Development of a Dengue Vaccine in Humans: So Near, Yet So Far

Dinesh Subedi1,2 and Andrew W Taylor-Robinson2*
1Department of Pharmacy, Little Buddha College of Health Sciences (Purwantchol University), Kathmandu, Nepal
2School of Medical & Applied Sciences, Central Queensland University, Rockhampton, Australia

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*Corresponding author: Prof Andrew Taylor-Robinson, School of Medical & Applied Sciences, Central Queensland University, Rockhampton, QLD 4702, Australia; Tel +61749232008; Email: a.taylor-robinson@cqu.edu.au

Abstract

Due to a combination of favourable factors promoting the population growth and geographical spread of the Aedes mosquito vector of dengue, the incidence of this rapidly emerging tropical infectious disease has increased over 30-fold in less than half a century. Today, there are around 100 million reported clinical cases each year. Co-circulation of multiple serotypes of the dengue virus causes a variety of manifestations ranging from mild symptoms, via debilitating morbidity, to a severe form that, although relatively rare, has a high mortality rate. As current dengue control programs are severely strained, an efficacious vaccine to prevent infection is an urgent global public health priority. However, production of a dengue vaccine is compromised by an incomplete understanding of compounding factors including the existence of five now known serotypes, duration of protection provided by vaccination, dosing regimen and period, cost of production, and determination of the immune status of vaccine recipients. All these issues need to be considered in successful vaccine design. Encouragingly, prior success with immunization strategies against the closely related viruses Japanese encephalitis and yellow fever provides a precedent to deliver a safe, effective and affordable dengue vaccine. At least seven candidates are presently in clinical trials. These preparations are based on: DNA; protein subunit; purified inactivated virus; classic live attenuated virus; live attenuated homologous dengue chimera; live attenuated deletion mutation. Of these, CYD-TDV, the three-dose live recombinant tetravalent chimeric vaccine based on a backbone of the yellow fever 17D vaccine strain, has advanced furthest and it is hoped to soon be granted a commercial license.

Keywords: Dengue; Virus; Immunity; Vaccine; Serotype; Antibody

Introduction

The dengue virus (DENV) is a small, enveloped, positive sense, single-stranded RNA virus of the genus Flavivirus and family Flaviviridae, which typically causes an acute fever in humans [1]. Characterized by neutralization assays, there are five recognized serotypes, DENV-1 to DENV-5, the last of which is newly discovered [2]. DENV is transmitted between humans by mosquitoes of the species Aedes aegypti and, less commonly, Aedes albopictus [1]. During the 19th century dengue was viewed as a sporadic disease, with long intervals between epidemics. However, this pattern has changed profoundly and now dengue ranks globally as the most significant arthropod-borne viral disease. The presentation of infection ranges from a mild self-limiting febrile illness to severe forms like haemorrhagic fever with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, increasing haematocrit (with decreasing platelets)), and life-threatening shock syndrome (with profound plasma leakage, severe bleeding, or organ failure) [3]. In recent decades, the worldwide prevalence of dengue has increased greatly, due in part to variance in serotype genetic diversity, geographic origin and distribution. The disease is now endemic in more than 100 countries in Africa, the Americas, the eastern Mediterranean, South East Asia and the Western Pacific, such that over 2.5 billion people face threat of infection. Current global annual estimates are that 390 million people are infected by dengue, of which 96 million present with clinical or subclinical severity [4]. Of those persons, 500,000 require hospitalization for life-threatening complications, with up to 20,000 fatalities [1].

Serotype-specific protection is typically achieved by infection with one dengue serotype conferring long-term resistance to re-infection by the same serotype. However, when cross-reactive antibodies [5] and/or cross-reactive T cells [6,7] are elicited through re-infection by a heterologous dengue serotype, the potential risk of antibody-dependent enhancement of disease increases. Secondary infection is the major risk factor for dengue haemorrhagic fever. Thus, a successful vaccine against DENV should attain pan-serotype immunity without stimulating associated pathology. Strategies have targeted tetravalent formulations that can provide collective protection to all four well-characterized DENV serotypes. Currently, a number of candidate tetravalent DENV vaccines are in different phases of preclinical and clinical evaluation [8]. Research on DENV-5 is only starting but this serotype will factor in future vaccine formulations.

