






Zika virus pathogenesis and current therapeutic advances

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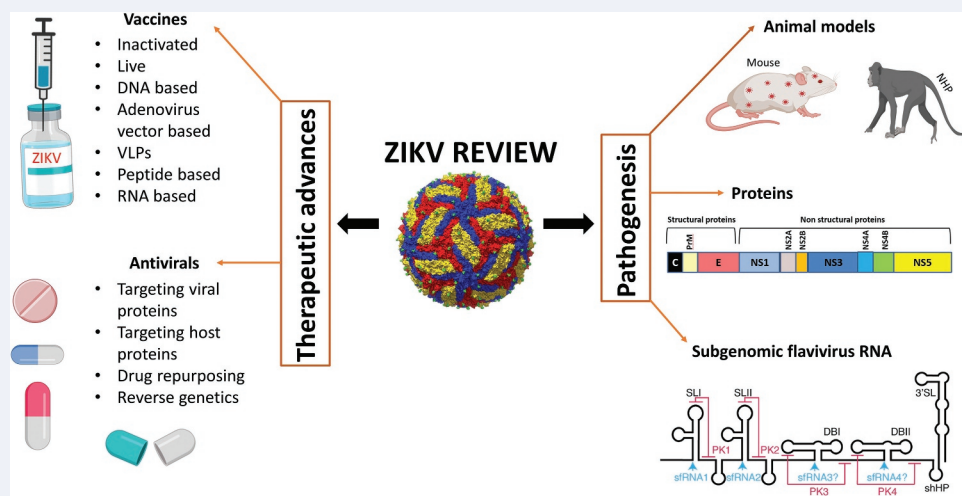
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ABSTRACT

Zika virus (ZIKV) is an emerging arthropod-borne flavivirus that, upon infection, results in teratogenic effects and neurological disorders. ZIKV infections pose serious global public health concerns, prompting scientists to increase research on antivirals and vaccines against the virus. These efforts are still ongoing as the pathogenesis and immune evasion mechanisms of ZIKV have not yet been fully elaborated. Currently, no specific vaccines or drugs have been approved for ZIKV; however, some are undergoing clinical trials. Notably, several strategies have been used to develop antivirals, including drugs that target viral and host proteins. Additionally, drug repurposing is preferred since it is less costly and takes less time than other strategies because the drugs used have already been approved for human use. Likewise, different platforms have been evaluated for the design of vaccines, including DNA, mRNA, peptide, protein, viral vectors, virus-like particles (VLPs), inactivated-virus, and live-attenuated virus vaccines. These vaccines have been shown to induce specific humoral and cellular immune responses and reduce viremia and viral RNA both *in vitro* and *in vivo*. Importantly, most of these vaccines have entered clinical trials. Understanding the viral disease mechanism will provide better strategies for developing therapeutic agents against ZIKV. This review provides a comprehensive summary of the viral pathogenesis of ZIKV and current advancements in the development of vaccines and drugs against this virus.

KEYWORDS




Antivirals; pathogenesis; sfRNAs; therapeutics; vaccines; zika virus



Introduction

Zika virus (ZIKV) is a mosquito-borne, single-stranded positive-sense, enveloped RNA virus that belongs to the *Flaviviridae* family [1]. ZIKV is closely related to other flaviviruses, such as Dengue virus (DENV), West Nile virus (WNV), Yellow Fever (YFV), and Japanese Encephalitis virus (JEV). Since its first isolation in Uganda (1947), sporadic global spread of ZIKV has

been observed over the last 7 decades. ZIKV was first isolated from a sentinel rhesus monkey [2], with the first human isolation reported in Nigeria in 1952 [3]. Despite the virus's long history and sporadic infection cases, it was not until 2016 that the WHO declared the virus to be a global public health emergency due to an expansive and major outbreak in Brazil [4]. Phylogenetic analysis of ZIKV shows that the virus is spread in 3 lineages: West African, East African, and Asian [5]. Currently, no

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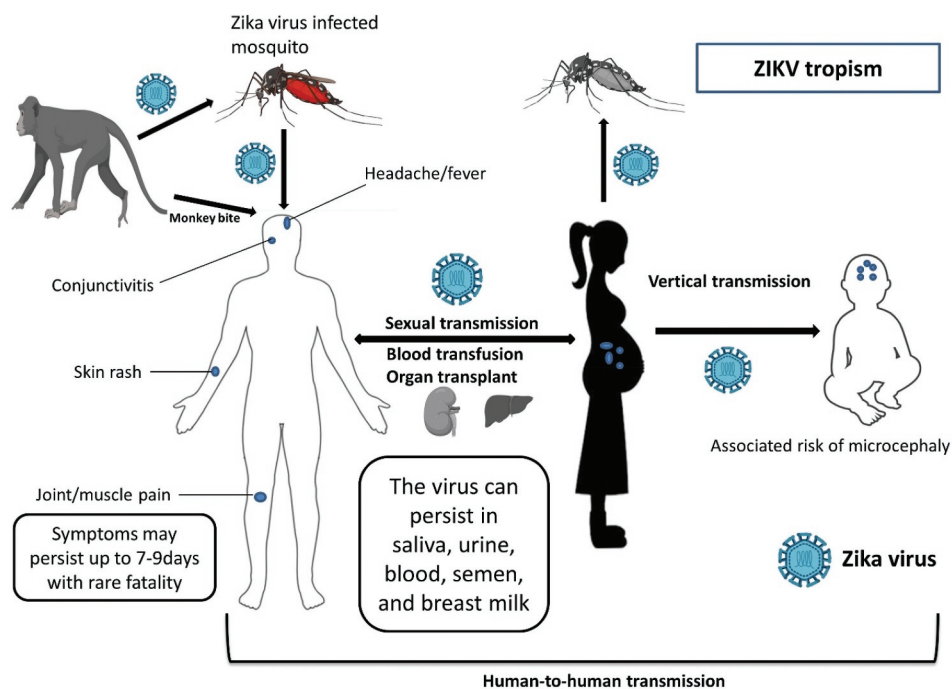


Figure 1. The biology of ZIKV, showing the transmission modes, tropism, and disease symptoms associated with ZIKV.

specific vaccines or drugs are approved for ZIKV; however, some are undergoing clinical trials. Of note, the pandemic currently seems to be declining despite the absence of therapeutic measures.

ZIKV is primarily transmitted by *Aedes* mosquitoes; however, other forms of transmission exist, including vertical [6], sexual [7], blood, and laboratory acquired [8]. There is also the possibility of acquiring ZIKV through body fluids, such as stool [9], semen [10], breast milk [11], urine [12] and saliva [13]. Contrary to other mosquito-borne flaviviruses, ZIKV is a unique flavivirus because it can persist for months in immune-privileged sites, such as the eyes and testes [14,15]. The wide range of tropism of ZIKV is summarized in Figure 1, which also explains the unique characters of this virus. Approximately 80% of ZIKV infections are asymptomatic, with the 20% of symptomatic infections resulting in ZIKV-associated infections, such as microcephaly in children [16] and Guillan Barre` syndrome in adults. Other congenital ZIKV-related diseases in children include cerebral malformations, ophthalmological and hearing defects, and arthrogyrosis [17]. Vector-borne transmission of ZIKV occurs in two mosquito-driven cycles: (i) a sylvatic cycle, in which the virus cycles between non-human primates (NHPs) and arboreal mosquitoes; (ii) an urban cycle, in which the virus cycles between humans and urban mosquitoes [18]. ZIKV is mostly maintained in the sylvatic cycle [19–21]. However, in this type of transmission, humans are usually incidental hosts. Currently, direct human to human transmission has become common in the urban infectious cycle as the virus can be spread perinatally [6], sexually [7], and

through breastfeeding [11] or blood transfusion [8], as mentioned above, making ZIKV a human pathogen. Despite zoonotic transmission being primarily maintained by monkeys and mosquitoes, ZIKV antibodies have also been detected in other animal species, including water buffalo, elephants, goats, hippos, impala, kongoni, lions, sheep, rodents, wildebeest, and zebras [22,23].

