

Prospects for a Zika Virus Vaccine

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A recent unprecedented outbreak of Zika virus (ZIKV) in the Americas has been associated with microcephaly and other congenital malformations in infants as well as Guillain-Barre syndrome in adults. The development of a safe and effective ZIKV vaccine is therefore an urgent global health priority. Promising data from preclinical vaccine studies in mice and monkeys suggest that an effective vaccine will likely be possible, but important scientific challenges remain. Here we review the current state of ZIKV vaccine development. We discuss different vaccination strategies and we highlight challenges facing clinical evaluation of ZIKV vaccine candidates.

Introduction

ZIKV is a flavivirus that was first isolated in 1947 from a rhesus monkey in the Zika forest of Uganda. It is an enveloped, positive-sense RNA virus that shares similarities with other flaviviruses, including dengue virus (DENV), West Nile virus (WNV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV). The unprecedented ZIKV outbreak in the Americas (Fauci and Morens, 2016; Petersen et al., 2016) prompted the World Health Organization to declare this epidemic a public health emergency of international concern from February 1 to November 18, 2016. ZIKV continues to spread in the Americas and in Asia, and the potential for explosive spread and the devastating clinical consequences in maternal-fetal infections underscore the urgency of the development of a ZIKV vaccine. ZIKV causes fetal microcephaly, intrauterine growth retardation, and other congenital malformations (Brasil et al., 2016a; Honein et al., 2017; Johansson et al., 2016; Melo et al., 2016; Mlakar et al., 2016; Rasmussen et al., 2016) and has also been associated with the neurologic disorder Guillain-Barre syndrome in adults (Brasil et al., 2016b; Cao-Lormeau et al., 2016; Dos Santos et al., 2016; Parra et al., 2016).

Animal models have been developed that recapitulate certain key aspects of ZIKV pathogenesis. Fetal pathology has been observed in ZIKV-infected pregnant female mice (Cugola et al., 2016; Li et al., 2016; Miner et al., 2016; Yockey et al., 2016) and monkeys (Adams Waldorf et al., 2016). ZIKV appears to target placental and fetal tissues in both humans and monkeys (Driggers et al., 2016; Dudley et al., 2016) as well as cortical neural progenitor cells in the central nervous system (Cugola et al., 2016; Garcez et al., 2016; Li et al., 2016; Miner et al., 2016; Osuna et al., 2016; Qian et al., 2016), which presumably contribute to fetal pathology and neuropathology.

In this review, we highlight recent advances in ZIKV immunology and vaccine development. Preclinical vaccine studies in mice and monkeys have suggested that multiple ZIKV vaccine platforms may be highly efficacious. Early-phase clinical trials have begun, and clinical efficacy trials are planned. We also review current challenges and key issues facing clinical ZIKV development.

Immunology of ZIKV Infection and Insights from Preclinical Vaccine Studies

Our understanding of the immune response to ZIKV is rapidly advancing but still far from complete. ZIKV is believed to induce both innate and adaptive immune responses that limit viral replication. The importance of innate immunity to ZIKV infection is evidenced by the enhanced susceptibility of mice lacking type I interferon (IFN) activity (Lazear et al., 2016; Yockey et al., 2016). ZIKV infection also induces IFN-induced transmembrane proteins 1 and 2 (IFITM1 and IFITM3) (Savidis et al., 2016), which have been shown to restrict viral replication. However, many questions remain as to the innate immune mechanisms that detect and respond to ZIKV.

There is an emerging consensus that ZIKV induces robust antibody responses that often cross-react with other flaviviruses, particularly DENV (Stettler et al., 2016), although it appears that there is only a single ZIKV serotype (Dowd et al., 2016a). ZIKV-specific neutralizing antibodies have been described, and monoclonal antibodies against various targets have been reported by several laboratories (Dejnirattisai et al., 2016; Sappapapu et al., 2016; Stettler et al., 2016; Swanstrom et al., 2016; Wang et al., 2016; Zhao et al., 2016). Recently, a potent neutralizing antibody, ZIKV-117, directed against a quaternary epitope on the ZIKV envelope (Env) protein dimer-dimer interface was shown to block ZIKV infection in mice and to limit early viral replication and fetal pathology when administered after ZIKV infection of pregnant mice (Sappapapu et al., 2016). Cross-reactive neutralizing antibodies against ZIKV and DENV have also been shown to bind conserved epitopes on the two viruses and have been shown to enhance viral replication in vitro (Charles and Christofferson, 2016; Dejnirattisai et al., 2016) and in mice (Stettler et al., 2016), raising the possibility that such cross-reactive antibodies might have the potential for antibody-dependent enhancement of disease.

Much remains to be learned about the cellular immune responses to ZIKV. CD4⁺ and CD8⁺ T cell responses to capsid (Cap) and Env have been observed in ZIKV-infected monkeys (Dudley et al., 2016; Osuna et al., 2016), and T cell responses to nonstructural protein 1 (NS1) and Env have been reported in

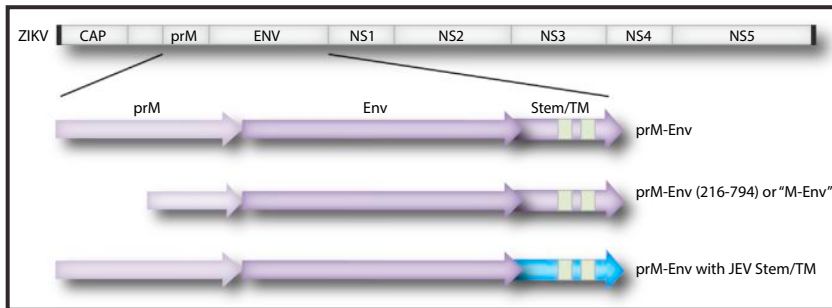


Figure 1. ZIKV Genomic Structure and Candidate Vaccine Immunogens

The pre-membrane (prM) and envelope (Env) proteins are shown in light and dark purple, respectively. Candidate immunogens that are currently being evaluated include full-length prM-Env, prM-Env (amino acids 216–794) with the “pr” cleavage peptide deleted (also termed “M-Env”) (Abbink et al., 2016; Larocca et al., 2016), and prM-Env with the JEV Env stem and transmembrane (TM) regions highlighted in blue (Dowd et al., 2016b).

