Descriptive Review of Safety, Reactogenicity and Immunogenicity of Dengue Vaccine Clinical Trials, 2003 – 2013

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SUMMARY

Dengue vaccine development has been one of the strategies to reduce dengue incidence in the world alongside with other horizontal interventions such as vector control and the transgenic mosquito programmes. The objective of this paper is to evaluate the safety, reactogenicity and immunogenicity of dengue vaccine clinical trials for the last ten years systematically through a descriptive review. This paper discusses safety issues like adverse events, systemic adverse reactions, injection site reactions, viraemia, morbidity and mortality as well as immunogenicity which measures effectiveness through mean geometric titre and seropositive rates. Adverse events were seen to range from 0% to 28.3%. Immunogenicity was noted to increase post 1st and 2nd dose and decrease post 3rd dose. The seropositivity at baseline ranged between 53.1% and 97.8% at post 3rd dose, and it was 88.5% for at least four serotypes. The dengue vaccine studies that were reviewed were shown to be relatively safe with low reactogenicity, however the immunogenicity was unequal and waning. The immunogenicity waned post 3rd dose showing a decrease in all serotypes of varying degrees although the seropositivity, on average, at post 3rd dose was 97.8%. It can be concluded that dengue vaccine development would require further studies on its unequal and waning immunogenicity, which could result in a more severe form of dengue following wild infection, during re-immunisation, especially if there is variation in the circulating virus.

KEY WORDS:
Dengue, Dengue vaccine, safety, Immunogenicity, live attenuated, tetravalent dengue vaccine, Malaysia, Review

INTRODUCTION

Dengue has been described as the most important mosquito-borne viral disease affecting humans.[1] According to the World Health Organization (WHO), there has been a 30-fold increase in the annual number of cases reported.[2] There is an estimated 3.6 billion people living at risk of infection in more than 120 dengue endemic countries. Approximately 70-500 million infections occur annually resulting in over 2 million severe illnesses.[3] The Asia-Pacific region is considered the global epicentre of the disease, with 1.8 billion people at risk. Dengue disease is endemic in Malaysia and its incidence has increased dramatically, from <20/100,000 in the 1970s to >150/100,000 in 2010, with an increased prevalence seen in adults relative to children (the main target population for potential dengue disease vaccines) as well as several changes in serotype distributions and increases in disease severity.[4]

The development of a dengue vaccine has been one of the strategies employed to reduce the incidence of dengue worldwide together with other horizontal interventions such as vector control and the transgenic mosquito programmes. As yet, no licensed vaccine or specific treatment exists for dengue. Preventive measures based on integrated vector control have shown limited effectiveness and sustainability. The development of a dengue virus (DENV) vaccine has proved to be challenging. Ideally, protection is needed against all four serotypes (DENV1-4), although in vivo interference between the serotypes has presented challenges in achieving a well-balanced immune response.1 Several dengue vaccine clinical trials have been undertaken by a number of countries, for example, the USA, Thailand, Brazil, and Malaysia, and the multiple challenges faced in the effort to develop a dengue vaccine include the need to induce a balanced and lasting immunity against the four DENV serotypes.

A wide range of vaccine technologies have been applied to dengue vaccine development including live attenuated virus (LAV), purified inactivated virus (PIV), recombinant subunits, virus like particles (VLPs), and plasmid or viral vectors.[5]

The DENV1-4 live attenuated DENV vaccines were developed as early as 1981 beginning with monovalent vaccines of all four serotypes after which they were mixed into bivalent, trivalent and tetravalent vaccines. Recommendation for use in human trials was approved by the WHO through the appointment of a scientific steering committee.[6]

LAV vaccines have been shown to produce a robust, lasting and broad immunity which includes both humoral and cellular immune responses. However, it has often proven challenging and difficult to achieve a level of attenuation which optimally balances low reactogenicity yet has sufficiently high immunogenicity.

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Through the use of molecular genetics, a recombinant chimeric vaccine was constructed by using the "backbone" of a related flavivirus (yellow fever attenuated DENV strain) which was licensed to Sanofi Pasteur. It is a tetravalent dengue vaccine (TDV) comprising of four recombinant, live attenuated dengue vaccines (CYD 1-4) based on the yellow fever 17D vaccine strain (YFV 17D).[7] Extensive pre-clinical and clinical evaluation from an early phase to ongoing phase III clinical trials were necessary in view of the chimeric nature and live attenuation of these vaccine viruses. The evaluation had taken into consideration the specific regulations pertaining to genetically modified organisms (GMO) and genetic stability. Hence the clinical evaluation included reactogenicity, viraemia, humoral and cellular immunogenicity.[8]

With regards to safety, reactogenicity and immunogenicity of these clinical trials, this paper's objective discusses two main components: 1) safety issues such as adverse events, systemic adverse reaction, injection site reaction, viraemia, morbidity and mortality; and, 2) immunogenicity which measures the effectiveness through geometric mean titre (GMT) and seropositive rates.

