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Development of dengue vaccines

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REVIEW

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ABSTRACT

Background

Dengue virus (DENV) causes up to 390 million infections yearly, of which 96 million are clinically manifested. Approximately 500,000 people with severe dengue require hospitalization each year and there are at least 25,000 deaths among children from Asian and Latin American countries. DENV is endemic in more than 100 countries. Chemical and biological controls have been implemented in targeting *Aedes aegypti* and *Aedes albopictus* mosquitoes, but these control practices failed to stem the dengue transmission. As a result, dengue vaccine has become a potential option recommended by WHO to be implemented in dengue endemic regions. Currently, several vaccine candidates are being evaluated in clinical studies. Amongst the vaccine candidates, live attenuated vaccines (LAV) are the furthest along the development pipeline. The most advanced vaccine, CYD-TDV (Dengvaxia) has been licensed in 19 countries. Several other live attenuated vaccines, as well as DNA, subunit, inactivated virus, viral-vectored and subunit-based vaccines, are under development and evaluation in preclinical or clinical studies. Each of the live-attenuated vaccine candidates targets on molecular determinants of virulence in DENV, with the emphasis on attenuating the DENV and inducing a balanced tetravalent immune response against all the four dengue serotypes.

Aims

This review presents several different vaccine approaches and their construction strategies, providing an insight into the development of future dengue vaccines such as live attenuated vaccines, DNA vaccines, sub-unit protein vaccines and viral vectored vaccines.

Methods

Recent development status of dengue vaccine candidates was reviewed based on the published data and an online registry for clinical trials (ClinicalTrials.gov) which is run by the U.S. National Library of Medicine, National Institutes of Health.

Results

Increasing burden of dengue necessitates the development of a safe and efficacious tetravalent dengue vaccine. Various vaccine strategies are being developed for disease prevention, each has its own strengths and limitations. Dengvaxia is a licensed dengue vaccine in 19 countries but it has been suspended in the Philippines in December 2017 due to its potential risks in children <9 years of age and seronegative vaccinees. TDV was able to elicit neutralizing antibodies as well as cross protective T cell responses against all four dengue serotypes and protected mice and nonhuman primates against challenge with wild type DENV. Seroconversion was achieved in both seronegative and seropositive adults and children <1.5 years of age with a single dose. TV003/TV005 was able to elicit multifunctional T cell response in addition to the humoral response. Seroconversion in 90 per cent of seronegative adults was observed with a single dose of TV005. However, eliciting a balanced immune response against all the four dengue serotypes remained the major impediment.

Conclusion

Dengvaxia has been launched in 11 countries but it has been withdrawn in the Philippines due to adverse effects in young children. Experimental vaccines such as TDV and TV003/TV005 are live attenuated vaccines which are currently in phase III clinical trials. Continued field trials will further our understanding of immune correlates of protection or risk.

Key Words
Dengue virus, dengue vaccine development, clinical trials, preclinical studies, seroconversion, efficacy

What this review adds:

1. What is known about this subject?
Dengue infection is a serious public health problem. Unmet medical need requires an understanding of the current vaccine and development of future dengue vaccines.

2. What new information is offered in this review?
This review addresses the strengths and limitations of live attenuated vaccines and other experimental vaccine candidates such as DNA, viral-vectored and subunit vaccines in development.

3. What are the implications for research, policy, or practice?
Further research should unveil more effective dengue vaccines and this requires a better understanding of the immune correlates of infection.

