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Review

An Update on Zika Virus Vaccine Development and New Research Approaches

Angie Lizeth Buitrago-Pabón [1,2,3,†], Salvador Ruiz-Sáenz [2,3,†], Alicia Jiménez-Alberto [1,2,3], Gerardo Aparicio-Ozores [1,3], Juan Arturo Castelán-Vega [1,2,3,*] and Rosa María Ribas-Aparicio [1,2,3,*]

1 Doctoral Program in Biomedicine and Molecular Biotechnology, Microbiology Department, Escuela Nacional de Ciencias Biológicas (ENCB), Instituto Politécnico Nacional (IPN), Mexico City 11340, Mexico; angiebuitrago23@hotmail.com (A.L.B.-P.); ajimeneza@ipn.mx (A.J.-A.); gaparico@ipn.mx (G.A.-O.)
2 Laboratorio de Producción y Control de Biológicos, Departamento de Microbiología, ENCB, IPN, Mexico City 11340, Mexico; sruizs2200@alumno.ipn.mx
3 Laboratorio de Biotecnología Molecular y Farmacéutica, Departamento de Microbiología, ENCB, IPN, Mexico City 11340, Mexico
* Correspondence: jcastelv@ipn.mx (J.A.C.-V.); rribas@ipn.mx (R.M.R.-A.); Tel.: +52-(55)-5729-6300 (ext. 62384) (R.M.R.-A.)
† These authors contributed equally to this review.

Abstract: Zika virus (ZIKV) is an emerging flavivirus that represents significant public health challenges, particularly in the Americas, and is a substantial risk to other parts of the world due to its rapid expansion and its established association with neurological disorders, including Guillain–Barré syndrome and an intrauterine fetal infection that can cause microcephaly, blindness, and other congenital neurological complications. To date, no vaccine to prevent ZIKV infections has been approved. Therefore, developing a safe and effective vaccine against this virus is a global health priority. This review analyzes the ZIKV outbreaks, as well as associated neurological complications, its genome, and immunological responses. The current vaccines in development have reported results from preclinical and clinical trials about novel approaches to obtain safer and more effective vaccines and the challenges faced by ZIKV vaccine development.

Keywords: vaccines; ZIKV; ADE; immunoinformatics; epitopes

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1. Introduction

Zika virus (ZIKV) is an emerging flavivirus that was first isolated in 1947 from the blood of Rhesus monkey 766 in Uganda [1]. The first evidence of human infection by this virus was detected in 1952 when neutralizing antibodies were observed in sera of patients from East Africa. In the years following, it became evident that ZIKV was confined mainly to areas in Africa [2–5]. However, in 2007, isolated outbreaks were reported on the island of Yap, resulting in a major epidemic that infected approximately three-quarters of Yap residents, with about 5000 of the 6700 residents affected [6]. Subsequently, in 2014, another significant outbreak occurred in French Polynesia [7]. At the end of 2015, the virus was detected for the first time in Brazil [8]; since then, it has attracted global attention due to its rapid expansion, particularly in tropical and subtropical regions. As of January 2018, the Centers for Disease Control and Prevention (CDC) had reported 223,477 confirmed cases in 87 countries and territories worldwide, of which 48 are from Latin America [9,10]. It has been reported that ZIKV transmission to humans can occur through sexual transmission, bodily fluids, blood transfusion, and vertical transmission from mother to fetus during pregnancy [11–15]. However, the most frequent form of transmission is through the puncturing of the skin during the feeding process of female mosquitoes of the genus *Aedes* (*A. aegypti*, *A. furcifer*, *A. taylori*, *A. luteocephalus*, and *A. africanus*) [16,17]. The incubation period of the virus is 3 to 12 days, and the most frequent clinical manifestations

seen in infected patients are high fever, skin rash, conjunctivitis, headache, malaise, and muscle-and-joint pain that lasts from 2 to 7 days and automatically resolves over time [10]. It should be noted that only 18% of ZIKV infection cases are symptomatic, and many people may never recognize that they have been infected [6]. Despite the enormous efforts of researchers, there is still no clinically approved drug to treat the disease; however, several drug candidate molecules have been discovered that have shown activity against ZIKV in vitro [18,19].

2. Neurological Complications

On 1 February 2016, the World Health Organization (WHO) declared ZIKV an international public health emergency [20] because the Zika outbreaks that have occurred in the Americas in recent years have been associated with Congenital Zika Syndrome (CZS). CZS is an intrauterine fetal infection that can cause congenital neurological complications such as microcephaly, ventriculomegaly, intracranial calcification, ocular abnormalities, and hearing loss [21,22]. CZS can also cause fetal development disorders such as growth restriction or fetal death when women are infected with the virus during the first trimester of pregnancy [21,23–27]. Cellular tropism and transmission mechanisms of ZIKV from mother to fetus during early pregnancy are still unknown in many aspects [28]. However, ZIKV has been shown to effectively target neural progenitor cells (NPCs), drastically shrinking them. In addition, it can cross the placenta and infect the amniotic fluid and fetal brain tissues, causing a significant impact on brain development [23–25]. ZIKV infection leads to cell cycle arrest, apoptosis, and inhibition of neural precursor cell differentiation, leading to cortical thinning and microcephaly [24]. In the context of ZIKV circulation, between 2015 and January 2018, more than 3720 cases of children born with congenital disabilities associated with previous ZIKV infections were reported in the Americas [28]. Likewise, during the epidemics in French Polynesia (2014) and the Americas (2015–2016), the incidence of Guillain–Barré syndrome (GBS) increased, and an association between GBS and ZIKV was established in a small proportion of those infected through epidemiological studies [29,30]. GBS is the most common cause of acute flaccid paralysis worldwide, with an incidence rate of approximately 1 for every 100,000 person-years [31]. GBS is an acute inflammatory polyradiculoneuropathy mediated by the immune system that classically presents progressive weakness, sensory changes, and hyporeflexia and is usually triggered by previous infection or other antigenic stimuli [32,33]. To date, 14 countries have reported an increased incidence of GBS associated with ZIKV [34]. In Brazil in 2015, 1708 cases of GBS with laboratory confirmation of prior ZIKV infection were reported [35]. In Colombia, among 381 cases of patients with neurological syndromes, 42 were confirmed as GBS associated with the virus [36]. In Mexico, there have been 95 cases in which the association between ZIKV and GBS was evaluated, and it was concluded that GBS occurs among patients with clinical symptoms [37,38].

3. ZIKV Genome

The ZIKV genome consists of a single chain of positive-sense RNA of approximately 11 kb delimited by two non-coding regions (5′- and 3′-NCR) and a single open reading frame encoding a polyprotein, which is processed into three structural proteins (capsid protein (C), precursor membrane (prM), and envelope protein (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [39–42]. The latter are involved in viral genome replication, virion assembly, polyprotein processing, and evasion from the host's antiviral responses [43,44].Results obtained by cryogenic electron microscopy (cryo-EM) have shown that the mature structure of ZIKV contains a capsid surrounded by a lipid bilayer that contains 180 copies of the E and M proteins in an icosahedral arrangement of 90 antiparallel heterodimers of E:M proteins, completely covering the viral surface (Figure 1A), like other flaviviruses [45–47]. The E protein consists of the following three domains: domain I (EDI), domain II (EDII), and domain III (EDIII) (Figure 1B) [40,41,46]. EDI acts as a bridge between EDII and EDIII, playing a crucial role in protein folding and the

recognition of cell receptors [41,45]. EDII is responsible for the dimerization of the protein and contains the fusion peptide, which helps in the fusion of viral and cell membranes during the entry of viruses into the cell and is highly conserved in flaviviruses [40,42]. EDIII is an immunoglobulin-like domain that binds the virus to cell receptors [47,48]. The E protein exhibits only one Asn154 glycosylation site (in the glycan loop), identified as relevant for neurovirulence (Figure 1C) [47–49].

