Detection and protection

Dr Sebastian Ulbert initiated a project in 2011 to try to prepare Europe for West Nile virus, a mosquito-borne disease experiencing range expansion for reasons yet to be understood

To begin, what is West Nile virus (WNV) and why is there a pressing need to study it in detail?

WNV normally infects birds but can be transmitted to mammals such as humans and horses. Although most infections lead to mild fever or remain asymptomatic, the virus can cause severe, even fatal, neurological disease.

Until the 1990s, WNV only sporadically caused outbreaks. Yet, during the last 15 years infections have been detected more and more frequently and, in 2012, WNV cases in the US and Europe were at their highest ever level. However, reasons for the continuous spread and the constant re-emergence of novel WNV variants remain to be understood.

Although there have been vaccines licensed that protect horses against the virus, no human vaccine is available today. The technologies underlying the veterinary vaccines are not directly transferrable to human use. In addition, it is very difficult to specifically diagnose a WNV infection. Therefore, research on WNV and the development of countermeasures, such as vaccines and diagnostic systems, are urgently needed.

Is there evidence to suggest WNV is now spreading more rapidly throughout Europe?

Annual WNV infections in Europe have been occurring for a couple of years now. 2012 was a peak year, but we need to study the spread over a longer period in order to find out its dynamics.

WNV is genetically very flexible (genetic plasticity) and each year novel variants are detected. Sometimes these mutations are negative and increase the virulence of disease. This has been studied in detail by Dr Luisa Barzon who is part of the West Nile Integrated Shield (WINGS) consortium. Her team has identified the strains responsible for outbreaks in Italy by sequencing these newly emerging viruses and modelling the mutations detected in WNV proteins. These proteins were then generated and studied in more detail.

The WINGS consortium is made up of nine international partners. Could you briefly explain how the work is divided among these collaborators? What is the significance of working with industry?

The major aims of WINGS are the development of vaccine candidates based on proteins and DNA for use in humans; creation of a specific diagnostic test; and expansion of the epidemiological knowledge base of the virus in Europe. The consortium unites experts from many different disciplines, including animal handlers, virologists and vaccination experts.

The rationale of the EC funding behind WINGS is to enhance Europe´s WNV preparedness by delivering tools to counteract the infection. But purely academic research bears the risk of developing techniques that might not be useful to the industries that will eventually produce and distribute the tools. Therefore, the project has had to be output-orientated, and consequently the participation of industrial partners brings a significant benefit to the consortium.

How is the virus currently detected? How would your diagnostic test differ?

The virus is either detected directly, mainly by looking for its RNA in the blood or nervous system, or indirectly by measuring antibodies that were produced in defence. The drawback of looking for RNA is that the virus disappears from the blood shortly after symptoms begin. As a consequence, WNV RNA is not detectable in all cases of symptomatic infection.

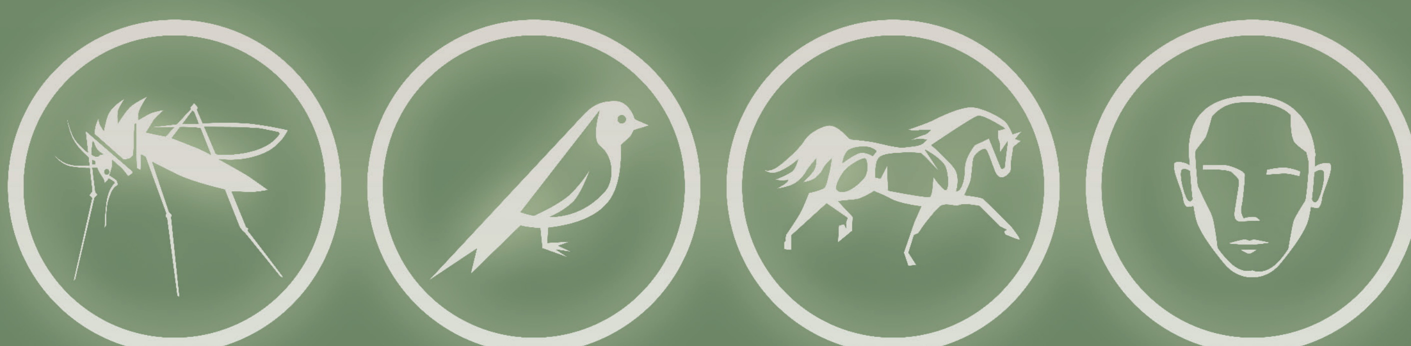
Our approach is to look for parts of the WNV proteins that are targeted by antibodies specific to a WNV infection. The resulting test will make confirmation experiments using live viruses obsolete and should greatly improve WNV diagnosis and surveillance.

What have been some of WINGS's most significant results to date?