Dengue vaccine development

In a coordinated attempt to meet the demands of producing an efficacious dengue vaccine to target disease prevention, a consortium of many research groups has been working together,
supported by vaccine manufacturers, clinical trial coordinators, policy-makers and funding agencies. Development of safe and effective vaccines to prevent infection with viruses that are close relatives of dengue such as yellow fever, Japanese encephalitis and tick-borne encephalitis has demonstrated a proof of principle for raising a protective vaccine against DENV [9,10]. However, unlike the above monotypic species, the existence of multiple serotypes of DENV which may be responsible for different episodes of mild to malignant disease confounds vaccine design. For example, repeated infection with heterotypic serotypes often results in severe dengue [11]. Hence, it is optimal to construct a vaccine that is simultaneously effective against all known serotypes; thus, the vaccine must be tetravalent (or arguably now pentavalent). All prototype vaccines under development are founded on the principle of eliciting a primary immune response that confers protection from illness by producing DENV-neutralizing antibodies, so vaccine candidates target a high concentration of neutralizing antibody to all DENV serotypes [9,12,13].

There are many challenges to developing multivalent dengue vaccines. An incompletely understood mechanism of protective immunity against dengue is the first hurdle. While neutralizing antibodies to DENV are recognized to provide protection against infection, the actual quantities required to remain to be determined. Also, little is known of the roles of other immune effector arms such as innate and T cell-mediated functions [14]. A second problem identified with vaccines is antibody-dependent enhancement (ADE) of dengue pathogenesis. Immune enhancement is possibly associated with non-neutralizing cross-reactive antibodies [15]. In order to address this issue, tetravalent vaccines which produce balanced immunity against four serotypes are being tested in field trials. However, antibody titers may decrease after vaccination and, again, ADE may become problematic. Lastly, currently no animal experimental model – in which valuable pre-clinical investigations may be performed – precisely duplicates human dengue infection. The published literature suggests that the use of mice and non-human primates (NHPs) produces promising results [8,13,16-18]. However, neither truly recapitulates DENV pathogenesis and the host immune response in humans. Efforts are focused on developing an improved mouse model for DENV infection and disease [19].

A variety of immunization strategies is being applied to develop a dengue vaccine. Each technology has its own strengths and weaknesses and which are in different stages of preclinical and clinical development (Table 1). Live attenuated candidates were in the front line of vaccine development, and their clinical evaluation is now well advanced. The next approach, a tetravalent DNA-based vaccine, contains a plasmid encoding prM and E genes of all four then known DENV serotypes. This was prepared as a joint venture between the Naval Medical Research Centre and the Walter Reed Army Institute of Research (WRAIR), USA, and is in phase 1 clinical trials. Due to the comparatively weak immunogenicity of DNA constructs, this is administered with adjuvant or in combination with a subunit vaccine [20]. A vaccine that incorporates full length prM and 80% E protein derived from each of four serotypes is another candidate in clinical evaluation. It was developed by Merck and has recently completed a phase 1 field trial [21]. In addition, a tetravalent purified inactivated vaccine has been developed in Vero cells by WRAIR, GlaxoSmithKline (GSK) and the Brazilian Oswaldo Cruz Foundation. Its advantages include reduced reactogenicity, no competition between components and its suitability for immunocompromised persons. However, the immune response is less broad, potent and durable. It was evaluated in phase 1 clinical trials in USA and Puerto Rico [22,23].

