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# The Regulation Requirement of Dengue Vaccines

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Additional information is available at the end of the chapter

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## Abstract

Dengue fever (dengue), a mosquito-borne disease caused by dengue viruses (DENVs), represents severe public health problems in Southeast Asia, Latin America, Africa and other subtropical regions. Many regulatory issues arise along with the development of dengue vaccines. It is required to follow the regulatory pathway for the license application. Dengue vaccines can be approved without local clinical phase III data. The national regulatory authorities (NRAs) must have the information, training and ability to review and approve the application. A novel vaccine product Dengvaxia® for dengue has been approved in many countries. The approval is based on clinical trials that show the vaccine could reduce about 60% dengue, prevented 90% of severe cases and 80% of hospitalizations. Several other DNA, live-attenuated, purified inactivated, subunit, vectored and chimeric vaccine candidates are currently developing in clinical phases. Although there are still some challenges for the development and regulation of vaccine, the prospects of dengue vaccines are promising provided that we can overcome the difficulty.

**Keywords:** dengue fever (dengue), dengue virus, dengue vaccine, clinical application

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## 1. Introduction

In this section, we describe some background about dengue fever (dengue).

### 1.1. Pathogens, vectors and symptoms

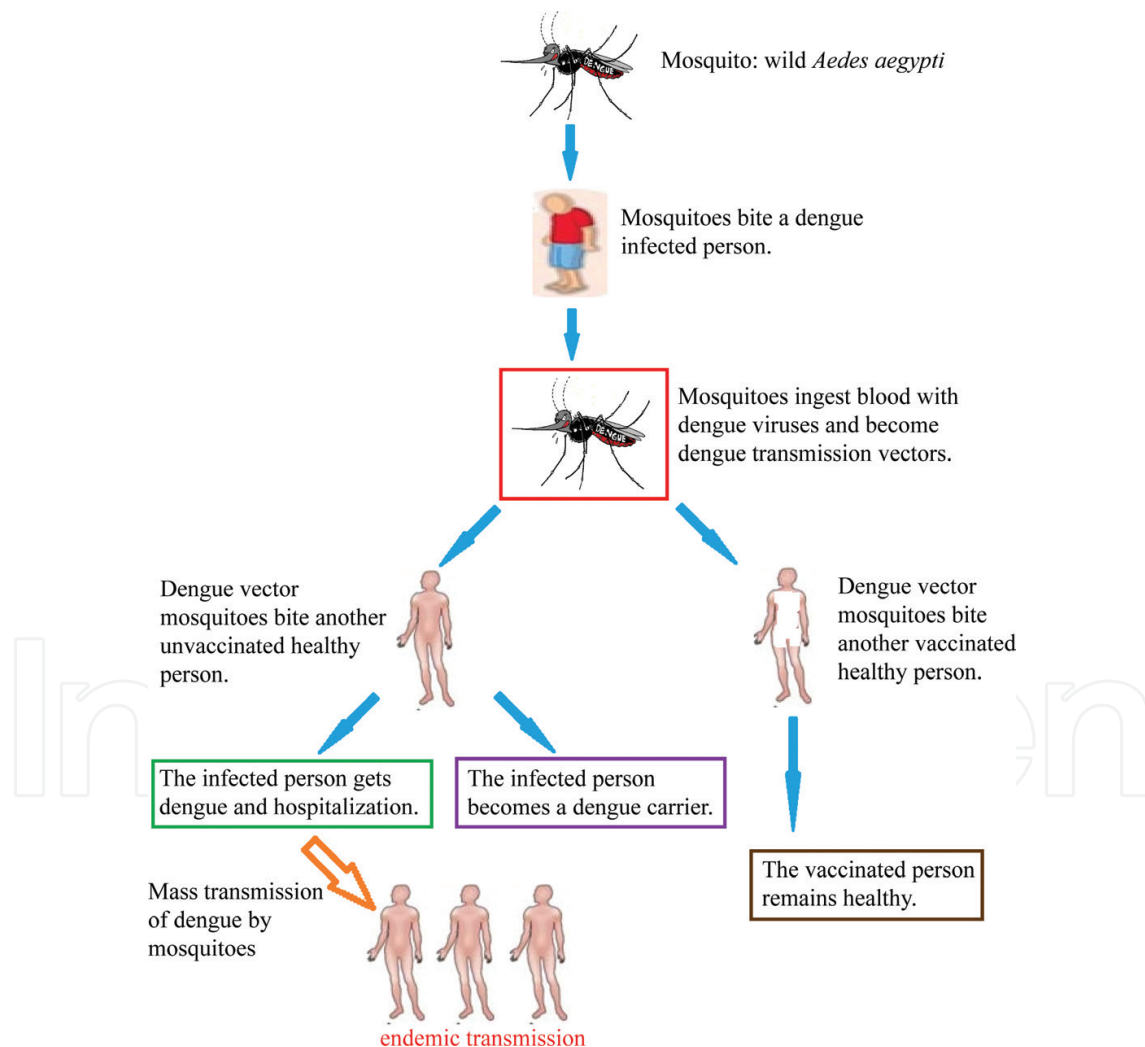
Dengue, a mosquito-borne disease, is widespread all over the world in recent years. It is caused by the dengue virus (DENV), which is transmitted by female *Aedes* mosquitoes of species *A. aegypti* and *A. albopictus*. The mosquito becomes infected when it bites a person with DENVs in their blood. DENVs are maintained in cycles that involve blood-sucking vectors

