

# Advances in Dengue Vaccine: A Review Study

## Abstract

Dengue is now a global burden with about 3.97 billion people living in 128 countries at risk and 400 million infections occurring every year. Four closely related serotypes have been implicated in the global burden: DENV 1-4. It has been reported that there are serotype specific differences in clinical manifestations. This systematic review is based on PRISMA guidelines. It discusses two types of DENV vaccine based on inclusion criteria: Tetravalent dengue vaccine (TDV) and dengue DNA vaccine that are in clinical studies. A number of studies evaluated the safety, reactogenicity, and immunogenicity of TDV especially Sanofi Pasteur's CYD-TDV. Most of the studies reported that the vaccine candidate is safe and elicit efficient neutralizing antibody. As part of the global effort to develop DENV vaccine, the US National Institute of Health also initiated a program which culminated in the development of a monovalent dengue vaccine candidate called rDEN4Δ30. Five different formulation were evaluated in clinical studies. Only a paper described a randomized controlled study of DENV DNA vaccine. It reported that the vaccine candidate is safe at the given dose. Studies are needed to evaluate the immunological profile of dengue vaccine in children and those in endemic areas. The future of DENV vaccine effort looks bright and it is hoped that soon an efficient DENV vaccine will be available for human use.

**Keywords:** Dengue virus; Dengue fever; Vaccine; DNA vaccine; Vaccine protection; Antibodies; T-cells; TDV

## Review Article

Volume 4 Issue 3 - 2016

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Received: November 22, 2016 | Published: December 30, 2016

**Abbreviations:** TDV: Tetravalent Dengue Vaccine; DENV: Dengue Virus; DSS: Dengue Shock Syndrome; DHF: Dengue Hemorrhagic Fever; DDV: DNA Vaccine

## Introduction

Dengue virus (DENV) is a vector-borne viral disease that cause more human morbidity and mortality worldwide than any other arthropod-borne virus. It is estimated that 3.97 billion people living in 128 countries are at risk of dengue, 400 million infections annually, and 96 million asymptomatic cases occurring every year [1-4]. Four closely related dengue serotypes cause the disease: DENV-1 to -4. Serotype specific differences in clinical manifestations have been reported. In a cross-sectional study, Balmaseda et al. [5] reported that among hospitalized children in Nicaragua over 3 years period DENV-2 was associated with more shock and internal hemorrhage while DENV-1 was associated with increased vascular permeability. Furthermore, DENV-1 was associated with more hospitalized primary dengue cases and more primary DENV infection with severe manifestations. Others reported that DENV-2 and DENV-3 may cause more severe diseases than the other serotypes and that DENV-4 is associated with milder illness [6-12]. Certain genotypes within particular serotypes are associated with epidemics of dengue hemorrhagic fever (DHF) vs. classic dengue [11,13]. *Aedes aegypti* mosquitoes are the primary vectors of DENV throughout the tropics [14]. DENV infection is categorized as asymptomatic infection, subclinical infection, undifferentiated fever, dengue fever, DHF with or without dengue shock syndrome (DSS) and other severe forms of dengue. DHF is characterized by fever, bleeding diathesis, and a

tendency to develop a potentially fatal shock syndrome. Clinical manifestations of dengue infection range from mild dengue fever to severe plasma leakage with hemorrhagic manifestations. Thrombocytopenia is an important finding in patients with dengue. A study reported that platelet count of dengue patients can be normal or abnormally high or low. This should be used for future diagnosis [15-17]. Management of dengue patient is to have good fluid replacement therapy until recovery of platelet. Multiple factors have been suggested to contribute to severe dengue such as secondary infections, age, viral load and infecting serotype and genotype [6-8]. Dengue infection is spreading at an alarming rate around the globe and statistics shows that the disease has increased dramatically since 1980, with epidemics occurring in both eastern and western Africa [18,19]. The WHO statistics indicates that 2.4% of the global burden of dengue hemorrhagic fever is in Africa and one-fifth of the populations are at risk [17].

