



Dengue Vaccines: An Update

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Abstract

Dengue is one of the most prevalent mosquito-borne diseases in the world, affecting an estimated 390 million people each year, according to models. For the last two decades, efforts to develop safe and effective vaccines to prevent dengue virus (DENV) infections have faced several challenges, mostly related to the complexity of conducting long-term studies to evaluate vaccine efficacy and safety to rule out the risk of vaccine-induced DHS/DSS, particularly in children. At least seven DENV vaccines have undergone different phases of clinical trials; however, only three of them (Dengvaxia[®], TV003, and TAK-003) have showed promising results, and are addressed in detail in this review in terms of their molecular design, efficacy, and immunogenicity. Safety-related challenges during DENV vaccine development are also discussed.

Key Points

Dengue vaccine development has been challenging because of the need to provide protection against all four dengue serotypes to avoid potentially causing antibody-dependent enhancement in further infections.

Denvaxia[®] is currently the only licenced vaccine, but phase III clinical trials with two other vaccines, TV-003/TV-005 and TAK-003, are currently ongoing, with promising results.

1 Introduction

Dengue fever represents a great burden for the public health systems worldwide and is considered the most prevalent mosquito-borne disease in tropical and subtropical regions of the world [1, 2], rapidly expanding every year due to several factors such as climate change, deforestation associated with uncontrolled urbanization, overpopulation, and

the emergence of mosquitoes resistant to common insecticides, amongst others [3–5]. Dengue fever is caused by dengue virus (DENV), which is classified under the genus *Flavivirus* of the family *Flaviviridae* and is transmitted by mosquitoes from the *Aedes* genus, mostly *Aedes aegypti* and *Aedes albopictus*. Four genetically distinct DENV serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) have been reported to co-circulate amongst humans worldwide [6, 7]. Dengue is characterized by a wide spectrum of clinical manifestations ranging from a mild febrile illness to severe dengue, increasing the risk of developing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). A secondary DENV infection (e.g., exposure to a heterotypic serotype) is the greatest risk factor for serious diseases due to the phenomenon of antibody-dependent enhancement (ADE) [8]. Briefly, cross-reactive antibodies generated after exposure to the first DENV serotype combine with the second DENV serotype to create infectious immune complexes that enter Fc-receptor-bearing cells. As a result, the number of infected cells and the amount of virus produced per cell increases. This is therefore imperative that DENV vaccines protect against infection from all four serotypes to avoid ADE.

At least seven DENV vaccines based on different platforms including live attenuated viruses, inactivated viruses, chimeric live attenuated viruses, DNA, and recombinant proteins have been developed and are currently undergoing different phases of clinical trials (Table 1) or are under preclinical investigation [6]. In this review we discuss recent progress of the three most advanced DENV vaccines

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Table 1 Candidate dengue vaccines in phase I or phase II clinical trials

Candidate	Platform	Phase/stage	References
TDEN-LAV (WRAIR/GSK)	Live-attenuated	Phase II (Discontinued)	[9]
TDENV-PIV (WRAIR/FioCruz/GSK)	Inactivated adjuvanted	Phase I (No recent reports)	[10]
D1ME100/TVDV (NMRC)	DNA vaccine	Phase I (No recent updates)	[11]
V180 (DEN-80E) (Merck/NIAD)	Recombinant (subunit)	Phase I (Published 2019)	[12]
DENV-1-LVHC	Live-attenuated	Phase I (Published 2021)	Clinicaltrials.gov [13]

Table 2 Dengue vaccines that have reach phase III or have been licensed

Vaccine	Manufacturer	Platform	Efficacy	Comments	References
Licensed					
CYT-TDV Dengvaxia®	Sanofi Pasteur	YFV Δ30 backbone	25–59%	Increases hospitalizations in seronegative vaccinees	[7]
Phase III					
TAK-003 (DENVax)	Takeda/Inviragen	Attenuated DENV-2 backbone for the four serotypes	73.3–85.3%	Well tolerated in adolescents and children	[14]
LATV TV003/TV005	NIAD/Butantan/Merck	DENV-1,3,4 Δ30 and rDENV2/4 Δ30	Not yet released	Single dose	[15]