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and vertebrate hosts. Dengue is transmitted by vectors and is not spread from one person to another person directly (**Figure 1**).

DENV, which belongs to genus *Flavivirus* of family *Flaviviridae*, is a small single-stranded RNA (ssRNA) virus comprising four distinct but closely related serotypes (DENV-1, 2, 3 and 4) [1, 2]. However, the fifth serotype DENV-5, which may be produced by genetic recombination, natural selection and genetic bottlenecks, has been discovered in 2013. This new variant follows the sylvatic cycle unlike the other four serotypes, which follow the human cycle [3].

Symptomatic DENV infections were classified into three categories: undifferentiated fever (UF), dengue fever (DF, dengue) and dengue haemorrhagic fever (DHF) (also called severe dengue). DHF was further classified into four severity grades, with grades III and IV being defined as dengue shock syndrome (DSS) [4]. Sometimes, the infection causes flu-like symptoms, which are mild, is called UF. DF begins 4–6 days after infection and its symptoms are



**Figure 1.** Dengue transmission: *Aedes* mosquitoes (*A. aegypti* or *A. albopictus*) bite a dengue-infected person and suck his blood. The mosquitoes ingest blood with dengue viruses and become transmission vectors after 8–10 days. The dengue vector mosquitoes bite another unvaccinated healthy person and the person gets dengue 4–13 days later. This may lead to endemic transmission if dengue is extensively spread by mosquitoes.

usually limited. However, serious problems can develop and occasionally result in a lethal complication called DHF. The symptoms may further progress to DSS, which is characterized by massive bleeding, shock and even lead to death.

Different DENV serotypes may cause a cross-serotypic immune response such as antibody-dependent enhancement (ADE). ADE is the most widely known example, which occurs in the setting of DENV infection. When an individual has been infected with one DENV serotype, the antibodies to the previous serotype will interfere with the immune responses to the new serotype to lead to more virus attack and entry. The disease varies in severity in humans from DF, which is usually limited in symptoms to lethal cases including DHF and DSS.

## 1.2. Epidemiology

Dengue is one of the most rapidly spreading mosquito-borne viral diseases in the world. The number of dengue cases reported annually to WHO increased from 0.4 to 1.3 million from 1996 to 2005 and reached 2.2 million and 3.2 million in 2010 and 2015, respectively [5]. The disease is currently common in more than 110 countries, mainly in Southeast Asia, Latin America, Africa, etc. Infections are usually acquired in the urbans, but they have expanded to villages, towns and cities recently.

Most people with dengue recover without any problems. The fatality rate is 1–5%, and less than 1% with adequate treatment. Because of urbanization, population growth, frequent international travelling and global warming, the incidence rate of dengue increased about 30-fold from 1960 to 2010 [6]. This disease inflicts a serious healthy, social and economic burden on the people of endemic areas.