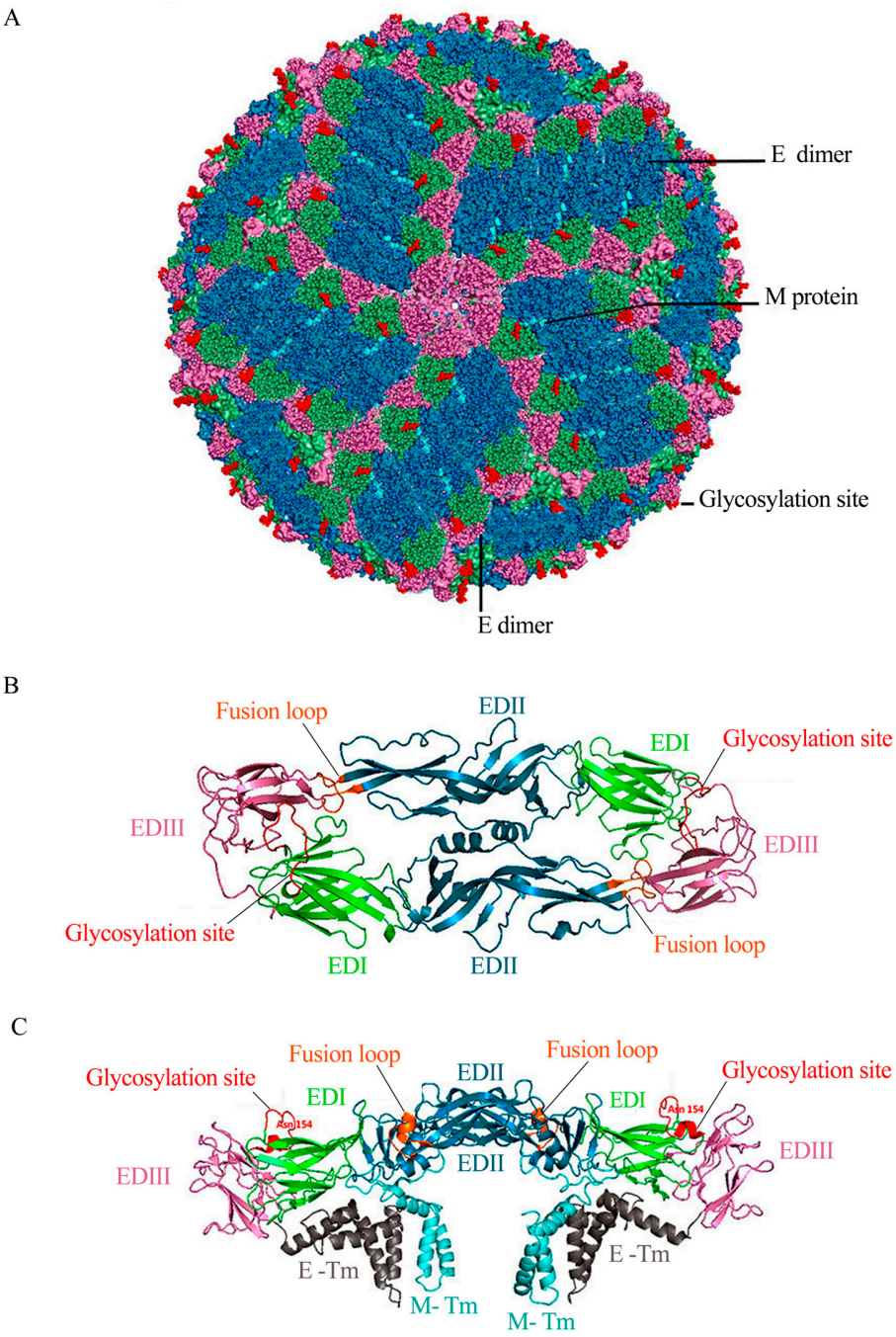

Figure 1. Structure of ZIKV. (**A**) Bioinformatics modeling of the mature structure of ZIKV (Based on Protein Data Bank [PDB] 5IRE, 3.8 Å resolution) [45]. E proteins are organized as dimers, and three parallel dimers form the raft configuration. Thirty of these herringbone-like rafts completely cover the viral surface. In the dimers of the E protein, EDI, EDII, and EDIII are shown in green, blue, and pink, respectively. The M protein, hardly seen in the lower layer (cyan), fills the space between the E-protein rafts. The conserved glycosylation site in Asn154 is highlighted in red. (**B**) Top view of the E-protein dimer. EDI, EDII, and EDIII are shown in green, blue, and pink, respectively. The fusion

loop located between residues 98–109 is shown in orange. (**C**) A side view of the E-protein dimer shows the transmembrane domains of E protein (E-TM) in gray and the transmembrane stem of M (M-Tm) in cyan.

Phylogenetic trees based on the entire coding region or NS5 region revealed the following three distinct genetic lineages of ZIKV reflecting the geographic origin: East Africa (Uganda prototype strain), West Africa (Senegal strains), and Asia (Yap Island strain). This study concluded that the Asian lineage diverged from a common ancestor and spread throughout Southeast Asia and the Pacific [50]. Congenital disabilities have only been reported in cases of infection by ZIKV strains belonging to the Asian lineage, including those identified during a 2016 outbreak in Angola. Therefore, it is crucial to note that the strains circulating in the Americas have been shown to belong to this lineage [9,24,27].

Similarly, a recent study demonstrated that these strains have a serine–asparagine substitution at prM position 139, leading to a more severe microcephalic phenotype and a thinner cortex than the ancestral strain in mice [50–52]. The occurrence of the S139N substitution is associated with increased reports of children born with congenital microcephaly and other serious neurological abnormalities, including GBS. This study suggested a possible explanation for the unexpected causal link between ZIKV and microcephaly and a probable understanding of how ZIKV evolved from a harmless mosquito-borne virus to a congenital pathogen with global impact [52,53].

4. Immune Response

Regarding the innate immune response, it has been shown that the activation and secretion of type I interferons (IFN-I) are critical because they can inhibit ZIKV replication in human cell lines and model mice [43,44,54–56]. In response to these mechanisms, ZIKV can antagonize IFN-I signaling during infection and disrupt the transcription of hundreds of IFN-stimulated genes (ISG), which encode the proteins that inhibit virus replication and spread [44,55,57,58]. Non-structural proteins NS1, NS3, NS4A, and NS5 have been identified as critical suppressors of IFN-I production [43,57,59]. The NS5 protein inhibits IFN-I signaling in human cells through the degradation of STAT2 in the ZIKV-infected host [54,56–58]. Therefore, knowing this mechanism of evasion of the immune response by the virus is essential to provide new venues for the development of antiviral drugs against ZIKV [59].

The adaptive immune response to ZIKV is protective and mediated by antibody-producing B cells in humoral immunity and T cells in cellular immunity [43,44,54,60]. In humoral immunity, viral proteins E, prM, and NS1 are the main targets of the neutralizing antibody response induced by ZIKV; these antibodies are essential for the generation of protection against virus infection [60–62]. Several isolated human monoclonal antibodies (mAbs) have demonstrated broad specificity against all ZIKV strains and potent protective activity both in vitro [62,63] and in vivo [64,65]. One of the most potent neutralizing antibodies, ZIKV-117, has demonstrated in vivo protective efficacy in mice against strains from Africa, Asia, and America. In addition, it decreased placental and fetal infection and reduced mortality in mice [65–67]. Likewise, it was found that there are highly specific neutralizing antibodies to ZIKV that recognize EDIII from E protein and have been reported to be the most potent neutralizers [65,68–70]. Another group of antibodies that recognize EDI and EDII has also been identified. They cross-reacted with DENV in vitro, which could cause antibody-dependent enhancement (ADE) and worsen the disease [71].

On the other hand, studies carried out in humans, non-human primates (NHPs), and mice infected by the virus have shown that the immunodominant response of ZIKV-specific CD8+ T cells is directed mainly towards structural proteins E, prM, and C, while CD4+ T response is directed toward proteins E, NS1, and NS5 [44,71–74]. Studies in mice demonstrated that CD8+ T has a protective role during infection, since the adoptive transfer of CD8+ T to Ifnar1−/− mice prevented central nervous system (CNS) infection and reduced weight loss and viral load [74–76]. Another study revealed that CD8+ T

responses in pregnant mice decreased compared to non-pregnant mice. These results suggest that lowered T-cell-mediated immunity during pregnancy may increase the spread of the virus to the fetus [77]. Finally, although the adaptive immune response to the virus has been attributed to CD8+ T cells, the participation of CD4+ T cells is not fully understood [78]. A study revealed that an immune response involving CD4+ T cells and IFNγ signaling promoted the generation of B cells and anti-ZIKV neutralizing antibodies. This response was correlated with reduced viral load in the CNS and survival [73,78]. Likewise, it showed that the adoptive transfer of purified CD4+ T cells prevented weight loss associated with the infection and protected mice from a lethal challenge with ZIKV. Therefore, it was concluded that CD4+ T cells could play a crucial role in the protective immune response to ZIKV [21,78,79].