Introduction

Dengue is a mosquito-borne disease of growing public health concern. An estimated 390 million infections occur annually, of which 96 million were manifested clinically.[1] With more than 40 per cent of the world's population now residing in dengue endemic countries, development of an effective dengue vaccine becomes a high priority. Dengue virus (DENV), being the causative agent of dengue infection, is classified under the genus *Flavivirus* within the family *Flaviviridae.* DENV has a single-stranded positive sense RNA genome, which is approximately 10.7kb in size. The open reading frame consisting of three structural genes (C, prM and E) and seven non-structural genes (NS), is flanked by 5' and 3' untranslated regions. There are four antigenically distinct DENV serotypes (DENV1-4) which lead to asymptomatic dengue or symptomatic dengue, ranging from dengue fever to dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Primary infection with a particular dengue serotype confers life-long immunity for that serotype while the immunity to the other dengue serotypes is short-lived. It has been proposed that secondary infection with a different serotype potentially increases the risk of developing DHF and DSS due to antibody enhancement effect (ADE) and reduced T-cell immune responses.[2] To control the dengue transmission, various combinations of chemical and biological controls had been implemented in targeting the dengue vectors, *Aedes aegypti* and *Aedes albopictus* mosquitoes and its breeding sites.[3] However, these control practices were less effective to control the dengue transmission. Nevertheless, biological control such as *Wolbachia*-infected mosquitoes appears to be a promising strategy. Collectively, this approach and vaccination are recommended by WHO to effectively prevent the dengue disease in dengue endemic regions.[4] Several vaccine candidates are being developed to prevent dengue disease (Table 1). Each of the vaccine strategies has its own strengths and limitations.

Vaccine Strategies

The development of dengue vaccine began as early as 1929. However, it has been hampered first by insufficient knowledge of the pathogenesis in dengue and later by the necessity to induce a balanced immune response against all the four dengue serotypes. In 1945, Sabin and Schlesinger reported the first successful dengue vaccine, whereby the 'Hawaiian' strain of DENV was attenuated in mouse brain by serial passages.[5] The attenuated strain was able to confer partial protection against DENV. However, its production was eventually terminated when DENV could be propagated in cell cultures, a theoretically safer production. Despite formidable challenges in developing dengue vaccines, remarkable progression towards clinical efficacy trials has been made in recent years. There have been several dengue vaccine candidates under development including live attenuated vaccines, inactivated vaccines, DNA vaccines, subunit vaccines and viral-vectored vaccines. Amongst these vaccine candidates, live attenuated vaccine (LAV) candidates are the furthest in the development, with the first ever vaccine showing some efficacy in phase III trials, licensed in December 2015.[6]

1) Live Attenuated Vaccines

Currently, there is no effective vaccine against all the four serotypes of DENV. The only licensed dengue vaccine is the chimeric YF17D-DENV tetravalent dengue vaccine, CYD-TVD, which was developed by Sanofi Pasteur. The CYD-TDV vaccine is licensed in 19 countries,[6] and sold as Dengvaxia. Dengvaxia is a tetravalent chimeric vaccine comprising the yellow fever attenuated 17D virus strain encoding the pre-membrane (prM) and envelope (E) structural genes of DENV1-4, respectively (Figure 1).[7]

Dengvaxia is recommended for use in individuals from 9–45 years old in dengue endemic regions. Efficacy of the vaccine has been evaluated in phase III clinical trials conducted in Asia (CYD14) and Latin America (CYD15), and a phase IIb trial (CYD23) in Thailand. However, based on the results of the phase III clinical studies, the overall efficacy of Dengvaxia was only 56.5 per cent, 60.8 per cent and 30.2

per cent in Asia, Latin America and Thailand, respectively.[8] Dengvaxia confers higher protection against DENV-3 (73.6 per cent) and DENV-4 (83.2 per cent), but lower protection against the two widely circulating dengue serotypes, DENV-1 (58.4 per cent) and DENV-2 (47.1 per cent) in vaccinees above nine years of age (pooled efficacy of two clinical trials conducted in Asia and Latin America, respectively). Dengvaxia showed substantially low efficacy against all serotypes (44.6 per cent) amongst those aged below nine years (Table 2). The vaccine efficacy in individuals above 45 years old has not been evaluated and remains unknown. The efficacy of Dengvaxia was found to be also dependent on the dengue serostatus of the vaccinees. The immunogenicity of Dengvaxia was lower (35.5 per cent) in individuals who were initially seronegative.[9]