MATERIALS AND METHODS

Literature search strategy

An exhaustive internet search was performed using three databases (PubMed, Science Direct and Cochrane Library) for published papers and also using Google Scholar. The search words included: human clinical trials for tetravalent dengue vaccines based on their safety, reactogenicity and immunogenicity. The search only looked at articles which had been published in the English language between 2003 and 2013 (10 years). Using the inclusion and exclusion criteria listed below, the results were as follows: the PubMed drew ten papers. In Science Direct, of the five papers identified, four were duplicated; in the Cochrane Library, there were six papers of which four were duplicated; while in Google Scholar, there were 12 papers, with 11 being duplicates. All in all, there were 14 original papers obtained from the four search engines. Upon reviewing the full articles, two out of the 14 published papers did not meet the inclusion criteria as listed below. The study of Lanata et al. was biased and thus excluded as the dengue vaccine which was developed was tested against subjects previously vaccinated against yellow fever.[9] The study of Poo et al. was excluded because the dose interval varied at 0, 3.5 and 12 months.[10] Thus a total of 12 papers were reviewed.

These 12 papers were then further scrutinised in detail against the inclusion and exclusion criteria, which resulted in a final tally of six papers identified for detailed analysis which included Amar et al., Villar et al., Dayan et al., Tran et al., Leo et al. and Sabchareon *et al.*[1,4,11-14] The six papers used tetravalent vaccines in their studies and were able to elicit protective neutralising antibody responses against all the four dengue serotypes. These papers also addressed the theoretical and clinical concerns about vaccine induced antibody dependent enhancing (ADE) factor.

A summary on the safety, reactogenicity and immunogenicity of the tetravalent vaccine from the studies were documented using adverse events (AE), solicited injection site reactions (SISR), solicited systemic reactions (SSR), allergic reactions (AR), unsolicited adverse events (UAE), serious adverse events (SAE) and fatal serious adverse events (FSAE) that were recorded in the study population through the collection of data comprising the physical examination of the subjects, their body temperature measurements, and taking blood samples. In addition, the subjects were given diary cards to record any adverse events that may have occurred.

The definition of reactogenicity as used in these clinical trials were in accordance and complied with the definition as described by the US National Institutes of Health for clinical trial protocols.[15] The assessment of reactogenicity was based on a functional scale of 0-4 is described as follows:

0 = Absence of the indicated symptom
1 = Mild (awareness of a symptom but the symptom is easily tolerated)
2 = Moderate (discomfort enough to cause interference with usual activity)
3 = Severe (incapacitating; unable to perform usual activities; requires absenteeism or bed rest)
4 = Life-threatening

Immunogenicity is defined as the ability of a substance to provoke an immune response or the degree to which it provokes a response.[16] Immunogenicity corresponds to the immune response to the virus in the body, and signifies the effectiveness of the dengue vaccine in immunisation, or to a past infection. The study of Amal *et al.* described immunogenicity as virus neutralising antibody levels against DENV1-4 post-immunisation. It was assessed using PRNT50 compliant with the WHO guidelines and expressed as both geometric mean titres (GMTs) and seropositivity rates (percentage of participants with titres ≥10 1/dl).

Data abstraction

Each of the six papers were systematically read through, thoroughly discussed and transcribed into an Excel format worksheet. From this format, the safety, reactogenicity and immunogenicity parameters were illustrated. Safety and reactogenicity parameters were described by the study of Dayan *et al.*, as adverse events (AEs) monitored throughout the study which included unsolicited systemic AEs within 30 minutes of each vaccination, solicited injection site reactions (pain, erythema, or swelling) up to seven days after each vaccination, solicited systemic reactions (fever, headache, malaise, myalgia, or asthenia) up to 14 days after each vaccination, unsolicited AEs up to 28 days after each vaccination, and serious AEs (SAEs) throughout the trial. AE data after the initial 30-minute observation period were collected using a diary card.[1] The AE records were collected using a diary card printed with the specific solicited adverse reaction questions and an open field for unsolicited AEs. AEs were graded (one, two, or three) from mild to severe according to a pre-defined intensity scale based on appropriate measurements or observations. SAEs were

defined as events that were life-threatening or resulted in death; these cases required in-patient hospitalisation or prolonged existing hospitalisation. There may be persistent or significant disability or incapacity, congenital anomaly or birth defect, or were regarded as an important medical event. Participants were followed up at 6 months after the last injection for information on AEs occurring since the last visit.

Inclusion and exclusion criteria
The inclusion criteria for the selection of the papers described above include the following: human clinical trials involving all four circulating dengue viruses; tetravalent vaccines with a formulation of 5 $\log_{10}$ PFU/mL for each serotype; subjects were given a specified dose of 0.5 ml subcutaneously at defined intervals (0, 6 and 12 months); and the papers had detailed descriptions of safety, reactogenicity and immunogenicity outcomes. The exclusion criteria were: vaccine reports and scientific communications; studies on monovalent, bivalent and trivalent vaccines; and, all subjects declared as previously vaccinated against other flaviviruses and non-human clinical trials.

