REVIEW

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Zika virus outbreak: a review of neurological complications, diagnosis, and treatment options

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Abstract

Zika virus (ZIKV) is an arbovirus transmitted mainly by mosquitos of *Aedes* species. The virus has emerged in recent years and spread throughout North and South Americas. The recent outbreak of ZIKV started in Brazil (2015) has resulted in infections surpassing a million mark. Contrary to the previous beliefs that Zika causes mildly symptomatic infections fever, headache, rash, arthralgia, and conjunctivitis, the recent outbreak associated ZIKV to serious neurological complications such as microcephaly, Guillain-Barré syndrome, and eye infections. The recent outbreak has resulted in an astonishing number of microcephaly cases in fetus and infants. Consequently, numerous studies were conducted using in vitro cell and in vivo animal models. These studies showed clear links between ZIKV infections and neurological abnormalities. Diagnosis methods based on nucleic acid and serological detection facilitated rapid and accurate identification of ZIKV infections. New transmission modalities such as sexual and transplacental transmission were uncovered. Given the seriousness of ZIKV infections, WHO declared the development of safe and effective vaccines and new antiviral drugs as an urgent global health priority. Rapid work in this direction has led to the identification of several vaccine and antiviral drug candidates. Here, we review the remarkable progress made in understanding the molecular links between ZIKV infections and neurological irregularities, new diagnosis methods, potential targets for antiviral drugs, and the current state of vaccine development.

Keywords Zika virus · Microcephaly · Zika vaccine · Aedes mosquito · Antiviral chemotherapy · Neuropathogen · Flavivirus · Guillain-Barre syndrome

Introduction

Zika virus (ZIKV) is an arbovirus (arthropod-borne) and belongs to *Flavivirus* genus within *Flaviviridae* family. Although first isolated in 1947 (Dick et al. 1952), it was not well studied until the recent outbreak in 2015. The massive outbreak in 2015 in Brazil has linked ZIKV infections with severe neurological complications such as microcephaly in the fetus and Guillain-Barré syndrome (GBS) in adults. Subsequently, World Health Organization (WHO) issued a public health emergency of international concern in 2016 (Gulland 2016; Heymann et al. 2016; WHO 2016a) and called for accelerated development of treatment strategies. A great emphasis was given to understanding the molecular links

between ZIKV infections and neurological complications, mechanisms of transmission, rapid diagnostic methods, vaccine, and antiviral drug development. The current review article discusses the latest advancements in the ZIKV research (Fig. 1).

Overview of ZIKV pandemic in North and South America

For more than 60 years, Zika infections were considered as mild infections since most ZIKV infections were either asymptomatic ($\sim 80\%$) or characterized by mild symptoms that were short-lived (Zammarchi et al. 2016). The symptoms resembled dengue (flavivirus) and chikungunya viral (alphavirus) infections and include acute fever, maculopapular rash, muscle aches, and eye pain. Until 2007, sporadic cases of ZIKV infections occurred in parts of Africa and Asia (Hayes 2009). From 2007, few outbreaks have been reported in Yap State, Federated States of Micronesia, Western Pacific, and

