



Recent Trands In Dengue Vaccine Development

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Abstract: Dengue is among the most prevalent and important arbovirus diseases of humans. In order to effectively control this rapidly spreading disease, control of the vector mosquito and a safe and efficacious vaccine are critical. Despite considerable efforts, the development of a successful vaccine has remained elusive. Multiple factors have complicated the creation of a successful vaccine, not the least of which are the complex, immune-mediated responses against four antigenically distinct serotypes necessitating a tetravalent vaccine providing long lasting protective immunity. Despite the multiple impediments, there are currently many promising vaccine candidates in pre-clinical and clinical development. Here we review the recent advances in dengue virus vaccine development and briefly discuss the challenges associated with the use of these vaccines as a public health tool

INTRODUCTION

Dengue is an infectious disease caused by a virus. The virus is transmitted by a type of mosquito (*Aedes aegypti*) that bites during daylight hours. The dengue virus belongs to the *Flaviviridae* family of viruses that cause diseases in humans. Dengue is the most common infection caused by viruses transmitted by mosquitoes (these are known as arboiral illnesses). Dengue causes severe flu-like symptoms, such as a high temperature (fever) of 40°C (104°F) or over, severe headache, muscle and joint pain, facial flushing, and skin rash. Anyone can catch dengue if the disease is common in that area (endemic). See the box, below left, for a list of high-risk countries. However, dengue is more common among older children, adolescents and adults. The risk of travellers catching dengue depends on several factors, including: the countries they visit, how long they stay in an endemic area (although even short-term visitors may

be vulnerable to dengue) the season of travel (mosquitoes breed in fresh-standing water, such as puddles and collected rainwater) the intensity of dengue transmission in that area.

Dengue is a self-limiting disease. Self-limiting means that it clears up by itself, usually within a couple of weeks. The incubation period (the time it takes for symptoms to show after infection) for dengue is five to eight days. There is no vaccine to prevent you becoming infected, although research into developing a vaccine to protect against dengue is in progress. The only way to prevent getting the virus is to avoid being bitten and to be particularly careful around the hours of dawn and dusk.

➤ Dengue fever also known as “BREAK BONE FEVER” is a mosquito borne viral infection.

➤ *Aedes aegypti* and *Aedes albopictus*

➤ Other species are –

➤ *A. Stegomyia*, *A. Polynesiensis*, *A. Scutellaris*, *A. Finalaya*

➤ The causative dengue viruses are members of the genus *Flavivirus*, within the family *Flaviviridae*.

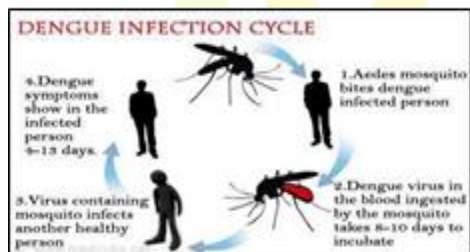
➤ WHO classifies dengue into two categories, dengue (with or without warning signs) and severe dengue.

➤ India also saw a doubling up of cases of dengue from 2014 to 2015 and the worst hit city was Delhi with over 1800 cases of the fever. 2016 isn't expected to be any better and this has become a cause of concern for the country.