Dengvaxia has been shown to reduce dengue hospitalizations of vaccinees, with an efficacy of 65.6 per cent among individuals aged above nine years and 44.6 per cent among individuals aged below nine years (Table 2). However, an updated efficacy study revealed that children younger than nine years old (particularly among those aged two to five years old) were associated with an elevated risk of hospitalization when they were infected naturally three years after the vaccination.[10] In fact, vaccinated children showed a 7.45-fold higher rate of hospitalizations when compared to non-vaccinated children.[11] It is possible that Dengvaxia elicited only transient antibody-mediated immune responses in the younger children. The subsequent waning antibody levels would predispose the vaccinated children to subsequent infections. The imbalance in immune responses elicited by the individual components of Dengvaxia might also explain the increased risk of hospitalization observed in year three follow-up of vaccinees younger than nine years old and seronegative individuals. Therefore, the World Health Organization (WHO) recommended the limited use of Dengvaxia to individuals aged from 9–45 years old in dengue endemic regions with high transmission intensity where the seroprevalence is more than 70 per cent at nine years of age.[4] However, following a declaration that Dengvaxia could pose health risks in people not previously infected, Dengvaxia was suspended in Philippines. Several children died from various complications allegedly attributed to Dengvaxia were reported.

Thus, Dengvaxia has some benefits but some serious caveats for its use. The number of serious adverse events in vaccinated individuals (62 per cent) was much higher than that of the control group (38 per cent).[12] Protection narrowly targeted only a certain age group (9-45 years old) of vaccinees and its low efficacy (35.5 per cent) for seronegative individuals, highlights the need for a more effective vaccine. Ultimately a dengue vaccine should be capable of conferring protection in seronegative individuals of all ages including the young children, irrespective of previous exposure to dengue or infecting serotype and thus pursuing alternative vaccine strategies is essential.

Collaborating with the Centers for Disease Control and Prevention (CDC), TDV (DENVax) is a tetravalent recombinant live attenuated dengue vaccine, comprising a live attenuated DENV-2 virus strain and three chimeric viruses expressing the prM and E protein genes for DENV-1, 3 and 4 in the DENV-2 genome (Figure 1).[13] TDV vaccine strain has three key attenuation mutations in the viral genome: 5'UTR-57 C-to-T; NS1-53 Gly-to-Asp and NS3-250 Glu-to-Val.

TDV was well tolerated in children and adults aged 1.5-45 years regardless of previous exposure to dengue infection. It was also found to be capable of inducing strong neutralizing antibodies and cross-reactive T-cell mediated responses to all four dengue serotypes. It showed favourable safety profile and mild adverse events in vaccine recipients, with varied seroconversion rates for the four different serotypes. However, it showed imbalanced seroconversion rates for the four different dengue serotypes. The seroconversion rate to DENV-4 (33 per cent) was much lower than the other three serotypes (>80 per cent) in a phase Ib randomised study.[14] The hurdle in achieving a balanced immune response from a tetravalent vaccine might be due to the viral interference between the four chimeric virus strains or the differences in virus replication kinetics. Despite this, the vaccine continues to be developed through ongoing phase II and phase III clinical trials. The clinical trials have been initiated in Brazil (ClinicalTrials.gov Identifier: NCT02406729). The recruitment process was initiated in 2016 and it is still ongoing.

For a live attenuated vaccine containing known molecular determinants of attenuation, it is of utmost importance to evaluate the genetic stability of the attenuation determinants. When the TDV vaccine strain was serially passaged ten times in Vero cells, there was no evidence of reversion at the NS1-53 Asp and NS3-250 Val loci. However, reversion was observed at the 5'UTR-57-U locus in viral strains from 10 of the 18 passages tested. The level of reversion increased with the passage number, reaching >50 per cent by passage 9, and >80 per cent of reversion levels by passage 10.[15] These data indicated the instability of the mutation at 5'UTR-57-U, rendering its propensity to revert

at low passage number. Due to the limited number of mutations, the probability of reversion to the wild type genotype is of concern as RNA viruses have a mutability rate of 10^{-4} to 10^{-6} mutations per nucleotide copied in a replication cycle.[16] Thus, there is a need to identify multiple attenuation determinant(s) such as in the E gene, non-structural genes and 3' UTR to generate a multiply-mutated vaccine strain with greater genetic stability.

