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Current Status of Vaccines against Dengue Virus

Jhon Carlos Castaño-Osorio,
Alejandra María Giraldo-Garcia and
Maria Isabel Giraldo

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Abstract

Dengue is a disease caused by the dengue virus (DENV), being the most important arbovirus in the world. About 3.97 billion people live in countries at risk and 400 million infections occur each year, of which 500,000 suffer from the most severe form of the disease and 25,000 of these die. The clinical spectrum of Dengue ranges from asymptomatic infection to severe Dengue characterized by increased vascular permeability, bleeding disorders, shock, and death. The increase in global cases of this disease is due in part to the absence of effective public intervention measures and lack of a specific treatment and vaccines licensed for human use. Therefore, in this review, we will present the different strategies known to date for the development of vaccines for this disease, as well as the results and limitations obtained in the different clinical studies.

Keywords: dengue, vaccine, tetravalent, immunopathogenesis

1. Introduction

Dengue is a mosquito-borne viral disease caused by four types of dengue viruses, which, in the recent years, has rapidly become widespread worldwide. Dengue virus transmission is attributed to female mosquitoes of the species *Aedes aegypti*, in the majority of cases, as well as *Ae. albopictus* to a lesser extent. Other diseases that are transmitted by this mosquito include chikungunya, yellow fever, and Zika infection [1]. Dengue is a very rapidly growing public health problem being currently faced by approximately 40% of the global population living in more than a hundred tropical and subtropical countries [2]. Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature as consequence of climate change, unplanned rapid urbanization, unprecedented population growth, increasing

movement of people (and consequently viruses), international travel and breakdown in public health infrastructure, and vector control programs. The actual numbers of dengue cases are underreported and many cases are misclassified. The prevalence of dengue is estimated at 3.9 billion people who are at risk of infection in 128 tropical and subtropical countries, mainly southeast and south Asia, Central and South America, and the Caribbean. A recent estimate indicates 390 million dengue infections per year (95% credible interval 284–528 million), of which 96 million (67–136 million) manifest clinically (with any severity of disease), with an estimated 500,000 cases each year of life-threatening disease in the form of severe dengue—including dengue hemorrhagic fever and dengue shock syndrome mostly in the pediatric population, with about 20,000 succumbing to it, and is the leading cause of childhood death in many countries [1–3]. Dengue is associated with considerable social, economic, and political consequences caused by urban epidemics, such as those seen in Delhi (1996), Cuba (1977–1979 and 1997), Taiwan (2002), and Brazil (2008). Furthermore, Dengue is currently also a major cause of morbidity in American and European travelers and military personnel [4]. This disease places a high economic burden on both governments and individuals; for instance, in America, Dengue illness costs US\$2.1 billion per year on average, excluding vector control, exceeding costs of other viral illnesses. In addition, in Southeast Asia, there is an estimate of 2.9 million dengue episodes and 5906 deaths annually, with an annual economic burden of \$950 million [3].

2. Natural clinical evolution

Dengue virus infections encompass a range of well-described clinical illnesses ranging from an asymptomatic infection to a self-limiting febrile illness, dengue fever, to severe dengue (shock and death), a clinical syndrome that typically presents with capillary permeability and can lead to dengue shock syndrome and dengue hemorrhagic fever. Among less common presentations of severe dengue are encephalitis, hepatitis, and renal dysfunction [4]. Infection by any dengue virus requires a 4- to 8-day incubation period and can produce a wide spectrum of illnesses, the majority of these being asymptomatic or subclinical. Although most patients are able to recover after a self-limiting (yet debilitating) illness, a small proportion develops a severe form of the disease, which is mainly characterized by plasma leakage with or without bleeding [3]. The acute illness is usually benign and self-limiting. Moreover, a secondary infection, corresponding to a subsequent infection with a different serotype is also characterized by acute fever and several other nonspecific signs and symptoms, usually indistinguishable from a range of other illnesses. However, in 2–3% of secondary infections with another serotype there is a higher risk of increased disease severity, causing life-threatening Dengue with Warning Signs (DWS+) and Severe dengue (SD), according to the revised WHO dengue case classification (DENCO) [2, 5]. Serotype-cross-reactive antibodies facilitate DENV infection in Fc-receptor-bearing cells by promoting virus entry via Fc γ receptors (Fc γ R), a process known as antibody-dependent enhancement (ADE) [6, 7]. Dengue without Warning Signs (DWS–) is more often observed in adults and adolescents and can manifest with only a mild fever only or a more disabling disease. This latter form is characterized by symptoms occurring mainly in the early febrile stage, such as the sudden onset of high fever, severe headache, retro-orbital pain, myalgia, arthralgia, and rash. In the critical phase, the skin is flushed with the appearance of a petechial rash, occurring predominantly around the time of defervescence,

when an increase in capillary permeability accompanied by increased hematocrit can occur, leading to hypovolemic shock that can result in organ impairment, metabolic acidosis, disseminated intravascular coagulation, and severe hemorrhage. If untreated, mortality can be as high as 20%, whereas appropriate case management and intravenous rehydration can reduce mortality to less than 1% [3]. SD usually affects children younger than 15 years of age, although it can occur in adults. SD is characterized by a transient increase in vascular permeability resulting in plasma leakage with high fever, bleeding, thrombocytopenia, and hemoconcentration, which can lead to shock [5]. Two factors, namely, antibody-dependent-enhancement (ADE) and inherent virulence of the DEN viruses, appear to contribute the most to disease pathogenesis [2].

3. Pathogenesis of Dengue virus infection

The pathophysiological basis for severe dengue is multifactorial, resulting from a complex interaction between the virus, the host, and, at least in part, immune-mediated mechanisms. Individuals with primary Dengue virus infection induce a lifelong protective immunity to the infecting serotype, accompanied by a short-term cross-immunity against other serotypes and development only leads to self-limited dengue fever characterized by high fever and debilitating joint pain, recovery from infection by one provides lifelong immunity against that serotype. However, subsequent infection with a different dengue serotype is more likely to cause DWS+/SD, and cross-immunity to the other serotypes after recovery is only partial and temporary. Epidemiological data suggest an increased risk of DWS+/SD in people with preexisting heterotypic dengue virus antibodies, and this has led to research in dengue pathogenesis focusing on the subsequent infections by other serotypes and an increased risk of developing severe dengue. Accordingly, antibodies against a given serotype can cross-react with, but not cross-protect against, the remaining three virus serotypes [4] during secondary infections. Immunopathological mechanisms have been proposed, such as the immune system improvement phenomenon (immunopotential), which contribute to the increased risk of DWS+ or SD. It is widely accepted that these cross-reactive antibodies can promote enhanced uptake of the heterologous virus into host cells, precipitating a hyperimmune reaction resulting in blood vessel leakage and potentially fatal hypovolemic shock [1, 2]. This increase occurs when nonneutralizing antibodies resulting from the primary infection favor the invasion of the second virus with a different serotype into the target cells, a phenomenon known as antibody-dependent amplification or antibody-dependent enhancement (ADE) [8, 9]. Subsequent infection with a different dengue serotype is more likely to cause DWS+/SD [8, 9]. According to the ADE hypothesis, a primary infection with DENV produces an insufficient concentration of antibodies or avidity to neutralize a secondary infection by DENV of a different serotype that differs in its amino acid sequence by 30–35%; and these sub-neutralizing antibody concentrations can promote infection in such cells by facilitating Fc-receptor-mediated entry [10]. These concentrations could be enough to opsonize the secondary virus and target it for Fc-receptor-mediated endocytosis into myeloid cells such as monocytes and macrophages (which constitute the main site of DENV replication) and in this manner promote higher viral loads. ADE can be observed *in vitro* and has also been proven to drive higher viral loads of DENV in animal models [11]. The following findings support the hypothesis of the antibody-dependent