ZIKV is a spherical, enveloped virus with icosahedral symmetry. The diameter of the mature virus is approximately 50 nm, and the diameter of the immature virus is closer to 60 nm [24–27]. ZIKV has a positive-sense single-stranded RNA genome approximately 10.7 kilobases in length. Located at the 5' and 3' ends of the genome are two flanking untranslated regions (UTRs), which in the ZIKV MR766 strain are 106 and 428 nucleotides long, respectively [28]. The ZIKV genome lacks a 3' poly (A) tract and ends with CU_{OH} , similar to other flaviviruses. The genome contains a single open reading frame (ORF) that encodes a polyprotein composed of approximately 3400 amino acids [28], which after being processed by host and viral proteases, yields three structural proteins (C, PrM, E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). In this review, we examine various studies to discuss the pathogenesis of ZIKV and the current development of therapeutics for treating infections of this virus.

ZIKV pathogenesis

Little is known about the pathogenesis of ZIKV since it involves complex interactions between viral and host factors. The molecular mechanisms of virus–host

interactions have been studied both *in vitro* and *in vivo* to provide more insight into the pathogenesis of ZIKV. The fact that ZIKV causes teratogenic effects conveys an urgent need for the rapid development of antiviral therapies [29]. WNV and Powassan virus (POWV) have also been shown to result in significant fetal injury in mice [30]. ZIKV infection begins with a single mosquito bite of a ZIKV-infected person. Viral replication begins in the epithelial cells of the mosquito midgut and proceeds to the salivary glands. The mosquito can spread the virus after a 10-day incubation period, when its saliva becomes infected [31]. The incubation period in humans is 3–12 days [32], and symptoms appear after 6–11 days. ZIKV is cleared within 24 days in 99% of patients [33].

Pathogenesis in humans

Following a mosquito bite from a ZIKV-infected mosquito, ZIKV infects and replicates in dendritic cells, spreading through the blood to other parts of the human body. In most cases, the virus is self-limiting; however, infections in pregnant women result in teratogenic effects [17]. In pregnant women, viral infection extends for a longer period since the virus replicates in the fetal brain for months with increasing effects during the early months of pregnancy [34].

ZIKV first binds to cellular receptors that are specific to different flaviviruses. ZIKV receptor members include DC-SIGN (dendritic cell-specific intracellular adhesion molecule 3-grabbing nonintegrin) and phosphatidylserine receptor proteins: TYRO 3, AXL, TIM, and TAM [35]. These receptors facilitate ZIKV entry into macrophages, monocytes, neural progenitor cells (NPCs), and fetal cells. These receptors play important roles in ZIKV infection, such as adhesion, migration, replication and evasion of the immune system, cytokine release, and antigen signaling pathways [35]. Experimentally, knocking out receptors does not result in total protection from viral infection because different flaviviruses use different receptors for entry [36].

AXL appears to play a major role in viral pathogenesis because it makes human skin fibroblasts permissive to ZIKV infection and replication [35]. AXL is highly expressed in the developing human cortex throughout neurogenesis and is overexpressed in glial cells. Surprisingly, studies by Wells *et al.* have shown that AXL is not required for ZIKV infection, even if it is highly expressed in NPCs: genetic ablation of AXL does not protect human NPCs or cerebral organoids from ZIKV infection [36] and an anti-AXL antibody does not reduce ZIKV infection in NPCs [37]. Hastings *et al.* also demonstrated that TAM receptors are not essential for ZIKV infection [38]. Generally, these studies suggest the existence of redundant entry receptors for ZIKV infection. TIM-1 (T cell immunoglobulin mucin domain 1) is a predominant ZIKV entry factor expressed in

human placental cells [6]. In skin fibroblasts, ZIKV induces the expression of pattern recognition receptors (PRRs), such as toll-like receptors (TLR3), RIG-1, and melanoma differentiation associated gene 5 (MDA5), enhancing the antiviral response against ZIKV infection [35].

Pathogenesis in animal models

Mouse models

Animal models have been used to study ZIKV infection in pregnant women and fetuses to optimize the development of vaccines and antiviral therapies [39]. Neonatal mouse models have been used to study the pathogenesis of ZIKV because they are more vulnerable to ZIKV infection than adult mice. Immunocompetent adult mice also demonstrate extensive ZIKV tropism in the brain, blood, spinal cord, spleen, kidney, and eye. Male *Ifnar*-deficient mice show high levels of ZIKV in their testes, providing more insights into the persistence of ZIKV in immunocompromised tissues [40]. *Rag-1* mice also exhibit pathogenesis of ZIKV in the epididymis and testicular cells [15].

The A129 mouse model, which is deficient in type-I IFN receptor, has also been used to study ZIKV pathogenesis. Different routes of viral administration have been used, including intraperitoneal and intravaginal. Three-week old mice infected with ZIKV (FSS13025) exhibited neurological disease and a high fatality rate from 6 days postinfection. However, older mice (11 week old) displayed 50% mortality in the 5th week and no mortality in the 11th week [41]. A129 mice infected with the MR766 (African) strain also exhibited weight loss and high mortality 6 days postinfection. Viral titers were highest in the brain and spleen [42], and a similar viral tropism was observed in an Asian lineage strain, except that it did not cause severe disease [43].

AG129 mice, with deficiencies in both type I and II IFN receptors, were injected intraperitoneally with ZIKV (FSS13025) and displayed neurologic disease with a high mortality rate 6 days postinfection [41]. However, intravaginal administration of the virus had a delayed lethality that manifested 22 days postinfection [44]. The H/PF/2013 (Asian) strain displayed lethality at 8 days postinfection, with the highest virus titers quantified on day 2 [45]. AG129 mice injected intraperitoneally with the MR766 (African) strain were characterized by a hunched back and hind-limb paralysis and succumbed 18 days postinfection [46]. In the same study, SCID mice were used in comparison with AG129 mice injected with the same strain. Surprisingly, lethality was delayed in SCID compared to AG129 mice. Mice succumbed 40 days postinfection on average [46].

The pathogenesis of ZIKV in the central nervous system (CNS) was also studied in the Swiss mouse model. One-day-old mice injected with ZIKV (SPH 2015) displayed paralysis and neurological disease

characterized by inflammation within the cerebral cortex [47]. BALB/c immunosuppressed mice injected with ZIKV PRVABC59 (Asian) displayed widespread viremia and inflammation of various tissues, especially orchitis, which may result in male infertility; however, treatment with Type 1 interferon (IFN 1) greatly reduced ZIKV infection [48]. Seven-week old C57BL/6 mice challenged with the ZIKV H/PF/2013 (Asian) and Senegal 1984 (African) strains also displayed widespread virus dissemination to immunosuppressed tissues, such as the testis and epididymis. Treatment with IFN 1 greatly improved the clinical outcomes of the mice [15].

Six-week-old TKO mice were used to study ZIKV dissemination to the CNS using ZIKV FSS13025 (2010 Cambodian isolate). Mice were more susceptible to ZIKV infection since they lacked 3 interferon regulatory factors (IRF3, IRF5, and IRF7), and ZIKV infection resulted in the death of neural progenitor cells [49]. Pregnant female mice have also been used to demonstrate infection by ZIKV in different trophoblasts and fetal endothelial cells of the placenta, resulting in congenital malformations [34].