The third vaccine candidate, TV003/TV005 developed by the National Institute of Allergy and Infectious Diseases (USA), utilized structural gene chimerization and deletion of 30 nucleotides in the 3' untranslated region (3' UTR) as the attenuation strategy. Chimeric DENV-2 component was created by substituting the prM and E genes of DENV-2 serotype into the DENV-4Δ30 backbone (Figure 1).[8] Amongst the different tetravalent formulations evaluated in clinical trials, both TV003 and TV005 showed promising results and were chosen for further evaluation and development. The TV005 has one log higher PFU in the dosage of rDEN2/4Δ30 than that of TV003 vaccine candidate. The overall tetravalent neutralizing antibody response induced by a single dose of TV005 (90 per cent) was also higher than TV003 (74 per cent).[17] The seroconversion to DENV-2 was observed to improve to 97 per cent in those who received TV005.

The protective efficacy of a single dose of TV003 was evaluated in ten healthy flavivirus-naïve individuals (NCT 01931176). No vaccinees developed dengue fever. However, 80 per cent of the vaccinees developed diffuse macular-papular rash and 40 per cent developed neutropenia. Vaccinees were then challenged with the rDEN2Δ30 vaccine six months after vaccination to evaluate the protective efficacy of TV003 (NCT02021968). TV003 was able to confer complete protection against viremia, rash and neutropenia. It will be further evaluated in dengue-endemic areas with a larger population. A clinical trial evaluating the protective efficacy of TV005 has been completed in the United States (NCT02317900).

The single dose vaccine candidate TV003 showed considerable promise, supporting its use in targeting populations unprimed by previous DENV infections. However, a primed population might respond even more robustly. A major caveat here is that the high tetravalent neutralizing antibody response may not equate with protection in the field, as shown by Dengvaxia in phase IIb trial.[18] Concern was also raised in a combined TV003 and TV005 trial, where the tetravalent seroconversion rate in African American volunteers were significantly lower (57 per cent) than the non-African American volunteers (86 per cent). This implies that the protective efficacy of the vaccine candidate might vary in different races.[19]

2) DNA Vaccines

A DNA vaccine comprises a plasmid vector harbouring the gene(s) of interest encoding for antigen(s). The intracellular expression and processing associated with DNA vaccines enable the antigen presentation to the host immune system in the context of MHC class I system, facilitating the stimulation of specific cytotoxic effector T cells. A series of cellular interactions are then initiated to activate and proliferate T cells and B cells, stimulating both humoral and cellular immune responses. However, there is a risk of nucleic acid integration into the host genomic DNA, potentially inactivating tumour suppressor genes or activate oncogenes, and leading to neoplastic transformation of host cells.[20] However, this risk appears to be a low probability event, occurring 3,000 times lower than rate of spontaneous mutation in mammalian cells. Another concern is that the host might produce anti-DNA antibodies when foreign DNA is detected, leading to autoimmune diseases.

The DNA vaccine approach has the advantage of potentially MHC-I presentation, as in a natural infection, and subsequent T-cell responses. Since E protein contains the immunological epitopes for inducing neutralizing antibodies, E proteins become the major target molecules for developing dengue DNA vaccine. Another principal molecular candidate for developing DNA vaccine is the prM which acts as a chaperonin that is essential for the proper folding of the E protein during viral maturation.[21]