ZIKV-infected humans (Stettler et al., 2016). In mice, CD8⁺ T cells appear to play a role in controlling ZIKV replication (Elong Ngono et al., 2017), but the importance of T cell responses in vaccine protection remains unclear.

Our group demonstrated the initial proof-of-concept that vaccines can protect against ZIKV challenge in both mice (Larocca et al., 2016) and rhesus monkeys (Abbink et al., 2016). We developed DNA vaccines expressing the ZIKV pre-membrane (prM) and Env proteins with the signal peptide deleted for enhanced expression (prM-Env amino acids 216–794; also known as “M-Env”) (Figure 1), adenovirus (Ad) vectors expressing this same immunogen, and purified inactivated virus (PIV) vaccines. All three vaccine platforms induced ZIKV-specific neutralizing antibodies and protected both mice and rhesus monkeys against challenge with ZIKV strains from Brazil and Puerto Rico (Abbink et al., 2016; Larocca et al., 2016). In rhesus monkeys, the ZIKV PIV vaccine and the Ad vector-based vaccine induced neutralizing antibodies after a single immunization and proved more immunogenic than the DNA vaccine, although two immunizations with DNA vaccines induced sufficient neutralizing antibody titers to protect. In a subsequent study, two immunizations of DNA vaccines expressing prM-Env (Figure 1) were similarly shown to protect rhesus monkeys against ZIKV challenge (Dowd et al., 2016b). Thus, multiple vaccine platforms can provide robust protection against ZIKV challenge in both rodents and primates. However, since these immunocompetent animal models generally do not develop clinical disease, the capacity of vaccines to prevent clinical complications of ZIKV infection remains to be determined. Nevertheless, these findings raise considerable optimism that the development of a ZIKV vaccine for humans will likely be possible.

Adoptive transfer studies have also shown that purified IgG from vaccinated animals confer passive protection in both mice and monkeys, providing further evidence that vaccine-induced antibodies represent a key mechanism of protection (Abbink et al., 2016; Larocca et al., 2016). Protection in passive transfer studies required serum microneutralization (MN50) titers of approximately 100 in both rodents and primates, and this correlate of protection provides a quantitative benchmark for future preclinical and clinical studies. Analogous correlates of protection based on neutralizing antibody titers have been reported in humans for other flavivirus vaccines, including JEV, TBEV, and YFV (Hombach et al., 2005; Kreil et al., 1997; Mason et al., 1973), suggesting the plausibility and generalizability of this correlate. Depletion of CD4⁺ and/or CD8⁺ T lymphocytes in vaccinated mice did not compromise protective efficacy

against ZIKV challenge (Larocca et al., 2016), suggesting that vaccine-elicited T cells were not required for protection in this model, although antiviral T cells may be relevant for virologic control in other settings (Elong Ngono et al., 2017).

These data suggest that neutralizing antibodies may be a generalizable immune correlate of protection for ZIKV vaccines. The specific MN50 titer required for protection in mice and monkeys may be dependent on the experimental specifics of the models, such as the challenge dose, but nevertheless this provides a potentially useful immunologic benchmark for early-phase clinical trials. Future preclinical studies will need to evaluate the potential impact of baseline immunity to DENV and other flaviviruses on the immunogenicity and protective efficacy of ZIKV vaccines, as well as the efficacy of candidate ZIKV vaccines to prevent congenital Zika syndrome in pregnant female animals.

Strategies for Clinical ZIKV Vaccine Development

Preclinical ZIKV vaccine studies have provided a strong proof-of-concept that the development of an effective ZIKV vaccine should be possible. Clinical development of ZIKV vaccines is now underway and is uniquely collaborative, focused, and efficient, with initiation of multiple phase 1 clinical trials within the first year of recognition of the ZIKV epidemic. PIV vaccines have previously been developed for other flaviviruses, including JEV and TBEV (Halstead and Thomas, 2011; Jarmer et al., 2014). DNA and PIV vaccines are particularly attractive for ZIKV, given the theoretical safety advantages of non-replicating vaccines in women who might be or might become pregnant. Other ZIKV vaccines that are in preclinical stages of development include RNA vaccines, recombinant vector-based vaccines, and purified protein subunit vaccines.

Vaccinations in a phase 1 clinical trial of the plasmid DNA vaccine (VRC-ZKADNA085-00-VP; VRC5288) produced by the Vaccine Research Center, National Institute of Allergy and Infectious Diseases (VRC/NIAID) (NCT02840487) started on August 3, 2016 in 80 flavivirus-naïve men and women between the ages of 18 and 35 at low risk of acquiring ZIKV infection in the United States. This plasmid DNA vaccine is based on the H/PF/213 ZIKV strain and expresses full-length prM-Env containing the JEV stem and transmembrane region (Figure 1). Inclusion of the JEV stem and transmembrane region led to increased secretion of subviral particles, but preclinical studies in rhesus monkeys showed that this modification (VRC5288) reduced immunogenicity and protective efficacy compared to the unmodified prM-Env (VRC5283) (Dowd et al., 2016b; Pierson and Graham, 2016). This ongoing phase 1

clinical trial will enroll four groups of 20 volunteers each who will receive 4 mg of the VRC5288 DNA vaccine by the intramuscular route at weeks 0 and 8; weeks 0 and 12; weeks 0, 4, and 8; or weeks 0, 4, and 20. The primary objective is safety, and the secondary objective is immunogenicity, including ZIKV-specific neutralizing antibody titers described in assays developed at Walter Reed Army Institute of Research (WRAIR) (Larocca et al., 2016) and at NIAID (Dowd et al., 2016b) and that were protective in preclinical studies. The VRC also plans to initiate a phase 1 clinical trial of the VRC5283 DNA vaccine.

Inovio Pharmaceuticals started vaccinations in phase 1 clinical trials of another candidate plasmid DNA vaccine expressing prM-Env (GLS-5700) in 40 flavivirus-naïve volunteers in the United States and Canada on July 26, 2016 and in 160 flavivirus-experienced volunteers in Puerto Rico on August 29, 2016 (NCT02809443, NCT02887482). This DNA vaccine demonstrated efficacy in immunocompromised mice with evidence that plasma IgG was the correlate of protection (Muthumani et al., 2016).

