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UPDATES ON DENGUE VACCINES; CURRENT STATUS, CHALLENGES AND FUTURE PERSPECTIVES

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Abstract

Dengue is a significant public health challenge worldwide. Vector management has become the prime intervention in dengue control. Although various attempts have been made to make a dengue vaccine during the last 3-4 decades, it has become challenging. This review explored the history of dengue vaccine development and its present status and prospects. A literature review from 2010- 2022 covering vaccine development approaches was considered in this study. Significant advancements in vaccine development are evident, with several candidates advancing through clinical trials. The live-attenuated CYD-TDV (Dengvaxia) vaccine by Sanofi Pasteur, the first dengue vaccine approved, has shown variable efficacy and raised safety concerns, especially in dengue-naïve populations. The TAK-003(Intertypic chimera-DENVax) (Qdenga®) has been approved by the European Medicines Agency (EMA) for use in individuals aged four years and older, based on national guidelines. The TV003/TV005 developed by the National Institutes of Health is currently in Phase III trials. These vaccines aim to address the challenges of antibody-dependent enhancement (ADE) and provide broad, serotype-specific immunity. The live-attenuated vaccines have shown potential, but they face issues like genetic instability and adverse reactions. Non-replicating platforms, such as DNA and subunit vaccines, offer safer alternatives but may need adjuvants to enhance immunogenicity. The review highlights the necessity for ongoing research and innovation to create vaccines that induce strong, protective immune responses without ADE. Despite the approval of Dengvaxia, its limited efficacy in some populations underscores the need for next-generation vaccines that offer comprehensive protection across various demographics.

Keywords: Dengue, Dengvaxia, Vaccine Development, Virus

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Introduction

Dengue represents an infectious condition stemming from any of the four dengue virus (DENV) serotypes, denoted as DENVs 1 – 4 (Murphy & Whitehead, 2011). This disease is transmitted primarily by the female Aedes mosquito. It is prevalent in tropical and subtropical regions, posing a substantial risk to nearly one-third of the global populace (Guzman *et al*., 2016). Infection with DENV can lead to a spectrum of pathological conditions, varying from mild asymptomatic dengue fever (DF) to severe forms such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), which can prove fatal (Rothman, 2011). The proliferation of DENV worldwide has been notably spurred by rapid urbanisation, increased international travel, inadequate mosquito control measures and the effects of globalisation (Tchuandom *et al*., 2019).

Dengue has emerged as one of the most prevalent re-emerging mosquito-borne diseases globally (Whitehorn & Farrar, 2010). The incidence of dengue has escalated by thirtyfold over the past five decades (Guzman *et al*., 2010). Presently, dengue is endemic to 128 countries, predominantly affecting developing nations, thus posing a threat to approximately 3.97 billion individuals annually (Faruk *et al.,* 2022). Recent modelling suggests an estimated 390 million cases of dengue infections annually, with approximately 96 million cases manifesting apparent symptoms (Hossain *et al*., 2021). The Indian subcontinent is the focal point of dengue transmission, although cases are considerably underreported (Faruk *et al.,* 2022). Consequently, there is an urgent imperative to enhance serosurveillance to enable authorities to adequately prepare for potential outbreaks (Jayawickreme *et al*., 2021).

No specific authorised antiviral drugs for treating dengue (Tantawichien, 2012) exist. Management usually involves symptom relief and supportive care (Hershan, 2023). For uncomplicated cases of dengue fever, treatment typically includes bed rest, oral rehydration therapy and the administration of paracetamol as an antipyretic and analgesic (Hershan, 2023). Advancements in Vaccine Technologies: Recent innovations in vaccine technologies, including recombinant subunit vaccines, DNA vaccines and virus vector vaccines, offer promising alternatives to conventional methods. Evaluating these emerging technologies is essential to assess their potential for enhancing vaccine efficacy and safety profiles. Global and Regional Variability: Vaccine effectiveness can differ based on regional epidemiology, circulating virus strains and demographic factors. A comprehensive review that includes data from diverse geographic regions and populations can shed light on how these variables impact vaccine performance and guide the development of tailored vaccination strategies.

Cross-reactivity significantly impacts dengue pathogenesis and vaccine development, notably through Antibody-Dependent Enhancement (ADE) (Zhang & Chen, 2020). This complicates vaccine development due to the risk of severe disease in individuals exposed to different dengue serotypes following vaccination. A significant challenge of ADE involves cross-reactive antibodies exacerbating infections with different serotypes (Halstead, 2003; Rothman, 2011; Monath & Hombach, 2018; Watanabe & Halstead, 2016).

The World Health Organization (WHO) has emphasised the need for new vaccine candidates that elicit strong, serotype-specific immunity without increasing disease risk (WHO, 2020). Reviewing current and emerging dengue vaccines in the context of WHO recommendations can help pinpoint research gaps and highlight opportunities for future development (WHO, 2020). This review aims to synthesise current knowledge on dengue vaccine candidates, address the challenges and obstacles in their development and offer recommendations for future research. By doing so, it seeks to contribute to advancing more effective and safer dengue vaccines, ultimately enhancing global public health efforts to combat this pervasive disease.

Method

The review is based on secondary sources, with data gathered from articles published between 2010 and 2024. This review employed a search strategy focused exclusively on the most recent vaccine and vaccine development updates against dengue. Data were gathered through an online literature search across databases such as Google Scholar, PubMed, NIH (National Library of Medicine) and Web of Science. The primary search terms included "vaccines against dengue," "vaccine candidates," "dengue treatment updates," "dengue case reports," and "dengue treatment options", among other related terms. Papers were selected for inclusion in this review after a comprehensive analysis of publication dates, abstracts, titles and journals. This review primarily examines recent studies highlighting significant advancements and ongoing research in dengue vaccine development (Khetarpal & Khanna, 2016; Malabadi, Chalannavar & Meti, 2017; Massey & Yulia, 2019).

Results

Approaches to Developing Vaccines for Dengue

Despite the challenges in creating an optimal dengue vaccine, significant advancements have been made in the past decade, with several vaccine candidates progressing to clinical trials in dengue-endemic and non-endemic regions (Deng *et al*., 2020) (Fig. 1).

