

Dengue vaccine: Global development update

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Abstract

The first licensed dengue vaccine, CYD-TDV (Dengvaxia[®]), has received regulatory approval in a number of countries. However, this vaccine has some limitations. Its efficacy against DENV2 was consistently lower than other serotypes. Protective efficacy also depended on prior dengue sero-status of the vaccinees. Lower efficacy was observed in children with < 9 years old and dengue-naïve individuals. More importantly, risk of hospitalization and severe dengue was increased in the youngest vaccine recipients (2-5 years) compared to controls. Thus, the quest of a better vaccine candidate continues. There are two live-attenuated vaccine candidates currently testing in phase III trial including DENVax, developed by US CDC and Inviragen (now licensed to Takeda) and TV003/TV005, constructed by US NIAID. In addition, there are several phase I–II as well as preclinical phase studies evaluating vaccines for safety and immunogenicity, this include other live-attenuated platform/strategy, purified-inactivated viruses formulated with adjuvants, DNA vaccine, subunit vaccine, viral vector and also heterologous prime/boost strategies. The major difficulties of dengue vaccine development are included the lack of the best animal model, various immune status of individual especially in endemic areas and clear cut off of protective immunity. Several research and development efforts are ongoing to find a better effective and accessible dengue vaccine for people needed.

Key words: Dengue vaccine, Live-attenuated virus, Inactivated virus, DNA vaccine, Subunit vaccine, Heterologous prime-boost

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Introduction

The worldwide distribution of dengue virus is expanding driving increases in morbidity, mortality and hospitalization. Previous studies estimate that there are approximately 58.4 million cases of symptomatic dengue infections annually leading to cause about 10,000 deaths per year.¹ In addition, the WHO reports that the incidence of dengue has increased 30 folds during the past five decades.² This rapid expansion

is the result of worldwide transportation and travel, increasing urbanization, as well as climate change which support the spreading of *Aedes* mosquitoes.³ There are four antigenic-related dengue serotypes (DENV1-DENV4) with approximately 65-70% amino acid homology. Infection with any serotypes of the virus can cause different clinical symptom range from a self-limiting febrile illness, dengue fever, dengue hemorrhagic fever to dengue shock syndrome.⁴

Theoretically, immune responses following infection provide long-term protection against homotypic re-infection and a short-term period of protection against heterotypic infection. Non-protective immune responses are also theorized to possibly exacerbate disease severity through antibody or T-cell dependent responses.⁵⁻⁷ Vector control strategies have had inconsistent success in impacting human infection and disease attack rates.⁸ Development and use of a safe and effective dengue vaccine could contribute greatly to reducing the global dengue burden. After several decades, the first dengue vaccine was licensed. A live attenuated chimeric yellow fever/dengue vaccine,⁹ named Dengvaxia[®] and developed by Sanofi Pasteur, has been approved in several countries such as Mexico, Thailand, Brazil, El Salvador and Costa Rica.¹⁰ In phase III trials the overall protective efficacy was moderate (56.7% and 60.8% in South East Asia and Latin America countries, respectively).^{11,12} Long-term safety follow up identified 2-5 year vaccine recipients had a higher relative risk of severe or

hospitalized dengue compared to controls in year 3 of the trial (two years after completion of the primary vaccination series).⁹ Two additional vaccine candidates (DENVax and TV003/TV005) are being tested in efficacy trials in both Asia and Latin America. Moreover, there are several vaccine candidates using various production platforms are on-going in early clinical and preclinical studies. This article reviews recent dengue vaccine development based on published literature searched from PubMed, abstracts presented at major related conferences, and clinical trials databases from the clinicaltrials.gov up to April 2018.

