Vaccine 32 (2014) 4885–4892

Contents lists available at ScienceDirect

Vaccine

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

Review

Safety, immunogenicity and efficacy of a recombinant tetravalent dengue vaccine: A meta-analysis of randomized trials

Vivaldo G. da Costa*, Ariany C. Marques-Silva, Vitor G. Floriano, Marcos L. Moreli*

Virology Laboratory, Federal University of Goiás, BR 364 Km 192. Pq Industrial 3800, 75801615 Jataí, GO, Brazil

ARTICLE INFO

Article history:
Received 14 May 2014
Received in revised form 17 June 2014
Accepted 8 July 2014
Available online 19 July 2014

Keywords:
Dengue vaccine
CYD-TDV
Meta-analysis
Safety
Efficacy
Immunogenicity

ABSTRACT

The World Health Organization has stipulated a target: reduce the mortality rate caused by dengue disease by 50% until 2020. Most likely, this goal can be achieved by means of a dengue vaccine. Accordingly, the recombinant and tetravalent dengue vaccine (CYD-TDV), developed by the Sanofi Pasteur Group, is in an advanced stage of human testing. Although there are multiple randomized, placebo-controlled trials evaluating the CYD-TDV, individual results may have little power to identify differences between the populations studied. Thus, we conducted a meta-analysis to determine a more precise estimate of the overall parameters of safety, immunogenicity and efficacy of CYD-TDV. A data search was conducted in the PubMed, Medline, Cochrane Central Register of Controlled Trials and SciELO databases with defined selection criteria. We included for meta-analysis seven randomized and placebo-controlled studies that included 6678 patients randomized to receive the CYD-TDV (4586) or placebo (2092). Regarding vaccine safety, it was found that there was no significant difference between treated and placebo groups, as only approximately 5.5% of patients were withdrawn from the study. Regarding immunogenicity, the levels of neutralizing antibodies were measured by weighted mean differences (WMD), which were always higher in the vaccinated group (WMD/DENV1 = 59.7, 95% confidence interval [CI] 57–61; WMD/DENV2 = 99, 95% CI 95–102; WMD/DENV3 = 138, 95% CI 133–142; WMD/DENV4 = 123, 95% CI 119–126). The clinical efficacy of the vaccine was 59% (95% CI 15–80; RR = 0.41, 95% CI 0.2–0.85, I^2 = 30.9%). In conclusion, safety and a balanced immune response to the CYD-TDV were found. However, to fully establish the clinical effectiveness and robustness of immunogenicity, it is necessary to perform further studies to assess the long-term effects of the vaccine.

1. Introduction

A dengue vaccine is a matter of priority for many countries due to increasing viral loads of dengue virus (DENV), to which half of the world's population is exposed, and due to the increasing number of severe disease cases. Moreover, a specific antiviral treatment does not exist, and the disease leads to high financial burdens [1,2]. Therefore, dengue is an important public health problem for approximately 120 countries [3,4].

DENV belongs to the genus *Flavivirus* (FV) and is classified into four serotypes (DENV1-4) which have distinct phylogenetic and antigenic characteristics [5,6]. This has hindered the development of a dengue vaccine. It is necessary that the new vaccine be tetravalent, because all four dengue serotypes can cause severe disease [7]. Additionally, it is necessary that vaccination would result in a balanced, protective and prolonged immune response [8,9]. In various parts of the world, there are several research projects aimed at developing the expected vaccine for dengue.

Several vaccine candidate have been developed and are in testing phases. Among these candidates, there are vaccines made of DNA (monovalent or tetravalent), of recombinant adenovirus, of alphavirus replicons, of protein sub-units and E protein domain III [10–17]. By means of recombinant DNA technology, there has been an emergence of chimeric vaccines for various viruses in recent years, including for DENV [18,19]. In this case, the chimeric vaccine for dengue is comprised of fragments of complementary DNA that encode the viral proteins (prM and E epitopes) of all four serotypes of DENV, which were inserted into the yellow fever vaccine cDNA (17D strain) [20].

Currently, the attenuated tetravalent dengue vaccine (TDV) is more advanced in clinical trials compared to other vaccines. Two TDV candidates have emerged: first, TDV has been developed by the Walter Reed Army Institute of Research (WRAIR) in collaboration

* Corresponding authors. Tel.: +55 64 3606 8350; fax: +55 64 3606 8200.
E-mail addresses: vivbiom@gmail.com (V.G. da Costa), mlmoreli@jatai.ufg.br (M.L. Moreli).

http://dx.doi.org/10.1016/j.vaccine.2014.07.008
0264-410X/

with GlaxoSmithKline Vaccines [21]; second, Sanofi Pasteur has developed a recombinant chimeric yellow fever/dengue vaccine from four recombinant strains of DENV, called CYD-TDV, including viral strains PUO-359 (DENV1), PUO-218 (DENV2), PaH881/88 (DENV3) and 1228 (DENV4). The CYD-TDV is the first to reach the final stages of human testing in different regions of the world [20].

Although there are multiple randomized controlled trials (RCT) evaluating the CYD-TDV, individual results may have reduced power to identify differences between the populations studied. Accordingly, we conducted this meta-analysis to determine an overall estimate of the safety, immunogenicity, and efficacy parameters of CYD-TDV.

2. Materials and methods

This meta-analysis adhered to the PRISMA protocol regarding guidelines for systematic reviews [22]. Additionally, we prospectively recorded the study protocol in PROSPERO, with the registration number: CRD42014007230, available at http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42014007230.

2.1. Search strategy

After defining our research protocol, we performed a systematic search in the PubMed, Medline, Cochrane Central Register of Controlled Trials (CENTRAL), and SciELO databases. In order to define our study of interest in these databases, the following descriptors were used: "dengue" OR "dengue virus" OR "DENV" AND "vaccine" OR "CYD-TDV" AND "randomized controlled trials" OR "clinical trial".