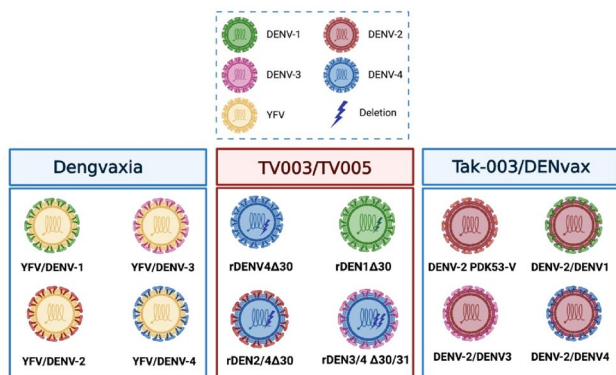


Fig. 1 Molecular design of the anti-dengue virus (DENV) vaccines in advanced stages of clinical development. **A** Dengvaxia® is based on a yellow fever backbone in which the pre-membrane (prM) and envelope (E) genes of YFV have been replaced by the homologous genes from each one of the four DENV serotypes [16, 17]. **B** TV003/TV005 was constructed by a deletion of 30 nucleotides (172–143) in the TL2 stem-loop of the 3'-UTR of DENV-4 and DENV-1 (rDEN4Δ30 and rDEN1Δ30), DENV-2 and DENV-3 components were constructed from the rDEN4Δ30 backbone [21, 22]. **C** Tak-003/DENVax is based on a live-attenuated DENV-2 strain (PDK-53-V) in which the pre-membrane (prM) and envelope (E) genes of YFV have been replaced by the homologous genes from each one of the four DENV serotypes [27]

(Dengvaxia®, TV003/TV005, and TAK-003) (Table 2), focusing on the molecular characteristics of each vaccine (Fig. 1) and the available clinical data on their efficacy and immunogenicity. Finally, we emphasize the safety-related

challenges associated with the risk of vaccine-induced DHS/DSS.

For this review we systematically searched PubMed, Web of Science, and clinicaltrial.gov to obtain results on specific topics such as vaccines, trials as well as safety on dengue vaccines, and immunopathogenesis. We selected the most appropriate bibliography for this document.

2 Development and Preclinical Evaluation of Dengue Virus (DENV) Vaccines

2.1 Dengvaxia®

Initially developed in the early 2000s by the National Institutes of Health (NIH), the University of St. Louis, and Acambis Inc, and subsequently licensed by Sanofi Pasteur, this vaccine takes advantage of the ChimeriVax™ technology. Based on a vaccine strain (17D) of yellow fever virus (YFV) in which the pre-membrane (prM) and envelope (E) genes of YFV have been replaced by the homologous genes from each one of the four DENV serotypes derived from DENV isolates obtained in Thailand and Indonesia between 1978 and 1988, this technology enabled the generation of four chimeric YF-DEN viruses that were used in the formulation of a tetravalent DENV vaccine (ChimeriVax™ DENV 1-4) [16, 17].

Preclinical evaluation of the safety and immunogenicity of the tetravalent ChimeriVax™ DENV 1-4 vaccine showed

a reduced neurovirulence profile in mice compared to the parental YFV vaccine strain (YF-VAX) [18]. Also, neurovirulence tests performed in *Macaca fascicularis* confirmed that the tetravalent ChimeriVax™ DENV 1-4 vaccine was significantly less neurovirulent than the parental YF-VAX strain [19]. In addition, the vaccine generated seroconversion and strong neutralizing antibody responses against all four DENV serotypes following one administration of either a high or a low dose of the vaccine in cynomolgus macaques and limited viremias compared to the parental DENV strains. Interestingly, challenge studies revealed that 92% of the vaccinated monkeys were protected against a challenge with wild-type DENV 1-4 [20].

2.2 TV003/TV005

The development of the live attenuated DENV vaccines called TV003/TV005 began in 1996 at the Laboratory of Infectious Diseases (LID) of the National Institute of Allergy and Infectious Diseases (NIAID). Considering the importance of the untranslated regions (UTRs) for the replication of DENV genome, the initial attenuation strategy focused on deleting 30 contiguous nucleotides (172-143) in the TL2 stem-loop from the 3'-UTR of DENV-4 (rDEN4 Δ 30) [20]. A mutant lacking the same homologous genomic region was also constructed for DENV-1 (rDEN1 Δ 30). Both mutants displayed an attenuated phenotype as demonstrated by their reduced infectivity, and exhibited their capacity to induce strong neutralizing antibody responses in rhesus macaques that correlated with the protection when challenged with wild types of DENV-1 and DENV-4 [21].