Three phase 1 studies have also recently been launched by the U.S. Army, NIAID, and Beth Israel Deaconess Medical Center (BIDMC) to evaluate the safety and immunogenicity of the ZIKV PIV (ZPIV) vaccine produced by WRAIR. This vaccine is based on the PRVABC59 ZIKV strain from Puerto Rico. It was grown in Vero cells and was formalin inactivated, purified, and adjuvanted with alum. WRAIR initiated vaccinations in the first-in-human study of ZPIV on November 7, 2016, using 5 µg of vaccine that will be given by intramuscular injection on days 0 and 28 in 25 flavivirus-naïve volunteers and subsequently in two additional groups of 25 volunteers that will be pre-immunized with licensed vaccines for JEV or YFV to assess safety and immunogenicity in flavivirus-naïve and flavivirus-experienced individuals (NCT02963909). St. Louis University started vaccinations in a parallel dose de-escalation study on November 14, 2016 (NCT02952833), and BIDMC initiated a regimen acceleration study on November 21, 2016 (NCT02937233). The BIDMC study will assess dosing schedules of days 0 and 28, days 0 and 14, days 0 and 7, and a single ZPIV immunization on day 0. Future plans include initiation of a phase 1 ZPIV trial in Puerto Rico early in 2017 in volunteers who have natural baseline exposure to other flaviviruses, including DENV. The VRC is also planning to boost volunteers who received their DNA vaccine with ZPIV, based on previous experience with DNA prime, inactivated virus boost regimens for influenza virus (Ledgerwood et al., 2011).

NIAID is planning to advance their ZIKV DNA vaccine into efficacy trials in the Americas in 2017 if the ongoing phase 1 studies demonstrate safety and immunogenicity with neutralizing antibody titers that have been shown to protect monkeys. However, the potency and durability of immune responses elicited by DNA vaccines in humans may prove limited. This effort is further challenged by the rapidly changing incidence and localization of the ZIKV outbreak in the Americas. Intensive epidemiologic surveys in both human and mosquito populations are underway to develop the most efficient network of clinical research sites in ZIKV-affected zones to execute ZIKV vaccine efficacy studies. Consideration is also being given to nonclassical study designs that would allow for rapid shifting of resources and focus from clinical sites with lower ZIKV incidence to those with higher ZIKV incidence.

WRAIR, NIAID, and BARDA are also working with Sanofi Pasteur, Emergent BioSolutions, and Takeda to advance the ZPIV vaccine into phase 2 and 3 studies if the phase 1 studies meet safety and immunogenicity benchmarks. Unlike gene-based vaccines such as plasmid DNA and RNA vaccines, the ZPIV vaccine requires a longer time for manufacturing to produce a sufficient amount for efficacy studies. It is likely that once ZPIV manufacturing has been completed, the ZPIV vaccine will also be tested in the clinical trial network that is being developed in the Americas (and perhaps Asia) for gene-based ZIKV vaccines.

Safety and immunogenicity data from the ongoing phase 1 clinical trials are expected soon. Given the timelines and inherent uncertainties of the clinical development process, it is important to develop a variety of vaccine platforms for ZIKV. In addition to ZPIV and DNA vaccines, RNA-based vaccines (Pardi et al., 2017), recombinant vector-based vaccines (Abbink et al., 2016), and purified protein subunit vaccines are also currently in development, with planned pre-clinical evaluation and potential advancement into phase 1 clinical trials.

Lessons from Other Flavivirus Vaccines

There is an extensive history of successful flavivirus vaccine development, and correlates and surrogates of protection have been defined for some of these vaccines. Human vaccines have been licensed for YFV, JEV, TBEV, and DENV (Ishikawa et al., 2014). The diversity of successful flavivirus vaccines provides important insights into ZIKV vaccine development. Three JEV vaccines have demonstrated clinical efficacy but deliver different antigenic components, including a whole live attenuated virus, a recombinant chimeric live attenuated virus, and a purified inactivated virus (Bista et al., 2001; Guirakhoo et al., 1999; Tauber et al., 2007). Each is suspected to generate varying levels of cell-mediated immunity, based on the fact that the majority of T cell epitopes are in the non-structural proteins. Each also varies in the doses and schedules used and differs in the durability of protection. What is common among all the vaccines is that all three generate a neutralizing antibody response against the Env protein that is believed to be important for protective efficacy (WHO, 2016).

Plotkin (2010) defined a correlate of protection or predictor of protection as an immune response that statistically correlates with and is responsible for protection. The response may be either a mechanistic or a non-mechanistic correlate of protection. A non-mechanistic correlate does not define how a specific immune response is protective but does predict protection through its relationship with an immune response that is mechanistically responsible for protection. A mechanistic correlate of protection has a causal relationship with protection. Correlates of protection have been identified in a number of ways, including passive administration of antibody, analysis of immune responses after natural infection, analysis of immune responses in experimental vaccine recipients who were protected versus not protected, evaluation of human infection and disease models, and evaluation of interventions in animal models. A surrogate of protection is an immune response that substitutes for the true immunologic correlate of protection (Plotkin, 2008). Thus, an immune function responsible for protection is a correlate, and a response that is measurable but not functional in a protective response is a surrogate.

JEV, TBEV, and YFV vaccines all have quantitative correlates or surrogates of protection. Neutralizing antibodies are the correlates for the YFV and JEV vaccines, while total binding antibody responses and neutralizing antibodies are correlates for the TBEV vaccine (Hombach et al., 2005; Kreil et al., 1997; Mason et al., 1973). For YFV, 0.7 neutralization units (equivalent to a 1:5 neutralizing antibody titer) is considered protective. Neutralizing units are generated by completing assays with a constant dilution of serum (antibody) while varying the amount of virus it is required to neutralize (Spector and Tauraso, 1968). Neutralizing antibody assays use the opposite approach: constant viral concentration with varying dilutions of serum. An actual sero-protective level for YFV has not yet been calculated. For JEV, a neutralizing antibody titer of 1:10 is considered protective, although 1:5 was initially considered as protective for the live attenuated virus vaccine. TBEV has ELISA and neutralizing antibody correlates of 125 ELISA units and 1:10, respectively (Venturi et al., 2006). A single DENV vaccine has now been licensed in more than 10 countries (not including the U.S.), but a correlate has not yet been determined for this vaccine. Cross reactivity and other DENV-specific assay limitations have proven challenging, including an apparent disconnect between robust neutralizing antibody titers after vaccination and clinical efficacy (Sabchareon et al., 2012).