Subsequently, we evaluated the studies found based on the following inclusion criteria: (1) RCT type studies that evaluated the use of CYD-TDV, (2) experiments involving humans, (3) studies evaluating the safety, immunogenicity, and preliminary efficacy parameters of the vaccine; and (4) studies that presented similar vaccination protocols, including the same formulation, the same number of doses (i.e., 0, 6 and 12 months) and the same sample collection period for evaluation of immunogenicity (28 days before and after each dose applied). The inclusion of only RCT type studies was necessary because of the possibility of bias that may arise in observational studies.

The selection of the studies of interest was performed by two researchers (V.G.C. and M.L.M.). Subsequently, data were extracted by three researchers (V.G.C., A.C.M.S. and V.G.F.) according to the data extraction protocol. Thus, studies chosen for meta-analysis had the following information extracted: study identification, year of publication, number of patients, mean age, age range, gender of patients, study site, date of recruitment, length of follow up, parameters of safety, immunogenicity (expressed as geometric mean titers (GMT)), and clinical efficacy of the vaccine, seropositivity for FV at baseline, and vaccine regimen of the test and control groups.

2.2. Data analysis

In order to obtain the overall estimated average of GMTs between placebo and vaccinated groups, weighted mean differences (WMD) were determined. In this case the variables are presented as continuous measures, and the WMD was calculated based on the average, standard deviation (SD), and sample size of each group [23]. However, as the sample SD was not disclosed, it was estimated by the values expressed by subgroups.

To evaluate the safety and efficacy of CYD-TDV, dichotomous data were extracted from each study and were inserted into a 2×2 contingency table, with subsequent individual determination of the risk ratio (RR), to obtain a summarized overall estimate.

Fixed-effects or random-effects models were chosen depending on whether there was an absence or presence of heterogeneity between studies. Heterogeneity was assessed by the I^2 statistic and Cochran Q test. I^2 values <25%, 25 to 50% and >50% indicate low, moderate and high heterogeneity, respectively [24]. Therefore, if significant heterogeneity existed, we adopted two strategies to reduce or identify the source of variability between studies: (1) analysis of the results in subgroups and; (2) continued exclusion of studies that had greater divergence of the results analyzed in relation to other studies [25–27]. In addition, we initially planned to use the Egger funnel plot to assess possible publication bias [28], but due to the limited number of selected studies and because of the significant heterogeneity among the studies, we chose not to use this method of analysis [29].

The study quality was assessed by a Jadad score [30], which was based on the following three subscales: randomization (2–0), blind (2–0), withdrawals and dropouts (1–0). For every answer of yes, unclear, or not, the values of 2 to 0 points were assigned, respectively. In our analysis, we judged that the studies evaluated that had a score ≥ 3 would be considered high quality.

We chose to use the random effects model during statistical analysis, because this model is applied when there is great variability between the results of studies, and because of this model typically provides a more conservative estimate of the significance of treatment [31]. To determine RR and WMD, we used a confidence interval (CI) of 95% with values of $p < 0.05$ considered statistically significant. The analysis results were presented in a table and a forest plot. All statistical analyses was performed using STATA IC/64 software (version 13.1, College Station, TX).

3. Results

We found 129 studies of reference in the databases using the descriptor combination previously established at the beginning of the research. After more detailed analysis, we refined the results by excluding eight studies [21,32–38]. The remaining seven studies were included for meta-analysis [39–45]. The flowchart of this study is shown in Fig. 1.

The studies included in this meta-analysis were conducted in Latin America [39,41,44] and Southeast Asia [40,42,43,45] and included a total of 6678 patients who received the CYD-TDV (4586) or placebo (2092). In total, researchers assessed the immunogenicity and safety of the vaccine, but only one study targeted analysis of the clinical efficacy of CYD-TDV [45]. The age of the enrolled patients varied from 2 to 45 years, among which 47.3% and 49.14% were males included in the vaccine and placebo groups, respectively. Great similarities were observed in the vaccination protocols used by researchers with the only difference being the administration of the placebo solution. The placebo solution was alternated in three doses with the CYD-TDV group, at months 0, 6 and 12. Most researchers utilized a saline solution (NaCl 0.9%) as a placebo, but some researchers also used standardized vaccines, such as tetanus/diphtheria/acellular pertussis [41], hepatitis-A, influenza [42], typhoid VI polysaccharide, pneumococcal polysaccharide [43,44], and rabies vaccine [45]. The characteristics of selected studies are shown in Table 1.

3.1. Safety

Of the patients recruited to participate in the studies, approximately 5.47% (251/4.586) did not fully complete the dengue vaccination protocol. In the control group, this rate was 5.6% (117/2.092). In both groups, the withdrawal occurred primarily by the choice of the individual, often after observation of discrete adverse reactions. In the vaccinated group (CYD-TDV), the main

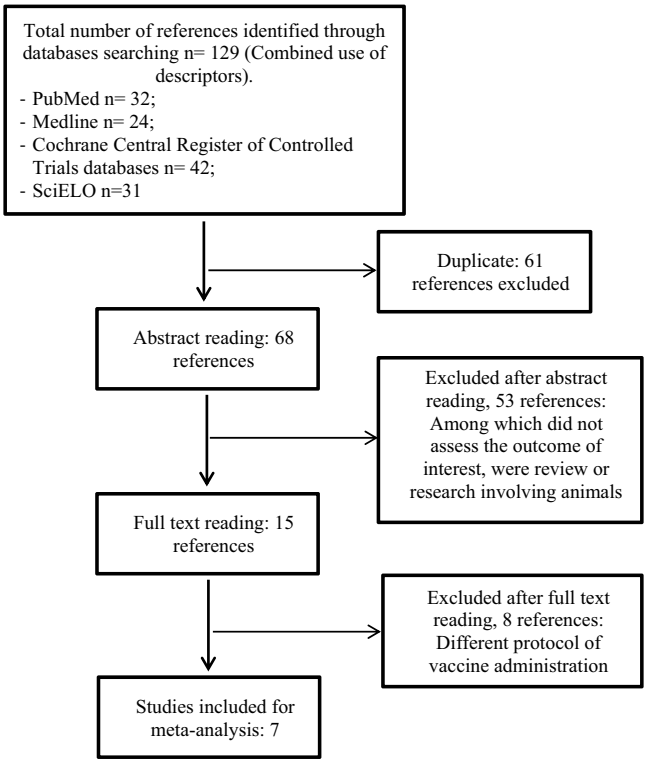

Fig. 1. Flow diagram of the selection process of studies.

reasons for withdrawal of the participants were voluntary (58.2%), although not related to adverse reactions, and by reason of non-compliance with the protocol (25%).

