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Review

Zika Virus Infection and Development of Drug Therapeutics

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Abstract: Zika virus (ZIKV) is an emerging flavivirus that is associated with neurological complications, such as neuroinflammatory Guillain Barré Syndrome in adults and microcephaly in newborns, and remains a potentially significant and international public health concern. The World Health Organization is urging the development of novel antiviral therapeutic strategies against ZIKV, as there are no clinically approved vaccines or drugs against this virus. Given the public health crisis that is related to ZIKV cases in the last decade, efficient strategies should be identified rapidly to combat or treat ZIKV infection. Several promising strategies have been reported through drug repurposing studies, de novo design, and the high-throughput screening of compound libraries in only a few years. This review summarizes the genome and structure of ZIKV, viral life cycle, transmission cycle, clinical manifestations, cellular and animal models, and antiviral drug developments, with the goal of increasing our understanding of ZIKV and ultimately defeating it.

Keywords: ZIKV; flaviviruses; microcephaly; antiviral drugs development

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1. Introduction

Zika virus (ZIKV) is a vector-borne *flavivirus* belonging to the *Flaviviridae* family and is closely related to several other flaviviruses that cause global disease, including dengue virus (DENV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), West Nile virus (WNV), and tick-borne encephalitis virus (TBEV) [1,2]. In 1947, ZIKV was first isolated from a febrile sentinel rhesus monkey in the Zika forest in Uganda and was detected in mosquitoes in the following year [3,4]. It was not until 1952 that the first human cases of ZIKV infection were reported in Uganda and the United Republic of Tanzania [5]. There was not much scientific awareness of ZIKV early after it was identified, and accordingly, it was understudied until the first reemergence of ZIKV occurred on the Yap Islands in Micronesia in 2007 [6]. Subsequently, an outbreak of ZIKV was reported in French Polynesia in 2013–2014 [7] and an epidemic in Brazil with ~130,000 suspected cases in late 2015 (Figure 1) [8,9]. In 2016, the World Health Organization declared the ZIKV epidemic as an international health emergency [10,11].

ZIKV infection is mostly asymptomatic and often causes a self-limiting febrile illness in 20% of adults [12]; however, it has been causally associated with congenital malformations and neurological disorders and has rapidly spread to more than 70 countries and territories, which has focused public attention on this emerging pathogen [13]. Of significant concern, no specific and effective vaccine or drug is currently available for ZIKV infection, which has motivated global and scientific efforts to develop countermeasures [14].

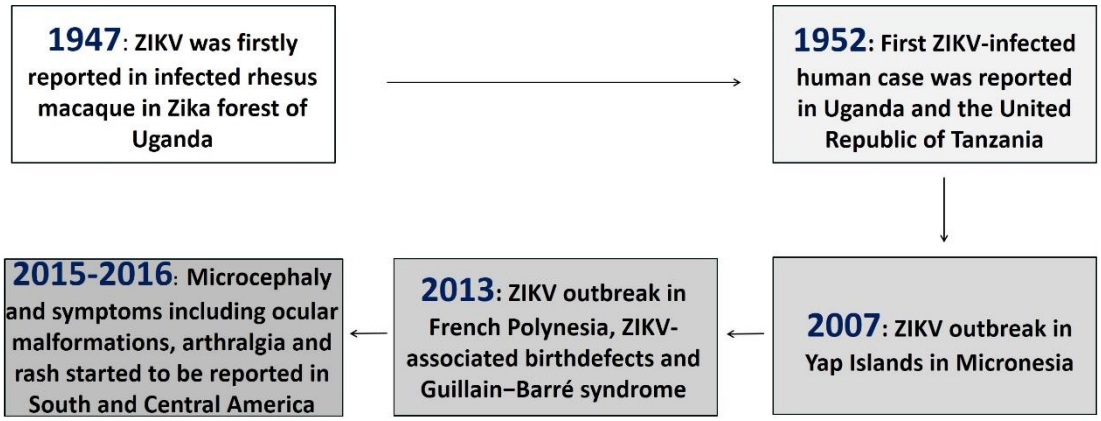


Figure 1. Discovery of ZIKV and its emergence as a global health threat.

2. Genome and Structure of ZIKV

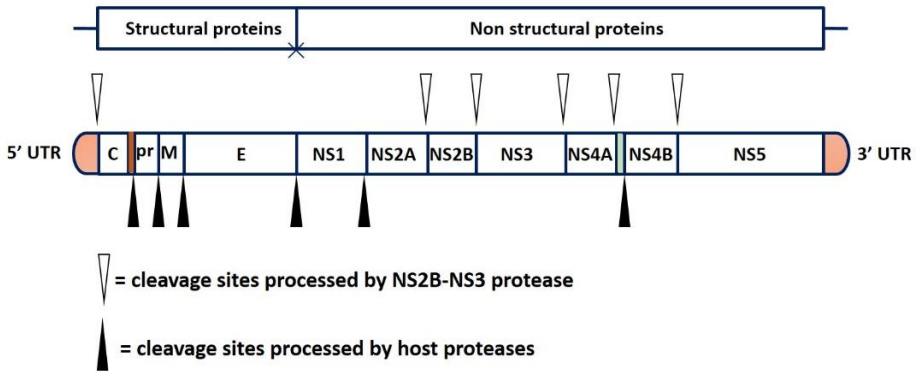
ZIKV is a single-stranded positive-sense RNA virus comprising of enveloped virions with a diameter of approximately 40–60 nm. The genome is approximately 10.7 kb in length and consists of open reading frames (ORFs) with flanking untranslated regions (UTRs) at both ends [15]. The ORF codes for a large polypeptide precursor, which is cleaved by host and virus proteases to generate structural and non-structural proteins from the N-terminus to the C-terminus (Figure 2A) [11].