Further efforts to achieve a tetravalent DENV vaccine led to the generation of the attenuated DENV-2 component by using the backbone of rDEN4 Δ 30 to generate two attenuated DENV4-DENV2 chimeric viruses in which the membrane and envelope genes (rDEN2/4 Δ 30 (ME)), or the capsid, membrane, and envelope genes (rDENV2/4 Δ 30 (CME)) of DENV-4 were replaced with the homologous genes of DENV-2 [21, 22]. Preclinical evaluation of the two chimeras showed that both display a highly attenuated phenotype in SCID-HuH-7 mice, and rhesus macaques in which the chimerization and the Δ 30 deletion was additive rendering the virus not infectious for monkeys [21]. A chimeric DENV3-DENV4 virus containing the original 30 nt deletion at the 3'-UTR was generated (rDEN3 Δ 30 (ME)) and further modified by introducing a non-continuous deletion of 31 nt (258-228) (rDEN3 Δ 30/31). Preclinical evaluation of the mutant virus rDENV3 Δ 30/31 in non-human primates revealed the desirable safety, undetectable viremia, and strong neutralizing antibody responses, which were sufficient to protect the vaccinated monkeys when challenged with wild-type DENV-3 [23].

2.3 TAK-003 (DENVax)

The development of the DENVax vaccine started in the late 1980s, when researchers from the Mahidol University in Bangkok, Thailand, isolated a DENV-2 strain (DENV-2 16681) from the serum of a patient with dengue hemorrhagic fever. The attenuation of the DENV-2 16681 strain by 53 serial passages in primary dog kidney cells (PDK cells) lead to the obtention of the DENV-2 PDK-53-V strain, which in contrast to the parental DENV-2 PDK-53 strain, which has attenuation-related mutations in the 5'UTR and NS1 gene, possesses an additional non-synonymous mutation in the NS3 gene. The DENV-2 PDK53-V strain displays reduced neurovirulence in suckling mice and lower replication rates in C6/36 cells [24–26], and was further used as the backbone to generate the DENVax vaccines.

The vaccine strains for DENV-1, DENV-3, and DENV-4 used to formulate tetravalent DENVax vaccine were generated by replacing the pre-membrane (prM) and envelope (E) genes from the DENV-2 PDK53-V strain, with the prM and E genes from wild-type DENV strains [27]. The chimeric DENVax viruses displayed “small plaque” and temperature-sensitive phenotypes when replicated in LLC-MK2 cells, in contrast to the parental wild-type strains. Tetravalent formulations containing the four DENVax vaccine strains (DENV 1-4) demonstrated reduced neurovirulence profiles in newborn ICR mice and were shown to be immunogenic in AG129 knockout mice, inducing high neutralizing antibody titers (1:320–1:2560) against the four DENV serotypes [27]. Preclinical evaluation of the tetravalent DENVax vaccine in cynomolgus macaques (*Macaca fascicularis*) showed a good vaccine safety profile and was well tolerated when administered by the subcutaneous route, while inducing protection against the four DENV serotypes (measured by the protection against viremia) after two immunizations with a high dose scheme of a 5:5:5:5 formulation of each DENVax strain (DENVax 1-4) [28].

3 Clinical Evaluation of DENV Vaccines

To date, Dengvaxia® is the only licensed DENV vaccine, yet phase III clinical trials with the TV-003/TV-005 and TAK-003 are currently ongoing with promising results. Nevertheless, as we discussed, differences between the age and serostatus of the vaccinees have been shown to have a direct impact on vaccine efficacy and safety. Most of the safety concerns regarding the DENV vaccines derive from phase III pediatric clinical trials to date, only Dengvaxia® and TAK003 (DENVax) have been tested in children, with mixed results (Fig. 2).




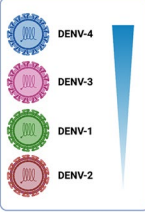
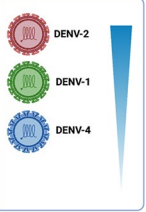
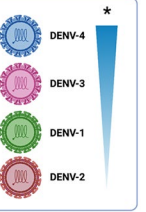
	Dengvaxia	DENVax	TV003/TV005
Backbone	 Yellow Fever Virus (17D)	 Cell culture attenuated DENV	 DENVΔ30
Serotype-specific efficacy			
Overall Efficacy (%)	**30.2% - 60.8%	62%	Data not available
Efficacy (%) seropositive	74.3-83.7%	52.3%-83.4%	Data not available
Efficacy (%) seronegative	35.5%-43.2%	***43.5%-91.9%	Data not available