In order to better determine the safety of the new vaccine, we calculated the RR (95% CI) of the groups during the study. The clinical pictures observed in the two groups are shown in Table 2. The clinical picture of the participants was grouped, for better disclosure and quantification, into the following subgroups: solicited injection site reaction (0–7 days), represented by symptoms of pain, swelling and erythema, solicited systemic reaction (0–28 days), including fever, headache, malaise and myalgia, serious adverse event, defined as severe symptoms requiring hospitalization (i.e., a condition that could be life threatening and may result in death), unsolicited adverse events, including upper respiratory tract infections, nasopharyngitis and dizziness, among others (occurring within 0–28 days after vaccination) and unsolicited allergic reactions.

According to the information presented in Table 2, the major solicited injection site reactions were pain (CYD-TDV: 50.5%, placebo: 60.4%) and erythema (CYD-TDV: 10.5%, placebo: 14.5%). A statistical significance between the two groups of $p = 0.00$ and $p = 0.03$, respectively, was found. In general, we observed greater safety in the CYD-TDV group than in the placebo group. Likewise, for all other items reported or observed in the patients, there was often a low RR for the vaccinated group. The exception was symptoms of fever, headache, dizziness and/or myalgia, which were more pronounced in the vaccinated group. However, in this case the p values ranged from 0.06 to 0.84 and $I^2 < 50\%$, indicating no statistical significance and low heterogeneity between the subgroups evaluated.

3.2. Immunogenicity

The GMT, which served as an index to determine the immunogenicity of groups (CYD-TDV and placebo), was obtained using plaque reduction neutralization tests ($PRNT_{50}$). GMTs were

Table 1
Description of the characteristics of included studies.

Author, Year	Country	Design of study	Date of enrolment	Sample size		Age range (y)	Mean age (y)	Male		Seropositive at baseline (FV)		Jadad score
				CYD-TDV	Placebo			CYD-TDV (%)	Placebo	CYD-TDV	Placebo	
Dayan, 2013	Brazil	Phase II RCT	19 Aug–20 Oct 2010	100	50	9–16	12.7	40	55	81% (69% DENV, 71% YF)	84% (71% DENV, 80% YF)	4
Amar-Singh, 2013	Malaysia	Phase III RCT	2 Dec–14 Aug 2012	199	51	2–11	6.4 (TDV), 6.5 (Pl.)	48.2	62.7	55.8% (DENV and/or JE)	60.8% (DENV and/or JE)	4
Villar, 2013	Colombia, Honduras, Mexico, and Puerto Rico	Phase II RCT	Oct 09–Feb 2010	401	199	9–16	12.6 (TDV), 12.5 (Pl.)	49.1	45.7	78.8% (75.1% DENV, 70.1% YF)	80.4% (77.9% DENV, 73.4%)	4
Leo, 2012	Singapore	Phase II RCT	7 April–09 Oct 2009	898	300	2–45	17.8 (TDV), 18.2 (Pl.)	44.1	54.4	26.5% DENV	32.4% DENV	4
Tran, 2012	Vietnam	Phase II RCT	Mar–Jul 2009	120	60	2–45	NR	48	58	76% (71% DENV, 5% JE)	80% (67% DENV, 13% JE)	4
Lanata, 2012	Peru	Phase II RCT	Sept 08–Feb 2009	199	99	2–11	D	50.7	46.5	37.2% DENV, 81.4% YF	48.5% DENV, 83.4%	4
Sabchareon, 2012	Thailand	Phase II RCT	Feb 09–Feb 2010	2669	1333	4–11	8.26 (TDV), 8.12 (Pl.)	48	48	70% DENV, 80% JE	69% DENV, 78% JE	4

RCT: Randomized controlled trial; Pl.: placebo; NR: Not reported; FV: Flavivirus; D.: Data presented in subgroups.

Table 2
Safety parameters between vaccine and placebo assessed by RR index.

	No of studies	Grade	CYD-TDV		Group placebo		RR (CI 95%)	Test heterogeneity	*p* Value
			n/*N*	%	*n*/*N*	%			
Solicited injection site reaction									
Pain	7	Any	1316/2605	50.5	628/1039	60.4	0.83 (0.79–0.89)	I^2 = 89.5%	0.00
	4	3	16/1470	1.1	15/628	2.4	0.53 (0.26–1.09)	I^2 = 8.7%	0.35
Erythema	6	Any	199/1889	10.5	107/739	14.5	0.66 (0.54–0.81)	I^2 = 59%	0.03
	4	3	0/1587	0	4/628	0.64	0.10 (0.01–0.89)	I^2 = 0%	0.73
Swelling	6	Any	144/2389	6	73/739	9.9	0.64 (0.49–0.83)	I^2 = 78%	0.00
	4	3	1/1588	0.06	4/628	0.64	0.19 (0.03–1.45)	I^2 = 0%	0.37
Systemic events									
Fever	5	Any	276/1709	16.15	79/692	11	1.51 (1.20–1.90)	I^2 = 0%	0.84
	6	3	57/1908	3	34/739	4.6	0.57 (0.37–0.89)	I^2 = 64%	0.01
Headache	6	Any	795/1907	41.2	254/756	33.4	1.19 (1.07–1.33)	I^2 = 46%	0.09
	4	3	76/1589	4.8	26/645	4	1.16 (0.74–1.82)	I^2 = 60%	0.06
Malaise	6	Any	734/1906	38.5	215/739	29	1.25 (1.10–1.42)	I^2 = 12.8%	0.33
	4	3	65/1587	4.1	17/628	2.7	1.51 (0.88–2.58)	I^2 = 0%	0.50
Myalgia	5	Any	630/1707	37	237/678	35	1.02 (0.90–1.14)	I^2 = 0%	0.44
	4	3	46/1587	2.9	17/628	2.7	1.08 (0.62–1.89)	I^2 = 0%	0.71
Serious adverse event	7	–	385/4583	8.4	218/2090	10.4	0.84 (0.72–0.98)	I^2 = 28.7%	0.21
Unsolicited adverse event	7	–	1053/2614	40	467/1271	36.7	1.09 (1.00–1.18)	I^2 = 72%	0.002
Unsolicited allergic reaction	3	–	29/518	5.6	9/215	4.2	0.95 (0.46–2.00)	I^2 = 0%	0.42

n = event; *N* = total. A *p* value less than 0.05 was considered as statistically significant.