Dengue infection, one of the most devastating

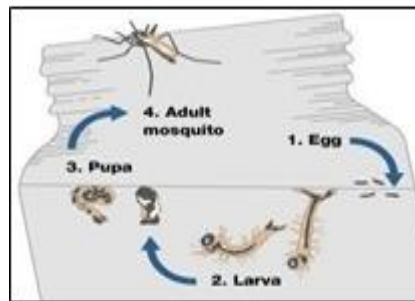
mosquito-borne viral diseases in humans, is now a significant problem in many countries. The causative dengue viruses are members of the genus *Flavi virus*, within the family *Flaviviridae*. There are four closely related serotypes, the dengue viruses (DENV) 1–4 and at least four genotypes within each serotype. Primary infection with a particular dengue serotype confers long-lasting immunity for that serotype (homotypic immunity). Immunity to other dengue serotypes (heterotypic immunity) lasts for a few months, after which patients are susceptible to heterotypic infection. All *flavi viruses* are lipid-enveloped, positive-strand RNA viruses. The RNA genome of dengue virus is about 10.7 kb and encodes three structural proteins, namely capsid protein (C), precursor membrane/membrane protein (PrM/M) and envelope protein (E). Besides the structural proteins, there are seven nonstructural proteins (NS) which are associated with viral replication and disease pathogenesis. The coding of the viral proteins is organized in the genome as C- prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5. The disease, caused by the four dengue virus serotypes, ranges from asymptomatic infection to undifferentiated fever, dengue fever (DF), and severe dengue hemorrhagic fever (DHF). DHF is characterized by fever, bleeding diathesis and plasma leakage with a tendency to develop a potentially fatal shock syndrome. Dengue infection with organ impairment mainly involves the central nervous system and liver. Consistent hematological findings include vasculopathy, coagulopathy, and thrombocytopenia. Laboratory diagnosis includes virus isolation, serology, and detection of dengue ribonucleic acid. Successful treatment, which is mainly supportive, depends on early recognition of the disease and careful monitoring for shock. A severity-based revised dengue classification for medical interventions has been developed and validated in many countries.

DENGUE CYCLE AND SYMPTOMS



- ❖ Bleeding, Low levels of blood platelets.
- ❖ Blood plasma leakage, Low blood pressure, Headache, Eye pain
- ❖ Muscle aches
- ❖ Back pain, joint pain.

❖ Gastrointestinal bleeding



Symptoms of dengue include:

feeling as if you have a severe case of flu, severe headache, high temperature (fever) of 40°C (104°F) or over (this can last for up to five days), severe aching in your joints (dengue is also known as 'breakbone fever'), severe muscle pain (myalgia) of the lower back, arms and legs, nausea and vomiting, chills (shivering), flat red skin rash (it may appear two to five days after the fever starts, and a second rash that looks similar to measles appears when the disease has progressed), facial flushing. These symptoms can be mild in some people and severe in others. All symptoms vary depending on age. Infants and young children usually have a high fever and a rash.

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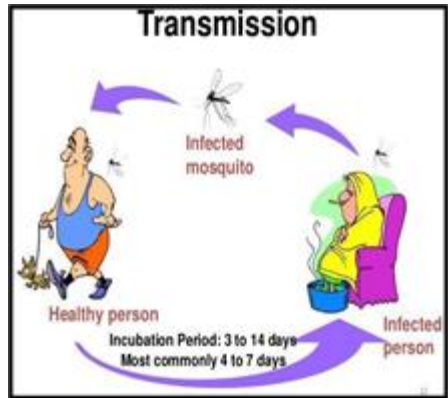
Remember the time when the epidemic of Dengue was spreading like wild fire across the tropical areas in 2008? The death tolls because of Dengue fever have been on the rise ever since.

However, the WHO and other health departments have done well in trying to curb the epidemic of Dengue fever by popularising the ill effects and symptoms of it.

The Dengue fever symptoms usually begin to show 4-6 days after getting infected and may last for up to 10 days. Some of the common symptoms include

- Sudden Fever
- Severe headaches
- Severe joint and muscle pain Fatigue
- Nausea
- Pain behind the eyes Vomiting
- Skin rashes, which may last for 2-5 days after the onset of fever
- Mild bleeding from the nose and the gums

TRANSMISSION CYCLES



- ❖ Extrinsic incubation period- 8 to 10 days.
- ❖ Intrinsic incubation period- 3 to 14 days.
- ❖ An average of 4 to 7 days.
- ❖ Cyclic nature of the disease-
- ❖ In endemic countries the cycle can be repeated at yearly interval.
- ❖ India is a endemic country for dengue fever.

Dengue Transmission

The risk of contracting dengue infection has increased dramatically since the 1940s. This upward trend is due to increases in long-distance travel, population growth and urbanization, lack of sanitation, ineffective mosquito control, and increases in the surveillance and official reporting of dengue cases. Dengue has spread through Southeast Asia, the Pacific Island countries, and the Middle East. Today, approximately 40% of people live in regions of the world where there is a risk of contracting dengue. Dengue is an endemic disease, which means that it occurs regularly, in tropical regions of the world. The disease is endemic in more than one hundred countries in Africa, the Americas, Asia, the Caribbean, and the Pacific.