Fig. 2 Overview of the efficacy trials of anti-dengue virus (DENV) vaccines in children in Latin America and Asia. Phase III clinical trials have been conducted for Dengvaxia and DENVax with mixed results. TV003/TV005 is currently undergoing phase III clinical trials. *The seroconversion rates for TV003/TV005 observed in phase II clinical trials are illustrated. **The lower value of the efficacy range depicted corresponds to the efficacy observed during the phase IIb trial conducted in Thailand. ***DENVax was only efficacious against DENV-1 and DENV-2 in seronegative individuals [38, 86]

3.1 Dengvaxia®

Early phase I and phase II clinical trials to evaluate the safety, immunogenicity, and reactogenicity of the tetravalent presentation of Dengvaxia® (TDV) in healthy adults, between 18 and 45 years, were carried out in USA, the Philippines, Australia, Mexico, Vietnam, Singapore, and India; some of these studies also included pediatric populations aged between 2 and 18 years [29].

The primary safety evaluation of the Dengvaxia® vaccine in adults showed that it was safe and well tolerated in a three-dose regimen at 0, 3–4, and 12 months, inducing mild to moderate transient local and systemic adverse events such as injection site pain, headache, malaise, and low-grade fever amongst others, with no vaccine-related severe adverse events (SAEs) reported. Assessment of the vaccine-induced cellular responses revealed that the vaccine did not induce the release of proinflammatory cytokines (IFN- γ , IL-1 β , IL-6, IL-8, IL-10, IL-12p70), while inducing DENV serotype specific T-helper responses [30, 31].

The CD8 responses against the NS3 protein of the YFV-17D strain used as the backbone for the ChimeriVax™

viruses were observed [32]. In individuals with pre-existing DENV immunity, the vaccine induced broader neutralizing antibody responses and boosted specific CD8+ responses against DENV non-structural proteins, particularly NS3, which are not elicited in naïve individuals. Even though most of the phase III clinical trials to evaluate the efficacy of Dengvaxia® have been conducted in the pediatric population (< 18 years), a randomized phase III clinical trial to evaluate the lot-to-lot consistency of the vaccine in healthy adults in Australia [33] revealed that even though naïve individuals developed neutralizing antibodies against all four DENV serotypes after receiving one dose of the vaccine, the only serotype-specific neutralizing antibodies produced were against DENV-4 [34]. Instead, DENV 1-3 were neutralized by cross-reactive antibodies, revealing the immunodominance of the DENV-4 component of the vaccine [34]. Moreover, differential rates of viral replication of each vaccine component were observed by RT-PCR in dengue-naïve individuals after receiving one vaccine dose: DENV-4 (44%), DENV-3 (12%), DENV-1 (7%), and DENV-2 (0%), which may pose a risk for seronegative individuals to develop vaccine-enhanced disease when infected with DENV-2.

The most informative study regarding the efficacy and safety of Dengvaxia® in the pediatric population was conducted in 35,000 children aged between 2 and 16 years. The study revealed that the efficacy was age dependent, ranging from 65% in children > 9 years to 45% in children 9 years or younger [35]. Worryingly, children under 9 years showed a tendency to develop severe dengue after immunization following natural exposure to the virus, an effect that was particularly seen in dengue-naïve children [36]. Pediatric studies to evaluate the efficacy of Dengvaxia® have shown that efficacy varies between DENV serotypes and age groups. Results from a phase IIb trial conducted in Thailand to evaluate the efficacy of Dengvaxia® in a cohort of 4002 children between 4 and 11 years revealed that vaccine efficacy was higher against DENV-4 and DENV-3 (100% and 75.3%, respectively) than for DENV-1 and DENV-2 (55.6% and 9.2%, respectively) [37].

In larger pediatric phase III trials conducted in five dengue endemic Latin America countries in a cohort of 20,869 healthy children between 9 and 16 years of age, efficacy against DENV-3 and DENV-4 was higher (74.0% and 77.0%, respectively) than for DENV-1 and DENV-2 (50.3% and 42.3%, respectively), confirming that the vaccine efficacy varies between serotypes and that it is age dependent [38]. The latter was later confirmed by a larger phase III clinical trial to evaluate the efficacy of Dengvaxia® in a cohort of healthy children from Latin America and Asia aged between 2 to 16 years. Results from this study revealed that the pooled rates of efficacy for symptomatic dengue in a follow-up period of 25 months were higher for children

older than 9 years (65.6%) than for children under 9 years (44.6%). Moreover, the pooled relative risk of dengue requiring hospitalization was higher for children under 9 years of age (1.58) than for children aged over 9 years (0.5) [39].

