

# DENGUE VACCINES

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**Abstract.** The uniqueness of the dengue viruses (DENVs) and the spectrum of disease resulting from infection have made dengue vaccine development difficult. Several vaccine candidates are currently being evaluated in clinical studies. The candidate currently at the most advanced clinical development stage, a live-attenuated tetravalent vaccine based on the chimeric yellow fever-dengue virus (CYD-TDV), has progressed to Phase 3 efficacy studies. Several other live-attenuated vaccines, as well as subunit, DNA, and purified inactivated vaccine candidates are at earlier stages of clinical development. Additional technological approaches, such as virus-vectored and Virus-Like Particles (VLP)-based vaccines are under evaluation in preclinical studies.

**Keywords:** dengue, vaccine

## INTRODUCTION

Dengue is a mosquito-borne flavivirus disease, which is currently an expanding global health problem. Four closely related viruses, the dengue viruses 1-4, cause the disease. In the Asia-Pacific region, dengue is a serious and growing threat to public health, and this region bears nearly 75% of the current global dengue burden. Specific antiviral medications are not available for dengue. Prevention using vector control has had only limited success. There is no specific dengue therapeutics, and prevention is currently limited to vector control measures. Despite dengue control programs, case management guidelines and

surveillance efforts, dengue virus transmission remains high, and prevention remains a public health priority. Development of an effective dengue vaccine would, therefore, represent a major advance in the control of the disease and is considered a high public health priority. While a licensed dengue vaccine is not yet available, the scope and intensity of dengue vaccine development has increased dramatically in the last decade, with the lead candidate currently in Phase III clinical trials. Dengue vaccine may be the major means to effectively control dengue with the high feasibility of a dengue vaccine (Thisyakorn, 2014).

## DEVELOPMENT OF DENGUE VACCINES

The first dengue vaccines were evaluated in 1929 (Thisyakorn and Thisyakorn, 2014). Development of safe and effective dengue vaccines faces many challenges.

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The pathogenesises of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are not clearly understood. One debated hypothesis concerning virus virulence is the immune enhancement hypothesis (Prommalikit *et al*, 2004). Although evidence suggests that dengue disease severity may be associated with genetic differences in dengue strains, virus virulence has been difficult to measure because of the lack of *in vivo* and *in vitro* models of disease (Prommalikit and Thisyakorn, 2015). Infection by one of the four dengue virus serotypes has been shown to confer lasting protection against homotypic re-infection, but only transient protection against a secondary heterotypic infection, which may lead to an increased risk of severe disease. Due to these dengue-specific complexities, vaccine development focuses on the generation of a tetravalent vaccine aimed at providing long-term protection against all 4 dengue virus serotypes. Additional challenges are posed by the lack of an adequate animal disease model and the resulting uncertainty around correlates of protection. In spite of these challenges, vaccine development has made remarkable progress in recent years, and the current dengue vaccine development is advanced, diverse, and overall promising (Thisyakorn and Thisyakorn, 2014).

A tetravalent dengue vaccine must be developed without the benefit of a full understanding of the pathogenesis of severe dengue disease or an adequate animal disease model. The concerns of disease enhancement and limited research funding for a disease that primarily affects the developing world limit dengue vaccine development. However, in the last decade, the efforts to develop dengue

vaccines have increased dramatically due to an increased awareness of the dengue pandemic and the development of new molecular techniques. At present, several dengue vaccines have been tested in human clinical trials, and a single candidate is now in phase III clinical trial (Table 1).

## DIFFERENT APPROACHES IN DENGUE VACCINE DEVELOPMENT

### Live attenuated virus

The first major effort for live attenuated dengue vaccine development began at the University of Hawaii using the traditional method of serial passage of virus in non-human host, and then it was transferred to Mahidol University in Bangkok, Thailand for further passage and development of candidate vaccines and testing (Thisyakorn and Thisyakorn, 2014). The candidate vaccine was used for Phases I and II clinical trials in Thai adults and children. Not all of the volunteers seroconverted to all four dengue serotypes, and some showed unacceptable reactogenicity. Consequently, further clinical testing was stopped. Although the development of this candidate vaccine was not successful, the initiative was responsible for the subsequent progress that has been made in developing a tetravalent dengue vaccine.