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Fig. 1 Schematic representation of ZIKV genome and its encoded proteins. **a** The 10,617 nucleotide ZIKV RNA encodes a polyprotein that gives three structural proteins envelope, premembrane/membrane, capsid protein and seven non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. **b** Structural proteins such as membrane and envelope proteins are displayed on the outer surface of the viral membrane and allow the lipid membrane to wrap around the nucleocapsid
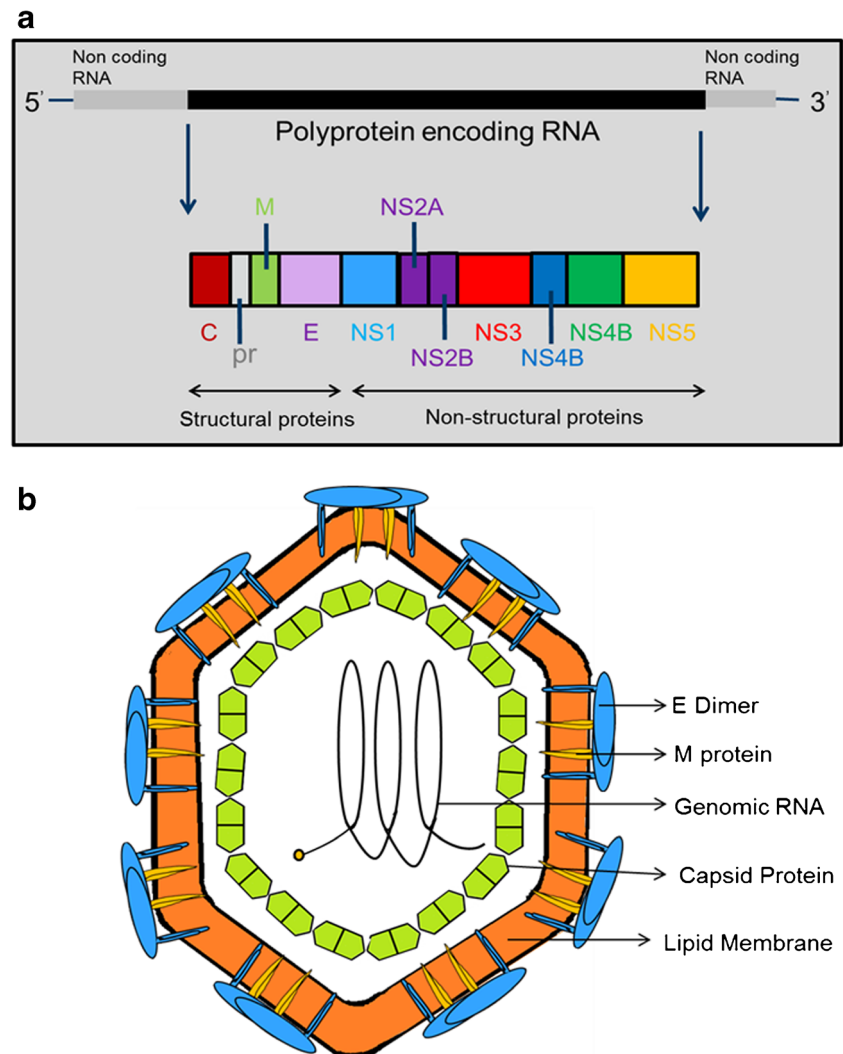
South East Asia (Duffy et al. 2009; Musso et al. 2014b). In 2014, a massive outbreak in French Polynesia with 32,000 cases of infection and a 40-fold rise in GBS was reported (Cao-Lormeau et al. 2016; Mallet et al. 2015).

The first ever evidence of Zika infections in Americas was documented in February 2015 in Brazil's northeastern state of Rio Grande do Norte (Zanluca et al. 2015). The patients reported maculopapular rash and intense pain much like chikungunya virus (CHIKV) infections. Although no deaths or severe complications were observed in these instances, Zika virus spread was predicted due to the naïve immunological status of the Brazilian population. By January 20, 2016, ZIKV infections spread to Puerto Rico (territory in the USA) and 19 other countries in South America (European Center for Disease Prevention and Control 2015; Pan American health Organization 2016). In Brazil alone, 500,000–1,500,000 suspected cases of Zika virus were confirmed by February 2016. In USA, four indigenous cases transmitted from local mosquitos were established in Florida in July 2016 (McCarthy 2016a). All the four cases in Florida were confined to a specific area in Miami because of an aggressive campaign conducted to prevent mosquito breeding and kill mosquito larvae. On the other hand, the situation in USA territory Puerto Rico reached to alarming levels with the total confirmed 5600 cases by July 2016, including 672 pregnant women (Adams et al. 2016). WHO reported a continuous vectorial transmission in 60 other countries including most of Central and South America, South East Asia, and Africa and hence declared ZIKV as a global public health emergency (European Center for Disease Prevention and Control 2016; WHO 2016e).