## 1.3. Treatment

The treatment is only supportive and there is no certain treatment for dengue. Current efforts are to develop antiviral drugs that would be used for the treatment of dengue and prevention of severe complications. There are several plausible therapeutic approaches such as the inhibition of the viral RNA-dependent RNA polymerase inhibitor, viral protease inhibitors, entry inhibitors that stop the virus entering cells or inhibitors of the 5' capping process, which is required for viral replication. Supportive care and adjuvant therapy may be needed in severe dengue (DHF and DSS).

## 1.4. Prevention and control

For dengue prevention, traditional method is only restricted to vector control measures. The best way is to inhibit the spread of the *Aedes* mosquitoes and avoid being bitten by them. This may be done by eliminating the mosquitoes, removing or covering standing water and wearing clothing that covers the body.

Integrated vector management (IVM) program is a strategy to control vectors recommended by the World Health Organization (WHO). IVM considers the following five key elements in the management process [5]:

- (1) **Advocacy, social mobilization and legislation:** The promotion of developmental policies, the establishment or strengthening of regulatory controls for public health and the empowerment of communities.
- (2) **Collaboration within the health sector and with others:** The consideration of all options for collaboration, strengthening communication among policy-makers, program managers and other key partners.
- (3) **Integrated approach to disease control:** The assurance of the rational use of available resources, integration of non-chemical and chemical control methods and integration with other disease control measures.
- (4) **Decision based on evidence:** The adaptation of strategies and interventions to local vector ecology, epidemiology and resources, guided by operational research and subject to routine monitoring and evaluation.
- (5) **Capacity-building:** The development of essential infrastructure, adequate finance and human resources.

Vaccination is a better measure than the vector control for dengue prevention. The development of a dengue vaccine actually represents a great achievement in the control of the disease. The impact of dengue is so enormous that its vaccine development is very crucial for public health. To develop a dengue vaccine, we have to realize the regulation requirements of vaccines such as basic research, animal studies and clinical trials, etc.

## 2. Regulation

Dengue vaccines, as all vaccine products regulated by national regulatory authorities (NRAs) and national control laboratories (NCLs), undergo a rigorous review of laboratory and clinical data to ensure their safety, efficacy and potency. The discovery and application of dengue vaccines should follow the frame work of vaccine research and development. The manufacturer has to comply with the related regulation, including nonclinical work, preclinical trials, human clinical trials and post-market surveillance [7]. In this section, we review the regulations that are specific and required for dengue vaccines, including basic requirements, regulatory pathways, special procedures, regulatory requirement summary and regulatory challenges.

### 2.1. Basic requirements

The WHO Initiative for Vaccine Research (IVR) focuses on the following objectives for dengue vaccine development:

- (1) **Knowledge and research:** Identification of knowledge gaps and research need to be related to the development, evaluation and implementation of dengue vaccines.
- (2) **Consensus and guidance:** The scientific consensus for dengue control and guidelines on the evaluation of dengue vaccines need to be established.

- (3) Review and evaluation: The evidence base for the policy recommendations related to the introduction of dengue vaccines need to be reviewed and evaluated previously.
- (4) Vaccination program: The guidance and program on dengue vaccine administration, including implementation strategies need to be strictly conducted.
- (5) Support of NRAs: The NRAs should be assisted and encouraged in the review of dengue vaccine registration files.

## 2.2. Regulatory pathways

The regulators of developing countries and developers for dengue vaccines had two meetings in 2007. The first meeting with Developing Countries' Vaccine Regulators Network (DCVRN) was held on April 2007 in Brazil [8]. Their topics included the nature and epidemiology of the dengue fever, the status of dengue vaccine development and the regulatory issues needed to be addressed. The second meeting was held in Thailand on December 2007 and several dengue vaccine developers participated in this meeting [8]. Each of these companies presented the development status of their candidates and outlined the issue that they regard important for testing and want ultimate regulatory approval of these vaccines. The summary points for their meeting report are as follows [8]:

- (1) Regulatory agencies need to address some issues related to multivalent vaccines because a dengue vaccine is tetravalent and provide protection against all the four DENV serotypes.
- (2) The potential risk of inducing antibody-enhanced diseases such as ADE should be verified by long-term safety assessment.
- (3) Dengue vaccines need to be assessed in diverse populations because the epidemiology and impact of dengue are varied in different countries.
- (4) The NRAs in developing countries may be involved in review of the applications of clinical evaluation and the marketing of vaccines and they should receive support as appropriate.
- (5) Manufacturers must submit a dossier to the NRAs for review.
- (6) The NRAs need to have access to the necessary expertise to review the quality and safety of dengue vaccines and consider accelerating their introduction.
- (7) The improved standardized tests should be introduced for the diagnosis of early infection and for the measurement of immune protection.

On October 2009, WHO and the Pediatric Dengue Vaccine Initiative (PDVI) convened a meeting in Thailand. The topics covered in this meeting considered the interactions between scientific regulatory reviews and ethics committee reviews of applications to undertake clinical trials of dengue vaccines. Their main conclusion was that it would be better if scientific and ethical reviews can work together well, but there was no alteration in scientific or technical views about the regulation of dengue vaccines.



On October 2011, WHO convened a consultation of experts in dengue vaccine regulations to review the current scientific data regarding safety concerns associated with the live attenuated dengue vaccine. It was emphasized that a complete plan and a suitable method for long-term safety assessment are required to ensure the introduction and continuous application of dengue vaccines.

On July 2016, WHO issued a position paper to mention that countries are encouraged to establish a functional pharmacovigilance system to monitor and manage adverse reactions following immunization when dengue vaccines were introduced. Countries considering vaccination should also have a dengue surveillance system which is capable of detecting hospitalization and severe dengue cases consistently [5].

### 2.3. Special procedures

At the dengue vaccine development stage, some clinical trials can be omitted, although many issues regarding implementation of dengue vaccines still need to be addressed. For example, a dengue vaccine can be approved without local clinical phase III data but adopt other country's data in some countries such as Indonesia. Some vaccine candidates have progressed from animal trials directly to human clinical phase II and III in some countries such as India.

### 2.4. Summary of regulation requirements

It is crucial to select suitable sites for clinical trials of dengue vaccine candidates. The key consideration for selecting sites in developing countries where dengue is endemic includes investigator experience, DENV prevalence, NRA competence, the implications between rural and urban sites, acquisition of multi-year data on dengue incidence and the ability to detect clinical dengue cases. The PDVI is working with these sites in developing countries to enhance their capabilities to undertake clinical studies and safety surveillance. Many important issues have been identified for regulatory review of dengue vaccines. Plans are being developed to provide appropriate training to the NRAs to build their review capacity. Based on the results of the PDVI/DCVRN meetings on dengue vaccines, the regulation requirement of dengue vaccines is summarized as follows [8, 9]:

- (1) Diligent safety surveillance: Because of possible immune enhancement in individuals who are only partially immunized and become naturally infected or who have been previously infected and receive a first vaccine dose, it is required to frequently monitor the safety of dengue vaccines in the initial stages of clinical trials. Improved definitions of adverse events following immunization are needed. In addition, methods to detect these events should be validated. It is necessary to perform improved safety surveillance and early viral analysis in cases of dengue fever.
- (2) Assurance a favourable risk-benefit for the specific country: The NRAs in dengue-prevalent countries must consider new approaches to accelerate vaccine development. They will be requested to discuss on licensure of a tetravalent dengue vaccine with demonstrated efficacy and safety against only one DENV serotype. However, under this condition, post-marketing surveillance for safety and efficacy against all four serotypes is required.