Challenges Facing Clinical Development of ZIKV Vaccines

Despite the unprecedented pace of preclinical and early clinical studies, important challenges remain for the clinical development of ZIKV vaccines. Development pathways and challenges for ZIKV vaccines have recently been reviewed (Marston et al., 2016; Thomas et al., 2016). The experience with other flavivirus vaccines combined with currently available preclinical data for ZIKV vaccines suggest that neutralizing antibodies will likely be important in protection against ZIKV (Abbink et al., 2016; Dowd et al., 2016b; Larocca et al., 2016). However, it remains to be determined whether a ZIKV vaccine will need to achieve true sterilizing immunity to protect a pregnant woman and her fetus from congenital ZIKV syndrome, which could be a higher bar than protecting a non-pregnant adult against ZIKV viremia. A more detailed understanding of potential viral reservoirs, such as in semen, will also be needed (Atkinson et al., 2017; Matheron et al., 2016).

Vaccine safety is critical for any prophylactic vaccine. ZIKV vaccine candidates will need to demonstrate an acceptable local and systemic safety profile. Injection site redness, swelling, and pain will need to be minimal, and the occurrence of fever, muscle aches, headache, and other systemic symptoms will need to be similar to or less than currently licensed vaccines. In addition, a ZIKV vaccine will also likely need to demonstrate safety across a broad age range, it should not cause Guillain-Barre Syndrome or other neurologic adverse events, and it should not be transmissible from vaccine recipients to non-recipients. Moreover, there should be no difference in the safety profile between baseline flavivirus-naïve and flavivirus-experienced recipients, and vaccination should not be contraindicated in pregnant women.

Assessment of vaccine immunogenicity will likely involve evaluation of both humoral and cellular responses, but it is

anticipated that most development decisions will be based on neutralizing antibody titers. Licensed vaccines for other flaviviruses have validated neutralizing antibody correlates of protection, suggesting that the same concept may apply for ZIKV, and preclinical studies support this correlate as discussed above (Abbink et al., 2016; Dowd et al., 2016b; Larocca et al., 2016).

Demonstrating vaccine efficacy may be challenging for a ZIKV vaccine. The ostensibly simple task of diagnosing ZIKV infection in the context of a clinical trial with participants at risk of ZIKV exposure is non-trivial. Only 20% of patients with ZIKV infection develop symptoms, and the typical clinical symptoms of rash and low-grade fever are non-specific (Duffy et al., 2009). Serologic testing for ZIKV infection is also confounded by cross-reactive antibodies to other flaviviruses, such as DENV (Rabe et al., 2016). Although detection of ZIKV RNA is highly specific, the rapid clearance of viremia complicates nucleic acid detection as the sine quo non for infection (Rabe et al., 2016). Technical solutions are being pursued for improved case definitions of ZIKV infection in the context of vaccine development, but these solutions will need to be developed quickly.

Most vaccine developers are focusing on an initial target product profile for a ZIKV vaccine that includes women of child-bearing age given significant fetal morbidity in the first trimester of pregnancy (Brasil et al., 2016a) and also includes men given the possibility of sexual transmission of the virus (D'Ortenzio et al., 2016). As a result of safety concerns for women who might become pregnant during or shortly after vaccination, the use of certain vaccine approaches, such as live attenuated vaccines, will likely not be initially favored and may be used in later target product profiles for populations such as children prior to sexual debut.

How will clinical trials of ZIKV vaccines unfold? Recent trials of DENV vaccines in the Americas built substantial clinical trial capability in regions where ZIKV is currently being transmitted (Hadinegoro et al., 2015; Villar et al., 2015). If ZIKV transmission intensity remains high in these areas, then these existing clinical trial sites could be re-purposed for ZIKV vaccine trials. However, if ZIKV transmission subsides by the time vaccine efficacy trials are initiated, then alternative study designs, such as the ring vaccination approach used in the Ebola ça Suffit! vaccine study in Guinea might need to be employed (Henao-Restrepo et al., 2015). It is also unclear whether ZIKV will become endemic and demonstrate seasonal transmission patterns, or if transmission will be epidemic and will become episodic with interim periods of relative quiescence. Moreover, the incidence of microcephaly and other major fetal abnormalities are relatively rare (Brasil et al., 2016a; Johansson et al., 2016), and thus efficacy studies would need to be exceedingly large to be powered to detect vaccine prevention of these important clinical endpoints. Designing efficacy trials only to measure prevention of ZIKV infection may be more tractable, but the mild clinical course of disease in the vast majority of cases and the challenges associated with serologic diagnosis pose unique difficulties. Moreover, there is also a concern regarding studies that measure vaccine efficacy with endpoints that do not substantially contribute to the public health burden.

ZIKV vaccine developers may seek regulatory approval to pursue less conventional clinical endpoint efficacy trials utilizing

adaptive trial designs. They may also seek licensure establishing the case for safety and efficacy outside the context of a randomized controlled clinical endpoint efficacy trial using accelerated approval mechanisms or the animal rule. In either instance, there would likely be a requirement for post-licensure demonstration of clinical benefit.

Can Antibodies Enhance Clinical Disease?

The possibility of antibody-dependent enhancement (ADE) of clinical disease is also a theoretical concern for ZIKV vaccines. The risk of severe DENV infection is significantly higher in people experiencing a secondary infection with a heterologous DENV serotype (Burke et al., 1988; Russell et al., 1967). Immune enhancement has been proposed as the pathologic mechanism for these rare (2%–4% of secondary infections) but severe clinical phenotypes. It has been suggested that antibodies from the first infection may fail to neutralize the second infecting virus and actually facilitate the second virus' infection of target cells such as monocytes and macrophages (Halstead, 2003). It is possible that when epitopes are insufficiently occupied, antibodies may increase viral uptake through interactions with immunoglobulin Fc receptors, resulting in increased levels of viremia (Rothman, 2011). The increased viral burden promotes a proinflammatory environment, coagulation dysfunction, and damage to vasculature endothelial cell linings. Epidemiologic observations, in vitro data, and limited small animal studies offer support for this theory, but data are conflicting and the relevance of these observations for humans remains unknown (Halstead et al., 1973; Kliks et al., 1988, 1989; Laoprasopwattana et al., 2005; Libraty et al., 2009; Pinto et al., 2015; Vaughn et al., 2000).

