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Zika Vaccine Development: Current Status

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Abstract

Zika virus outbreaks have been explosive and unpredictable and have led to significant adverse health effects—as well as considerable public anxiety. Significant scientific work has resulted in multiple candidate vaccines that are now undergoing further clinical development, with several vaccines now in phase 2 clinical trials. In this review, we survey current vaccine efforts, preclinical and clinical results, and ethical and other concerns that directly bear on vaccine development. It is clear that the world needs safe and effective vaccines to protect against Zika virus infection. Whether such vaccines can be developed through to licensure and public availability absent significant financial investment by countries, and other barriers discussed within this article, remains uncertain.

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While discovered in 1947, Zika virus (ZIKV) remained unimportant and unnoticed until the Yap Island outbreaks in 2007. Even then, it scarcely raised interest and concern quickly abated as the outbreak dwindled without further outbreaks occurring. This situation changed dramatically with the unexpected and large outbreaks that began in 2015 in Brazil—eventually resulting in an estimated 200,000 identified cases.[1] As a result, the world has had to play "catch-up" by rapidly investigating ZIKV immunology, pathophysiology, and its short- and long-term effects, as well as the full spectrum of pathology the virus may cause in different populations.

As of the time of this writing, no effective drug to treat—or vaccine to prevent—Zika infection is licensed and available. Both are needed, yet neither was a priority to scientists or funders until the 2015 outbreaks. Now, as of this writing, some 18 known vaccine candidates are in various stages of preclinical and clinical development. Research funding has been made available, although it is clearly too little, is contributed by too few countries, and may not be sustained. In this regard, recent calls for global funding of new vaccines have proven timely.[2] Lastly, there is still much to be learned about the virus's mechanism of disease action and how it causes specific pathology across different ages, sexes, medical conditions (eg, pregnancy), and other issues—some undoubtedly yet to be defined.

Despite the large outbreaks in 2015-2016, subsequent years have been relatively quiet in terms of ZIKV. In the United States and US territories, there were over 41,000 cases in 2016, fewer than 1200 cases in 2017, and only 220 cases in 2018.[3] The rest of the world has experienced a similar situation; nonetheless, much of the world is at risk, and the many people who travel are also at risk. Travelers from endemic areas can also introduce ZIKV into the new territories.[4-6] This scenario begs the question of what the future holds for this virus—continued low-level circulation, outbreaks in new locations, or recurrent outbreaks once population immunity wanes or as the percentage of naive individuals in a population increases?[7-11] The current unpredictability and lack of future ZIKV outbreaks is a major impediment to creating effective public health policies, disease surveillance, and control measures (including continued clinical vaccine development and studies).

In this review, we will briefly summarize current knowledge about ZIKV and outbreaks, while primarily focusing on issues

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related to Zika vaccine development and the current status of these efforts. Finally, we provide a "look ahead" regarding the future of Zika vaccine development.

ZIKA DISEASE/OUTBREAKS

Zika virus was first isolated in Uganda in 1947 in monkeys and spread beyond its historical endemic range in equatorial Africa and Asia to areas of Micronesia in 2013-2014.[12] Up until 2007, when a large epidemic in the Yap Islands resulted in the infection of three-quarters of the approximately 270,000 residents,[13] ZIKV infections remained limited to periodic cases or small-scale epidemics. Since being detected in 2015 in Brazil, with reported cases of congenital malformations and other neurologic disorders linked to ZIKV infection (in French Polynesia and Brazil), ZIKV has spread to many countries in South America, Central America, the Caribbean, and briefly into the United States. On February 1, 2016, the World Health Organization (WHO) declared a Public Health Emergency of International Concern regarding microcephaly, Guillain-Barré syndrome (GBS), and other neurologic conditions associated with the appearance of ZIKV in the Americas. On November 18, 2016, the WHO ended this Public Health Emergency of International Concern. It is projected that nearly 400 million individuals can be potentially infected with ZIKV annually (similar to dengue virus [DENV] infection and burden),[14] as the distribution of the *Aedes* mosquito species (*Aedes aegypti*, *Aedes albopictus*, *Aedes polynesiensis*, and *Aedes hensilli*) associated with ZIKV transmission is widespread. A recent study estimates that 22.7 million people in the United States live in areas with active year-round populations of the *Aedes* mosquito vectors, setting the stage for possible year-round ZIKV transmission.[15] Local transmission has already occurred in US territories in the Caribbean and Pacific regions, demonstrating the unexpected ability of ZIKV to quickly spread to nonepidemic regions. Countries with a documented presence of ZIKV (as of March 2018) are shown in Figure A, and countries suitable for *A aegypti* and *A albopictus* are shown in Figure B.