The three N-terminal structural proteins, capsid protein (C), precursor of membrane (prM), and envelope protein (E) are the skeletal elements for the formation of virus particles. Among them, C combines with viral genomic RNA to form a nucleocapsid core, whereas prM and E are viral surface glycoproteins that are adsorbed to the host cytoplasmic membrane [16]. The M protein is expressed as a glycosylated prM and attached to the host-derived lipid envelope, whereas the E protein may or may not be glycosylated and this is a determinant of neuroinvasion, acting to increase both transepithelial and axonal transportation [17].

There are seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) at the C-terminus of the genome that participate in multiple stages and functions of the viral life cycle, such as RNA replication, virus particle assembly, and immune escape. Among the nonstructural proteins, NS1 has been identified to possess multiple functions in the viral life cycle including the viral particle complex formation, immune evasion, and pathogenesis, it is the most enigmatic protein of the flaviviruses [18,19]. Additionally, NS1 is a glycoprotein and its glycosylation is crucial for effective secretion, virulence, and viral replication [20,21]. Recent publications reported that the overall structure of the ZIKV NS1 proteins is highly similar to the WNV and DENV2-NS1 structures, with the same protein fold and domain arrangement [22–24]. Different flaviviruses may exert their NS2A functions in a virus-specific manner [25–27]; ZIKV NS2A has a single segment that inserts the ER membrane and six segments that are peripherally connected with the ER membrane that is essential for viral RNA synthesis and virion assembly [28]. NS2A also regulates the evasion of innate immunity, exhibiting an inhibitory effect on Type-I IFN induction at the step of TANK binding kinase 1 (TBK1) complex formation [29]. The flaviviral NS3 protein is divided into two functional domains: a C terminal domain, which contains RNA triphosphatase and helicase activities, and an N terminal protease domain, requiring a cofactor for appropriate function [30]. The protease cofactor, NS2B, is a small crucial transmembrane protein and is integral for proper folding and functions of the NS3 protease [31]. The viral protease active holoenzyme is the trypsin-like NS3 protease in complex with NS2B [32]. The two cleavage sites, NS2A/NS2B and NS2B/NS3, are mediated via NS3 in cis [33], while processing both NS3/NS4A and NS4A/NS5 are mediated by NS3 and NS3 in trans [34–36], as well as at internal sites with C, NS2A, NS3,

and NS4A [37,38]. Additionally, the mature NS4A and NS4B proteins are produced by cleavage in two sites by NS2B/NS3 protease [34].

A



B

ZIKV Protein Name	ZIKV Protein Function
C	Production of nucleocapsid
prM	Protects E during assembly
E	Membrane binding and fusion
NS1	Viral particle complex formation and immune response regulation
NS2A	Viral replication and capsid assembly
NS2B	NS3 cofactor
NS3	RNA helicase and serine protease NTPase
NS4A	Viral membrane formation
NS4B	Inhibits antiviral state
NS5	RNA dependent RNA polymerase

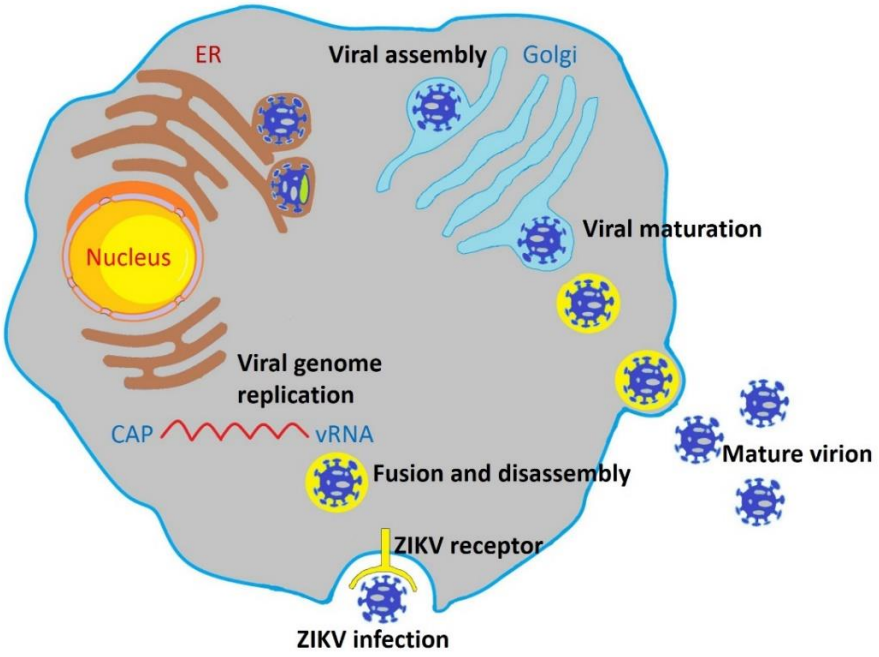
Figure 2. Genome structure and proteins of ZIKV. (**A**). ZIKV RNA codes for a polyprotein that is co-translationally cleaved into yield 10 protein: three structural proteins (C, prM/M, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The white and black triangle represents cleavage sites that are processed by NS2B-NS3 protease and cleavage sites that are processed by host proteases, respectively. (**B**). The table summarizes the names and biological functions of structural and non-structural proteins from the 5′ to the 3′ untranslated regions. The three structural proteins are responsible for the virus particle formation and are involved in virus entry, assembly, and the release of virions into host cells. The other seven are nonstructural proteins play roles in viral genome replication and host immunity evasion.

