


## West Nile virus vaccines – current situation and future directions

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### ABSTRACT

West Nile virus (WNV) is a widely spread human pathogenic arthropod-borne virus. It can lead to severe, sometimes fatal, neurological disease. Over the last two decades, several vaccine candidates for the protection of humans from WNV have been developed. Some technologies were transferred into clinical testing, but these approaches have not yet led to a licensed product. This review summarizes the current status of a human WNV vaccine and discusses reasons for the lack of clinically advanced product candidates. It also discusses the problem of immunological cross-reactivity between flaviviruses and how it can be addressed during vaccine development.

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### Introduction

West Nile virus (WNV) is a single-stranded RNA virus which belongs to the family Flaviviridae, genus Flavivirus. This family includes several important human arthropod-borne pathogens, such as dengue (DENV), Zika (ZIKV), tick-borne encephalitis (TBEV), Yellow fever (YFV) or Japanese encephalitis (JEV) viruses.<sup>1</sup> WNV circulates in birds and is transmitted by many different mosquito species. These can infect other animals including horses and humans, which, although dead-end hosts, can develop disease symptoms. In humans, most infections remain asymptomatic or may lead to mild fever or headache.<sup>2</sup> Mainly older or immunocompromised individuals are at risk for more severe forms of WNV-induced disease, which occur in up to 1% of the infections.<sup>2</sup> Symptoms include high fever, but also neurological complications like encephalitis or meningitis. Fatality rates reach 10% among the severe cases.<sup>3</sup> Since its first description in 1937 WNV has caused several outbreaks in Africa, Asia and South Eastern Europe.<sup>4</sup> In 1999 the virus received much of public attention when its introduction into the USA was detected, which was followed by a rapid spread over the entire American continent. In the following years, thousands of people needed to be hospitalized, and more than 1,500 fatalities have been recorded until today. In addition, WNV led to countless severe infections among horses and caused the decline of local bird populations.<sup>5,6</sup> WNV outbreaks occur in unpredictable magnitude and localization. In Europe, a peak in WNV circulation was experienced in 2018, in total, 1,503 cases were counted. In addition to countries such as Italy and Greece, where WNV is endemic already for several years, the virus was detected for the first time in Germany.<sup>7,8</sup>

This increase in endemic areas over the last decades has made WNV the arthropod-borne human pathogenic flavivirus with the largest distribution worldwide.<sup>9</sup> WNV can be divided into five genetic lineages.<sup>10</sup> Lineage 1 has caused major outbreaks in the 1990s, including the epidemic in the

USA. In contrast, lineage 2 was traditionally associated with less pathogenicity. However, recently emerging variants of lineage 2 viruses have gained substantial epidemic potential and are responsible for many outbreaks in Europe and Africa.<sup>11,12</sup> Several mutations have been identified which can be linked to increased virulence in both genetic lineages, illustrating the high capacity of this single-stranded RNA virus to mutate its genome.<sup>13</sup>

### WNV vaccine development

Since the (re-)emergence of WNV in the late 1990, substantial research has been invested in the development of vaccines for human and veterinary use. For horses, this was successful, and several equine vaccines have been licensed.<sup>14,15</sup> For humans, no vaccine is yet available. To develop WNV vaccines, different technological platforms were employed, including those from the licensed vaccines for TBEV, JEV and YFV (attenuated strains and chemically inactivated viruses) in addition to novel and highly sophisticated technologies. These include recombinant proteins, virus-like particles, RNA-replicons, chimeric flaviviruses, viral vectors expressing WNV genes, DNA- and RNA vaccines. Several recent reviews have described these different approaches, so they will not be discussed herein further detail.<sup>9,16–19</sup> WNV immunization studies used different experimental setups, such as viral strains, immunization schedules or animal models. But taken together, they have led to critically important general conclusions:

- protection from WNV can be achieved by a large variety of immunization techniques
- major component of the protective immune response are antibodies against the E-protein. In fact, the E-protein is major or even single component of all vaccine candidates described to be successful

- E-protein-based WNV vaccines are protective against genetic lineages 1 and 2

Some of the vaccine candidates which proved to be protective in animal models were transferred to clinical testing in humans (Table 1). To date, clinical trials have been performed with a DNA vaccine, chimeric flaviviruses using the yellow fever vaccine strain or an attenuated DENV as backbones and a recombinant, insect-cell-derived E protein ectodomain.<sup>22-26</sup>

In addition, two inactivated whole virus vaccine candidates have been evaluated clinically. Firstly, two doses of a hydrogen peroxide inactivated WNV vaccine led to detectable neutralizing antibodies in approx. 50% of the study participants. The authors indicate that this result might be improved by adding a third dose or by using an alternative inactivation protocol which combines hydrogen peroxide and formaldehyde.<sup>20</sup> Secondly, three doses of formaldehyde inactivated WNV particles induced high titers of neutralizing antibodies.<sup>21</sup>

None of these WNV vaccine trials reported any adverse events or safety concerns which could impede further clinical testing.