Currently there is no specific antiviral agents and vaccine for treating and/ or preventing dengue infection [2,4]. The prevention technique is based on control of *Ae aegypti* which is failing in most countries as a result of lack of resources and apathy. Taking into consideration the global burden of dengue, the best preventive intervention is the use of vaccines. Studies has shown that long-term homotypic immunity is generated after infection with a single DENV serotype and pre-existing immunity to one DENV serotype is a risk factor for severe disease upon secondary heterotypic infection. Based on these, a potential DENV vaccine must induce long-lasting protective immunity against all four DENV serotypes [20-25]. The aim of this study is to summarize current data available on dengue vaccines and provide an insight

into the safety-efficacy profile and the potential drawbacks of dengue vaccines based on safety-efficacy studies.

### Method of Data Collection

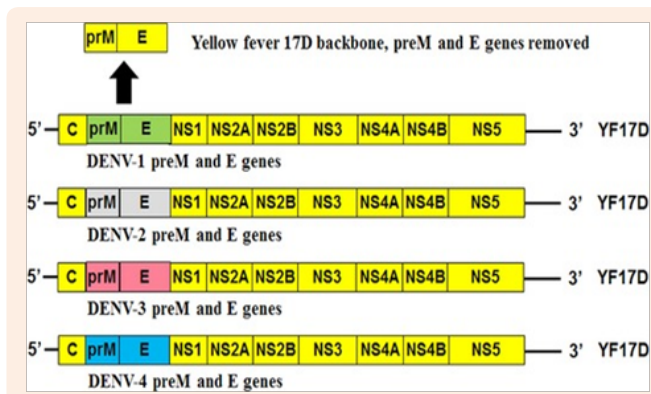
A systematic review based on PRISMA guidelines [20] was performed. The search was conducted across the following electronic databases: PubMed, Embase, WHO Library information, and Google scholar using different combinations of search terms (with synonyms and closely related words) such as: “dengue virus”, “dengue vaccine”, “randomized controlled trials” “clinical study”, “efficacy”, and “effectiveness”. The titles and abstract of all articles were screened to determine their eligibility. Other articles that showed up as related during the search were screened. The full text of eligible articles were retrieved and further assessed for inclusion. Conferences proceedings and agency reports were searched to identify ongoing or unpublished. Authors were contacted to share preliminary summaries or unpublished studies to assess their eligibility for inclusion in the review. Searches were limited to between January 2010 and January 2016, and limited to English language. Eligible studies were randomized controlled trials that assessed the efficacy of a dengue vaccine and possess clinical trial registration number.

A total of 29,188 articles were identified by searching PubMed (255), Embase (280), WHO library of information (453), and Google scholar search (28,200) respectively. The identified studies were screened on the basis of original study and its relevance to the aim of this review. Studies that did not meet the inclusion criteria were excluded.

Of the 29,188 screened reports, 11 articles were finally selected on the basis of inclusion criteria to describe tetravalent Dengue vaccine (TDV) and Dengue DNA vaccine (DDV). Additional studies were obtained through citation tracking of review and original articles.

**Tetravalent Dengue Vaccine (TDV):** A live attenuated, tetravalent DENV vaccine candidate was generated using reverse genetics which is able to provide immunity to each of the four serogroup of DENV. Attenuation was achieved by generating recombinant DENV which are modified by deletion, or alteration, by antigenic chimerization between two related DENV using the following two strategies: 1. Introduction of an attenuating 30 nucleotide deletion (Delta 30) mutant into the 3' untranslated region of DENV-1 and DENV-4, and 2. Replacement of structural proteins of the attenuated rDENV Delta 30 vaccine candidate with those of DENV2 or DENV3. Attenuation of the four monovalent vaccine candidate was achieved for rhesus monkeys or humans. One of the candidate that is showing promise is recombinant, live-attenuated tetravalent dengue disease vaccine (CYD-TDV) produced by Sanofi-Pasteur, Lyon France. CYD-TDV is in the late stage of clinical development and has been evaluated in different populations and age groups. It is made of four recombinant (CYD-1 to -4) which expresses the dengue pre-membrane and envelope proteins of one of the four DENV serotypes and the non-structural as well as capsid protein of an attenuated yellow fever (YF) vaccine in YF-17D (Figure 1) [26,27]. Hadinegoro et al assessed