4.1. Antibody-Dependent Enhancement (ADE) of ZIKV Infection

There is a complex serological interaction between ZIKV and other human pathogenic flaviviruses, mainly with DENV [80]. The consequence of this interaction is the production of cross-reactive antibodies generated by previous infections with heterologous viruses, which can cause disease-enhancing activity through ADE [63,66,71,80,81]. The cell biology of ADE is still unknown [81]. However, it is believed to increase the efficacy of infection within the host after secondary infection (especially after reinfection by DENV) [82]. This occurs due to the presence of high concentrations of non-neutralizing antibodies that cross-react against the structural proteins of the secondary virus, facilitating the uptake of the virus by cells that express FcγR receptors and, therefore, the stimulation of higher viral loads [81,83–85]. Different in vitro studies have demonstrated the phenomenon of ADE among certain flaviviruses because specific antibodies against DENV [83,84,86] and WNV [87,88] increased the infectivity of ZIKV in cell culture. Likewise, in vivo studies have shown that DENV disease is lethally potentiated in mice [68,71,87,89] and NHPs when previously exposed to ZIKV [90,91]. On the other hand, analyses of mAbs generated from sera from donors infected with ZIKV and DENV also demonstrated cross-reactivity between these flaviviruses [71,86,88,92,93].

Antibodies prone to ADE were found to target epitopes of the prM and E protein fusion loop (FLE), a highly cross-reactive antigenic site [93,94]. These antibodies had little neutralizing activity. However, they potently promoted ADE [86,94,95].

It is unclear whether ZIKV antibodies could cause ADE in reinfection with DENV in humans. However, the first known patient with a fatal case of DENV strongly associated with prior exposure to ZIKV was recently reported in the United States [96]. This finding suggests that pre-existing immunity to ZIKV likely played a role in triggering ADE and intensified the DENV1 infection, leading to a fatal case of dengue hemorrhagic fever/Down syndrome consistent with ADE [95,96]. Therefore, given that the ZIKV outbreaks in recent years have occurred in DENV-endemic areas, the possibility that pre-existing antibodies may cause ADE or that an increased risk of fetal transmission of a ZIKV infection exists are among the most critical concerns in flavivirus vaccine design. The most recent research suggests that the mutation or deletion of prM and FLE in the design of new vaccines against ZIKV should help to reduce the risk of the induction of antibodies prone to ADE [95,96].

4.2. Evaluation of ZIKV Vaccines

ZIKV has challenged the scientific community to address a relatively poorly characterized pathogen that represents a substantial threat to global public health due to its rapid expansion throughout the Americas and a close association with neurological complications such as Guillain–Barré syndrome and congenital microcephaly in neonates. Research is currently underway to develop a prophylactic vaccine against the virus. Strategies under study span multiple vaccine platforms, including live attenuated and inactivated whole-virus vaccines, nucleic acid vaccines (DNA and RNA vaccines), viral vector-based vaccines, peptide subunit vaccines, recombinant proteins, and virus-like particles (Figure 2). Although no licensed vaccines or antivirals are yet available to prevent and control ZIKV, several

vaccine candidates are undergoing clinical trials. For this reason, the rapid development of safe and effective vaccines against the virus is a public health priority. In the following section, we will offer an in-depth overview of the current ZIKV vaccine candidates and platforms undergoing clinical trials.

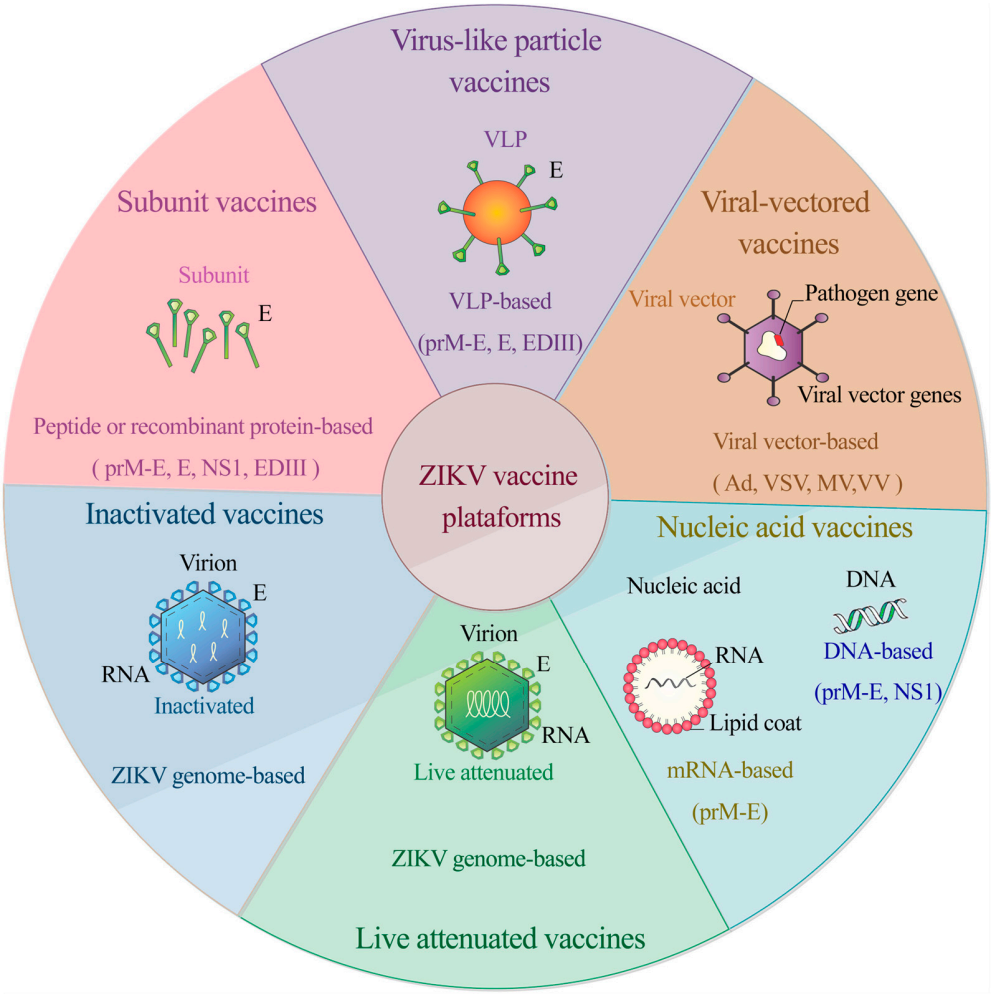

Figure 2. Vaccine platforms under development against ZIKV. Strategies span multiple vaccine platforms, including live attenuated and inactivated whole-virus vaccines, nucleic acid vaccines (DNA and RNA), viral vector-based vaccines, peptide subunit vaccines, recombinant proteins, and virus-like particles.