Non-human primate (NHP) models

Rhesus macaques have been used to study ZIKV pathogenesis in the brain using a French Polynesian ZIKV strain [50], a finding reported across New World and Old World macaque species [51]. ZIKV RNA accumulates in the brain, cerebrospinal fluid, urine, and saliva for at least 3 weeks. This study showed that ZIKV infection elicited host immune responses that include ZIKV-specific T-cells and nAb responses. Prolonged

detection of viral RNA in urine and saliva, even after viral clearance in the blood, showed that ZIKV persisted in certain tissues [50]. Pigtail macaques infected with a Cambodian ZIKV isolate (FSS13025) were shown to develop fetal brain lesions during their pregnancy [52]. This was the first reported case of a fetal brain injury in a NHP after maternal infection with ZIKV infection. The pigtail macaque model provides a novel model for testing vaccines and other therapeutics against ZIKV [52]. Another study involving rhesus and cynomolgus macaques infected with 2 ZIKV isolates of Thai and Puerto Rican origin showed that ZIKV also persisted for more than 3 weeks in the saliva and semen, even after no traces of virus remained in the blood. This study demonstrated that ZIKV infection elicited rapid innate and adaptive immune responses in macaques, protecting them from reinfection [53].

NHPs have provided an ideal model for studying infections during pregnancies since they mimic humans in many ways, including placenta and brain development. These models have also shown susceptibility to many other flaviviruses, making them excellent models for ZIKV studies.

ZIKV protein functions and role in viral pathogenesis

The ZIKV genome is translated into 3 structural proteins (C, PrM and E) and 7 nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5), which are summarized in Figure 2. The structural proteins are essential for genomic replication and host immunity [54].

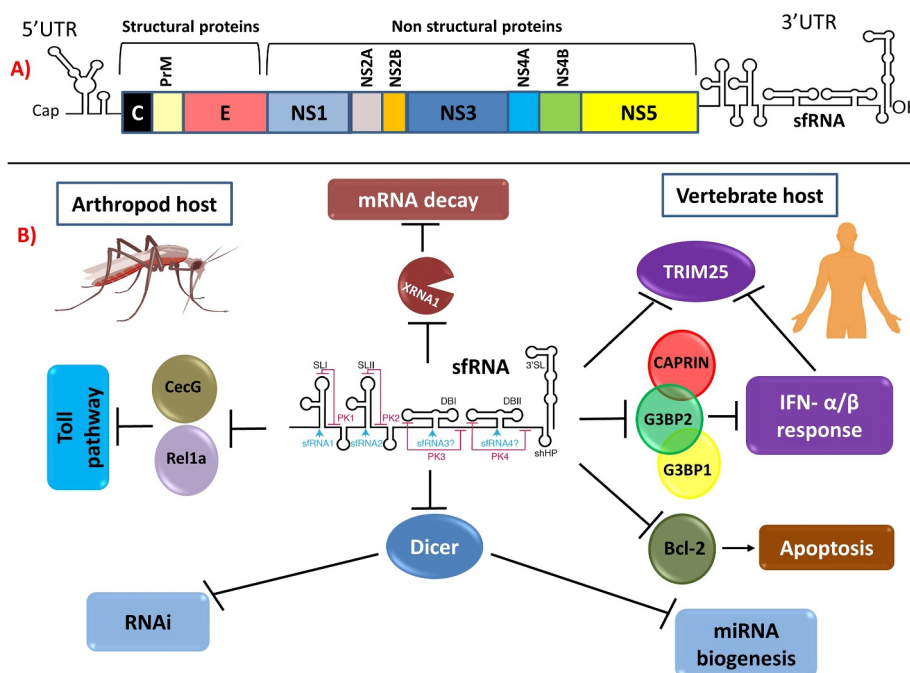


Figure 2. Subgenomic flaviviral RNA location and its roles in pathogenesis. (a) ZIKV genome scheme showing the location of sfRNA at the 3'UTR. (b) sfRNA pathogenic functions in arthropod and vector hosts. (a) and (b) are reproduced with permission and minor modifications from Slonchak and Khromykn [78].

Glycoprotein E is a major target for antibodies as it is responsible for facilitating viral entry into the host. ZIKV is capable of mediating the antibody response by the changing amino acids that surround the Asn154 glycosylation site in the virus E protein [55], which implicates glycosylation as an important player not only in the structure and tropism of the virus but also in its pathogenesis and evasion of the immune system.

The primary roles of PrM and capsid in ZIKV pathogenesis are still not well defined. However, the PrM protein has a conserved region that is responsible for viral maturation, egression and secretion [56]. Therefore, inhibiting the function of PrM will interfere with viral infectivity and pathogenicity. The capsid protein has been shown to play an important role in viral assembly since it is the primary structural protein that interacts with the viral genome within the viral particle [57]. Generally, flavivirus capsids have been shown to localize in the nucleoli in addition to their association with the phospholipid membranes of the ER and lipid droplets. Localization of the capsid in the nucleoli may indicate that it plays a role in pathogenesis apart from viral packaging [57].

NS are important for entry, translation, replication and pathogenesis of the virus. However, little information is known about the functional significance of ZIKV NS proteins or their role in ZIKV-induced pathogenesis. NS proteins play a major role in evading host immune responses specifically by interfering with the interferon (IFN) I response [58]. NS1 has been described as a pathogenicity factor and the most enigmatic protein of flaviviruses since it plays a major role in replication, as well as in immune evasion. NS1 is secreted from infected cells in the form of hexamers (sNS1), which are the hallmark of flavivirus NS1 [59]. Notably, sNS1 has been used as a ZIKV diagnostic marker and incorporated into vaccine development [59].

ZIKV NS1 and NS4B inhibit IFN I expression, leading to autophagic degradation of NS2B and NS3 through the autophagy pathway, inhibiting viral replication [58]. NS1 and NS4B suppress IFN I signaling by targeting TBK1, consequently inhibiting the production of interferons. NS2B3 (NS2B-NS3) has been shown to inhibit the JAK-STAT pathway by enhancing degradation of Jak-1. It is therefore clear that the co-operation among NS1, NS2B3 and NS4B proteins generally helps to enhance viral replication by evading IFN I responses.

The NS5 protein is the largest (approximately 900 amino acids) and most conserved of the flavivirus proteins. The NS5 protein contains a methyltransferase for RNA capping and a polymerase for viral RNA synthesis [60]. NS5 is also an IFN antagonist that degrades STAT2, which in turn, limits type I IFN signaling and leads to increased viral replication. STAT2 is a signaling molecule required in the IFN I pathway. The mechanism of STAT2 degradation in ZIKV by the NS5 protein is distinct from that in DENV. Expression of ZIKV NS5 alone results in

STAT2 degradation and does not require maturation of the N terminus of NS5 and does not involve UBR4 [61]. The interaction is also host-specific since NS5 is unable to degrade murine STAT2, leading to susceptibility to ZIKV infection in immunocompetent mice. ZIKV has been shown to bind and degrade STAT2 through proteasomal degradation. Antagonism of STAT1 and STAT2 phosphorylation results in ZIKV disease [62].

ZIKV NS2A has been shown to play a central role in recruiting viral RNA, the structural protein prM/E, and the viral NS2B/NS3 protease to the virion assembly site and in engineering virion morphogenesis [63]. A single mutation interfering with these interactions did not significantly affect viral RNA replication but selectively abolished virion assembly, demonstrating the specific role of these interactions in viral morphogenesis [64].

The flavivirus NS4A and NS4B proteins have also been shown to inhibit JAK/STAT and RLR signaling through multiple mechanisms [65]. In ZIKV, overexpression of NS4A and NS4B in fetal neuronal stem cells (fNSCs) reduces neurosphere formation and inhibits differentiation [66]. The effects of these two proteins were further linked to increased mediated Akt-mTOR signaling and were ZIKV-specific because DENV failed to show a similar effect [66].

sRNA functions and role in viral pathogenesis

Subgenomic flavivirus RNA (sRNA) is a highly structured 0.3–0.7 kb long noncoding RNA and is said to be the most abundant viral RNA species in infected cells. sRNA is produced in all arthropod-borne flaviviruses during viral infection and represents the 3'-terminal highly conserved region of the 3' UTR (untranslated region) [67]. sRNAs have many isoforms with different 5' ends, including sRNA-1, sRNA-2, sRNA-3, sRNA4, and other new isoforms yet to be described.