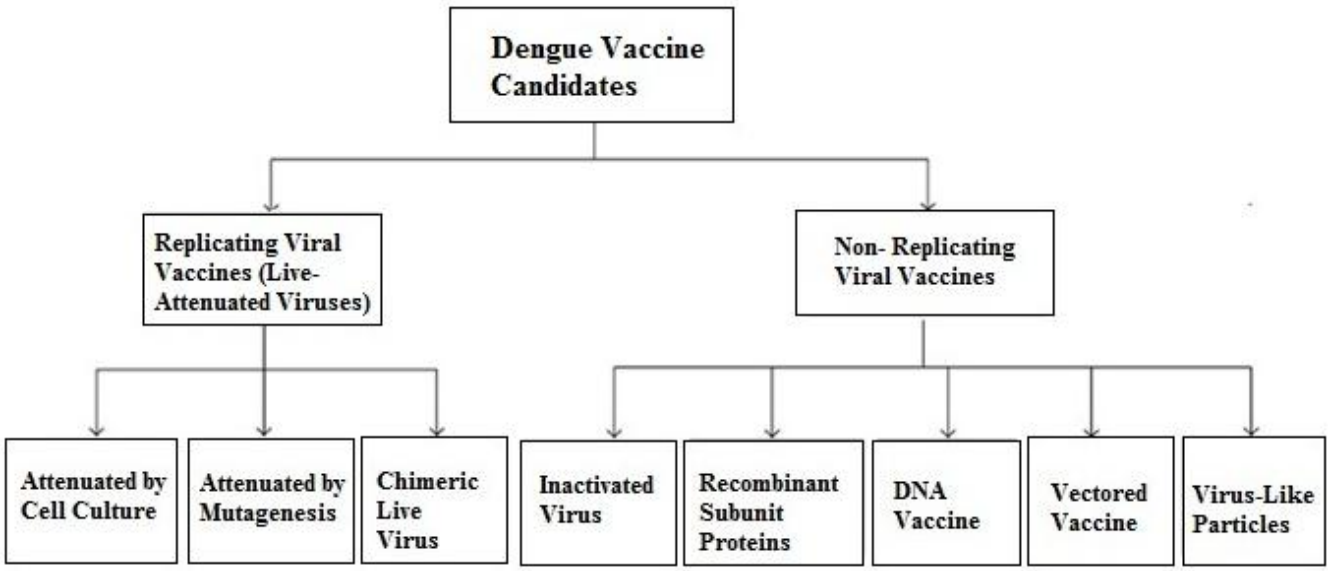

Figure 1: Categorisation of potential dengue vaccine options (Khetarpal & Khanna, 2016)

Replicating Viral Vaccines

Replicating viral vaccines, including live-attenuated viruses (LAV), are designed to reduce the pathogen's virulence while maintaining its viability (Whitehead *et al*., 2007). These vaccines are created through serial passage in cell lines, targeted mutagenesis, and the development of chimeric vaccine viruses (Guy *et al*., 2010). The advantages of replicating viral vaccines include their ability to induce robust, long-lasting, broad-spectrum immunity and lower production costs (Khetarpal & Khanna, 2016). However, they face challenges such as difficulties in achieving adequate attenuation, genetic instability, potential reversion to virulence, and complications in multicomponent vaccines (Guy *et al*., 2010).

Live-attenuated vaccines Produced through Cell Culture Passage

The development of live-attenuated vaccines (LAV) via serial passage in cell lines originated at Mahidol University in Bangkok, Thailand (Bhamarapravati & Sutee, 2000). Initially, a tetravalent formulation attenuating all four DENV serotypes was attempted. However, this vaccine failed to evoke

a balanced immune response despite adjustments to viral concentrations (Innis, 2010). Moreover, an increase in adverse reactions, particularly associated with the DENV-3 vaccine strain, was noted, leading to the cessation of further development of these LAV strains (Bauer *et al*., 2015).

Another LAV, developed at the Walter Reed Army Institute of Research (WRAIR) in Maryland, USA, utilises cell culture passage for attenuation. This vaccine is currently undergoing clinical trials in collaboration with GlaxoSmithKline (GSK) (Durbin *et al*., 2011). By passaging all four DENV serotypes in primary dog kidney (PDK) cells, a tetravalent formulation named F17/Pre was created. However, during phase II clinical trials, F17/Pre resulted in DENV-4 vaccine-induced viremia (Bauer *et al*., 2015; Durbin *et al*., 2011). Subsequently, in a separate phase II trial involving 86 healthy flavivirus-naive adults in the USA, F17/Pre DENVs were received and passaged in foetus rhesus lung cells to obtain seed viruses with higher purity (Bauer *et al*., 2015; Durbin *et al*., 2011). The resultant formulations, F17 and F19, exhibited similar safety profiles and immunogenicity after two doses of LAV (Durbin *et al*., 2011). Notably, while F19 was intended to contain 10-fold less DENV-4, its actual reduction was only fourfold at the time of vaccine release (Bauer *et al*., 2015; Durbin *et al*., 2011). Although both F17 and F19 demonstrated comparable neutralisation titers against DENV-4, the incidence of DENV-4 vaccine-induced viremia decreased potentially due to rederivation and passage, which further attenuated the DENV-4 strain (Bauer *et al*., 2015). However, a similar phase II trial conducted in healthy individuals in Puerto Rico revealed discrepancies in the in vitro potency of DENV-4 in F19, indicating issues with the storage stability of the DENV-4 strain (Bauer *et al*., 2015; Durbin *et al*., 2011).

Live-attenuated vaccines Developed through Targeted Mutagenesis

The Laboratory of Infectious Disease initially pioneered this approach at the National Institute of Allergy and Infectious Disease (NIAID) and the National Institutes of Health (NIH) in Maryland, USA (Whitehead *et al*., 2003). The NIH has forged nonexclusive licensing agreements with manufacturers in Brazil (Instituto Butantan), Vietnam (Vabiotech) and India (Serum Institute of India and Panacea Biotech) for its advancement (Bhamarapravati & Sutee, 2000). The vaccine comprises a blend of four DENV strains attenuated through site-directed mutagenesis, involving the deletion of 30 nucleotides in the 3′UTR (Murphy & Whitehead, 2011). Designated as DEN1Δ30 and DEN4Δ30, the attenuated strains correspond to DENV-1 and DENV-4, respectively (Whitehead, 2015).

