)] | 4 (4.8) | 1 (2.3) | 2.05 (0.20-100.84) | 2 (3.0) | 0 (0.0) | NA | 2 (11.1) | 1 (5.9) | 1.89 (0.10-111.44) |
| Dehydration[*n* (%)] | 63 (74.1) | 30 (74.1) | 1.14 (0.72-1.82) | 55 (82.1) | 23 (82.1) | 1.00 (0.60-1.70) | 8 (44.4) | 7 (38.9) | 1.14 (0.36-3.70) |

*Not all participants were evaluable for shock as no blood pressure result (all participants: CYD-TDV *n*=84, control *n*=43; <9 years: CYD-TDV *n*=66, control *n*=26; ≥9 years: CYD-TDV *n*=18, control *n*=17); IQR, interquartile range; risk ratio is calculated as the ratio between the CYD-TDV and placebo group of the number of cases with specified clinical signs and symptoms among participants with hospitalized VCD. CYD-TDV: tetravalent dengue vaccine. VCD: virologically-confirmed dengue.

### 3.4. Clinical signs and symptoms of hospitalized VCD

Of the 138 participants diagnosed with dengue by a physician, 114 (82.6%) were VCD; representing 75/89 (84.3%) and 39/49 (79.6%) of diagnoses in the CYD-TDV and control groups, respectively. The remaining 24 participants diagnosed with dengue by a physician were not VCD. In addition, there were 17 VCD cases that were not diagnosed as dengue by a physician; physician diagnoses included cellulitis, gastroenteritis, bronchitis, systemic infection, pharyngitis, tonsillitis, viral infection, and gastritis.

No meaningful differences were noted in dengue symptomatology between the groups during the hospital phase, or by age group (Table 5). Clinical shock was reported in four cases in the CYD-TDV group (two in each of the age groups), and in one case in the control group (in the ≥9 years age group); two of the cases of shock in the CYD-TDV group (in the <9 years age group) were classed as dengue hemorrhagic fever grade Ⅲ. All cases with clinical shock fully recovered.

### 3.5. Vaccine-related SAEs and deaths

CYD-TDV had an acceptable safety profile, with no vaccine-related SAEs or vaccine-related deaths reported during the hospital phase. Five deaths not related to treatment were reported during the hospital phase; two road traffic accidents, two gunshot wounds, and one drowning. There were no fatalities due to dengue in the entire six-year follow-up.

## 4. Discussion

This long-term safety follow-up showed CYD-TDV significantly reduced the RR of hospitalized VCD by 72% (during the active phase) and 58% (during the entire six-year follow-up) in those aged ≥9 years. There was a trend towards reduced hospitalized VCD during the hospital phase alone in this age group. Participants aged <9 years gained no benefit from receipt of CYD-TDV, but had no increased risk of dengue hospitalization compared with controls during follow up. A large proportion (80%) of participants in the original phase IIb study agreed to participate in this follow-up extension, and almost 95% of them continued through Year 4, reflecting a remarkable commitment to follow up from the community, and a major achievement by the study team in Thailand.

The observations from this study are consistent with data from the four-year safety follow-up in the two pivotal phase Ⅲ studies (CYD14 and CYD15). The CYD15 study showed CYD-TDV reduced the RR of hospitalized VCD by 71% over four years of follow-up in children aged 9-16 years in Latin America[15,16]. The CYD14 study showed CYD-TDV reduced the RR of hospitalized VCD over the four years of follow-up by 61% in those ≥9 years of age at the time of first injection in the Asia Pacific region[16,17]. In this six-year follow-up, CYD-TDV did not significantly reduce the RR of hospitalized VCD in those aged <9 years[16,17].

The reasons for the apparent lack of efficacy against hospitalized VCD among those aged <9 years during the hospital phase remains to be fully determined. It has been suggested younger children are more likely to be seronegative, and they have lower levels of vaccine-induced immune responses prone to waning more rapidly below protective levels, or a reduced qualitative response dependent on age-related factors, including a less-developed vascular physiology and partially immature immune responses[4,18,19]. In addition, CYD-TDV vaccination may partially mimic primary dengue virus infection where subsequent exposure to wild-type virus may trigger a secondary-like infection, which is associated with an increased risk of symptomatic or severe disease[18]. This latter hypothesis is supported by the *post-hoc* case-cohort reanalysis of three CYD-TDV trials (including the current study)[5]; the onset of a higher risk of hospitalization for dengue and severe dengue occurred during the third year after the first vaccination in those seronegative aged ≥9 years, but started earlier in those seronegative aged < 9 years. Updated recommendations from Strategic Advisory Group of Experts on Immunization include the use of a pre-vaccination screening strategy, so that only dengue seropositive individuals are vaccinated, as outlined by the WHO[20].

