

Recent advances in human flavivirus vaccines

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Abstract

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.

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.

The most recent flavivirus outbreak was caused by Zika virus (ZIKV) after its appearance in South America in 2015[3]. 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[4]. However, symptoms can be more severe for pregnant women as ZIKV infection may result in microcephaly in fetuses and newborn infant[5]. Furthermore, recent epidemics linked ZIKV to Guillain-Barre syndrome in adults[6]. Due to the rapid increase in neurological cases, ZIKV was declared a public health emergency in February 2016.

Dengue virus (DENV) is the most common mosquito-borne viral disease, resulting in approximately 100 million symptomatic cases annually [7]. The clinical spectrum of dengue disease is broad ranging from fever to severe disease with evidence of plasma leak, which can lead to death [8]. 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[9]. This is believed to be mediated by immune-mediated enhancement such as antibody dependent enhancement (ADE)[10, 11]. 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 [12-14]. These antibodies are weakly neutralising due to the incomplete cleavage of prM in infected host cells[12]. Furthermore, the structural flexibility of E proteins on the virion only allows limited accessibility of these antibodies to their epitopes[15-17].

WNV, ZIKV and DENV 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]. Due to 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, 3 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 Centre (VRC) in collaboration with Vical developed a DNA plasmid vaccine (VRC-302) expressing the WNV proteins prM and E. In 2005, a successful phase I clinical trial demonstrated the safety and immunogenicity of the vaccine in humans [41]. Furthermore, all subjects developed neutralising antibodies at levels comparable to those seen in horses known to be protective. To enhance the immunogenicity of the vaccine, the promoter was modified and tested during a second phase I trial (VRC-303)[42]. VRC-303 induced a T cell response of greater magnitude when compared with VRC-302.

Hawaii Biotech focused on the development of a purified recombinant WNV subunit vaccine (HBV-002) containing the proteins prM and a truncated E (WN-80E). Immunising mice, hamsters and rhesus macaques with HBV-002 induced high titres of neutralising antibodies and protected animals against WNV challenges[43-45]. A phase I clinical study was initiated in 25 flavivirus-naïve volunteers. The vaccine was well tolerated and induced high neutralising antibody titers[46]. Further studies are planned by Hawaii Biotech to assess the safety of HBV-002 in young and elderly subjects.

Dengue Virus

The development of a DENV vaccine is challenging as it needs to be equally protective against each of the four serotypes to reduce the risk of immune-mediated enhancement. In 2015, the first licensed vaccine (Dengvaxia; CYD-TDV) for dengue was authorised for use and has now been approved in 11 countries. Dengvaxia was developed by Sanofi Pasteur and is a tetravalent live attenuated vaccine[47]. The DENV proteins prM and E for each of the four serotypes were inserted into the backbone of the YFV 17D vaccine strain. Two large phase III clinical trials have been completed in over 30,000 children resulting in an overall vaccine efficacy of 66% [48, 49]. However, the serotype-specific efficacy for DENV2 was poor with less than 43%. This stands in discrepancy with earlier work showing that the vaccine produced high

neutralising antibody titers against DENV2. One explanation for this mismatch might be due to the lack of an effective T cell response against DENV as Dengvaxia contains non-structural proteins from YFV. Further analysis of the trials also revealed a much lower efficacy in seronegative individuals compared to those who were seropositive for DENV at the time of vaccination. A danger signal was also observed during the long-term safety study which reported an unexplained increase in hospitalisation for severe dengue among children younger than 9 years old when compared to unvaccinated subjects [50]. As children below 9 years old are likely to be enriched for dengue-naïve subjects, Dengvaxia vaccination in these children might mimic a primary infection which may result in ADE upon a natural DENV infection. Thus, Dengvaxia is only recommended to be used in individuals aged 9 to 45 years as well as in dengue endemic regions with a seroprevalence of 70% [51].