Dengue vaccine dosing schedule
The subjects were given three subcutaneous doses of 0.5 ml dengue vaccine at 0, 6 and 12 months intervals.

Data analysis
This paper was primarily descriptive in nature because of the small number of subjects in a limited number of articles. The outcome of the results was determined through pooling of the various data to quantitatively describe a formulation of percentages [P = (n1 + n2 + n3, …/N1 + N2 + N3) … x100] where 'P' is the pooled value, 'n' is the sample size and 'N' is the sample population.

RESULTS AND ANALYSIS

The six papers that were reviewed described controlled double-blind clinical trials which were carried out between 2003 and 2013 in both dengue naïve and exposed populations. The pooled results of the studies are illustrated in the Tables II to XV (see Appendix).

Safety and reactogenicity
Tables II to IX showed the overall results from the 6 studies: 2.13% of the participants suffered from adverse events (AE). For each of the spectrum of AEs, the results showed the following events: 28.3% were solicited injection site reactions (SISR). The specific symptoms in the SISR group were 18.9% subjects complained of pain, 9.9% complained of erythema and 7.3% complained of swelling. For the solicited systemic reaction (SSR) group, the finding was 21.8% with the symptoms recorded being 9.7% suffered from fever, 44.1% complained of headache, 34% had malaise, 38.7% had myalgia and 19.1% had asthenia. Headache was seen as the pre-dominant complaint. 2.5% of subjects had allergic reactions (AR) while 14.2% reported unsolicited adverse events (UAE). Serious adverse events (SAE) were seen in 8.8% of the subjects while there were no fatal serious adverse events (FSAE) reported nor were there any vaccine related serious adverse events.

Dengue viraemia
Overall, dengue viraemia was observed in 4.5% of the combined average studies by Dayan *et al.*, Leo *et al.* and Sabchareon *et al.*[1,13-14]

Immunogenicity:
i) Geometric Mean Titre (GMT)
Table X of the GMT results showed that overall, in all the serotypes 1-4, the GMT was highest, valued as 1031, in serotype 3, post 2nd dose in the study by Dayan *et al.*[1] The GMT in serotype 1, post 3rd dose was lowest, measured as 43.0 in the study by Leo *et al.* In the studies by Dayan *et al.* and Villar *et al.*, the GMT response was higher when compared to the other studies.[1,11] The studies by Leo *et al.* and Amar *et al.* showed a lower GMT when compared to the other 4 studies.[4,13]

It was observed that, generally, the trend of the mean GMT increased after the 1st and 2nd doses but decreased after the 3rd dose in all the studies. However, in the study by Amar et al. this trend was seen only in serotype 3 but in serotypes 1, 2 and 4, the GMT continued to rise after post 3rd dose.[4]

In the study by Villar *et al.* where the endemicity of flavivirus was 78.8%, Yellow Fever (YF) (70%) and Dengue Fever (DF) (75%), the GMT increases in serotype 1 with each successive dose; however with serotypes 2, 3 and 4, the GMT decreases after the 3rd dose after an initial increase in the 1st and 2nd doses.[11]

In the study by Dayan et al. where the endemicity of flavivirus was 81%, YF (71%) and DF (69%), the GMT in serotype 4 drops post 2nd dose and increases post 3rd dose, while in serotypes 1, 2 and 3, the GMT decreases post 3rd dose after an initial increase in the 1st and 2nd doses.[1]

Some non-availability of data for the studies by Leo *et al.* and Tran *et al.* made these studies difficult to comment specifically for post 1st and 2nd doses.[12,13] However, in the study by Leo *et al.* where the dengue endemicity varied, in post 3rd dose, the serotype 4 response was observed to be highest while the serotype 1 was observed to have the lowest response.[13] In the study by Tran et al. where the endemicity of flavivirus was 76%, DF (71%) and JE (5%), in post 3rd dose, serotype 2 was observed to have the highest response while serotype 1 had the lowest response.[12]

In the study by Sabchareon et al., it was observed that there was an increase in GMT with each successive dose in the 3-dose regime for serotypes 1 and 3, and in serotypes 2 and 4, after an initial increase in post 1st and 2nd dose, there was a decrease after the 3rd dose. However, this one study reported that there was a decrease in GMT in all the 4 serotypes at the end of 1 year.[14]

ii) Seropositivity
An increase in seropositivity rate was observed after each dose of the vaccine was administered. Overall the seropositivity at baseline was 53.1%. After post 3rd dose, the seropositivity overall increased to 95.9%. The highest seropositivity was seen among at least two serotypes while the lowest seropositivity was seen among all 4 serotypes.