Whether or not ADE will prove clinically relevant for ZIKV infection in humans remains to be determined. It has been speculated that severe clinical manifestations of ZIKV, when they occur, may be due to immune enhancement. It has also been postulated that primary infection with DENV or another flavivirus may induce antibodies that cross-react with a subsequent "secondary" infection with ZIKV and result in increased viral burden and a cascade of deleterious immunologic and clinical events (Dejnirattisai et al., 2016). It has also been suggested that ZIKV antibodies may impact subsequent DENV infection by an analogous mechanism (Kawiecki and Christofferson, 2016). Definitive data addressing these concepts in humans are currently not available.

Recent studies have shown that DENV-specific sera and monoclonal antibodies can increase ZIKV replication both in vitro (Barba-Spaeth et al., 2016; Charles and Christofferson, 2016; Dejnirattisai et al., 2016) and in mice (Stettler et al., 2016). However, passive transfer of sub-protective doses of neutralizing antibodies prior to ZIKV challenge did not result in enhancement of ZIKV replication or disease in mice or monkeys (Abbink et al., 2016; Larocca et al., 2016). Moreover, we have recently observed that monkeys that were primed with DENV or YFV and subsequently challenged with ZIKV did not exhibit increased ZIKV replication or adverse clinical outcomes as compared to flavivirus-naïve controls (unpublished data), and similar data have been reported online by the University of Wisconsin (<https://zika.labkey.com/project/OConnor/ZIKV-013/begin.view>). Large prospective clinical trials will likely be required to evaluate the possibility of immune enhancement to ZIKV in humans.

Conclusions

The pace of preclinical and early clinical development of ZIKV vaccines has been unprecedented. In less than a year, proof-of-concept preclinical studies have demonstrated that multiple vaccine platforms, including nucleic acid vaccines, purified inactivated virus vaccines, and recombinant vector-based vaccines, provide robust protection in rodent and primate challenge models. Moreover, adoptive transfer studies have shown that protection can be mediated by vaccine-elicited antibodies. Multiple phase 1 clinical trials have been initiated, and clinical efficacy trials are planned. However, unique challenges will need to be addressed in the clinical development of ZIKV vaccines. Key issues facing ZIKV vaccine development include the unclear durability of protective immune responses in humans, the potential impact of baseline cross-reactive antibodies to other flaviviruses, the need to protect against congenital Zika syndrome, and the difficulty designing and conducting efficacy trials in the context of a rapidly changing epidemic. As clinical development of a variety of ZIKV vaccine candidates moves forward, basic and preclinical research will undoubtedly continue to increase our understanding of ZIKV immunology and pathogenesis.

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REFERENCES

- Abbink, P., Larocca, R.A., De La Barrera, R.A., Bricault, C.A., Moseley, E.T., Boyd, M., Kirilova, M., Li, Z., Ng'ang'a, D., Nanayakkara, O., et al. (2016). Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. *Science* 353, 1129–1132.
- Adams Waldorf, K.M., Stencel-Baerenwald, J.E., Kapur, R.P., Studholme, C., Boldenow, E., Vornhagen, J., Baldessari, A., Dighe, M.K., Thiel, J., Merillat, S., et al. (2016). Fetal brain lesions after subcutaneous inoculation of Zika virus in a pregnant nonhuman primate. *Nat. Med.* 22, 1256–1259.
- Atkinson, B., Thorburn, F., Petridou, C., Bailey, D., Hewson, R., Simpson, A.J., Brooks, T.J., and Aarons, E.J. (2017). Presence and persistence of Zika virus RNA in semen, United Kingdom, 2016. *Emerg. Infect. Dis.* 23, 23.
- Barba-Spaeth, G., Dejnirattisai, W., Rouvinski, A., Vaney, M.C., Medits, I., Sharma, A., Simon-Lorière, E., Sakuntabhai, A., Cao-Lormeau, V.M., Haouz, A., et al. (2016). Structural basis of potent Zika-dengue virus antibody cross-neutralization. *Nature* 536, 48–53.
- Bista, M.B., Banerjee, M.K., Shin, S.H., Tandan, J.B., Kim, M.H., Sohn, Y.M., Ohrr, H.C., Tang, J.L., and Halstead, S.B. (2001). Efficacy of single-dose SA 14-14-2 vaccine against Japanese encephalitis: a case control study. *Lancet* 358, 791–795.
- Brasil, P., Pereira, J.P., Jr., Moreira, M.E., Ribeiro Nogueira, R.M., Damasceno, L., Wakimoto, M., Rabello, R.S., Valderramos, S.G., Halai, U.A., Salles, T.S., et al. (2016a). Zika virus infection in pregnant women in Rio de Janeiro. *N. Engl. J. Med.* 375, 2321–2334.
- Brasil, P., Sequeira, P.C., Freitas, A.D., Zogbi, H.E., Calvet, G.A., de Souza, R.V., Siqueira, A.M., de Mendonça, M.C., Nogueira, R.M., de Filipis, A.M., and Solomon, T. (2016b). Guillain-Barré syndrome associated with Zika virus infection. *Lancet* 387, 1482.
- Burke, D.S., Nisalak, A., Johnson, D.E., and Scott, R.M. (1988). A prospective study of dengue infections in Bangkok. *Am. J. Trop. Med. Hyg.* 38, 172–180.
- Cao-Lormeau, V.M., Blake, A., Mons, S., Lastère, S., Roche, C., Vanhomwegen, J., Dub, T., Baudouin, L., Teissier, A., Larre, P., et al. (2016). Guillain-Barré

Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 387, 1531–1539.

Charles, A.S., and Christofferson, R.C. (2016). Utility of a dengue-derived monoclonal antibody to enhance Zika infection in vitro. *PLoS Curr.* 8, 8.

Cugola, F.R., Fernandes, I.R., Russo, F.B., Freitas, B.C., Dias, J.L., Guimarães, K.P., Benazzato, C., Almeida, N., Pignatari, G.C., Romero, S., et al. (2016). The Brazilian Zika virus strain causes birth defects in experimental models. *Nature* 534, 267–271.

D'Ortenzio, E., Matheron, S., Yazdanpanah, Y., de Lamballerie, X., Hubert, B., Piorkowski, G., Maquart, M., Descamps, D., Damond, F., and Leparco-Goffart, I. (2016). Evidence of sexual transmission of Zika virus. *N. Engl. J. Med.* 374, 2195–2198.