enhancement (ADE): undiluted sera obtained early from patients with secondary infection was shown to enhance dengue virus infection *in vitro*, infants born from dengue-immune mothers displayed a higher viral burden than infants born to dengue nonimmune mothers and further demonstrated immune activation associated with disease severity, and lethal antibody-dependent enhancement has been shown in dengue mouse models, and virus-antibody complexes bind to Fc γ receptor-bearing cells, resulting in increased infected cell mass and a rise in viremia. The cardinal feature of DWS+ is plasma leakage believed to arise from pro-inflammatory cytokine-inflicted damage to the vascular endothelium. Although the cause of severe form of dengue infection is not clear, it has been revealed that secondary infection with a different serotype of DENV, or even a homotypic reinfection, are major risk factors for SD probably due to antibody-dependent enhancement. The phenomena of original antigenic sin, as well as immune evasion that inhibits interferon (IFN)- α and IFN- β signaling by suppressing Jak-Stat activation, a cytokine storm, and autoimmune responses are thought to contribute to the pathogenesis of severe form of dengue disease [12, 13], see **Figure 1**. Dengue virus (DENV) can inhibit both type I IFN production and signaling in susceptible human cells, including dendritic cells (DCs). The proteolytic activity of the NS2B3 protease complex of DENV allows it to function as an antagonist of type I IFN production. Other DENV proteins that antagonize type I IFN signaling include NS2A, NS4A, NS4B, and NS5 by targeting different components of this signaling pathway, such as STATs [15]. During a primary infection, serotype-specific as well as cross-reactive memory T-cell responses are produced. On the other hand, during a secondary dengue virus infection, viral epitopes expressed on infected cells trigger activation of serotype-cross-reactive memory T-cells, with the production of pro-inflammatory cytokines. The latter ultimately lead to plasma leakage in the vascular endothelium. The specific cellular

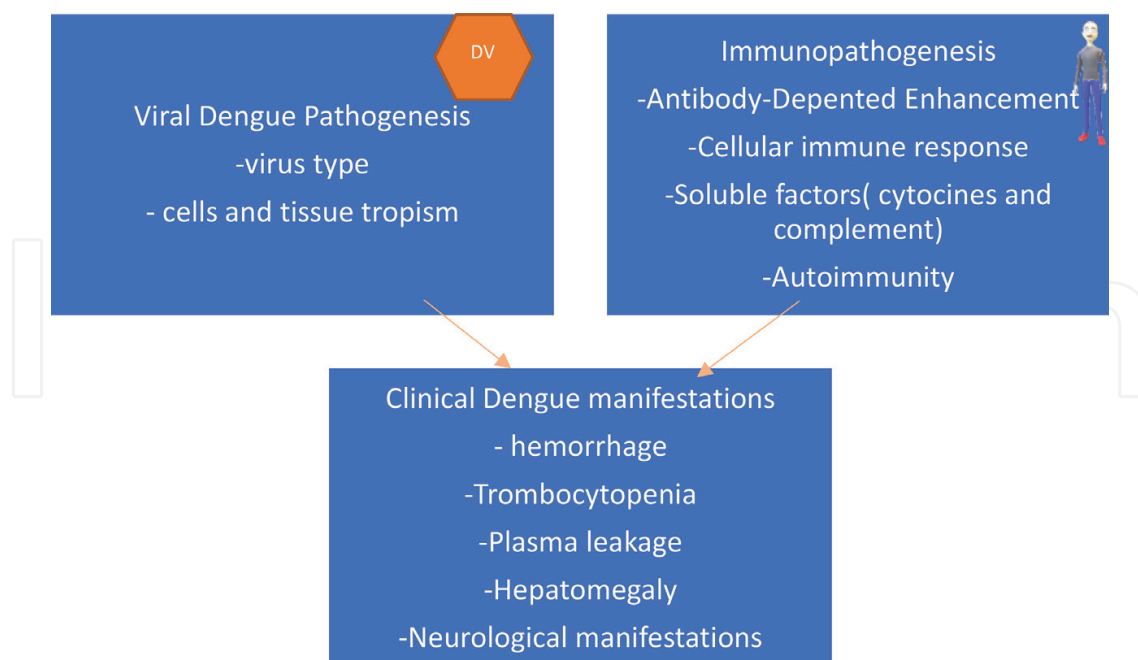


Figure 1. A hypothetical model of dengue pathogenesis. Viral and immunological factors contribute to clinical manifestations, including severe hemorrhage, thrombocytopenia, plasma leakage, hepatomegaly, and neurological compromise. DV: dengue virus. Adapted from Wan et al. [14].

response against DENV begins with the activation of CD4⁺ T-cell during viremia and subsequently the activation of CD8⁺ T-cell. In individuals with DHF or DWS⁺ due to secondary infections, the presence of CD4⁺/CD8⁺ memory T-cell and cytotoxic CD4⁺/CD8⁺ T-cell has been demonstrated [16], and the activation of T-cell and the production of cytokines are important factors in the pathogenesis of DWS⁺ [17]. Likewise, the cellular immune response, in the case of DWS⁺, exacerbates the activation and release of cytokines, which is related to the greater severity of the clinical picture. The activation of the complement system has also been demonstrated in DHF, and high concentrations of C3 and C1q proteins can be detected in severe cases. It is suggested that complex virus-circular antibodies could activate the cascade reaction of the complement [18], see **Figure 2**. Regulatory immune pattern in homologous versus a pro-inflammatory pattern in heterologous dengue virus secondary infection has been reported. Several soluble factors produced by T cells, monocytes, macrophages, and mast cells have been proposed to increase vascular permeability in primary endothelial cells. These factors include TNF α , interleukin 6, interleukin 8, interleukin 10, interleukin 12, macrophage migration inhibitory factor, HMGB1, MCP-1, and matrix metalloproteinases. Endothelial permeability can also be influenced by the maturation state of NS4B, which modulates the cytokine response in monocytic cell lines. In addition, secreted NS1 protein, along with anti-NS1 antibodies and complement activation, might be involved in dengue virus-induced vascular leakage. Moreover, around defervescence, when plasma leakage is apparent, high levels of complement activation products C3a and C5a are detected in plasma, followed by accelerated consumption and large reduction of complement components in patients with dengue shock syndrome. Activation of the complement system can stimulate the production of inflammatory cytokines associated with DWS⁺/SD, and trigger local and systemic effects implicated