Aedes Mosquitoes

Can any type of mosquito carry dengue? The dengue virus is carried and spread by mosquitoes in the genus *Aedes*, which includes a number of mosquito species. Of these species, the primary vector of the dengue virus is the species *Aedes aegypti*. It is the principal dengue vector responsible for dengue transmission and dengue epidemics. Other mosquito species in the genus *Aedes* — including *Aedes albopictus*, *Aedes polynesiensis*, and *Aedes scutellaris* — have a limited ability to serve as dengue vectors.

A photograph shows a mosquito on human skin at high magnification. The skin looks very dry, and pores are visible. The mosquito has a black thorax, wings, and head; two fuzzy antennae; and six segmented legs. Its abdomen is orange. An elongated tube-like proboscis extends from the anterior end of the mosquito's head

and is shown penetrating the top layer of human skin. *Aedes aegypti* is the principal vector responsible for dengue transmission. Courtesy of James Gathany/PHIL/CDC. Some rights reserved.

How Is Dengue Transmitted to Humans?

A circular diagram of arrows and illustrations shows the dengue virus transmission cycle. A mosquito from the genus *Aedes* is shown at the top of the diagram. Its body is mostly black with an orange abdomen. An arrow points from the mosquito to a simplified illustration of a human body. A second arrow points from the human body to a mosquito at the bottom of the diagram. A third arrow points from the mosquito at the bottom of the diagram to a second illustration of a human body. A fourth arrow points from the second illustrated human body to the mosquito at the top of the diagram, completing the cycle.

The dengue virus is spread through a human-to-mosquito-to-human cycle of transmission. The dengue virus is spread through a human-to-mosquito-to-human cycle of transmission. Typically, four days after being bit by an infected *Aedes aegypti* mosquito, a person will develop viremia, a condition in which there is a high level of the dengue virus in the blood. Viremia lasts for approximately five days, but can last as long as twelve days. On the first day of viremia, the person generally shows no symptoms of dengue. Five days after being bit by the infected mosquito, the person develops symptoms of dengue fever, which can last for a week or longer.

VACCINES DEVELOPMENT WHO GUIDELINE FOR DENGUE VACCINES DEVELOPMENT

Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated) (update of TRS932, in press).

❖ Guidelines for the evaluation of dengue vaccines in endemic areas (WHO/IVB).

❖ Guidelines for plaque reduction neutralization testing of human antibodies to dengue virus (WHO/IVB/07.07, and Viral Immunology 21:123-132).

❖ Cell-mediated immunity in dengue vaccine development (Vaccine 27:355- 368).

❖ Next generation dengue vaccines (Vaccine 29:7276-7).

There is a pressing need for guidelines focused on the clinical evaluation of dengue vaccines in exposed populations, because Phase 1 and 2 clinical trials of dengue vaccines have begun, and Phase 3 field trials may be warranted soon. In response to

this need, the WHO Initiative for Vaccine Research (IVR) has conducted a series of expert consultations resulting in guidelines published as a WHO; 2008[Report No.: WHO/IVB/08.12]. This document, directed toward national regulatory authorities (NRAs), vaccine developers and the scientific community at large, provides guidance for the evaluation and registration of dengue vaccines in dengue-endemic countries. The new document builds on a previous guidance document published in 2002 [WHO. Guidelines for the evaluation of dengue vaccines in populations exposed to natural infection. Geneva, Switzerland: WHO; 2002 [Report No.: TDR/IVR/DEN/02.1]]. This report summarizes the recommendations in the new guidelines, which emphasize the many unique aspects of dengue vaccine trials. These include strong recommendations to collect accurate dengue incidence data across multiple transmission seasons, and to conduct Phase 2 or 3 bridging studies, post-Phase 3 follow-up safety studies and Phase 4 post-licensure trials to better elucidate vaccine immunogenicity, protective efficacy, or safety in endemic areas where multiple dengue types and other flaviviruses circulate and where flavivirus vaccines are widely used.