CYD-TDV in two phases three randomized efficacy trials involving 21 000 children between the ages of 2 and 14 year in the Asia-Pacific region and between the ages of 9 and 16 years in Latin America. The vaccine was administered in three doses at baseline, 6 months, and 12 months. Vaccine efficacy against virologically confirmed dengue while safety was assessed during a 25 months efficacy surveillance phase. Reactogenicity and immunogenicity were also assessed in a subgroup of participants. They reported that the vaccination significantly reduced the incidence of virologically confirmed dengue and showed acceptable and safer reactogenicity profiles [28]. In another randomized, placebo-controlled study, Durbina et al. [29] evaluated the safety and immunogenicity of four different tetravalent dengue vaccines was evaluated in 113 flavivirus-naïve adults. The serum neutralizing antibody levels to all four dengue vaccines were measured on days 0, 28, 48, and 180. A single dose of the vaccine was administered resulted in induction of trivalent or better neutralizing antibody response in 75%- 90% of vaccines. It was also found that trivalent or better response correlated with rash and with non-black race. Black race was significantly associated with reduced incidence of vaccine viremia [29]. This reflects the effect of race on disease severity in natural dengue infection.



**Figure 1:** Structural representation of CYD-TDV (Source: Thomas, 2014).

In a randomized controlled phase I study in Philippines, Capeding et al. [30] reported that a vaccine regime of either 3 TDV vaccination administered over a year or two TDV vaccination given more than 8 months apart led to a balanced antibody response to all four dengue serotypes among flavivirus-exposed populace that included children [31]. The reactogenicity did not increase with successive vaccination and was not higher in children than in adults and adolescents. Others have also reported of the efficacy of TDV in clinical studies [32-36]. The safety profiles of this vaccine have been evaluated. The study by Hadinegoro et al reported that the vaccine was safe in all age groups in the first year of the study but during the third year, higher cases of hospitalizations were reported in the vaccinated group in comparison to the unvaccinated group for those individuals below the age of 9. There was no report of increased risk among subjects aged 9 and above with a reported more stable and sustained hospitalization reduction into the third year although reduction in efficacy was reported from the first to third year. Leo et al reported that in

children aged 9 and above, efficacy against virologically confirmed dengue of any severity was 64% while against hospitalization 81% as well as severe disease 93%. Efficacy was seen to decline over the years which could be attributed to waning vaccine efficacy or increase in enhanced disease reported among the seronegatives during the third year. Due to the reported higher efficacy and lack of any safety signal, the best age groups to benefit from this vaccine are individuals from the age of 9 to 1635. There is data on safety and immunogenicity for individuals aged up to 45 but there is no efficacy data for individuals above the age of 1636, [37]. Osorio et al. [31] reported that there is acceptable tolerability and immunogenicity of the tetravalent DENVAX formulation in healthy, flavivirus-naïve adults but more clinical studies are needed to test DENVAX in different age groups in DENV-endemic areas. Although TDV is safe, a number of adverse events have been reported. Sirivichayakal et al. [34] reported of the following solicited adverse events (AEs): injection site pain, itching and erythema which was mostly mild while Rupp et al reported of the following unsolicited AEs: headache, fatigue, myalgia and injection site pain [38].

The U.S. National Institute of Health (NIH) as part of the global effort to develop a DENV vaccine initiated a program with the aim of developing a live attenuated tetravalent dengue vaccine which could induce protection against all four DENV serotypes [39]. Recombinant technology was used in two primary attenuation strategies: deletions in the 3' untranslated region and structural gene chimerization. A prototype monovalent candidate vaccine termed rDEN4Δ30 that contains all the structural and non-structural proteins of wild type DENV-4 was developed. It was attenuated at 3'UTR. When the analogues 30 nucleotide was removed, it resulted in the creation of additional vaccine candidate. Furthermore, chimeric vaccine candidate were developed by the substitution of prM and E genes from each of the rDEN4Δ30 vaccine candidates. Five of these formulations, designated as TV001-TV005 (Figure 2) were evaluated in clinical studies [29,41].

During the clinical studies, a very important observation was made: chimerization of the viruses was highly attenuating and even in some cases over-attenuating. The first DENV-3 vaccine candidate that was analyzed was a chimeric virus construction which had DENV-3 and rDEN4Δ30 background. The strategy was similar in the Sanofi-Pasteur vaccine but the prM and E of DENV-3 was substituted with another DENV virus rather than the YF 17D virus. Upon evaluation in human, the developed virus, rDEN3/4Δ30 was found to be poorly infectious because it induced seroconversion to DENV-3 in 20% of the subjects [40]. The failure of rDEN3/4Δ30 led to the evaluation of two other DENV-3 vaccine candidates: rDEN3Δ30/31 and rDEN3-3'D4Δ30. The first vaccine candidate includes 31 nucleotide deletion in the 3' UTR plus addition to the 30 nucleotide deletion homologues to that found in rDEN1Δ30 and rDEN4Δ30. The latter is a chimeric virus in which the entire 3' UTR of rDEN4Δ30 replacing that of DENV-3. It was found that both viruses induced seroconversion to DENV-3 in more than 80% of subjects when evaluated as monovalent viruses. The latter candidate appeared to be less infectious than the former. This resulted in rDEN3Δ30/31 been chosen as the DENV-3 component of the tetravalent vaccine.