4.3. Inactivated Vaccines

Since the outbreaks of 2015 in Brazil, the development of inactivated vaccines has been the primary strategy used by various research groups. ZPIV is a whole-virus, formalin-inactivated, alum-adjuvanted vaccine derived from the Puerto Rican strain PRVABC59 developed by the Walter Reed Army Institute of Research [97]. Preclinical studies in BALB/c mice showed that after a single immunization administered intramuscularly with alum, high levels of neutralizing antibodies were generated, conferring complete protection against viremia [97]. In a subsequent study, protective efficacy was also demonstrated in Rhesus macaques. After administration of two doses of vaccine in a month, specific binding antibodies to ZIKV-Env and neutralizing specific antibodies against ZIKV produced protection, which was subsequently confirmed with a second challenge to the monkeys one year later [64]. The safety and immunogenicity of ZPIV in humans were tested in three placebo-controlled clinical trials of two doses of 5 µg 28 days apart (NCT02963909, NCT02952833, and NCT02937233), and a fourth dose reduction study examining safety and immunogenicity was completed in August 2021 (NCT03008122) [60,98]. The results showed

that the ZPIV vaccine is safe and well tolerated in a two-dose regimen at either weeks 0 and 4 or weeks 0 and 2. The ZPIV candidate generated potent neutralizing antibody titers in healthy adults, thus demonstrating its ability to induce a sufficient immune response that could offer potential clinical benefit [98,99]. Alternatively, Takeda Pharmaceuticals is developing a similar candidate that relies upon a purified inactivated virus vaccine called TAK-426. A Phase 1 clinical trial (NCT03343626) was completed in November 2020. The trial demonstrated that TAK-426 was well tolerated, had an acceptable safety profile, and was immunogenic in both flavivirus-naive and flavivirus-primed adults aged 18–49. Because of the limitations of the trial, there was not enough information on the durability of the response. The results showed that the 10 µg dose had the best immunogenicity and safety profile, justifying its selection for use in the Phase 2 clinical trial that was scheduled for June2023 (NCT05469802) to describe the side effects and the immunogenicity of TAK-426 in two different age groups [100]. VLA-1601, another inactivated vaccine, is also being evaluated. Although the Phase 1 clinical trial (NCT03425149) concluded in November 2018, the official final report has not been published yet. BBV121, developed by Bharat Biotech, Telangana, India, is another purified inactivated viral vaccine candidate. This vaccine formulation, containing the African prototype strain MR766 [101], has successfully completed a Phase 1 clinical trial (NCT04478656). The trial focused on assessing the safety, tolerability, and immunogenicity of two doses across three escalating cohorts of BBV121, compared to a placebo. It concluded in November 2018, but the report has not been published yet.

4.4. Live Attenuated Vaccines

The attenuated chimeric vaccine rZIKV/D4Δ30-713 uses ZIKV prM-E in a DENV-4 backbone, with a 30-nucleotide deletion in the 3 'UTR to attenuate viral replication. The Phase 1 clinical trial (NCT03611946) evaluated safety and immunogenicity in 56 adults with no previous flavivirus infection. This trial finished in March 2022 and concluded that the vaccine was safe and well tolerated, but the seroconversion was poor because chimerization can render highly attenuated viruses. Therefore, these results demonstrate that rZIKV/D4Δ30-713 is over-attenuated and will not continue being a vaccine candidate [102–105]. One of the drawbacks of live attenuated vaccines is that over-attenuation can compromise vaccine efficacy.

4.5. DNA Vaccines

Another popular strategy is the use of DNA vaccines. In 2016, three studies entered clinical trials for VRC5283 and VRC5288 vaccines, which are derived from French Polynesian strain H/PF/2013 of Asian lineage that encodes the prM and E proteins developed by the Vaccine Research Center (VRC) and the National Institute of Allergies and Infectious Diseases (NIAID) [106]. The design consisted of inserting the prM and E protein sequences into a vector containing the cytomegalovirus promoter (VRC8400), which has been clinically evaluated in previous studies, showing an excellent safety profile [107,108]. For VRC5283, prM was exchanged for prM from JEV, and for VRC5288, the final 98 amino acids of the E protein were substituted by the JEV analog sequences [106,107]. Preclinical studies in BALB/c mice and Rhesus macaques demonstrated that the two constructs could induce strong neutralizing specific antibody responses against ZIKV after two administrations four weeks apart [106]. In a separate trial, vaccines also reduced and eliminated viremia in some animals after a viral challenge, providing protection against the virus [60,106]. Based on these findings, both vaccines advanced to phase 1 clinical trials to evaluate safety, tolerability, and immunogenicity in humans (NCT02996461 and NCT02840487, respectively) [109]. The two evaluated DNA vaccines were safe and well-tolerated, and most adverse events were mild. Both vaccines were immunogenic, but the most remarkable effects were seen with VRC5283. This result is promising because 100% (14 of 14) of the participants who received it by needle-free injection in divided doses had detectable antibody responses and greater neutralizing and T-cell antibody responses. Therefore, VRC5283 advanced to the

next phase of clinical trials (NCT03110770). The Phase 2 clinical trial concluded in October 2019. It aimed to assess safety, immunogenicity, and efficacy in populations in South and Central America, the Caribbean, and the USA endemic zone for ZIKV, using a vaccination regime at 0, 4, and 8 weeks with needle-free delivery using a Stratis device. The conclusions have yet to be published, but the results have already been uploaded to the NCT03110770 case record online. It was observed that after the 28-day analysis, the titers of Zika antigen-specific neutralizing antibodies were high. The best regime was four injections of 4 mg of vaccine administrated IM by a needle-free injection device, producing 187.629 geometric mean titers (GMT) of Zika antigen-specific neutralizing antibodies, which suggests a robust immune response. No severe adverse events were reported, and the most important reaction was at the injection site, causing pain; malaise; and, in some cases, headache and myalgia [110]. The fourth study, GLS-5700, used a vaccine constructed from the consensus sequence of prM genes and ZIKV E protein, using several isolates of the infection-causing virus in humans [111]. Preclinical studies demonstrated that this synthetic DNA vaccine administered by the CELLECTRA-3P electroporation device generated cellular and humoral immune responses, including the production of neutralizing antibodies in mice and NHPs, providing complete prevention against viremia [111]. The GLS-5700 DNA vaccine was evaluated in two clinical trials involving different study subjects. One was conducted in a healthy population (NCT02809443); in contrast, the other evaluated a population of adults seropositive for dengue virus (NCT02887482). Both trials have finished already, but only the results of the first trial have been published (2021). In the first trial, it was reported that the GLS-5700 vaccine did not produce any severe adverse events, and local minor reactions at the injection site included pain, redness, swelling, and itching. Remarkably, 62% of participants developed neutralizing antibodies, as demonstrated in a Vero-cell assay; a neuronal cell assay also showed that 70% and 95% of participant sera blocked infection by 90% and 50%, respectively. All these results suggest that vaccine-induced humoral responses are protective [112]. This candidate will be entering future trials to corroborate its efficiency.

4.6. RNA Vaccines

mRNA-1325 and mRNA-1893 are modified mRNA vaccines encapsulated in lipid nanoparticles (NPLs) encoding prM and E proteins derived from a 2007 Micronesia strain developed by Moderna Therapeutics, a biotechnology company in Cambridge, MA, USA. [113]. They were designed by replacing prM sequences with human IgE signal or JEV sequences [113,114]. Preclinical studies showed that after two administrations, both induced high levels of protecting neutralizing antibodies against ZIKV in multiple mouse models, including some in immunocompetent and immunosuppressed pregnant mice [113]. The protective responses elicited by mRNA-NPL vaccines were durable, even 14 weeks after the booster dose, and exposed mice showed no morbidity or mortality [113]. Given these results, both vaccines advanced to Phase 1 clinical trials for human testing (NCT03014089 and NCT04064905) [113]. By 2021, both clinical trials had already finished, and a complete report was published in 2023, remarking on the effectiveness and safety of both candidates. The data showed that mRNA-1325 did not elicit good titers of ZIKV-specific neutralizing Ab (NAb) responses, so it was discarded to participate in future clinical trials. In contrast, mRNA-1893 was well tolerated and only produced grade 1 and 2 adverse reactions at higher doses. No severe adverse events were reported. A 100 μg dose induced the best ZIKV-specific NAb response in flavivirus-seronegative participants and a 10 μg dose in flavivirus-seropositive participants. A two-dose schedule elicited a Nab response against ZIKV that persisted for at least 12 months after the last dose. One limitation of the study was that the antibody-dependent enhancement with dengue virus was not measurable. However, the mRNA-1893 Phase 1 clinical trial provided good results and will advance to a Phase 2 study in approximately 800 flavivirus-seropositive and -seronegative adults from the USA and Puerto Rico (NCT04917861); this study has an estimated completion date of April 2024 [115].