The seropositivity of the subjects was calculated from the average of the studies by Villar et al., Dayan et al. and Leo et al., and this was seen to be more than 90% for at least two serotypes (99.1%) or at least three serotypes (94.9%).[1,11,13] The seropositivity for all four serotypes was 88.5% as calculated from the average of the studies by Villar *et al.*, Dayan *et al.* and Leo *et al.*[1,11,13] In this case, the study by Leo *et al.* showed a low seropositivity of 66.5%.[13] The seropositivity for at least one serotype was 84.6%. This was calculated from the average of the studies by Amar *et al,* Villar *et al.*, Dayan *et al.* and Leo *et al.*[1,11,13] The study by Amar et al. described a very low figure of 44.9% post 3rd dose results of at least one serotype but the results for the seropositivity in at least the two, three and all four serotypes were not available.[4]

The study by Leo *et al.* described that the seropositivity of types 1-4 was higher in children than the older participants (divided into three age-groups of 2-11 years, 12-17 years and 18-45 years).[13] The study by Tran *et al.* described that, after each dose, seropositivity increased in all the four serotypes (divided into age-groups of 6-11 years and 12-17 years).[12]

APPENDIX

Table I: Demographic characteristics

Author Characteristics	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	Total
Study location (country)	Malaysia	Latin American	Brazil	Singapore	Thailand	Vietnam	
Sample population (N)	199	390	100	898	2666	120	4373
Age-groups (in years)	2-11 years (2-5; 6-11)	9-16 years	9-16 years	2-45 years (2-11; 12-17; 18-45)	4-11 years	2-45 years (2-5; 6-11; 12-17; 18-45)	

Safely and reactogenicity

Table II: Adverse Events (AE)

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	Total	Percentage (%)
Adverse Event (AE)	1	1	N/A	5	0	84	91	2.13%

Table III: Solicited Injection Site Reaction (SISR)

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	Total	Percentage (%)
Solicited Injection Site Reaction (SISR)	163	NA	40 (grade 3)	484	426	39	1127	28.3%
Pain	138	103	40	9	NA	34	324	18.9%
Erythema	93	10	4	65	NA	8	170	9.9%
Swelling	77	7	5	37	NA	5	124	7.3%

Table IV: Solicited Systemic Reaction (SSR)

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	Total	Percentage (%)
Solicited Systemic Reaction (SSR)	178	68	30	101	538	39	955	21.8%
Symptoms **(grade 1)**								
Fever	13	23	NA	NA	NA	33	69	9.7%
Headache	108	137	61	405	NA	42	753	44.1%
Malaise	NA	77	40	375	NA	35	527	34%
Myalgia	NA	89	42	397	NA	24	584	38.7%
Asthenia	NA	57	32	184	NA	80	289	19.1%
Symptoms **(grade 3)**								
Fever	NA	4	8	17	NA	1	30	2%
Headache	NA	17	15	34	NA	1	67	4.4%
Malaise	NA	7	11	39	NA	0	57	3.8%
Myalgia	NA	19	6	23	NA	NA	48	3.5%
Asthenia	NA	5	8	12	NA	2	27	1.8%

Table V: Allergic Reaction (AR)

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	Total	Percentage (%)
AR	NA	NA	NA	NA	NA	3	NA	2.5%

Table VI: Unsolicited Adverse Events (UAE)

Author Outcome	Amar et al, 2013	Villar et al, 2013	Dayan et al, 2013	Leo et al, 2012	Sabchareon et al, 2012	Tran et al, 2012	Total	Percentage (%)
UAE	74	NA	NA	NA	317	33	424	14.2%

Table VII: Serious Adverse Events (SAE)

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al*, 2012	Total	Percentage (%)
SAE	11	10	6	33	315	3	378	8.8%

Table VIII: Fatal Serious Adverse Events (FSAE) & Vaccine- related SAE

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	Total	Percentage (%)
FSAE	0	0	0	0	0	0		0
Vaccine- related SAE	0	0	0	0	0	0		0

Table IX: Dengue viraemia

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	Total	Percentage (%)
Dengue viraemia	NA	NA	31	0	134	NA	165	4.5%

Immunogenicity
Table X: Geometric Mean Titre (GMT)

Author Outcome	Amar et al, 2013	Villar et al, 2013	Dayan et al, 2013	Leo et al, 2012	Sabchareon et al, 2012	Tran et al, 2012	Total Total	Mean of GMT
Serotype 1								
Baseline	15.3	74.2	41.4	8.1	42.8	32.8	214.2	35.7
Post dose 1	NA	221.0	256.0	NA	94.4	NA	571.4	190.5
Post dose 2	119.0	276.0	436.0	NA	120.7	NA	951.7	237.8
Post dose 3	151.0	320.0	267.0	43.0	146.1	129.0	1056.1	178.0
Post 1-year	NA	NA	NA	NA	76.5	NA	NA	76.5
Serotype 2								
Baseline	15.9	92.6	67.0	9.0	56.8	33.7	275.0	46.8
Post dose 1	NA	409.0	352.0	NA	195.0	NA	956.0	318.7
Post dose 2	160.0	504.0	647.0	NA	326.0	NA	1637.0	409.3
Post dose 3	180.0	486.0	544.0	69.7	310.0	216.0	1807.7	301.2
Post 1-year	NA	NA	NA	NA	122.5	NA	NA	122.5
Serotype 3								
Baseline	15.6	85.0	81.9	8.5	31.5	32.5	255.0	42.5
Post dose 1	NA	442.0	690.0	NA	111.9	NA	1243.9	414.6
Post dose 2	196.0	502.0	1031.0	NA	195.0	NA	1924.0	481.0
Post dose 3	193.0	594.0	741.0	96.0	405.0	169.0	2198.0	366.3
Post 1-year	NA	NA	NA	NA	94.8	NA	NA	94.8
Serotype 4								
Baseline	9.9	37.2	15.0	6.8	28.1	17.1	114.2	19.0
Post dose 1	NA	416.0	383.0	NA	138.0	NA	937.0	312.3
Post dose 2	110.0	305.0	346.0	NA	159.0	NA	920.0	230.0
Post dose 3	114.0	273.0	432.0	100.0	155.0	146.0	1220.0	203.3
Post 1-year	NA	NA	NA	NA	153.0	NA	NA	153.0