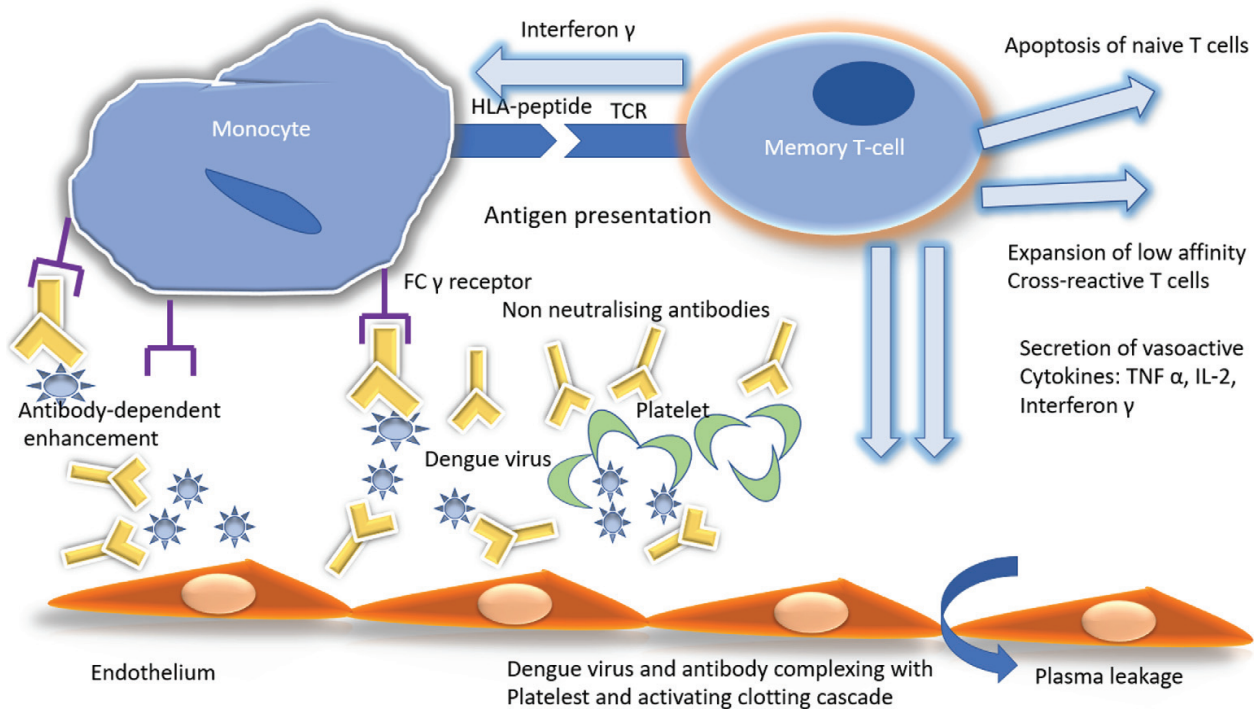


Figure 2. Schematic representation of the immunopathogenesis of severe dengue disease. Adapted from Webster et al. [4].

in intravascular coagulation. Finally, although controversial, the role of autoimmunity in the pathogenesis of dengue is mentioned, as autoantibodies resulting in platelet and endothelial cell dysfunction might be involved in severe dengue pathogenesis. Not all cases of DWS+ occur in people who experience a secondary infection, since in some cases the virus' own virulence, added to the characteristics of the host, leads to the complication of the disease [19], which may be due to the presence of antibodies against viral proteins that have cross-reactivity with platelets and coagulation factors [18]. Certain nonstructural proteins such as NS1, NS2, and NS3 appear to have a certain structural homology with coagulation factors, platelets, integrins, and adhesins of human endothelial cells, allowing the activation of autoreactive T lymphocytes that participate in the pathology of dengue [16, 20]. Anti-NS1 antibodies correlate with disease severity, and cross-reaction of anti-NS1 antibodies with liver and endothelial cells are also implicated in affecting the integrity of the vascular endothelium and platelets has been proposed to trigger these cells to express nitric oxide and undergo apoptosis [2, 3, 14]. Certain antibodies to some E protein epitopes can bind to human plasminogen and inhibit plasmin activity (see **Figure 3**). Recently, it has been reported that Tropomyosin (TPM)-1 may play an important role in the pathogenesis of SD. It is plausible that the elevation of TPM-1 in the plasma of SD patients can be due to excessive cell death, thereby releasing TPM into the circulation as DAMPs, and leading to mast cell activation. Moreover, the insulin pathway may play a role in the pathogenesis of SD, hence, regulating the insulin

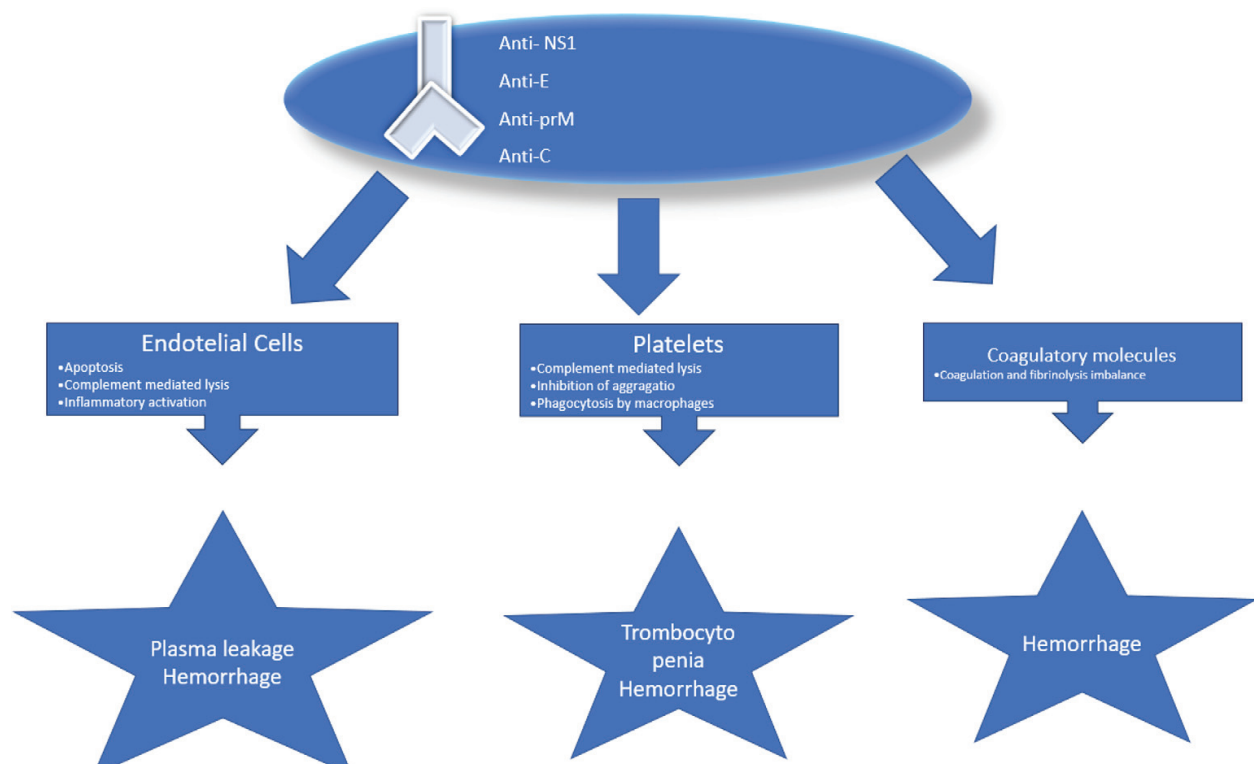


Figure 3. A schematic model of autoantibody-mediated immunopathogenesis in DENV infection. Molecular mimicry between platelets, endothelial cells, and coagulatory molecules with NS1, prM, E, and C proteins underlies the cross-reactivity of anti-NS1, anti-prM, anti-E, and anti-C Abs, respectively, to host proteins. Abs Z antibodies; C Z capsid protein; DENV Z dengue virus; E Z envelope protein; NS Z nonstructural protein; prM Z precursor membrane protein. Adapted from Wan et al. [14].

signaling pathway may be a key intervention to reduce plasma leakage in patients with SD [12], see **Figures 1–3**.