In designing a monovalent DNA vaccine, the prM and E genes of DENV-1 serotype were cloned into a plasmid vector (pVR1012) under the control of the human cytomegalovirus promoter, generating the recombinant DNA vaccine, D_1ME^{100}. To enhance the immunogenicity of D_1ME^{100}, it was formulated with a lipid-based adjuvant, Vaxfectin and higher neutralizing antibody titres were demonstrated. The neutralizing antibody titres to the Vaxfectin-adjuvanted vaccine were at least 2–2.5 fold higher than the non-adjuvanted vaccine. However, the average neutralization titres against each of the dengue serotypes were observed to vary greatly, being the highest against DENV-2 and lowest against DENV-4.[22] With the same adjuvant, Danko and colleagues conducted dose escalation Phase I clinical trial of a tetravalent dengue DNA vaccine (TVDV). No anti-DENV IgG antibody responses were detected in vaccinees administered with unadjuvanted TVDV. Minimal vaccine-induced neutralizing antibody

responses were detected by screening enzyme-linked immunosorbent assay (ELISA) among the vaccinees administered with low dose (1mg) adjuvanted TVDV or high dose (2mg) adjuvanted TVDV. Despite the poor neutralizing antibody responses, high dose adjuvanted TVDV elicited anti-dengue IFNγ T-cell responses in 79 per cent of vaccinees.[23] It was concluded that the rate of response appeared to be a dose-dependent trend.

In a recent study, Zheng and colleagues (2017) inoculated mice with a new dengue DNA vaccine candidate ($pVAX_1$-D_1ME) to evaluate its protective efficacy against DENV-1 and DENV-2. The monovalent DNA vaccine which expressed the prM and E proteins of DENV-1 induced both humoral and cellular responses after three doses (100μg each) of DNA vaccine delivered by intramuscular (IM) injection.[24] The $pVAX_1$-D_1ME vaccine was able to confer protection to mice against lethal DENV-1 challenge. Similar result was observed for $pVAX_1$-D_2ME. The bivalent DNA vaccine candidate consisting of $pVAX_1$-D_1ME and $pVAX_1$-D_2ME was found to generate a balanced immunogenic response to both the dengue serotypes (DENV-1 and DENV-2) in BALB/c mice. Nevertheless, the end-point titres of neutralizing antibodies in the bivalent vaccine-immunized mice were lower when compared to those in the monovalent vaccine-immunized mice.[25] Since the vaccine comprises two recombinant $pVAX_1$ plasmids, there might be viral interference between the DENV-1 and DENV-2 candidates in the bivalent formulation. This finding should be an important consideration in further research on the development of tetravalent dengue DNA vaccine. The safety and protective efficacies of the vaccine should be also further evaluated in *in vivo* studies involving non-human primates before proceeding to clinical trials.

3) Viral-Vectored Vaccines

Viral-vectored vaccines are based mostly on non-flavivirus backbones. In this approach, a virus serves as a vector to carry antigenic genes that are capable to elicit both humoral and cellular immune responses. Viral-vectored vaccines are capable of enhancing immunogenicity without an adjuvant and induce cytotoxic T lymphocyte response, leading to the elimination of virus-infected cells. Despite their efficacies, viral-vectored vaccines present some unavoidable problems. Previous exposure to the vector virus induces production of neutralization antibodies against the vector virus as shown in the use of cAdVax-DenTV which was later discontinued.[26] Vaccination in such individuals might be ineffective as the pre-existing neutralization antibodies against the recombinant vector virus may eliminate them before they are able to induce an immune response.

Venezuelan equine encephalitis (VEE) virus replicon particles (VRPs) have been demonstrated to induce both innate and adaptive immune responses in mice. White and colleagues constructed E85-VRP by cloning a C-terminally truncated soluble form of E, representing 85 per cent of the E protein into the VEE replicon plasmid, pVKE.[27] It was demonstrated that E85-VRP was capable of inducing serotype-specific antibodies, predominantly targeting EDIII which has been identified as a major target of murine monoclonal antibodies. The E85-VRP elicited robust neutralizing antibody responses following two doses of a tetravalent vaccine administered to macaques 6 weeks apart induced 100 per cent seroconversion against all four dengue serotypes. The neutralizing antibodies protected all the rhesus macaques from all the four dengue serotypes.[27] To be selected as the priming vaccine in a prime-boost strategy with live-attenuated vaccine as the booster, it is important to conduct longer-term immunogenicity studies to address the duration of the neutralizing antibody responses induced by the vaccines after the final booster.[27]

