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Next generation dengue vaccines: A review of the preclinical development pipeline

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ABSTRACT

Dengue represents a significant and growing public health problem across the globe, with approximately half of the world's population at risk. The increasing and expanding burden of dengue has highlighted the need for new tools to prevent dengue, including development of dengue vaccines. Recently, the first dengue vaccine candidate was evaluated in Phase 3 clinical trials, and other vaccine candidates are under clinical evaluation. There are also a number of candidates in preclinical development, based on diverse technologies, with promising results in animal models and likely to move into clinical trials and could eventually be next-generation dengue vaccines. This review provides an overview of the various technological approaches to dengue vaccine development with specific focus on candidates in preclinical development and with evaluation in non-human primates.

1. Introduction

Dengue is a mosquito-borne flaviviral disease that has spread to most tropical and many subtropical areas, creating a significant burden of disease and economic costs in endemic countries [1,2]. One recent prediction of the global burden suggests approximately 390 million dengue infections each year (95% credible interval 284–528), of which 96 million (C.I. = 67–136) are clinically apparent [2]. To date, specific dengue therapeutics are not available and disease prevention is limited to vector control and personal protective measures with little data to support their impact on clinical disease [3]. Thus, the development of a safe and effective dengue vaccine would represent a major advancement in the control of the disease. One candidate has been evaluated in Phase 3 trials in Asia and Latin America. The vaccine, a three-dose live recombinant tetravalent dengue vaccine based on the YF17D backbone (CYD-TDV), demonstrated efficacy in the first year of the observation period (from 28 days after the third dose) of 56.7% in Asia [4] and 60.8% in Latin America [5]. In longer-term hospital-based follow up, a signal of increased risk of severe and hospitalized dengue was identified in the 2–5 year age group in Asia, with a relative risk of hospitalized dengue in year 3 post-dose 1 of 7.45 (95% CI 1.15–313.80) [6]. The mechanism behind this increased risk is not understood [7], and the sponsor has recommended an indication for individuals 9+ years of age [8]. Second generation vaccines may improve on the range of the age indication, dose-scheduling, or efficacy, as well as contribute to vaccine supply security.

This is an update of a 2011 review focusing on dengue vaccine candidates in preclinical development [9]. It is based on published data and written updates solicited from vaccine developers and researchers. Primary focus was given to candidates who are in active development and have been evaluated in non-human primate (NHP) models (Table 1). The first part of the review presents an overview of dengue immunity, challenges to vaccine development and the various technologies used, while the second part provides a more detailed description of specific vaccine projects in preclinical development.

2. Overview of dengue vaccine development

2.1. Dengue immunity and challenges to vaccine development

The disease dengue is caused by four serotypes of dengue virus (DENV), DENV-1 to DENV-4. Multiple serotypes of DENVs co-circulate in endemic areas [10]. Infection by one serotype confers lasting protection against disease, and possibly infection, following re-exposure to the same serotype, but only transient protection against secondary infection by one of the three heterologous serotypes (reviewed in [11]). Dengue vaccine development efforts therefore aim for a vaccine which simultaneously

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Table 1
Active dengue vaccine candidates in preclinical development that have been evaluated in NHP models.