Dejnirattisai, W., Supasa, P., Wongwiwat, W., Rouvinski, A., Barba-Spaeth, G., Duangchinda, T., Sakuntabhai, A., Cao-Lormeau, V.M., Malasit, P., Rey, F.A., et al. (2016). Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus. *Nat. Immunol.* 17, 1102–1108.

Dos Santos, T., Rodriguez, A., Almiron, M., Sanhueza, A., Ramon, P., de Oliveira, W.K., Coelho, G.E., Badaró, R., Cortez, J., Ospina, M., et al. (2016). Zika virus and the Guillain-Barré syndrome - case series from seven countries. *N. Engl. J. Med.* 375, 1598–1601.

Dowd, K.A., DeMaso, C.R., Pelc, R.S., Speer, S.D., Smith, A.R., Goo, L., Platt, D.J., Mascola, J.R., Graham, B.S., Mulligan, M.J., et al. (2016a). Broadly neutralizing activity of Zika virus-immune sera identifies a single viral serotype. *Cell Rep.* 16, 1485–1491.

Dowd, K.A., Ko, S.Y., Morabito, K.M., Yang, E.S., Pelc, R.S., DeMaso, C.R., Castilho, L.R., Abbink, P., Boyd, M., Nityanandam, R., et al. (2016b). Rapid development of a DNA vaccine for Zika virus. *Science* 354, 237–240.

Driggers, R.W., Ho, C.Y., Korhonen, E.M., Kuivanen, S., Jääskeläinen, A.J., Smura, T., Rosenberg, A., Hill, D.A., DeBiasi, R.L., Vezina, G., et al. (2016). Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. *N. Engl. J. Med.* 374, 2142–2151.

Dudley, D.M., Aliota, M.T., Mohr, E.L., Weiler, A.M., Lehrer-Brey, G., Weisgrau, K.L., Mohns, M.S., Breitbach, M.E., Rasheed, M.N., Newman, C.M., et al. (2016). A rhesus macaque model of Asian-lineage Zika virus infection. *Nat. Commun.* 7, 12204.

Duffy, M.R., Chen, T.H., Hancock, W.T., Powers, A.M., Kool, J.L., Lanciotti, R.S., Pretrick, M., Marfel, M., Holzbauer, S., Dubray, C., et al. (2009). Zika virus outbreak on Yap Island, Federated States of Micronesia. *N. Engl. J. Med.* 360, 2536–2543.

Elong Ngono, A., Vizcarra, E.A., Tang, W.W., Sheets, N., Joo, Y., Kim, K., Gorman, M.J., Diamond, M.S., and Shresta, S. (2017). Mapping and role of the CD8(+) T cell response during primary Zika virus infection in mice. *Cell Host Microbe* 21, 35–46.

Fauci, A.S., and Morens, D.M. (2016). Zika virus in the Americas—yet another arbovirus threat. *N. Engl. J. Med.* 374, 601–604.

Garcez, P.P., Loiola, E.C., Madeiro da Costa, R., Higa, L.M., Trindade, P., Delvecchio, R., Nascimento, J.M., Brindeiro, R., Tanuri, A., and Rehen, S.K. (2016). Zika virus impairs growth in human neurospheres and brain organoids. *Science* 352, 816–818.

Guirakhoo, F., Zhang, Z.X., Chambers, T.J., Delagrave, S., Arroyo, J., Barrett, A.D., and Monath, T.P. (1999). Immunogenicity, genetic stability, and protective efficacy of a recombinant, chimeric yellow fever-Japanese encephalitis virus (ChimeriVax-JE) as a live, attenuated vaccine candidate against Japanese encephalitis. *Virology* 257, 363–372.

Hadinegoro, S.R., Arredondo-García, J.L., Capeding, M.R., Deseda, C., Chotpitayasunondh, T., Dietze, R., Muhammad Ismail, H.I., Reynales, H., Limkittikul, K., Rivera-Medina, D.M., et al.; CYD-TDV Dengue Vaccine Working Group (2015). Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N. Engl. J. Med.* 373, 1195–1206.

Halstead, S.B. (2003). Neutralization and antibody-dependent enhancement of dengue viruses. *Adv. Virus Res.* 60, 421–467.

Halstead, S.B., and Thomas, S.J. (2011). New Japanese encephalitis vaccines: alternatives to production in mouse brain. *Expert Rev. Vaccines* 10, 355–364.

Halstead, S.B., Chow, J.S., and Marchette, N.J. (1973). Immunological enhancement of dengue virus replication. *Nat. New Biol.* 243, 24–26.

Henao-Restrepo, A.M., Longini, I.M., Egger, M., Dean, N.E., Edmunds, W.J., Camacho, A., Carroll, M.W., Doumbia, M., Draguez, B., Duraffour, S., et al. (2015). Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. *Lancet* 386, 857–866.

Hombach, J., Solomon, T., Kurane, I., Jacobson, J., and Wood, D. (2005). Report on a WHO consultation on immunological endpoints for evaluation of new Japanese encephalitis vaccines, WHO, Geneva, 2–3 September, 2004. *Vaccine* 23, 5205–5211.

Honein, M.A., Dawson, A.L., Petersen, E.E., Jones, A.M., Lee, E.H., Yazdy, M.M., Ahmad, N., Macdonald, J., Evert, N., Bingham, A., et al.; US Zika Pregnancy Registry Collaboration (2017). Birth defects among fetuses and infants of US women with evidence of possible Zika virus infection during pregnancy. *JAMA* 317, 59–68.

Ishikawa, T., Yamanaka, A., and Konishi, E. (2014). A review of successful flavivirus vaccines and the problems with those flaviviruses for which vaccines are not yet available. *Vaccine* 32, 1326–1337.

Jarmer, J., Zlatkovic, J., Tsouchnikas, G., Vratskikh, O., Strauß, J., Aberle, J.H., Chmelik, V., Kundi, M., Stiasny, K., and Heinz, F.X. (2014). Variation of the specificity of the human antibody responses after tick-borne encephalitis virus infection and vaccination. *J. Virol.* 88, 13845–13857.

Johansson, M.A., Mier-y-Teran-Romero, L., Reefhuis, J., Gilboa, S.M., and Hills, S.L. (2016). Zika and the risk of microcephaly. *N. Engl. J. Med.* 375, 1–4.

Kawiecki, A.B., and Christofferson, R.C. (2016). Zika virus-induced antibody response enhances dengue virus serotype 2 replication in vitro. *J. Infect. Dis.* 214, 1357–1360.