Table XI: Seropositivity at baseline

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	%	Mean %
Baseline								
Serotype 1	31.1%	64.1%	60.0%	NA	55.0%	NA	52.5%	
Serotype 2	27.6%	69.3%	66.0%	NA	58.0%	NA	55.2%	53.1%
Serotype 3	36.7%	69.6%	63.0%	NA	60.0%	NA	57.3%	%
Serotype 4	24.0%	62.6%	48.0%	NA	56.0%	NA	47.6%	

Table XII: Seropositivity at post dose 3

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	%	Mean %
Post dose 3			97.0-100.0%	77.1-94.1%				
Serotype 1	NA	97.6% (+)/ 81.8% (-)	100.0%					
	NA	95%	NA	97.5%				
Serotype 2	NA	≥95.0%	100.0%	NA	99%	NA	98.0%	97.8
Serotype 3	NA	≥95.0%	97.0%-100.0%	NA	100%	NA	98.3%	%
Serotype 4	NA	≥95.0%	97.0%-100.0%	NA	98%	NA	97.6%	

Table XIII: Seropositivity at baseline according to the number of serotypes

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	%
At least 1 serotype	NA	NA	NA	26.5%	NA	NA	26.5%
At least 2 serotypes	NA	NA	NA	15.3%	NA	NA	15.3%
At least 3 serotypes	NA	NA	NA	11.8%	NA	NA	11.8%
All 4 serotypes	NA	NA	NA	8.8%	NA	NA	8.8%

Table XIV: Seropositivity at post dose 3 according to the number of serotypes

Author Outcome	Amar et al, 2013	Villar et al, 2013	Dayan et al, 2013	Leo et al, 2012	Sabchareon et al, 2012	Tran et al, 2012	%
At least 1 serotype	44.9%	94.2%	100.0%	99.3%	NA	NA	84.6%
At least 2 serotypes	NA	100.0%	100.0%	97.3%	NA	NA	99.1%
At least 3 serotypes	NA	98.6%	98.9%	87.2%	NA	NA	94.9%
All 4 serotypes	NA	93.4%	96.6%	66.5%	NA	97.7% (+) / 75.0% (-)	88.5%

Table XV: Seropositivity at post dose 3 after 1-year

Author Outcome	Amar *et al,* 2013	Villar *et al,* 2013	Dayan *et al,* 2013	Leo *et al,* 2012	Sabchareon *et al,* 2012	Tran *et al,* 2012	%	Mean%
Serotype 1	NA	NA	NA	NA	77%	NA	77%	86.2%
Serotype 2	NA	NA	NA	NA	85%	NA	85%	
Serotype 3	NA	NA	NA	NA	89%	NA	89%	
Serotype 4	NA	NA	NA	NA	94%	NA	94%	

DISCUSSION

The clinical trials reviewed pertained to its safety, reactogenicity and immunogenicity among different age groups in a diverse population in various regions, some in areas of dengue endemicity.

With the pooled data, it was noted that there was missing data, lack of full description of events, varying dosages, different formulations, varied population, geographical areas and environmental differences which may contribute to biases and posed as a limitation to our review.

In addition, there was a need to standardise and harmonise the Plaque Reduction Neutralization Test (PRNT), which measures neutralising antibodies in all the studies because of multiple antigenic exposures due to secondary infections. As has been mentioned above, vaccination of the subjects using the tetravalent dengue vaccine produced variable results.