Current dengue vaccines in the developmental pipeline Vaccine candidates in clinical phases (see also in Table 1)

1. Live-attenuated virus

Among all of dengue vaccine candidates, tetravalent CYD vaccine (also known as Dengvaxia[®]) is the only vaccine that has recently been licensed for clinical use in some countries. The construct is based on a Yellow fever 17D vaccine virus backbone chimerized with prM and E proteins from DENV-1-4 replacing the YF prM and E.¹³ Efficacy was variable in Phase 3 trials in Asia and the Americas differing according to serotype, age, and dengue serostatus at baseline. There was a safety signal in 2-5 year olds in year 3 of the phase 3 trial in Asia.⁹ On-going evaluation of the booster (4th dose) is underway in Singapore (NCT02824198)¹⁴ and Latin America countries (NCT02623725)¹⁵ to evaluate the boosting effect in participants who previously completed 3 doses regimen. Recent study on safety and immunogenicity of a tetravalent CYD vaccine is being tested in HIV-positive adults (NCT02741128).¹⁶

Due to Dengvaxia[®] is not yet a perfect vaccine against dengue, further questing for a better vaccine is therefore on going. The US NIH has been developing their vaccine candidates named TV003 and TV005 (Figure 1). NIH vaccine is based on directed mutagenesis inducing attenuation without losing immunogenicity. Mutations at the 3' end have been induced in

DENV-1, -3, and -4 strains and a DENV-2/-4 chimera has been made using the DENV-4 backbone with DENV-2 prM and E replacing the DENV-4 prM and E.¹⁷ Balanced neutralizing antibody responses are matched with diverse but variable viremia measured following vaccination. Failure to measure viremia or neutralizing antibody boost following a second vaccination or with experimental human infection with a less attenuated DENV strain offers hope the vaccine has the potential for clinical benefit.¹⁸ A phase 2 in Taiwan (NCT03485144)¹⁹ and phase 3 trial are underway in Brazil by Butantan (NCT02406729).²⁰ The latest study of TV005 in 50-70 years old adults demonstrated that the vaccine is well tolerate and highly immunogenic in elderly population.²¹

The other promising tetravalent recombinant live-attenuated dengue vaccine is DENVax, originally developed by US-CDC in cooperation with Inviragen, now licensed to Takeda. It is live virus vaccine utilizing chimerization with DENV-2 PDK-53 as the backbone with DENV-2/-1, -2/-3, and -2/-4 chimeras created by replacing the DENV-2 prM and E genes with the respective genes from the other DENV serotypes.²² Trials demonstrated tetravalent neutralizing antibody profiles after vaccination with DENV-2 consistently having higher titers.^{23,24} A large phase 3 is underway and powered to demonstrate efficacy against any dengue of any severity caused by any dengue virus type. Secondary objectives will assess less common clinical endpoints such as hospitalization and severe disease (NCT02747927).²⁵ More importantly, the study that aim to assess the immunogenicity and safety of the concomitant and sequential administration of YF vaccine and DENVax is ongoing in healthy participants (NCT03342898).²⁶

Among these vaccine candidates, the major difference in vaccine components that is Dengvaxia[®] lack of dengue non-structural (NS) proteins. Two other live-attenuated vaccine candidates still incorporate DENV NS in their constructs, TV003/TV005 has whole attenuated virus for three of four serotypes while DENVax contains whole NS of attenuated

