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Recent advances in human flavivirus vaccines

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Dengue (DENV), West Nile (WNV) and Zika (ZIKV) viruses are mosquito-transmitted flaviviruses that cause thousands of human deaths and millions of illnesses each year. In the last decades, epidemic outbreaks of all three flaviviruses emerged and caused a major health and economical problem in many parts of the world. The increasing and expanding burden of flaviviruses has highlighted the need for effective human vaccines against all three viruses. This review provides an overview of the recent progress in DENV, WNV and ZIKV vaccines development with specific focus on candidates in human clinical development.

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Introduction

West Nile virus (WNV) is a neurotropic virus infecting birds, horses and humans. Infection of humans is associated with a febrile illness that can progress to a lethal encephalitis with symptoms including cognitive dysfunction and flaccid paralysis [1]. Although the majority of infections are asymptomatic, elderly and very young people as well as immune-compromised individuals are at high risk of severe disease. Since its sudden appearance in North America in 1999, WNV has spread globally and is now endemic in Asia, Europe, the Middle East and the United States (U.S.) [1]. From 1999 to 2015, a total of 43 937 cases have been reported which resulted in 1911 deaths [2]. To date, WNV is the main cause of encephalitis in the U.S.

Dengue virus (DENV) is the most common mosquito-borne viral disease, resulting in approximately 100 million symptomatic cases annually [3]. The clinical spectrum of dengue disease is broad ranging from fever to severe disease with evidence of plasma leak, which can lead to death [4]. Dengue can be caused by any of four related viruses, termed serotypes (DENV1–4). Epidemiologic studies have determined that a secondary infection with a heterologous serotype is the greatest risk factor for developing severe dengue [5]. This is believed to be mediated by immune-mediated enhancement such as antibody dependent enhancement (ADE) [6•,7•]. The ADE hypothesis proposes that during a secondary infection, with a previously unencountered DENV serotype, the presence of heterotypic antibodies promotes viral uptake in monocytes and macrophages, via Fc receptors, leading to an increase in viral load. The major players of ADE are cross-reactive antibodies elicited against the precursor membrane protein (prM) and the fusion loop domain in the envelope (E) protein [8–10]. These antibodies are weakly neutralising due to the incomplete cleavage of prM in infected host cells [8]. Furthermore, the structural flexibility of E proteins on the virion only allows limited accessibility of these antibodies to their epitopes [11–13].

The most recent flavivirus outbreak was caused by Zika virus (ZIKV) after its appearance in South America in 2015 [14]. ZIKV was first discovered in 1950 in the forest of Uganda and has subsequently spread to Africa, Asia and the Pacific. The majority of infections are asymptomatic with some cases resembling dengue fever [15•]. However, symptoms can be more severe for pregnant women as ZIKV infection may result in microcephaly in fetuses and newborn infant [16]. Furthermore, recent epidemics linked ZIKV to Guillain-–Barre syndrome in adults [17]. Due to Because of the rapid increase in neurological cases, ZIKV was declared a public health emergency in February 2016.

WNV, DENV and ZIKV are members of the genus *Flavivirus*. The viral RNA genome encodes a polyprotein composed of three structural proteins (E, capsid protein and prM) and seven non-structural proteins [18]. Because of the fact that E proteins mediate viral attachment as well as viral fusion to host cells, most neutralising antibodies are generated against epitopes on the E protein [18–20]. The E protein is therefore considered as an optimal target for flavivirus vaccine development. Analysis of panels of human anti-DENV monoclonal antibodies

have identified potent neutralising antibodies which bind conformational epitopes on the E protein [21]. However, even though these conformational antibodies show potent neutralisation they are mostly serotype-specific. An exciting recent development by our group is the discovery of a new epitope for conformational quaternary antibodies, the E dimer epitope (EDE), which is conserved across all four DENV serotypes [13,22]. Thus, most EDE antibodies are capable to potently neutralise all serotypes. This finding has major implications for the future development of vaccines against DENV and perhaps other flaviviruses.

In this review, we address the current stage of vaccine development for WNV (Table 1), DENV (Table 2) and ZIKV (Table 3) with specific focus on candidates in human clinical development.

West Nile virus

Currently there are no licensed human vaccines to protect against WNV infection. However, several WNV vaccines have been licensed for veterinary use and are reviewed in [23]. A major reason for not developing a human vaccine is probably due to the lack of a substantial commercial interest. However, the need to develop an effective human vaccine emerged during the two largest WNV outbreaks in 2003 and 2012. A human WNV vaccine should induce high titers of neutralising antibodies as preclinical studies demonstrated a crucial role for antibodies in terminating viremia and preventing WNV dissemination [24,25]. T cells were also shown to play an important role in clearing WNV and limiting disease severity [26–28]. However, the protective role of T cells during infection has been questioned in several studies [28–30].

Vaccines under clinical trials

Live attenuated

The ChimeriVax-WN02 vaccine is a live attenuated human vaccine developed by Sanofi Pasteur. Genes encoding the WNV prM and E protein were inserted into the backbone of the yellow fever virus (YFV) 17D vaccine strain. To reduce neurovirulence and further attenuate the virus, three additional mutations were added on the E protein [31]. ChimeriVax-WN02 induced strong neutralising antibody and T cell responses as well as protected hamsters, mice and rhesus macaques against WNV challenges [31,32]. A phase I study in volunteers revealed that ChimeriVax-WN02 was well tolerated and highly immunogenic [33]. Two phase II clinical trials have also been completed with promising results in younger adults and in two older age groups [34•,35•].

Another live attenuated vaccine was developed by the National Institute of Allergy and Infectious Diseases (NIAID) and consists of the WNV prM and E genes inserted into an attenuated DENV vaccine strain (DENV4Δ30). The chimerisation of WNV with a non-neuroinvasive flavivirus and a 30 nucleotide deletion in the 3′UTR, highly attenuated the virus but still induced a strong immunogenic response in mice, geese and rhesus macaques [36,37]. This vaccine candidate was tested in two phase I clinical trials inducing a seroconversion rate against WNV above 80% in volunteers [38].

Inactivated

A research team at the Oregon Health & Science University created an inactivated WNV vaccine (HydroVax-001) using a novel, hydrogen peroxide-based process [39•]. In preclinical studies, immunised young and aged mice showed robust antibody and T cell responses and were protected against a lethal WNV challenge [40]. With the financial support of the NIAID, HydroVax-001 entered a phase I clinical trial in 2015.

Recombinant protein and DNA

The Vaccine Research Center (VRC) in collaboration with Vical developed a DNA plasmid vaccine (VRC-302) expressing the WNV proteins prM and E. In

Table 1

Human WNV vaccines currently in clinical development

Vaccine candidate	Developer	Vaccine type	Approach	Vaccination	Current stage
rWN/DEN4Δ30	NIAID	Live attenuated	DENV4Δ30 backbone expressing WNV prM-E	2 doses (0/6 months)	Phase I
ChimeriVax-WN02	Sanofi Pasteur	Live attenuated	YFV17D backbone expressing WNV prM-E	1 dose	Phase II
HydroVax-001	Oregon Health & Science University/ NIAID	Inactivated	Hydrogen peroxide-inactivated WNV Kunjin strain	2 doses (0/29 days)	Phase I
HBV-002	Hawaii Biotech	Recombinant subunit	Soluble WNV prM-E protein	3 doses (0/30/60 days)	Phase I
VRC 302/VRC 303	Vical/NIAID	DNA	Plasmid DNA expressing WNV prM-E	3 doses (0/28/56 days)	Phase I

National Institute of Allergy and Infectious Diseases (NIAID).