NS4A has a small molecular weight compared to other non-structural proteins and antagonizes interferons [39]. NS4B protein is a glycosylated membrane-associated viral protein that is critical for viral replication [40], it has been reported to interact with NS3, suggesting potential interactions with the replication complex [41]. In particular, NS5 is the most conserved and largest of the flavivirus proteins, it encodes an RNA-dependent RNA polymerase (RdRp) for genome replication, methyltransferase for 5′ RNA cap formation and methylation, and a Type I interferon antagonist (Figure 2B) [42].

3. Life Cycle of ZIKV

Similar to other known flaviviruses, the viral life cycle of ZIKV could be roughly divided into stages of binding, entry, translation, replication, assembly, and release (Figure 3) [43]. Briefly, the E protein is involved in the attachment of virus to receptors on the host cell membrane, and the virus is internalized through endocytosis that is mediated by clathrin proteins in the acid pH environment [20,44]. Several cell surface receptors facilitate ZIKV

viral entry, such as Tyro3, DC-SIGN, AXL, and TIM-1 [45]. Subsequently, fusion of viral membrane with endosome membrane occurs, the positive-strand viral genomic RNA releases from nucleocapsid into cytoplasm, and the synthesis of negative-strand genomic RNA using viral positive-strand genome as template by RdRp [9]. On surface of endoplasmic reticulum, the negative-strand RNA is used to synthesize new viral positive-strand genomes and viral mRNAs, which are further translated into viral polyproteins via host machinery. The resulting polyprotein is cleaved into the three nonstructural and seven structural proteins by the viral protease as mentioned above [42]. The newly-synthetized positive RNAs could be recruited for further activities of translation/replication or incorporated into the virions, which initiates their assembly [46]. Immature virions are assembled in the endoplasmic reticulum and transfer to the Golgi apparatus through budding for maturation, until their final release into the extracellular space [15,46].



Antivirals	Drug targeting
Duramycin, Nanchangmycin	entry
Emetine, NITD008, BCX4430, 2′-C-methylated nucleosides, DMB213, Fidaxomicin, Lycorine, UA, and TPB	translation/transcription
Ribavirin, TH6744, TH5487, Manidipine, Cinidipine, Benidipine, and Sofosbuvir	replication
Cavinafungin	endoplasmic reticulum

Figure 3. Life cycle of ZIKV. Virions attach to the cell surface receptors and subsequently enter cells by receptor-mediated endocytosis and are internalized into clathrin-coated vesicles. Endosome acidification triggers conformational changes, fusion of viral membrane with endosome membrane, and particle disassembly and the viral genomic RNA is released from nucleocapsid into the cytoplasm. The ssRNA then is translated into a single polyprotein that is further processed co-translationally and post-translationally by viral and cellular proteases. This cleavage makes three structural proteins and seven nonstructural proteins. Viral genome replication initiates with negative-strand RNA synthesis, and further serves as a template for the synthesis of the positive-strand genomic RNA. Virus assembly take places in the endoplasmic reticulum (ER) surface by budding, and immature virus particles travel along the host secretory pathway through the trans-Golgi network, where virion maturation occurs followed by release from the cell via exocytosis. Additionally, the below table shows where the inhibitor/antivirals of ZIKV act during the ZIKV life cycle.

4. Transmission Cycle of ZIKV

Similar to DENV and YFV, ZIKV directly circulates between *Aedes* mosquitos and humans, and thus is capable of epidemic transmission. ZIKV circulation has been documented in two evolutionarily and ecologically distinct transmission cycles: an urban or human cycle, between peridomestic/domestic *Aedes* spp. mosquitoes and humans; and an enzootic, sylvatic cycle, where ZIKV circulates between non-human primates and *Aedes* spp. mosquitoes [1,47,48]. Epidemiological evidence suggests that *Aedes aegypti* is the main vector, and it spreads the virus after sucking the blood of ZIKV-infected individuals. It has high kinship behavior characteristics and is one of the most difficult vectors to control in the urban transmission cycle of ZIKV [49].

In addition to insect-borne transmission, ZIKV can also be transmitted by non-vector means, such as sexual transmission, mother–child transmission, and blood transfusion. ZIKV is generally transmitted between people through sexual contact, mostly from men to women, and viral RNA can be detected in the semen of men [50]. When ZIKV infects pregnant women, it infects the fetus through vertical transmission, and the virus is distributed in the placenta, amniotic fluid, and fetal brain, resulting in fetal intrauterine developmental restriction, congenital malformation, microcephaly, and miscarriage [51,52]. Further, Brazil published a case report of ZIKV transmission through blood transfusion [53]; most patients infected with ZIKV are asymptomatic, whereas approximately 3% of blood donors have positive ZIKV test results, and the virus can survive in whole blood for 2 months, making it easy to spread to blood donors. Because of the diversity and complexity of ZIKV transmission patterns, it is difficult to formulate strategies against ZIKV infection [54]. In addition to human-to-human transmission, it is unclear whether domestic animals could transmit ZIKV via sexual behavior among non-human primates or through mosquitoes, and whether it can be characterized by human sexual transmission needs to be further explored [55,56].