Table 1. Dengue vaccine candidates currently evaluate in clinical trials

Vaccine type	Vaccine name/Strategy	Developer	Clinical Trial Phase
<i>Attenuated chimera</i>	CYD, Dengvaxia[®]: Yellow fever 17D vaccine virus backbone chimerized with prM and E proteins from DENV-1-4	Sanofi-Pasteur	Licensed, Post licensed evaluation is on-going
	TV003/TV005: Attenuated by deletion of 30 nucleotides from 3' UTR of DENV-1, DENV-3 DENV-4, and a chimeric DENV-2/DENV-4	US NIH	Phase III
	DENVax: Use attenuated DENV-2 PDK-53 as the backbone and replace with prM and E of other serotypes (DENV-2/-1, -2/-3, and -2/-4 chimeras)	US CDC/Inviragen/ Takeda	Phase III
<i>Inactivated virus</i>	Purified formalin-inactivated virus (PIV) formulated with adjuvants	WRAIR/GSK	Phase I
<i>DNA vaccine</i>	Monovalent DENV-1 prME delivered by needle-free biojector Tetravalent prM/E formulated with Vaxfectin	US NMRC	Phase I
<i>Subunit vaccine</i>	V180: 80% of N-terminal E protein produced in insect cell formulated with ISOCOMATRIX and alhydrogel	Hawaii Biotech Inc. and Merck	Phase I
<i>Heterologous prime/boost</i>	TLAV-prime/PIV-boost and vice versa	US Army Medical Research and Materiel Command	Phase I

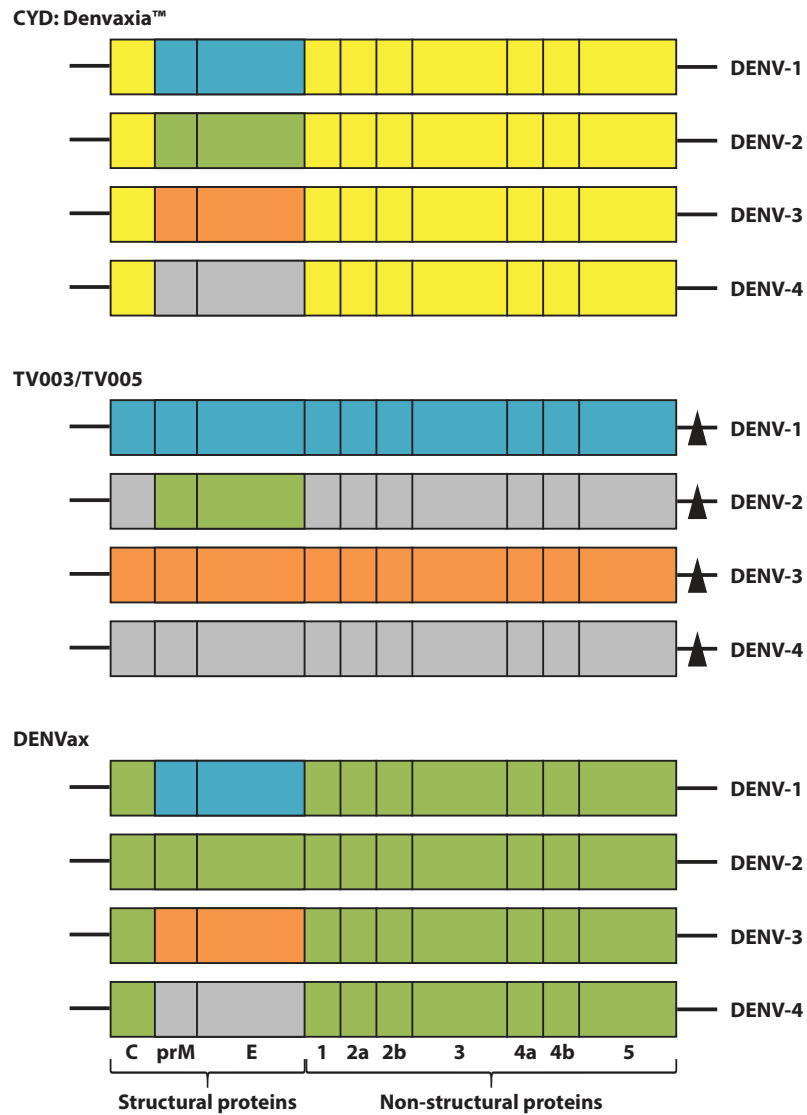


Figure 1. Schematic picture for comparison of developmental strategies of three live-attenuated dengue vaccines (CYD or Denvaxia™, TV003/TV005 and DENVax). Yellow = yellow fever 17D virus, blue = DENV-1, green = DENV-2, red = DENV-3, grey = DENV-4, triangle represents mutations at 3'UTR.