- (3) The vaccines used in testing and clinics should be the same: Vaccines being tested in subsequent studies should be the same as those being used in earlier clinical stages. Any changes in manufacturing processes or formulation are critical and could result in the need to repeat the clinical trials or require complex studies.
- (4) “Standard definitions” should be commonly understood: Standardized testing methods for antibody responses and virus typing are essential, especially for laboratory-based serological tests. The validated international reference standards need to be established. In addition, phase IIa, phase IIb and phase III trials should be clearly defined.
- (5) An animal model is not necessarily required: The development of an animal model would be of great benefit but likely not essential for licensure. Clinical trials could provide data to help the NRAs to understand the science of the possible severe immune-enhanced disease and identify correlates of immunity/protection that would assist vaccine development.
- (6) Characteristics of clinical trial design should be defined: An effort to design clinical trial and consult the NRAs is the duty of the vaccine manufacturers. These issues include target age group for immunization, vaccine dosage schedule, trial duration and follow-up, possible immune responses to other flaviviruses, diagnosis and case definition and long-term safety surveillance. Additional considerations for assessing potential trial sites include the prevalence of the viral strains, influence of concurrent mosquito control programs, community involvement, and virological and diagnostic services.
- (7) Phase III trials data can be conditionally omitted: For vaccine approval, the responsible NRAs need to establish special procedures to review and accept other country data. Phase III trials may be undertaken in some countries based on the safety and efficacy data from Phase II (a or b) trials in other countries.
- (8) Joint review of license applications is recommended: The DCVRN prefers the formal procedures for collaboration and joint review of clinical trial monitoring including good clinical practice (GCP) inspections by the responsible NRAs, European Medicine Agency (EMA) and/or United States Food Drug Administration (US FDA) with facilitation by WHO. The NRAs would need to have access to the necessary expertise and advisory committee to review the quality, efficacy and safety aspects of the license application.

## 2.5. Regulatory challenges

The development of dengue vaccines in developing countries has been recently accelerated due to the substantial funding increase from public institutions and private sources. Along with the progress of vaccine development, two complex regulatory issues arise as follows [8]:

- (1) Clinical trials data are limited in developing countries: Dengue is prevalent only in tropical countries where there is poor public health. It is almost impossible to perform clinical trials for dengue vaccines in developed countries. Clinical trials to assess the safety and efficacy of dengue vaccines are usually carried out in developing countries where dengue is prevalent, and the first licensure of dengue vaccines will occur there.



- (2) Regulatory review and license approval are challenging in developing countries: It is required to ensure that developing countries have the ability to undertake appropriate regulatory review of proposed clinical studies and of applications for licensure. The NRAs of these developing countries must have the information, training and capabilities to review clinical trials and approve applications eventually.

### 3. Product

In this section, we introduce the newly licensed dengue vaccine in endemic countries and some developing dengue vaccines based on the regulation requirements.

#### 3.1. Philosophy of development

Dengue has become an increasing threat around the world along with the transmission of the disease-carrying mosquitoes and the itinerary to dengue-endemic areas. To reduce the social and economic burden of dengue, before 2020, WHO aims to decrease the overall dengue mortality and morbidity by 50 and 25%, respectively. In the absence of specific antiviral therapy, effective vector control is the only strategy to mitigate the incidence rate of dengue. Vector control interventions have not been satisfied in reducing dengue transmission due to increasing rural-urban migration, rapid population growth, unplanned urbanisation and insecticide resistance in mosquitoes. The need for an effective dengue vaccine is obvious because a safe, efficacious and economic dengue vaccine can be a supplementary measure for dengue prevention and control. Vaccine development focuses on the generation of a tetravalent vaccine to provide long-term protection against the four DENV serotypes (DENV-1, 2, 3 and 4). Additionally, vaccination of target groups such as travellers and migratory population may be a suitable strategy to prevent the spread of dengue to non-endemic areas or dengue-free regions. However, there was no commercially available dengue vaccine before 2015.

#### 3.2. Successful product: Dengvaxia®

The first dengue vaccine, known as Dengvaxia® (CYD-TDV) by Sanofi Pasteur, was registered in Mexico on December 2015 and has been licensed in many countries, including Mexico, Brazil, Philippines, El Salvador, Costa Rica, Guatemala, Peru, Indonesia, Paraguay and Singapore till 2016. It is approved for use for those aged 9–45 and recommended to prevent four DENV serotypes (DENV-1, 2, 3, and 4) [10–12].

The significant reduction in disease burden using Dengvaxia® has been demonstrated in recent research. For individuals that have been already exposed to at least one DENV, the vaccination program is most effective. Immunological screening of the population prior to vaccination is recommended. When the vaccine is given only to partial immune individuals, disease burden decreases considerably. Vaccination strategies must be planned based on epidemiological disease dynamics for each specific endemic region [10].