Vaccines under clinical trials

Live attenuated

The NIAID developed a tetravalent live recombinant dengue vaccine (TV003). TV003 consists of all 4 full-length DENV serotypes which lack 30 nucleotides in the 3'UTR. In addition, the DENV2 component is a chimera in which the prM and E proteins of DENV4 were replaced by those of DENV2. Earlier clinical trials demonstrated robust tetravalent antibody and T cell responses in 74% of the subjects after a single dose of TV003 [52, 53]. The lowest serotype-specific response was obtained against DENV2 [53]. To assess the protective efficacy of TV003 towards DENV2, a human challenge model was performed using a DENV2 strain, rDEN2Δ30[54]. TV003 immunised individuals showed complete protection against rDEN2Δ30 challenge as judged by viremia. Since February 2016 a large-scale phase III trial has been launched in Brazil.

Another tetravalent live recombinant dengue vaccine was developed by Takeda (DENVax). DENVax consists of an attenuated full-length DENV2 component and three chimeras containing the prM and E genes of DENV1/3/4 expressed in a DENV2 backbone. The safety and immunogenicity of this vaccine were

demonstrated in phase I trials[55-57]. Using a two dose schedule, phase II studies were conducted in Puerto Rico, Colombia, Singapore and Thailand in individuals aged 1.5 to 45 years[58]. The seropositivity rate in all age groups was over 95% for DENV1-3 and 72.7-100% for DENV4. Furthermore, DENVax induced neutralising antibody responses in both seropositive and seronegative individuals. Since September 2016, Takeda started a phase III trial in Latin America and Asia.

Inactivated

GlaxoSmithKline in collaboration with Fiocruz and the Walter Reed Army Institute of Research (WRAIR) developed a tetravalent purified inactivated virus (TDENV-PIV). All four DENV serotypes were produced in insect cells and inactivated with formalin. Two phase I trials were conducted in Puerto Rico and the U.S. using dengue-naïve and dengue-primed subjects[59].

Recombinant protein and DNA

Merck's V180 is a tetravalent recombinant subunit protein vaccine consisting of prM and a truncated E protein (DEN-80E) [60]. In preclinical studies, this vaccine induced high titers of neutralising antibodies in mice and rhesus macaques [60]. A monovalent version of the vaccine, DEN1-80E, was studied in a phase I clinical trial [61]. Although the vaccine induced DENV1 neutralising antibodies, titers were modest and waned over time. Using V180 with the adjuvant ISCOMATRIX resulted in improved seroconversion rates [62].

The U.S. Naval Medical Research Center developed a tetravalent DNA plasmid vaccine (TVDV) with genes encoding prM and E proteins from each DENV serotype[63, 64]. The safety and immunogenicity of this vaccine was evaluated in a phase I clinical trial using the monovalent DENV1 candidate in flavivirus-naïve adults[65]. However, the vaccine induced a poor neutralising antibody response in humans. Work is currently ongoing to improve the immunogenicity of TVDV using the adjuvant Vaxfectin [66].

Zika Virus

There is no human ZIKV vaccine yet available despite its potential for causing severe neurological diseases. However, the sudden ZIKV outbreak in South America alerted health authorities and the public to develop a vaccine. Preclinical studies have demonstrated a protective role of CD8 T cells and antibodies against ZIKV infection, the latter being sufficient for complete protection [67-70]. However, co-circulation of DENV, WNV and ZIKV has been reported in several countries and previous studies suggested an immunological cross-reactivity between viruses, which may drive immune-mediated enhancement [71-73]. This hypothesis was recently confirmed in mice demonstrating that DENV or WNV specific antibodies are cross-reactive to ZIKV and enhance disease severity in whole organisms [74]. Thus, several aspects have to be considered for developing a ZIKV vaccine.

Vaccines under clinical trials

Inactivated

Using a formalin-inactivation vaccine approach, the WRAIR developed a human vaccine candidate for ZIKV (ZPIV). ZPIV was tested in preclinical studies and demonstrated robust antibody responses against ZIKV in mice and rhesus macaques as well as completely protected animals against ZIKV challenges[67, 68]. With the financial support of the NIAID, ZPIV vaccine entered its first human phase I clinical trial in November 2016.