Further trials have suggested that the observer age-dependent risk of dengue requiring hospitalization in children under 9 years of age might be related to pre-existing anti-DENV immunity. A cohort study that analyzed data from three different efficacy trials revealed a higher incidence of hospitalization due to virologically confirmed dengue (VCD) for seronegative vaccinees aged between 2 and 16 years (3.06%) than for seronegative controls (1.87%), a trend that was also observed for seronegative vaccinees aged between 9 and 16 years (1.57% in seronegative individuals vs. 1.09% in seropositive individuals) [36].

Despite the high vaccine efficacy observed with Dengvaxia® in different clinical trials, recent mathematical models using data of more than 800,000 children vaccinated in the Philippines estimate there will be more than 1000 hospitalizations due to severe dengue in a period of 4 years post-vaccination, in both seronegative and seropositive individuals [40]. These models along with the high rates of hospitalization observed in children under 9 years of age, that were wrongly considered as vaccine failure cases, highlight the importance of conducting enhanced phase IV surveillance studies in children vaccinated with Dengvaxia® for a better assessment of the effectiveness of the vaccine in dengue endemic countries, even before the approval and deployment of the vaccine.

3.2 TV003/TV005

During the early phases of its clinical development, several phase I clinical trials were conducted using monovalent formulations of the DENVax vaccine candidates to evaluate the safety profile, replication capacity of the individual DENVax viruses, and transmissibility from vaccinated individuals to *Toxorhynchites splendens* mosquitoes [41–45].

After these initial studies, six monovalent DENVax vaccine candidates were selected for further clinical evaluation formulated as five tetravalent mixtures (TV-001 to TV-005). Two vaccine candidates (TV003 and TV005) formulated with rDEN1D30 rDEN2/4D30, rDEN3D30/31, and rDEN4D30, but with different amounts of the rDEN2/4D30 component (10^3 PFUs/mL in TV003 and 10^4 PFUs/mL in TV005) were selected for further clinical evaluation after inducing the most balanced neutralizing antibody responses against the four DENV serotypes [46].

The safety and immunogenicity of TV003 and TV005, evaluated in two randomized placebo-controlled trials [47, 48] in flavivirus-naïve subjects, revealed that both TV003 and TV005 were well tolerated and showed a good safety profile, with low-grade rash reported as the most frequent

adverse event in 76% of the participants in both groups. A single dose of TV003 induced balanced neutralizing antibody responses against the four DENV serotypes with seroconversion rates between 64% (DENV-2) and 100% (DENV-4), while the specific response against DENV-2 improved with the TV005 vaccine (84%) after a single dose.

The lower proportion of participants that seroconverted to DENV-2 after receiving one dose of TV003 led to the development of a DENV-2 challenge model in which the original rDEN2D30 was used as a challenge virus 6 months post-vaccination, since it induced viremias 100-fold higher than the vaccine strain (rDEN2/4D30). The results showed that all the participants developed protective DENV-2 responses and induced specific neutralizing antibodies against DENV-2 [21].

These results led to the licensing of the TV003 vaccine by the Butantan Institute in Brazil under the name Butantan-DV, which was manufactured as a lyophilized tetravalent DENV vaccine and subjected to a two-step, double-blind, randomized, placebo-controlled, phase II clinical trial in 155 DENV-naïve and 145 DENV-exposed healthy individuals, aged between 18 and 59 years [48]. The vaccine was safe and well tolerated and induced robust balanced neutralizing antibody responses with seroconversion frequencies above 78% for the four DENV serotypes, in both DENV-naïve and DENV-exposed participants. Significant T-CD8 responses were observed in DENV-naïve and DENV-exposed participants 91 days after receiving one dose of the Butantan-DV vaccine, suggesting that this vaccine elicits broader protective immune responses in adults, in comparison to tetravalent vaccines based on the expression of structural DENV proteins. A randomized, multicenter, double-blind, placebo-controlled phase III clinical trial for the Butantan-DV vaccine is currently being conducted in Brazil with 16,944 participants divided into three age groups (18–59 years, 7–17 years, and 2–6 years); results for this trial are still to be published.

3.3 TAK-003 (DENVax)

The clinical evaluation of DENVax started with a randomized, double-blind, dose-escalation phase I clinical trial conducted by Takeda in Rionegro, Antioquia, Colombia, aimed to evaluate the safety and the immunogenicity against the four DENV serotypes of a two-dose scheme of DENVax administered intradermally (ID) or intramuscularly (IM) to DENV-naïve adults between 18 and 45 years of age [49]. The vaccine was safe and well tolerated among the participants in the study, inducing transient local reactogenicity and mild systemic adverse events. Vaccination induced the production of neutralizing antibodies against the four DENV serotypes, yet antibody titers against DENV-3 and DENV-4 were lower among the participants [50].