Two vaccine candidates have shown complete protection from infection in preclinical trials and are now being developed further.

We have gained important insight into the human antibody response to WNV infections, which has led to the identification of epitopes (antigenic determinants) suitable for specific diagnostic tests. We are in close contact with the diagnostics industry to bring this test to market.

In addition, detailed investigations of the epidemiology of WNV, particularly in Northern Italy, has led to several important findings on WNV variants.



Clipping WNV's wings

The **West Nile Integrated Shield** project is an EU Seventh Framework Programme initiative working to halt the spread of a potentially deadly disease. Utilising modern techniques, the international team seeks to develop effective control methods and diagnostic tools

IN AUGUST 1999, an outbreak of 59 cases of encephalitis and meningitis was reported to the New York City Department of Health. After extensive analysis, West Nile virus (WNV) was identified as the perpetrator. This was the first documented incidence of WNV in the Western Hemisphere. In the years that have followed, more than 30,000 cases of infection have affected the US alone.

In Europe, an upward trend in WNV virulence is a growing concern for health experts and the public, but compared to North America, information on emerging European strains is limited. An international consortium was therefore set up in 2011 to try to abate further outbreaks. The West Nile Integrated Shield (WINGS) project, which is coordinated by Project Manager Dr Sebastian Ulbert from the Fraunhofer Institute for Cell Therapy and Immunology (IZI), includes nine institutions from eight different countries, and has already published new research on the epidemiology, diagnosis and prevention of WNV to increase Europe's preparedness for WNV infection in the years to come.

WEST NILE VIRUS

WNV was thought to have originated in Uganda more than 60 years prior to the outbreak in New York. In 1937, during research into yellow fever virus in the Western Nile District of Uganda, a 37-year-old woman from Omogo presented with febrile illness. Subsequent tests led to the first isolation of WNV. Since then, the virus has been identified in Egypt (1942), India (1953) and by the early 1960s had spread north to France. Reports of WNV have helped to build a fuller picture of the origins of the disease, and recent developments in phylogenetics have now dated the emergence of WNV as a distinct virus to around 1,000 years ago.

WNV primarily infects birds and is transmitted by mosquitoes. However, it possesses the ability to transmit to and infect a wide array of species, including humans. Approximately 80 per cent of WNV infections are subclinical and therefore cause no symptoms. Within the remaining 20 per cent, individuals develop flu-like symptoms with high fever, and in rare circumstances – about 1 per cent – severe neuroinvasive disease (eg. encephalitis) can ensue. Within this group, mortality rates can be as high as 20 per cent, with elderly and immunodeficient subpopulations at particularly high risk.

> Collaboration with other West Nile virus-related consortiums is enabling WINGS to spread its reach and effectiveness in confronting the virus head on

Whilst mosquitoes (particularly those from the widely spread *Culex* genus) are the primary WNV vectors, compared to most mosquito-borne viruses WNV has an incredibly diverse vector and host range. For instance, more than 300 avian species are susceptible and a substantial amount of these belong to the Passeriformes, which accounts for more than half of all bird species and includes sparrows and crows.

Recently, new strains of WNV have evolved – a real challenge for researchers seeking to develop a vaccine or a specific diagnostic test. WINGS is closely examining the epidemiology of WNV in Europe to monitor these changes and develop control tools in a timely fashion. A 2013 study led by WINGS member Stefan Chabierski from Fraunhofer IZI, studied the human antibody response to the European WNV strain responsible for outbreaks in Italy and Greece in 2010. Antibodies in blood from WNV-infected humans were analysed, revealing that whilst the humoral immune response is largely heterogeneous, several dominant peptides are strongly recognised by human sera.

Antibodies are detectable quickly after WNV infection and maintained for a long time. However, as Ulbert explains: "The problem with detecting antibodies against WNV is the structural similarity of WNV to other members of the flavivirus family, such as dengue or tick-borne encephalitis virus".

DIAGNOSIS AND BEYOND

Laboratory diagnosis is often based on the detection of WNV RNA in blood and cerebrospinal fluid (CSF) by nucleic acid amplification (NAAT), but these tests can be challenging due to low-level or absent viremia at the time of onset (ie. the virus has already left the bloodstream). The WINGS consortium has identified the necessity for an improved protocol and methods for WNV diagnosis and screening.

In 2013, a study led by Dr Luisa Barzon from the University of Padova, Italy examined the excretion of WNV in urine during symptomatic infection. Using a method known as real-time reverse-transcription polymerase chain reaction (RT-PCR), the assay revealed that detection of WNV could be achieved more effectively by testing the urine of patients as compared to testing blood. In addition, the WINGS consortium is looking for antigens (proteins of the virus) that are targeted by WNV-specific antibodies and therefore allow diagnosis without cross-reactivity towards similar infections. Several antigens have already been found and are currently being transformed into a serologic test.