WNV induced disease is most severe in the elderly; hence, the major target population for a vaccine has an aged immune system. This represents a challenge for vaccine developers with respect to immunogenicity and safety.<sup>27</sup> Antigens which are highly immunogenic in a fully functional immune system might need higher or more doses during immunosenescence. In addition, an immune system with reduced T-cell function might be unable to restrict replication of a vector or a virus which is normally attenuated. Some of the pre-clinical developments address these issues by using aged animals.<sup>28</sup> Likewise, clinical trials included groups with aged individuals. Both live virus vaccine candidates demonstrated high immunogenicity in individuals >50 years of age, and similar findings were reported from a phase I trial using the DNA vaccine.<sup>22,26,29</sup>

### Current absence of WNV vaccine candidates in late stages of clinical development

In light of these successful developments, a human WNV vaccine should already be on the market or at least in the final phases of clinical testing. But in contrast, none of the clinical studies proceeded after stage II, most strategies have stopped already several years ago.<sup>30</sup> Today, there is still no

human WNV vaccine, and there is no candidate even close to licensure. Current prevention strategies rely mainly on mosquito control programs.<sup>31</sup> Although these are much cheaper than vaccines, which require long and expensive clinical developments, their effects are transient. Immunization would clearly be a sustainable, long-lasting method to protect humans from disease caused by WNV.

In general, the reasons for the lack of a human vaccine may include scientific challenges (mainly in obtaining protective immunity), safety concerns, difficulties in clinical study design or economic considerations. As discussed above, it is possible to obtain protective immune responses to WNV by using a variety of different immunization technologies. Existing flavivirus vaccines also use different approaches: the YFV vaccine consists of an attenuated virus, whereas TBEV and JEV vaccines are made of inactivated pathogens. In addition, first clinical tests in humans have provided solid evidence for the capability of at least some of the candidates to elicit robust neutralizing antibody responses, which normally correlate well to protection against flaviviruses. Problems in obtaining protective immunity are therefore unlikely to be responsible for the poor outcome of clinical WNV vaccine development to date.

Initial clinical trials have provided good safety profiles for the WNV candidate vaccines. The existing flavivirus vaccines against YFV and TBEV are in use for decades and are generally well tolerated. On the other hand, a recently developed and (at least in some countries) licensed DENV vaccine has caused substantial discussion on vaccine safety. The tetravalent vaccine CYD-TDV was designed to protect against all four major DENV serotypes and consists of the YFV vaccine virus genetically modified to express the DENV prM/E proteins. Large clinical efficacy trials revealed a small, but significant, increase in the risk for hospitalization due to DENV in individuals naïve to the virus. In contrast, those with pre-existing DENV-immunity were protected from DENV-caused disease.<sup>32</sup> The exact reasons for these observations still have to be determined. Achieving vaccine-mediated protection against DENV is intrinsically challenging due to the need to equally protect against each of the four serotypes. It is debatable to what extent similar problems would occur with a WNV vaccine. Nevertheless, the only WNV candidate vaccine that underwent a phase II clinical trial so far consists of the same backbone-technology as CYD-TDV. It can, therefore, be assumed that the manufacturers first want to fully

**Table 1.** WNV vaccine candidates in clinical testing until today.

Candidate vaccine	Type	Key data to date	Most advanced clinical stage	References
Hydrovax-001	Inactivated using hydrogen peroxide	Neutralizing antibodies in 50% of individuals after two doses.	I	20
Inactivated WNV ChimeriVax-WN02	Inactivated using formaldehyde Recombinant yellow fever vaccine strain expressing the prM/E-fragment of WNV	Neutralizing antibodies after three doses. Neutralizing antibodies (>90%) in younger and older age groups after one dose	I/II II	21 22
rWN/DEN4Δ30	Recombinant attenuated DENV expressing the prM/E-fragment of WNV	Neutralizing antibodies in 89% of individuals after two doses.	I	23
HBV-002	Recombinant truncated E-protein	Neutralizing antibodies in all individuals after three doses	I	24-25
VRC WNV	DNA plasmid expressing the prM/E fragment	Neutralizing antibodies (>90%) in younger and older age groups after three doses	I	26

clarify the causes of the problems associated with CYD-TDV before the technology is further expanded to other infections.