Due to the lower antibody responses against DENV-4, DENVax (now named TDV) was reformulated to increase the amount of the DENVax4 component, and different formulations and dosing schedules were tested in a randomized, multicenter, phase 1b study carried out in the USA with 140 DENV-naïve individuals between 18 and 45 years of age [51]. The seroconversion rates observed among the participants in the study were 84–100% (DENV-1), 96–100% (DENV-2), DENV-3: 83–100% (DENV-3), and 33–77% (DENV-4).

The evaluation of the safety and immunogenicity of a two-dose scheme (0, 90 days) of the TDV vaccine in DENV-exposed individuals during a randomized, double-blind, placebo-controlled phase II clinical trial carried out in Puerto Rico, Colombia, Singapore, and Thailand using a “high-dose” vaccine with higher titers of the DENVax3 and DENVax4 components, revealed that the vaccine induced lower neutralizing antibody responses against the DENVax4 component regardless of prior DENV exposure [52].

A large-scale phase III clinical trial to evaluate the efficacy of DENVax in a cohort of 20,071 healthy children, between 4 and 16 years in dengue-endemic countries from Latin America and Asia, is currently being conducted. Primary results at 12 months post vaccination showed efficacy variations according to DENV serotype: 97.7% for DENV-2, 73.7% for DENV-1, and 62.6% against DENV-3, while the efficacy results for DENV-4 were inconclusive. Interestingly, the overall vaccine efficacy was similar between participants who were seronegative at baseline (74.9%) and those who were seropositive at baseline (82.2%), and were independent of age range. Overall vaccine efficacy against dengue leading to hospitalization was shown to be 95.4% amongst seronegative individuals and 94.4% for seropositive individuals [14].

Data from the same trial at 18 months post-vaccination revealed an overall vaccine efficacy of 76.1% in seropositive individuals and 66.2% in seronegative individuals, with an overall efficacy against different DENV serotypes ranging from 95.1% against DENV-2 to 48.9% against DENV-3. The overall efficacy against dengue requiring hospitalization was 90.4%, and 85.9% against DHF. Yet, when stratified by age group, the vaccine efficacy against requiring hospitalization was significantly lower in those previously seronegative children aged between 4 and 5 years (59.1%), as the efficacy to prevent hospitalization in seropositive children the same age was 51.6% [53].

The cumulative efficacy data of the DENVax vaccine 3 years post-vaccination were recently published, showing an overall vaccine efficacy of 62% against VCD, significantly lower than the one observed at 18 months post-vaccination. A similar effect was observed with the overall efficacy against dengue requiring hospitalization, which reduced from 90.4 to 83.6%. In baseline seropositives, vaccine efficacy against VCD ranged between 52.3% for DENV-3 and

83.4% for DENV-2, yet, in baseline seronegatives the vaccine was only efficacious against DENV-1 and DENV-2 (43.5% and 91.9%, respectively), while no efficacy was observed for DENV-3. The efficacy against dengue requiring hospitalization remained high amongst seropositive individuals but not in seronegative individuals, when the vaccine was only efficacious against DENV-1 and DENV-2. Interestingly, the vaccine efficacy did not show any clear patterns that associated the efficacy observed with the age of the participants [54].

These significant variations in vaccine efficacy, assessed 18 months apart, should be further analyzed during long-term phase IV surveillance trials to rule out that the protective efficacy observed is due to cross-protection and might decline over time, as has been observed in pediatric populations during natural infections [55].

4 Challenges in DENV Vaccine Development

Immunopathological events are a common feature of DENV infections, with several underlying mechanisms such as an overreactive proinflammatory immune response (cytokine storm) characterized by an elevation of the plasmatic concentrations of several proinflammatory cytokines such as granulocyte-macrophage colony-stimulating growth factor (GM-CSF), macrophage inflammatory protein 1 beta (MIP-1 β), interferon gamma (IFN- γ), and Interleukin 10 (IL-10) [56], which eventually leads to vascular leak, hemorrhages [56, 57], and some other thrombotic events [58, 59]. Molecular mimicry involving E and NS1 viral proteins has also been documented to activate cross-reactive antibodies against platelets and endothelium leading to severe dengue [60–62]. Moreover, naturally infected individuals with one DENV serotype, showing low levels of anti-DENV antibodies (<1:80) suffer complications during secondary infections with a heterologous DENV serotype [63]. Specific antibodies produced during infections constitute a significant part of immune response to neutralize invaders; however, for pathogens such as DENV, under certain conditions antibodies generated in an initial encounter may enhance further infections [64, 65]. Interestingly, patients who develop high titers of anti-DENV antibodies (>1:320) exhibit protection against further symptomatic DENV infections.