determined 28 days before and after each vaccine dose. After the last dose of CYD-TDV there was a variation in the GMT from 43 to 741, while in the placebo group values were lower and ranged from 8.17 to 140. Fig. 2A shows a forest plot with the values of WMD coming from GMTs obtained from CYD-TDV and placebo groups. For analysis, the data were stratified according to serotypes, and we generally observed a significant mean difference which was higher in vaccinated groups (WMD = 59.7, 95% CI 57–61, $p < 0.00$ [DENV1]; WMD = 99, 95% CI 95–102, $p < 0.00$ [DENV2]; WMD = 138, 95% CI 133–142, $p < 0.00$ [DENV3]; WMD = 123, 95% CI 119–126, $p < 0.00$ [DENV4]). In summary, the values of WMD in the vaccine group (CYD-TDV) were higher, in descending order, for DENV3, 4, 2 and 1.

Fig. 2B shows the results of GMTs in association with the geographical origin of samples collected. It is evident that WMD, ranging from 180 to 242 in the countries of Latin America, was higher than samples of patients from Southeast Asia (WMD = 50–116), although there was high heterogeneity ($I^2 > 97\%$) [39–45].

3.3. Clinical efficacy of the vaccine

Although the main focus of most researchers was to determine the immunogenicity and safety of CYD-TDV, we decided to determine the clinical efficacy of the vaccine. Thus, Fig. 3 shows a forest plot, with an overall pooled RR of 0.41 (95% CI 0.2–0.85, $I^2 = 30.9\%$, $p = 0.2$), i.e., the vaccine provided a 59% protection (95% CI 15–80), for preventing symptomatic DENV infections compared to the placebo group. All suspected dengue cases were virologically confirmed by RT-PCR.

3.4. Sensitivity analysis of heterogeneity

In order to reduce heterogeneity or determine its source, we performed an analysis of the meta-data by dividing into subgroups and excluding individual studies that contributed to a greater divergence of results. Thus, as studies included in this meta-analysis had a similar study design, they were analyzed in the following subgroups: age of patients enrolled, baseline FV serostatus and type of placebo used in the control group. In view of this, we cannot determine the heterogeneity source between studies in terms of immunogenecity. However, for subgroups that reported symptoms of pain, fever, swelling, erythema and unsolicited adverse events, which were variable, we observed that the source of heterogeneity was related to the different types of placebo solutions, as groups with the same type of placebo solution did not show significant heterogeneity ($I^2 = 0.0\%$ and $p > 0.05$ [data not shown]).

4. Discussion

Meta-analysis of the seven RCT studies shows that the CYD-TDV is definitely a safe vaccine, because although some serious side effects occurred, these were not related to the vaccination. Furthermore, when standardized vaccines were used in the control groups, for example, tetanus, diphtheria, or acellular pertussis, there were often milder symptoms in CYD-TDV group. These results are encouraging, because safety is one of the main requirements for the development of new vaccines. The safety of a vaccine is also associated with a balanced immune response. Due to this, GMTs were increased to four DENVs. There was a mean fold increase from baseline levels of GMTs of 3.5 to 14-fold for DENV1, 5 to 16-fold for DENV2, 5 to 13-fold for DENV3, and 5 to 28-fold for DENV4. These results were expressed in the forest plot for the continuous variable WMD that represented the individual values of GMTs and combined estimates [39–45].

The difficulty in developing dengue vaccines was based on the necessary complexity of a vaccine that could generate balanced and protective immunogenicity against four serotypes of DENV [46]. There has been concern that if the vaccine does not produce a balanced immune response, with the generation of neutralizing antibodies, a more severe clinical picture could occur in vaccinated patients secondary to an exaggerated immune response and, consequently, result in a severe form of dengue [47,48]. However, so far this outcome has not been observed, again reflecting that the CYD-TDV can be considered safe.

Another factor that shows that CYD-TDV has generated balanced immune responses is that this vaccination protocol has been implemented in various endemic areas for dengue. It may be noted that during the application of CYD-TDV, cases of DENV infections have been constant. This is because in the control groups, levels of GMTs at baseline showed a certain value, and after the last collection of samples, the levels were slightly higher, showing that