ZIKV-infected cells produce sRNAs in their 3'-untranslated regions that accumulate during infection and resist degradation by host 5'-3' exonucleases in infected cells, such as XRN1. ZIKV-infected cells have been suggested to play a role in the replication cycle of the virus and in evasion of the immune system [68,69].

Flavivirus sRNAs contain stem-loop (SL) and dumbbell (DBL) structures made up of nucleotides that form pseudoknots (PK). During ZIKV infection, two XRN1 resistant RNA are produced, xrRNA1 as a result of XRNA stalling at SL1 and xrRNA2 as a result of XRNA stalling at SL2 [68,70]. These two RNAs form three-way junctions of coaxial stacking of helices P1 and P2, while P3 is located at the acute angle of P1. Three-way junctions are highly structured elements of nucleic acids, such as rRNA, and have a unique topology in ZIKV. These two different resistant RNAs can occur as the result of cellular mechanisms [70].

sRNAs have several functions in flavivirus infections, as summarized in Figure 2. First, these RNAs are

directly linked to cytopathic effects (CPEs). In a cell culture experiment of the pathogenicity of WNV and DENV, genomes harboring mutations that interfered with the formation of full-length sfRNA produced no visible plaques, and replication was inefficient in both insect and mammalian cell lines [67]. Funk et al., demonstrated the role of sfRNA in pathogenesis both *in vitro* (Vero cells) and *in vivo* (mice). All mutant viruses that did not produce sfRNA1 were highly attenuated in mice and can be used as potential vaccine candidates [71].

The second function of sfRNAs is suppression of IFN responses in vertebrates. ZIKV sfRNA functions both as a RIG-1 and MDA-5 agonist and has stronger activity than DENV serotype 2, which only affects RIG-1 [68]. In WNV, sfRNAs directly antagonize IFN-stimulated gene (ISG) products, such as protein kinase R and RNase L, which bind RNAs [69]. DENV sfRNA antagonizes proteins that modulate viral infection, which include G3BP1, G3BP2, and CAPRN1, colocalizing with them. Most flaviviruses produce and use this type of RNA in their mechanisms of interacting with the host. sfRNA was first characterized in Murray Valley Viral infections [72] and was later found in Japanese Encephalitis Virus (JEV) [73] and WNV [74]. sfRNAs help flaviviruses evade the innate immune system [69,75,76]. Mutations in sfRNA, such as deletions, lead to significant effects on the viral replication and life cycle of DENV and WNV in cells that exhibit IFN I responses [69,77]. These mutations may also be the cause of the emergence of new pathogenic viral strains as a result of viral evolution [78].

Third, sfRNAs have been shown to induce apoptosis in cells through the Bcl-2 mediated PI3k/Akt signaling pathway [79]. However, overexpression alone did not induce apoptosis, indicating that its action requires flavivirus replication.

Fourth, several studies have shown that sfRNA also plays an important role in the flaviviral life cycle and dissemination in infected insects [80–82]. sfRNAs also determine the infection and transmission rates of WNV and DENV in mosquitoes [80,83]. Their fifth function entails the ability of sfRNAs to suppress RNAi and miRNA pathways [82].

The sixth function includes the generation of sfRNA by XRN1, which represses the activity of the exoribonuclease that plays a major role in cellular mRNA decay, dysregulating host mRNA stability. During flavivirus infections, changes in the half-life of mRNA have a significant effect on the expression of normal short-lived mRNAs that encode cytokines compared to long-lived transcripts. Flaviviruses can therefore escape cellular-mediated immunity by taking advantage of this dysregulation [84].

Lastly, sfRNAs play a role in the replication of flaviviruses through downregulation of RNA synthesis and translation [85]. Transfection of JEV infected cells with a -sfRNA to counter effect of +sfRNA elevated the

antigenome levels implicating that sfRNAs inhibits antigenome synthesis.

Development of ZIKV vaccines

Following the emergence of ZIKV, rapid and promising vaccine development has been ongoing by incorporating the lessons learned from the design of other flavivirus vaccines. Despite the development of several ZIKV vaccines, a few challenges have arisen considering the possibility of crossreactivity and adverse effects in immunocompromised individuals. Therefore, development of a safe and efficacious ZIKV vaccine is of great importance. Currently, multiple vaccine platforms are being incorporated to create new vaccines, such as DNA, mRNA, peptide, protein, viral vectors, virus-like particles (VLPs), inactivated-virus, and live-attenuated virus. These platforms and the current vaccines are briefly discussed below and summarized in Table 1.

Inactivated virus and live-attenuated virus vaccines

Traditionally, the development of vaccines has involved the use of inactivated and attenuated organisms. A similar approach has been used to design vaccines for several flaviviruses, including DENV, TBEV, JEV, and YFV.

For ZIKV, Shan *et al* developed a vaccine using an infectious cDNA clone with 10 nucleotide deletions within the 3' untranslated region of the viral genome. The vaccine was immunogenic and protected both immunodeficient AG129 and CD-1 mice, causing higher T-cell responses than the wild-type virus. Mechanistically, the attenuated vaccine also elicited increased sensitivity to type-I IFN and downregulated RNA synthesis. The attenuated 10-del ZIKV did not infect mosquitoes after oral feeding with spiked-blood meals, representing an additional safety feature of the vaccine [86]. Sumathy *et al.* designed an alum-absorbed inactivated virus vaccine using the Ugandan ZIKV strain MR766. The inactivated vaccine was shown to protect AG129 and BALB/c mice from viral infection. Two doses of the vaccine-elicited high titers of neutralizing antibodies in BALB/c mice. Importantly, vaccine antisera were able to protect passively immunized mice against virus challenge [87].

Xie *et al.* used a reverse genetics approach to develop two chimeric viruses (CHV) by swapping the prM-E (premembrane and envelope) structural genes between DENV-2 and ZIKV, incorporating a full-length cDNA clone. Chimeric ZIKV with DENV-2 prM-E (Chimeric Virus 1) and Chimeric DENV-2 with ZIKV prM-E genes (Chimeric Virus 2) were highly attenuated in AG129 mice and were immunogenic as indicated by their decreased viremia and weight loss. These chimeric viruses also protected mice against DENV-2 and

ZIKV challenge. Notably, this study demonstrated that the PrM-E genes are major determinants of DENV and ZIKV thermostability and that the hydrogen-bond interaction between Q350 and T351 in the CD loop of the ZIKV E protein is not required for virion thermostability. Additionally, the conformation of the extended CD loop is important for viral assembly or release. Potentially, these viruses can be developed into effective vaccines against ZIKV [88]. Currently, 5 of 7 inactivated virus vaccines have completed phase 1 clinical trials. ZPIV is a purified, formalin-inactivated ZIKV vaccine candidate that has been completed and has been demonstrated to be well-tolerated and immunogenic in a phase 1 human clinical trial [89]. In all the vaccines, formalin was used to inactivate the virus [87,89,90].

DNA-based vaccines

DNA-based vaccines offer several advantages compared to other vaccine platforms. DNA-based vaccines are easy to manufacture, transport, and store. Importantly, DNA-based vaccines have been shown to produce both antibody and T-cell immune responses, exhibiting an added advantage over other protein based-vaccines [91]. However, DNA-based vaccines have a major disadvantage with respect to administration, requiring special equipment for electroporation to facilitate DNA entry into the cell [92].