A similar vaccine has been developed by another research group, synthesized with a modified mRNA, nucleoside 1-methylpseudouridineand encapsulated in NPLs [61,116]. Their results showed that the vaccine elicited robust and long-lasting neutralizing antibody responses in mice and NHPs. In C57BL/6 mice, a single intradermal 30 µg immunization protected against immune challenges at two weeks and five months after vaccination, and a single dose of 50 µg was enough to protect NHPs against a challenge five weeks after vaccination [116]. These data demonstrated that nucleoside-modified mRNA-NPL produces rapid and long-lasting protective immunity; therefore, it is a promising new vaccine candidate against ZIKV [116].

4.7. Viral-Vectored Vaccines

Another vaccine approach involves expressing ZIKV genes along various viral vectors, including adenovirus, vesicular stomatitis virus (VSV), and live attenuated measles virus (MV). This strategy allows for the production of heterologous antigens after immunization [117–119]. MV-ZIKA is a vector of the Schwarz MV vaccine expressing the soluble prM and E proteins (MV-Zika-sE) developed by Themis Bioscience GmbH, Wien, Austria [120].

Preclinical studies in an allogeneic mouse pregnancy model showed that vaccination with this candidate induces robust humoral and cellular immune responses directed against the E protein of ZIKV [116,120]. In addition, it reduced the virus load in different organs and prevented fetal infection. Therefore, this vaccine has promising immunogenicity and protective capacity against infection and was evaluated in a Phase 1 clinical trial completed in 2018; the results have not been published yet (NCT02996890) [120]. A second, similar candidate vaccine (MV-ZIKV-RSP) against ZIKV was also evaluated in a Phase 1 clinical trial (NCT04033068) completed in June 2020. An official report has yet to be published, but the results are available online on the case record page (NCT04033068). Although no serious adverse effects were observed, there was no sign of significant induction of anti-ZIKA-RSP antibodies respecting the placebo groups [114,119].

Recombinant chimpanzee adenoviruses are currently being explored as vaccine vectors for multiple pathogens. ChAdOx1 is a replication-deficient chimpanzee adenoviral vaccine against ZIKV developed by the University of Oxford [121]. The vaccine design consisted of an expression cassette for prM and E proteins (without the transmembrane domain) [121]. In preclinical trials, a single dose without adjuvant elicited adequate levels of protective responses in mice exposed to ZIKV, as well as a specific, long-lasting immune response against ZIKV-Env without in vitro evidence of an ADE against DENV [121]. Due to its safety and immunogenicity profiles in humans, paired with its suitability for large-scale production under Good Manufacturing Practices (GMP), this vaccine was evaluated in two clinical trials—one of them in Oxford, England (NCT04015648), and the other in Monterrey, Mexico (NCT04440774). Both cases evaluated healthy volunteers aged 18–50. Also, both Phase 1 clinical trials are already completed, but to this date, no one has published the results. A similar ChAdOx1 vaccine against dengue virus (ChAdOx1 Chik) is being developed. A Phase 1 clinical trial (NCT03590392) showed that a single dose induced IgG and T-cell responses against CHIKV structural antigens. In addition, it showed excellent safety, tolerability, and 100% PRNT50 seroconversion after a single dose [121,122]. We need to await the results for ChAdOx1 Zika, expecting similar results. There is another adenoviral vector-based vaccine called Ad26.ZIKV.001. This vaccine took its design from a non-replicating adenoviral vector, type 26 (Ad26), that encodes the ZIKV M-Env antigens and was evaluated in preclinical stages in mice and NHPs; in these assays, the vaccine produced complete protection against viremia from ZIKV challenge [123]. Later, the Ad26.ZIKV.001 vaccine advanced to a Phase I clinical trial (NCT03356561) concluded in 2019 with favorable results. This clinical trial showed that all regimens were well tolerated, and in both regimes, ZIKV neutralizing titers peaked 14 days after the second vaccination. Neutralizing antibodies persisted for at least 1 year. A one-dose regimen of 1×10^{11} vp Ad26.ZIKV.001 induced seroconversion in all participants 56 days after vaccination. In addition, Env-specific cellular responses were induced. All these results

show Ad26.ZIKV.001 as a promising candidate for further development [124]; however, no Phase 2 clinical trial has been scheduled yet.

In contrast, a promising example of a chimeric vaccine utilizing insect viruses is the ARPV/ZIKV vaccine, which incorporates genetic material encoding the prM and envelope E proteins of ZIKV onto an arbovirus (ARPV) backbone. This construct relies upon the idea that insect-specific viruses are incapable of replication within vertebrate hosts, so these vaccines are reliably safe. However, the immunogenicity is not good enough yet, and it will be necessary to improve this vaccine to elicit durable and protective immune responses against ZIKV infection [125].

Table 1 shows a summary of ZIKV vaccine candidates in ongoing clinical trials. Furthermore, in addition to the vaccine candidates being tested in clinical trials, there are also vaccine designs that use novel strategies for vaccine construction. These proposals will be described next.

Table 1. ZIKV vaccine candidates in clinical development.

Vaccine Platform	Candidate Name	Sponsor	Antigen	Clinical Trial Data		References
				Phase I	Phase II	
DNA Vaccines	VRC5283	NIAID/VRC	prM-E	NCT02996461	NCT03110770	[109,110]
	VRC5288	NIAID/VRC	prM-E	NCT02840487		[109]
	GLS-5700	GeneOne Life Science, Inc. Inovio Pharmaceuticals	prM-E	NCT02809443		[111,112]
Inactivated virions	ZPIV	NIAID/WRAIR/ BIDMC	Virion	NCT02937233		[98,99]
				NCT02952833		
				NCT02963909		
				NCT03008122		
	BBV121	Bharat Biotech International	Virion	CTRI/2017/05/ 008539		[101]
	PIZV (TAK-426)	Takeda Pharmaceuticals	Virion	NCT03343626		[100]
	VLA1601	Valneva Austria GmbH Emergent BioSolutions	Virion	NCT03425149		
Live attenuated	rZIKV/D4Δ30-713	NIAID		NCT03611946		[104]
mRNA	mRNA-1325	Moderna Therapeutics	prM-E	NCT03014089		[113,114]
	mRNA-1893			NCT04064905		
Viral vector-based	MV-ZIKA (Measles-vectored)	Themis Bioscience GmbH	prM-sE	NCT02996890		[120]
	MV-ZIKA RSP		prM-E	NCT04033068		
	ChAdOx1 Zika	University of Oxford	prM-E	NCT04015648		[121]
	Ad26.ZIKV.001	Janssen Vaccines and Prevention B.V.	M-Env	NCT03356561		[12]

Notes: NIAID, National Institute of Allergy and Infectious Diseases; VRC, The Dale and Betty Bumpers Vaccine. Research Center at the National Institutes of Health, USA; GmbH, from German, limited liability company.

4.8. Recombinant and Subunit Vaccines

Recombinant and subunit-based vaccines have been developed as an alternative platform due to their notable safety, relative ease of antigen production, and modest cost [126]. Two research groups have proposed constructions of recombinant subunit vaccines using 80% and 90% of the gene encoding the ZIKV E protein's N-terminal region in *Escherichia coli* and *Drosophila*, respectively [126,127]. The vaccines demonstrated immunogenicity and protective efficacy in immunocompetent mice by eliciting both humoral and cellular immune responses and protecting them against ZIKV in an in vivo challenge [126,127]. Another study described the production of EDIII in *E. coli* by inclusion bodies [70]. The vaccine was evaluated through subcutaneous immunization of mice with 25 and 50 µg of EDIII for 11 weeks. It stimulated virus-specific neutralizing antibody responses with titers above the threshold and correlated with protective immunity against ZIKV. An E-protein dimer was constructed in a recent study without prM or the immunodominant fusion loop epitope [128]. Mice immunization induced dimer-specific antibodies that protected them against ZIKV during pregnancy, with no evidence of ADE or antibody cross-reaction with those of DENV [128]. Recently, one of the most novel approaches was developing an intranasal vaccine that uses EDIII fused to the FLIPr protein, a formyl I receptor inhibitor, and an FcγR antagonist. The vaccine was tested in immunodeficient AG129 mice and induced protective immune responses against ZIKV. These results promise safer vaccines for high-risk populations, such as pregnant women, immunosuppressed infants, immunocompromised individuals, and the elderly [129]. Another attractive vaccine candidate is zDIII-F, which consists of a bacterial ferritin-based nanoparticle fused in frame with ZIKA envelope protein domain III at the amino terminus of ferritin. Immunization of mice with a single dose of zDIII-F resulted in the robust induction of neutralizing antibodies, protecting them from the lethal viral challenge. The vaccine induced the production of CD4 T cells and CD8 T cells, suggesting a complete immune response. The passive transfer of neutralizing antibodies from vaccinated subjects to naïve animals protected them against the lethal viral challenge [130].