5. Clinical Manifestations of ZIKV Disease

ZIKV is related to other human flaviviruses that cause significant pathology including DENV, YFV, JEV, WNV, TBEV, and Saint Louis encephalitis virus (SLEV) and is most closely related to Spondweni virus (SPONV) [57]. In comparison to the encephalitic flaviviruses such as WNV and TBEV, ZIKV generally is less neuroinvasive in adult patients, and rarely leads encephalitis and meningitis [58]. Regarding the early prevalence of ZIKV infection in humans, approximately 20% of patients show influenza-like symptoms that are similar to self-limited fever, which can subside within a few days. The ZIKV pandemic in 2016 was associated with a variety of serious diseases including thrombocytopenic purpura and dyspnea, which might be related to pathogenic changes in the evolution of ZIKV [59].

In May 2015, the largest ZIKV outbreak in history occurred in Northeastern Brazil, and caused great public panic [60,61]. ZIKV can be transmitted from mother to child; the clinical phenotypes of the latter include cerebral calcification, cortical malformation, ventricular enlargement, delayed myelination [62], and cerebellar brainstem dysplasia [63]. A series of studies has shown that ZIKV has a tendency to infect neural progenitor cells, which might affect the development of the fetal brain, and obstruction is the main mechanism of microcephaly and fetal death [64,65]. Apart from its neurophilic features, ZIKV also results in visceral infection that is similar to DENV and YFV, which attacks the immune system in the body. During the ZIKV epidemic in French Polynesia, Guillain Barré Syndrome was reported many times. This suggests that ZIKV infection targets neurons and glial cells to mediate demyelination of the peripheral nervous system and the pathogenesis of axon malformation; thus, Guillain Barré Syndrome can occur simultaneously with or after ZIKV infection [62].

6. Cellular Models of ZIKV

A great deal of human primary cells and cell lines have been used to deeply explore the tropism and pathogenic mechanisms of ZIKV [4]. Primary cell infection has been

demonstrated for neonatal keratinocytes, adult dendritic cells, amniotic epithelial cells, sertoli cells, trophoblast progenitor cells, astrocytes and microglial cells, cytotrophoblasts, and human endothelial stromal cells [66]. Recently, studies demonstrated that ZIKV directly infects human cortical neural progenitor cells with high-level efficiency, causing stunted cell growth and transcriptional dysregulation [67]. In term of cell lines, nonhuman primate cell lines, as well as various human cell lines and small animals cell lines have been used to study ZIKV infection and potential drugs. With regard to human cell lines, such as SF268, Caco-2, and JEG-3 cells, allowed ZIKV replication and formed cytopathic effects (CPE). Moreover, some cell lines, including Hela, Hek, and LNCaP cells, effectively infected ZIKV but did not show CPE [68,69]. *Aedes aegypti* and albopictus-derived cell lines have also been tested to date with scientific topics on the life cycle of ZIKV [70]. Another recently developed alternative is to culture human neurospheres and brain organoids, which permit ZIKV infection and exhibit reduced viability and growth resembling microcephaly [71,72].

7. Animal Models of ZIKV

An intensive effort by the global scientific community has resulted in the deployment of animal models for the study of multiple aspects of ZIKV biology. Similar to animal models of JEV infection, rodents and non-human primates are common animal models to study ZIKV. After ZIKV infects the human body, it partially acts on the STAT2 molecule of the downstream signaling pathway of the Type I interferon receptor through the NS5 protein, resulting in its degradation, which antagonizes the human immune response. However, ZIKV infection has no such effect on mice, which could explain why immunized mice cannot serve as the host of ZIKV [73,74]. Therefore, immunocompetent adult mice have a certain tolerance to lethal ZIKV strains. Newborn mice can be infected by Senegal and Puerto Rico ZIKV strains, which cause 30% mortality, whereas the latter causes a sharp decline in body weight, serious damage to the nervous system, and the death of newborn mice. In addition, T-cells of ZIKV-infected mice infiltrate the central nervous system, which is consistent with other neuroinvasive flavivirus infection phenotypes [73,75].

In adult mice with Type I interferon receptor deficiency (*Ifnar1*$^{-/-}$ mice), after infection with ZIKV, viruses are enriched in the brain, blood, spleen, kidney, liver, and eyes, which can recapitulate the characteristics of the vertical transmission of ZIKV through the placenta, limb paralysis, and invasive diseases of the nervous system. After ZIKV infection in male *Ifnar1*$^{-/-}$ mice, the structures of the testicular stroma and vas deferens are seriously damaged [73]. This mouse model is also applicable to the study of the WNV infection mechanism; specifically, *Ifnar1*$^{-/-}$ mice show severe pathological signs after infection with WNV and generally die within 3–5 days [76]. Adult mice with Type I and Type II interferon receptor knockout (AG129 mice) were infected with French Polynesia, Cambodia, or Porto strains via subcutaneous, intradermal, or peritoneal routes and showed signs of ataxia, tremor, and paralysis, and all three strains had lethal effects. Among them, the French Polynesia strain at a dose of only 1 PFU could cause the death of AG129 mice [77–79].

Immunodeficient mice with IRF3/IRF5/IRF7 interferon genes are more susceptible to the ZIKV strain Cambodia, which causes neurological damage, the apoptosis of neural progenitor cells, hind limb weakness, paralysis, and other signs, until the death of ZIKV-infected mice [80]. In addition, after injecting the interferon antibody MAR1-5A3 into wild-type pregnant mice, pregnant mice in this state could be infected with ZIKV. Viral load can be detected in both maternal and fetal bodies, and this animal model is suitable for exploring the mechanism of ZIKV infection [73]. It is worth noting that although this kind of immunodeficient mouse model is helpful for understanding the disease characteristics that are caused by ZIKV infection, it still has some limitations for comprehensively exploring the pathogenic mechanism and immune response to flaviviruses. For example, IFNα/β-dependent viral tropism and the initiation of lymphoid T- and B-cell pathways are not obvious in such animal models. To further explore the immune mechanism that is associated with ZIKV, more animal models need to be studied.