In addition to the rapid increase in Zika cases, the current outbreak was considered explosive due to its association with neurological irregularities. In the pregnant women, Zika infection has recently been associated with congenital birth defects. Brazilian Ministry of Health reported a 20-fold increase in patients with microcephaly in 2015 in comparison to historical statistics. The observation of high number of microcephaly cases in the 2015 outbreak may partly be attributed to the availability of more sensitive surveillance protocols and underdiagnosis bias in previous years (Schuler-Faccini et al. 2016;

Victora et al. 2016). A recent study suggested that nearly one third of ZIKV-infected pregnant females develop neurological complications such as microcephaly, central nervous system alterations, and fetal cerebral calcifications (Brasil et al. 2016b). Risk of microcephaly was reported to be 1–13% during the first trimester and negligible during second and third trimesters (McCarthy 2016b). Zika virus infection is a pandemic in progress, and many important questions about its teratogenicity, links to microcephaly, and treatment strategies need answers. Yet, it reinforces the possibility that mild infectious agents can emerge as severe infections for human beings.

Virology of ZIKV

ZIKV is an 11 kb positive single-stranded RNA virus that belongs to *Flavivirus* genus. ZIKV is related to other flaviviruses, more closely with dengue virus (DENV), Japanese encephalitis virus (JEV), yellow fever virus (YFV), and distantly to West Nile virus (WNV). Two important lineages, African and Asian, were identified based on the phylogenetic analyses (Faye et al. 2014).

ZIKV genome consists of two non-coding regions and one coding region. The coding region encodes a polyprotein that makes three structural proteins (capsid, envelope, membrane precursor) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Structure of ZIKV is similar to other flaviviruses, except at Asn180 glycosylation site in glycoproteins that make up the icosahedral shell. The glycosylated moiety at Asn180 was required for viral attachment of host cells and varies among ZIKV strains and flaviviruses (Sirohi et al. 2016). Like other flaviviruses, ZIKV may likely infect host cells by endocytosis after the interaction of virus particles with host cell surface receptors. Receptors such as C-type lectin, phosphatidylserine, T cell immunoglobin and mucin (TIM) and AXL, Tyro3, and Mertk (TAM) are identified as entry factors for various flaviviruses. In case of DENV, AXL receptor was considered as a major receptor for viral entry. AXL activates a cascade of events such as activation of AXL kinase, recruitment of interferon receptor (INFAR), inhibition of innate immune response, and replication of the virus inside the host cells. Role of AXL as a receptor required for the entry of ZIKV is contradictory since in vitro results support and most in vivo mice results question its role as a receptor mediating the viral entry (Hamel et al. 2015; Hastings et al. 2017; Nowakowski et al. 2016; Tabata et al. 2016; Wang et al. 2017; Wells et al. 2016).

ZIKV pathogenicity

ZIKV infections are associated with severe neurological manifestations such as microcephaly and Guillain-Barré syndrome. ZIKV infections are also being linked to other neurological disabilities such as meningoencephalitis, fetal cerebral calcification, central nervous system alterations, and myelitis. However, molecular evidences linking ZIKV infections to these pathological manifestations are largely unknown. Since the recent WHO declaration of ZIKV as a global health concern (Heymann et al. 2016), rapid improvements were made in understanding the molecular mechanisms of the ZIKV pathogenesis (Zhou et al. 2017).

ZIKV transmission

ZIKV transmission can be classified into vector-borne and non-vector borne. In vector-borne transmission, several species of *Aedes* mosquitos were reported. In non-vector borne human transmission, routes such as transplacental transmission, blood transfusion, and sexual transmission were reported (Besnard et al. 2014; Centers for Infectious Disease Research Policy 2016; Foy et al. 2011; Patino-Barbosa et al. 2015; Sharma and Lal 2017).