**Live attenuated dengue vaccine**

A live attenuated vaccine (LAV) contains a formulation of the viable microorganism that has been weakened under laboratory conditions as a result of which it can cause no or only very mild disease symptoms [24]. It may be genetically modified such that one mildly infective organism is manipulated to carry antigens from an unrelated pathogen [25]. Since LAVs can replicate they elicit an elevated antigen-specific immune response in a vaccinee. Moreover, their mode of infection of an immunized person is similar to that of an infection with the wild-type virus. They stimulate strong cellular and antibody responses and often confer life-long immunity after only a single dose. The first human viral vaccine, for yellow fever, was based on LAVs and to date they have been used successfully against many viruses like measles, mumps, rubella (MMR combined vaccine), varicella (chickenpox), influenza (nasal spray) and rotavirus [25-28].

Due to the effectiveness of preparations based on virus attenuation, LAVs are one of the most appealing strategies for dengue vaccine design. Moreover, this method has proved successful for its close relatives yellow fever and Japanese encephalitis [24,25]. However, difficulty in achieving proper attenuation, altered growth patterns, doubtful genetic stability and unbalanced immune responses against multiple DENV serotypes are major difficulties encountered during development of this type of vaccine [14]. This approach was first adopted by Sabin in 1945 by passaging DENV-1 in mouse brain but the degree of attenuation varied with virus strain and side effects developed in human volunteers [26]. Later, Halstead and Marchette attenuated the virus in a primary dog kidney (PDK) cell line. This tetravalent preparation was shown to be safe in phase 1 and phase 2 clinical trials [29,30]. Unfortunately, unpredictable molecular changes due to replication inside the vaccinated host were noted, following which plans for phase 3 studies were suspended [31-33].

**Live attenuated yellow fever/ dengue (CYD) vaccine (chimeric YF 17D/DENV)**

As an alternative approach to vaccine development, genetic engineering has been applied to create effective tetravalent dengue LAVs. These include a live attenuated hybrid yellow fever/tetravalent dengue vaccine (CYD-TDV) that is considered a leading candidate since it is currently at the most advanced stage, phase 3, of clinical development [10]. This research pathway was started at the US National Institutes of Health and St. Louis University and was progressed further by Acambis, now part of Sanofi Pasteur [10,34,35]. CYD-TDV is based on the
The vaccine is named ChimeriVAX®.

DCs followed by a controlled limited inflammatory cytokine profile 

In vitro and phenotypically, YF 17D demonstrated good stability both in vitro and in vivo. Moreover, when compared in vitro with their wild-type parents, CYD 1-4 showed an acceptable clinical safety profile and promising immunogenicity [39-41]. Vigorous viraemia together with production of neutralizing antibodies that provides immunity to each serotype of wild-type virus. Studies also indicated that the immunization regimen should be spaced sufficiently far apart to prevent the potential for interference between serotypes. A regimen of 0-6-12 months has been proposed [38,43]. Prior immunity to yellow fever did not interfere with DENV-specific immune responses, and did not induce significant YF 17D NS3-specific CD8+ T cell or dengue serotype-specific helper CD4+ T cell responses [38,43].

CYD-TDV was tested in a series of clinical trials in the US, Philippines and Mexico using cohorts of adults, adolescents and children. The first evaluation used a preparation of monovalent CYD-2 administered to healthy Flavivirus-naïve US adults. The results reported that CYD-2 is as safe as the yellow fever vaccine (YF-VAX®, Sanofi Pasteur) [36]. Moreover, viraemia developed in only a very short period while an antibody response to wild-type DENV-2 was detected in most subjects [36,37]. Individuals exposed previously to YFV 17 D showed stronger, broader and longer lasting production of antibody. In a subsequent tetravalent study, a mixture containing 5 log10 CCID50 of each serotype was

Table 1: Dengue vaccine candidates in clinical development.