A protective versus pathological outcome depends on the balance between the host's genetic and immunological background and viral factors. Vaccine development has been slowed by fears that immunization might predispose individuals to the severe form of dengue infection [3, 4]. There are four distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3, and DEN-4).

No DENV-specific therapies are available, while a DENV vaccine that elicits protection in people with prior DENV exposure but not in naive individuals and that is not equally protective against all four serotypes has recently begun to be licensed on a country-by-country basis. This is mostly due to an incomplete understanding of the interplay between viral and host factors that contribute to DENV pathogenesis. On the virus side, some DENV lineages are more virologically and epidemiologically fit than others and are thus associated with DWS+/SD manifestations. On the host side, DENV infection history is the primary determinant associated with the development of more severe dengue disease, with potential contributions from other factors such as genetic variation, age, and sex.

Several studies have demonstrated that DENV-specific antibodies can protect against infection and, under certain conditions, enhance infection and disease severity, whereas the role of T cells remains unclear. Thus, to avoid the risk of enhancement, a safe vaccine against dengue virus will need to confer protective immunity against all four serotypes [10]. Consequently, the adaptive immune response to dengue can be both protective and pathogenic, which complicates vaccine development, as discussed in this chapter.

4. Dengue vaccines

Dengue virus is widespread throughout the tropics, representing an important, rapidly growing public health problem with an estimated 2.5–3.9 billion people at risk of dengue fever and the life-threatening severe dengue disease. Therefore, the need for a safe and effective vaccine for dengue is immediate. Vaccine development has been slowed by fears that immunization might predispose individuals to the severe form of dengue infection [4]. The characteristics and challenges that the ideal vaccine for the dengue virus must have are described in the following.

4.1. Characteristics of an ideal dengue vaccine and challenges to its development

4.1.1. Characteristics

- Safe in children and adults [3, 4]
- Avoids ADE (antibody-dependent enhancement) and pathogenesis
- Rapid immunization regime requiring a single vaccine or two that fit in with established vaccine programs

- Induces a balance between reactogenicity and immunogenicity
- Suitable for use in target age groups
- Genetically stable
- Stimulates neutralizing antibodies and Th1 cell-mediated immunity
- Induces long-lasting immunity, safety, and protection
- Generates neutralizing immunity to all four serotypes
- Does not contribute to immunopathogenesis (vaccine-induced enhancement)
- Easy storage and transportation
- Affordable and cost effective

4.1.2. Challenges

- Existing possibility of triggering ADE
- Vaccine must be tetravalent
- Dengue virus serotypes do not induce long-lasting heterotypic immunity
- No suitable or ideal animal model exists for immunization studies
- No well-established viral virulence markers are available
- Correlates of protection are not well defined
- Subsequent infection (especially, after a long-time interval) may lead to severe dengue
- Vaccine candidates should be evaluated in geographic areas with different transmission patterns [3].

To date, there are several DENV vaccines under development, with some in phase 3 safety and efficacy testing. These include inactivated, live attenuated, recombinant subunit, viral vectored, and DNA vaccines. Dengue vaccine development has aimed to elicit a neutralizing antibody response, as T cells are assumed to contribute a minor or secondary role in dengue vaccine-mediated protection. Next, we will describe each of these vaccines.

4.2. Vaccine types

4.2.1. Live-attenuated virus (LAV)

The fundamental aim of vaccination is to promote protective immunity while avoiding disease from the vaccine itself. The first generation of viral vaccines was based on empirical attenuation by repeated passage in cultured cells. Several LAVs are eligible vaccines as they meet the following criteria; they elicit a strong and protective immune response with a low risk of disease from the vaccine itself. In the present regulatory environment, the use of LAVs has

also been limited by safety concerns, including reversion to wild-type virulence. Because LAVs are shed from vaccines, they sometimes present a risk to unvaccinated individuals with impaired immunity. Although LAV vaccines have been developed for many RNA viruses, the mutability of these pathogens presents unique challenges for vaccine design [21].

4.2.2. *Purified inactivated virus (PIV)*

It is widely believed that inactivated dengue virus vaccines are impractical given the difficulty in obtaining sufficiently high titers of the virus in a suitable cell substrate. However, this was challenged when dengue type-2 (dengue-2) virus was adapted to replicate to high titers in certified Vero and fetal rhesus lung (FRhL-2) cell cultures and used to make prototype purified, inactivated virus (PIV) vaccines. In addition, in formulation with an aluminum hydroxide adjuvant, these vaccines elicit virus-neutralizing antibodies in mice and rhesus macaques and provide at least partial protection against virus challenge [22].

4.2.3. *Recombinant subunits*

Recombinant subunit-based vaccines may prove to be significantly advantageous compared to other approaches currently being implemented for development of a dengue vaccine. First of all, the lack of a replicating virus helps to ensure the safety of the product by avoiding the possibility for inadequate attenuation or reversion in the context of live virus approaches, or inadequate inactivation in the context of killed virus vaccines. Furthermore, under a tetravalent formulation, the ability to induce a balanced immune response may be more easily manipulated through dose adjustments using recombinant subunits compared to four replicating viruses. Finally, in terms of yield and cost effectiveness, and since the dengue vaccine mainly targets developing areas, a high yielding, highly immunogenic, recombinant subunit could prove to be an attractive alternative to vaccines based on virus replication, (live attenuated or killed) where yields may be lower than required [23].

Recombinant subunit vaccines stand as one of the safest alternatives, as a means to bypass the issue of viral interference, offering the possibility to administer a tetravalent formulation on an accelerated schedule. An advantage of an accelerated schedule is that full protective immunity could be induced more quickly, thus avoiding the potential of exacerbated disease due to partial immunity during an extended immunization course. Among other advantages of an accelerated schedule are better general compliance, more suitability for travelers and military personnel, easier integration into existing immunization schedules, and the potential for use in an outbreak setting. A balanced tetravalent immune response may also be more readily accomplished through simple dose adjustments for each of the four recombinant proteins, in comparison to live virus vaccines where the interactions between viruses can be complex and unpredictable [24].