Mosquito-borne transmission

Transmission dynamics of ZIKV via mosquitoes is complex, mainly due to the involvement of several mosquito species in ZIKV maintenance. To date, ZIKV has been isolated from 17 *Aedes* mosquito species as well as *Anopheles gambiae*, *Anopheles coustani*, *Culex perfuscus*, and *Mansonia uniformis* mosquitoes (Ayres 2016; Saiz et al. 2016; Slavov et al. 2016). Among these several mosquito types, ZIKV transmission to humans has been reported only for female *Aedes aegypti*, *Aedes albopictus*, *A. hensilli* (responsible for Yap Island outbreak), and *A. polynesiensis* (responsible for French Polynesia outbreak) (Imperato 2016; Lazear and Diamond 2016). *A. albopictus* mosquitoes are suspected of broader transmission of virus due to their extensive geographic distribution in tropical, sub-tropical, and temperate regions (Thomas et al. 2012). In Americas, *A. aegypti* and *A. albopictus* are identified as the major vectors for human transmission (Petersen et al. 2016b). It is presumed that uninfected mosquitoes acquire ZIKV through infected humans, although further studies are needed to confirm whether the viral titer of infected humans are sufficient to infect mosquitoes (Grard et al. 2014). It was suggested that the incubation period for ZIKV in mosquitoes for viral transmission is ~10 days (Boorman and Porterfield 1956). An important strategy to prevent mosquito-borne transmission is avoidance of exposure to mosquitos. When possible, pregnant women should delay the travel to the areas of active ZIKV transmission. If travel is unavoidable, women are encouraged to use insect repellents such as DEET and permethrin.

Transplacental transmission

Accumulating evidences suggest that ZIKV is capable of crossing placenta to infect fetal nervous system. This transplacental transmission is supported by the detection of ZIKV RNA and antigens in amniotic fluid, placenta, and visualization of ZIKV in fetal brain tissues (Calvet et al. 2016b; Lazear and Diamond 2016; Petersen et al. 2016b). The mechanism used by ZIKV to cross placental barrier is yet to be known. However, many active studies are being carried to understand this mechanism. Pregnant mice when injected with ZIKV have shown poor intrauterine growth and poor fetal brain development (Microcephaly) suggesting that the virus can cross the placenta (Cugola et al. 2016; Li et al. 2016a, b; Miner et al. 2016a; Wu et al. 2016). In addition, immune-deficient mice injected with ZIKV showed viral particles and RNA in the fetal placenta (Miner et al. 2016a; Quicke et al. 2016). ZIKV isolates are capable of replicating in various cell lines including neuronal, placental, muscle, retinal, pulmonary, colonic, and hepatic cells. The ability of ZIKV isolates to grow in various cell lines explains that the virus can survive and move across cells (Chan et al. 2016). Accumulating evidence as shown above suggest the ZIKV movement across the placenta to cause neuronal tissue damage in infants.

Sexual transmission

ZIKV is also capable of trasmitting through sexual intercourse. Multiple cases of male to female transmission of ZIKV have been reported (Hills et al. 2016; Lazear and Diamond 2016; Mansuy et al. 2016; Moreira et al. 2017; Petersen et al. 2016a; Rowland et al. 2016; Venturi et al. 2016). Several case studies of ZIKV infection in the USA claimed sexual intercourse with infected male partners (returned from ZIKV endemic areas) as a causal factor for transmission to females (Foy et al. 2011). Observations such as replication of ZIKV in testicular lineage cell lines support the survival and prolonged shedding of virus from this anatomical site to spread the infection (Atkinson et al. 2016; D'Ortenzio et al. 2016; Foy et al. 2011; Lazear et al. 2016; Musso et al. 2015; Rossi et al. 2016). The female genital tract cell lines are also susceptible to ZIKV infections, suggesting a possible transmission of the virus from infected males to females through sexual intercourse. Studies using mouse models of vaginal ZIKV infection indicated that transcription factors IRF3 and 7 and type I interferon alpha receptor IFNAR may play a role in viral replication in these sites (Yockey et al. 2016). Recently in 2016, other transmission routes such as male-male and female to male were also reported, thereby raising concerns that ZIKV can spread widely (CDC Prevention Newsroom 2016; Davidson et al. 2016; Deckard et al. 2016; Santora 2016).