Larroca *et al.* designed a vaccine expressing full-length prM-E genes using a Brazilian strain, BeH815744. The vaccine elicited high Env-specific neutralizing antibodies and good T cell immune responses. A single-dose immunization provided complete protection in BALB/c, SJL and C57BL/6 mice [93]. Dowd *et al.* developed a DNA vaccine candidate, VRC ZKADNAO85-00-VP, using a vector (VRC 5288) incorporating a full-length prM-E gene from the ZIKV strain H/PF/2013 with a JEV stem and transmembrane regions to facilitate protein expression. This vaccine is currently undergoing phase 1 and 2 clinical trials [94]. Gaudinski *et al.* compared two vectors, VRC5288 and VRC5283, which differ in their final 98 amino acids of the envelope protein. VRC5283, which encodes the wild-type ZIKV sequence, showed higher immunogenicity and was more efficient than VRC5288. Nonetheless, both vaccines were tolerable in healthy individuals. Currently, VRC5283 is in Phase 2 investigation undergoing clinical trials to optimize its safety, immunogenicity, and dose delivery regimens [95].

Tebas *et al.* evaluated the safety and immunogenicity of a DNA vaccine, GLS-5700, which is currently under development. The vaccine is composed of a prM-E sequence from an infectious ZIKV clone and was tested in 40 participants with no adverse effects. Inoculated individuals developed ZIKV specific antibodies, and this trial successfully demonstrated the safety

and efficacy of the vaccine [96]. Development of a synthetic DNA vaccines is appropriate for emerging infectious diseases such as ZIKV since they can be manipulated for the rapid design of novel antigens. This platform can be used for the rapid development of vaccines, using relevant antigens expressed in the emergent pathogen [97].

Adenovirus vector-based vaccines

Recombinant adenoviral vectors have been widely used in the development of vaccines, offering several advantages, including safety, ease of manufacturing, and broad and strong immune responses. Human and chimpanzee adenoviruses have been extensively explored in the design of vaccine vectors.

Abbink *et al.* designed a rhesus adenoviral vector (RhAd52) expressing prM-Env using a ZIKV Brazilian strain and tested its efficacy by intramuscular immunization in monkeys. A single dose of immunization-induced neutralizing antibodies protected monkeys against ZIKV [98]. Similar work with chimpanzee adenovirus vectors (ChAd) has demonstrated protective immune responses in the A129 mouse model [99].

Kim *et al.* designed a recombinant E1/E3-deleted adenoviral vector (pAd.ZIKV-Efl) from the ZIKV strain BeH815744 that expressed a codon-optimized Env-antigen. The extracellular region of the ZIKV envelope was linked to the T4 fibrin trimerization domain, facilitating protein expression. Additionally, the vector was designed with a polyhistidine tag and a Tobacco Etch Virus (TEV) to increase protein folding and facilitate purification. The vaccine was shown to protect C57BL/6 mice from lethal challenge with the ZIKV DAKAR41542 strain [100]. Notably, in these vaccine studies, the ZIKV E subunit protein production yield was very low, a finding that has also been highlighted by Larroca *et al.* [93]. The low yield of the E protein was probably due to the absence of preM, which is important for protein stability. Therefore, preM is an indispensable factor in the development of ZIKV E protein-based vaccines.

Virus-like particles

Virus-like particles (VLPs) have emerged as a powerful platform for the development of vaccines due to their ability to produce broad and strong immune responses. VLPs are self-assembling platforms that resemble viruses but are noninfectious and nonreplicating. Boigard *et al.* used VLPs to develop a ZIKV immunogenic vaccine by coexpressing the C-prM-Env with the NS2B/NS3 protease to test their efficacy. The ZIKV VLP immunogen was tested in BALB/c mice and elicited high antibody titers against ZIKV FSS13025 and MR-766 strains; however, VLP immunization did not enhance DENV infection when ADE tests were conducted [101].

Peptide-based vaccines

Immune-informatics approaches are becoming an interesting platform for the development of vaccines as they can allow the manipulation of conformational or linear epitopes to optimize epitope-based vaccines. The availability of information in the genome sequence has simplified the prediction of T-cell epitopes in developing ZIKV epitope-based vaccines, which elicit immune responses. Dikhit *et al.* used this *in silico* approach to predict 9 promiscuous epitopes in 5 proteins by combining human leukocyte antigen-binding specificity and population coverage [102]. These epitopes consisted of capsid (MVLAILAFL), Env (RLKGVSYSL and RLITANPVI), NS2A (AILAALTPL), NS4B (LLVAHYMYL and LVAHYMYLI), and NS5 (SLINGVVRL, ALNTFTNLV and YLSTQVRYL). Altogether, these epitopes elicited immune responses from human CD8 + T cells and bound to at least one HLA molecule from the majority of the population. Moreover, further research needs to be performed both *in vitro* and *in vivo* to understand the immunological relevance of these epitopes in the development of vaccines against ZIKV [102].

RNA-based vaccines

RNA provides a good platform for developing ZIKV vaccines since it can be easily modified and incorporated into vaccines to improve their immunogenicity and eliminate side effects. RNA vaccines have an added advantage over DNA vaccines in that they have no risk of possible integration into the human genome. Specifically, mRNA has proven to be a highly effective platform in the design of ZIKV vaccines.

Pardi *et al.* developed a novel anti-ZIKV vaccine in which the prM-E proteins were encoded by mRNA. The mRNA was encapsulated using lipid-nanoparticles (mRNA-LNPs), which subsequently enhanced protein expression. The vaccine-elicited antibodies that protected C57BL/6 mice from a lethal ZIKV H/PF/2013 challenge [103].

Richner *et al.* also used this approach to develop a full-length prM-E RNA sequence of ZIKV into LNPs to increase shuttling between cells. The immunodominant fusion loop in domain II (DII-FL) was mutated, crippling the reactivity of the antibodies targeting this region and resulting in no ADE in response to DENV infection. The vaccine protected immunodeficient AG129 and BABL/c mice, as well as immunocompetent C57BL/6 mice, with the production of high and durable neutralizing antibodies [104].

Chahal *et al.* also employed RNA nanotechnology in designing an RNA vaccine candidate expressing prM-E as an open reading frame using the Asian ZIKV isolate Z1106033. Immunization through intramuscular injection elicited ZIKV E protein-specific IgG responses and protected C57BL/6 mice. Additionally, immunization

also resulted in good CD8 + T cell responses against the peptide (IGVSNRDFV) derived from the envelope protein [105].

ZIKV drug development

Currently, there are no specific drugs to treat or prevent ZIKV infections, and treatment involves the administration of fluids and plenty of rest by infected individuals. Painkillers (e.g., Paracetamol) are used to alleviate headaches, fever, and myalgia. Four strategies have been employed in the development of ZIKV antiviral drugs, including targeting viral proteins (Table 2), targeting host proteins (Table 3), repurposing of clinically approved drugs, and using reverse genetic systems, such as infectious cDNA clones and replicons.

Drug(s) repurposing has rapidly emerged as a strategy for developing ZIKV drug(s) from clinically approved drug(s). Drug repurposing is faster and most cost-effective than other platforms of drug design. These drugs include the antibiotic azithromycin [106], the antiparasitic drug nitazoxanide [107], the antimalarial drug chloroquine [108], the antiprotozoal drug emetine [109], the antiviral drug ribavirin and favipiravir [110], and many other FDA-approved drugs. High-throughput screening has also been extensively performed for both repurposed drugs and compound screening.

Importantly, the reverse genetic system has also been incorporated into the design of antivirals by using infectious cDNA clones, ZIKV replicons, and VLPs. The use of reporter genes, such as luciferase and EGFP, in designing recombinant ZIKV has provided a novel platform in determining the mechanisms of drug inhibition. The flavivirus replicon has been proven to be safe when working with the virus since it is not infectious. VLPs have also been used in drug and vaccine development for several flaviviruses, such as WNV, JEV, YFV, DENV, and ZIKV [111].

ZIKV infections are usually characterized by mild illness and uneventful recovery; therefore, when designing anti-ZIKV strategies, the primary target population should be highly considered, namely, immunocompromised individuals, pregnant women and their fetuses [112]. Currently, five pregnancy categories for anti-ZIKV drugs have been defined: Category A, B, C, D and X, all of which have considerations for use in pregnancy [113]. For Category A, adequate and well-controlled studies have failed to demonstrate a risk to fetuses in the first trimester or later of pregnancy. In Category B, animal reproduction studies have failed to demonstrate a risk to fetuses, but there are no adequate or well-controlled studies in pregnant women. For Category C, animal reproduction studies have shown an adverse effect on fetuses, and while there are no adequate or well-controlled studies in humans, their potential benefits may warrant use of

Table 2. ZIKV Drugs Targeting Viral Proteins.