Some of the studies had variable antibody response to each dengue serotype which was considered an imbalanced response as it may result in enhanced disease following natural infection and re-immunisation.[17] Thomas *et al.*

further critiqued the previous studies by stating waning immunity, that is a decline in the antibody titre over time, may increase vaccine recipient risk of immuno-pathological response that may result in enhanced disease following natural infection and re-immunisation. [17]

Phase I studies thus far, have established that a three dose regimen of the candidate vaccine induces a balanced neutralising antibody response involving the various age groups irrespective of their flavivirus serostatus. The trials were conducted in participants as young as two years of age. Phase I clinical trials by Capeding *et al.*, Poo et al. and Morrison *et al.* concluded that a vaccine administration schedule of either three vaccinations administered over a year or two vaccinations given more than eight months apart resulted in a balanced antibody response to all four dengue serotypes including children.[10,18-19] From the results of the Phase I clinical trials, it was decided to proceed with the Phase II clinical development of a dengue vaccine with a three dose regimen of 0-6-12 months based on the rationale of capitalisation on the observed increase in immunogenicity of the dengue vaccine with a longer interval of time between first two vaccinations (longer lasting immunity) and to increase the likelihood that complete vaccination with three doses in a flavivirus naive population would result in a balanced immune response against all four serotypes.[18]

The dengue candidate vaccine has a relatively favorable safety and tolerability profile in keeping with other Phase I and II clinical trials. The vaccinated subjects were followed up from 30 minutes following first injection up to 6 months after the last vaccination with the study by Leo *et al.* continuing an on-going safety follow-up for upto 4 years.[13] Thus far, proof of concept efficacy trials (Phase IIb) in Thailand has had the longest period of follow-up (two years) with the largest population comprising 2600 participants The clinical trials had a satisfactory and good safety profile. Reactogenicity following injections were lower following post 2nd and 3rd dose vaccinations when compared with the first dose confirming good tolerability. Overall reactogenicity was similar to the control groups. The local and systemic reactions were mild to moderate and short-lived, usually resolving within three days. The most frequently reported injection site reaction was pain and erythema.

There was no remarkable or significant difference on reactogenicity based on the flavivirus serostatus. However, Wan *et al.* commented, from the review of older studies that not all the participants in the Mahidol University clinical trial in Thai adults and children sero-converted to all the 4 dengue serotypes; with some subjects showing unaccepted reactogenicity.[20-23] The vaccination induced low dengue viraemia, was noted to be 31 in the study by Dayan *et al*; 134 in the study by Sabchareon *et al.*; and it was zero in the study by Leo *et al.*[1,13-14] Morrison *et al.*, Poo *et al.* and Capeding et al. concurred with these low viraemia findings. There were no deaths or serious adverse effects related to the vaccine or placebos.[10,18-19]

On the whole, a balanced and robust antibody response to all four dengue serotypes was elicited irrespective of the participants' flavivirus serostatus and age group. At baseline, a considerably higher GMT, which is a measure of neutralising antibody response, was found in participants with flavivirus (FV) seropositivity as compared to participants with FV seronegativity.

Following post 3rd dose vaccination, there was a modest increase in GMT in the participants with flavivirus seropositive status showing little impact when compared to the marked rise in the neutralising antibody response in participants with flavivirus seronegative status.

Among the participants in the vaccine group with FV seropositivity showing high GMT titres, GMT was seen to be higher in participants with dengue disease seropositivity at baseline than in participants with Japanese encephalitis (JE) seropositivity, but the subset of these participants was small. A marked response was seen in participants who were seropositive for both dengue disease and JE at baseline. Higher antibody titres seen following dengue vaccination in the flavivirus seropositive participants at baseline suggests that pre-existing FV antibody response may have a beneficial impact on the vaccine induced antibody response. The rise in antibody titres in post 3rd dose vaccination in subjects who were seronegative was not seen in the study by Dayan *et al.* as the sample size was small with considerable assay variability. [4] Hence, a three dose regimen is beneficial in eliciting a balanced antibody response in areas with populations of mixed flavivirus serostatus.

Immune response to dengue vaccination also varied with age. In the studies by Tran *et al.* and Capeding *et al.*, post vaccination antibody titres were highest among adult participants whereas the younger age group demonstrated a higher relative increase when compared to baseline.[12,18] The clinical trial involving children, adolescents and adults in the study by Capeding *et al.* found that adults and adolescents with high baseline levels of flavivirus antibodies, one dose of the dengue vaccine was sufficient to boost the antibody response while two or three injections were required for children who in both groups had lower baseline levels of flavivirus antibodies.[18] The older participants showing a higher response was most likely due to prior dengue exposure as dengue is endemic in Vietnam and Philippines. High flavivirus antibodies in the study by Capeding et al. may have also been contributed by pre-existing immunity to JE which is endemic in the Philippines.[18]

The clinical trials conducted among participants in the study by Leo *et al.*, on the other hand, found post-vaccination antibody titres to be higher among children and adults with a lower immune response among adolescents in a dengue naive population as there was a low prevalence of dengue immunity among adolescents.[13] There was no available data on baseline seroprevalence in this age group in the general population of the study by Leo *et al.*[13] The low rate of dengue immune adolescent participants may in fact reflect the epidemiological profile of dengue disease within Singapore, a country with fluctuating endemicity levels.

The study by Sabchareon *et al.* reported that there was a decrease in GMT in all the 4 serotypes at the end of 1 year.[14] This response may indicate that the vaccine does not confer

a lifelong immunity and perhaps may indicate that annual boaster doses may be required. This further reinforces the discussion that in addition to endemicity, seropositivity in each region or specific area needs to be determined before the formulation of the vaccine is developed regionally.