<table>
<thead>
<tr>
<th>Technology</th>
<th>Vaccine developer</th>
<th>Developmental stage</th>
<th>Principle</th>
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<tbody>
<tr>
<td>DNA vaccine [20]</td>
<td>Naval Medical Research Center, USA</td>
<td>Phase 1</td>
<td>Plasmid encoding prM/E genes from each of DENV 1-4 serotypes</td>
</tr>
<tr>
<td>Protein subunit vaccine [21]</td>
<td>Merck, USA</td>
<td>Phase 1</td>
<td>Truncated E protein derived from each of DENV 1-4 serotypes</td>
</tr>
<tr>
<td>Purified inactivated virus vaccine [22,23]</td>
<td>WRAIR, USA GlaxoSmithKline (GSK), UK Oswaldso Cruz Foundation, Brazil</td>
<td>Phase 1</td>
<td>Uses killed wild-type virion containing all structural proteins and viral RNA</td>
</tr>
<tr>
<td>Classic live attenuated vaccine (LAV) [31-33]</td>
<td>Mahidol University, Thailand Sanofi Pasteur, France</td>
<td>Completed phase 2, but no further testing</td>
<td>Tetravalent LAV for genetic and phenotypic study, a mixture containing 5 log10 CCID50 of each serotype</td>
</tr>
<tr>
<td>Live attenuated YF17D/DENV chimeric virus [36,38]</td>
<td>Acambis, USA (acquired by Sanofi Pasteur in 2008)</td>
<td>Phase 3</td>
<td>Uses Yellow Fever 17D vaccine strain as a backbone, replacing the prM and E protein coding sequences in YF virus with DENV serotype sequences</td>
</tr>
<tr>
<td>Live attenuated DENVax chimeric virus [47,49]</td>
<td>Centers for Disease Control and Prevention, USA Inviragen, USA Takeda, Japan</td>
<td>Phase 2</td>
<td>Inserts structural protein genes from DENV-1/3/4 into DENV-2 PDK-53</td>
</tr>
<tr>
<td>Live attenuated DENV Δ30 deletion mutation vaccine [49, 57]</td>
<td>National Institute for Allergy and Infectious Diseases (NIAID), USA</td>
<td>Phase 2</td>
<td>Uses reverse genetics, including a 30 nucleotide deletion in the 3’ untranslated region of the genome of DENV 1-4 serotypes</td>
</tr>
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Aventis-Pasteur 17D yellow fever virus vaccine (YF 17D), which is used as a genetic backbone for carrying four recombinant dengue LAVs (DENV 1-4) [36]. It was constructed by inserting structural genes prM and E from each of four wild-type dengue virus serotypes into the corresponding genes of YF 17D. The serotypes used were PU0-359/TVP-1140 (serotype 1), PU0-218 Thai strain (serotype 2), Pah 881/88 Thai strain (serotype 3) and TVP-980 1228 Indonesian strain (serotype 4) [37]. The yellow fever virus retains capsid and non-structural proteins, as well as 5’ and 3’ untranslated regions (UTRs) which allows replication of recombinant virus, while the dengue components prM and E provide antigenicity. The vaccine is then prepared by electroporation of Vero cells with an RNA transcript prepared from viral cDNA of each of the DENV serotypes. The CYD-TDV was produced by combining the four CYD viruses into a single vaccine preparation containing 5 log10 CCID50 of each serotype [37,38]. The vaccine is named ChimeriVAX®.