Using DEN4Δ30 as a backbone, DENV-2 and DENV-3 attenuated strains were generated by replacing their structural prM and E genes with the respective serotypes (Bauer *et al*., 2015; Whitehead *et al*., 2007). However, chimerisation led to over-attenuating rDEN2/4Δ30 and rDEN3/4Δ30 strains (Whitehead, 2015). A variation in the DENV-3 component resulted in selecting the rDEN3Δ30/31 strain, where an additional 31 nucleotides were deleted from rDEN3Δ30 (Whitehead *et al*., 2007). In the tetravalent formulation TV005, the infectivity of the DENV-2 component was enhanced by utilising the DENV-2 attenuated rDEN2/4Δ30 strain at a 10-fold higher dose compared to other components (Kirkpatrick *et al*., 2016). Conversely, TV003 contains 103 pfu of each of the four components (Whitehead, 2015). Notably, a single dose of TV005 has demonstrated efficacy in sterilising immunity. The TV003/TV005 is currently undergoing evaluation in a human challenge model to facilitate a more rigorous assessment of their protective efficacy (Kirkpatrick *et al*., 2016; Bauer *et al*., 2015). The TV003 has exhibited protective effects against challenges with the DENV-2 attenuated rDEN2Δ30 strain (Whitehead *et al*., 2007). Similar evaluations of protective efficacy are underway for TV005, and human challenge experiments for DENV-3 are being planned (Gubler, 2006). Phase III trials of this vaccine candidate have commenced in Brazil (Murphy & Whitehead, 2011).

Chimeric Live Viruses

Chimeric dengue vaccines have been developed through two main approaches: (i) using another attenuated flavivirus and (ii) utilising an attenuated DENV strain (intertypic chimera) (Durbin *et al*., 2011). One notable example of the former is the chimeric yellow fever-dengue (CYD) vaccine being developed by Sanofi Pasteur and marketed as "Dengvaxia" (Guy *et al*., 2010). In this vaccine, the prM and E genes of the attenuated yellow fever LAV strain 17D have been substituted with the corresponding genes from DENV (Guy *et al*., 2010).

The rationale behind this design stems from the understanding that the humoral response against structural proteins of the DENV confers protective immunity during natural infection; hence, these chimeras are expected to elicit a protective immune response in recipients (Bhamarapravati & Sutee, 2000). Extensively evaluated in clinical trials, a tetravalent mixture of the four chimeric viruses has recently been approved in several countries, including Mexico, Brazil, El Salvador, and the Philippines (Murphy & Whitehead, 2011).

Another example is DENVax, developed by Inviragen Inc. in Fort Collins, CO, USA. Using a backbone of DENV-2 strain attenuated by 53 passages in PDK cells, the prM and E genes of this strain were swapped with corresponding genes from DENV-1, DENV-3, and DENV-4 (Coller *et al*., 2011). As the mutations in the attenuated strain were primarily in the non-structural proteins, it was utilised as such for the DENV-2 component in the tetravalent formulation (Kirkpatrick *et al*., 2016).

These chimeric viruses exhibited a temperature-sensitive phenotype, reduced replication in mosquito cell lines, genetic stability, and lacked neurovirulence in suckling mice (Bauer *et al*., 2015). Three tetravalent formulations with variable doses of each component were tested in non-human primates, revealing that DENV-2 dominated and its replicative potential decreased with increasing doses of DENV-3 and DENV-4 components (Whitehead *et al*., 2007). Despite significantly lower neutralising antibody titers against DENV-4, macaques were protected against the DENV-4 challenge (Whitehead, 2015).

A phase I clinical trial in healthy subjects in Colombia confirmed the safety and immunogenicity of DENVax, highlighting the lowest neutralising antibody titers against DENV-4 and the highest against DENV-2, consistent with findings from non-human primate studies (Gubler, 2006). Further efficacy insights will emerge from phase II clinical trial evaluation, with phase III evaluation of this vaccine now underway (Coller *et al*., 2011).

Non-replicating Viral Vaccines

These vaccine candidates lack the ability to replicate, offering the advantage of providing immunity without the risk of causing infection. Various strategies are employed to develop this type of vaccine, including DNA vaccines, subunit proteins, VLPs and others (Wu *et al*., 2022). The advantages of these vaccines are that they typically exhibit reduced reactogenicity, making them safer for individuals with compromised immune systems (Akter *et al*., 2024). They also tend to induce a balanced immune response, especially in tetravalent formulations. The disadvantage is that non-replicating viral vaccines often generate a less broad, potent, and long-lasting immune response than replicating viral vaccines (Akter *et al*., 2024). This may increase the risk of antibody-dependent enhancement (ADE). Additionally, adjuvants are often required to enhance their immunogenicity (Thomas & Endy, 2011; Wiersma *et al*., 2017).

Purified Inactivated Virus (PIV)

The Walter Reed Army Institute of Research (WRAIR) in Maryland, USA, developed a monovalent dengue vaccine using formalin treatment to inactivate the virus. Studies in mice and Rhesus macaques confirmed its safety and ability to induce an immune response (Coller *et al*., 2011; Sun *et al*., 2013). Despite not being prone to viral interference or reverting to a pathogenic form, these vaccines have limitations due to structural changes caused by formalin treatment and their inability to replicate (Khetarpal & Khanna, 2016; Raina, 2020). However, they have been investigated as priming vaccines in combination with live-attenuated vaccines (LAV) in a prime-boost immunisation strategy, showing complete protection in macaques (Khetarpal & Khanna, 2016). Phase I trials assessing the safety of different doses of the DENV-1 component have been completed in the USA among flavivirus-naive individuals (Thomas, 2015). Additionally, two Phase I trials are underway, evaluating a tetravalent mixture of the four PIVs (TPIV) in combination with various adjuvants in healthy adults in the USA and Puerto Rico (Meyer *et al*., 2020). Another Phase I study is recruiting participants in the USA to evaluate TPIV in prime-boost vaccination with a tetravalent LAV developed by WRAIR and GlaxoSmithKline (GSK) (Zhang *et al*., 2019; Chen *et al*., 2021).