DENV-2 (figure 1). This may attribute to the vaccine immunogenicity as well as protective efficacy. CD8+ T cell epitopes mainly reside in NS3 and NS5. In addition, CD4+ T cells probably serve the function of maintaining B- and T-cell memory for anamnestic antibodies response during repeated natural DENV infection.²⁷

2. Inactivated virus

The major advantage of inactivated virus is its safety profile. Moreover, it could avoid viral interference between live viruses in tetravalent formulation. However, the development of inactivated dengue virus is hindered by a low immunogenicity. Currently, there are few numbers of purified formalin-inactivated virus (PIV) entered the clinical trials. DENV-1 PIV, developed by WRAIR, adjuvanted with aluminum hydroxide has been evaluated in Phase I. The results demonstrated that the vaccine was safe and immunogenic when measure the antibody responses by ELISA and neutralization tests in a small number of volunteers.²⁸ Tetravalent formulation of PIV

was further studied in Phase I to evaluate the optimal dose and adjuvants (alum vs AS01_E vs AS03_B). All of the formulations were safe, and showed balance antibody responses. Interestingly, the strong anamnestic responses was detected after received a booster dose in the second year after primary vaccination.²⁹ This finding demonstrated the adjuvant could strengthen the efficacy of inactivated viral vaccine to vaccine meet the requirement of long-term protection.

3. DNA vaccine

Progress with DNA vaccines go slower than other approaches mainly due to low-immunogenicity of DNA vaccine itself. For example, monovalent DENV-1 DNA vaccine (D1ME100) developed by the US NMRC had evaluated in phase 1 clinical trial. However, low anti-dengue neutralizing antibody was detected in only minor proportion of vaccinated individuals.³⁰ As a result, an approach that formulated the plasmid DNA with adjuvant, Vaxfectin® (cationic lipid:neutral lipid combination) was attempted and showed promising

results in NHPs.³¹ Phase 1 study of this tetravalent DNA vaccine formulated with Vaxfectin® has been completed (NCT01502358).³² Another strategy to improve DNA vaccine immunogenicity could be use efficient delivery technique such as electroporation. Our study demonstrated that tetravalent dengue DNA delivered by intramuscular electroporation could induced moderate/high neutralizing antibody against all four dengue serotypes both in mice³³ and NHPs (unpublished data). Moreover, DNA vaccine might be used in combination with other vaccine platform in prime/boost strategy to maximize vaccines immunogenicity and protective efficacy (discussed below). (Figure 2 and Table 2)

4. Subunit vaccine

Subunit DENV protein vaccine is mainly focused on recombinant E protein by using various expression strategies including *E.coli*, yeast and insect cells. Example of candidate that has been evaluated in non-human primate were performed by Hawaii Biotech Inc and Merck, 80% of amino-terminal E protein was produced in insect cell, *Drosophila* S2 expression system.³⁴ Proteins were purified and formulated with adjuvants ISCOMATRIX and alhydrogel. This vaccine (called V180) was evaluated in phase 1 clinical trial with dose escalation and formulated with adjuvants (NCT01477580).³⁵