One day after rhesus monkeys are infected with the Polynesia strain of ZIKV, viral RNA could be isolated from their plasma, cerebrospinal fluid, saliva, and urine [81]. In the cynomolgus monkey model, viral RNA is present in the semen and saliva for more than 3 weeks [82]. In addition, ZIKV replication is active, but it does not cause embryo death when inoculating chicken embryos at embryonic age E13 with ZIKV. When the embryonic age was increased to E15 and E20, pathological characteristics such as brain malformation, a decline in brain growth and development, and increased ventricular volume were observed. The chicken embryo model might provide an important basis to study the pathogenic mechanism of ZIKV in developing embryos [83].

8. Antiviral Drug Advancement

8.1. Host-Targeting Antivirals

Specific and well-studied viral enzymes are the target of the majority of successful antiviral drugs; however, this target-based research strategy often takes many years, is costly to develop, and easily misses other possible and key targets, such as cellular factors that are essential for virus infection. There are many types and quantities of drugs that target diverse cellular proteins to treat human diseases, resulting in opportunities for them to be rapidly repurposed, and bypassing the initial stages of the safety assessment [84]. Host-acting inhibitors can be directed to the viral life cycle, including binding, entry, fusion, replication complex formation, viral maturation, and release, and they often exhibit broad-spectrum antiviral effects on multiple viruses with the benefit of a higher barrier to drug resistance [85]. Thus, targeting the host is an attractive broad-spectrum antiviral strategy.

To maintain persistent and appropriate viral replication, viruses depend on the provision of nucleosides from the host cells. The guanosine analog, ribavirin exerts broad-spectrum antiviral activity against several DNA and RNA viruses both in vitro and in vivo. Direct and indirect antiviral mechanisms can explain the antiviral effect of ribavirin; the former includes lethal mutagenesis, polymerase suppression, and interference with RNA capping, whereas the latter includes immunomodulatory effects and inosine monophosphate dehydrogenase inhibition [86]. Ribavirin shows inhibitory effects on ZIKV replication without cytotoxic effects in mammalian cells and blocks viremia in ZIKV-infected STAT-1-deficient mice, which were highly sensitive to ZIKV infection [87]. A dose-dependent decrease in gene expression at non-toxic concentrations was observed in ZIKV-infected cell lines, including human neural progenitor cells (hNPCs), A549 cells, and Vero cells [88]. A recent study established a cerebral organoid model with ZIKV infection and demonstrated that TH6744 and TH5487 could limit ZIKV propagation and ZIKV-induced neurotoxicity. TH6744 treatment inhibited the late steps of the ZIKV life cycle, reducing the number of ZIKV-infected cells during secondary infections without affecting ZIKV genome replication [89].

The skin epidermis is the initial barrier for ZIKV to enter the host cell, and three cell lines, epidermal keratinocytes, dermal fibroblasts, and skin dendritic cells, have been shown to be permissive to ZIKV infection [45]. TIM-1 was found at the uterine–placental interface, is present in the primary placental cells, and was found to play a key role in ZIKV entry in the placenta. Duramycin, a specific pharmacological inhibitor of TIM, blocks ZIKV infection at the uterine–placental interface [90]. AXL is highly expressed in the neural and radial cells of the developing brain throughout neurogenesis and is considered a strong candidate receptor for ZIKV entry into the developing brain [45,91]. The natural product nanchangmycin, an inhibitor of AXL, was generated in *Streptomyces nanchangensis*. Rausch et al. screened a library of bioactive compounds with antiviral activity against ZIKV infection in three distinct cell lines and revealed that nanchangmycin abrogates viral internalization through clatherin-coated vesicles. Moreover, the authors showed that nanchangmycin also suppresses WNV, DENV, and Chikungunya virus, which employ similar host pathways for entry. These studies will ultimately inform therapeutic strategies for antiviral interventions against important flaviviruses and alphaviruses [92].

The host endoplasmic reticulum-localized signal peptidase (ER-SPase) plays a key role in processing flavivirus prME, which can be efficiently inhibited by cavinafungin [93]. This compound is an alaninal-containing lipopeptide that is isolated from *Colispora cavincola* that shows potent inhibition of the growth of ZIKV-infected cells (CC_{50} = 1.65 μM and IC_{50} = 0.15 μM) [94]. Targeting ER-Spase could be a potential method for the development of novel ZIKV inhibitors.

Voltage-gated calcium channel (VGCC) couples depolarization of the cytomembrane with the entry of calcium, which triggers gene expression, neurotransmission, secretion, contraction, and other physiological processes. An increasing number of selective calcium channel blockers has been used as novel therapeutic interventions [95]. A high-throughput screen of an FDA-approved library of inhibitors of JEV was performed by Wang and co-workers, and five hit drugs were identified against JEV, WNV, and ZIKV infection; three of the hits (manidipine, cinidipine, and benidipine) were determined to be DHP VGCC inhibitors. The authors confirmed that intracellular Ca^{2+} is crucial for flavivirus infection, and cytoplasmic calcium is an attractive target for the treatment of flavivirus infection [96].