Recombinant Subunit Vaccine

The expression of dengue virus envelope (E) proteins through recombinant methods in yeast and insect systems has been explored for their potential as vaccines, with studies conducted in mice and monkeys. These investigations primarily targeted the 80% amino-terminal portion of the DENV E protein, known as the ectodomain (Costin *et al*., 2013). By deleting 20% of the protein at the C-terminal, which includes the transmembrane region, extracellular secretion and easy purification were achieved while preserving antigenicity (Costin *et al*., 2013). The resulting recombinant 80% E proteins, referred to as r80E, for all four DENV serotypes, are being manufactured by Hawaii Biotech Inc., HI, USA and Merck and Co., NJ, USA (Costin *et al*., 2013; Manoff *et al*., 2019). Monovalent DEN2 80E was assessed in mice with various adjuvants, demonstrating ISCOMATRIX™ as the most immunogenic, while alum showed poor immunogenicity (Manoff *et al*., 2019). Subsequent evaluation in macaques with a tetravalent formulation containing ISCOMATRIX showed weaker titers against DENV-4 (Manoff *et al*., 2019). Strategies to enhance the immunogenicity of DEN4 80E, such as using its dimeric form or administering a double dose, were explored in macaques, resulting in improved neutralising titers against DENV-4, albeit lower than those against other serotypes (Costin *et al*., 2013; Manoff *et al*., 2019).

Consequently, a tetravalent mixture of the four r80Es was tested in both flavivirus-naive and dengue-primed macaques, revealing a more balanced immune response against all four serotypes with a 0-, 1-, and 6-month immunisation schedule compared to a 0-, 1-, and 2-month schedule (Coller *et al*., 2011; Innis *et al*., 2018). Additionally, the safety of two doses (10 μg and 50 μg) of DEN1 80E/alum was confirmed in flavivirus-naive adults. However, it induced modest neutralising titers for DENV-1, which waned substantially after 26 weeks (Madhun *et al*., 2019). A Phase I study has been completed assessing the safety and immunogenicity of a tetravalent formulation with and without adjuvants (alum and ISCOMATRIX) in healthy adults (Gromowski *et al*., 2019).

Meanwhile, recombinant antigens based on DENV envelope domain III (EDIII) have been generated by various research groups using different expression hosts such as *Escherichia coli* (*E. coli*) and yeast (Hoffmann *et al*., 2017). These antigens, either expressed independently or fused with carriers like maltose-binding protein and the *Neisseria meningitidis* p64k protein, have demonstrated the ability to elicit immune responses against DENV in both mice and non-human primates (Hoffmann *et al*., 2017; Zhang *et al*., 2020). Currently, these vaccine candidates are in the preclinical phase of development.

Dengue DNA Vaccine

The dengue DNA vaccine operates through a plasmid vector containing the gene(s) responsible for encoding an antigen. Upon vaccination, this vector is taken up by antigen-presenting cells (APCs). Once inside the cell, the plasmid instructs the production of the antigen, which then binds to MHC class I molecules and is presented on the cell surface, triggering a protective cytotoxic immune response (Sarkar *et al*., 2018). The Naval Medical Research Centre (NMRC), USA, has developed a candidate DENV-1 DNA vaccine (D1ME100) by introducing the prM and E genes of the DENV-1 serotype into a plasmid vector (Carter *et al*., 2017). This vaccine underwent extensive evaluation in mice and macaques without adjuvants before progressing to phase 1 trials in healthy adults. Although the DENV-1 DNA vaccine was well-tolerated, it exhibited low levels of neutralising antibody titers and responders (Sarkar *et al*., 2018). To address this limitation, a lipid-based adjuvant called Vaxfectin was investigated to enhance its immunogenicity (Gromowski *et al*., 2020).

A tetravalent dengue DNA vaccine (TVDV) was subsequently assessed for immunogenicity both with and without Vaxfectin in macaques. Results indicated that the addition of Vaxfectin led to higher and more sustained neutralisation titers, with average titers against DENV-1, DENV-2, DENV-3 and DENV-4 approximately 200, 270, 170 and 70, respectively, a month after the final boost (López *et al*., 2021). Six months later, while titers against DENV-2 and DENV-3 decreased, those against DENV-1 and DENV-4 experienced marginal increases. Conversely, in the group without Vaxfectin, only titers against DENV-2 were detectable six months post-final boost (López *et al*., 2021). Furthermore, Vaxfectin enhanced protection against viremia upon DENV-2 challenge (Carter *et al*., 2017).

Replication-Defective Virus Vectored Vaccines

In this approach, a virus is used as a carrier to deliver antigenic genes that can induce a neutralising antibody response. Various viral vectors, such as adenovirus vectors, Venezuelan equine encephalitis virus vectors, and attenuated measles virus, have been employed for this purpose (Meyer *et al*., 2020). One example of this method is the cAdVax vaccine, which consists of bivalent constructs expressing prM and E proteins from two dengue serotypes each (DENV-1 and DENV-3 in one construct and DENV-2 and DENV-4 in another). Research conducted in non-human primates demonstrated that this vaccine generated neutralising antibodies specific to the respective dengue serotypes (Zhang *et al*., 2019). As a result, a tetravalent formulation known as cAdVax-DenTV was developed by combining these bivalent constructs, showing effectiveness against all dengue serotypes in Rhesus macaques (Chen *et al*., 2021).

Virus-Like Particle (VLP) Vaccines

The co-expression of prM and E proteins of DENVs in different hosts has demonstrated their ability to co-assemble into VLPs. This suggests the feasibility of developing a vaccine comprising physical mixtures of four monovalent DENV VLPs to create a tetravalent formulation (Sun *et al*., 2022). Regarding the utilisation of VLPs for vaccine development, the yeast system may be more advantageous due to its potential for higher yields and its ability to glycosylate the antigens. Recent research indicates that DENV E ectodomain expressed in yeast can form VLPs even without prM (Sharma *et al*., 2023). Another strategy involves presenting DENV EDIII on VLPs generated by hepatitis B virus core antigens (Liu *et al*., 2024).