4.9. Virus-Like Particle (VLP) Vaccines

Among the platforms currently under study, virus-like particles (VLPs) are a promising alternative for vaccine development because they are nanostructures that mimic the organization and conformation of viruses but lack the viral genome that allow their production and subsequent antigen presentation. They can trigger a strong humoral and cellular immune response due to their repetitive structures [131]. In the last four years, several studies have described strategies to assemble and produce VLPs of ZIKV using various virus proteins and testing their efficacy in animals. In 2017, a research group described the production in the Expi293 cell line of the joint expression of structural (CprME) and non-structural (NS2B/NS3) virus proteins. Immunization of BALB/c mice with the VLP vaccine stimulated significantly higher neutralizing antibody titers than an inactivated vaccine [132]. Another study proposed ZIKV-VLPs utilizing the hepatitis B core antigen (HBcAg) fused to EDIII, which proved capable of rapid production in large, readily purifiable quantities in *Nicotiana benthamiana* [133]. HBcAg-zDIII VLPs are highly immunogenic, as two doses elicited potent humoral and cellular responses in C57BL/6 mice and did not show evidence of ADE against DENV [133]. Immunologically optimized VLPs generated from the cucumber mosaic virus with a Th-cell epitope of tetanus toxin (CuMVtt) were also produced using EDIII and tested in BALB/c mice [134]. The vaccine induced high levels of specific IgG after a single injection, and such antibodies neutralized ZIKV without potentiating DENV infection in vitro [134].

In recent studies, the production of ZIKV-VLP in recombinant immune complexes (RICs) derived from plants and cloned in *Pichia pastoris* GS115 has been described using the EDIII fused to the hepatitis B surface antigen (HBcAg) protein [135]. This construct was shown to be immunogenic in BALB/c mice [136]. Furthermore, VLPs have been produced in the baculovirus expression system using prM and E proteins. They have

demonstrated good immunogenicity in immunized mice, as they stimulate high levels of virus-neutralizing antibody titers, ZIKV-specific IgG titers, and potent memory T-cell responses [137]. A novel vaccine strategy has been reported using a phage display-produced mimetic envelope dimer epitope (EDE) docked on adeno-associated (AAV) capsids (VLP), which induces neutralizing antibodies and does not elicit ADE. This mimetic protein, called mimotope, is designated to be a dual-vaccine candidate for ZIKV and DENV. After immunization with one of these mimotopes, antibodies that recognized both viruses and were protective against the infection were induced, in addition to not eliciting ADE [138].

4.10. Epitope-Based Peptide Vaccines: An Immunoinformatics Approach

Currently, computational methods are an essential tool in developing new-generation vaccines. Due to the advances in computational immunoinformatics and immunogenomics, isolated epitopes that may stimulate specific humoral or cellular immune responses can be predicted [139]. Epitope-based vaccines are considered a cost-effective approach in developing vaccines meant to avoid whole-pathogen use. Some tools allow researchers to identify B-cell and T-cell epitopes using protein sequences or sequence alignments [139,140]. The prediction of B-cell epitopes is based on artificial neural networks that identify epitopes using mathematical algorithms and classify them according to the obtained score. The higher the peptide score, the greater the probability that it becomes an epitope [140]. The prediction of T-cell epitopes determines the binding affinity of a peptide to alleles of the major histocompatibility complex (MHC and HLA in humans), as they would be presented on the surface of the cell through the cross-presentation pathway, as well as the binding affinity of epitopes that interact with the transporter associated with antigen processing (TAP) [140–142].

In recent years, epitope-based peptide vaccines have been advertised as a new approach to obtaining more effective or safer vaccines against ZIKV because of the associated neurological disorders and autoimmunity syndromes [140]. The most recent designs of vaccines are based on peptides [143–146], T-cell epitopes [147,148], epitopes that bind to MHC-I and MHC-II [144,145], B-cell epitopes [147,148], and multi-pathogenic vaccines (that can simultaneously protect against CHIKV) [146]. In addition, vaccines based on phage VLPs that carry potential B-cell epitopes have been explored [149]. Satisfactory results have been reported with this type of vaccine in mice and cell lines. Several studies adopting immunoinformatics approaches are underway to find a candidate vaccine against ZIKV soon.

As an example of epitope-based vaccines, in 2020, Shahid et al. published an in silico design of a multi-epitope-based peptide vaccine, which was constructed by exploring the ZIKV proteome using immunoinformatic tools [147]. They used the ZIKV proteome to predict B-cell, T-cell, and IFN-γ epitopes and constructed a final sequence linked by AAY and GPGPG linkers. The final construct was of 435 amino acids and was evaluated in simulations of toxicity and immunogenicity. This strategy's effectiveness relies on producing a complete immune response because the selected epitopes should trigger humoral and cellular immune responses. Even though there was no in vitro construct, the researchers concluded it could be a suitable candidate if validated experimentally [147].

In Table 2, we present a descriptive summary of promising strategies used to develop new ZIKV vaccines.

In summary, the development of vaccines against ZIKV has been the subject of intense research, encompassing various vaccine platforms in phases of development and evaluation. Next, a comparative evaluation of the main vaccine candidates will be presented, considering the results obtained in preclinical and clinical trials.

Inactivated vaccines: Clinical trials show that ZPIV has a tolerable safety profile and has been demonstrated to generate a sufficient immune response that could provide a potential clinical benefit. TAK-426 has also demonstrated safety and immunogenicity in clinical trials. It is expected to progress to Phase 2 trials to evaluate the long-term efficacy and durability of the immune response to determine its utility in broader populations [99,100].

Table 2. Promising strategies used to develop new ZIKV vaccines.

Vaccine Platform	Name	Technology	Strategy Objective	Results	Limitations	Cross-Reactive Antibodies	Year	Reference
Recombinant and subunit vaccines	ZIKV E80	80% N-terminal ZIKV E protein	NAb; specific T-cell response	Both E80 proteins elicited robust binding and neutralizing antibody responses after three immunizations and protected mice against experimental ZIKV challenge	Should be proven in NHPs before human trials	Minimal (1:160 titers)	2018	[126]
	ZIKV E90	N-terminal 90% E protein	NAb	Abs levels reached 1:10,000 35 days post immunization and remained constant until day 42 post immunization. Mice inoculated with vaccine antisera exhibited a 100% survival rate.	Absence of glycosylation	Approximately 1:1000 titers	2017	[127]
	E dimer	Triple-mutant ZIKV PF13 ED123 dimer	To avoid cross-reaction with DENV	Stable ZIKV E dimers are immunogenic and protect against ZIKV challenge and infection of the placenta and fetus in pregnant mice.	Large-scale manufacturing concerns and storage stability requirements	Cross-reactivity to DENV is limited	2019	[128]
	rZEIII-FLIPr	Recombinant ZIKV E-protein-domain III-FLIPr fusion protein	Mucosal immunity and systemic immune responses via intranasal administration (dendritic cells)	rZEIII-FLIPr alone induces not only serum IgG and IgA but also sIgA in the vagina, effectively reducing the viral load in the blood and vagina following ZIKV infection in immunodeficient AG129 mice.	Cross-reactive Abs with DENV and cellular immune response were not assessed	N/A	2021	[129]
	zDIII-F	ZIKV E-protein-domain III fused in frame at the ferritin N terminus	NAb, (IFN)-γ-positive CD4 T, and CD8 T; avoid DENV infection ADE	Elicits robust humoral and cell-mediated immune responses. Vaccinated mice exhibit robust protection against lethal viral challenge.	A single-dose, single-immunization regimen and scaling up to human trials	N/A	2023	[130]