Sofosbuvir is an FDA-approved nucleotide polymerase inhibitor for the treatment of hepatitis C virus infection and has anti-ZIKV activity in multiple human tumor cell lines and isolated human fetal-derived neuronal stem cells [14]. Furthermore, Gardinali et al. used a rhesus monkey model to test the efficacy of sofosbuvir against ZIKV infection, which is relevant to congenital syndrome and vertical transmission. Compared to those in ZIKV-infected animals, all sofosbuvir-treated dams had a shorter period of viremia and undetectable viral RNA loads in tissue and blood samples from four of the five neonates [97]. These results confirmed that sofosbuvir has the potential to be a therapeutic agent against ZIKV infection in humans.

8.2. RdRp Inhibitor

RdRp enzymes of flaviviruses are crucial for viral replication; thus, they are attractive targets for the development of antiviral therapeutic strategies. The FDA-approved antiprotozoal drug emetine exhibits antiviral effects against ZIKV infection by binding to the NS5 protein, with IC_{50} values between 9 and 53 nM. Further animal experiments showed that emetine significantly reduces the viral RNA load in the liver and serum of ZIKV-infected *Ifnar1*$^{-/-}$ immunocompromised mice [98].

Yin et al. reported that the adenosine analog NITD008 has antiviral activity against DENV infection in vitro and in vivo [99]. NITD008 has also been shown to inhibit ZIKV in cell culture, and the treatment of ZIKV-infected A129 mice with the compound suppresses peak viremia and provides protection against ZIKV-induced death [100]. In addition to DENV and ZIKV, NITD008 blocks other flavivirus infections, including YFV, WNV, and Powassan virus [99]. Similarly, another adenosine analog, BCX4430, was reported to be a selective inhibitor of RdRp, and it exhibits broad-spectrum activity against a wide range of RNA viruses, including YFV, Ebola virus, and Marburg virus in animal models [101,102]. Julander et al. confirmed that BCX4430 consistently reduces the viral cytopathic effect that is induced by Puerto Rican, Malaysian, and Ugandan isolates of ZIKV in various cell lines with a low μg/ml range. Furthermore, the authors established an AG129 mouse model of severe ZIKV infection, and the therapeutic treatment of ZIKV-infected mice with BCX4430 improved the survival rate and protected against weight loss [103]. Moreover, active recombinant ZIKV RdRp was expressed in *Escherichia coli*, and the authors demonstrated that another adenosine triphosphate analog, 2′-C-methylated nucleosides, has effective inhibitory activity against ZIKV infection by suppressing RdRp. Novel nucleoside triphosphates could thus be new and promising therapeutics for ZIKV based on rational design [104].

Xu et al. purified recombinant ZIKV NS5 polymerase and performed an RdRp assay to facilitate the development of ZIKV RdRp inhibitors. Enzyme assays showed that a pyridoxine-derived non-nucleoside analog compound, DMB213, could inhibit RNA synthesis by competing with natural nucleoside triphosphate substrates and suppressing ZIKV

RdRp activity with an IC_{50} of 5.2 μM. Furthermore, the authors used cell-based assays confirming the anti-ZIKV activity of DMB213 with an IC_{50} of 4.6 μM [105].

A first-in-class macrocyclic antibiotic, fidaxomicin, has been used to treat *Clostridium difficile* infection clinically. Yuan et al. reported that fidaxomicin prominently inhibits Asian and African lineage ZIKV in multiple cell lines and efficiently reduces the viral load in the brain and testes of ZIKV-infected *Ifnar*$^{-/-}$ mice, ultimately alleviating ZIKV infection-associated pathological damage, including neuronal necrosis, paralysis, and hunching. Mechanistic studies confirmed that fidaxomicin could suppress the RNA synthesis-catalyzing activity of ZIKV RdRp and directly bind the ZIKV NS5 protein [106].

A benzyl phenethylamine alkaloid, lycorine, was initially isolated from *Narcissus pseudonarcissus* in 1877, and its chemical structure was elucidated in 1956 [107]. Chen et al. revealed that lycorine decreases ZIKV viral RNA, viral protein expression, and progeny virus counts. To explore the antiviral mechanism of lycorine, the authors performed a time-of-addition assay and confirmed that lycorine acts against ZIKV post-infection and suppresses RdRp activity. In addition, lycorine was found to protect ZIKV-infected AG6 mice from death by reducing the viral load in the blood. As lycorine exhibits effective and potent antiviral activity against ZIKV infection in vitro and in vivo, it might be a good candidate inhibitor to effectively combat ZIKV infection in the future [108].

Lin et al. performed a fluorescence-based polymerase assay to assess the antiviral effect of different drugs on ZIKV; they showed that 10-undecenoic acid zinc salt (UA) could inhibit ZIKV RdRp activity with an IC_{50} ranging from 1.13 to 1.25 μM, and this had an antiviral effect that was comparable to that of sofobuvir. Additionally, molecular docking analyses and site-directed mutagenesis confirmed that D535 is the crucial amino acid in the interaction between UA and RdRp. Owing to the attractive antiviral mechanism of UA, it might be a promising candidate to treat ZIKV infections [109]. A structure-based approach targeting the ZIKV RdRp was employed by Pattnaik et al., who demonstrated that 3-chloro-N-[({4-[4-(2-thienylcarbonyl)-1-piperazinyl]phenyl}amino)carbonothioyl]-1-benzothiophene-2-carboxamide (TPB) strongly inhibits ZIKV replication at sub-micromolar concentrations by binding to the RdRp catalytic active site. Moreover, a 25 mg/kg body weight dose of TPB treatment significantly reduces viremia in the ZIKV-infected BALB/c mouse model, suggesting that TPB is a promising candidate inhibitor of ZIKV infections [110].