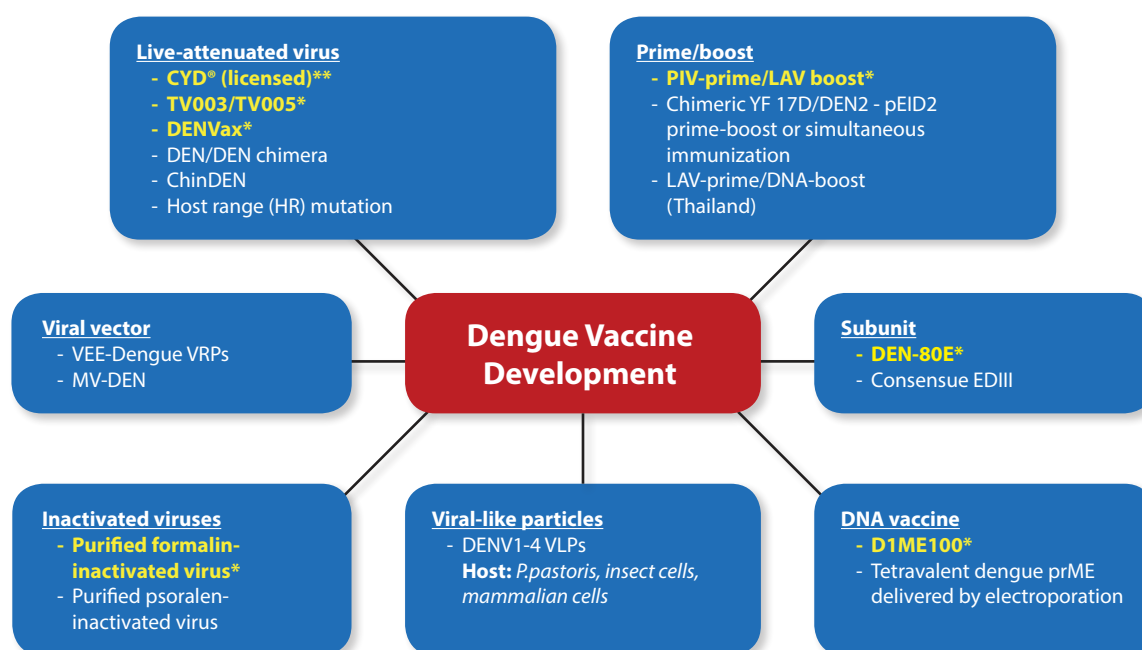


Figure 2. Current dengue vaccine candidates and their evaluating status. Abbreviations: CYD; Chimeric Yellow Fever Dengue viruses, EDIII; envelope domain III, LAV; live-attenuated virus, MV; measles virus, PIV; purified inactivated virus, VEE; Venezuelan equine encephalitis, VRPs; virus replicon particles

* Evaluating in clinical trials

** Licensed

Table 2. Ongoing dengue vaccine candidates in preclinical phase

Vaccine type	Strategy ^(Ref)	Developer	Animal	Comments
Live-attenuated virus	DEN/DEN chimeric viruses⁴⁴: replacing the prM/E of recent clinical isolates with pr-M cleavage enhancing into the genetic background of attenuated DENV-2	Chiang Mai University, Mahidol University, NSTDA, BioNet Asia	Rhesus macaques (<i>Macaca mulatta</i>)	<ul style="list-style-type: none"> - Chimeric virus with pr-M cleavage enhancing mutation (cD1-4pm) showed higher protection against DENV-1 and DENV-2 when compared to those without pr-M cleavage - Robust NAb responses after immunized with 10⁵ PFU of chimeric virus cD1-4pm - Complete protection was observed in all animal immunized with cD1-4pm.
	Chin/Den⁴⁵: chimeric DENV based on the JE live vaccine strain SA 14-14-2 as a backbone	Beijing Institute of Microbiology and Epidemiology, the Chengdu Institute of Biological Products	Rhesus macaques (<i>Macaca mulatta</i>)	<ul style="list-style-type: none"> - A single immunization of ChinDen-2 elicited strong neutralizing antibodies in a dose-dependent manner (10³, 10⁴, 10⁵ PFU). - 10⁴ and 10⁵ PFU immunized NHPs were fully protected from wild-type DENV-2 challenge.