Kliks, S.C., Nimmanitya, S., Nisalak, A., and Burke, D.S. (1988). Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am. J. Trop. Med. Hyg.* 38, 411–419.

Kliks, S.C., Nisalak, A., Brandt, W.E., Wahl, L., and Burke, D.S. (1989). Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *Am. J. Trop. Med. Hyg.* 40, 444–451.

Kreil, T.R., Burger, I., Bachmann, M., Fraiss, S., and Eibl, M.M. (1997). Antibodies protect mice against challenge with tick-borne encephalitis virus (TBEV)-infected macrophages. *Clin. Exp. Immunol.* 110, 358–361.

Laoprasopwattana, K., Libraty, D.H., Endy, T.P., Nisalak, A., Chunsuttiwat, S., Vaughn, D.W., Reed, G., Ennis, F.A., Rothman, A.L., and Green, S. (2005). Dengue Virus (DV) enhancing antibody activity in preillness plasma does not predict subsequent disease severity or viremia in secondary DV infection. *J. Infect. Dis.* 192, 510–519.

Larocca, R.A., Abbink, P., Peron, J.P., Zanolto, P.M., Iampietro, M.J., Badamchi-Zadeh, A., Boyd, M., Ng'ang'a, D., Kirilova, M., Nityanandam, R., et al. (2016). Vaccine protection against Zika virus from Brazil. *Nature* 536, 474–478.

Lazear, H.M., Govero, J., Smith, A.M., Platt, D.J., Fernandez, E., Miner, J.J., and Diamond, M.S. (2016). A Mouse model of Zika virus pathogenesis. *Cell Host Microbe* 19, 720–730.

Ledgerwood, J.E., Wei, C.J., Hu, Z., Gordon, I.J., Enama, M.E., Hendel, C.S., McTamney, P.M., Pearce, M.B., Yassine, H.M., Boyington, J.C., et al.; VRC 306 Study Team (2011). DNA priming and influenza vaccine immunogenicity: two phase 1 open label randomised clinical trials. *Lancet Infect. Dis.* 11, 916–924.

Li, C., Xu, D., Ye, Q., Hong, S., Jiang, Y., Liu, X., Zhang, N., Shi, L., Qin, C.F., and Xu, Z. (2016). Zika virus disrupts neural progenitor development and leads to microcephaly in mice. *Cell Stem Cell* 19, 120–126.

Libraty, D.H., Acosta, L.P., Tallo, V., Segubre-Mercado, E., Bautista, A., Potts, J.A., Jarman, R.G., Yoon, I.K., Gibbons, R.V., Brion, J.D., and Capeding, R.Z. (2009). A prospective nested case-control study of dengue in infants: rethinking and refining the antibody-dependent enhancement dengue hemorrhagic fever model. *PLoS Med.* 6, e1000171.

Marston, H.D., Lurie, N., Borio, L.L., and Fauci, A.S. (2016). Considerations for developing a Zika virus vaccine. *N. Engl. J. Med.* 375, 1209–1212.