The long-term objective and current focus of WINGS is the development and creation of a WNV vaccine. Although several veterinary vaccinations have been commercialised, which include inactivated virus or viral vector systems,

Kick-Off Meeting WINGS, February 2011 at Fraunhofer IZI, Leipzig, Germany. Back row from left to right: Orsolya Lorincz, Zsolt Lisziewicz, Bärbel Kaufmann and Kurt Bürki. Front row from left to right: Stefan Chabierski, Gerd Rundstrom, Niek Sanders, Ernst Verschoor, Sebastian Ulbert, Luisa Barzon, Karin Lövgren and Uwe Liebert.

none exist for human use. In 2011, researchers from Fraunhofer IZI, including Principal Investigator Ulbert´s team, tested a DNA vaccine in murine models of WNV. By generating a DNA plasmid that coded for the WNV viral envelope (E) protein, the group was able to show that vaccination stimulated anti-WNV T cell responses and neutralising antibodies, which protected mice against a lethal dose of the virus. Importantly, vaccination-induced protection was vastly enhanced by a recombinant protein boost. This synergistic effect was somewhat of a breakthrough for the WINGS researchers and will be further developed and tested over the coming months. "Our candidate vaccines have just successfully completed evaluation in non-human primates, meaning that they have now reached late-term preclinical status," elaborates Ulbert. "The next step will be clinical testing, which we are currently preparing for by planning partnerships and organising funding."

Collaboration with other WNV-related consortia – including fellow EU Seventh Framework Programme (FP7)-funded projects EuroWestNile (EWN); EDENext, Biology and control of vector-borne infections in Europe; and Vector-borne Risks for Europe (VECTORIE) – is enabling WINGS to spread its reach and effectiveness in confronting the virus head on. For example, in late 2012, European WNV project coordinators Ulbert (WINGS), Antonio Tenorio and Annapaola Rizzoli (EWN) and Byron E Martina (VECTORIE) held a summit in which they discussed collaborative initiatives. Through this meeting of minds, they agreed to publish a joint paper on WNV research and recently held a workshop at the 5th European Congress of Virology (ECV) in Lyon, France. It is hoped that through cross-fertilisation of ideas and expertise, researchers can find the best way forward in fighting the virus in Europe.

WINGS aims

- **Development of a specific diagnostic test** – the protein components of WNV are very similar to other flaviviruses. Hence, antibodies produced upon infection with WNV show a substantial degree of crossreactivity to antigens from related viruses, which complicates a specific serologic diagnosis. The WINGS project is looking for antigens in structural and non-structural proteins of WNV that allow a specific diagnosis of the virus
- **Vaccine development** – to date, there is no vaccine to protect humans from WNV infection. WINGS scientists are using several different innovative techniques of antigen design, vaccine delivery and adjuvants to develop vaccine candidates for efficient and longlasting protection of humans against WNV. Immunisation strategies are rapidly adaptable to newly emerging WNV strains
- **Epidemiology of WNV in Europe** – the epidemiologic situation of WNV in Europe is changing quickly. Novel strains (of both major WNV lineages) are detected now every year, with numerous cases of severe neurological symptoms among humans. Research on these novel WNV isolates as well as close collaboration between the WINGS consortium and national and international surveillance agencies helps to understand the distribution of WNV throughout Europe

INTELLIGENCE

WINGS

OBJECTIVES

- To discover antigens in structural and non-structural proteins of West Nile virus (WNV) that allow a specific diagnosis
- To develop vaccine candidates for the efficient and longlasting protection of humans against WNV, using several different innovative techniques of antigen design, vaccine delivery and adjuvants
- To understand the spreading of WNV throughout Europe

KEY PARTNERS

Fraunhofer Institute for Cell Therapy and Immunology (IZI) (coordinator), Germany • **Institute of Virology**, Leipzig University, Germany • **Biomedical Primate Research Centre**, The Netherlands • **Department of Molecular Medicine**, Padova University, Italy • **Institute of Laboratory Animal Science**, Zürich University, Switzerland • **Laboratory of Gene Therapy**, Ghent University, Belgium • **Division of Infectious Diseases**, Department of Medicine, School of Medicine, Washington University, USA • **Novavax AB**, Sweden • **Genetic Immunity Kft.**, Hungary

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SEBASTIAN ULBERT studied biology in Tübingen, Germany and obtained his PhD from Amsterdam University, The Netherlands where he studied the phenomenon of antigenic variation of the parasite causing sleeping sickness in the lab of Piet Borst. After a postdoc at the European Molecular Biology Laboratory in Heidelberg he joined Fraunhofer IZI in Leipzig where he currently heads a research group investigating vaccine technologies and diagnostics of infectious diseases.

West Nile Shield Project
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