Targets	Inhibitors	IC ₅₀ μM	ZIKV Strains	Cell lines tested	Biochemical Assays	Animal tests	Pregnancy Category	Reference
Envelope	EGCG	21.4	ZIKV _{BR} , MR 766	Vero E6	Cell viability	Wistar (SPF) rats	-	[115,156]
	Crotoxin Pinocebrin	0.23, 0.32 μg/μl 17.4	PRAWBC59	Vero E6, C6/36 JEG-3	WB WB, QRT-PCR, IFA, TOA	BALB/c	-	[114] [117]
	<i>Psiloxylon mauritianum</i>	19.5 μg/ml	MR766, ZIKV PF-13	Vero, A549	TOA, IFA, RT-qPCR, FISH, Flow cytometry, COMET	-	Phase 1 Trials	[157]
	Porphyrins (Co-protoporphyrin IX (CoPPIX) and Sn-protoporphyrin IX (SnPPIX))	2.49, 5.78	MR766, ZIKV _{BR}	BHK-21, Vero, HeLa,	TOA, IFA, TEM, WB, ELISA	Rats and humans	-	[116]
	Suramin	47	Beh233339	-	DSC, NMR	C57BL/6	-	[118,119]
	Ivermectin	~5.00	MEX_1_7/2015	Huh-7, HeLa, NPC, JEG3, HAEC	Flow cytometry, IFA	C57BL/6 J	-	[120,121]
NS5 polymerase	BCX4430	3.8 ± 2.5, 4.7 ± 0.6, 4.7 ± 2.2	PRVABC59	Vero76, Huh-7, RD	Immunohistochemistry(IHC), qRT-PCR	AG129 mice, Rhesus macaques	Phase 1 trials	[158]
	7DMA Emetine	1.3 0.0298	MR766 MR 766, PRAWBC59, FSS13025	Vero, BHK, C6/36 SNB-19, HEK 293, Vero E6, hNSC	TOA, IFA, RT-PCR Western blot(WB), IF	AG129 mice	-	[46] [109]
	Cephaeline	0.0189	MR 766, PRAWBC59, FSS13025	SNB-19, HEK 293, Vero E6, hNSC	WB, IF, NS5 polymerase inhibition	AG129 and SJL mice	-	[109]
	Ribavirin	Unknown	MR766, PRVABC59, and PC-740	A549, hNPCs, HDfs and Vero	MTT assay, qRT-PCR, western-blot	129 SV	-	[110,135]
NS5 Methyltransferase	Sofosbuvir	unknown	ZIKV _{BR} , MR766	VeroE6	Cell viability, Caspase assay	Swiss Albino mice	B	[131]
	Favipiravir	-	PRVABC59, Pe-740	Vero, hNPCs	qRT-PCR, WB, IFA	129 SV	-	[110,135]
	SAM analog sinetungin, S-adenosyl-L-homocysteine (SAH F3043-0013, F0922-0796, F1609-0442, and F1750-0048)	1.18, 0.43	H/PP/2013	unknown	Radioactive methyltransferase assay	ND	-	[159]
	Lopinavir-ritonavir	4.8 ± 2.4, 12.5 ± 7.4, 17.5 ± 8.4 and 17.6 ± 3.1	-	Vero	Plaque Reduction Assay	ND	-	[132]
NS2B/NS3	Novobiocin	4.78 ± 0.41 μg/ml	PRVABC59	Vero, Huh-7, <i>in silico</i>	NS2B-NS3 protease activity	-	B	[123]
	Aprotinin	42.63, 62.24	PRVABC59	Vero, Huh-7, <i>in silico</i>	NS2B-NS3 protease activity	BALB/c mice	C	[123]
	Bromocriptine	0.361 ± 19	Z1106033	<i>in silico</i>	X-ray crystallography	-	B	[125]
	NSC135618	13.04 ± 2.00	PRVABC59	Vero	NS2B-NS3 protease assay	-	B	[126]
	Peptide Z2	1.0	PRVABC59	A549, hNPCs, HPEC,	IFA, qRT-PCR, WB, mass spectrometry	-	-	[160]
	Erythrosin B	0.62 ± 0.12	SZ01/2016, GZ01/2016	C6/36, Vero, Huh-7	IFA, flow cytometry, RT-qPCR,	C57BL/6 mice	-	[161]
	Temoporfirin, Niclosamide, Nitazoxanide	0.024, 0.48, 1.48	PRVABC59	HPECs, A549, HNPC	IFA, qRT-PCR, protein thermal shift assay, WB	-	B	[124]
	NSC157058, NSC86314, NSC716903	0.82 ± 0.09, 1.12 ± 0.11, 3.01	PRVABC59	A549, HPECs, hNPCs	IFA, WB, qRT-PCR, SPR	-	-	[107]
	Myricetin, Quercetin, Luteolin, Isorhamnetin and Apigerin	1.3 ± 0.1, 2.4 ± 0.2, 2.7 ± 0.3, 15.5 ± 0.7, 56.3 ± 0.9 and 3.5 ± 0.2	FSS13025	hNPCs	Fluorescent peptide, IF, RT-PCR	-	-	[162]
			ZIKV 8375	-	NMR	-	-	[122]

Table 3. ZIKV Drugs targeting host proteins.