Zika virus infection typically causes no or mild clinical symptoms (such as mild fever, arthralgia, headache, conjunctivitis, rash, muscle and joint pain) in adults and was not considered a major public health threat until recently. These symptoms usually last for 2 to 7 days and have significant similarities with DENV-like disease. The incubation period of ZIKV is not clear but is likely to range from 3 to 12 days.[18] Only 18% of ZIKV infection cases are reported to be symptomatic, and many individuals might never recognize that they have been infected with ZIKV.[13]

Strong evidence now indicates that ZIKV infection can cause severe fetal neurologic malformations, including microcephaly or fetal death (referred to as congenital Zika syndrome).[19,20] The most severe spectrum of Zika syndrome pathology occurs in the unborn, including musculoskeletal, ocular, craniofacial, genitourinary, pulmonary, and other abnormalities.[21] Zika virus has also been linked to an increased incidence of a potentially life-threatening GBS (an inflammatory autoimmune disorder that affects the peripheral nervous system) in adults, as well as other neurologic abnormalities.[22] Guillain-Barré syndrome occurs at a frequency of 1.1 to 1.8 cases per 100,000 people per year and occurs predominantly in older adults.[23] Since the beginning of the ZIKV epidemic in French Polynesia in 2013, the incidence of ZIKV-associated GBS has increased by 20-fold,[24] with 42 patients diagnosed with Zika-associated GBS between November 2013 and February 2014.[25] Presently, Brazil is the most affected country, with estimates of 1.5 million cases of ZIKV infection reported since the outbreak began.[26] In 2015, the WHO defined a 19% increase in incidence of GBS in Brazil in comparison with the prior year.[27]

Zika virus is transmitted by *Aedes* genus mosquito bites; however, clinical cases of

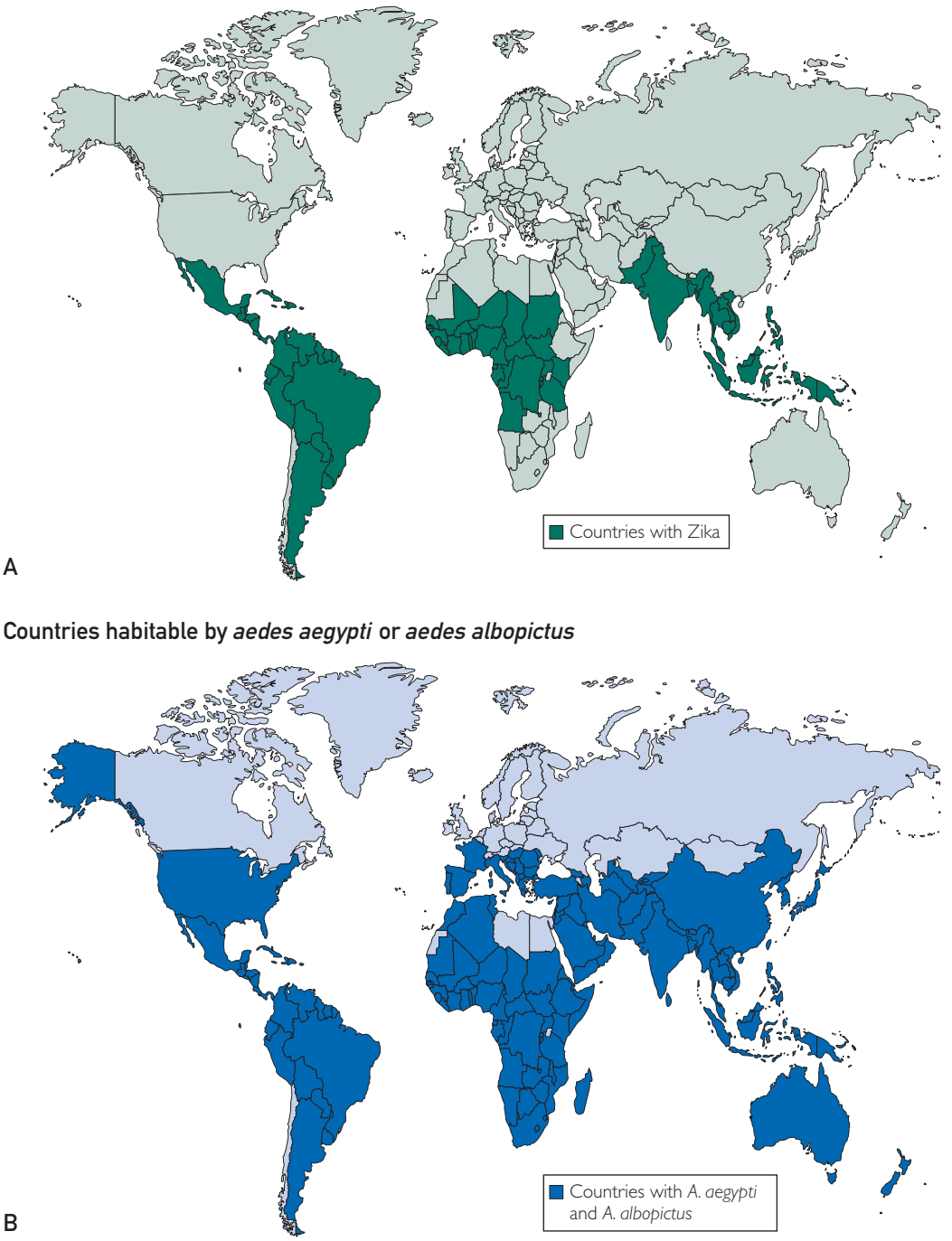

FIGURE. Countries at risk for Zika virus infection. A, The world map shows locations with known Zika virus presence in dark green (current as of March 2018). Data from the Centers for Disease Control and Prevention.[16] B, Countries suitable for *Aedes aegypti* and *Aedes albopictus*. Data from *Int J Infect Dis*.[17] Created with https://mapchart.net