Most of the cases of severe hospitalized VCD in the current study occurred in those aged <9 years. However, there was no difference in the risk of severe hospitalized VCD between the two study groups in this age group during the hospital phase or the entire six-year follow-up. For severe hospitalized VCD, there were no clinically meaningful differences in severity or symptomatology between the treatment groups. A study of the profile of 38 cytokines/chemokines and dengue viremia in children hospitalized with VCD during the two phase Ⅲ studies was not indicative of increased disease severity in the CYD-TDV group[21].

The incidence of hospitalized VCD fluctuated widely over the four-year hospital phase. Since 2011, outbreaks of dengue were observed in 2013 and 2015 in Thailand[22]. These years broadly correspond with Years 1/2, and 4 of the extension study, and may in part explain the variation observed during follow-up, in particular the higher number of cases in the final year relative to the other years.

The dominant circulating dengue serotype in Thailand has varied over time[23]. In the initial phase Ⅱb study, dengue serotype 2 had the highest prevalence in terms of symptomatic VCD[1], which was also the case for hospitalized VCD during this study. Although CYD-TDV is effective against each of the four dengue serotypes[24], vaccine efficacy varies by serotypes and is lowest against serotype 2[4]. Of note, the RR of hospitalized VCD during entire six-year period was highest with serotype 2 in those aged <9 years but not those aged ≥9 years.

No vaccine-related SAE or death due to dengue was reported over the entire six-year period of our study. Only one vaccine-related SAE (acute febrile illness) was reported in the control group during the active phase of this study, and none were reported in the CYD-TDV group[1]. The current study confirms CYD-TDV has an acceptable long-term safety profile, consistent with that reported in an integrated safety analysis[25].

This study has a number of limitations. It was conducted at one center/site in one country; as such, the results may not be applicable to other settings with different dengue endemicity. Passive surveillance of hospitalized dengue cases was undertaken, so there is the potential for under-reporting. However, focusing only on hospitalized cases minimizes the potential for under-reporting compared with ambulatory cases. In addition, the occurrence of SAEs was also not actively followed throughout the six years of follow-up; this information was gathered retrospectively during study contact with the participants.

In conclusion, the risk of hospitalized VCD among children in Thailand vaccinated with CYD-TDV is reduced in those aged ⩾9 years over six years of follow-up. Additional analyses of CYD-TDV studies have shown vaccine efficacy is highest in those seropositive at baseline, and there is an increased risk for hospitalized and severe dengue in those seronegative. Only individuals with previous dengue exposure are recommended for dengue vaccination.

## Conflict of interest statement

The authors declare that there is no conflict of interest.

## References


[1] Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: A randomised, controlled phase 2b trial. *Lancet* 2012; **380**(9853): 1559-1567.

[2] Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, Chua MN, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: A phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 2014; **384**(9951): 1358-1365.

[3] Villar L, Dayan GH, Arredondo-Garcia JL, Rivera DM, Cunha R, Deseda C, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. *N Engl J Med* 2015; **372**(2): 113-123.

[4] Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N Engl J Med* 2015; **373**(13): 1195-1206.

[5] Sridhar S, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T, et al. Effect of dengue serostatus on dengue vaccine safety and efficacy. *N Engl J Med* 2018; **379**(4): 327-340.

[6] SAGE Working Group on dengue vaccines and WHO Secretariat. *Background paper on dengue vaccines*. 2016. [Online]Available from: http://www.who.int/immunization/sage/meetings/2016/april/1_Background_Paper_Dengue_Vaccines_2016_03_17.pdf.[Accessed on 29th August 2019].

[7] Halstead SB. Licensed dengue vaccine: Public health conundrum and scientific challenge. *Am J Trop Med Hyg* 2016; **95**(4): 741-745.