The second viral-vectored dengue vaccine candidate (MV-DEN), developed by Themis Bioscience and Institut Pasteur, was constructed on the basis of expression of a single tetravalent DENV antigen construct from a live-attenuated measles virus vaccine vector. MV-DEN construct harboured the EDIII domains of all four DENV serotypes and the DENV-1 M protein ectodomain (ectoM). The protective efficacy of the vaccine against all four DENV serotypes is currently being evaluated in macaques.[28]

4) Subunit Protein Vaccines

Recombinant subunit protein vaccines are composed of defined protein antigens derived from the pathogen of interest. Recombinant subunit vaccines have advantages and disadvantages over the other types of vaccines and have been successfully used for vaccination against HBV. The strategy for constructing dengue subunit protein vaccines were based on the successful HBV vaccines by expressing viral antigens in expression system. The principal disadvantage of subunit vaccine is the necessity of large amounts of purified antigens in creating the vaccine. Similar to purified inactivated vaccines, subunit protein vaccines require adjuvants to enhance their immunogenicity. Requirement of purified antigens and adjuvants increase the cost of the vaccine. The potential of contamination with pathogenic expression vectors that are used to produce the dengue antigens tend to lead to lower antigen yield.[29] The primary advantages of a subunit vaccine include minimal reactogenicity and induction of balanced antibody responses, conferring complete protection against the four

dengue serotypes.[29] This was demonstrated by Clements and colleagues[30] who showed that their truncated recombinant DENV E protein subunits (80E), conferred protection against viral challenge in both the mouse and monkey challenge models. The E subunit truncated at amino acid 395 of the E protein was cloned into pMttΔXho vector. Expression of this gene construct resulted in the production of a truncated E protein subunit comprising 80 per cent of the N-terminal of the E protein (DEN-80E).

Low doses of the tetravalent antigens administered with ISCOMATRIX® adjuvants were highly immunogenic and capable of inducing protective immune responses in both murine and non-human primate models. However, when mice were administered with higher doses of the tetravalent antigen formulation, viremia breakthrough was observed in these animals. Although neutralizing antibodies demonstrated *in vitro* neutralization capacity, they did not have the sufficient neutralizing activity, avidity or specificity to control the viral infection in the animal models. This could be due to a shift in the antibody avidity or subclass, which have impacted the capability of the vaccine to neutralize the viruses.[30] The complexities of neutralization highlight the multifactorial nature of neutralizing antibodies and the molecular mechanisms by which they confer protection against dengue viral infection.

Govindarajan and colleagues[31] evaluated the immunogenicity and efficacy of low, medium and high doses of the ISCOMATRIX™ adjuvanted tetravalent DEN-80E subunits in non-human primates. Antibody responses were found to be balanced against all four dengue serotypes, except that the DEN4-80E responses remained low at all dosage levels. In terms of protective challenge, the vaccinated animals were protected upon challenge with 10^5 PFU of each of the four DENV serotypes for 12 months.[31]

A small proof-of-concept phase I clinical trial (NCT00936429) was then initiated to evaluate the safety, tolerability and immunogenicity of a monovalent DEN1-80E adjuvanted with 1.25mg Alhydrogel™ (HBV-001 D1).[32] The neutralizing antibody titters in majority of the subjects waned over time. Alternative formulations or vaccination schedules are required to achieve high titre and durable neutralizing antibody responses that could confer long term protection against DENV.

Conclusion

Although Dengvaxia has been licensed to be marketed in 19 countries, its efficacy against DENV-1 and DENV-2 which are predominant in Asia remained significantly low. Its limited efficacy based on the serostatus and age of the vaccinees, highlights the need to develop a universal dengue vaccine which confers a balanced immune response against all four dengue serotypes regardless of the serostatus and age of the individuals. TDV and TV003/TV005 are live attenuated vaccines which are currently in phase III clinical trials. An ideal live attenuated vaccine should show sufficient genetic stability. Larger pools of the human population from different ethnic groups in different geographical areas should be recruited in clinical trials to evaluate seroconversion across different ethnicities and of all age groups including seronegative subjects. The other vaccine strategies such as DNA vaccines, viral-vectored vaccines and subunit protein vaccines appeared to be potential vaccine candidates, but require more extensive evaluation in non-human primates before initiating clinical trials. Improving downstream processes and antigen formulations may yield a cost-effective and affordable second generation dengue vaccines, which are less dependent on cold chain storage and distribution.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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ETHICS COMMITTEE APPROVAL