Preclinical evaluation of CYD-TDV for genetic and phenotypic stability as well as for immunogenicity was performed in vitro on primary and transformed cells and in vivo in NHPs. Genetically and phenotypically, YF 17D demonstrated good stability both in vitro and in vivo. Moreover, when compared in vitro with their wild-type parents, CYD 1-4 showed an acceptable clinical safety profile and promising immunogenicity [39-41]. Vigorous growth of CYD 1-4 in monocyte-derived human dendritic cells (DC) illustrated that these viruses induce maturation of DCs followed by a controlled limited inflammatory cytokine response with consistent expression of type I interferons [38,42]. Immunogenicity tests in NHPs including rhesus (Macaca mulatta) and cynomolgus (Macaca fascicularis) macaques following primary immunization showed a short-lived, low level viraemia together with production of neutralizing antibodies that provides immunity to each serotype of wild-type virus. Studies also indicated that the immunization regimen should be spaced sufficiently far apart to prevent the potential for interference between serotypes. A regimen of 0-6-12 months has been proposed [38]. Furthermore, poor replication of CYD in the Aedes mosquito vector, high level seroconversion to three of the four tested serotypes, and mild to moderate and transient adverse effects were reported [38,43]. Prior immunity to yellow fever did not interfere with DENV-specific immune responses, and did not induce significant YF 17D NS3-specific CD8+ T cell or dengue serotype-specific helper CD4+ T cell responses [38,43].
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given to both dengue-naive and dengue-exposed US volunteers in a regimen of three injections at 0-3-12 months. This showed that for immunization across mixed age groups CYD-TDV is non-infective and antigenic for all serotypes; this is irrespective of whether the location is endemic or epidemic for dengue [37,38].

This favourable outcome of phase 1 studies prompted a phase 2 trial involving a three dose regimen inoculated six months apart with the aim of determining safety and immunogenicity of the tetravalent vaccine in larger populations with varying Flavivirus exposure and vaccination histories [38,44]. A secondary objective was to evaluate a possible alternative formulation. Conducted in Peru, the first phase 2 study indicated good safety and immunogenicity in children. A follow-up phase 2 study then evaluated the efficacy of any dengue vaccine candidate against clinical disease in a population naturally exposed to dengue [38]. In this protocol, the primary efficacy analysis was based on the number of dengue cases in vaccinated and control subjects over a one year period following completion of the vaccination schedule. The results were not significant and hence the efficacy of the current formulation remains in question [38]. A WHO advisory group of experts emphasised that to prove or disprove efficacy of CYD-TDV, further evaluation in larger populations and in different epidemiological settings is mandatory [14,45].

Recently, two separate phase 3 clinical trials of CYD-TDV were started and are currently ongoing. One study is assessing safety, efficacy and immunogenicity in South East Asia: Indonesia, Malaysia, Philippines, Thailand and Vietnam. In parallel, a second trial is taking place in Latin America. Preliminary results are encouraging; during the first two years following vaccination, there was no sign of an increase in serious adverse events. This extensive study provides further reassurance of the safety of CYD-TDV. If complete evaluation is favourable, it is widely anticipated that Sanofi Pasteur will apply for commercial licensure from global health regulatory authorities [14,38].

DENVax chimeras

This vaccine design is predicated on a DENV-2 construct that was developed in the 1980s at Mahidol University, Thailand. The recombinant chimeric vaccine contains an attenuated strain of DENV-2 that was attained by 53 serial passages in PDK cells of wild-type DENV-2 16681 recovered from a patient with severe dengue infection. Popularly known as DENV-2 PDK-53, it exhibits attenuated replication in mammalian and insect cell culture in vitro, attenuated neurovirulence in newborn ICR mice and attenuated replication in NHPs in vivo [46]. Furthermore, the DENV-2 PDK-53 strain has undergone intensive clinical investigation both as monovalent and multivalent preparations with attenuated DENV strains of other serotypes. The outcomes were very promising, showing no severe clinical symptoms, high immunogenicity and mild viraemia. The US Centers for Disease Control and Prevention are working collaboratively on this candidate with the biotechnology companies Inviragen and Takeda [47-49].

The chimeric DENV-2 PDK-53 tetravalent vaccine is based on the DENV-2 PDK-53-V variant, which possesses an extra unique mutation in NS3 gene. Mutation for attenuation of DENV-2 PDK-53 depends on the 5’ non-coding region and on genes encoding non-structural proteins 1 and 3 but is independent of structural genes prM and E. Thus, DENV-2 PDK-53-V variants were engineered to express structural genes of wild-type DENV-1(16007), DENV-3(16562) and DENV-4(1036) serotypes. The candidate preparation containing three chimeric viruses (DENV-1/3/4) plus the original DENV-2 PDK-53 strain is known as DENVax [50].