The molecular docking approach is a time-saving, direct, and clear analysis, which was applied to rationally screen a library of 5000 phytochemicals to assess inhibitors against ZIKV RdRp by Rehman and collaborators. Polydatin was identified as the leading phytochemical compound, with a high docking score of −18.71 (kcal/mol), and this successfully binds inside the ZIKV RdRp pocket. Further studies showed that polydatin exhibits better attachment to the receptor than the control drug, sofosbuvir. A deep exploration of polydatin biocompatibility and effectiveness will contribute to the development of cost-effective ZIKV inhibitors [111].

8.3. Protease Inhibitors

The NS2B-NS3 protease of flaviviruses plays a key role in the maturation and replication of the viral cycle; therefore, it could be used for drug development and design. Lim et al. expressed the NS2B-NS3 protease (NS2B-NS3^{pro}) of ZIKV in *E. coli* BL21 (DE), and the purified NS2B-NS3^{pro} was used to determine the antiviral activities of 22 polyphenol compounds by performing inhibition and kinetic assays, which revealed that myricetin exhibits mixed-type inhibitory pattern against ZIKV NS2B-NS3^{pro}, with IC_{50} and Ki values in the micromolar range [112].

An agonist of dopamine receptors 2 and 3, bromocriptine, which is used to treat Parkinson's disease and is considered safe during pregnancy (FDA-approved pregnancy category B), has been reported to have antiviral activity against DENV1-4 and TBEV [113]. Recently, Chan and co-workers performed a fluorescence-based enzymatic assay and docking model analysis, and revealed that bromocriptine inhibits ZIKV NS2B-NS3 protease by interact-

ing with several active residues in the proteolytic cavity [114]. Moreover, bromocriptine showed a synergistic effect with interferon-α2b against ZIKV infection in vitro; thus, it might be a promising and available therapy for ZIKV infection in pregnant patients [114]. Similarly, Cui et al. also conducted a fluorescence-based screening assay to identify inhibitors targeting ZIKV NS2B-NS3 protease. A natural active compound derived from black tea, theaflavin-3,3′-digallate (ZP10), interacts with several key residues in the proteolytic cavity of ZIKV NS2B-NS3 and shows a dose-dependent inhibitory effect on ZIKV infection, indicating its promising application for ZIKV therapy [115].

NSC135618 is an allosteric inhibitor of DENV2 protease in vitro with an IC_{50} value of 1.8 μM, which was also identified as a broad-spectrum antiviral compound against flaviviruses through virus titer reduction assays, and this could significantly suppress viral titers of ZIKV (IC_{50} = 1.0 μM), WNV (IC_{50} = 1.27 μM), and YFV (IC_{50} = 0.28 μM) in A549 cells in the sub-micromolar concentration range [116]. ZIKV targets hNPCs and neurons [67,117] and also infects placental epithelial cells (hPECs) and fetuses during pregnancy resulting in microcephaly in newborns [118]. Further testing revealed that NSC135618 effectively inhibits ZIKV replication in hNPCs and hPECs in a dose-dependent manner [118], which could provide a valuable therapeutic strategy for fetal microcephaly that is relevant to ZIKV infection.

ZIKV NS2B-NS3 protease was used as a biochemical screen for small-molecule inhibitors by Abrams and co-workers. They found that the five-lipoxygenase-activating protein blocker MK-591 inhibits ZIKV protease and infection in neural stem cells, and the most potent inhibitors of ZIKV infection were determined to be tetracycline and methacycline, which are members of the tetracycline family of antibiotics. Moreover, an immunocompetent mouse model was used to further assess the antiviral activities of the two compounds; they were confirmed to reduce the viral load in the brain and relieve ZIKV-induced motor deficits, providing a potential treatment option for the neurological complications of ZIKV infection [119].

Yuan et al. conducted an in silico structure-based screen of a chemical compound library to identify promising ZIKV NS2B-NS3 protease inhibitors. Novobiocin was found to inhibit ZIKV activity by binding the NS2B-NS3 protease with high stability, and novobiocin treatment significantly increased the survival rate of ZIKV-infected dexamethasone-immunosuppressed mice. Moreover, there were fewer severe histopathological changes and lower viral loads in the major tissues and blood in novobiocin-administered mice compared to those in the untreated controls [120].

Li et al. developed a split luciferase screening assay to identify flavivirus NS2B-NS3 orthosteric inhibitors and reported that temoporfin blocks ZIKV infection in human neural progenitor and placental cells and exerts inhibitory effects on viremia and mortality in ZIKV-infected mice. Moreover, molecular docking analysis showed that temoporfin interacts with NS3 pockets that harbor key NS2B residues, thus non-competitively inhibiting ZIKV polyprotein processing activity. Owing to its potential antiviral activity against ZIKV in placental cells, temoporfin could be an easily developed and promising therapy to combat ZIKV and other flaviviruses that are relevant to infections [121]. Suramin, an anti-parasitic drug with a long half-life was approved by the FDA, and it exerts a potent inhibitory effect on ZIKV infection with IC_{50} values of ∼1.93–3.85 μM and an SI index of 500–1000. Further mechanistic studies found that suramin blocks the stage of viral adsorption, cellular entry, and replication and exhibits stronger interactions with ZIKV NS3 helicase than the envelope protein. However, the mechanisms that are related to suramin resistance, found with ZIKV mutants, have not been clarified for unknown reasons, and the precise antiviral mechanism of suramin remains to be explored [122].