Not applicable

Table 1: Development status of current dengue vaccine candidates

Vaccine approach	Candidate name/Identifier	Developer	Phase	Valency	References
Live attenuated vaccines	Chimeric yellow fever virus dengue vaccine CYD-TDV	Sanofi Pasteur	Licensed	Tetravalent	33
	Intertypic chimera DENVax	CDC-Inviragen/Takeda	Phase III	Tetravalent	34
	TV003/TV005	US National Institutes of Health and Butantan	Phase III	Tetravalent	8
DNA vaccines	DNA vaccine expressing prM and E protein	Naval Medical Research Centre (NMRC), USA	Phase I	Tetravalent	35
Inactivated vaccines	Purified inactivated vaccine (TDENV-PIV)	GSK/US WRAIR/Fiocruz	Phase I	Tetravalent	36,37
	TLAV-TPIV Heterologous prime-boost with LAV and tetravalent purified inactivated vaccine Adjuvant: Alum	WRAIR, USA	Phase I	Tetravalent	38
Viral-vectored vaccines	EDIII and DENV-1 ectoM expressed from live-attenuated measles virus vector	Themis Bioscience / Institute Pasteur	Preclinical (Mice)	Tetravalent	28
	E85 expressed from single-cycle VEE virus vector	Global Vaccines	Preclinical (Macaques)	Tetravalent	27
Subunit protein vaccines	Recombinant subunit vaccine-V180	Hawaii Biotech, Merck and Co.	Phase I	Tetravalent	32
	EDIII-p64k fusion proteins and EDIII-capsid fusion proteins Expression system: *Escherichia coli*	Pedro Kourí Tropical Medicine Institute (IPK) / Center for Genetic Engineering and Biotechnology (CIGB)	Preclinical (Monkeys)	Monovalent	39
	Bivalent 80E-STF2 fusion proteins Expression system: baculovirus/insect cells	VaxInnate	Preclinical (Mice and monkeys)	Tetravalent	40
	Consensus EDIII protein Expression system: *Escherichia coli*	Taiwanese National Health Research Institute (NHRI)	Preclinical (Monkeys)	Tetravalent	41
Virus-like particles	EDIII-HBsAg VLPs or ectoE-based VLPs Expression system: *Pichia pastoris*	The International Centre for Genetic Engineering and Biotechnology (ICGEB)	Preclinical (Macaques)	Tetravalent	42

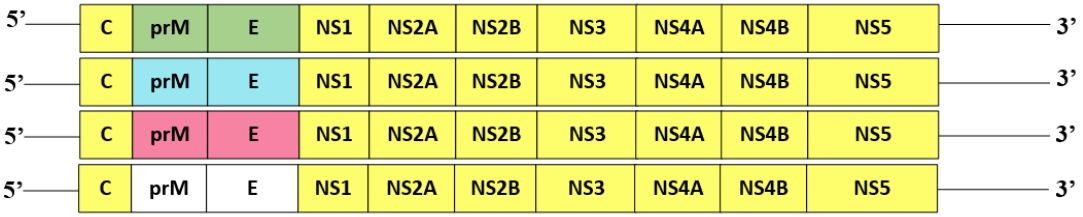
Table 2: The vaccine efficacy against dengue serotypes in the CYD14 (Asia) and CYD15 (Latin America) clinical trials