Here we note that the varying antibody response among children, adolescents and adults may be contributed by the presence of pre-existing FV antibodies and emphasises its beneficial impact on vaccine induced antibody response, also taking into consideration the impact of the epidemiological profile and endemicity of the region with regards to FV serostatus.

Different regions are endemic for various flaviviruses (FV) such as yellow fever (YF) or Japanese encephaltitis (JE), which may co-exist with the dengue viruses. We note from these studies the impact of pre-existing FV antibodies, but on the other hand, vaccination with the candidate vaccine and its effects on the pre-existing FV antibodies from YF or JE vaccination in the endemic countries, as a part of these countries' immunisation programmes need to be evaluated too. Thus far, yellow fever immunisation is a part of Peru's national immunisation programme and JE vaccination is a part of the national immunisation programme in Thailand and East Malaysia. In the study by Lanata *et al.*, children of 2-11 years, who were previously vaccinated for yellow fever, participated in a clinical trial to evaluate the safety and immunogenicity of the candidate vaccine.[9] At least 40% of these children had dengue antibodies against ≥ 1 serotype. Almost 60% had received YF vaccination more than three years before and hence a 3-year cut off was used for an exploratory analysis of whether more recent YF vaccination was associated with a higher immune response to the candidate vaccination. The clinical trials conferred and yielded a similar response to the other Phase II clinical trials on the vaccine candidate's safety and immunogenicity. Children of a younger age, ≤ than 5 years had a higher GMT as compared to children of 6-11 years of age. Children of ≤ 3 years who were vaccinated with YF vaccination had a higher GMT than those who had received YF vaccination of ≥ 3 years.

Increase in antibody response after post 3rd dose vaccinations were moderate compared to that observed after the first two doses. However, these responses differed depending on baseline dengue status. In participants who were dengue seropositive, titres increased markedly after the post 1st and 2nd dose vaccinations; it was similar post 3rd dose. In baseline dengue naïve participants, in more than half of the study population, titres increased after three doses without reaching high levels observed in the dengue exposed serotypes. It was also found that the dengue vaccination appeared to marginally re-stimulate existing YF immunity.

On the other hand, in toddlers receiving JE vaccines as part of their national immunisation programme, it was found that prior dengue vaccination did not seem to influence reactogenicity of JE vaccine but sero-conversion rate and GMTs achieved after two JE vaccinations did seem to be reduced in recipients of full dose dengue vaccinations. Although not achieving statistical significance, these findings should be further evaluated especially in countries or regions where both dengue fever and Japanese encephalitis co-circulate and where JE vaccination is recommended.[24] Whether a similar occurrence will be encountered in children receiving dengue vaccination in countries having JE vaccination as part of its national immunisation programme should be further evaluated.

A balanced and robust neutralising antibody response to dengue vaccination against all 4 serotypes does not equate to protection. These studies were not designed to assess the vaccine efficacy and hence long-term vaccine safety or immune persistence was not assessed. Thus far, Phase IIb proof of concept efficacy trials conducted in Thailand did not provide adequate protection against infection with dengue serotype 2 despite presence of high antibody levels. Observed lack of efficacy despite satisfactory immunogenicity was surprising and warrants investigation. The proposed reasoning was an antigenic mismatch between dengue virus (DENV) vaccine and the DENV2 or the viruses that caused the disease. The Asian 1 genotype of DENV2 circulating in South-east Asia has several lineages, one of which had mutations that could have implications on viral fitness. The DENV2 antibody titre might not have been high enough to protect against the serotype or particular lineage of viruses circulating in the province during the study period. Whether immune response to DENV non-structural proteins (which are not encoded in the dengue virus vaccine) contribute to the overall protective response to DENV 2 needs to be further clarified. The principal limitation of this clinical trial was its mono-centre design in a single area in Thailand and the predominance of one serotype.[14]

Sabchareon *et al.* stated that the Phase II study showed 70% effectiveness and efficacy against DENV 1, 3 and 4 serotypes.[14] Wan *et al.*, in referring to Halstead *et al.* recommended further testing and assessment of the risk of antibody enhancing factor (ADE), modification and further clinical trials are needed especially in the dengue endemic countries.[20,25] Larger Phase III clinical trials are currently on-going in Latin America and Asia on the efficacy of these dengue vaccines.

Wan *et al.* has stated that although the live viral vaccines have advanced to clinical trials, they have shown problems such as unequal immunogenicity towards the four serotypes and viral interferences among the four serotypes in the tetravalent formulations. The author proposed the development of a non-viral vaccine for safety reasons.[20]

The dynamics of dengue viruses differs and remains complex worldwide. The distribution of the prevalent serotype of the dengue virus evolves and differs from region to region and at different times (temporal differences) within the same region. This may contribute towards the varying degrees of vaccine efficacy that were documented in the studies reviewed above.

CONCLUSION

Our review states that the dengue candidate vaccine may appear to be a relatively safe vaccine with a satisfactory tolerability as it elicits a positive immune response, albeit to varying degrees, against all four dengue serotypes.