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nonvector transmission, such as sexual transmission from infected males and mother to child transmission, have been well documented.[28] Zika virus has been detected in bodily fluids (eg, semen, urine, saliva, vaginal fluids, breast milk, and tears) following infection; the longest reported period from symptom onset to detection of ZIKV RNA was 9 months following infection in semen from 184 ZIKV-infected men.[29] Data from the largest cohort study to date revealed that infectious ZIKV RNA shedding in semen declines during the first 3 months after symptom onset.[29] Notably, a recently developed synthetic DNA vaccine encoding ZIKV premembrane/membrane (prM) and envelope (E) proteins entirely protected mice against ZIKV-induced testis and sperm damage.[30] New research using pregnant rhesus macaques revealed long-standing persistence of ZIKV in both maternal and fetal tissues, enhanced maternal and fetal immune responses, and fetal inflammation.[31] It is not yet clear if pathologic effects are different or more severe among immunocompromised persons. Given ZIKV pathogenesis and cell and tissue tropism, there is an urgent need to develop Zika vaccines that have the potential to protect the public—especially pregnant women and their fetuses—from ZIKV infection.[32]

ZIKA VIRUS

Zika is an emerging, mosquito-borne, enveloped, nonsegmented, 10-kilobase, single-stranded, positive-sense RNA flavivirus of global significance. Three main genetic lineages of ZIKV (2 from Africa [African I and African II lineages] and 1 from Asia) have been identified.[33-35] Genetic analyses indicate that its geographic circulation was limited to the areas around Africa and Asia by the 2000s; ZIKV then evolved into 2 distinct lineages with differential pathogenesis—African (ie, African I lineage) and Asian.[36-38] A study published in July 2018 by Beaver et al[39] revealed that the African lineages were more virulent than the Asian and induced stronger inflammatory responses. However, it is not clear why neurologic disorders and congenital malformations throughout the Americas are mainly associated with the Asian lineage strains.[40] The Asian strains have been found to activate innate immune interferon (IFN) regulatory factors via NS1, NS4B, and NS5 ZIKV nonstructural proteins while African lineage strains have not, demonstrating evolutionary differences in pathogenesis and molecular responses between Asian and African lineages.[39,41]

The Zika genome is analogous to that of other Flaviviridae RNA viruses (ie, yellow fever, Japanese encephalitis, West Nile, tick-borne encephalitis, and dengue type 1-4 viruses) and encodes for 3 major structural proteins (ie, prM, capsid [C], and E [a primary target of neutralizing antibody development]) and 7 nonstructural proteins (ie, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Notably, the nonstructural NS5 protein is implicated in RNA synthesis and viral replication. Importantly, the proteins documented to have significance in terms of immune response include nonstructural protein NS5 that has been shown to suppress type I IFN signaling through STAT2-mediated degradation in the ZIKV-infected host.[42] During viral shedding, prM is cleaved by furin protein to generate mature infectious virions. Zika virus E protein is the main surface protein that participates in host cell receptor attachment and virus lipid bilayer fusion, while the nonstructural NS1 through NS5 proteins are engaged in virus propagation. Robust adaptive immune responses to ZIKV E, NS1, NS3, NS4B, and NS5 proteins such as production of neutralizing antibodies and T-cell responses have been detected in animal models of ZIKV infection, which indicates that vaccination may protect against ZIKV disease.[43-46]