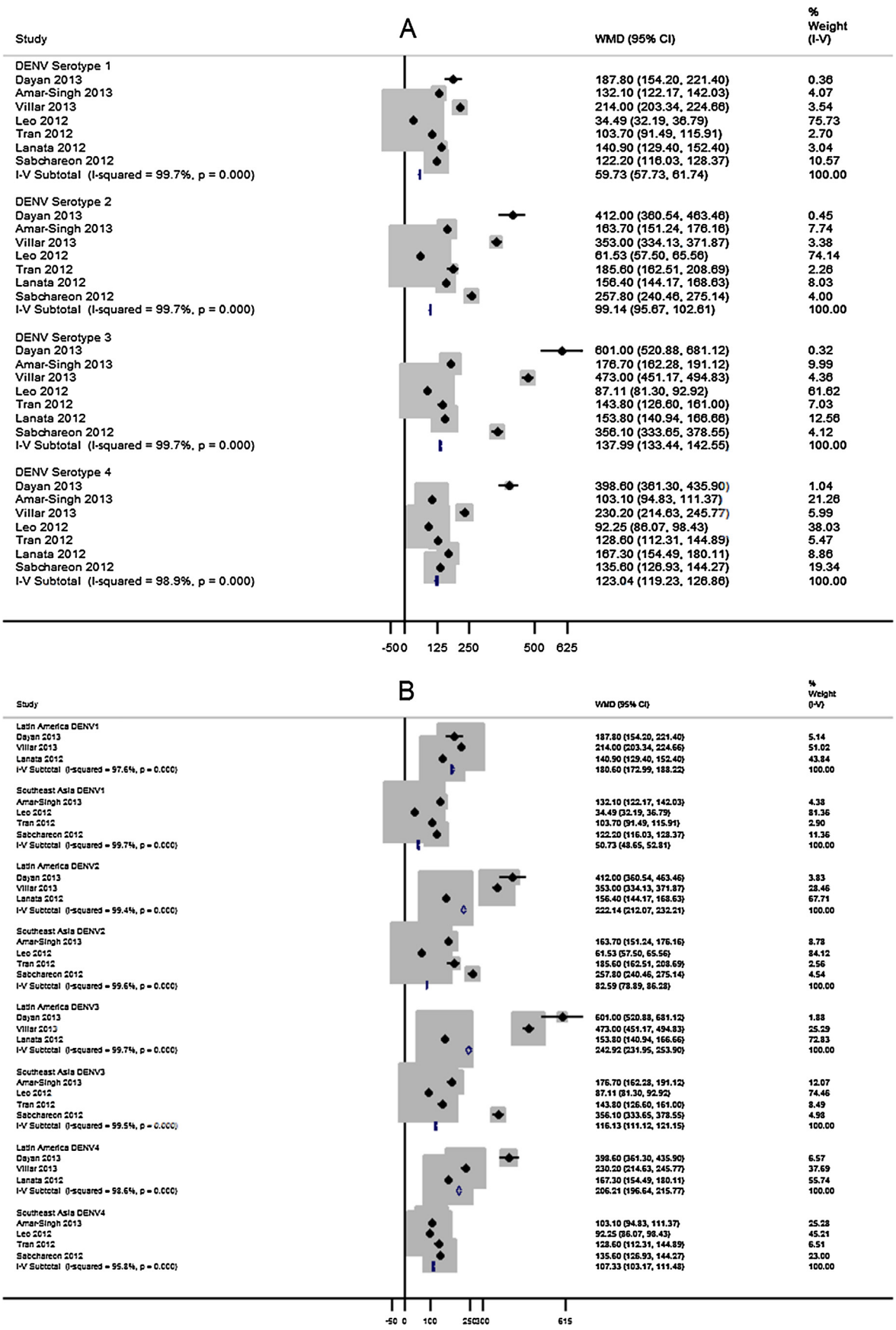

Fig. 2. (A) Forest plot of the levels of GMTs that were analyzed by the weighted mean differences (WMD), which were computed using a random effects model. Data were grouped according to the DENV serotype; (B) shows the results according to the origin of the patients enrolled in the studies.

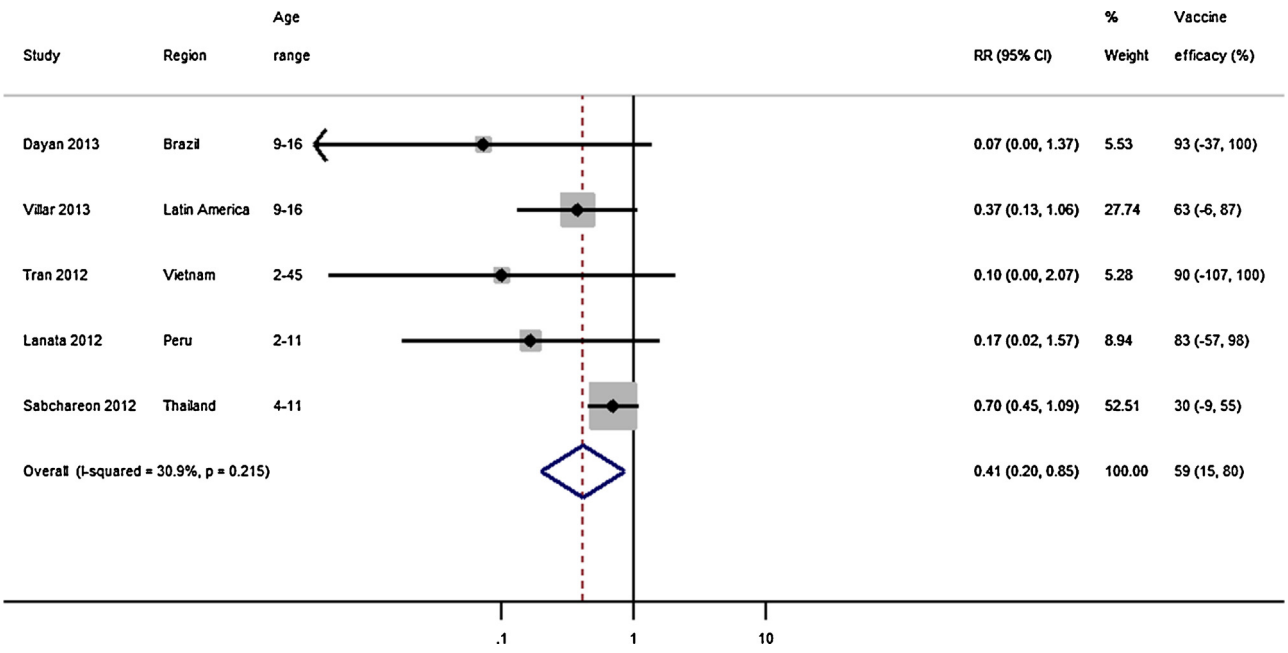

Fig. 3. Forest plot of CYD-TDV efficacy in dengue disease. The diamond represents the pooled estimate with a 95% CI.

the control group is being exposed to wild-type DENV. Similarly, during the study period the vaccinated group also may have been exposed to DENV. On the one hand, this shows a balanced immune response generated by CYD-TDV, but on the other hand it can be controversial, why does the vaccine only increase the levels of GMT? Asymptomatic infections by DENV may also be contributing to increased levels of GMTs. However, in contrast to this reasoning, a study conducted by Dayan et al. [38] observed high levels of GMTs after the third dose of CYD-TDV in population considered FV-naive. These contradictory factors show the complexities involved in developing a dengue vaccine, and more studies are needed to clarify these controversial points.