Table 2. *Cont.*

Vaccine Platform	Name	Technology	Strategy Objective	Results	Limitations	Cross-Reactive Antibodies	Year	Reference
VLP Vaccines	VLPs-ZO2/ZO3	ZKIV VLPs assembled with co-expressed structural (CprME) and non-structural (NS2B/NS3) proteins	Self-assembly of VLPs and production of protective Ab titers	Most of the VLP vaccine formulations stimulated neutralizing antibody titers higher than those induced by an inactivated vaccine control.	Adjuvant incorporation into a vaccine raises safety concerns	4G2 MAb significantly enhanced DENV infection	2017	[132]
	HBcAg-zDIII VLPs	Hepatitis B core antigen that displays the ZIKV E protein domain III	NAb; avoid DENV infection ADE	Two doses elicited potent humoral and cellular responses in mice that exceed the threshold correlated with protective immunity against multiple strains of ZIKV.	Concerns related to pre-existing immunity or immune tolerance to HBV	Ab did not enhance the infection of DENV in Fcγ receptor-expressing cells	2017	[133]
	CuMVttVLP-EDIII	Cucumber mosaic virus enhanced with universal Th-cell epitope fused with ZIKV E protein DIII and DOPS adjuvant	NAb; avoid DENV infection ADE	Induces strong antibody response, and the use of adjuvant can skew the immune response in a Th1 direction.	Cannot exclude ADE in other serotypes or genotypes of DENV	Did not induce enhancing antibodies against DENV-2	2019	[134]
	Hbc-ZE3/ Hbche-ZE3	Hepatits B core VLPs fused with ZIKV E protein DIII	Codelivery system of VLPs and improved immune complex as new vaccine strategy	VLPs contain potent T-cell epitopes that effectively activate macrophages and may stimulate Toll-like receptors (TLRs) through the presence of encapsidated nucleic acid.	VLP assembly is a heterogeneous process which may be affected by the electrostatic and steric properties of each antigen insertion	N/A	2020	[135]
	ZSV VLP	In-frame fusion of ZIKV E domain III with the hepatitis B virus Surface antigen	NAb; avoid DENV infection ADE	The titers of elicited Abs, though modest, are indicative of protective efficacy in mice.	ZSV VLPs elicited only modest ZIKV NAb titers despite having four copies of ZIKV EDIII	Abs did not enhance a sub-lethal DENV-2 challenge in AG129 mice	2019	[136]

Table 2. *Cont.*

Vaccine Platform	Name	Technology	Strategy Objective	Results	Limitations	Cross-Reactive Antibodies	Year	Reference
VLP Vaccines	Vac-prME	prME into a baculovirus expression system	Induce humoral and cellular responses	The VLP-based vaccine is highly immunogenic (inducing a wide-ranging and balanced immune response); however, the neutralization antibody titers and cytokine levels were relatively weak compared with those elicited by the inactivated virus control.	The conformation of VLPs potentially slightly differs compared with the conformation of the authentic virus	Will be investigated further in a suitable animal model	2018	[137]
	AAV2 VLP	Evelope mimotopes displayed on adeno-associated virus (AAV) capsids (VLP)	Bivalent vaccine targeting ZIKV and DENV without inducing ADE	Immunization with the VLP modified with mimotope 2 induced antibodies that recognized ZIKV and DENV, thus, providing a proof of concept of the isolation of the EDE structure to be used as a vaccine.	Denatured VLPs were not recognized by the Ab, indicating that the structure adopted by the peptides on the particle is necessary for their correct folding	The generated Abs do not induce ADE with DENV.	2023	[138]
Epitope-based peptide vaccines	MEBP	14 CTL epitopes and 11 HTL epitopes fused with β-defensin as adjuvant at the N end	Stimulation of B-cell, T-cell (HTL and CTL), and IFN-γ epitopes	The predictions demonstrate that in silico, the construction was antigenic and non-allergenic and showed binding affinity to the TLR-3 molecule.	Only in silico predictions of immunogenicity	N/A	2020	[147]
	Multiepitope vaccine construct	17 CTL epitopes and 8 MHC class II epitopes fused with β-defensin as adjuvant at the N end	Stimulation of B-cell, T-cell (HTL and CTL), and IFN-γ epitopes	After all prediction results, the multiepitope subunit vaccine may have the ability to induce cellular, as well as humoral, immune response.	Needs experimental validation	N/A	2018	[148]
	MS2-Zika-E377-388 PP7-Zika-E241-259 PP7-Zika-E346-361	ZIKV B-cell epitopes on MS2 and PP7 bacteriophage coat proteins	Stimulation of B cells	Immunization with VLPs displaying a single B-cell epitope minimally reduces ZIKV infection, whereas immunization with a mixture of VLPs displaying a combination of B-cell epitopes neutralizes ZIKV infection.	A single B-cell epitope does not protect against ZIKV infection challenge	N/A	2018	[149]

Ab: antibody; NAb: neutralizing antibody.

DNA/RNA vaccines: VRC5283, GLS-5700, and mRNA-1893 have been demonstrated to induce strong neutralizing antibody responses in preclinical and clinical trials. VRC5283 achieved a 100% antibody response, as strong neutralizing antibody and T-cell responses were produced. Additionally, GLS-5700 exhibited a strong protective response, as participants developed antibodies to neutralize the infection. Both vaccines were well tolerated, with only minor local reactions reported. Furthermore, mRNA-1893 induced robust and long-lasting immune responses without serious adverse events [111,118].

Viral vector-based vaccines: ChAdOx1 and Ad26.ZIKV.001 have immense potential based on the results of preclinical and clinical trials. They have demonstrated the induction of specific immune responses against ZIKV, confirming their safety and tolerance in Phase 1 clinical trials. However, further research is needed to fully evaluate their long-term efficacy and ability to prevent ZIKV infection in diverse populations [123,124].

Although these vaccines present promising results, further research is required to assess the persistence of the provided protection and determine their effectiveness against natural exposure to ZIKV. This process involves conducting additional clinical trials in later stages and implementing long-term follow-up of participants to gain a more comprehensive understanding of the long-term effectiveness of these vaccines. In addition to safety and efficacy, it is essential to consider logistical and economic aspects in evaluating vaccines, such as ease of large-scale production, product stability, storage and distribution requirements, and the cost associated with each vaccine. These factors can significantly impact a vaccine's implementation and long-term success in clinical practice. Table 3 provides a comprehensive comparison of all mentioned vaccine platforms, highlighting their primary advantages and disadvantages.

Table 3. Benefits and drawbacks of vaccine platforms.

Vaccine Platform	Advantages	Disadvantages	References
DNA	Ability to rapidly test multiple candidate antigen designs, rapidly produce GMP material, and established safety profile in humans. Induction of both humoral and cellular immune responses.	Limited immunogenicity, concerns regarding genomic integration, reliance on vectors for efficient gene transfer and nucleic acid delivery, and safety concerns related to potential long-term effects	[106,150]
RNA	High immunogenicity, no risk of integration into the host genome, and rapid development and scalability.	Limited clinical data for some candidates, safety concerns regarding strong inflammation events, requires strict cold-change storage at very low temperatures, and potential interferon responses.	[106,150]
Virus-Like Particles	High immunogenicity due to repetitive structures, no risk of genome insertion or	Complex manufacturing process, instability, environmental sensitivity, particle conformation could be different to that of the wild	[133,137,150]
Epitope-Based	High specificity to target pathogen-specific epitopes, enhancing the immune response; fast and accurate design; time-/cost-effective formulations; desired immunogenicity with minimized adverse effects; and suitable for certain vulnerable groups.	Requires multiple peptides for broad protection, needs experimental validation, multiepitope constructions may not be correctly recognized by immune cells, limited durability of the immune response, and potential epitope variability.	[139,148,149]
Attenuated	Mimics natural infections, eliciting robust B- and T-cell responses, inducing a strong and lasting immune response; possible single dose.	Safety concerns regarding virulence reversion and potential to cause infection; adverse events in immunocompromised individuals; and over-attenuation, which may compromise vaccine efficacy.	[150,151]