TREATMENT OF DENGUE

"Aspirin and other NSAID's (like Ibuprofen, Diclofenac) should not be given to patients. These will cause severe bleeding. Hence, to take paracetamol to relieve muscle and joint aches, fever and headache,"

❖ Maintenance of the patient's body fluid volume is critical to severe dengue care

Dengue fever facts:

❖ Dengue fever is a disease caused by a family of viruses that are transmitted by mosquitoes.

Symptoms of dengue fever include severe joint and muscle pain, swollen lymph nodes, headache, fever, exhaustion, and rash. The presence of fever, rash, and headache (the "dengue triad") is characteristic of dengue fever. Dengue is prevalent throughout the tropics and subtropics. Dengue fever is caused by a virus, and there is no specific medicine or antibiotic to treat it. For typical dengue fever, the treatment is directed toward relief of the symptoms (symptomatic treatment). Papaya leaf extract can be used to treat dengue fever.

The acute phase of the illness with fever and muscle pain lasts about one to two weeks. Dengue hemorrhagic fever (DHF) is a specific syndrome that tends to affect children under 10 years of age. This complication of dengue causes abdominal pain, hemorrhage (bleeding), and circulatory collapse (shock). The prevention of dengue fever requires control or eradication of the mosquitoes carrying the virus that causes dengue. A vaccine for dengue fever was approved in April 2016 for use in dengue-endemic areas. Dengue fever is a

disease caused by a family of viruses transmitted by mosquitoes. It is an acute illness of sudden onset that usually follows a benign course with symptoms such as headache, fever, exhaustion, severe muscle and joint pain, swollen lymph nodes (lymphadenopathy), and rash. The presence of fever, itchy rash, and headache (the "dengue triad") is characteristic of dengue. Other signs of dengue fever include bleeding gums, severe pain behind the eyes, and red palms and soles. Dengue (pronounced DENG-gay) can affect anyone but tends to be more severe in people with compromised immune systems. Because it is caused by one of five serotypes of the dengue virus, it is possible to get dengue fever multiple times. However, an attack of dengue produces immunity for a lifetime to that particular viral serotype to which the patient was exposed. A 2009 outbreak of dengue fever in the Florida town of Key West involved three patients who did not travel outside of the U.S. and contracted the virus. Subsequent testing of the population of Key West has shown that up to 5% of the people living in the area have antibodies to dengue. In total, 28 people were diagnosed with dengue fever in this outbreak. In 2015, 210 people were diagnosed with dengue on the Big Island of Hawaii. This is the largest outbreak in Hawaii since 2001, when 122 people were diagnosed with dengue.

Dengue fever is common in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. Thailand, Vietnam, Singapore, and Malaysia have all reported an increase in cases. According to the World Health Organization (WHO), there are about 390 million cases of dengue fever worldwide, and 96 million require medical treatment. Five hundred thousand cases of dengue hemorrhagic fever, the most severe form of dengue, require hospitalization each year. Nearly 40% of the world's population lives in an area endemic with dengue. The World Health Organization (WHO) estimates 22,000 deaths occur yearly, mostly among children.

The virus is contracted from the bite of a striped *Aedes aegypti* mosquito that has previously bitten an infected person. The mosquito flourishes during rainy seasons but can breed in water-filled flower pots, plastic bags, and cans year-round. One mosquito bite can cause the disease.

The virus is not contagious and cannot be spread directly from person to person. It is mosquito-borne, so there must be a person-to-mosquito-to-another-person pathway. The full life cycle of the virus involves the mosquito as the vector (transmitter) and the human as the source of infection.

After being bitten by a mosquito carrying the virus, the incubation period for dengue fever ranges from

three to 15 (usually five to eight) days before the signs and symptoms of dengue appear in stages.