Dengue Vaccine Candidates

Of the listed candidates, only Dengvaxia and TAK-003 have progressed to late-stage clinical trials and received conditional approvals. The dengue vaccine candidates that have advanced to clinical trials are

listed in Table 1. Among the vaccine candidates listed, only Dengvaxia and TAK-003 have advanced to late-stage clinical trials and received conditional approvals.

Table 1: Dengue Vaccine Candidates Currently in Different Phases of Clinical Trials (Khetarpal & Khanna, 2016; Raina, 2020)

Vaccine Candidate	Developer	Vaccine Type	Phase of Clinical Trials	Key Findings and Notes
Chimeric yellow virus dengue vaccine - Dengvaxia (CYD-TDV)	Sanofi Pasteur	Tetravalent chimeric live-attenuated virus	Approved (Mexico, Brazil, El Salvador, Philippines)	Licensed in several countries. Moderate efficacy, with concerns about safety and efficacy in children <9 years and dengue-naïve individuals.
TAK-003 (Intertypic chimera-DENVax) - Qdenga®	Takeda Pharmaceutical	Tetravalent live-attenuated virus	Recently completed Phase III (Approved by the European Medicines Agency (EMA) for individuals aged four years and older)	Demonstrated high efficacy and safety profile in phase II trials. Current phase III trials have recently been completed.
TV003/TV005 (Targeted mutagenesis-based LAV-TetraVax-DV)	National Institutes of Health (NIH)	Tetravalent live-attenuated virus	Phase II	Phase I and II trials have shown the vaccine to be safe and immunogenic. Phase III trials are underway to evaluate efficacy.
V180	Merck & Co.	Recombinant subunit	Phase I/II	Early trials show promise in safety and immunogenicity. Further trials are needed to assess efficacy.
DENVax	Inviragen (now Takeda)	Tetravalent live-attenuated virus	Phase II	Positive results in early trials. Currently in phase II to evaluate broader efficacy and safety.
TDENV-PIV	GlaxoSmith Kline	Purified inactivated virus	Phase I	Initial trials indicate a good safety profile. Further phases are needed to assess immunogenicity and protection.
DNA Vaccine	US Naval Medical Research Center	DNA-based vaccine	Phase I	Shows safety and immune response in preclinical studies.

				Phase I trials are ongoing.
V180	Merck	Recombinant subunit	Phase I	Early trials suggest good immunogenicity. More research is needed to confirm efficacy.

Currently, a minimum of seven dengue virus (DENV) vaccines have been developed, employing various platforms such as live attenuated viruses, inactivated viruses, chimeric live attenuated viruses, DNA, and recombinant proteins. These vaccines are either in various stages of clinical trials or are still in the preclinical investigation phase (Tables 2 & 3) (Guzman *et al*., 2016).

Table 2: 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)	(Lin *et al*., 2021)
TDENV-PIV (WRAIR/FioCruz/GSK)	Inactivated adjuvanted	Phase I (No recent reports)	(Fernandez *et al*., 2015)
D1ME100/TVDV (NMRC)	DNA vaccine	Phase I (No recent updates)	(Danko *et al*., 2018)
V180 (DEN-80E) (Merck/NIAD)	Recombinant (subunit)	Phase I (Published 2019)	(Man of *et al*., 2019)
DENV-1-LVHC	Live-attenuated	Phase I (Published 2021)	Clinicaltrials.gov (Endy *et al*., 2021)

Table 3: Dengue vaccines that have reached phase III or have been licensed

Vaccine	Manufacturer	Platform	Efficacy	Comments	References
Licensed					
CYT-TDV Dengvaxia®	Sanofi Pasteur	YFV A30 backbone	25-59%	Increases hospitalisations in seronegative vaccinees	(da Silveira, Tura & Santos 2019)
Phase III					
TAK-003 (DENVax) Qdenga®	Takeda/Inviragen	Attenuated DENV-2 backbone for the four serotypes	73.3-85.3%	Well tolerated in adolescents and children	(Biswal *et al*., 2019)

LATV TV003/TV005	NIAD/Butantan/ Merck	DENV-1,3,4 A30 and rDENV2/4 A30	Not yet released	Single dose	(Whitehead, 2016)

Dengvaxia Dengue Vaccine

Initially developed in the early 2000s by the National Institutes of Health (NIH), the University of St. Louis and Acambis Inc., and later licensed by Sanofi Pasteur, this vaccine utilises ChimeriVax™ technology. The chimeric yellow fever dengue vaccine (CYD-TDV) has recently gained approval under the name Dengvaxia® in several countries, including Mexico, Brazil, El Salvador and the Philippines (Guy *et al*., 2019). In Mexico, Dengvaxia received regulatory approval from the Mexican government in December 2015 (Thomas, 2015). It was approved for use in various other countries, including Brazil, the Philippines, and Indonesia (Thomas, 2023). Dengvaxia has received regulatory approvals in these countries for administration in adults aged 9-45 years due to the increased risk of hospitalisation in children under nine years old and its poor efficacy in dengue-naïve individuals (Guy *et al*., 2019). This vaccine is based on the yellow fever virus (YFV) 17D strain, where the pre-membrane (prM) and envelope (E) genes of YFV were replaced by those of the four dengue virus (DENV) serotypes. This tetravalent formulation, known as ChimeriVax™ DENV 1-4, demonstrated reduced neurovirulence in preclinical models, including mice and non-human primates, and induced neutralising solid antibody responses against all four DENV serotypes (Guy *et al*., 2015). Despite promising preclinical results, including high protection rates in cynomolgus macaques, the vaccine's performance in subsequent clinical trials it revealed varying levels of efficacy, particularly among different age groups and serostatus, leading to its restricted use based on prior DENV exposure (Guzman *et al*., 2016; Hadinegoro *et al*., 2015).