The infection enhancement process known as antibody-dependent enhancement (ADE) in dengue is well documented [66], but the details of this event in dengue remain elusive. Virus entry is known to be facilitated by Fc γ (Fig. 3) and mediates T-cell activation and release of TNF- α and other cytokines that cause endothelial dysfunction. Hence, effective DENV vaccines must induce a strong and highly neutralizing response against all four DENV serotypes to avoid vaccine-induced immunopathologic events.

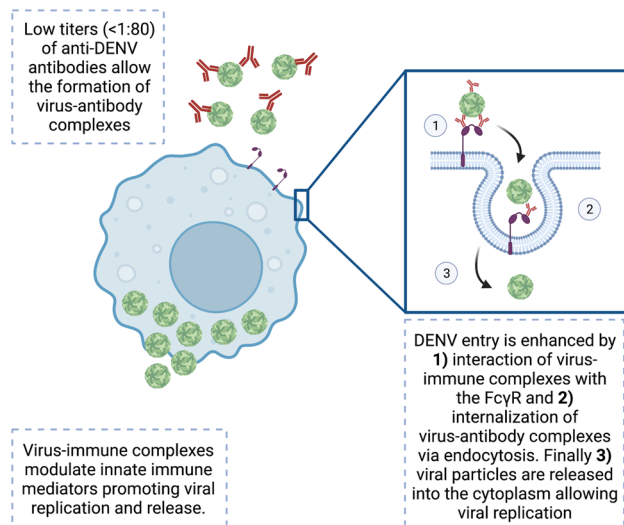


Fig. 3 Schematic representation of antibody-dependent enhancement (ADE) in dengue virus (DENV) infection. Low levels of anti-DENV antibodies (<1:80) against one DENV serotype promote the formation of virus-immune complexes during secondary infections with a heterologous DENV serotype. These virus-immune complexes are internalized into monocytes, macrophages and dendritic cells via the Fc γ receptor, promoting viral release into the cell cytoplasm. Virus-immune complexes modulate innate immune pathways promoting viral replication and release

Evidence shows that this process might be potentiated mostly by anti-prM antibodies. prM is present in immature virus particles. Antibodies like anti-prM not only facilitate Fc uptake, but these antibodies in high concentrations also poorly neutralize virus [67]. Different IgG subclasses (IgG1–4) exist and exhibit dissimilar properties regarding half-lives, levels in serum, complement activation, and binding to Fc receptors. Therefore, not only the targeted viral protein, but also the IgG subclass could help to better understand how neutralization/protection can be favored rather than ADE/immunopathogenesis. ADE occurrence in vaccinees must be examined regarding not only the IgG subclasses, but also the glycosylation stage [68, 69] to separate protective from immunopathogenic events. This is because glycosylation might alter affinity to Fc receptors as has been documented [70].

It seems all the live attenuated vaccines against dengue show capacity to induce antibodies against prM and fusion loop epitope resulting in serotype cross-reactivity and increasing the risk of ADE [71]. A new and important concern is how individuals will react to dengue vaccines, which arises in regions affected by the Zika virus (ZIKV). ADE has also been reported in individuals infected by DENV-2 who were previously infected by ZIKV [72]. Evidence in animal models show this event for both DENV and ZIKV when using live attenuated vaccines [73].

An inefficient innate immune response followed by production of subneutralizing antibodies and hyper-reactive T-cell response might contribute to immunopathologic events [67] in both dengue-infected individuals and vaccinees, but these events must be carefully analyzed in both vaccinees and naturally infected individuals to assess biological significance. Maps of epitopes targeted by highly neutralizing antibodies have been developed in macaques [74]; however, similar studies are needed in humans and these data must be considered for future vaccine development.

5 Safety Issues with Dengue Vaccines

To develop vaccines against dengue it is important for these to recognize the four existing serotypes; the three more advanced vaccines have considered this for their design (Table 3). An important consideration in natural infection is that a primary infection with one DENV serotype would establish a long-term memory against that specific serotype, but might result in a short-term, subneutralizing, and enhancing response for the other serotypes [75–77]. The enhancing phenomenon in subsequent dengue infections with a different DENV serotype could result due to previous heterotypic exposure, and avoiding these events is a constant concern in the field of dengue vaccines [78, 79].