4.2.4. *Virus-like particles (VLPs)*

VLP vaccines are virus-like particles that do not contain replicative genetic material, but permit presentation of antigen in a repetitive, ordered array similar to the virion structure, which is thought to increase immunogenicity [25]. Thus, the safety concerns of virus vaccines regarding reversion mutants and immunocompromised individuals are obviated. The recombinant of

VLP allows these vaccines to be usually manufactured large-scale in a cost-effective manner, following current good manufacturing practices. They induce quick and fulminant humoral immune responses by displaying antigens in an ordered and repetitive way. Their particulate nature and dimensions allows an efficient assimilation by dendritic cells (DCs) and transportation to lymph nodes, followed by presentation and induction of optimal immune responses. VLPs are renowned for inducing rapid and strong antibody responses. This trait is attributed to their dense, highly repetitive, quasi-crystalline structures [26], see Dengue vaccine candidates in **Table 1**.

Candidate name/ identifier	Antigen	Vaccination	Developer	Preclinical	Phase I	Phase II	Phase III
CYD Live recombinant based on a yellow fever vaccine 17D backbone	DENV-1-4 prM/E	3 doses (0/6/12 months)	Sanofi Pasteur	X	X	X	X
TV003/TV005 Tetravalent live, attenuated/recombinant (whole virus DENV1–3 and recombinant DENV2 in DENV4 backbone)	DENV-1,3,4 whole genome, DENV-2 prM/E	1 dose	US National Institutes of Health and Butantan (with licenses to other manufacturers)	X	X	X	X
DENVax Tetravalent live, attenuated/recombinant (whole virus DENV2 and recombinant DENV1/3/4 in DENV2 backbone)	DENV-2 whole genome, DENV-1, -3, -4 prM/E	2 doses (0/90 days)	Takeda	X	X	X	
DPIV Tetravalent purified inactivated vaccine	DENV-1–4 whole genome	2 doses (0/28 days)	GSK/US WRAIR/Fiocruz	X	X		
DEN-80ETetravalent E protein subunit vaccine	Soluble DEN 1/2/3/4 prM/E protein	3 doses (0/1/2 months)	Merck	X	X		
TVDVTetravalent “shuffled” prM/E expressed from plasmid vector DNA vaccine	Plasmid DNA expressing DENV 1/2/3/4 prM-E	3 doses (0/1/3 months)	US Naval Medical Research Center	X	X		
TLAV-TPIV Heterologous prime-boost with live-attenuated tetravalent, live-attenuated vaccine and tetravalent alum-adjuvanted purified inactivated vaccine	Purified inactivated DENV or plasmid vector expressing prM/E (prime) and live-attenuated DENV (boost)		US WRAIR	X	X		

Dengue vaccine candidates; adapted from Kirsten et al. [27].

Table 1. Dengue vaccine candidates.

4.3. Vaccines under clinical trials

4.3.1. CYD-TDV *Dengvaxia*

Sanofi Pasteur's CYD vaccine is a live-attenuated tetravalent chimeric vaccine. In this vaccine, the pre-membrane and envelope proteins from a wild-type dengue virus corresponding to each of the four serotypes are substituted into the yellow fever (YF) 17D vaccine backbone. A strong neutralizing antibody response to DENV2 was elicited in the first CYD clinical trial in healthy adults, which evaluated only the serotype 2 vaccine strain. Participants previously given YF vaccine seroconverted to all four dengue serotypes [28]. The first licensed dengue vaccine, a live, attenuated, tetravalent dengue vaccine (CYD-TDV; *Dengvaxia*), has recently been registered in 15 countries as a three-dose immunization schedule administered subcutaneously at 6-month intervals [29]. In the case of *Dengvaxia*, vaccination of children with no previous infection (seronegative) may mimic an initial infection during the first step in the development of ADE. Because vaccine protection is incomplete and unequal against the four serotypes, a natural infection later in life can complete the sequence of events, causing ADE and severe, life-threatening dengue fever [30].

Following CYD-TDV introduction, it should be administered as a three-dose series given on a 0-/6-/12-month schedule. However, additional evidence is required in order to determine whether equivalent or better protection may be obtained through simplified schedules. In response to a delay in a vaccine dose for any reason, the vaccine course should be resumed (not restarted), maintaining the 6-month interval between subsequent doses. Given the 12-month duration of the immunization schedule and to enable better vaccine monitoring, countries should have vaccine tracking systems implemented. CYD-TDV is not recommended for use in children under 9 years of age, consistent with current labeling, in view of the association of CYD-TDV with increased risk of hospitalized and severe dengue illness in the 2- to 5-year age group. The target age for routine vaccination should be defined by each country, intended to maximize the vaccination impact and programmatic feasibility of targeting specific age groups. For instance, some countries may present the highest incidence of dengue illness among the adult age population and may consider vaccinating people up to 45 years of age in routine programs. The implementation of a routine CYD-TDV vaccination program at 9 years of age in settings meeting the criteria mentioned above is expected to contribute to a 10–30% reduction in symptomatic and hospitalized dengue illness over 30 years [31], see **Table 2**. This vaccine will be reviewed further in a separate section since, differently to other vaccines in this section, *Dengvaxia* has already been registered.

4.3.2. TV003 and TV005 *Dengue vaccine*

The Laboratory of Infectious Diseases at the U.S National Institutes of Health has evaluated numerous monovalent and tetravalent dengue candidate vaccines to identify candidates with the most acceptable safety, infectivity, and immunogenicity profile. Among these, TV003 is an admixture of four live-attenuated recombinant dengue vaccine candidate viruses (rDEN1D30, rDEN2/4D30, rDEN3D30/31, and rDEN4D30) [36]. Various monovalent candidates were initially tested in Phase 1 trials in order to optimize each of the four vaccine virus strains. Vaccine virus serotypes 1, 3, and 4 are based on complete viruses, while serotype 2 is a recombinant

Reference	Lead author /year	Conclusion
Four-year safety follow-up of the tetravalent dengue vaccine efficacy randomized controlled trials in Asia and Latin America,	Arredondo-García et al. 2018 [32]	Data from the clinical trials for up to year 4 after first vaccination indicate a positive benefit–risk profile for the CYD-TDV vaccine for the population aged 9 years old.
A multi-country study of dengue vaccination strategies with Dengvaxia and a future vaccine candidate in three dengue-endemic countries: Vietnam, Thailand, and Colombia.	Lee et al. 2018 [33]	Given the absence of efficacy and half-life data for any of the second-generation vaccine candidates, it was assumed that NVC is 80% efficacious with a half- life of 8 years.
Dengue vaccination during pregnancy—an overview of clinical trials data.	Skipetrova et al. [34]	In the small dataset assessed, no evidence of increased adverse pregnancy outcomes has been identified from inadvertent immunization of women in early pregnancy with CYD-TDV compared with the control group. The conclusions are limited to vaccination in CYD-TDV in the first trimester, since no data are available on pregnancy outcome for administration of this vaccine in the second or third trimester. The data described here, and those continuing to emerge from the on-going clinical development and post-marketing of CYD-TDV, provide a valuable contribution to the currently limited available information on the use of the dengue vaccine in pregnant women.
Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue-endemic country: Randomized controlled phase I trial in the Philippines.	Capeding et al. 2011 [35]	The safety profile of TDV in a flavivirus endemic population was consistent with previous reports from flavivirus-naïve populations. A vaccine regimen of either three TDV vaccinations administered over a year or two TDV vaccinations given more than 8 months apart resulted in a balanced antibody response to all four dengue serotypes in this flavivirus-exposed population, including children.