ISSUES IN VACCINE DEVELOPMENT

The ideal vaccine against Zika would require a single dose, be capable of being

TABLE 1. WHO Target Product Profile

Variable	Preferred profile	Minimal profile
Indication for use	Prevent clinical illness in individuals ≥9 y	
Contraindications	None for pregnant/lactating women	
Target population	Women of reproductive age Adolescent girls ≥9 y Boys/men of the same age range	
Exclusions	Depends on vaccine platform: Neurologic disorders Autoimmune disorders Immunosuppression	
Vaccine type	Nonreplicating platforms • Inactivate whole virus • Subunit-based Platforms with licensed vaccines	Single-cycle replicating vectors with robust safety data Live attenuated vaccine or replication competent with safety data
Safety/reactogenicity	Comparable to WHO-recommended vaccines Low risk of high fever	Tolerable reactogenicity and acceptable safety profile
Efficacy	Demonstrated prevention of ZIKV illness	Meets an established surrogate of immunity in animal models or cohort studies (ie, neutralizing Ab titers in >70% of recipients)
Dose	Single dose	Multiple dose
Durability	Long-lasting protection of more than 1 y and can be maintained by a single booster dose	Protection lasts at least 1 y and can be maintained by booster doses
Route	Injectable (IM or SC)	
Virus coverage	Monovalent with documented neutralization of Asian and African lineages	
Product stability	At least 12 mo at −20°C At least 6 mo at 2°-8°C	At least 12 mo at −20°C Stability for 6 h at 2°-8°C
Coadministration with other vaccines	Can be administered with other vaccines with no loss of immunogenicity or safety	Stand-alone vaccine
Presentation and packaging	Liquid product in single-dose or multidose packages. Maximal volume of 0.5 mL	Lyophilized product in single-dose or multidose packages. Maximal volume of 0.5 mL

Ab = antibody; IM = intramuscular; SC = subcutaneous; WHO = World Health Organization; ZIKV = Zika virus.
Data from the World Health Organization.[47]

administered to anyone regardless of age or medical condition (including pregnancy), result in durable (if not lifelong) immunity, prevent medically significant outcomes of infection in both the immediate recipient (eg, GBS) and in the fetus (eg, microcephaly and other congenital conditions), be safe and highly effective, and ideally not require either a cold chain or complex logistics to store and administer. Given the observation of intermittent outbreaks thus far, the ability to store vaccine stockpiles for long periods of time is also necessary. Such a product profile has yet to be achieved for any vaccine. The WHO has defined a more limited target product profile (Table 1), at least for a first-generation vaccine product.[47] Vaccine development will also face several challenges, including the lack of an established correlate of protection (in children, adults, and the fetus), current unpredictability and lack of outbreaks, the large number of subclinical infections, the marked variability in clinical manifestations during symptomatic illness, the need to test multiple vulnerable populations, the uncertain effects of prior immunity to ZIKV or other flaviviruses (ie, DENV), and the lack of animal models that recapitulate important features of human disease.[48] These and other factors have been discussed in a recent WHO landscape analysis.[49]

Zika Immunology

Both type I and type III IFNs are produced in response to ZIKV infection and can limit viral replication.[50] Zika virus proteins can actively inhibit the IFN response during infection.[51] As we learn more details about these virus-host interactions, the results may provide important information regarding appropriate adjuvant selection for vaccine candidates. Many of the animal models used for ZIKV work have defects in innate immune pathways; therefore, results must be interpreted with caution, as they may not reflect what will occur in humans. Nonhuman primate, guinea pig, and chicken embryo models are also being developed, as are models to study vaccine outcomes in pregnancy.[31,52-55] Efforts are also under way to develop a controlled human infection model for Zika, much like what has been done with influenza, malaria, and DENV.[56]

The adaptive immune response to flaviviruses (including ZIKV) involves both humoral and cell-mediated components. Neutralizing antibody (considered a major contributor to protective immunity) responses target the E protein and are cross-reactive with other flaviviruses.[57-59] This cross-reactivity has been an issue for diagnostic tests in areas where diseases such as dengue fever, West Nile virus, and yellow fever are endemic. In these geographic areas, coinfection with multiple flaviviruses is common and represents a complication in terms of immune response analysis that must be considered during the development and deployment of a ZIKV vaccine.[60]

Monoclonal antibody treatment protects mice against infection,[61] and depletion of T cells in previously vaccinated animals does not abrogate protective immunity.[62] These experiments suggest that humoral immunity can protect against disease and that vaccine-induced immunity can be protective. Despite this, there are several questions that remain unanswered: (1) in DENV, suboptimal antibody responses can enhance disease severity—a phenomenon known as antibody-dependent enhancement (ADE), and we do not know if ADE could also be an issue for ZIKV vaccines as it has been in the use of DENV vaccines; (2) vaccine responses are likely to be quite different in individuals who have no prior exposure to Zika or other flaviviruses compared with those with preexisting immunity, and these population groups may require fundamentally different vaccine formulations for both safety and optimal protection; and (3) it is likely that optimal humoral responses to ZIKV will require T-cell help, and a solitary focus on antibody titers as the only immunologic end point may be counterproductive.

Dengue virus infection also serves as a good model of cellular immunity to ZIKV. Robust T-cell responses develop after DENV infection, with $CD4^+$ T-cell responses targeting the C, E, and NS1 proteins while $CD8^+$ T-cell responses preferentially target the nonstructural proteins.[63,64] In contrast, for ZIKV, $CD4^+$ and $CD8^+$ T-cell epitopes have also been found in the prM, C, and E proteins.[65] This has also been observed for Japanese encephalitis virus.[66] In a mouse model, both vaginal and subcutaneous infection with ZIKV led to the proliferation of virus-specific T cells.[67] Adoptive transfer of these T cells into naive mice led to a significant reduction in viral titers upon infection, indicating that T-cell responses contribute to protective immunity. The evidence thus far suggests that an optimal vaccine would elicit both humoral and cellular immunity.