Table 2. (Continued)

Vaccine type	Strategy ^(Ref)	Developer	Animal	Comments
<i>Live-attenuated virus (Continued)</i>	Dengue with host range (HR) mutation⁴⁶ : transmembrane domain I truncation to select the viruses that replicate only in insect cells	Arbovax	African Green monkey (<i>genus Chlorocebus</i>)	<ul style="list-style-type: none"> - High seroconversion rate in NHPs against all four serotypes after single injection of 4×10^6 infectious centers (1×10^6 IC/serotype) - At 2 months post-vaccination, NHPs were divided into 4 subgroups and challenged each of WT DENV serotype, animals vaccinated with HR-Tet had lower magnitude and duration of viremia compared with mock-vaccinated animals
	KD382^{47,48} : attenuated by serial passages in non-natural host	KAKETSUKEN and Mahidol University	Cynomolgus monkey	<ul style="list-style-type: none"> - All NHPs seroconverted against four serotypes after single dose of KD382 administration (10^5 FFU of each serotype) either in naïve or in dengue pre-existing immunity NHPs - Tetravalent neutralizing antibodies were last for at least 2 years
<i>Inactivated virus</i>	Purified psoralen-inactivated virus⁴⁹	US Naval Medical Research Center (NMRC)	<i>Aotus nancymaae</i>	<ul style="list-style-type: none"> - Three doses (10 ng each) of inactivated DENV-1 formulated with in alum were immunized intradermally. - All NHPs developed neutralizing antibody against DENV-1 and the duration of viremia was reduced after challenged with 1.1×10^4 PFU of WT DENV-1
<i>Recombinant protein</i>	Consensus EDIII expressed in <i>E. coli</i>⁵⁰⁻⁵²	Taiwanese National Health Research Institutes (NHRI).	BALB/c mice and NHPs (<i>Macaca cyclopis</i>)	<ul style="list-style-type: none"> - 20 µg of consensus EDIII formulated with aluminum phosphate (2 or 3 doses) elicits NAb against all four serotypes in mice but NAb against DENV-2 was predominated - Study in NHPs in 2 doses (200 µg of cED III subunit vaccine with aluminum phosphate), 8 weeks apart, 2/3 NHPs developed NAb against only DENV-2 - The results in mice demonstrated that lipidated EDIII is essential to improve DENV-4 immunogenicity without adjuvant needed
<i>DNA vaccine</i>	Tetravalent dengue prME⁵³ : prM/E consensus sequence of each serotype delivered by electroporation	ChulaVRC, Chiang Mai University, NSTDA	ICR mice and NHPs (<i>Macaca fascicularis</i>)	<ul style="list-style-type: none"> - Intramuscular electroporation delivery of tetravalent DNA vaccine induced NAb responses against four serotypes with anamnestic NAb responses after boosting - Evaluation in NHPs both in tetravalent DNA/DNA regimen and LAV/DNA heterologous prime/boost is currently ongoing (manuscript in preparation)
<i>Viral vector vaccine</i>	VEE-Dengue VRPs⁵³ : Infectious single cycle VEE expressing dengue antigens	University of North Carolina at Chapel Hill (UNC)	Rhesus macaque	<ul style="list-style-type: none"> - Two dose of tetravalent E-85 VRPs (10^8 IU of each serotype) immunization showed 100% seroconversion - NHPs were completely protected from DENV3 and DENV4 and significantly reduced the viremic duration of DENV1 and DENV2 at 18 weeks after the last dose
<i>Virus like particles</i>	MV-DEN⁵⁴ : A single live attenuated measles virus expressing EDIII of DEN1-DEN4	Themis Bioscience, the Institut Pasteur	Transgenic mice (susceptible to measles virus infection)	<ul style="list-style-type: none"> - 2 doses of 10^5 TCID50 of recombinant MV elicited neutralizing antibody against four serotype of virus in mice
	DSV⁴⁵ : Chimeric VLPs using hepatitis B surface antigen to display envelope domain III of four DENV serotypes expressed in <i>P. pastoris</i>	NCR Biotech Science Cluster/International Centre for Genetic Engineering and Biotechnology, India and Emory University	Immunogenicity: BALB/c mice and Rhesus macaque Challenge model: AG129 mice	<ul style="list-style-type: none"> - The chimeric VLPs formulated with alhydrogel or alhydrogel+MPL induced tetravalent neutralizing both in mice and Macaque - No antibody-dependent enhancement was observed after transferred immunized sera to AG129 mice