Hence, from the beginning, the need for tetravalent, equally effective immunization for all the four DENV serotypes was recognized as the most important challenge for design and development. Other concerns emerged later, such as the stimulation of high titers of neutralizing antibodies over those that sensitized individuals and enhance infection by DENV [71].

Dengvaxia[®], one of the most advanced developments in vaccines against dengue, raised serious safety concerns when data showed an increased risk of hospitalization in naïve vaccinees when they were exposed to natural infection [80]. Takeda vaccine TAK-003 is still in phase III trials and has been shown to be safe and to induce protection against dengue-related hospitalization with efficacies around 75–80% independent of serostatus of vaccinated individuals [9]. Numerous phase II studies are still being carried out for this live-attenuated vaccine over the globe and in different age-group populations [31, 43, 81]. However, a phase III trial is still ongoing and the final conclusions on efficacy, side or rare adverse effects for this vaccine have not been drawn.

Regarding TV003/TV005, another live-attenuated vaccine developed in the National Institute of Allergy and Infectious Disease, data show these do not allow immunodominant expression of any of the four serotypes, resulting in a desirable immune response. Moreover, initial reports showed that a single dose is protective and nearly sterilizing

Table 3 Dengue vaccine characteristics

	Molecular design	Efficacy	Main side effects	Immunogenicity
Dengvaxia®	DENV-1 (prM-E) YFV-17D genome DENV-2 (prM-E) YFV-17D genome DENV-3 (prM-E) YFV-17D genome DENV-4 (prM-E) YFV-17D genome [16, 17, 85]	45–65% 44.6% under 9 yo 65.6% over 9 yo [35, 38, 39]	Injection site pain, headache, malaise, low-grade fever, among others [30, 31]	CD8+ reponse mostly to NS3 and neutralizing antibodies mostly against DENV-4 [34, 81]
TV003/TV005	Complete DENV-1 Δ30 (3'UTR) DENV-2 (prM-E) in DENV-4 Δ30 (3'UTR) Complete DENV-3 Δ30 (3'UTR) Complete DENV-4 Δ30 (3'UTR) [21, 22, 85]	Data not available	Low grade rash [47, 48]	Reported as close to sterilizing Strong neutralizing antibodies in rhesus macaques [21, 46, 81]
TAK003 (DENVax)	DENV-1 (prM-E) in DENV-2 genome* DENV-2 complete genome* DENV-3 (prM-E) in DENV-2 genome* DENV-4 (prM-E) in DENV-2 genome* *Referring to genetic construction [27, 85]	74.9–76.1% in seropositive (previously exposed) 66.2–82.2% in seronegative (<i>naïve</i>) [14, 53]	Transient local reactivity and mild systemic adverse events [49, 50]	Neutralizing antibodies against all four serotypes [50, 52]

immunity is achieved in a second dose 6–12 months after the first immunization [10, 82, 83]. This vaccine has been licensed by different manufacturers and the Instituto Butantan started a phase III trial to evaluate its efficacy [47].

Data of clinical trials for other promising developments using different vaccine platforms are still necessary, but much work is directed towards improving the existing vaccines. Developers aim to assure vaccines raise a broad and long-lasting highly neutralizing antibody response against all four serotypes [84]. Nevertheless, cellular response must also be evaluated and be efficient in vaccinees along with the antibody responses to assure the desirable protection. Most of all, vaccines should not pose any risk for developing severe disease in either naïve or previously exposed to DENV individuals. Thus, the scientific community is still working towards the safe and universal dengue vaccine the world needs.

6 Challenges and Opportunities

Reviewing clinical data on current dengue vaccines, clearly these pose a singular challenge for developers since protection must be achieved equally for all four serotypes without causing any potentially immunopathogenic event in further DENV encounters. Despite all efforts, dengue

epidemics have intensified in number and frequency, and are affecting new geographical areas, thus it is critical to greatly improve control measures in dengue areas, and to develop a universal and highly effective vaccine to counteract dengue as one of those important measures. As safety remains a concern, the race to get an effective and safe dengue vaccine continues to be an imperative necessity.

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Authors contributions JM TF and MIS designed the content and wrote the first draft, ARS reviewed the manuscript and made suggestions, JM TF produced the figures and revised the manuscript, MIS revised and submitted the manuscript.

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