Vaccine Indications and Target Audience

Although Zika infection typically causes no or self-limited symptoms, more severe symptomatology can and does occur. This may be particularly true in pregnant women and women of childbearing age, infants/children, older adults, and those who are immunocompromised. Given the destructive fetal effects of Zika infection and the need to protect unborn children, it seems reasonable that the group requiring the most immediate protection is women, followed by men, of childbearing potential. This will likely be followed by the desire and need to protect all ages. Guillain-Barré syndrome may be particularly more likely and severe among older adults, and hence this represents a different spectrum of risk group. Such considerations may drive vaccine development efforts. For example, women who are pregnant cannot receive a live virus vaccine, while older immunosenescent adults may require vaccine adjuvants to boost immune response.

Ethical Issues

While not the intent of this review, we briefly comment on ethical issues related to vaccine development and policy. A significant ethical issue is how and under what conditions should pregnant women be included in Zika vaccine trials, given the unknown possible effects on the unborn developing child. Recent opinions on this question have been published.[68] Other issues include how to provide vaccine to those in lower income countries who cannot afford it and, given historic new vaccine pricing, how a vaccine can be utilized in poorer countries where outbreaks may occur. If vaccine supplies are limited, who shall be prioritized?

Animal Testing

Animal models provide an important testing ground for developing new vaccines. With the obvious caveat that these are models that only partially recapitulate human anatomy, physiology, and immune response, animal models of infection provide important insights because capturing the complexity and systemic interactions required for immune function requires a living organism. These models provide complete control over baseline characteristics (eg, age, sex, pregnancy status, preexisting immunity), timing and dose of infection, sampling time points, biospecimen collection (eg, blood, tissues, organs), and the ability to examine gonadal tissues and products of conception.

Mouse models are inexpensive, are available on multiple genetic backgrounds, are conducive to large sample sizes, and have a vast array of relevant immunologic tools (ie, antibodies and sequence information) available. Mouse models of ZIKV infection are relatively common, and a number of strains have been used to examine aspects of ZIKV disease.[69] Zika virus has variable replication efficiency in wild-type mouse strains (BALB/c greater than C57BL/6) but does not produce clinical illness in adult mice. Disruption of IFN responses is required for disease manifestations. The most common models are inbred strains on the 129 background that lack the IFNAR1 (A129) or both the IFNAR1 and IFNGR1

TABLE 2. Zika Vaccines in Development

Institute/company	Status	Vaccine(s) platform(s)
Inovio Pharmaceuticals, Inc	In phase I clinical trials	DNA vaccine
NIH	In phase I clinical trials	DNA vaccine Live VSV recombinant (early R&D) Live attenuated ZIKV (early R&D)
WRAIR/Sanofi Pasteur Limited	In phase I clinical trials	Whole, purified, inactivated virus
Butantan Institute	In phase I clinical trials Early-stage research	Live, DENV-vectored vaccine expressing premembrane/membrane and envelope proteins Purified inactivated virus
Bharat Institute of Science and Technology	Preclinical/animal studies	Purified inactivated virus VLP-expressing polyprotein
NewLink Genetics Corporation	Preclinical/animal studies	Purified inactivated virus
PaxVax, Inc	Preclinical/animal studies	Purified inactivated virus
Novavax, Inc	Preclinical/animal studies	Protein nanoparticle vaccine
Replikins Ltd	Preclinical/animal studies	Synthetic peptide vaccine
Pharos Biologicals LLC	Preclinical/animal studies	DNA vaccine
Bio-Manguinhos	Early-stage research	Purified inactivated virus YF17DD chimera VLP DNA
US CDC	Early-stage research	VLP expressing ZIKV DNA Live adenovirus recombinant
CureVac AG	Early-stage research	Thermostabile mRNA-based vaccine
Geovax Labs, Inc	Early-stage research	Live MVA recombinant
GlaxoSmithKline plc	Early-stage research	Self-amplifying mRNA platform Whole, inactivated virus
Hawaii Biotech Inc	Early-stage research	Aluminium hydroxide gel (Alhydrogel) + recombinant protein
University of Oxford	Early-stage research	Live adenovirus recombinant
Protein Sciences Corporation	Early-stage research	Recombinant envelope protein
Sanofi	Early-stage research	YF17D chimera
Sementis	Early-stage research	Live poxvirus recombinant
Themis Bioscience GmbH	Early-stage research	Live measles recombinant
Valneva SE	Early-stage research	Purified inactivated virus
Mayo Clinic Vaccine Research Group	Early-stage research	Naturally processed and HLA-presented ZIKV peptides packaged with biodegradable nanoparticles
Moderna, Inc	Early-stage research	Lipid nanoparticle–delivered mRNA
Emergent BioSolutions Inc	Early-stage research	Inactivated, whole virus
Institut Pasteur of Shanghai	Early-stage research	Recombinant subunit VLP
Takeda Pharmaceutical Company Limited	Early-stage research	Alum adjuvanted, inactivated whole virus
Edward Jenner Institute for Vaccine Research	Early-stage research	Simian adenovirus vector
VBI Vaccines Inc	Early-stage research	VLP containing envelope and NS1 proteins
Vaxart, Inc	Early-stage research	Recombinant oral vaccine

CDC = Centers for Disease Control and Prevention; DENV= dengue virus; mRNA = messenger RNA; MVA = modified vaccinia virus ankara; NIH = National Institutes of Health; R&D = research and development; VLP = virus-like particles; VSV= vesicular stomatitis virus; WRAIR = Walter Reed Army Institute of Research; ZIKV = Zika virus.