The PRNT method is commonly used to evaluate the immunogenicity of a vaccine [49]. This method measures the level of neutralizing antibodies to the virions which prevents the virions from infecting cells in in vitro cultures. All studies included in this meta-analysis used the PRNT50% method (titer $\geq$ 10 1/dil) and expressed the results in GMTs. However, there are detriments to associating protective antibodies with neutralizing antibodies [50]. First, the classification of neutralizing antibodies originates from neutralization tests. Second, protective antibodies are described in vivo by preventing the development of a specific disease. In the case of dengue, the finding of protective antibodies is hampered by the lack of an animal model that reliably mimics the disease observed in humans. One of the most controversial studies regarding the clinical efficacy of CYD-TDV was conducted by Sabchareon et al. [45]. In this study, the authors reported higher GMTs for the four DENV after vaccination but did not observe significant protection from disease caused by serotype DENV2. In this case, several hypotheses can be formulated, however, we call attention to a study by Sirivichayakul et al. [51] which recently showed that the PRNT method may not be a good test to correctly measure levels of protective antibodies. So then, how do we determine whether the antibodies generated are protective, if the gold standard PRNT test might fail? The most likely answer is found in a study conducted by Mammen et al. [52]. In this study, the authors used several slightly attenuated strains of dengue vaccine candidates which were inoculated subcutaneously into healthy FV-naive adults. Approximately 42% of these volunteers developed dengue fever caused by the DENV1/3 strains. The authors believe that this model can provide early indications of the protection of vaccine candidates before employing them in large experimental field trials involving human beings.

Regarding the low clinical efficacy of CYD-TDV for DENV2 observed in the study of Sabchareon et al. [45], additional studies will be important to clarify what types of protective immunity the vaccines are providing [50]. Do they provide monotypic, heterotypic or multitypic immunities as observed in natural DENV infections? Another important point is that while Sabchareon et al. evaluated the clinical efficacy of the vaccine, there is also efficacy against infection [53]. If the vaccine reduced the levels of asymptomatic infections, this would be an important point, because as described by Endy et al. [54], asymptomatic dengue infection is a key component to the global burden of the dengue infections. Therefore, reducing DENV infections directly reduces the chances of mosquitoes of the *Aedes* sp. of becoming infected and transmitting the virus.

Regarding the variation in the clinical efficacy of the vaccine between the study conducted in Thailand [45] and the others [39,41,43,44], we cannot make a direct association with the other studies included in our metadata due to a peculiarity of this study: it was designed specifically to evaluate the clinical efficacy of the vaccine. Why was the efficacy of the vaccine higher in other regions compared with the study conducted in Thailand? The answers are complex and there are several: the small sample size in the other studies reflected a decreased probability of the occurrence of the outcome of interest (in other words, there were reduced chances for the dengue disease to be observed, consequently increasing the clinical efficacy of the vaccine); the patients could have been exposed to different loads of DENV, with some having been exposed after the end of the first, second or third vaccine dose; the introduction during the study of a new serotype of DENV2 in which levels of neutralizing antibodies generated by vaccine would not result in sufficient protection against dengue disease; finally, the levels of neutralizing antibodies reflecting protection could be higher than anticipated and could vary depending on the serotype. One potential solution to increase the clinical efficacy of the vaccine would be to make minor changes to the formulation; the number of infectious doses was the same among all four DENV ($\sim$5 $\log_{10}$ 50% cell culture infectious dose).

Regarding FV seropositivity at baseline, the recruited patients had high levels of dengue, yellow fever and Japanese encephalitis seropositivity. In general, patients coming from Latin America had GMT higher levels than those from Asia. Additionally, there was a greater number of FV-seropositive patients at baseline coming from Latin America. It is known that, in patients seropositive for FV, there is the possibility that CYD-TDV will induce high levels of antibodies [55]. However, it is important to note that there is a possibility of cross-reactions occurring between FV [56], so additional studies will be important to clarify if the results of the PRNT for dengue in the vaccinated patients was masked by other FV.

The observed variability of the WMD was associated to geographic region, we think that this difference could be directly connected to the serological status of the patients with previous infections by flaviviruses. However, this is perhaps not the only factor causing the variability of WMD between regions; because RCT type studies have long durations, it is likely that the patients have been exposed to various viral loads of DENV after the first vaccination. In view of this, the patient selection also may have caused variation in WMD occurrence because in an endemic country, the circulation of DENV is variable; thus, the patients could have been selected from highly endemic areas or those of medium endemicity, consequently influencing the values of WMD.

The results of this meta-analysis should be interpreted with some caution because our study had limitations. First, there was significant heterogeneity among the studies that evaluated immunogenicity, and we were not able to determine the source of the heterogeneity. Second, clinical efficacy was based on studies with a small number of samples, in which the assessment of efficacy was a secondary objective. Consequently, larger multicenter studies regarding vaccine efficacy are ongoing in the Americas (NCT01374516) and Asia (NCT01373281), which involve a total of approximately 29 thousand volunteers. Thirdly, the WMD was based on standard deviations of the group, and if it had been based on individual samples it might have produced more accented values than those observed.

5. Conclusion

Despite the limitations discussed above, the results of our meta-analysis show that CYD-TDV is considered safe and provided a balanced and robust immune response, while levels of GMTs were only evaluated for a relatively short period. Regarding clinical efficacy, there was a 59% protection, but this result was based on a small sample population. Therefore, more studies are needed to evaluate the clinical efficacy and immunogenicity of this vaccine in the long term.