Dengue DNA Vaccine (DDV): Only one paper carried randomized controlled study on Dengue DNA vaccine. In a clinical study by Beckett et al. [41], a candidate Dengue DNA vaccine was construct was developed through the incorporation of pre-membrane and enveloped genes into a plasmid vector. Their phase 1 clinical trial was conducted using the DENV-1 vaccine construct (DIME 100) which involved 22 healthy flavivirus-naïve adults who were assigned to one of two groups. Each of the group was given three intramuscular injections at 0, 1, and 5 months of either high dose of 5.0mg or low dose of 1.0mg DNA vaccine utilizing the needle-free Biojector® 2000. They reported of a number of solicited signs and symptoms which included local mild pain or tenderness, local swelling, muscle pain, and fatigue. Five subjects in the high dose group and none in the low dose group developed detectable anti-dengue neutralizing antibodies. T-cell IFN gamma responses were found in 50% and 83.3% of subjects in the low and high dose groups respectively. This means the safety profile of the vaccine candidate is acceptable at both doses. The authors therefore concluded that the result of the study highlights the safety profile and favorable reactogenicity of a DENV-1 DNA vaccine.

**Conclusion**

There is an urgent need for an efficacious and affordable tetravalent DENV vaccine. However, lack of adequate animal disease model and identifying immune correlation of disease protection are some of the major obstacles impeding the development of a successful DENV vaccine. The Sanofi Pasteur CYD has been licensed in some few countries but it recorded poor efficacy in dengue naïve individuals during phase III clinical study. This could be attributed to the yellow fever virus backbones which lack the essential dengue T cell epitopes of the nonstructural region which have been shown to play important role in providing protection against dengue [42,43]. Other studies also implicated immunity to dengue NS1 as essential in providing

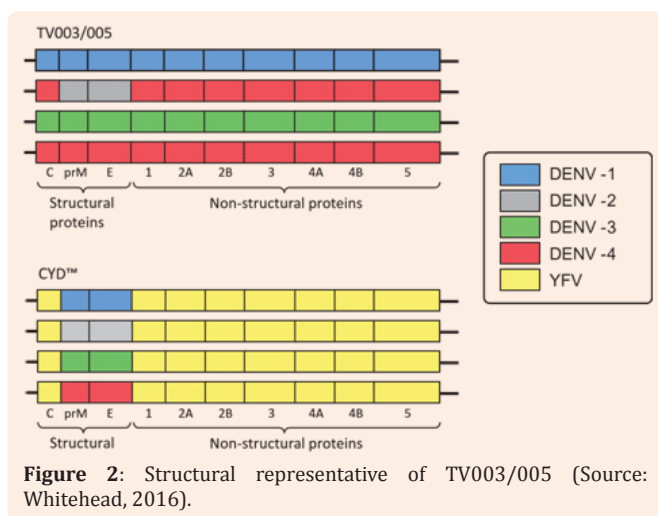


Figure 2: Structural representative of TV003/005 (Source: Whitehead, 2016).

protection which CYD-TDV lacks. It has also been reported that it generated lots of cross-reactive nonneutralizing/ enhancing antibodies. It has therefore been proposed that it is not only the presence of dengue neutralizing antibodies that may hold the key to a successful dengue vaccine candidate but also DENV serotype-specific neutralizing antibodies [44,45]. Therefore an effective dengue vaccine must be designed with the capability of inducing predominantly serotype-specific neutralizing antibodies (protective) in the absence of serotype cross-reactive neutralizing antibodies (disease enhancing) [46]. Studies are recommended to elucidate the immunological profile of dengue vaccine in children and those in endemic areas. The list of potential dengue vaccine is growing and despite the low efficacy recorded so far, it is hoped that soon an efficacious dengue vaccine will be available.

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