### 3.3. Production of Dengvaxia®

Dengvaxia® is designed to induce the immune system to produce antibodies against all four DENV serotypes (DENV-1, 2, 3, and 4). It is a live attenuated tetravalent chimeric vaccine developed using recombinant DNA technology by replacing the PrM (pre-membrane) and E (envelope) structural genes of the yellow fever live attenuated vaccine [10, 11]. For the vaccine, the virus is genetically engineered to include genes encoding for dengue proteins. Its production is based on a weakened combination of the yellow fever virus and each of the four DENV serotypes.

### 3.4. Efficacy of Dengvaxia®

Dengvaxia® was given as a three-dose series on a 0/6/12 month schedule and has been evaluated in two Phase III clinical trials. The results have been proved for each trial and pooled. The recent research has found that the vaccine was effective in reducing about 60% dengue, 65.6–81.9% hospitalization and in preventing 80–90% of DHF cases in individuals 9–45 years old [2, 10, 11].

### 3.5. Disadvantages of Dengvaxia®

The transmission dynamic model and clinical trial data have demonstrated that Dengvaxia® effectiveness depends mainly on the age group vaccinated and local transmission intensity [10]. Therefore, the new vaccine still has some disadvantages as follows:

- (1) Reduction rate is low: In clinical trials, the vaccine only reduced the chances of developing the disease by about 60%.
- (2) Not effective for all ages: The vaccine is only approved for use in people 9–45 years old. In fact, the vaccine seems to be least effective in children younger than 9 who need the vaccines most.
- (3) Side effects possibly occur: Vaccinated individuals potentially have more severe cases of dengue if they contact DENVs later in life.

### 3.6. Developing products

Several other DNA vaccine, live-attenuated vaccine, inactivated vaccine, subunit vaccine and chimeric vaccine candidates are being developed at early stages of clinical trials by some manufacturers (Table 1) [13–15]. Other vaccines using virus vectors and virus-like particles (VLP) are being evaluated in preclinical studies [15].

- (1) DNA vaccine: DNA vaccine candidates have been used to induce cellular immunity against various antigens using *in vitro* or animal models. This technology is based on cloning a specific gene into a bacterial plasmid containing a strong promoter for expression in mammalian cells.

- (2) Live-attenuated vaccine: Live attenuated vaccine candidates, made of weakened viruses, are excellent immunogens because they can induce both humoral and cellular immune responses similar to a natural infection, but their virulence is not enough to cause diseases.
- (3) Inactivated vaccine: Inactivated vaccine candidates, made from viruses inactivated by heating or formaldehyde, are safer than live-attenuated candidates because of their inability of virulence reversion and no harm to immunocompromised individuals. A synergistic formulation with another live attenuated vaccine candidate (prime-boost strategy) is being evaluated.
- (4) Subunit vaccine: Recombinant subunit vaccine candidates, made of viral protein subunits, have wider safety profile. They will not produce the same immune responses as live attenuated vaccines because they contain only one or few viral proteins. The DENV envelope protein is the most immunogenic and usually used for vaccine production.
- (5) Chimeric vaccine: Chimeric yellow fever/dengue virus vaccine candidates are the most advanced vaccine, being evaluated on large-scale clinical trials worldwide. These are categorised as follows:
  - a. The chimeric live attenuated DENV-2/DENV vaccine (DENVax): The DENVax was constructed using the backbone of attenuated DENV-2 (PDK-53 strain) in cell culture, in which the prM and E genes of DENV-2 PDK-53 were substituted for those of wild-type DENV-1, 3 or 4.
  - b. The chimeric live attenuated DENV/DENV vaccine (TetraVax-DV): The TetraVax-DV is a tetravalent vaccine candidate for which attenuation was achieved by deleting 30 nucleotides on 3' untranslated region of wild-type DENV-1 and DENV-4. This approach did not result in any attenuation for the other two serotypes: DENV-2 and DENV-3.
- (6) Vectored vaccine: The vectored vaccine candidates, a recombinant poxviruses and adenoviruses expressing foreign proteins, have been demonstrated to induce strong humoral and cellular responses in humans. Several live virus vectors such as adenovirus, alphavirus and vaccinia virus have been engineered to express DENV E protein for further evaluation as dengue vaccine candidates.