Targets	Inhibitors	IC ₅₀ μM	ZIKV Strains	Tested Cell Lines	Animal tests	Biochemical Assays	Pregnancy Category	Reference
IMP dehydrogenase inhibition	Merimepodib	0.6	FSS13025, MR766	Vero E6	-	qRT-PCR.	-	[163]
Pyrimidine synthesis inhibitor	Cyclosporine A	unknown	MEX_1_7/2015, ZIKV DAK_41525	Huh-7, hNSC	-	IF, Flow cytometry	C	[120]
Purine biosynthesis inhibitor	6-methylmercaptapurine riboside (6MMPr)	24.5, 20.3	ZIKV PE243	Vero, SH-SY5Y	-	qRT-PCR, TOA, Flow cytometry, IFA	-	[141]
Purine biosynthesis inhibitor	Methotrexate	0.28	PRVABC59, H/PAN/2016/BEL-259,634	Vero, hNPCs	-	IFA	-	[164]
Nucleoside metabolic inhibitor	Palonosetron	16.3 ± 7.7	ZIKV _{BR} (KX197192.1)	Huh-7	-	IFA	B	[165]
Cholesterol metabolism inhibitor	Mevastatin	3.42	MR766	Vero 76	ND	WB, RT-qPCR	-	[166]
Cholesterol metabolism inhibitor	Imipramine	5.05	PRVABC59					
Cholesterol metabolism inhibitor	Imipramine	-	PF-25-013-18	HFF1, Vero	C57BL/6 N	IFA, WB, RT-QPCR, Flow cytometry	C	[167,168]
Cholesterol metabolism inhibitor	Benzamil	-	PRVABC59	Vero, RPE (Retinal Pigment Epithelial)	-	qRT-PCR, IFA	B	[169]
Cholesterol metabolism inhibitor	Gsw4869	-	PRVABC59	Human fetal astrocytes, NPCs	-	TEM, WB, IFA, IHC, qRT-PCR	-	[143]
Cholesterol metabolism inhibitor	7-KC	4.064	ZIKV SPH, ZIKV Ibh 365	Vero, Human neurons	-	Autophagy TOA, QRT-PCR,	-	
SREBP pathway inhibitor	Nordihydroguaiaretic acid (NDGA)	9.1	ZIKV PA259459	Vero	ND	ELISA, WB, qPCR	Not approved	[145]
SREBP pathway inhibitor	Tetra-O-methyl nordihydroguaiaretic acid (M4N)	5.7	ZIKV PA259459	Vero	ND	ELISA, WB, qPCR	-	[145]
caspase 3 activity inhibitor	PHA-690,509	1.72	MR766, FSS13025, PRVABC59	SNB-19, hNPCs	-	WB, IHC, TOA, caspase-3 assay,	D	[146]
caspase 3 activity inhibitor	Bithionol		PRVABC59	Vero, RAW264.7	-		-	[147]
caspase 3 activity inhibitor	Phloretin	22.85, 9.31	MR766, PRVABC59	Vero	-	IFA, WB	-	
Caspase inhibitor	Emricasan	0.17, 0.13, 0.19	MR766, PRVABC59, FSS13025	SNB-19, hNPCs	3D brain organoids	WB, IHC, TOA, caspase-3 assay,	-	[146]
Protein Metabolism inhibitor	Celgosvir	50	MR766, PRVABC59	Vero 76	ND	WB, RT-qPCR	-	[166]
Protein Metabolism inhibitor	Fatostatin	≤1.2	PA259459	Vero	-	WB, IFA, ELISA, RT-QPCR	-	[145]
Protein Metabolism inhibitor	Metformin		PA259459	Vero, BHK-21	ND	Lipidomics, IFA, RT-QPCR	-	[170]
Protein metabolism inhibitor	Cyclosporine A	1	MEX_1_7/2015, ZIKV DAK_41525	Huh-7, hNSC	C57BL/6	IFA, Flow cytometry	C	[120]
Endocytosis and Endosomal Fusion inhibitors(EEF)	25-hydroxycholesterol (25HC)	0.188	ZIKV SZ01	Vero	A129 mice, Rhesus macaque	Plaque assay, IFA,	-	[134]
EEF	Arbidol	12.09 ± 0.77, 10.57 ± 0.74	MR66, ZIKV Paraba-01	Vero, PS, HBCA, UKF-NB-4	129 SV	IFA	-	[135,171]
EEF	K22	2.1-2.5	PR-2015; PRVABC59	Vero Huh-7	-	IFA, TEM, TOA	-	[136]
EEF	Tenovin 1	0.7	Mex2-81, FSS13025, MR766	Jeg3, BHK-21, Vero, Human U2OS	-	RT-QPCR, TOA, IFA	-	[137]
EEF	Peptide EV37	-	PRVABC59	Huh-7, Huh 7.5.1, BHK-21, HEK293T	-	TOA, QRT-PCR, WB, Flow cytometry	-	[138]
EEF	Chlorpromazine	-	Beh19015, PE243/2015	BHK-21, Huh-7, Vero E6	C57BL/6	IFA	-	[172,173]
EEF	Amodiaquine	-	ZIKV (strain PLCal_ZV)	Vero, Huh-7	SCID-beige mice	QRT-PCR, Flow cytometry	-	[139]
EEF	Chloroquine	9.82, 14.20, 12.36	MR766, ZIKV _{BR}	Vero, hBMECs, hNPC	A129, SJL mice	qRT-PCR, Flow cytometry, IF	-	[108]

(Continued)

Table 3. (Continued).

Targets	Inhibitors	IC ₅₀ μ M	ZIKV Strains	Tested Cell Lines	Animal tests	Biochemical Assays	Pregnancy Category	Reference
Apoptosis and vATPase inhibitor	Obatoclax, Saliphenylhalamide (Saliphe)	0.04 \pm 0.01, 0.05 \pm 0.02	ZIKV FB-GWUH-2016, MR766, H/PF/2013	RPE	3D brain organoids	TDA, IFA, qRT-PCR,	-	[174]
Inhibits mTOR, STAT3 and NF- κ B signaling pathways	Nidosomalide	0.28	MR766, PRVABC59, FSS13025	SNB-19, hNPCs	Chick embryos	WB, IF, TOA, caspase-3 assay,	B	[146,175,176]
Innate immunity and Epigenetics	GSK-126	-	H/PF/2013	Vero, MRC-5	C57BL/6 J	RT-QCR, OPCR, WB, CHIP, IFA, IHC	-	[150]
TNF- α neutralizing antibody	Infliximab	-	-	hNPCs	Swiss mice	-	B	[177]
NMDA receptor blocker	Memantine	-	H5-2015-BA-01	HPSCs, U87 glial cell	SV129	IHC	B	[178]
Unknown	Azithromycin	-	SPH2015, PRVABC59, FSS13025	HPSCs, U87 glial cell	ICR mice	-	B	[106]
Unknown	Ebelsen	-	PRVABC59	AG129	AG129	IFA, IHC, WB, qRT-PCR, Histology	-	[179]

ND – Not Done DSC- Differential Scanning Calorimetry CPE -Cytopathic Effects PRNT-Plaque Reduction Neutralization Test SPR-Surface Plasmon Resonance MMR-Nuclear Magnetic Resonance

these drugs in pregnant women, despite the potential risks. In Category D, there is positive evidence of a human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but the potential benefits of these drugs may warrant their use in pregnant women, despite these potential risks. For Category X, studies in animals or humans have demonstrated fetal abnormalities, and/or there is positive evidence of a human fetal risk based on adverse reaction data from investigational or marketing experience. Therefore, the risks involved in the use of these drugs in pregnant women clearly outweigh their potential benefits. In this section, we summarize drug development using host and viral proteins as targets for drug design.

Antivirals targeting viral proteins

Flavivirus host proteins (E, NS1, NS2B-NS3, NS3 and NS5) have been used as targets for developing therapeutics against ZIKV. Different compounds act directly by binding to the E protein and impairing E-mediated membrane fusion. Most of these compounds have been effective *in vitro*; however, only a few of them have reached clinical trials. These compounds include Crotoxin, a venom from *Crotalus durissus terrificus*, and epigallocatechin gallate (EGCG), a polyphenol that inhibits ZIKV entry into host cells [114,115]. Co-protoporphyrin IX (CoPPIX) and Sn-protoporphyrin IX (SnPPIX) have also been shown to exhibit anti-ZIKV activity by interfering with viral morphology and entry into host cells [116]. Pinocembrin (natural flavonoid) evidenced efficacy against ZIKV and is now in Phase 1 trials [117]. Suramin and Ivermectin have also shown anti-ZIKV activity both *in vitro* and *in vivo* [118–121].

The ZIKV NS2B-NS3 protease and NS3-helicase play a major role in viral replication. Consequently, several compounds have been developed to target these two proteins. Natural flavonoids, including myricetin, quercetin, luteolin, isorhamnetin and apigenin, have been shown to noncompetitively inhibit ZIKV protease [122]. Lopinavir-ritonavir and novobiocin have also been shown to exhibit anti-ZIKV effects [123]. Novobiocin was shown to protect mice against a lethal ZIKV challenge, reducing viremia and histopathological damage [123]. Erythrosin B, a category B drug, was found to noncompetitively inhibit DENV2 and ZIKV NS2B-NS3 proteases [124] and was also shown to reduce viral titers in YFV, JEV, and WNV, with low cytotoxicity and a micromolar potency [124]. In a screening of 2816 approved drugs, 3 potent drugs, temoporfin, niclosamide, and nitazoxanide, were identified as inhibitors of ZIKV protease [107]. Moreover, temoporfin was shown to inhibit ZIKV replication in human placental cells and protected mice from succumbing to ZIKV infection [107]. Aprotinin, used to reduce bleeding during

complex surgery [125], and bromocriptine [126] were found to be potent inhibitors of ZIKV NS2B-NS3. In both, molecular models were developed to predict binding with the NS2B-NS3 protease.