Table 2. Some CYD-TDV Dengue vaccine safety and immunogenicity studies in different populations.

virus based on the serotype 4 vaccine strain with the structural proteins replaced by those of serotype 2. A single dose of TV005 elicits seroconversion rates above 90% against each serotype, and 90% of flavivirus-naïve recipients displayed a tetravalent response. TV003 or TV005 has been licensed to several manufacturers, including Butantan, VaBiotech, and Merck. Phase 2 studies are underway in Brazil and Thailand, and a Phase 3 trial led by Butantan began in February, 2016, in Brazil [27], see **Table 3**.

4.3.3. DENVax

Takeda's live tetravalent dengue vaccine (TDV) candidate is based on a molecularly characterized attenuated serotype 2 strain (TDV-2). The DENV-2 PDK-53 virus was initially obtained through 53 serial passages of the wild-type (wt) DENV-2 16681 in primary dog kidney (PDK)

Reference	Lead author /year	Conclusion
In a randomized trial, the live-attenuated tetravalent dengue vaccine TV003 is well-tolerated and highly immunogenic in subjects with flavivirus exposure prior to vaccination	Whitehead et al. 2017 [37]	In summary, the authors demonstrated that the NIH tetravalent dengue vaccine TV003 is well-tolerated in flavivirus-experienced individuals and elicits robust post-vaccination neutralizing antibody titers.
The live-attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model	Kirkpatrick et al. 2016 [36]	TV003 induced complete protection against challenge with rDEN2Δ30 administered 6 months after vaccination. TV003 will be further evaluated in dengue-endemic areas.

Table 3. Some TV003 vaccine safety and immunogenicity studies.

cells. The DENV-2 PDK-53 virus has proved to be safe, well-tolerated, immunogenic, and elicits long-term humoral and cellular immune responses to DENV-2, based on clinical trials conducted in the United States and Thailand [38]. Three chimeric strains (TDV-1, TDV-3, and TDV-4) were engineered by substituting the premembrane (prM) and envelope (E) structural genes of the respective DENV strains into the attenuated TDV-2 backbone [39]. TDV is designed to promote humoral and cellular protective immune responses against all four dengue serotypes, as it contains the premembrane and envelope proteins unique to each serotype. These specific proteins are needed to induce neutralizing antibodies. The use of DENV-2 as a backbone for TDV may confer additional protection against dengue. In particular, TDV contains the genes encoding the conserved nonstructural (NS) proteins within the dengue backbone; and these proteins have been shown to be important in generating T-cell-mediated responses to dengue infection. Furthermore, anti-NS1 antibodies have been associated with cross-protective humoral immune responses [40]. **Table 4** shows some of the studies conducted to determine the effectiveness of this vaccine.

4.3.4. DPIV tetravalent purified inactivated vaccine

The Walter Reed Army Institute of Research (WRAIR) in collaboration with GlaxoSmithKline Vaccines (GSK) developed a live-attenuated tetravalent dengue virus vaccine candidate comprised of four live virus strains representing each of the four DENV types. These strains were attenuated by serial passage in primary dog kidney (PDK) cells [44]. The US Navy Naval Medical Research Center (NMRC) has developed a tetravalent DNA vaccine (TVDV), formulated with Vical's Vaxfectin adjuvant, containing genes encoding the premembrane (prM) and envelope (E) proteins for all four serotypes of dengue virus. Both Vaxfectin-formulated and unformulated vaccines are currently being evaluated in Phase I human testing [45].

Inactivated vaccines are assumed to provide acceptable safety profiles across a wide age range as well as in immunocompromised hosts. In addition, these can be co-administered with other vaccines. Shortened vaccination schedules and rapid immunization are also feasible using this type of vaccines. For these reasons, a safe and efficacious tetravalent DENV PIV could be suitable for national immunization programs across broad age ranges and baseline health

status, as well as an active immunization option for travelers and military personnel, and a potential tool for outbreak response [46]. **Table 5** shows several DPIV vaccine safety and immunogenicity studies.

Reference	Lead author /year	Conclusion
Safety and immunogenicity of a live-attenuated tetravalent dengue vaccine candidate in flavivirus-naive adults: a randomized, double-blinded Phase 1 clinical trial	George et al. 2015 [41]	TDV was generally well-tolerated, induced trivalent or broader neutralizing antibodies to DENV in most flavivirus-naive vaccines, and is undergoing further development.
Safety and immunogenicity of a recombinant live-attenuated tetravalent dengue vaccine (DENVax) in flavivirus-naive healthy adults in Colombia: a randomized, placebo-controlled, phase 1 study	Osorio et al. 2014 [42]	The authors emphasize the acceptable tolerability and immunogenicity of the tetravalent DENVax formulations in healthy, flavivirus-naive adults. Further clinical testing of DENVax in different age groups and in dengue-endemic areas is warranted.
Development of DENVax: A chimeric dengue-2 PDK-53-based tetravalent vaccine for protection against dengue fever	Osorio et al. 2011 [43]	The DENVax vaccine is considerably different from previously tested tetravalent vaccines in that all four strains contain the same attenuating mutations as the DEN-2 PDK-53 strain, a strain that has been shown to be both safe and immunogenic in humans. Such vaccine is critically needed to protect people from the threat of dengue infection and improve public health worldwide.

Table 4. Some TDV(DENVax) vaccine safety and immunogenicity studies.

Reference	Lead author /year	Conclusion
Phase I randomized study of a tetravalent dengue purified inactivated vaccine in healthy adults from Puerto Rico.	Diaz et al. 2018 [47]	Results from this first phase I study of a new vaccine candidate with inactivated DENV in a dengue-primed population showed that all four DPIV vaccine formulations were well-tolerated and immunogenic. This new investigational DPIV vaccine had an acceptable safety profile in a small number of flavivirus-primed healthy adult subjects and all formulations boosted neutralizing antibodies (Nab) responses, with complex adjuvants increasing immunogenicity versus alum adjuvantation. Nab titers remained high (and above baseline titers) through M13. These results encourage continuation of DPIV clinical development.
Phase 1 randomized study of a tetravalent dengue purified inactivated vaccine in healthy adults in the United States	Lepine et al. 2017 [48]	All DPIV formulations were well-tolerated. No vaccine-related serious adverse events were observed through 12 months after the second vaccine dose. In all DPIV groups, geometric mean antibody titers peaked at Day 56, waned through 6 months after the second vaccine dose, and then stabilized. In the nine subjects where boosting was evaluated, a strong anamnestic response was observed. These results support continuation of the clinical development of this dengue vaccine candidate.

Table 5. Some DPIV vaccine safety and immunogenicity studies.