### 3.7. Challenges of vaccine development

Though Dengvaxia® has been successfully developed and licensed in many countries, dengue vaccine development is time-consuming, costly and difficult. The following challenges are still ahead of us for the development of new dengue vaccines [15, 16].

- (1) Restriction of virus growth: The DENV is growing poorly in cell culture.
- (2) Limitation in immunization: Infection by one of the four DENV serotypes will provide lasting protection against homotypic reinfection, but only transient protection against a secondary heterotypic infection.

Vaccine candidate	DEN-Vax	TetraVax-DV	TDEN PIV	V180
Type	Chimeric vaccine	Chimeric vaccine	Inactivated vaccine	Subunit vaccine
Trait	A recombinant chimeric vaccine with DENV-1, 3 and 4 components on DENV-2 backbones	A tetravalent admixture of monovalent vaccines that were tested separately for safety and immunogenicity	An inactivated tetravalent vaccine	A recombinant subunit vaccine expressed in <i>Drosophila</i> cells
Ongoing clinical trial	Phase I and II	Phase I and II	Phase I and II	Phase I
Testing country, company or institute	United States, Colombia, Puerto Rico, Singapore and Thailand	Thailand and Brazil	GSK and the Walter Reed Army Institute of Research	Merck

**Table 1.** Dengue vaccine candidates under development and on clinical trials.

- (3) Incidence of antibody-enhanced diseases: The existence of four distinct DENV serotypes is capable of eliciting cross-reactive and ADE against the remaining three serotypes.
- (4) Lack of an animal model: A suitable animal for dengue vaccine studies is currently not available and may result in uncertainty for the correlates of protection.
- (5) Variation of the efficacy evaluation data: The neutralising antibody response to a specific DENV serotype is traditionally detected by plaque reduction neutralisation test (PRNT), but PRNT variations often occur in the collaborative studies. The variation may account for erroneous results in vaccine efficacy evaluation. Standardised guidelines need to be established by regulatory bodies like WHO for conducting PRNT.
- (6) Complexity of tetravalent vaccines: Dengue is mainly caused by four DENV serotypes; thus, an effective dengue vaccine must be tetravalent. Theoretically, it is possible for inducing immune responses in individuals not protected against all four serotypes. However, the tetravalent vaccine development is difficult and complicated because of interference among the viruses.
- (7) Emergency of more DENV serotypes: Discovery of DENV variants such as a sylvatic strain DENV-5 may impede the dengue vaccine initiative. Further genetic variability, ecology and epidemiology studies of these new strains are needed for the effective dengue vaccine development.

## 4. Perspectives

For successful vaccine introduction, it is essential to have early preparation and understanding of the true burden of dengue. Although a licensed dengue vaccine-Dengvaxia® has been available, there is need to ensure that appropriate surveillance is maintained to monitor its efficacy, safety and effectiveness during the post-licensure period. An evidence-based

approach is recommended to enhance and harmonize critical characteristic of dengue vaccines including case classification, data analysis and laboratory testing. The strengthening vaccination policy will require more investment in current public health systems; furthermore, R&D, advocacy and regulation requirements of vaccines should be emphasized. The increasing knowledge and technology will provide more insights to improve vaccine design and quality. If the following prospective comes true, the effect and impact of dengue vaccines will be significant.

#### **4.1. Enhancement of efficacy and safety**

Vaccine efficacy against dengue seemed to vary according to the serotype of the infecting DENV [17]. Additional pooled efficacy, integrated safety analyses from the clinical phase III efficacy studies and the ongoing safety studies should be used to confirm the efficacy and longer term safety of dengue vaccines.

#### **4.2. Enlargement of preventive age range**

In recent studies, age-related patterns for dengue vaccine efficacy were observed in the pooled estimated efficacies against severe dengue [17]. The prevention of dengue infection caused by four DENV serotypes in individuals mainly aged 9–45 years. It would be better if the vaccine can be improved to provide significant protection to those whose age is below 9 and over 45.