Table 2. (Continued)

Vaccine type	Strategy ^(Ref)	Developer	Animal	Comments
<i>Virus like particles</i> (Continued)	DENV-2 VLPs ⁵⁶ : express in mosquito cells	Chiang Mai University, Mahidol University, NSTDA, Thailand	BALB/c mice and NHPs (<i>Macaca fascicularis</i>)	<ul style="list-style-type: none"> - Two doses of VLPs formulated with adjuvants induced strong NAb response in mice - Without adjuvants, VLPs with enhanced prM cleavage induced higher levels of neutralizing antibody than those without enhanced prM cleavage - VLPs +adjuvant (AbISCO-100) strongly augmented neutralizing and EDIII-binding Abs in DENV-2-prime NHPs
Heterologous prime/ boost	Chimeric YF 17D/ DEN2- prime/pE1D2- boost or vice versa and simultaneous immunization⁵⁷	FIOCRUZ	Preclinical (mice)	<ul style="list-style-type: none"> - Prime boost prime/booster protocols or simultaneous immunization provided a synergistic effect on the eliciting neutralizing antibody response. - Simultaneous immunization increased protection against a lethal dose of DENV2, when compared to the separated immunization

5. Heterologous prime/boost

Heterologous prime/boost probably provide advantages than conventional immunization because it combines more than one vaccine platforms in the immunization schedule. Each vaccine will overcome or improve the weakness of another vaccine platform. There are several advantages of using this approach including, i) probably overcome the imbalance immune responses of live-virus vaccines, ii) expand the breadth of humoral and/or cellular immune responses depending on the constructs used and iii) could improve the target product profile with shorter immunization schedules. However, there are some limitations or concerns of using this approach such as the costs of vaccines may increase and the logistics involved in packaging and use may not be feasible for widespread use. Currently, heterologous regimen using tetravalent live attenuated-prime followed by tetravalent PIV-boost and vice versa is under investigation in phase I trial (NCT02239614, NCT03141138).^{36,37} We and colleagues, under financial support from Thai BIOTEC, NSTDA have evaluated heterologous prime/boost using tetravalent LAV and DNA. The preliminary results found that LAV-prime/DNA-boost protocol provided complete protection against DENV2 challenge in NHPs (manuscript in preparation). The study for evaluation of protective efficacy against all four dengue serotypes is underway (**Figure 2 and Table 2**).

Vaccine candidates in preclinical phases

Although there are several vaccine candidates have been evaluating in clinical trials. However, those vaccines still need further improvement. Various vaccine platforms including, live-attenuated virus, inactivated virus, recombinant protein, DNA vaccine, viral vector vaccine and heterologous prime/boost vaccines are being tested in preclinical as described in **Table 2**

Remaining challenges and new strategic approaches

Dengue vaccine development process requires several steps to achieve a licensed vaccine. Lack of suitable animal models which display symptoms as seen in human is the major problem. Only special mouse strains, for example, mice that

deficient in IFN α/β and IFN γ receptors (AG129) support dengue replication *in vivo*.³⁸ Moreover, this mouse strain could develop DENV specific Abs and displayed protection against both homologous and heterologous viral challenge.³⁹ More recently, development of humanized mice by engraftment of human hematopoietic progenitors in immunodeficient mice such as NOD/*scid*/*IL2R γ ^{null}* (NSG) or BALB/*c-Rag2^{null} IL2r γ ^{null}* (BRG) seem promising for vaccine and pathogenesis studies.^{40,41}

These mouse models carry human immune cells that could serve as target for dengue infection (monocytes, dendritic cells) as well as immune effector cells (B and T cells). Using humanized mice, we can preliminarily observe human immune responses which is the important evidence to support the potential of each new vaccine candidates before move to the next evaluation steps. In addition, humanized mice could probably reduce (or skip) the usage of large animal model (eg. NHPs) that need special housing conditions, high cost and concern about ethical issues. Nonetheless, it is also needed to take into account that humanized mouse is not yet had a perfect human immune system and might not provide fully reliable results.