The genetic stability of DENV-2 PDK-53-based chimeras was investigated in detail by sequencing the genomes of DENVax virions, the results of which showed no reversion in attenuation properties for these chimeras [51]. Moreover, preclinical evaluation of DENVax has demonstrated safety and efficacy, with no mortality, neurovirulence or other signs of illness in newborn IRC mice. In testing for immunogenicity, AG129 mice were immunized with DENVax, resulting in production of neutralizing antibodies, titers of which were similar to those stimulated by each serotype of the wild-type dengue virus. This indicates a lack of interference among the components of the tetravalent formulation in the AG 129 mouse model [52]. In a separate study, chimeric DENVax was tested for safety, immunogenicity and efficacy in cynomolgus macaques using three different virus combinations. Each formulation was well tolerated and viraemia was very low. Dengue-specific antibody responses were also quite promising but levels varied between formulations. Overall, the potential shown by DENVax has informed the choice of this tetravalent preparation for progression to subsequent clinical evaluation [47].

The first phase 1 clinical trial, conducted on US healthy adult volunteers in collaboration with St. Louis University, assessed safety and tolerability of DENVax as well as evaluating neutralizing antibody titers to each of the four then known dengue serotypes following two doses administered 90 days apart [47]. DENVax induced neutralizing antibodies to all serotypes in a majority of samples and demonstrated to be safe and well tolerated. Furthermore, a second phase 1 evaluation is being performed on healthy Flavivirus-naïve adults in a high altitude, low endemic region of Columbia. Encouraging initial indications facilitated approval for a current phase 2 study that has enrolled individuals from US, Puerto Rico, Columbia, Singapore and Thailand. Full results of this trial will be published shortly but preliminary outcomes show that DENVax is both safe and effective, thus endorsing it as a potent dengue vaccine candidate [47].

Live attenuated DENV Δ30 vaccines

The capability of nucleotide deletions in the 3’-untranslated region (3’-UTR) to attenuate dengue viruses has been exploited to generate another live attenuated candidate. Researchers at the US National Institutes of Health produced a vaccine by two distinct recombinant methodologies [49]. Initially, a 30 nucleotide deletion from 3’-UTR (10478-10507) of a cDNA clone of the 814669 strain of DENV-4 Dominica (rDENV-4 Δ30) was safe and immunogenic in both rhesus macaques and humans [13,53]. However, attenuation was not consistently reliable. Therefore, a
second chimerization step was introduced in which the rDENV-4 Δ30 backbone was used to create intertypic recombinant viruses by replacing prM and E genes with those from each of the DENV 1-3 serotypes. These chimeric vaccines showed low infectivity to mosquitoes and high attenuation in macaques [13,49].

In preclinical evaluation, at high doses rDENV-4 Δ30 does does, however, elicit mild clinical effects and hepatotoxicity. Thus, in order to reduce infectivity and increase attenuation, two derivative mutants of rDENV-4Δ30 were genetically engineered; chimeric DENV-4 Δ 30 NSS-K_{200}A, H_{201}A(200,201) and chimeric DENV-4 Δ 30 NSS-S_{4995}R(4995) [54]. Both were attenuated in NHPs and in severe combined immunodeficiency (SCID) mice in which human hepatoma (HuH) cells are engrafted (the SCID-HuH-7 mouse model). The chemical mutagenesis protocol adopted was distinct for these two viruses. Mutation at amino acid positions 200 and 201 of the DENV-4 NSS polymerase gene, resulting in a paired change to alanine, causes a 40-fold reduction of viraemia compared to its parent rDENV-4 Δ 30 virus, as determined in SCID-HuH-7 mice [54]. Moreover, it was also highly attenuated in rhesus macaques, producing neutralizing antibody titers comparable to those induced by rDENV-4 Δ 30 virus. This promising preclinical profile has made rDENV-4 Δ30 NSS-S_{4995}R(4995), a suitable vaccine candidate for testing in human volunteers. For chimeric DENV-4 Δ 30 NSS-S\_4995R(4995), mutagenesis at nucleotide position 4995, resulting in a serine to leucine change at amino acid residue 158 of the NS3 polymerase, reduced virus replication in human liver cells in vitro. Hence, this has been considered as an alternative candidate for clinical evaluation [54,55].