4.3.5. DEN 80E vaccine

This vaccine (developed by Hawaii Biotech and now licensed to Merck) is composed of a recombinant truncated protein corresponding to 80% of the N-terminal DENV E protein (DEN-80E). The C-terminal truncation of the E protein at amino acid 395 removes the membrane anchor sequence of the protein, resulting in a recombinant E protein with improved secretion, purification and immunogenicity. The DEN-80E protein for each of the four dengue serotypes has been expressed in the *Drosophila* S2 expression system to produce a tetravalent vaccine [49], which induces a high level expression of proteins of interest. Specifically, the system was chosen to express a plasmid containing the prM and N-terminal 80% of the E gene sequence of DENV-2. The resulting polyprotein undergoes cleavage by endogenous proteases and the 80E protein with a native-like N terminus is released. Two doses of the DENV-2 subunit 80E protein were administered to rhesus macaques in combination with one of seven different adjuvants at a 3-month dosing interval. Following this administration, animals were challenged with wild-type DENV-2 2 months after the last dose of vaccine. Neutralizing antibodies were detected in all study animals after the first dose and this response was boosted by the second dose. The highest neutralizing antibody titers were produced by the r80E protein formulated with the adjuvants AS05 or AS08, and protection against viremia was correlated with a higher neutralizing antibody titer at challenge. The same system was employed to generate recombinant subunit E proteins (80E) of the other DENV serotypes. A tetravalent formulation of the recombinant 80E proteins was evaluated in mice and nonhuman primate experiments. In some instances, the NS1 protein of DENV-2 was included in the formulation to potentially enhance the immune response to the vaccine. Macaques were immunized with the tetravalent formulation four times (day 0, 28, 67, and 102) and were challenged 5 months after the last dose. Due to the limited number of monkeys in each group, monkeys were only challenged with DENV-2 or DENV-4. Monkeys developed a robust neutralizing antibody response to all four DENV serotypes and were completely protected from DENV-2 challenge [50]. **Table 6** shows some of the studies conducted to determine the effectiveness of this vaccine.

4.3.6. TVDV tetravalent “shuffled” prM/E expressed from a plasmid vector DNA vaccine

The U.S. Naval Medical Research Center (NMRC) developed a tetravalent plasmid DNA vaccine candidate using prM and E protein genes expressed in a plasmid vector. A DENV-1 monovalent candidate of this vaccine was evaluated for safety and immunogenicity through a phase I clinical trial on healthy flavivirus-naïve adults using a three-dose schedule at 0/1/5 months. The results showed poor immunogenicity. Although it is possible that TVDV may have a role as a travel vaccine in the future, the available data is currently insufficient to anticipate its potential use as a travel vaccine [52].

The TVDV is a mixture of equal amounts of four monovalent double-stranded plasmid DNA vaccines produced under current Good Manufacturing Practices conditions in the United States. Each monovalent plasmid contains the prM and E genes of dengue 1, 2, 3, or 4 viruses cloned into the backbone plasmid VR1012 (Vical Incorporated, San Diego, CA) [53]. **Table 7** shows some of the studies conducted to determine the effectiveness of this vaccine.

Reference	Lead author/ year	Conclusion
Preclinical development of a dengue tetravalent recombinant subunit vaccine: Immunogenicity and protective efficacy in nonhuman primates	Govindarajan et al. 2015 [51]	Overall, the subunit vaccine was demonstrated to induce strong neutralization titers resulting in protection against viremia following challenge even 8–12 months after the last vaccine dose.
The development of recombinant subunit envelope-based vaccines to protect against dengue virus induced disease	Coller et al. 2011 [24]	The DEN-80E recombinant subunit proteins for all four dengue virus types are expressed at high levels and have been shown to maintain native-like conformation. When formulated with a variety of adjuvants the antigens are potent immunogens and induce high titer virus-neutralizing antibody responses. Furthermore, the antigens have been shown to protect against viral challenge in both mouse and nonhuman primate models. Tetravalent vaccine formulations have also been evaluated in preclinical models with no evidence of immune interference or competition between the four DEN-80E antigens being observed. These proofs of concept preclinical studies led to the advancement of a monovalent DEN1-80E vaccine candidate into clinical testing.
Development of a recombinant tetravalent dengue virus vaccine: immunogenicity and efficacy studies in mice and monkeys	Clements et al. 2010 [23]	The production of recombinant dengue 80E proteins in <i>Drosophila</i> S2 cells that are capable of eliciting potent immune responses in mice and nonhuman primates represents a major achievement in the effort to develop a recombinant dengue vaccine. The S2 cell expression system efficiently produces 80E from all four dengue serotypes. Our data show that co-administration of the subunits from the four serotypes results in a balanced immune response, equivalent to that observed when the four individual components are administered separately. Furthermore this response can be induced in a relatively short period of time (2–3 months).

Table 6. Some DEN 80E Vaccine safety and immunogenicity studies.

4.4. Vaccine candidates under preclinical assays

There are numerous vaccine candidates that are being studied in preclinical trials, as can be seen in **Table 8**.

4.4.1. EDIII-p64k fusion proteins and EDIII-capsid fusion proteins expressed in *E. coli*

Te Pedro Kourí Tropical Medicine Institute (IPK) in collaboration with the Center for Genetic Engineering and Biotechnology (CIGB) in Cuba have led the development of various recombinant subunit vaccine candidates. One approach is based on fusion of DENV EDIII to the carrier protein p64k of *Neisseria meningitidis*, and this EDIII-p64k fusion protein is then expressed in *E. coli*. Evaluations in mice showed that monovalent vaccine candidates for all

Reference	Lead author/ year	Conclusion
Safety and immunogenicity of a tetravalent dengue DNA vaccine administered with a cationic lipid-based adjuvant in a Phase 1 clinical trial	Thomas et al. 2018 [53]	TVDV-Vaxfectin was safe and well-tolerated in this early Phase 1 human clinical trial. Whereas anti-dengue IFN γ T-cell responses occurred in most of the study subjects, anti-dengue neutralizing antibody responses were poor. Utilization of alternative delivery methods as well as examining prime-boost approaches may result in a more robust and long-lasting humoral immune response.
A dengue DNA vaccine formulated with Vaxfectin® is well-tolerated, and elicits strong neutralizing antibody responses to all four dengue serotypes in New Zealand white rabbits	Raviprakash et al. 2012 [54]	The formulated vaccine and the adjuvant were tested for safety and/or immunogenicity in New Zealand white rabbits using a repeat dose toxicology study. The formulated vaccine and the adjuvant were found to be well-tolerated by the animals. Animals injected with formulated vaccine produced strong neutralizing antibody response to all four dengue serotypes.

Table 7. Some TVDV vaccine safety and immunogenicity studies.

DENV serotypes were able to induce neutralizing antibodies and protect against viral challenge. DENV-1 and DENV-2 monovalent candidates have also been evaluated in NHPs. Monkeys were immunized subcutaneously with four doses of the monovalent vaccine (50–100 g protein per dose, formulated in Freund’s adjuvant), which proved to be immunogenic and provided protection against viral challenge. Adjuvants suitable for human use are under evaluation, including *N. meningitidis* serogroup A capsular polysaccharide (CPSA) adsorbed on aluminum hydroxide [25].