(AG129) and a strain on the B6 background lacking IFNAR1. Severe combined immunodeficiency mice also develop disease but at a delayed rate compared with AG129. Mice lacking INF response factors (IRFs) (eg, IRF3, IRF5, IRF7) also exhibit disease symptoms on experimental infection. Unfortunately, the immunosuppressed nature of these animals complicates vaccine development and testing. The use of INF receptor–blocking antibodies can be utilized in wild-type mice to recapitulate disease susceptibility. The use of neonatal mice and/or intracranial inoculation can produce some disease symptoms (eg, neurologic signs or paralysis). A model of intrauterine infection has been established in Swiss Jim Lambert (SJL) mice.[70]

Although immune responses to ZIKV have been detected in a large number of larger animals (water buffalo, goats, lions, sheep), nonhuman primates are the model of choice for ZIKV research. Rhesus and cynomolgus macaques are most commonly used. Clinical symptoms include erythema, fever, and lymphadenopathy, and virus can be found in multiple body fluids.[71] New World monkeys have more severe clinical symptoms after flavivirus infection than Old World monkeys and may represent a more restrictive animal model.[72] It should be noted that different ZIKV strains induce different symptoms in different animals, suggesting that human disease manifestations may also be strain-dependent. The nonhuman primate studies have provided important insights into ZIKV immunity, as well as efficacy testing for developmental vaccines. Together with the mouse strains, these animal models will continue to be useful platforms for testing and evaluating ZIKV vaccines.

CURRENT STATUS OF VACCINE DEVELOPMENT

The recent outbreaks of ZIKV in the Pacific and South America significantly heightened public health awareness of Zika as well as increased research interest in Zika virology, pathology, epidemiology, and immunology. In March 2016, the WHO reported 18 ZIKV vaccine programs, and the number may have grown since then. A wide variety of formulations are being studied; among those being tested are live virus vaccines, inactivated vaccines, whole-virus vaccines, subunit vaccines, and messenger RNA (mRNA)–, DNA-, protein-, and vector-based formulations. Table 2 lists the most developed of these candidates. Herein, we will highlight a few of the vaccine approaches.

The Walter Reed Army Institute of Research has developed a whole-virus, formalin-inactivated vaccine.[62] This vaccine has been tested with and without alum, utilizing multiple immunization routes, and in both mice and nonhuman primates. Vaccination elicits high-titer neutralizing antibodies as well as cellular immune responses and produces sterile immunity in rhesus macaques.[73] This vaccine has undergone phase 1 clinical trials (ClinicalTrials.gov Identifier: NCT02937233, NCT02952833, NCT02963909), and another phase 1, dose de-escalation study examining safety and immunogenicity is ongoing (NCT03008122). The results from the first 3 trials indicate that recipients developed robust neutralizing antibody titers that on transfer into mice significantly reduced or eliminated viral titers following ZIKV challenge.[74] Adverse effects were reported by 84% of recipients but were relatively minor (eg, pain or tenderness at injection site, fatigue, headache, malaise).

rZIKV/D4Δ30-713 is a live attenuated vaccine being tested in flavivirus-naive adults (NCT03611946). This vaccine is constructed on the backbone of DENV serotype 4 and expresses ZIKV surface proteins. The virus vector has been attenuated through the deletion of a 30 nucleotide sequence in the 3′ untranslated region that drastically reduces viral replication. The resulting vaccine vector has been administered to rhesus macaques and to humans with no serious adverse events reported.[75,76] In addition to

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protein and live virus vaccines, a number of nucleic acid vaccines have demonstrated promising results.

Messenger RNA 1325 (NCT03014089) is a non—self-amplifying mRNA-based vaccine expressing a prM-E protein. The antigen sequence has been modified to remove epitopes in regions known to contribute to DENV ADE.[77] Uridines have also been replaced with 1-methylpseudouridine in order to avoid innate immune responses to the nucleic acid backbone. AG129 mice developed neutralizing antibody following a single vaccination, and those titers increased significantly after a booster immunization. Animals receiving 10 ug of vaccine (with or without the booster immunization) were protected against a lethal ZIKV challenge. A lower vaccine dose (2 ug) resulted in 60% protection after a single immunization and 90% protection after a prime-boost immunization. The effects of the vaccine in immunocompetent mice (C57BL/6) were also studied. Neutralizing antibody titers were low but detectable after a single immunization and were significantly increased following the booster immunization. Mice were pretreated with IFNAR-blocking antibody and then challenged with ZIKV (Dakar 41519). Unimmunized mice developed high levels of viremia, lost weight, and experienced a 25% to 30% mortality rate following challenge. In contrast, mice given a prime-boost immunization exhibited no viremia, no weight loss, and 100% survival. These studies also demonstrated that removal of the E-DII-FL epitope thought to be involved in ADE did not affect the development of neutralizing antibodies or vaccine-induced protection.