TV003/TV005

TV003/TV005 are live attenuated dengue vaccines developed by the National Institute of Allergy and Infectious Diseases (NIAID) (Whitehead *et al*., 2017). The attenuation strategy involved deleting 30 nucleotides from the 3' untranslated region (UTR) of the DENV-4 genome, which was then used as a backbone for generating attenuated chimeric viruses for the other three serotypes. Preclinical studies in rhesus macaques demonstrated that these vaccines elicited neutralising solid antibody responses and provided robust protection against all four DENV serotypes (Durbin *et al*., 2016; Whitehead *et al*., 2017). TV003/TV005 is undergoing Phase III trials, with promising results indicating its efficacy in preventing dengue (Possas *et al*., 2024).

TAK-003 (DENVax) Qdenga®

TAK-003, also known as DENVax, recently gained approval under the brand name Qdenga® by the European Medicines Agency (EMA) for individuals aged four years and older, following national recommendations for dengue prevention (Angelin *et al*., 2023). Therefore, Qdenga® is another promising dengue vaccine candidate from a DENV-2 strain isolated in Thailand. This strain underwent serial passage in primary dog kidney cells, leading to the development of the attenuated DENV-2 PDK-53-V strain (Saez-Llorens *et al*., 2018). TAK-003 uses this DENV-2 backbone to create chimeric vaccines for the other three serotypes by replacing the prM and E genes. Preclinical studies showed that TAK-003 induced robust immune responses and provided protection against all four DENV serotypes in cynomolgus macaques (Tricou *et al*., 2020). The vaccine demonstrated a good safety profile and is undergoing further evaluation in clinical trials to determine its efficacy across diverse populations (Biswal *et al*., 2019).

Clinical Evaluation of Dengue Vaccines

Presently, Dengvaxia® is the only dengue vaccine that has received official approval (Whitehead, 2016). However, phase III clinical trials for other vaccines, such as TV-003/TV-005 and TAK-003, are currently underway and show promising results (da Silveira, Tura, & Santos, 2019). Despite these advancements, the efficacy and safety of dengue vaccines are influenced by factors such as the age and serostatus of the vaccine recipients (Lin *et al*., 2021; Fernandez *et al*., 2015). Safety concerns primarily stem from phase III paediatric trials, where only Dengvaxia® and TAK-003 (DENVax) have been tested in children, leading to mixed results (Biswal *et al*., 2019). These findings underscore the need for continued research to address safety and efficacy issues across different age groups and serostatus (Fig. 2) (Endy *et al*., 2021; Danko *et al*., 2018).

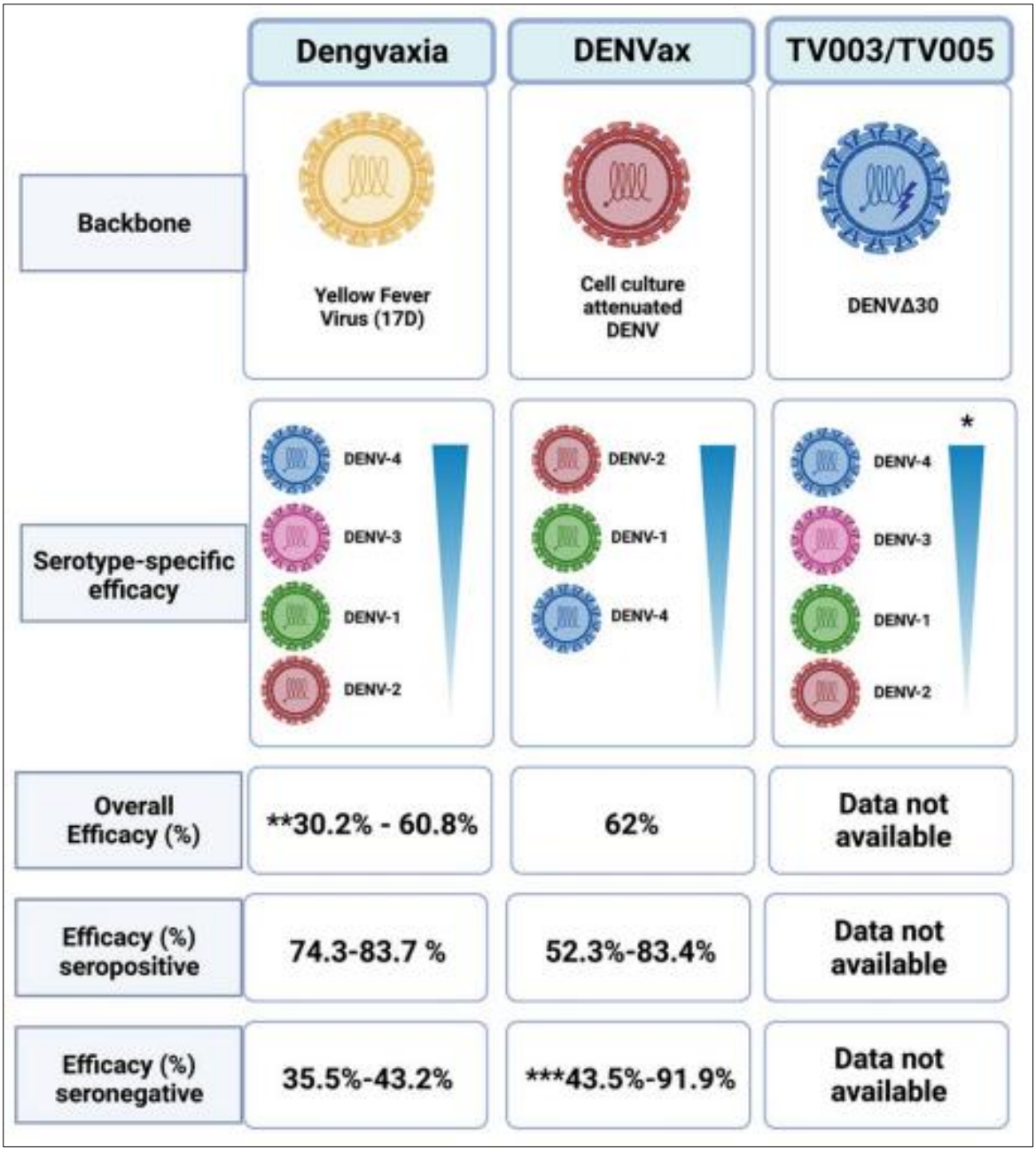

Figure 2: Summary of Efficacy Trials for Anti-Dengue Virus (DENV) Vaccines in Children across Latin America and Asia. Phase III trials for Dengvaxia and DENVax have yielded mixed results. TV003/TV005 is currently undergoing Phase III trials, with promising results indicating its efficacy in preventing dengue. The figure highlights that the efficacy range for TV003/TV005 includes results from