5. Final thoughts

Finally, we want to reflect on the implications of the co-circulation of the dengue virus and the Zika virus, as well as on the new indications for the use of the Dengvaxia vaccine.

First, we will analyze the fact that the appearance of the infection by the Zika virus (another flavivirus) in zones of high prevalence for dengue constitutes an interesting challenge for the development of the ideal vaccine for both viruses.

5.1. Zika virus infection means new challenges in dengue vaccine development

Among pathogenic human flaviviruses, DENV and ZIKV are most closely related to each other, with 55.1–56.3% amino acid sequence identity. Zika virus is closer to dengue virus than to any of the other flaviviruses and indeed is almost close enough to think of it as a fifth serotype [10]. Accordingly, emerging literature indicates many similarities between these two viruses in terms of interactions between the virus and host immune system. For both viruses, the interferon system is the central mediator of host defense and target of a viral counterattack,

Techonological approach	Antigen	Vaccine developer	Valency under evaluation or evaluated in NHP
Recombinant subunit vaccines	EDIII-p64k fusion proteins and EDIII-capsid fusion proteins expressed in <i>E. coli</i>	IPK/CIGB	Monovalent
	Bivalent 80E-STF2 fusion proteins expressed in baculovirus/insect cells	VaxInnate	Tetravalent
	Tetravalent consensus EDIII protein expressed in <i>E. coli</i> .	NHRI	Tetravalent
DNA vaccine	prM/E expressed from plasmid vector DNA vaccine	US CDC	Tetravalent
VLP Vaccines	EDIII-HBsAg VLPs or ectoE-based VLPs expressed in <i>P. pastoris</i>	ICGEB	Tetravalent
Virus-vectored vaccines	Tetravalent EDIII and DENV-1 ectoM expressed from live-attenuated measles virus vector	Themis Bioscience/Institut X Pasteur	Tetravalent
	E85 expressed from single-cycle VEE virus vector	Global Vaccines	Tetravalent
Purified inactivated virus vaccine	Psoralen-inactivated DENV	US NMRC	Monovalent
Purified inactivated DENV	Purified inactivated DENV	WRAIR/GSK/FIOCRUZ	Tetravalent
	Inactivated virus (+VEE-particle adjuvant)	Global Vaccines	Tetravalent
Live-attenuated virus vaccines	DEN/DEN chimeric viruses, live, attenuated	Chiang Mai University/ Mahidol X University/ NSTDA/BioNet-Asia	Monovalent
	DEN host range mutations	Arbovax	Tetravalent

Table 8. Active dengue vaccine candidates in preclinical development that have been evaluated in NHP models.

whereas complex interplays between antibody and T cell responses likely determine the outcome of infection in flavivirus immune settings [55]. Dejnirattisai et al. found that most mAbs to DENV also bound to ZIKV, yet the antibodies targeting the major linear fusion-loop epitope (FLE) did not neutralize ZIKV, whereas they showed neutralizing activity against DENV. ZIKV virus infection was found to be potently enhanced by DENV-immune plasma and mAbs to DENV, suggesting the possibility that preexisting immunity to DENV might increase ZIKV replication; thus, this data indicate that immunity to DENV might drive greater ZIKV replication and have clear implications for disease pathogenesis and future vaccine programs for ZIKV and DENV [11]. There have been safety concerns related to Dengvaxia resulting from long-term vaccine trials. In patient groups under 9 years of age, hospitalization from DENV infection was greater for vaccinated children than for the nonvaccinated control group. These findings suggest ADE of infection in DENV naive children at the start of the study trial and who had been primed by but not protected by the vaccine. Consequently, the vaccine is not licensed for use in children under 9 years of age and, furthermore, it is recommended for use only in populations with a seroprevalence of 70% or greater of prior DENV exposure in the age group to be vaccinated [56].

Currently, there is a high pressure to produce a vaccine against ZIKV, and in this context, the extensive serological cross-reaction between DENV and ZIKV must be considered. It is likely necessary that the vaccine be used in areas with high seroprevalence for DENV and raising de novo ZIKV-neutralizing responses in such a setting might be challenging. It is likewise possible that vaccination of DENV-naive subjects against ZIKV might promote ADE of DENV infection and, conversely, that vaccination against DENV might promote ADE of ZIKV infection. In summary, cross-reaction of antibodies to DENV with ZIKV and promotion of ADE of infection can occur due to the existing similarities between the two viruses, even though ZIKV differs in sequence identity from DENV by around 41–46% (in the sequence of the envelope protein). In this context, ZIKV could be considered a fifth member of the DENV serocomplex, a factor that must be considered in vaccine approaches to these two viruses [11]. The results of Barba-Spaeth group suggest that the epitope targeted by the EDE1 bnAbs is more adequate for developing an epitope-focused vaccine for viruses of the ZIKV/DENV super serogroup than is the FLE, which induces poorly neutralizing and strongly infection-enhancing antibodies [57].

5.2. The Dengvaxia future

Dengvaxia is the only vaccine licensed to date for use in humans, which is why epidemiologists, health professionals, clinical physicians, and basic researchers (virologists, immunologists, molecular biologists, etc.) should be concerned about the future of this vaccine, which has had a reverse according to the latest publications of its results, so we will end this chapter with the following reflection based on the publications from 2016 to date.

Since April 2016, Dengvaxia has been licensed for use in 19 countries, and was recommended by the WHO Strategic Advisory Group of Experts (SAGE) on immunization to be used in regions with high endemicity, as defined by a prevalence of dengue antibodies of more than 50% in the targeted age group of people aged 9–45 years. Nevertheless, Guiar's mathematical model finds that a significant reduction of hospitalizations can be only achieved when the vaccine is directed exclusively to seropositive individuals [58]. Along this same line, this group of researchers in 2017 predicted a significant reduction in dengue virus infection-related hospital admissions resulting from the administration of Dengvaxia only to dengue seropositive individuals, based on the analysis of an age-structured model using the available vaccine trial data. Moreover, the researchers predicted a significant increase in the number of dengue-related admissions, over a 5-year period, if the vaccine is to be administered without previous population screening for serostatus. The take-home message is that individual serostatus is the most important feature when implementing this vaccine and that only individuals of any age who have experienced at least one dengue virus infection will benefit from vaccination [59, 60]. New data by Sanofi in November 2017 showed that Dengvaxia could increase the risk of severe dengue in people who had not been previously exposed to the virus. For any countries considering vaccination as part of their dengue control program, the WHO recommends a "prevaccination screening strategy," in which only dengue seropositive people are vaccinated. The prescreening process could be achieved by conventional serological testing for dengue virus to identify people who have had previous dengue infections. As Sanofi stated, "We are confident in Dengvaxia's safety and its proven potential to

reduce dengue disease burden in endemic countries. We will continue to work with the international public health community and endemic countries, to ensure the best usage of the vaccine to increase protection for populations at risk of subsequent dengue infections [that are] potentially more debilitating” [61].

Author details

Jhon Carlos Castaño-Osorio^{1*}, Alejandra María Giraldo-García¹ and Maria Isabel Giraldo²

*Address all correspondence to: jhoncarlos@uniquindio.edu.co

1 Quindio University, Armenia, Quindio, Colombia

2 Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA

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