#### **4.3. Improvement of quality control**

The quality control of dengue vaccines is as important as the evaluation of their efficacy. Dengue vaccines currently are not included in any pharmacopoeia. Their production, characterization, identification, test and assay should be defined in the official pharmacopoeia. Vaccine manufacturers, testing laboratories and the NRAs must strictly follow the compendial definition, methods and guidance.

#### **4.4. Development of multivalent vaccines**

If someone exposes to only one DENV serotype and specific immunity is induced to protect against this serotype, the individual protected against only one serotypes may be subjected to a severe immune response such as ADE once the individual exposes to the other serotypes. The major hindrance of dengue vaccine development is the complex immune responses to DENVs and the difficulty in eliciting concomitant protection against all distinct DENV serotypes. It would be the best to develop a multivalent vaccine against all the DENV serotypes including DENV-1, 2, 3, 4 and 5 or more, not only restricted to some serotypes.

#### **4.5. Implementation of post-market surveillance**

The dengue vaccine should be closely monitored to ensure its persistent efficacy and safety after marketing. The vaccine approved for clinical application may be required to perform additional studies to give further evaluation and often address specific questions such as



safety, efficacy or possible side effects and contraindications. Local and global capacity for assessing the long-term safety of the dengue vaccine in post-licensing surveillance must be strengthened to meet the challenges imposed by its potentially complex performance [17].

#### **4.6. Revision or enactment of related regulations**

Regulatory requirements of dengue vaccine must be flexible and specific in developing countries. Continuous revision or enactment of guidelines, regulations and laws for dengue vaccines is needed to expedite new vaccine discovery. Conditional license approval should be set up and clearly defined in that the dengue transmission is diverse in different countries and local areas. In addition, the mechanisms of professional review and international cooperation for vaccine clinical application must be established.

### **5. Conclusions**

Although many developmental and regulatory challenges are confronted, the dengue vaccine discovery is rapid and efficient. The product Dengvaxia® has been approved in several countries and a number of vaccine candidates are being developed in different phases of clinical trials. Efficacy of the vaccine candidates is variable due to varying epidemiology of the disease in diverse populations. The situation may further be complicated by the emergency of a new serotype of DENV such as DENV-5 which is different from the original four serotypes (DENV-1, 2, 3 and 4) was isolated. Sustained transmission of a new serotype may become another obstacle in the future development of dengue vaccines. Hence, it is required to strengthen the surveillance of the disease prior to any dengue vaccine is introduced into the clinical application. The safety of vaccines in clinical trials should be evaluated for longer period of time and larger populations should be involved because of its potential risk of inducing immune enhancement such as ADE. In addition, it is urgent to develop and standardise diagnostic approaches for better prediction of the protective immune responses in dengue.

Regulations are the most important criteria for the development and marketing of dengue vaccines. Regulatory science is evidence-based and any improvement or change should be dependent on scientific data. Therefore, more epidemiological and clinical data for DENV are essential for dengue vaccine research and development (R&D). Continuous revision or establishment of flexible and specific laws, regulations and guidance for dengue vaccines will expedite new vaccine discovery. Dengue is usually prevalent in developing countries with diverse social, cultural, economic and scientific development. Also, the dengue epidemiology and disease burden are quite different in these countries. Every country has specific conditions for dengue transmission, prevention and control, regulations for dengue vaccines should be enacted and implemented in accordance with the needs of individual countries and the situation of local circumstances.

In spite of some limitations, the collaborative effects of regulatory bodies such as WHO, PDVI and policy makers with vaccine manufacturers to facilitate vaccine R&D and standardize field trials are significant. The NRAs are working together to cooperate with the vaccine



manufacturers to accelerate dengue vaccine development and standardise their testing. The current object is to strengthen the dengue surveillance network, explore the social and economic burden of dengue, identify clinical trial areas and support basic research to produce safe and effective dengue vaccines. In the near future, dengue vaccination may become an effectively preventive measure to substitute for vector control to suppress the increasing global burden of dengue.

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