NS5 is an RNA-dependent RNA polymerase that plays an important role in viral genome replication. Several inhibitors have been developed using NS5 as a therapeutic target. BCX4430, an adenosine analogue, has broad-spectrum activity against a wide range of RNA viruses with potent *in vivo* activity against YFV, WNV, Tick-borne Encephalitis virus (TBEV), Marburg and Ebola viruses [127–129]. The compound is now in clinical trials and is a promising antiviral agent against ZIKV infection [130]. Other compounds tested in mice (AG129) include 7-deaza-2'-C-methyladenosine, a potent inhibitor of ZIKV replication, which was shown to delay ZIKV pathogenesis in a robust mouse model (AG129) [46]. Emetine and cephaeline also inhibited ZIKV polymerase both *in vitro* and in AG129 mice [109]. Favipiravir and ribavirin also inhibited ZIKV NS5 in both Asian and African strains [110]. Finally, sofosbuvir protected ZIKV-infected mice from mortality by decreasing viral RNA levels in different tissues, preventing acute neuromotor and long-term memory sequelae [131]. Flavivirus NS5 methyltransferases are also central players in viral replication and have also been used as targets for drug design. Methyltransferase inhibitors include S-adenosyl-L-methionine (SAM) and/or S-adenosyl-L-homocysteine (SAH) analogues. Other NS5 inhibitory compounds include F3043-0013, F0922-0796, F1609-0442, and F1750-0048 [132].

Antivirals targeting host proteins

Antivirals acting on host proteins interfere with different parts of the ZIKV life cycle, impairing viral replication. The first step in the ZIKV life cycle is binding of the virus to receptors (DC-SIGN, TYRO 3, AXL, TIM, and TAM) [35], followed by internalization by endocytosis to reach endosomes [37]. Different compounds have been developed using this target. Endocytosis and endosomal fusion (EEF) inhibitors include 25-hydroxycholesterol (25HC), a natural product of lipid metabolism shown to reduce viremia in mice and monkeys. Moreover, 25HC protected infected fetal mice from microcephaly [134]. Chloroquine, an antimalarial and anti-inflammatory drug, reduced ZIKV titers in ZIKV-infected cells and protected mouse neurospheres from morphological damage [108]. Arbidol, also known as umifenovir, an approved drug both in Russia and China, was shown to reduce viral titers in ZIKV with a micromolar effect [135]. Additionally, the drug also reduced viral multiplication in WNV and tick-borne encephalitis virus (TBEV) with a strong cell-dependent effect [135]. K22, a small compound inhibitor with potential activity against a broad range of coronaviruses, was also shown to inhibit ZIKV. K22 efficiently interfered with the replication of other

flaviviruses, including JEV, WNV and, to a certain extent, Usutu virus (USUV), Wesselsbron virus (WESSV), hepatitis C virus (HCV), and bovine viral diarrhoea virus (BVDV) [136]. Another EEF inhibitor is Tenovin-1, which inhibits ZIKV multiplication in primary fibroblasts [137]. A venom peptide, Ev37, from the scorpion *Euscorpions validus* inhibited ZIKV, DENV-2, hepatitis C virus (HCV) and herpes simplex virus type 1 (HSV-1) infections in a dose-dependent manner. The drug showed low cytotoxic effects *in vitro*; however, it had no effect on Sendai virus (SeV) or adenovirus (AdV) [138]. Finally, Amodiaquine, an antimalarial drug, was shown to exhibit antiviral activity in both ZIKV and DENV at a micromolar concentrations *in vitro* [139].

Different drugs have been developed by targeting lipid metabolism, the endoplasmic reticulum (ER), and nucleoside biosynthesis, impairing ZIKV replication. Pyrimidine synthesis inhibitors include cyclosporine A, which inhibits ZIKV in different cell lines [120]. Cyclosporin A was also shown to be effective against the WNV NS5 protein [140]. Purine synthesis inhibitors include 6MMP, which decreases ZIKV infectious titers by more than 99% in a dose and time-dependent manner [141]. Methotrexate (MTX) decreases ZIKV titers in a dose-dependent manner in Vero and hNSCs cells by antagonizing dihydrofolate reductase (DHFR) [142]. Cholesterol metabolism inhibitors include GW4869, a neutral sphingomyelinase-2 (nSMase2) inhibitor that effectively inhibits ZIKV propagation in human astrocytes and decreases extracellular vesicle (EV) levels [143]. 7-Ketocholesterol (7-KC) inhibited ZIKV replication specifically in viral budding, release from the host, and viral integrity [144]. Nordihydroguaiaretic acid (NDGA) and its methylated derivative tetra-O-methyl nordihydroguaiaretic acid (M4N) were shown to inhibit ZIKV and WNV infections by interfering with the sterol regulatory element-binding protein (SREBP) pathway [145]. In the same line, fatostatin was also shown to inhibit the SREBP pathway in ZIKV [145]. Caspase-3 activity inhibitors include PHA-690509, which reduces ZIKV multiplication *in vitro* [146]. Bithinol, a broad spectrum compound, also inhibited ZIKV caspase activity, hence inhibiting ZIKV pathogenicity [147]. Additionally, metformin was shown to inhibit ZIKV replication by inactivating adenosine monophosphate-activated protein kinase (AMPK), a regulator of lipid metabolism [148]. Other inhibitors include compounds that inhibit the NMDA receptor, such as memantine, a blocker of the N-methyl-D-aspartate receptor (NMDAR) that was shown to inhibit neuronal damage as a result of ZIKV infection [149]. Finally, the innate immune response has been used as drug target in the case of GSK-126, which is currently in clinical trials [150].

Currently, a few drugs, including Pinocembrin and BCX4430, also known as Galidesivir, have completed phase 1 clinical trials performed by Biocryst Pharmaceuticals. Galidesivir was administered intravenously in 24 healthy volunteers. In trials, this drug was

shown to be safe and tolerable. Intramuscular administration and animal models have also been used to show that this drug has survival benefits against several pathogens, including Ebola [129], Marburg, YFV, WNV [127] and ZIKV [129]. Additionally, Galidesivir has shown broad-spectrum activity *in vitro* against more than 20 RNA viruses in nine different families, including flaviviruses, togaviruses, bunyaviruses, arenaviruses, paramyxoviruses, coronaviruses, and flaviviruses [151]. Nevertheless, this drug has been shown to confer resistance as a result of an E460D substitution in the NS5 protein of TBEV [128], which represents a major challenge in the development of viral replication inhibitors since the viruses are rapidly evolving.

In summary, many ZIKV antiviral drugs have been developed despite the fact that most of them never reach clinical trials. As an RNA virus, ZIKV is prone to rapid evolution as a result of mutations in both structural and nonstructural proteins, possibly leading to drug resistance. Therefore, additional research is needed to understand this hypothesis. Drug combinations should be considered for effective treatment of ZIKV.

Conclusions and future perspectives

Since the reemergence of ZIKV, several studies have been performed to understand the pathogenesis of the virus, including the use of mice and other animal models, such as macaques. However, questions remain to be answered concerning the different mechanisms through which the virus escapes the immune system and causes disease in mammalian hosts. Having many routes of transmission and a wide range of tropism, ZIKV has become a crucial human pathogen in need of quick therapeutic measures and vaccines that convey long-term immunity. Understanding ZIKV interactions with the host will give more insights into the development of these therapeutic measures. Despite the rapid development of ZIKV vaccines, more research is needed to complete clinical trials for ZIKV therapeutics. Importantly, more studies should be performed on the development of vaccines that are safe, especially for pregnant women, unborn fetuses, infants, and elderly individuals. The role of sRNAs is still not well-defined in ZIKV pathogenesis and disease; therefore, future studies in this field will be of novel importance.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Ministry of Science and Technology of China [2018ZX10101004, 2013FY113500]; the Science and Technology Bureau of Wuhan, China [2018-201261638501]; the Chinese Academy of Sciences

[153211KYSB20160001, ZDRW ZS-2016-4]; the Wuhan Institute of Virology, China [WIV-135-PY2], and Sino-Africa Joint Research Center, Chinese Academy of Sciences [SAJC201605].

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