Technological approach	Vaccine developer	Antigen	Valency under evaluation or evaluated in NHP
Recombinant subunit vaccines	IPK/CIGB	EDIII-p64k fusion proteins and EDIII-capsid fusion proteins expressed in *E. coli*	Monovalent
	VaxInnate	Bivalent 80E-STF2 fusion proteins expressed in baculovirus/insect cells	Tetravalent
	NHRI	Tetravalent consensus EDIII protein expressed in *E. coli*	Tetravalent
DNA vaccines	NMRC	Tetravalent "shuffled" prM/E expressed from plasmid vector	Tetravalent
	CDC	prM/E expressed from plasmid vector	Tetravalent
VLP Vaccines	ICGEB	EDIII-HBsAg VLPs or ectoE-based VLPs expressed in *P. pastoris*	Tetravalent
Virus-vectored vaccines	Themis Bioscience/Institut Pasteur	Tetravalent EDIII and DENV-1 ectoM expressed from live-attenuated measles virus vector	Tetravalent
	Global Vaccines	E85 expressed from single-cycle VEE virus vector	Tetravalent
Purified inactivated virus vaccines	NMRC	Psoralen-inactivated DENV	Monovalent
	WRAIR/GSK/FIOCRUZ	Purified inactivated DENV	Tetravalent
	Global Vaccines	Inactivated virus (+VEE-particle adjuvant)	Tetravalent
Live attenuated virus vaccines	Chiang Mai University/Mahidol University/NSTDA/BioNet-Asia	DEN/DEN chimeric viruses	Monovalent
	Arbovax	DEN host range mutations	Tetravalent
	Beijing Institute of Microbiology and Epidemiology	DEN-SA 14 14 2	Monovalent
	Novartis Institute for Tropical Diseases/Agency for Science, Technology and Research, Singapore	DEN targeted mutation (2′-*O*-methyltransferase mutant)	Bivalent
Heterologous prime-boost approaches	NMRC/WRAIR	Purified inactivated DENV or plasmid vector expressing prM/E (prime) and live attenuated DENV (boost)	Tetravalent
Simultaneous administration	FIOCRUZ	DENV prM/E expressed from live attenuated chimeric YF 17D/DEN virus with DNA vaccine	Monovalent

provides long-term protection against all DENV serotypes, and current approaches focus on the development of tetravalent vaccines.

The mechanism of protective immunity against dengue is not fully understood. There is considerable evidence for a major role of antibody-mediated DENV neutralization in protection against infection and disease [11]. However, it is unclear what quantity and/or quality of neutralizing antibody is needed for protective immunity. Further support for this conclusion is the apparent dissociation of the protective efficacy of CYD-TDV and the elicited virus-neutralizing antibody of individuals in vaccine clinical trials, when measured in Vero cell-based $PRNT_{50}$ assays [12,13]. Recent studies have begun to better define the cell-specific characteristics associated with *in vitro* virus-neutralization assays [14]. In addition, contributions of other immune mechanisms such as cytotoxic T cell responses are less clear (reviewed in [11,15]). Further research is needed to better define and validate immune correlates of protection for vaccine development.

Another challenge for vaccine development is the potentially detrimental role of immune enhancement in dengue pathogenesis. Severe disease is most commonly observed in secondary, heterologous DENV infections. Antibody-dependent enhancement (ADE) of infection has been proposed as the primary mechanism of dengue immunopathogenesis [16–18]. *In vitro* studies suggest that the capacity of DENV antibodies to contribute to neutralization or enhancement of infection is determined by multiple factors, including antibody specificity, antibody affinity, antibody titre and epitope accessibility (reviewed in [19,20]).

The potential risk of immune enhancement of infection and disease underscores the importance of developing dengue vaccines which produce long-lasting immunity to all four DENV serotypes. A tetravalent vaccine eliciting protective, neutralizing antibody responses against all serotypes should address theoretical concerns about vaccine-induced immune enhancement. However, vaccine-induced immune enhancement might again become problematic as antibody titres wane post-vaccination. The applicability of an immune enhancement hypothesis to explain the latest results in younger children in the CYD-TDV longer term follow up is unclear with currently published data [6].

DEN virions are composed of a lipid envelope modified by the insertion of envelope (E) proteins and premembrane/membrane (prM/M) proteins (depending on the maturation state [21]), surrounding a nucleocapsid composed of capsid (C) proteins and the viral RNA genome (reviewed in [22]). Human antibodies raised against the DEN virion are mostly targeted at the E and prM proteins [23]. Research to determine the most suitable target epitopes for vaccines is ongoing, and while considerable progress has been made in the characterization of the humoral immune response to DENV, important knowledge gaps still exist. There is evidence suggesting that anti-E antibodies have higher type-specific neutralization capacity and lower ADE potential than anti-prM antibodies. Moreover, most potent DENV neutralizing murine monoclonal

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