[8] Halstead SB. Critique of World Health Organization recommendation of a dengue vaccine. *J Infect Dis* 2016; **214**(12): 1793-1795.

[9] World Health Organization. Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated). *Tech Report Series* 2013; **979**: 53-135.

[10] Vannice KS, Wilder-Smith A, Barrett ADT, Carrijo K, Cavaleri M, de Silva A, et al. Clinical development and regulatory points for consideration for second-generation live attenuated dengue vaccines. *Vaccine* 2018; **36**(24): 3411-3417.

[11] World Health Organization. *WHO guidelines for the clinical evaluation of dengue vaccines in endemic areas. Initiative for Vaccine Research (IVR) of the Department of Immunization, Vaccines and Biologicals*. Geneva: World Health Organization; 2008.

[12] World Health Organization. *Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control*. Geneva: World Health Organization; 1997.

[13] Newcombe RG. Two-sided confidence intervals for the single proportion: Comparison of seven methods. *Stat Med* 1998; **17**(8): 857-872.

[14] Breslow NE, Day NE. *Statistical methods in cancer research. Volume II–The design and analysis of cohort studies*. Lyon: IARC Scientific Publications; 1987, p. 1-406.

[15] Cortés M, Zambrano B, Humberto R, Langevin E, Haney O, Noriega F. *Long–term safety of a CYD–TDV dengue vaccine in Latin American dengue endemic countries*. Atlanta: American Society of Tropical Medicine and Hygiene; 2016, p. 13-17.

[16] Arredondo-García JL, Hadinegoro SR, Reynales H, Chua MN, Rivera Medina DM, Chotpitayasunondh T, et al. Four-year safety follow-up of the tetravalent dengue vaccine efficacy randomized controlled trials in Asia and Latin America. *Clin Microbiol Infect* 2018; **24**(7): 755-763.

[17] Wartel TA, Frago C, Zambrano B, Langevin E, Gailhardou S, Noriega F, et al. Long-term safety of a CYD-TDV dengue vaccine in Asia dengue endemic countries. 5th Pan American Dengue Reseearch Network Meeting, 20-23 April 2016, Panama; 2016.

[18] Guy B, Jackson N. Dengue vaccine: Hypotheses to understand CYD-TDV-induced protection. *Nat Rev Micro* 2016; **14**(1): 45-54.

[19] Wilder-Smith A, Massad E. Age specific differences in efficacy and safety for the CYD-tetravalent dengue vaccine. *Expert Rev Vaccines* 2016; **15**(4): 437-441.

[20] World Health Organization. Dengue vaccine: WHO position paper-September 2018. *Wkly Epidemiol Rec* 2018; **93**(36): 457-476.

[21] Harenberg A, de Montfort A, Jantet-Blaudez F, Bonaparte M, Boudet F, Saville M, et al. Cytokine profile of children hospitalized with virologically-confirmed dengue during two phase ||| vaccine efficacy trials. *PLoS Negl Trop Dis* 2016; **10**(7): e0004830.

[22] Ministry of Public Health. *Dengue disease (dengue fever, dengue heamorrhagic fever, dengue shock syndrome)*. 2015. [Online]Available from: http://www.boe.moph.go.th/Annual/AESR2015/aesr2558/Part%201/02/dhf.pdf.[Accessed on 29th August 2019].

[23] Limkittikul K, Brett J, L'Azou M. Epidemiological trends of dengue disease in Thailand (2000-2011): A systematic literature review. *PLoS Negl Trop Dis* 2014; **8**(11): e3241.

[24] Agarwal R, Wahid MH, Yausep OE, Angel SH, Lokeswara AW. The immunogenicity and safety of CYD-tetravalent dengue vaccine (CYD-TDV) in children and adolescents: A systematic review. *Acta Med Indones* 2017; **49**(1): 24-33.

[25] Gailhardou S, Skipetrova A, Dayan GH, Jezorwski J, Saville M, Van der Vliet D, et al. Safety overview of a recombinant live-attenuated tetravalent dengue vaccine: Pooled analysis of data from 18 clinical trials. *PLoS Negl Trop Dis* 2016; **10**(7): e0004821.