The second tissue-culture-passaged dengue vaccine was developed at the Walter Reed Army Institute of Research (WRAIR). The WRAIR-produced tetravalent dengue vaccine formulation also showed problems of unbalanced immunogenicity and reactogenicity. New formulations seemed to be safe and immunogenic in a phase II study; however, the protective efficacy needs to be further

Table 1  
Development of dengue vaccines.

Type of vaccines	Dengue virus genes (N)	Stage of development
Live attenuated virus (traditional)	10	Phase II tetravalent (WRAIR & GSK)
Live attenuated virus (molecular)	10	Phase II monovalent (USNIH) Phase I tetravalent (USNIH) Protects nonhuman primates (USFDA)
Yellow fever chimera	Chimera 2+8 yellow fever virus	Phase III tetravalent (SP)
Dengue chimera	Chimera 2+8 DEN-2	Phase II tetravalent (Inviragen)
Purified inactivated	3	Protects monkeys (GSK, WRAIR)
Recombinant subunit	< 1	Phase I DENV1 (Hawaii Biotechnology)
DNA	2 +	Protects monkeys (Naval Medical-Research Center and Maxygen)

Source: Halstead and Thomas (2013).

evaluated. Further testing has been delayed by manufacturing complexities and the determination of the optimal dose and schedule of the vaccine.

The US National Institutes of Health introduced a new era of dengue vaccine research with direct mutagenesis technology. Dengue and other flavivirus genomes were readily altered genetically, resulting in attenuated variants while US Food and Drug Administration created a molecularly attenuated dengue vaccine. Both techniques provide an alternative approach to constructing a live attenuated tetravalent dengue vaccine.

There are several important safety issues for live dengue vaccines. Principal among these concerns is the theoretical risk of enhanced disease following dengue vaccination. The rationale for a tetravalent vaccine is the perceived requirement to simultaneously induce primary-type immune responses to all four dengue viruses. The simultaneous production of neutralizing an-

tibodies specific to each of the four dengue viruses is predicted to minimize the risk of disease enhancement following natural infection. However, antibody-dependent enhancement appears to occur with neutralizing antibodies at sub-neutralizing concentrations, so a vaccine that induces protection for a period of time might later increase the risk for enhanced disease.

This is particularly a concern for vaccines that induce low levels of neutralizing antibodies, but enhanced disease might occur with any vaccine given enough time. To adequately assess this risk, the risk of incomplete immunization, and waning antibody titers, dengue vaccine clinical development plans must include flavivirus-primed and flavivirus naïve volunteers and sufficiently long-term follow-up to make statistically powered conclusions regarding the safety of dengue vaccination in flavivirus-endemic areas. However, neither live attenuated nor live chimeric dengue vaccines inoculated into dengue-immune

children or adults have resulted in enhanced disease caused by a vaccine virus.

Other safety concerns with live attenuated virus vaccines include cell-culture-derived adventitious agents, community spread of the vaccine virus by resident vector mosquitoes, vaccine virus neurovirulence, and the effects of vaccine administration to immunocompromised hosts. Numerous dengue vaccine developers have also performed risk assessments of vector transmission by vaccine recipients. Results of published studies indicate a very low likelihood that a vaccine could transmit vaccine-derived dengue viruses to a mosquito.

Molecular clone-based strategies for a tetravalent dengue vaccine offer important advantages over traditional attenuation in cell culture. These include a reduced risk of adventitious agents, which will also reduce product quality assurance costs, and a molecular explanation for attenuation. Interference observed when mixtures of four dengue viruses are inoculated in susceptible human volunteers must also be studied in genetically modified vaccine viruses.

### **Chimeric virus**

Chimeric dengue vaccine viruses can be derived by inserting serotype-specific dengue antigen genes into a single attenuated dengue genomic construct. A different approach was taken to insert dengue structural genes into the infectious cDNA backbone of the well-established yellow fever vaccine virus strain 17D. This was started at Washington and St Louis University Medical Schools. These yellow fever chimeras are being further developed commercially by Acambis, Inc and are licensed

for manufacture to Sanofi Pasteur. Vero cells serve as the substrate for vaccine virus production.