phase IIb trials conducted in Thailand and notes that DENVax demonstrated efficacy only against DENV-1 and DENV-2 in individuals who were seronegative prior to vaccination (Capeding *et al*., 2014; Villar *et al*., 2015)

Discussion

Dengvaxia and TAK-003 (Qdenga®) are currently licensed dengue vaccines. Qdenga® has been approved for use in some countries (Angelin *et al*., 2023). Clinical trials of Qdenga® have shown high efficacy in protecting against virologically confirmed dengue and severe dengue among children aged 4 to 16 years living in endemic regions (Biswal *et al*., 2019). However, only serological data are available for individuals aged 16 to 60 years, with no efficacy data reported for those over 60 years (Sáez-Llorens *et al*., 2018). The role of Qdenga® as a travel vaccine remains unclear. This review presents the studies supporting Qdenga®'s approval and offers recommendations for travellers, as issued by the Swedish Society for Infectious Diseases Physicians (WHO, 2022). Dengvaxia®, early phase I and II trials conducted across various countries, including the USA, the Philippines, Australia, Mexico, Vietnam, Singapore and India, evaluated its safety, immunogenicity and reactogenicity. These trials included both adults and children aged 2 to 18 years (Whitehead, 2016).

The vaccine was generally well-tolerated in adults, administered in a three-dose regimen (0, 3-4 and 12 months). Common adverse effects were mild to moderate and included injection site pain, headache, malaise and low-grade fever, with no severe adverse events reported (Lin *et al*., 2021). The vaccine did not induce pro-inflammatory cytokines but did produce serotype-specific T-helper responses (Fernandez *et al*., 2015). In individuals with pre-existing dengue immunity, Dengvaxia® generated broader neutralising antibody responses and enhanced specific CD8+ responses against dengue non-structural proteins, particularly NS3 (Danko *et al*., 2018).

Phase III trials predominantly focused on paediatric populations, revealing that vaccine efficacy varied by age and dengue serotype. In a study involving 35,000 children aged 2 to 16 years, efficacy ranged from 65% in those older than nine years to 45% in younger children. Notably, children under nine years showed an increased risk of severe dengue following natural exposure, especially if they were dengue-naïve prior to vaccination (Endy *et al*., 2021; Biswal *et al*., 2019).

Further trials in Latin America and Asia demonstrated varying efficacy across serotypes and age groups. In a phase IIb trial in Thailand, Dengvaxia® showed higher efficacy against DENV-4 (100%) and DENV-3 (75.3%) compared to DENV-1 (55.6%) and DENV-2 (9.2%) (da Silveira *et al*., 2019). More extensive phase III trials in Latin America showed similar patterns, with higher efficacy against DENV-3 (74.0%) and DENV-4 (77.0%) compared to DENV-1 (50.3%) and DENV-2 (42.3%) (Whitehead, 2016).

A recent phase III trial revealed that efficacy for symptomatic dengue was higher in children older than nine years (65.6%) than those under nine years (44.6%). The risk of hospitalisation for dengue was also higher in younger children (relative risk of 1.58) compared to older children (relative risk of 0.5) (Endy *et al*., 2021). Additional studies indicate that seronegative children under nine years had a higher incidence of hospitalisation due to dengue compared to seropositive children (Biswal *et al*., 2019).

Mathematical models based on data from over 800,000 vaccinated children in the Philippines predict significant hospitalisations due to severe dengue, both in seronegative and seropositive individuals, highlighting the need for enhanced phase IV surveillance. These findings stress the importance of

ongoing evaluation to better assess Dengvaxia®'s effectiveness and safety in dengue-endemic regions before further vaccine deployment (da Silveira *et al*., 2019).

The TV003/TV005 are tetravalent dengue vaccine candidates developed from early phase I trials that evaluated monovalent formulations of the DENVax vaccines. These initial trials assessed safety, replication capacity and the potential to transmit vaccine viruses to *Toxorhynchites splendens* mosquitoes (Vega *et al*., 2020). Following these trials, six monovalent DENVax vaccine candidates were advanced to phase II trials as five tetravalent formulations (TV-001 to TV-005). TV003 and TV005, which include the rDEN1D30, rDEN2/4D30, rDEN3D30/31 and rDEN4D30 components but differ in the amount of rDEN2/4D30 (10^3 PFUs/mL for TV003 and 10^4 PFUs/mL for TV005) were selected for further evaluation due to their ability to induce balanced neutralising antibody responses against all four dengue virus serotypes (Guzman *et al*., 2017). In randomised placebo-controlled trials, TV003 and TV005 were well-tolerated, with low-grade rash reported as the most common adverse event. TV003 induced balanced neutralising antibody responses with seroconversion rates ranging from 64% for DENV-2 to 100% for DENV-4. TV005 showed improved seroconversion against DENV-2 (84%) (Lindenbach *et al*., 2018).

A challenge model using the rDEN2D30 virus demonstrated that all participants who had not seroconverted to DENV-2 with TV003 developed protective responses and neutralising antibodies against DENV-2, indicating the vaccine's effectiveness (Valdes *et al*., 2021). These promising results led to the licensing of TV003, branded as Butantan-DV, by the Butantan Institute in Brazil. A phase II clinical trial involving 300 participants (155 DENV-naïve and 145 DENV-exposed) demonstrated that Butantan-DV was safe and well tolerated. It induced robust neutralising antibody responses with seroconversion frequencies above 78% for all four DENV serotypes and significant T-CD8 responses (Guzman *et al*., 2017).