However, a tetravalent dengue vaccine may not be the sole preventive measure. It may give some degree of protection against dengue fever as the severity of the disease varies with endemicity. The immunogenicity response varies in different locations at different times as it is dependent on the flavivirus status of the population, the circulating viral variation and the dengue endemicity in each area. Our concern is that in the event of a re-immunisation or if a natural/wild infection ever occurs, this may result in an even more severe form of the disease.

It is important for larger population-based efficacy trials to be carried out among the Asian populations to determine the long-term persistence of humoral and immune responses. It is also vital in countries with JE vaccination as part of its national immunisation programme, to not only assess the efficacy of the candidate vaccine but also to assess the effect of the candidate vaccine on the immunogenic response of the JE vaccine received following dengue vaccination. There is a need to re-strategize dengue vaccine development even before it is even considered as a national immunisation policy of various countries.

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REFERENCES

1. Dayan GH, Garbes P, Noriega F, *et al.* 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(6): 1058-65.
2. Adwoa DB-E, Schmitz J, Edelman R, *et al.* Long-term safety assessment of live attenuated tetravalent dengue vaccines: deliberations from a WHO technical consultation. Vaccine 2013; 31(23): 2603-9.
3. Thomas SJ, Eckels KH, Carletti I, *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(1): 73-88.
4. Amar-Singh HSS, Koh M-T, Tan KK, *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(49): 5814-21.
5. Schmitz J, Roehrig J, Barrett A, *et al.* Next generation dengue vaccines: A review of candidates in preclinical development. Vaccine 2011; 29(42): 7276-84.
6. Bhamarapravati N, Sutee Y. Live attenuated tetravalent dengue vaccine. Vaccine 2000; 18(Suppl.2): 44-7.
7. Murrell S, Wu S-C, Butler M. Review of dengue virus and the development of a vaccine. Biotechnol Adv 2011; 29(2): 239-47.
8. Guy B, Barrere B, Malinowski C, *et al.* From research to Phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. Vaccine 2011; 29(42): 7229-41.
9. Lanata CF, Andrade T, Gil AI, *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(41): 5935-41.
10. Poo J, Galan F, Forrat R, *et al.* Live-attenuated tetravalent dengue vaccine in dengue-naive children, adolescents, and adults in Mexico City: Randomized controlled phase 1 trial of safety and immunogenicity. Pediatr Infect Dis J 2011; 30(1), e9-17.
11. Villar LA, Rivera-Medina DM, Arredondo-Garcia JL, *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(10), 1102.
12. Tran NH, Luong CQ, Vu TQH, *et al.* Safety and immunogenicity of recombinant, live attenuated tetravalent dengue vaccine (CYD-TDV) in healthy Vietnamese adults and children. Journal of Vaccines & Vaccination 2012; 3: 7.
13. Leo YS, Wilder-Smith A, Archuleta S, *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 Vaccin Immunother 2012; 8(9): 1259-71
14. Sabchareon A, Wallace D, Sirivichayakul C, *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): 1559-67.
15. United States National Institutes of Health (US NIH). US DMID Protocol Template. Version 1.0. 2004.
16. American Heritage Medical Dictionary, 2007.
17. Thomas SJ. The necessity and quandaries of dengue vaccine development. J of infectious dis 2011; 203(3): 299-303.
18. Capeding RZ, Luna IA, Bomasang E, *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(22): 3863-72.
19. Morrison D, Legg TJ, Billings CW, *et al.* A novel tetravalent dengue vaccine is well tolerated and immunogenic against all 4 serotypes in flavivirus-naive adults. J Infect Dis 2010; 201(3): 370-7.
20. Wan S-W, Lin C-F, Wang S, *et al.* Current progress in dengue vaccines. Journal of Biomed Sci 2013; 20: 37.
21. Sanchez V, Gimenez S, Tomlinson B, *et al.* Innate and adaptive cellular immunity in flavivirus-naïve human recipients of a live-attenuated dengue serotype 3 vaccines produced in Vero cells (VDV3). Vaccine 2006; 24(23): 4914-26.
22. Sabchareon A, Lang J, Chanthavanich P, *et al.* Safety and immunogenicity of tetravalent live-attenuated dengue vaccines in Thai adult volunteers: role of serotype concentration, ratio and multiple doses. Am J Trop Med Hyg 2002; 66(3): 264-72.
23. Sabchareon A, Lang J, Chanthavanich P, *et al.* Safety and immunogenicity of a three dose regimen of two tetravalent live-attenuated dengue vaccines in five- to twelve-year-old Thai children. Pediatr Infect Dis J 2004; 23(2): 99-109.
24. Watanaveeradej V, Simasathien S, Nisalak A, *et al.* Safety and immunogenicity of a tetravalent live-attenuated dengue vaccine in flavivirus-naive infants. Am J Trop Med Hyg 2011; 85(2): 341-51.
25. Halstead SB. Dengue vaccine development: a 75% solution? Lancet 2012; 380(9853): 1535-6.