Acknowledgements

Funding: VGC received financial support (fellowship no. 201310267000308) from Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG). The funding had no role in study design, data analysis, assembly and decision to submit this manuscript.

Contribution of authors: Study conception and design (VGC, MLM), data acquisition (ACMS, VGF, VGC), analysis and interpretation of data (ACMS, VGF, VGC, MLM). All authors read and approved the final version of the manuscript.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

[1] Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: a continuing global threat. Nat Rev Microbiol 2010;8(Suppl):s7–16.
[2] Shepard DS, Undurraga EA, Halasa YA. Economic and disease burden of dengue in southeast Asia. PLoS Negl Trop Dis 2013;7:e2055.
[3] World Health Organization. Dengue: Guidelines for diagnosis, treatment, prevention and control 2009. Available at ⟨http://www.who.int/tdr/publications/documents/dengue diagnosis.pdf⟩.
[4] Lopes AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global burden of disease and risk factors. Oxford: Oxford University Press; 2006.
[5] Holmes EC, Burch SS. The causes and consequences of genetic variation in dengue virus. Trends Microbiol 2000;8:74–7.
[6] Monath TP, Tsai TF. Flaviviruses. In: Richman DD, Whitley RJ, Hayden FG, editors. Clinical virology. New York, NY: Churchill Livingstone Inc.; 2000. p. 1133–79.
[7] Halstead SB. Dengue. Lancet 2007;370:1644–52.
[8] Murphy BR, Whitehead S. Immune response to dengue virus and prospects for a vaccine. Annu Rev Immunol 2011;29:587–619.
[9] Whitehead SS, Blaney JE, Durbin AP, Murphy BR. Prospects for a dengue virus vaccine. Nat Rev Microbiol 2007;5:518–28.
[10] Durbin AP, Whitehead SS. Next-generation dengue vaccines: novel strategies currently under development. Viruses 2011;3:800–14, http://dx.doi.org/10.3390/v3101800.
[11] McArthur MA, Sztein MB, Edelman R. Dengue vaccines: recent developments, ongoing challenges and current candidates. Expert Rev Vaccines 2013;12:933–53.
[12] Zellweger RM, Miller R, Eddy WE, White LJ, Johnston RE, Shresta S. Role of humoral versus cellular responses induced by a protective dengue vaccine candidate. PLoS Pathog 2013;9:e1003723, http://dx.doi.org/10.1371/journal.ppat.1003723.
[13] del Angel RM, Reyes-del VJ. Dengue vaccines: strongly sought but not a reality just yet. PLoS Pathog 2013;9:e1003551, http://dx.doi.org/10.1371/journal.ppat.1003551.
[14] Torresi J, Tapia-Conyer R, Margolis H. Preparing for dengue vaccine introduction: recommendations from the 1st dengue v2V international meeting. PLoS Negl Trop Dis 2013;7:e2261, http://dx.doi.org/10.1371/journal.pntd.0002261.
[15] Züst R, Dong H, Li XF, Chang B, Balakrishnan T, Toh YX, Jiang T, et al. Rational design of a live attenuated dengue vaccine: 2′-*o*-methyltransferase mutants are highly attenuated and immunogenic in mice and macaques. PLoS Pathog 2013;9:e1003521, http://dx.doi.org/10.1371/journal.ppat.1003521.
[16] Huang CY, Kinney RM, Livengood JA, Bolling B, Arguelo JJ, Luy BE, et al. Genetic and phenotypic characterization of manufacturing seeds for a tetravalent dengue vaccine (DENVax). PLoS Negl Trop Dis 2013;7:e2243, http://dx.doi.org/10.1371/journal.pntd.0002243.
[17] Zhao H, Jiang T, Zhou XZ, Deng YQ, Li XF, Chen SP, et al. Induction of neutralizing antibodies against four serotypes of dengue viruses by MixBiEDIII, a tetravalent dengue vaccine. PLoS One 2014;9:e86573, http://dx.doi.org/10.1371/journal.pone.0086573.
[18] Azevedo AS, Yamamura AM, Freire MS, Trindade GF, Bonaldo M, Galler R, Alves AM. DNA vaccines against dengue vírus type 2 based on truncate envelope protein or its domain III. PLoS One 2011;6:e20528, http://dx.doi.org/10.1371/journal.pone.0020528.
[19] Azevedo AS, Gonçalves AJ, Archer M, Freire MS, Galler R, Alves AM. The synergistic effect of combined immunization with a DNA vaccine and chimeric yellow fever/dengue virus leads to strong protection against dengue. PLoS One 2013;8:e58357, http://dx.doi.org/10.1371/journal.pone.0058357.
[20] Guy B, Barrere B, Malinowski C, Saville M, Teyssou R, Lang J. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. Vaccine 2011;29:7229–41.
[21] Thomas SJ, Eckels KH, Carletti I, Barrera RDL, Dessy F, Fernandez S, et al. A phase II, randomized, safety and immunogenicity study of a re-derived, live-attenuated dengue virus vaccine in healthy adults. Am J Trop Med Hyg 2013;88:73–88.
[22] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009;339:332–6.
[23] Cappuccio FP, Kerry SM, Forbes L, Donald A. Blood pressure control by home monitoring: meta-analysis of randomized trials. BMJ 2004;329:145–8.
[24] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.
[25] Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: a comparison of methods. Stat Med 1999;18:2693–708.
[26] Sterne JA, Egger M, Smith DG. Systematic reviews in health care: investigating and dealing with publication and other biases in meta-analysis. BMJ 2001;323:101–5.
[27] Thompson SG. Why sources of heterogeneity in meta-analysis should be investigated. BMJ 1994;309:1351–5.
[28] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
[29] Lau J, Ioannidis JP, Terrin N, Schmid CH, Olkin I. The case of the misleading funnel plot. BMJ 2006;333:597–600.
[30] Jadad AR, Moore RA, Caroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary. Control Clin Trials 1996;17:1–12.
[31] Harris RJ, Bradburn MJ, Deeks JJ, Harbord RM, Altman DG, Sterne JAC. Metan: fixed- and random-effects meta-analysis. Stata J 2008;8:3–28.