A phase III trial of Butantan-DV is underway in Brazil, with 16,944 participants across three age groups (2-6 years, 7-17 years and 18-59 years). Results from this trial are anticipated (Lindenbach *et al*., 2018). The Qdenga® vaccine, formerly known as DENVax, has undergone extensive clinical evaluation (Vega *et al*., 2020). Initial phase I trials conducted by Takeda in Rionegro, Colombia, assessed the safety and immunogenicity of a two-dose regimen administered either intradermally (ID) or intramuscularly (IM) to DENV-naïve adults aged 18 to 45 years. The vaccine demonstrated a good safety profile with transient local reactions and mild systemic adverse events. However, antibody responses to DENV-3 and DENV-4 were less robust than other serotypes (Vega *et al*., 2020).

The vaccine formulation was adjusted to address the suboptimal responses to DENV-4, increasing the amount of the DENV-4 component. Subsequent phase 1b studies in the USA involving 140 DENV-naïve adults showed improved seroconversion rates across all serotypes, with notable increases in DENV-4 responses (Guzman *et al*., 2017). In a phase II trial conducted across Puerto Rico, Colombia, Singapore, and Thailand, the updated formulation (TDV) demonstrated variable efficacy. The vaccine elicited lower neutralising antibody responses against DENV-4 but remained effective overall (Lindenbach *et al*., 2018).

A large-scale phase III trial involving 20,071 children aged 4 to 16 years in dengue-endemic regions of Latin America and Asia is underway (Valdes *et al*., 2021). Initial results at 12 months showed high efficacy against DENV-2 (97.7%), moderate efficacy against DENV-1 (73.7%) and DENV-3 (62.6%), with inconclusive results for DENV-4. The overall vaccine efficacy was similar between seronegative (74.9%) and seropositive (82.2%) participants, with high efficacy against dengue-related hospitalisation

(95.4% in seronegative and 94.4% in seropositive individuals) (Valdes *et al*., 2021). However, 18-month follow-up data revealed a decreased overall efficacy of 76.1% in seropositive and 66.2% in seronegative individuals (Vega *et al*., 2020). Efficacy varied significantly by serotype, with rates ranging from 95.1% against DENV-2 to 48.9% against DENV-3. The efficacy against dengue requiring hospitalisation was 90.4%, but was notably lower in seronegative children aged 4 to 5 years (59.1%) compared to seropositive children (51.6%) (Vega *et al*., 2020).

Recent cumulative data over three years indicated a reduction in overall vaccine efficacy to 62% against virologically confirmed dengue (VCD) and 83.6% against hospitalisation (Guzman *et al*., 2017). For baseline seropositive, efficacy varied significantly by serotype, with the highest efficacy observed against DENV-2 (83.4%) and the lowest against DENV-3 (52.3%). For seronegative, efficacy was noted only for DENV-1 and DENV-2 (43.5% and 91.9%, respectively) (Guzman *et al*., 2017). These variations underscore the need for continued phase IV surveillance to assess long-term efficacy and cross-protection, particularly given the observed declines in vaccine effectiveness over time (Lindenbach *et al*., 2018).

Conclusion

In summary, the current landscape of dengue vaccination demonstrates significant advancements and ongoing challenges. Dengvaxia® and Qdenga® (TAK-003) have shown efficacy in protecting against dengue, with Qdenga® displaying high efficacy in children aged 4 to 16 years living in endemic regions (Angelin *et al*., 2023). However, data on its efficacy for individuals over 16 years old and its role as a travel vaccine remain limited. Dengvaxia® has shown variable efficacy across different age groups and dengue serotypes, with concerns about reduced efficacy and increased risk in younger, dengue-naïve children (Endy *et al*., 2021; Biswal *et al*., 2019).

Recent trials indicate that while Qdenga® and Dengvaxia® offer substantial protection, there is a need for continuous surveillance to address the variability in vaccine efficacy and safety, particularly in diverse demographic groups and long-term settings (Vega *et al*., 2020; Guzman *et al*., 2017). The ongoing evaluation of new vaccine candidates like TV003/TV005 shows promise, though further data is required to confirm their long-term effectiveness and safety (Lindenbach *et al*., 2018; Valdes *et al*., 2021). Overall, these findings underscore the importance of continued research and phase IV surveillance to understand the long-term impact of dengue vaccines better and to optimise their use in both endemic regions and for travellers (Guzman *et al*., 2017; da Silveira *et al*., 2019). The progress made thus far is encouraging, but addressing the remaining gaps in knowledge will be essential for improving public health outcomes related to dengue fever.

Future perspectives

The landscape of dengue vaccination is poised for significant advancements, yet several challenges must be addressed to optimise public health outcomes. As vaccine candidates like Qdenga® and Dengvaxia® continue to be evaluated, there is a clear need for comprehensive long-term studies to fully understand their efficacy and safety profiles across diverse populations. Future research should focus on improving vaccine formulations to enhance protection, especially against less responsive serotypes such as DENV-4 and addressing concerns related to reduced efficacy in younger, dengue-naïve children. Additionally, expanding studies to include older age groups and travellers from non-endemic regions will be crucial for understanding the broader applicability of these vaccines.

Mathematical models and real-world data will be vital in predicting and managing potential outcomes, including severe dengue hospitalisations. Integrating phase IV surveillance will be essential for

monitoring long-term vaccine performance and ensuring continued safety. Researchers and public health officials must also prioritise the development of new vaccine candidates, such as TV003/TV005, which show promise in generating balanced immune responses against all four dengue virus serotypes. As these new vaccines advance through clinical trials, they will need to be evaluated for their effectiveness in diverse settings and populations.

Moreover, global collaboration will be key in addressing dengue fever, particularly in endemic regions where vaccine coverage and accessibility remain challenges. Efforts should also be directed towards enhancing community awareness and vaccine acceptance to achieve higher immunisation rates. Addressing these future research directions and public health strategies can move closer to achieving comprehensive dengue control and prevention, ultimately reducing the burden of this debilitating disease worldwide.

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