The first Phase III efficacy trial for a recombinant, live, attenuated tetravalent dengue vaccine (CYD-TDV) in highly dengue-endemic area in five Asian countries in 10,275 children demonstrated that this dengue vaccine is efficacious when given as a 0-6-12 month schedule to 2-14-year-old children. The vaccine showed a 56.5% (95% CI: 43.8-66.4) overall efficacy with the contribution of each of the 4 serotypes, and more than 80% of severe dengue episodes were avoided with a two-thirds reduction in hospitalization. Higher efficacy was observed in the immunogenicity subset seropositive at baseline.

The safety profile was consistent with the good safety profile observed in previous studies. Over the 25-month follow-up period, no evidence of antibody dependent enhancement in partially or completely vaccinated individuals was observed. The interesting finding of this trial was that vaccine efficacy was higher for participants who were seropositive for dengue than for those who were seronegative. Furthermore, vaccine efficacy increased with age, which could be a marker of previous exposure to dengue (Capeding *et al*, 2014; Wilder-Smith, 2014).

A second Phase III clinical trial done in Latin American countries in 20,875 children and adolescents aged 9-16 years demonstrated a 60.8% (95% CI: 52.0-68.0) overall efficacy with the contribution of each of the 4 serotypes. Additional observation of the results showed a significant reduction of the risk of hospitalization by 80.3%. Higher efficacy was observed in the immu-

nogenicity subset seropositive at baseline. The safety profile was consistent with the good safety profile observed in previous studies, showing no evidence of antibody dependent enhancement in partially or completely vaccinated individuals. Results confirm the potential public health impact of the vaccine and support the vaccine's potential to reduce the public health burden of dengue. It should be recognized as the dawn of a new era of dengue control, because the potential use of this vaccine could be a major turning point for global dengue control. The interesting finding of this trial was that vaccine efficacy was higher for participants who were seropositive for dengue than for those who were seronegative (Dengue Vaccine Initiative, 2014). The results from Latin America complement those in Asia and provide a more global picture of the vaccine's potential to contribute to reaching the 2020 WHO target of reducing the global burden of dengue by decreasing morbidity by 25% and mortality by 50% (WHO, 2012; Thisyakorn *et al*, submitted).

### **Inactivated virus**

Inactivated whole virus vaccines have two advantages: they cannot revert to a more pathogenic phenotype, and they are unlikely to interfere with each other in combination. Moreover, induction of cell-mediated and humoral immune responses has been demonstrated with inactivated flavivirus vaccines. Conversely, inactivated vaccines express only the part of the viral genome that encodes structural proteins. In the context of dengue immunity and immunopathology, raising antibodies that are not fully protective may lead to breakthrough infections or enhance infections with

wild-type dengue viruses. Other potential disadvantages of these vaccines are the cost per dose and their usual requirement for multiple immunizations.

Newer complex adjuvant systems have been shown to mediate long-lasting antibody response. Two, AS03 and AS04, have been incorporated into vaccines licensed for human use and are being explored by the WRAIR/GSK/Oswald Cruz Foundation Killed Dengue Vaccine Initiative.

### **Subunit vaccines**

Recombination subunit approaches offer the advantages of anticipated minimal reactogenicity, freedom from adventitious agents, and low cost. However, incomplete post-translational processing of proteins can lead to proteins that differ from native proteins and antibody responses. Production in mammalian cells may reduce some of these concerns. A Phase I study to assess the safety and tolerability of a DEN-1 candidate in healthy US adults has been completed and the publication of the results is pending. Risk of enhanced disease upon exposure to wild-type viruses post-vaccination would need to be assessed as for all other approaches to dengue vaccines. Vaccines that elicit a cytotoxic T-cell memory response may lower this risk.

### **DNA vaccines**

Dengue DNA vaccines offer a possible method to raise protective immunity, which bypasses the problem of interference seen with multivalent live virus vaccines. DNA vaccines are composed of a plasmid or plasmids containing dengue genes. These are reproduced to a high copy number in bacteria such as *E. coli*. The plasmid contains a eukaryotic promoter and termination

sequence to drive transcription in cells after being inoculated into a vaccine recipient. The transcribed RNA is translated to produce proteins that are processed and presented to the immune system in the context of the host's own MHC molecules. New genes such as intracellular trafficking and immunostimulatory sequences can be added to the plasmid, producing expressed antigens, which lead to B- and T-cell responses.