A similar mRNA-based vaccine also incorporates a modified nucleoside (1-methylpseudouridine) and encodes the prM-E glycoproteins of the H/PF/2013 strain of ZIKV.[45] This vaccine construct contains modified nucleosides intended to boost antigen production and reduce innate immune responses to the construct. Mouse studies revealed that the vaccine elicited neutralizing antibody, antigen-specific $CD4^+$ T-cell responses and protected against viral challenge. Vaccination of nonhuman primates (rhesus macaques) resulted in the development of E protein—binding antibody and neutralizing antibody, and 80% of immunized animals were protected against viral challenge (ie, no detectable viremia).

MV-ZIKA, a recombinant measles virus expressing the prM and E proteins (NCT02996890), has been developed by Themis Bioscience GmbH. This construct is based on the measles virus vaccine backbone, which is an approach that has also shown promise with chikungunya virus. The measles vaccine has an excellent safety record and results in robust immunity to measles. It is hoped that these characteristics are retained when novel antigens are added to the measles vaccine virus genome.

The most advanced vaccine candidates to date include VRC-ZKADNA090-00-VP, a plasmid-based DNA vaccine encoding the prM and E proteins of a French Polynesian strain of ZIKV (H/PF/2013). It is currently undergoing a phase 2 clinical trial in healthy adults aged 18 to 35 years (NCT03110770), along with VRC-ZKADNA085-00-VP, a similar DNA-based plasmid vaccine also encoding the prM and E proteins of the H/PF/2013 strain of ZIKV (NCT02840487).[78,79] Animal studies demonstrated that these vaccine candidates were highly immunogenic in BALB/c and C57BL/6 mice, eliciting high-titer neutralizing antibodies at 2-, 10-, and 50-ug doses. A 2-dose vaccination schedule in rhesus macaques elicited virus-binding and virus-neutralizing antibody responses. The vaccines also reduced—and, in some animals, eliminated—viremia after viral challenge. The study also found a correlation between protection from challenge- and prechallenge-neutralizing antibody titer.

VLA1601 is a purified, inactivated, whole-virus, alum-adsorbed vaccine being

tested in flavivirus-naive adults (NCT03425149). Early study results indicate that the vaccine elicits neutralizing antibodies in a dose- and schedule-dependent manner.[80] This vaccine was developed using the same platform as the IXIARO Japanese encephalitis vaccine.

After 2016, the number of ZIKV cases has decreased to the point that phase 3 field trials of the previously mentioned vaccines may be difficult or impossible to conduct.[48] Nevertheless, evaluating the effectiveness of these and other vaccine candidates is essential if we are to be prepared for a reemergence of the virus. This reemergence may occur in regions with prior epidemics once population immunity wanes and/or among naive populations due to shifts in geographic range of the *Aedes* vectors. Governmental agencies such as the US Food and Drug Administration and the European Medicines Agency are exploring pathways for regulatory approval that provide the needed flexibility to address ZIKV-specific considerations.[48,81]

A wide variety of other preclinical-stage vaccine candidates are currently being developed. The Mayo Clinic Vaccine Research Group is using an established mass spectrometry approach to isolate ZIKV-derived peptides from infected cells for use as a peptide-based vaccine. Peptide-based vaccines have a number of significant advantages that would be ideally suited for use as a ZIKV vaccine: inexpensive to manufacture, safe for universal administration, no cold chain requirements, easily stored and administered, can contain T- and/or B-cell epitopes, and have a defined epitope selection in order to avoid ADE. Preclinical studies utilizing such peptides in combination with biodegradable nanoparticles in mice are currently in progress.

EXPERT COMMENTARY AND A LOOK AHEAD

We believe that safe and effective Zika vaccines can be developed and approved; however, major concerns exist. First, because Zika outbreaks have essentially ceased and are unpredictable, it is likely that vaccine development may be stalled and timely efforts curtailed. Recently, the results of a WHO/National Institutes of Health conference on this issue have been published.[48] This problem may require the use of the Food and Drug Administration animal rule, human challenge studies, passive transfer animal studies, and other methods in order to continue to advance vaccine development and licensure.

Historically, such has been the case when an emerging pathogen causes substantial concern and threatens public health, emergency funding from governments results, only to not be sustained as the epidemic wanes. The recent lessons of severe acute respiratory syndrome, Middle East respiratory syndrome, Lassa fever, and to a lesser extent Ebola and others, loom large in this regard. When financial costs and profit are uncertain, the private sector will be reluctant to invest the magnitude of dollars necessary to bring such a vaccine to the point of licensure. If so, sustainable models for funding must be developed before the next large outbreaks of Zika.