Serotype	Efficacy of CYD14 (Asia) (10275 participants)		Efficacy of CYD15 (Latin America) (20869 participants)	Pooled efficacy of CYD14 + CYD15 (9 years old and above)
	9 years old or above	Below 9 years old		
DENV-1	65.7% (46.6-78.2)	46.6% (25.7-61.5)	54.8% (40.2-65.9)	58.4% (47.7-66.9)
DENV-2	36.8% (-10.1-63.3)	33.6% (1.3-55.0)	50.2% (31.8-63.6)	47.1% (31.3-59.2)
DENV-3	69.5% (31.9-87.0)	62.1% (28.4-80.3)	74.2% (63.9-81.7)	73.6% (64.4-80.4)
DENV-4	87.9% (75.5-94.6)	51.7% (17.6-71.8)	80.9% (70.9-87.7)	83.2% (76.2-88.2)
All serotypes	67.8% (57.7-75.6)	44.6% (31.6-55.0)	64.7% (58.7-69.8)	65.6% (60.7-69.9)

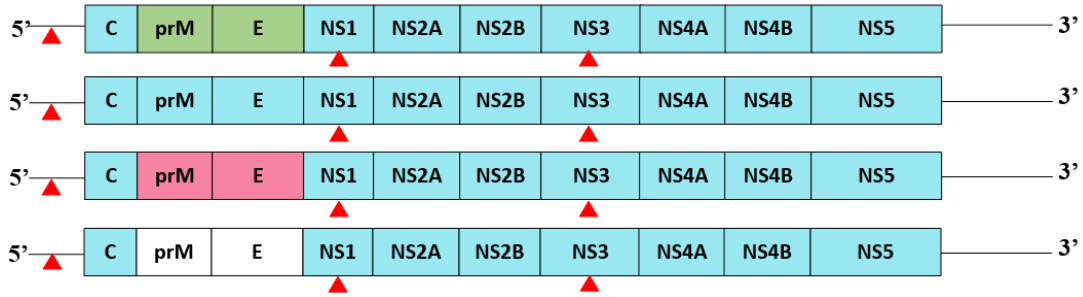
The efficacy of CYD14 (Asia) and CYD15 (Latin America) against dengue virus serotypes in individuals who aged 9 years and above and in individuals aged below 9 years. The Latin American trial involved only individuals above 9 years old (9-16 years old).[10] The numbers in brackets indicate the 95% confidence interval.

Figure 1: The genetic structure and design of the three live-attenuated dengue vaccines furthest in development

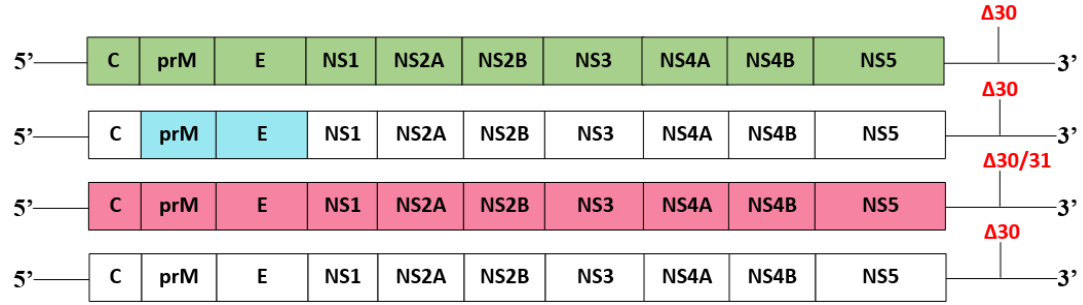
Sanofi Pasteur (Dengvaxia):

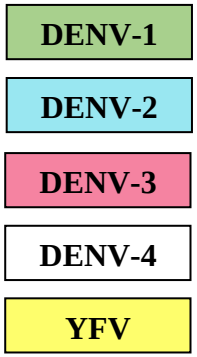


CDC-Inviragen/Takeda (TDV):



US National Institutes of Health (TV003/TV005):





The individual serotypes are designated by color (Green = DENV-1, Blue = DENV-2, Pink = DENV-3, White = DENV-4) and Yellow = Yellow Fever Virus (YFV)). Red Arrowheads represent the three key mutations that attenuated the virulence of the vaccine strains.