TREATMENT WITH CARICA PAPAYA LEAVES

Contents:

- Papain, Chymopapain, Cystatin, Tocopherol.
- Ascorbic acid, Flavonoids, Cyanogenicglucosides & Glucosinolates, Vitamins.
- Minerals (calcium, magnesium, sodium, potassium, manganese, iron), Saponins, Cardiac glycosides & Alkaloids

Clinical study



The main objective of the current study is to investigate the potential of Carica papaya leaves extracts against Dengue fever in 45 year old patient bitten by carrier mosquitoes. For the treatment of Dengue fever the extract was prepared in water. 25 ml of aqueous extract of C. papaya leaves was administered to patient infected with Dengue fever twice daily i.e. morning and evening for five consecutive days. Before the extract administration the blood samples from patient were analyzed. Platelets count (PLT), White Blood Cells (WBC) and Neutrophils (NEUT) decreased from $176 \times 103/\mu\text{L}$, $8.10 \times 103/\mu\text{L}$, 84.0% to $55 \times 103/\mu\text{L}$, $3.7 \times 103/\mu\text{L}$ and 46.0%. Subsequently, the blood samples were rechecked after the administration of leaves extract.

VACCINE DEVELOPEMENT

The first dengue vaccine, **Dengvaxia**(CYD-TDV) by **Sanofi Pasteur**, was first registered in Mexico in December, 2015. CYD-TDV is a live recombinant tetravalent dengue vaccine that has been evaluated as a 3-dose series on a 0/6/12 month schedule in Phase III clinical studies.

It has been registered for use in individuals 9-45 years of age living in endemic areas.

This vaccine has been evaluated in two large pivotal Phase 3 trials in 5 countries in Asia and 5 countries in Latin America, in participants aged 2–16 across the two trials One vaccine is currently approved for the prevention of dengue infection. Sanofi Pasteur registered Dengvaxia (CYD-TDV), a live recombinant tetravalent vaccine, in several countries in late 2015-2016, with Mexico being the initial country to register the vaccine in December 2015 The vaccine is given in 3 doses at age 0, 6, and 12 months. It underwent testing in

more than 02030,000 volunteers and was shown to reduce the risk of severe illness and hospitalization by as much as 30% in individuals previously infected with one or more strains. The vaccine proved less effective in persons who were not previously exposed to dengue and in areas with a lower burden of disease. Owing to concern that the vaccine may act like an initial dengue infection in this second group of individuals not previously infected with the virus, with additional exposure to a second serotype placing these individuals at increased risk of severe dengue.

TECHNICAL AND REGULATORY ASSESSMENT

Dengue vaccine trials are conducted under US Investigational New Drug (IND) supervision, dengue vaccines have been considered independently by NRAs in endemic countries for clinical trials and registration without reliance on WHO pre-qualification.

WHO has provided support to NRAs that first received the CYD-TDV dengue vaccine dossier to assist informed decision-making for vaccine registration

a dengue vaccine should be tetravalent in nature and provide protection against all four dengue serotypes, regulatory agencies need to address additional issues associated with multi-valent vaccines such as interference between the vaccine serotypes.

Dengue vaccine research

Dengue is a mosquito-borne flavivirus disease that has spread to most tropical and many subtropical areas. The disease is caused by four closely related viruses, the Dengue viruses 1-4. There are no specific dengue therapeutics and prevention is currently limited to vector control measures. A dengue vaccine would therefore represent a major advance in the control of the disease.

Status of vaccine development

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Preclinical developments:

❖ The preclinical pipeline for dengue vaccines includes both conventional as well as novel technological approaches, including • recombinant subunit vaccines

- DNA vaccines
- VLP vaccines
- virus-vectored vaccines

- purified inactivated
- virus vaccines
- live attenuated virus vaccines.

CURRENT STATUS IN INDIA

The Indian authorities requested us to perform a study in adults. The results will be an important part of database as to consider additional clinical trials in India with vaccine and the results will also play a part to support our licensure application.

This would be the first time a vaccine developed entirely in India for a vector-borne disease has been advanced for clinical development in India.

India's largest pharmaceutical company, Sun Pharma, has signed a deal with the Delhi unit of the International Centre for Genetic Engineering and Biotechnology (ICGEB) to develop a dengue vaccine.

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