Clinical vaccine development includes different phases, represented by studies of increasing scale. Whereas initial phase I and II testing for safety and immunogenicity can be performed with dozens to hundreds of participants, vaccine efficacy has to be demonstrated via large phase III studies in populations affected by the pathogen. As WNV-outbreaks occur sporadically and the affected regions vary, it is extremely difficult to evaluate the impact of vaccine-mediated protection in such an efficacy trial, which is planned for a specific region during a specific time span. Given the detailed epidemiological information available for WNV outbreaks in the USA since 1999, it might nevertheless be possible to identify hotspots with relatively high WNV incidence, which could be suitable areas for efficacy trials, but it is hard to predict for how long such a trial would need to run.<sup>18,30</sup> However, uncertainty in planning efficacy studies is not a WNV-specific problem, in fact, many emerging viruses show highly variable patterns in magnitude and localization of their outbreaks. Clinical ZIKV-vaccine developments are confronted with strongly decreasing case numbers in the areas affected most during the 2016 epidemic. As a consequence, vaccine developers are discussing alternative licensure models with regular authorities.<sup>33</sup> Such strategies include accelerated licensure pathways or the FDA animal rule, i.e. the licensure of a vaccine if suitable animal models for efficacy testing exist, developed primarily to encounter potential threats of bioterrorism.<sup>34,35</sup>

For most of these alternative licensure strategies, a correlate for protection is required. This means that a (usually immunological) marker which is indicative for the successful induction of a protective immune response has been established. A key result from vaccination studies with WNV is the capability of antibodies to mediate complete protection. This is highly similar to other flaviviruses, and in the cases of YFV or JEV, specific titers in neutralizing antibodies serve as a correlate for protection.<sup>18</sup> In case of vaccines for YFV, this threshold was established via experiments in non-human primates.<sup>36</sup> However, non-human primates are much less susceptible to WNV than they are to YFV, which complicates the establishment of such a threshold in these animals.<sup>37</sup> Likewise, the fact that most of the people infected with WNV do not develop clinical symptoms makes the identification of an antibody-mediated correlate of protection via clinical data challenging, even during larger scale trials.

Nevertheless, it cannot be excluded that a WNV vaccine could obtain market approval by using one of the alternative strategies instead of classical efficacy trials, however, this would require that the vaccine leads to clear clinical and also socioeconomic benefits. WNV can induce severe, even fatal disease. It has caused more than 1,500 deaths since 1999 in the USA alone and 181 deaths in Europe during 2018. Consequently, a WNV vaccine would save lives and would avoid the suffering of many people. However, economically it is debatable whether a vaccine would be beneficial to the health systems or whether the costs would be unacceptably high. Two published studies have addressed the cost-effectiveness of WNV vaccination in the USA, one in

2006,<sup>38</sup> the other one in 2017.<sup>39</sup> Both investigations conclude that a WNV vaccine is unlikely to save costs. As case numbers are relatively low, and the assumed costs per vaccine dose are high, the amount of money necessary to avoid a single case is consequently very high. Cost-effectiveness increases when only specific age groups are immunized as compared to universal vaccination.<sup>39</sup> However, even with targeted immunization, it would still be markedly lower as compared, for example, to the herpes zoster vaccine, which also targets aged individuals.<sup>40</sup> Obviously, an increase in case numbers and a vaccine which is cheap and ideally only needs a single dose could put this scenario upside down. In this context, it is relevant that almost all clinically tested vaccine candidates until now require more than one dose to achieve elevated titers of neutralizing antibodies in a significant proportion of study participants. Many also rely on sophisticated, but expensive, technologies. WNV is constantly expanding its endemic area, and case numbers, e.g. in Europe, have increased enormously in 2018. However, it is hard to predict whether this will lead to a significant change in outbreak characteristics which could influence the planning of vaccine efficacy trials.

On the other hand, there is evidence for different genetic variations that are associated with severe forms of WNV disease, which could lead to more targeted vaccination approaches.<sup>40</sup> This might have an impact on cost effectiveness, provided that genetic tests are available and accepted by persons willing to get vaccinated.

### Potential problems arising with flavivirus vaccines in areas of co-circulation

Flaviviruses are structurally very similar; hence, many antibodies elicited during an infection can bind to other flaviviruses as well. Especially the E-protein contains highly conserved parts, most prominently the fusion loop domain, which is recognized by most of the cross-reacting antibodies.<sup>41</sup> This is a significant problem for the specificity of flavivirus serological tests.<sup>42</sup> In addition, in the case of DENV, it can also have a direct impact on the course of an infection, as antibodies against one of the four DENV serotypes can enhance infection with another serotype. The phenomenon of antibody-dependent enhancement (ADE) is believed to arise from the binding of antibodies which are unable to neutralize the virus but lead to increased uptake into host cells, e.g. via Fc-receptor-mediated endocytosis into macrophages. This would lead to more severe symptoms during secondary, heterologous dengue infection.<sup>43,44</sup> Although ADE has been demonstrated using cell-culture-based assays, its role in the pathogenesis of human dengue infections remains controversial.<sup>45</sup> The emergence of ZIKV in DENV endemic areas has led to investigations to test potential ADE between both virus infections, which might be responsible for some of the severe symptoms of ZIKV infections, for example, fetal neurological malformations. ADE between DENV and ZIKV could be demonstrated in cell culture experiments and in animal models, and a large proportion of cross-reactive antibodies from secondary infections due to co-circulation map to the E-protein.<sup>46,47</sup>