- Mason, R.A., Tauraso, N.M., Spertzel, R.O., and Ginn, R.K. (1973). Yellow fever vaccine: direct challenge of monkeys given graded doses of 17D vaccine. *Appl. Microbiol.* 25, 539–544.
- Matheron, S., d'Ortenzio, E., Leparco-Goffart, I., Hubert, B., de Lamballerie, X., and Yazdanpanah, Y. (2016). Long-lasting persistence of Zika virus in semen. *Clin. Infect. Dis.* 63, 1264.
- Melo, A.S., Aguiar, R.S., Amorim, M.M., Arruda, M.B., Melo, F.O., Ribeiro, S.T., Batista, A.G., Ferreira, T., Dos Santos, M.P., Sampaio, V.V., et al. (2016). Congenital Zika virus infection: beyond neonatal microcephaly. *JAMA Neurol.* 73, 1407–1416.
- Miner, J.J., Cao, B., Govero, J., Smith, A.M., Fernandez, E., Cabrera, O.H., Garber, C., Noll, M., Klein, R.S., Noguchi, K.K., et al. (2016). Zika virus infection during pregnancy in mice causes placental damage and fetal demise. *Cell* 165, 1081–1091.
- Mlakar, J., Korva, M., Tul, N., Popović, M., Poljšak-Prijatelj, M., Mraz, J., Kolenc, M., Resman Rus, K., Vesnaver Vipotnik, T., Fabjan Vodusek, V., et al. (2016). Zika virus associated with microcephaly. *N. Engl. J. Med.* 374, 951–958.
- Muthumani, K., Griffin, B.D., Agarwal, S., Kudchodkar, S.B., Reuschel, E.L., Choi, H., Kraynyak, K.A., Duperret, E.K., Keaton, A.A., Chung, C., et al. (2016). In vivo protection against ZIKV infection and pathogenesis through passive antibody transfer and active immunisation with a prMEnv DNA vaccine. *NPJ Vaccines*. Published online November 10, 2016. <http://dx.doi.org/10.1038/npjvaccines.2016.21>.
- Osuna, C.E., Lim, S.Y., Deleage, C., Griffin, B.D., Stein, D., Schroeder, L.T., Orange, R., Best, K., Luo, M., Hraber, P.T., et al. (2016). Zika viral dynamics and shedding in rhesus and cynomolgus macaques. *Nat. Med.* 22, 1448–1455.
- Pardi, N., Hogan, M.J., Pelc, R.S., Muramatsu, H., Andersen, H., DeMaso, C.R., Dowd, K.A., Sutherland, L.L., Searce, R.M., Parks, R., et al. (2017). Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature*. <http://dx.doi.org/10.1038/nature21428>.
- Parra, B., Lizarazo, J., Jiménez-Arango, J.A., Zea-Vera, A.F., González-Manrique, G., Vargas, J., Angarita, J.A., Zuñiga, G., Lopez-Gonzalez, R., Beltran, C.L., et al. (2016). Guillain-Barré syndrome associated with Zika virus infection in Colombia. *N. Engl. J. Med.* 375, 1513–1523.
- Petersen, L.R., Jamieson, D.J., Powers, A.M., and Honein, M.A. (2016). Zika virus. *N. Engl. J. Med.* 374, 1552–1563.
- Pierson, T.C., and Graham, B.S. (2016). Zika virus: immunity and vaccine development. *Cell* 167, 625–631.
- Pinto, A.K., Brien, J.D., Lam, C.Y., Johnson, S., Chiang, C., Hiscott, J., Sarathy, V.V., Barrett, A.D., Shresta, S., and Diamond, M.S. (2015). Defining new therapeutics using a more immunocompetent mouse model of antibody-enhanced dengue virus infection. *MBio* 6, e01316–e15.
- Plotkin, S.A. (2008). Vaccines: correlates of vaccine-induced immunity. *Clin. Infect. Dis.* 47, 401–409.
- Plotkin, S.A. (2010). Correlates of protection induced by vaccination. *Clin. Vaccine Immunol.* 17, 1055–1065.
- Qian, X., Nguyen, H.N., Song, M.M., Hadiono, C., Ogden, S.C., Hammack, C., Yao, B., Hamersky, G.R., Jacob, F., Zhong, C., et al. (2016). Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell* 165, 1238–1254.
- Rabe, I.B., Staples, J.E., Villanueva, J., Hummel, K.B., Johnson, J.A., Rose, L., Hills, S., Wasley, A., Fischer, M., and Powers, A.M.; MTS (2016). Interim guidance for interpretation of Zika virus antibody test results. *MMWR Morb. Mortal. Wkly. Rep.* 65, 543–546.
- Rasmussen, S.A., Jamieson, D.J., Honein, M.A., and Petersen, L.R. (2016). Zika virus and birth defects—reviewing the evidence for causality. *N. Engl. J. Med.* 374, 1981–1987.
- Rothman, A.L. (2011). Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nat. Rev. Immunol.* 11, 532–543.
- Russell, P.K., Udomsakdi, S., and Halstead, S.B. (1967). Antibody response in dengue and dengue hemorrhagic fever. *Jpn. J. Med. Sci. Biol.* 20 (Suppl), 103–108.
- Sabchareon, A., Wallace, D., Sirivichayakul, C., Limkittikul, K., Chanthavanich, P., Suvannadabba, S., Jiwariyavej, V., Dulyachai, W., Pengsaa, K., Wartel, T.A., et al. (2012). Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet* 380, 1559–1567.
- Sapparapu, G., Fernandez, E., Kose, N., Bin Cao, Fox, J.M., Bombardi, R.G., Zhao, H., Nelson, C.A., Bryan, A.L., Barnes, T., et al. (2016). Neutralizing human antibodies prevent Zika virus replication and fetal disease in mice. *Nature* 540, 443–447.
- Savidis, G., Perreira, J.M., Portmann, J.M., Meraner, P., Guo, Z., Green, S., and Brass, A.L. (2016). The IFITMs inhibit Zika virus replication. *Cell Rep.* 15, 2323–2330.
- Spector, S., and Tauraso, N.M. (1968). Yellow fever virus. I. Development and evaluation of a plaque neutralization test. *Appl. Microbiol.* 16, 1770–1775.
- Stettler, K., Beltramello, M., Espinosa, D.A., Graham, V., Cassotta, A., Bianchi, S., Vanzetta, F., Minola, A., Jaconi, S., Mele, F., et al. (2016). Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science* 353, 823–826.
- Swanstrom, J.A., Plante, J.A., Plante, K.S., Young, E.F., McGowan, E., Gallichotte, E.N., Widman, D.G., Heise, M.T., de Silva, A.M., and Baric, R.S. (2016). Dengue virus envelope dimer epitope monoclonal antibodies isolated from dengue patients are protective against Zika virus. *MBio* 7, 7.
- Tauber, E., Kollaritsch, H., Korinek, M., Rendi-Wagner, P., Jilma, B., Firbas, C., Schranz, S., Jong, E., Klingler, A., Dewasthaly, S., and Klade, C.S. (2007). Safety and immunogenicity of a Vero-cell-derived, inactivated Japanese encephalitis vaccine: a non-inferiority, phase III, randomised controlled trial. *Lancet* 370, 1847–1853.
- Thomas, S.J., L'Azou, M., Barrett, A.D., and Jackson, N.A. (2016). Fast-track Zika vaccine development - is it possible? *N. Engl. J. Med.* 375, 1212–1216.
- Vaughn, D.W., Green, S., Kalayanaroj, S., Innis, B.L., Nimmannitya, S., Suntayakorn, S., Endy, T.P., Raengsakulrach, B., Rothman, A.L., Ennis, F.A., and Nisalak, A. (2000). Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J. Infect. Dis.* 181, 2–9.
- Venturi, G., Mel, R., Marchi, A., Mancuso, S., Russino, F., Pra, G.D., Papa, N., Bertiato, G., Fiorentini, C., and Ciufolini, M.G. (2006). Humoral immunity and correlation between ELISA, hemagglutination inhibition, and neutralization tests after vaccination against tick-borne encephalitis virus in children. *J. Virol. Methods* 134, 136–139.
- Villar, L., Dayan, G.H., Arredondo-García, J.L., Rivera, D.M., Cunha, R., Deseda, C., Reynales, H., Costa, M.S., Morales-Ramírez, J.O., Carrasquilla, G., et al.; CYD15 Study Group (2015). Efficacy of a tetravalent dengue vaccine in children in Latin America. *N. Engl. J. Med.* 372, 113–123.
- Wang, Q., Yang, H., Liu, X., Dai, L., Ma, T., Qi, J., Wong, G., Peng, R., Liu, S., Li, J., et al. (2016). Molecular determinants of human neutralizing antibodies isolated from a patient infected with Zika virus. *Sci. Transl. Med.* 8, 369ra179.
- WHO (2016). Japanese Encephalitis Vaccines: WHO position paper, February 2015—Recommendations. *Vaccine* 34, 302–303.
- Yockey, L.J., Varela, L., Rakib, T., Khoury-Hanold, W., Fink, S.L., Stutz, B., Szigeti-Buck, K., Van den Pol, A., Lindenbach, B.D., Horvath, T.L., and Iwasaki, A. (2016). Vaginal exposure to Zika virus during pregnancy leads to fetal brain infection. *Cell* 166, 1247–1256.e4.
- Zhao, H., Fernandez, E., Dowd, K.A., Speer, S.D., Platt, D.J., Gorman, M.J., Govero, J., Nelson, C.A., Pierson, T.C., Diamond, M.S., and Fremont, D.H. (2016). Structural basis of Zika virus-specific antibody protection. *Cell* 166, 1016–1027.