Table 3. *Cont.*

Vaccine Platform	Advantages	Disadvantages	References
Inactivated	Elicits strong immune responses, potent induction of NAb, and safe due to pathogen inactivation.	Potential epitope alteration during the inactivation process, which could render the vaccine ineffective and usually requires booster vaccinations.	[150,152]
Viral-vectored	Potent immune responses due to vector fusion reminiscent of natural infection dynamics and induction of both humoral and cellular immunity.	Complex manufacturing processes, risk of genomic integration, pre-existing immunity against vectors may diminish vaccine effectiveness, and limited data on long-term safety and efficacy.	[150]
Recombinants and Subunit	Safe and well-characterized antigen, cost-effective production, safe, stable, can be easily scaled-up for manufacturing, suitable for individuals with compromised immunity, and lower risk of adverse events compared to whole-virus vaccines.	Possible weak immune response that requires adjuvants or conjugates for optimal efficacy, multiple doses required for robust immunity, and cross-reactivity and limited durability of the immune response.	[150,153]

Selecting the most suitable vaccine against ZIKV will require a comprehensive evaluation considering each population's specific needs, circumstances, and epidemiological situation. It may be necessary to implement a comprehensive vaccination strategy that combines different vaccines and approaches to effectively address the prevention and control of the disease in various contexts and populations. It is crucial to continue research and surveillance to obtain more data on the safety, efficacy, and durability of vaccines against ZIKV.

5. Challenges in ZIKV Vaccine Development

Efforts to develop a vaccine began quickly after the close association of ZIKV with severe neurological disorders and the increase in children born with congenital malformations among populations affected by the virus. Since then, a wide variety of strategies have been developed by researchers around the world to produce safe and effective vaccines against the virus. However, despite the efforts, several challenges must be solved and encompass several key areas. Firstly, pre-existing immunity to flaviviruses poses significant challenges due to the potential ADE effect, which could impact vaccine safety, immunogenicity, and clinical efficacy. Secondly, the possible association between ZIKV and CZS highlights the need to understand the immune responses necessary to prevent fetal infection and the devastating consequences of the virus. Thirdly, there is the theoretical concern that vaccine-induced immune responses might cause GBS. Finally, there are considerable challenges associated with vaccinating pregnant women and vulnerable populations. Vaccine safety is a priority, so thorough evaluations of possible adverse effects are required to evaluate long-term safety and effectiveness [154]. However, major challenges arise from the crucial need for well-characterized animal models that accurately reflect human diseases and from the significant decline in human ZIKV infection cases. The unpredictable nature of future outbreaks compounds this issue. In the absence of sustained viral transmission, evaluating the efficacy of candidate vaccines against the virus through traditional clinical trials becomes exceedingly complex [154].

To address this challenge, in 2016, the National Institute of Allergy and Infectious Diseases (NIAID) and the Walter Reed Army Institute of Research (WRAIR) engaged in discussions regarding the potential utilization of a ZIKV Controlled Human Infection Model (CHIM) to facilitate vaccine development. They deliberated on the conditions under which such a model could be ethically justified. It was emphasized that using a CHIM posed considerable risks due to the limited understanding of the virus at that time [155]. The declining number of natural Zika cases and challenges in conducting traditional clinical trials led to reconsideration of a ZIKV CHIM. Identifying critical endpoints for vaccine

efficacy evaluation necessitated a comprehensive plan to address potential risks, including fetal infection, mosquito-borne transmission, sexual transmission, and GBS. Any study must exclusively recruit non-pregnant women to ensure fetal safety. Housing participants in a controlled environment can eliminate mosquito-borne transmission during the viremia period. This isolation also offers significant protection against sexual transmission. Furthermore, to reduce the risk of sexual transmission, all participants can be required to use barrier methods following discharge from the controlled environment. As an additional safety measure, the study must only enroll women because sexual transmission is more common in males. Finally, to mitigate the risk of GBS, participants should be limited to those aged 50 or younger with no history of GBS or autoimmune diseases [156].

Recently, as an alternative to this problem, the possibility of using new innovative biological modeling approaches has been raised, such as the use of organoids that simulate the infection to study the pathogenesis; detect new drugs; and, in the future, serve to test the vaccines that are under development against ZIKV [157]. Organoids are an exciting alternative, as they are three-dimensional scale models that can physiologically mimic the actual organ. In addition, data generated in vivo and in vitro to study the mechanisms of infection by pathogens using them exist. However, there are still limitations in developing organoids to fully model host immune responses [157]. It is unclear whether pre-existing antibodies to ZIKV could cause ADE after DENV reinfection in humans. However, the first patient with a fatal case of DENV associated with prior exposure to ZIKV was recently reported in the United States [96]. This suggests that pre-existing immunity to ZIKV may be highly determinant in causing ADE and increasing the risk of severe disease due to DENV. Therefore, future vaccines must consider the use of antigens that prevent ADE. Also, it is crucial to determine whether pre-existing cross-reactive antibodies may be involved in the fetal transmission of the virus or if they may modulate the immune response triggered by the virus and cause fetus malformations. The association of the virus with CZS remains a critical concern for vaccine development, as there is a need to develop a vaccine against the virus that can be administered independently to different target populations. An alternative would be nasal vaccines based on recombinant proteins because they are not infectious, can induce protective immune responses against ZIKV, and can be safe and effective options for high-risk populations, such as pregnant women, immunosuppressed individuals, and the elderly.

Despite declining reported cases, the true prevalence of ZIKV infections has been underestimated due to decreased vigilance in surveillance and monitoring efforts worldwide. A recent study conducted in Merida City, Yucatán, Mexico, during 2021–2022 revealed strong evidence of ongoing ZIKV transmission, estimating an incidence of 2.8–5.2 per 1000 person-years, with most cases being symptomatic. Moreover, this may still underestimate the actual prevalence due to potential waning of antibodies over time or undetectable antibodies in asymptomatic individuals because of test sensitivity. These findings highlight the importance of maintaining vigilance and awareness regarding the ongoing risk of ZIKV outbreaks. Furthermore, they emphasize the critical need for public health authorities to implement appropriate measures to prevent future outbreaks and improve diagnosis in transmission areas. Therefore, the development of vaccines against ZIKV remains a public health necessity [158].

6. Conclusions

In conclusion, this review underscores the imperative for the urgent development and licensing of an effective ZIKV vaccine despite a decline in reported cases since 2018 [154]. Persistent evidence indicates ongoing ZIKV transmission in high-risk areas, necessitating heightened concern within the public health system regarding potential future outbreaks. It is imperative to implement preventive measures capable of mitigating potential risks and improve diagnosis [158]. Various vaccine platforms have been explored, yet the outcomes of Phase I and Phase II clinical studies remain inconclusive, with some trials yet to publish their results. While endpoints for assessing immunogenicity and the durability

of protective antibodies exist, additional considerations in ZIKV vaccine development include addressing safety concerns inherent in human clinical trials and eliciting robust innate and adaptive immune responses [77,155].

To achieve these goals, vaccine candidates must undergo rigorous animal testing before progressing to human trials. However, the limited global incidence of Zika infections poses challenges for large-scale clinical trials. Recognizing the urgency of addressing this infection disease as an imperative public health concern and the need to prevent future outbreaks, one promising approach involves establishing a controlled human infection model for ZIKV adhering rigorous risk assessment protocols, facilitating continued testing and data collection on ZIKV infections and immune responses. This model offers a significant advantage in expediting vaccine licensure [154,156]. Alternatively, exploring organoids as a surrogate for human participants in trials presents a potential avenue, although the technology's limitations warrant further characterization [157].

Ongoing trials promise to provide additional insights to inform the selection of the most promising vaccine candidate and advance it toward Phase III clinical evaluation. Continued collaboration and innovation in vaccine development are essential to addressing the constant threat of ZIKV infection and safeguarding public health, preventing further outbreaks.

This exhaustive and meticulous review provides an in-depth understanding of all aspects of ZIKV and offers a detailed analysis of the current state of vaccines against the virus. From describing various vaccine platforms to identifying challenges in their development, this research stands out for its thoroughness and meticulousness in gathering and presenting relevant data.

Similarly, the information presented in this study is paramount to the scientific community, healthcare professionals, and policymakers, as it underscores the importance of international collaboration and continuous action in the fight against ZIKV to protect public health and prevent future health crises related to this emerging virus.

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