9. Conclusions and Future Perspective

Viral pandemics have seriously affected human production and life. Over the past 50 years, more than 300 new or recurrent infectious diseases have been reported as epidemics [123]. In recent years, outbreaks that are caused by ZIKV, DENV, severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2), severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) have caused concern; however, there are no approved specific therapeutic drugs for these viruses.

YFV, DENV, WNV, and JEV cause an arthropod-borne disease in humans, known as Zika fever. Since the ZIKV outbreak in Brazil in 2015, several scientific communities have engaged in efforts to understand and control ZIKV infection. ZIKV can be transmitted through infected mosquitos, blood transfusion, urine, sexual behavior, and from mother to fetus. ZIKV causes congenital neurological disorders and replicates effectively in reproductive tissues, with the following symptoms: conjunctivitis, skin rash, joint pain, and fever, with an incubation period of approximately 7 days [124]. Some patients also have edema, diarrhea, and body weakness. Moreover, severe phenotypes, including congenital abnormalities in fetuses, microcephaly, and Guillain Barré syndrome are relevant to ZIKV infection, which greatly affect human health [125,126].

While ZIKV cases have dropped from their peak during the 2015–2017 outbreak, the threat of a resurgence of ZIKV due to vector migration, virus mutation, and human travel remains. Given this situation, there is an urgent need to explore novel antiviral targets and develop effective compounds for ZIKV infection [11]. The life cycle of ZIKV depends on host cytokines, cellular signals, and metabolic pathways targeting virus or cell components that are necessary for virus replication; thus, the design of specific ZIKV inhibitors is the primary core goal of modern drug research and development.

As discussed in this perspective, in recent years, extensive novel chemical entities and preclinical investigations of existing compounds have been performed to investigate their antiviral effect on ZIKV in vitro and in vivo system [127], for instance, anti-inflammatory and anticancer molecules, anti-parasitics, and antibiotics. The Table 1 is the summary of the antiviral strategies develop for ZIKV. However, few candidates with inhibitory activity of ZIKV in animal models have advanced into clinical trials, the result would be difficult to extrapolate to humans, it is hard for mostly the test antivirals to accomplish the entire drug development pipeline [128]. Further development of novel compounds as well as effective combination therapies may provide innovative avenues for the treatment of ZIKV infection. Additionally, as the emphatic target population for anti-ZIKV therapeutic strategies would be pregnant women and patients with other medical complications [129]. Therefore, there remains an urgent need for the development of additional effective candidates.

Furthermore, the DENV and YFV outbreaks also affect broad swaths of several endemic areas for ZIKV. Based on this situation, the development of antivirals that could broadly inhibit multiple flaviviruses infections, may represent an efficient and cost-effective strategy for the treatment of co-infections in endemic regions. Since flaviviruses entry and efficient propagation requires require a series of host factors and cellular metabolic pathways, this offers a valuable way to explore host targets as therapeutic tools that, in some instances, could be broad spectrum inhibitors, whose effect would be less prone to emergence of viral mutants [12,130]. Additionally, for prospective antiviral drug researchers, it is essential to contrast ZIKV with other flaviviruses relatives, with respect to viral pathogenesis and replication, which could lead to the development of antiviral drugs and vaccines for the treatment of ZIKV infection. In the increasingly competitive new drug market environment, it is particularly necessary to use short-term, efficient, and low-cost technology to obtain ideal drugs.

Table 1. Summary of the antiviral strategies develop for ZIKV.

Antiviral Drug	No.	Drug Name	Comments
Host-targeting antivirals	1	Ribavirin	Show inhibitory effects on ZIKV replication
	2	TH6744	Inhibit the last steps of the ZIKV life cycle
	3	TH5487	Limit ZIKV propagation and ZIKV-induced neurotoxicity
	4	Duramycin	Specific pharmacological inhibitor of TIM
	5	Nanchangmycin	AXL inhibitor
	6	Cavinafungin	Target the ER-Spase
	7	Manidipine	DHP VGCC inhibitor
	8	Cinidipine	DHP VGCC inhibitor
	9	Benidipine	DHP VGCC inhibitor
	10	Sofosbuvir	Nucleotide polymerase inhibitor
RdRp inhibitor	11	Emetine	By binding to the NS5 protein
	12	NITD008	Adenosine analog
	13	BCX4430	Selective inhibitor of RdRp
	14	2′-C-methylated nucleosides	Adenosine triphosphate analog
	15	DMB213	Inhibit RNA synthesis
	16	Fidaxomicin	Directly bind the ZIKV NS5 protein
	17	Lycorine	Inhibit ZIKV post-infection and RdRp
	18	UA	Inhibit ZIKV RdRp activity
	19	TPB	Inhibit ZIKV replication
Protease inhibitors	20	Myricetin	Exhibit mixed-type inhibitory pattern
	21	Bromocriptine	Inhibit ZIKV NS2B-NS3 protease
	22	ZP10	Interact with key residues in proteolyic cavity
	23	NSC135618	Allosteric inhibitor of DENV protease
	24	MK-591	Five-lipoxygenase-activating protein blocker
	25	Tetracycline	Reduce the viral load in the brain and motor deficits
	26	Methacycline	Reduce the viral load in the brain and motor deficits
	27	Novobiocin	Bind NS2B-NS3 protease
	28	Temoporfin	Interact with NS3 pockets that harbor key NS2B residues
	29	Suramin	Block viral adsorption, cellular entry, replication and exhibit stronger interactions with ZIKV NS3 helicase

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