Besides using animal models, dengue human infection model (DHIM) was considered as the most potent tool for dengue vaccine evaluation. It provides important information for clinical development by testing in small number of participants. The vaccine developer could early evaluate vaccine safety, immunogenicity of each vaccine formulation or intervention by using DHIM. There are two DHIM have been developed currently. First, US NIH, used a modified DENV-2 strain DEN2 Δ 30 that caused only very mild clinical symptoms but induced viremia in 100% of the 30 participants (infectious model). Second, WRAIR developed DHIM which aims to identify DENV strain that cause symptomatic dengue fever (infection and disease model).^{42,43} This could be the fast track for down-selection of best vaccine candidate, formulation or intervention. Moreover, in order to mimic the evaluation in dengue-endemic areas, pre-existing dengue infection could be set up by using DHIM. More importantly, DHIM probably provide the surrogate parameters (eg. neutralizing antibody titers, type of T cell responses) which highly correlate with

immune protection. However, the primary objective of DHIM is for the selection of the most suitable vaccine candidate or viral challenge strain, DHIM is different from regular phase I trial as it must perform in early-phase, strictly control clinical study. In addition, the virus developed by WRAIR which aim to identify the strain that cause symptomatic dengue fever need to be performed as inpatient setting.

Conclusions

The first dengue vaccine, CYD-TDV (Dengvaxia®) has been licensed in 2015/2016 in a number of countries. However, its efficacy against DENV-2 was consistently lower than other serotypes in all large clinical trials (CYD23, CYD14 and CYD15). Protective efficacy also depended on prior sero-status of the vaccinees. Lower efficacy was observed in children with < 9 years old and dengue-naïve individuals. More importantly, risk of hospitalization is increased in those participants (< 9 years old vaccinated individuals). Thus, questing for a better vaccine candidate is continuing. There are two live-attenuated vaccine candidates currently testing in phase III trial. First, DENVax, developed by USCDC and Inviragen (now licensed to Takeda), it showed promising results in phase II trial in endemic area, but the neutralizing antibody titers against DENV-3 and DENV-4 were lower than DENV-1 and DENV-2. Second, TV003/TV005, constructed by US NIAID, single administration of these vaccine admixtures were highly immunogenic and could protect the second vaccine dose at 6 months after first dose immunization (no viremia and increased of neutralizing titer). Evaluation of these vaccine admixtures in endemic area in Brazil (by Butantan Inc.) is ongoing. In addition, there are several phase I–II as well as preclinical phase studies evaluating vaccines for safety and immunogenicity, this include other live-attenuated platform/strategy, purified-inactivated viruses formulated with adjuvants, DNA vaccine, subunit vaccine, viral vector and also heterologous prime/boost strategies. The major difficulties of dengue vaccine development are included the lack of the best animal model, various immune status of individual especially in endemic areas. Moreover, the clear cut-off of the protective immunity is not clearly established yet. For example, both NAb levels and seroconversion rate against DENV-2 of CYD vaccine were comparable or higher than other serotypes but the protective efficacy against DENV-2 is the lowest. Other protective parameters such as T cells responses profile and/or specific neutralizing epitope may need to be explored comprehensively.

With all those challenges, it is interesting to see all research and development are advancing with very promising results; and to see the quest for better effective and accessible dengue vaccine for people needed is very likely to achieve in the next few years.

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