However, recent epidemiological studies on the effect of pre-existing DENV immunity on ZIKV infection in regions

endemic for both viruses report correlations both with protection and with enhancement, which demonstrates the need for further detailed investigations.<sup>48,49</sup>

As the enhancement of flavivirus infections due to ADE would be a danger signal for the development of flavivirus vaccines including WNV, this issue needs to be addressed and excluded during vaccine development. Immunological cross-reactivity between WNV and other flaviviruses in serological diagnosis is well described.<sup>50</sup> Potential infection-enhancing effects of WNV-antibodies have only been subjected of few studies until now, mainly in the context of ZIKV emergence. Plasma samples from convalescent human WNV- (but not TBEV-) infections were demonstrated to enhance ZIKV infections both in vitro and in vivo.<sup>51,52</sup> On the other hand, no ADE was observed in mice previously infected with ZIKV and then challenged with WNV. Depending on the ZIKV strain used, these animals were even better protected against WNV than those naïve to ZIKV.<sup>53</sup> The complementary animal experiment (ZIKV challenge after WNV infection) has not yet been described. In addition, vaccine-induced ADE between different members of the JEV-serocomplex (WNV, JEV, Murray Valley encephalitis virus and Saint Louis encephalitis virus) is not very probable in light of existing data.<sup>54</sup> Nevertheless, the finding that WNV-antibodies can enhance ZIKV-infections is of relevance, since both viruses have overlapping distribution.<sup>9</sup> It highlights the need to investigate potential ADE caused by a given vaccine candidate in detail, ideally by using clinical data in addition to laboratory-based studies. For existing flavivirus vaccines, e.g. the licensed TBEV or YFV vaccines, the availability of large sets of human sera from immunized individuals greatly facilitates such analyses.<sup>52</sup>

A potential way to address the issue of cross-reactivity-induced ADE is the elimination of some of the conserved sequences from the vaccine. It has been demonstrated that by inserting point mutations near and into the fusion loop domain of the E protein, the binding of antibodies from heterologous flavivirus infections is significantly diminished.<sup>55,56</sup> A vaccine candidate for ZIKV, consisting of an RNA coding for a ZIKV virus-like particle, contains four such point mutations and did not induce ADE for DENV.<sup>57</sup> A similar strategy could be used for a WNV vaccine. Mutant WNV E-proteins have been developed and shown to significantly diminish cross-reactive binding of antibodies against heterologous flaviviruses.<sup>58</sup>

Within the E-protein of WNV, many neutralizing antibodies target the domain DIII, which is structurally less conserved among flaviviruses. Using only the DIII domain of WNV has been shown to fully protect mice from lethal infection, and recent data suggest that this antigen does not induce ADE for DENV and ZIKV.<sup>59–61</sup> Alternatively, a vaccine could predominantly induce protective T-cell responses and thereby avoid ADE. WNV vaccine approaches based on T-cell epitopes have been described, but protection in animal models reached 75%, so the combination with neutralizing antibody-inducing antigens still seems necessary.<sup>62,63</sup>

## Conclusions

WNV remains a significant threat to humans in many parts of the world. Its potential to cause outbreaks in newly endemic areas, as exemplified in Europe 2018, is alarming. Its ability to acquire mutations which lead to increased virulence, paired with its flexibility in using various mosquito species as vectors and birds as amplifying hosts make epidemics extremely unpredictable. Global warming and the ever increasing traffic of humans, animals and goods are additional factors favoring the further spread of WNV. Consequently, a human vaccine would be essential to encounter this global threat. Technologies for WNV vaccines have been developed, they are protective and safe. Some issues have prevented the transformation of these candidate vaccines into marketable products until now. However, there are possible ways to address and overcome these obstacles: to increase cost-effectiveness, the vaccine candidates need to be optimized for low production costs and long lasting effectiveness upon a single dose vaccine regimen. To avoid potential interference of immune responses to different flaviviruses in areas of co-circulation, vaccine candidates should be lacking epitopes which lead to binding of cross-reactive antibodies. Finally, due to the difficulties in planning efficacy trials, licensing procedures need to be adapted, similar to vaccines against other (re-) emerging infections.

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## Disclosure of potential conflicts of interest

There are no conflicts of interest.

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