In theory, DNA vaccines afford numerous advantages over conventional vaccines, including ease of production, stability and transport at room temperature, the ability to add new genes to the vaccine, and the possibility of vaccinating against multiple pathogens in a single vaccination with reduced reactogenicity.

Tetravalent DNA vaccines have been created by shuffling the envelope genes from the four dengue serotypes and transfecting the resulting chimeric genes into human cells by DNA plasmids. The transfected human cells were then incubated with type-specific dengue antibodies and subjected to flow cytometry. Antibody markers permitted rapid screening of libraries and identification of novel expression of C-terminal truncated antigens that combined envelope and pre-membrane epitopes from all four dengue serotypes when inoculated in mice and monkeys that had successfully raised neutralization antibodies. Monkeys resisted challenge with DEN-1 but not DEN-2.

A DENV-1 DNA vaccine was evaluated in flavivirus-negative volunteers with a three-dose series at day 0, and at 1 and 5 months. None of the low-dosage recipients and half of the high dosage recipients

developed neutralizing antibodies. More recently, protection has been achieved in a rhesus monkey model by boosting tetravalent DNA vaccination with a tetravalent live attenuated dengue vaccine.

The DNA approach also carries unique risks. The first is the theoretical risk of nucleic acid integration into the host's chromosomal DNA to potentially inactivate tumor suppressor genes or activate oncogenes. This risk appears to be well below the spontaneous mutation frequency for mammalian cells. However, if a mutation due to DNA integration is a part of a multiple hit phenomenon leading to carcinogenesis, it could take many years for this problem to become evident. Another concern is that foreign DNA may induce anti-DNA antibodies leading to autoimmune diseases such as systemic lupus erythematosus. However, to date, studies on lupus-prone mice, normal mice, rabbits, and people have not validated this concern (Thisyakorn and Thisyakorn, 2014).

Although no licensed dengue vaccine is yet available, the increasing knowledge of dengue vaccine development is providing more insights into improved vaccine design. Several promising dengue vaccine candidates are in preclinical and clinical development, and one is moving into Phase III testing. If the safety concerns can be surmounted, economic forces and technologic advances should soon bring one or more dengue vaccines into the market. It remains for the vaccine community to develop and implement plans for the strategic use of dengue vaccines by developing evidence-based policies to target high risk groups and decrease virus transmission.

Early preparation and understanding

of the true burden of disease will be essential for successful vaccine introduction and, with this in mind, the ASEAN Member States Dengue Vaccination Advocacy Steering Committee (ADVASC) convened a Regional Workshop to review the current status of dengue surveillance and diagnostics in the ASEAN Region (Thisyakorn, 2012). The ADVASC have recommended an evidence-based approach to strengthening and harmonizing key attributes of dengue surveillance, including case classification, data collection, data analysis and laboratory testing. Strengthening vaccination policy will require further investment in existing health systems, and recommendations for research and advocacy are also outlined here (Thisyakorn *et al*, submitted).

## CONCLUSION

Dengue virus is the causative agent of a wide spectrum of clinical manifestations, ranging from mild acute febrile illness to classical dengue fever, DHF, and DSS. DHF and DSS are the potentially fatal forms of dengue virus infection, which has become an intractable global health problem.

Vector control has achieved only limited success in reducing the transmission of dengue and there are currently no licensed antivirals to treat dengue. The most effective way to control dengue diseases in the future will be through the use of a safe and effective vaccine. Dengue is a unique and complex disease; developing a dengue vaccine has proven equally complex. Although no licensed dengue vaccine is yet available, several vaccine candidates are under development, including live attenuated virus vaccines, live chimeric virus vac-

cines, inactivated virus vaccines, and live recombinant, DNA and subunit vaccines. The CYD-TYD live attenuated dengue vaccine, being developed by Sanofi Pasteur, has demonstrated clinical efficacy and a good safety profile.

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## DENGUE VACCINES

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