[32] Morrison D, Legg TJ, Billings CW, Forrat R, Yoksan S, Lang J. A novel a tetravalent dengue vaccine is well tolerated and immunogenic against all 4 serotypes in Flavivirus-naive adults. J Infect Dis 2010;201:370–7.
[33] Guy B, Nougarede N, Begue S, Sanchez V, Souag N, Carre M, et al. Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. Vaccine 2008;26:5712–21.
[34] Guy B, Saville M, Lang J. Development of Sanofi Pasteur tetravalent dengue vaccine. Hum Vaccines 2010;6:696–705.
[35] Qiao M, Shaw D, Forrat R, Wartel-Tram A, Lang J. Priming effect of dengue and yellow fever vaccination on the immunogenicity, infectivity, and safety of a tetravalent dengue vaccine in humans. Am J Trop Med Hyg 2011;85:724–31.
[36] Poo J, Galan F, Forrat R, Zambrano B, Lang J, Dayan GH. Live-attenuated tetravalent dengue vaccine in dengue-naïve children, adolescents, and adults in Mexico City. Pediatr Infect Dis J 2011;30:e9–17.
[37] Capeding RZ, Luna IA, Bomasang E, Lupisan S, Lang J, Forrat R, et al. Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue endemic country: randomized controlled phase I trial in the Philippines. Vaccine 2011;29:3863–72.
[38] Dayan GH, Thakur M, Boaz M, Johnson C. Safety and immunogenicity of three tetravalent dengue vaccine formulations in healthy adults in the USA. Vaccine 2013;31:5047–54.
[39] Dayan GH, Garbes P, Noriega F, Sadovsky ADI, Rodrigues PM, Giuberti C, Dietze R. Immunogenicity and safety of a recombinant tetravalent dengue vaccine in children and adolescents ages 9–16 years in Brazil. Am J Trop Med Hyg 2013;89:1058–65.
[40] Amar-Singh HSS, Koh MT, Tan KK, Chan LG, Zhou L, Bouckenooghe A, et al. Safety and immunogenicity of a tetravalent dengue vaccine in healthy children aged 2–11 years in Malaysia: a randomized, placebo-controlled phase III study. Vaccine 2013;31:5814–21.
[41] Villar LA, Rivera-Medina DM, Arredondo-García JL, Boaz M, Starr-Spires L, Thakur M, et al. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9-16 year olds. A randomized, controlled, phase II trial in Latin America. Pediatr Infect Dis J 2013;32:1102–9.
[42] Leo YS, Wilder-Smith A, Archuleta S, Shek LP, Chong CY, Leong HN, et al. Immunogenicity and safety of recombinant tetravalent dengue vaccine (CYD-TDV) in individuals aged 2–45 years−−Phase II randomized controlled trial in Singapore. Hum Vaccines Immunother 2012;8:1259–71.
[43] Tran NH, Luong CQ, Vu TQH, Forrat R, Lang J, Vu QD, et al. Safety and immunogenicity of recombinant, live attenuated tetravalent dengue vaccine (CYD-TDV) in healthy Vietnamese adults and children. J Vaccines Vaccin 2012;3:162, http://dx.doi.org/10.4172/2157-7560.1000162.
[44] Lanata CF, Andrade T, Gil AI, Terrones C, Valladolid O, Zambrano B, et al. Immunogenicity and safety of tetravalent dengue vaccine in 2–11 year-olds previously vaccinated against yellow fever: randomized, controlled, phase II study in Piura, Peru. Vaccine 2012;30:5935–41.
[45] 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 randomized, controlled phase IIb trial. Lancet 2012;380:1559–67.
[46] Hombach J, Cardosa MJ, Sabchareon A, Vaughn DW, Barrett AD. Scientific consultation on immunological correlates of protection induced by dengue vaccines report from meeting held at the World Health Organization 17–18 November 2005. Vaccine 2007;25:4130–9.
[47] Thomas SJ. The necessity and quandaries of dengue vaccine development. J Infect Dis 2011;203:299–303.
[48] Thomas SJ, Endy TP. Critical issues in dengue vaccine development. Curr Opin Infect Dis 2011;24:442–50.
[49] Roehrig JT, Hombach J, Barrett AD. Guidelines for plaque-reduction neutralization testing of human antibodies to dengue viruses. Virol Immunol 2008;21:123–32.
[50] Halstead SB. Identifying protective dengue vaccines: guide to mastering an empirical process. Vaccine 2013;31:4501–7.
[51] Sirivichayakul C, Sabchareon A, Limkittikul K, Yokasan S. Plaque reduction neutralization antibody test does not accurately predict protection against dengue infection in Ratchaburi cohort, Thailand. Virol J 2014;11:48, http://dx.doi.org/10.1186/1743-422X-11-48.
[52] Mammen MP, Lyons A, Innis BL, Sun W, McKinney D, Chung RCY, et al. Evaluation of dengue virus strains for human challenge studies. Vaccine 2014;32:1488–94.
[53] Rodriguez-Barraquer I, Teran-Romero LM, Burke DS, Cummings DAT. Challenges in the interpretation of dengue vaccine trial results. PLoS Negl Trop Dis 2013;7:e2126, http://dx.doi.org/10.1371/journal.pntd.0002126.
[54] Endy TP, Anderson KB, Nisalak A, Yoon IK, Green S, Rothman AL, et al. Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand. PLoS Negl Trop Dis 2011;1:e975, http://dx.doi.org/10.1371/journal.pntd.0000975.
[55] Bancroft WH, Top Jr FH, Eckels KH, Anderson JH, McCown JM, Russell PK. Dengue-2 vaccine: virological, immunological, and clinical responses of six yellow fever-immune recipients. Infect Immun 1981;31:698–703.
[56] Mansfield KL, Horton DL, Johnson N, Li L, Barrett ADT, Smith DJ, et al. Flavivirus-induced antibody cross-reactivity. J Gen Virol 2011;92:2821–9.