Recombinant protein, DNA and RNA

Two DNA based vaccines expressing the ZIKV proteins prM and E are currently tested in healthy volunteers [67, 75]. Preclinical studies demonstrated that both vaccines induced high neutralising antibody titers and completely protected animals against ZIKV challenges [67, 68, 75].

Recently, a new messenger RNA (mRNA) vaccine candidate encoding the ZIKV proteins prM and E was generated by a research group at the University of

Pennsylvania [76]. A single dose of this vaccine elicited a potent and durable neutralising antibody response in mice and rhesus macaques as well as protected these animals from ZIKV challenges. The protective efficacy of this mRNA vaccine was also confirmed by an independent research group [77]. Furthermore, this group showed that the risk of ADE to DENV was reduced by introducing additional mutations in the fusion loop domain in the E protein. These modified mRNA vaccines show promising results for future human trials.

Conclusions

WNV, DENV and ZIKV have become a major health and economic burden in today's society. To prevent diseases and decrease viral spread, effective human vaccines are needed. There are currently no licensed human WNV and ZIKV vaccines. However, several vaccine candidates show promising results and may soon become available. The first licensed DENV vaccine, Dengvaxia, was recently approved in 11 countries. However, Dengvaxia is only recommended to be used in endemic regions as well as in individual aged above 9 years. Thus, the need for a more global and effective vaccine is still present. Two other DENV vaccine candidates (TV003 and DENVax) are currently assessed in phase III clinical trials and show promising results.

Highlights

- No WNV and ZIKV vaccines are available for human use.
- Some WNV vaccine candidates show promising results in phase II clinical trials.
- First licensed DENV vaccine (Dengvaxia – Sanofi Pasteur) available in 11 countries.
- Dengvaxia can only be used in dengue endemic countries and in individuals aged 9-45 years.
- DENV vaccine candidates (NIAID/Takeda) are currently in phase III clinical trials.

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Tables

Vaccine candidate	Developer	Vaccine type	Approach	Vaccination	Current Stage
rWN/DEN4Δ30	NIAID	live attenuated	DENV4Δ30 backbone expressing WNV prM-E	2 doses (0/6 mths)	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).

Table 1 Human WNV vaccines currently in clinical development.

Vaccine candidate	Developer	Vaccine type	Approach	Vaccination	Current Stage
CYD-TDV (Dengvaxia)	Sanofi Pasteur	live attenuated	YFV17D backbone expressing DENV1/2/3/4 prM-E	3 doses (0/6/12 mths)	Licensed
TV003	NIAID	live attenuated	full-length DENV1/2/3/4, lacking 30 nucleotides in the 3'UTR	1 dose	Phase III
DENVax	Takeda	live attenuated	full-length DENV2 & DENV2 backbone expressing DENV1/3/4 prM-E	2 doses (0/90 days)	Phase III
TDENV-PIV	GSK, Fiocruz & WRAIR	inactivated	formalin-inactivated DENV1/2/3/4	2 doses (0/28 days)	Phase I
V180	Merck	recombinant subunit	soluble DENV1/2/3/4 prM-E protein	3 doses (0/1/2 mths)	Phase I
TVDV	U.S. Naval Medical Research Center	DNA	Plasmid DNA expressing DENV1/2/3/4 prM-E	3 doses (0/1/3 mths)	Phase I

GlaxoSmithKline (GSK); National institute of allergy and infectious diseases (NIAID); Walter Reed Army Institute of Research (WRAIR).

Table 2 Human DENV vaccines currently licensed or in clinical development.

Vaccine candidate	Developer	Vaccine type	Approach	Vaccination	Current Stage
ZPIV	WRAIR/NIAID	inactivated	formalin-inactivated ZIKV	2 or 3 doses	Phase I
VRC-ZKADNA085-00-VP	NIAID	DNA	Plasmid DNA expressing ZIKV prM-E	2 or 3 doses	Phase I
GLS-5700	Inovio GeneOne	DNA	Plasmid DNA expressing ZIKV prM-E	3 doses (0/1/2 mths)	Phase I

National institute of allergy and infectious diseases (NIAID); Walter Reed Army Institute of Research (WRAIR).

Table 3 Human ZIKV vaccines currently in clinical development.

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