Second, given current realities, it is likely that more than one Zika vaccine type may need to be developed. For example, an inactivated vaccine will be required for pregnant women and perhaps women of childbearing age. Live attenuated vaccines could be deployed in children, males, and older adults. Higher-potency or adjuvanted vaccines may be needed for the immunosenescent elderly and those who are immunocompromised. DNA- or vector-based vaccines may prove more immunogenic and result in longer-lasting immunity.

Third, more research is necessary to determine what the Zika infection outcomes of interest are that we want vaccine to prevent. Is it GBS? Zika congenital conditions? Latent infection and persistence in gonadal organs? Other? Fourth, issues of vaccine-induced immunity are significant. How many doses will be required and over what time periods?

How durable and long-lasting will vaccine-induced immunity be? Are there risks for ADE in humans resulting in inadvertent harm? Fifth, ethical issues such as human challenge studies that include pregnant women in vaccine development clinical trials and include children in clinical trials, among others, must be defined. These and other important questions remain and must be solved. Although we believe they are solvable, it may require considerable time—even decades—to do so. In the meantime, the most advanced candidate vaccine in clinical development appears to be the National Institutes of Health-developed vaccine. It is likely, assuming no safety concerns arise in future phase 3 studies, that this vaccine will be the first licensed. This is likely to be followed by additional vaccines and effective antiviral drugs to treat and prevent infection over the short term.

CONCLUSION

Public health urgency, advances in genetics-based design of vaccines, and newer insights in immunology have combined to accelerate vaccine development against Zika virus. Multiple vaccine candidates are already in preclinical through phase 2 studies, with some poised to enter phase 3 studies of efficacy. This is remarkable given the fact that Zika virus went relatively unnoticed until the large outbreaks in Brazil starting in 2016. On the positive side, this extraordinary progress has resulted from collaborations among academia, biotech start-ups, pharmaceutical companies, nongovernmental organizations, and a variety of funders. On the negative side, inadequate research funding, late funding given the initial outbreaks in the Yap Islands in 2007, and a variety of difficulties in moving vaccine candidates down the development pipeline, have slowed progress. In particular, governments must invest the research and development dollars and provide industry incentives for rapid clinical development to occur. This must be a shared burden but should particularly have intense and sustained involvement by the countries where mosquito abatement has been "too little too late" and where outbreaks of this disease have developed. Additionally, these outbreaks are an excellent example of the need for a global funding entity—as called for in a recent publication. It is likely that in the private sector, the costs of Zika vaccine development cannot be recouped due to the relative lack of current and ongoing large-scale outbreaks. Thus, industry will need incentives or government-provided development dollars to invest resources into the multimillion dollar enterprise needed to advance a vaccine candidate through to approval by regulatory authorities.

In the meantime, limited history thus far suggests that ZIKV outbreaks can strike and abate relatively rapidly, with limited ability to predict where the next outbreak(s) will occur. Other unknowns include possible further mutational divergence of strains and the development of new Zika clades that may require more than a monotypic vaccine for full protection. Lastly, thoughtful decisions must be made regarding whom and with what priority different groups should receive vaccine, at what ages, and to prevent which outcomes. Other issues such as whether pregnant women can be enrolled in vaccine studies and receive specific vaccine types will need to be debated and considered.

The health and well-being of the globe requires a safe and effective Zika vaccine. For couples of childbearing age desiring to have children, the prospect of Zika infection is terrifying. Maternal infections can and do lead to a spectrum of congenital consequences—some of which are severe and much of which is yet to be fully unraveled. The global community must pay attention and remain undeterred by the tyranny of the next urgency that detracts attention away from the goal of a vaccine. It is not simply an infection "over there"—this is a global issue that requires a global solution. The future and health of the most vulnerable among us—the yet unborn—depends on our efforts.

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Abbreviations and Acronyms: **ADE** = antibody-dependent enhancement; **C** = capsid; **DENV** = dengue virus; **E** = envelope; **GBS** = Guillain-Barré syndrome; **IFN** = interferon; **IRF** = IFN response factor; **mRNA** = messenger RNA; **prM** = premembrane/membrane; **WHO** = World Health Organization; **ZIKV** = Zika virus

Potential Competing Interests: Dr Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories and is a consultant on vaccine development to Merck & Co. Inc, Avianax LLC, Adjuvance, Valneva SE, Medicago Inc, Sanofi Pasteur Limited, GlaxoSmithKline plc, and Emergent BioSolutions Inc. Drs Poland and Ovsyannikova hold 3 patents related to vaccinia and measles peptide vaccines. Dr Kennedy holds a patent related to vaccinia peptide vaccines and has received funding from Merck Research Laboratories to study waning immunity to mumps vaccine. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

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The Thematic Reviews on Vaccines will continue in an upcoming issue.

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