In pursuance of a tetravalent formulation, the previously successful strategy of deleting 30 nucleotides from UTRs was applied to the remaining three serotypes. Attenuation of DENV-1 was as effective as for rDENV-4 Δ 30 virus, but suboptimal in DENV-2 and DENV-3. Consequently, prM and E genes from DENV-2 and DENV-3 replaced the corresponding genes of the rDENV-4 Δ 30 virus backbone (rDENV-2/-4 Δ 30 and rDENV-3/-4 Δ 30, respectively) [56]. These exhibited tolerable attenuation, safety and immunogenic profiles in rhesus macaques. In addition, each individual candidate triggered seroconversion but without noticeably harmful effects in humans. Various tetravalent formulations (TetraVAX-DV) were examined in a phase 1 clinical trial [56]. A formulation containing each of 3 log_{10} plaque-forming units of all four included serotypes induced the most balanced antibody responses. Seroconversion rates of each of DENV-1 to -4 were also encouraging in this initial evaluation, following which a further two-site clinical trial using an identical formulation is underway in Brazil and Thailand [56,57].

**Conclusion**

Although a dengue vaccine has yet to be released to the public, a wealth of data indicates that substantial progress has been made on the path to a first licensed product for use in humans. However, for this to be realized many issues remain to be resolved through further research. One important factor is virus-virus interaction as dengue may be caused by any of the now five recognized serotypes and hence for previously vaccinated individuals the possibility exists of natural challenge by one or more serotypes. Studies to date do not signify precisely the extent of any potentially harmful effects caused to vaccinees since immune enhancement is also associated with recurrent severe infection with different serotypes. In addition, the impact of prior infection with, and/or vaccination against, other *Flavivirus* members with which common epitopes are shared should be defined better in order to ensure dengue vaccine safety and efficacy in the general population. Most of the clinical trial data indicate that for the leading vaccine candidate the dosing schedule is long (up to one year), which arguably casts doubt on its safety in endemic areas. How to optimize safety, tolerance and efficacy of immunization regimens in infants and immunocompromised patients presents a particular challenge to vaccinologists.

As dengue occurs primarily in developing countries, from what source(s) the expense of licenced vaccine manufacture and distribution will be met is also a matter for consideration. Even if scientists succeed in constructing a commercially approved vaccine, will it be available to all people at low cost? In this context the final hurdles of vaccine production may be higher than one would hope. Nevertheless, many candidates are in the development pipeline. The possibility exists of introducing more than one generation of vaccine within a short time frame. However, complementary control strategies to be utilized in combination with a vaccine to combat spread of this globally re-emerging disease should be explored. For instance, controlling the *Aedes* vector by introduction of *Wolbachia*, a naturally occurring and harmless symbiotic bacterium of other insects that naturally reduces the ability of mosquitoes to pass dengue between people, holds considerable potential to reduce dengue transmission in epidemic areas [58]. A valid experimental model would mimic effectively the way in which DENV multiplies and causes pathology in its human host; this would greatly accelerate our understanding of the mechanism of protective immunity on which vaccination is predicated. The availability of such a powerful investigative tool to perform basic preclinical research would likely shorten the currently extensive period of time required for applied preclinical and clinical evaluation.

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