Transmission by breastfeeding and blood transfusion

Zika RNA and infective Zika viral particles have been detected in high loads in breast milk of infected mothers (Dupont-Rouzeyrol et al. 2016). ZIKV RNA was also detected in breast milk and serum of two mothers and the sera of their infants (Besnard et al. 2014). However, ZIKV replication was not found in Vero cells inoculated with breast milk. ZIKV infection was also not seen in infants despite the presence of detectable maternal viremia in four mothers, making the transmission via breast milk as inconclusive at the moment (Cavalcanti et al. 2017).

During ZIKV outbreak in French Polynesia, approximately 3% of blood donors were tested positive for ZIKV (CDC 2016; Musso et al. 2014a). The first confirmed case of blood transfusion-related ZIKV infection was reported in Brazil in 2016 (Motta et al. 2016) Subsequently, WHO issue strict guidelines for blood transfusions in areas where ZIKV infections are endemic (Imperato 2016). Until now, there are no reported cases of ZIKV infection due to blood transfusion in the USA; however, as a safety precaution, blood donors are being instructed to report any ZIKV symptoms during 2-week post blood donation.

ZIKV-associated neurological disorders

Microcephaly

Microcephaly is a rare neurological abnormality characterized by poor brain development in infants. Microcephaly infants develop smaller head compared to healthy infants of same age and sex. The ZIKV outbreak in Brazil in 2016 had received much attention because of the surge of ZIKV-associated microcephaly cases, suspecting a possible association (Brasil et al. 2016a, b; Duarte et al. 2017). Microcephaly was reported to occur either alone or as congenital syndrome and is characterized by intellectual disabilities, developmental delay, seizures, vision, and hearing problems in the affected children. Microcephaly can be diagnosed in utero or postnatally (Calvet et al. 2016a; Oliveira Melo et al. 2016). In February 2016, WHO declared a public health emergency due to clusters of association of microcephaly in areas affected by ZIKV infections (WHO 2016c).

In vitro models to understand ZIKV induced microcephaly
The molecular mechanisms underlying the microcephaly and brain damage in infants following ZIKV infection is under intense investigation. Early reports have drawn links between ZIKV infection and microcephaly based on observations on cell tropism of the virus in vitro. ZIKV was found to infect and reduce the cell viability of cultured neuronal progenitor cells and human cortical neurospheres as evidenced by the decrease in expression of cell cycle genes, upregulation of apoptosis,

and dysregulation of neuronal cell proliferation (Tang et al. 2016; Cugola et al. 2016; Qian et al. 2016).

Several in vitro studies speculated AXL (a TAM family receptor) as a potential entry receptor for ZIKV. A close correlation of AXL expression and ZIKV tissue tropism made researchers highlight the role of AXL as an entry receptor for ZIKV (Nowakowski et al. 2016). It was suggested that ZIKV preferentially binds to AXL overexpressed cells such as radial glial cells, placental cells, and neural stem cells (astrocytes and neuron precursors) that form the cerebral cortex of the developing fetus brain (Nowakowski et al. 2016; Tabata et al. 2016). AXL expression is also found to be abundant in other anatomical sites such as stem cells of the retina, dermal fibroblasts, and cells of immune and reproductive tissues (Bauer et al. 2012; Graham et al. 2014; Hamel et al. 2015). Hamel et al. tested many receptors of flaviviruses and reported that AXL plays a major role in ZIKV infection of multiple human non-neural cell types (Hamel et al. 2015). Modulation of AXL response through incubation of host cells with AXL neutralizing antibodies, *axl* siRNA, and CRISPR/Cas9 *axl* gene deletion reduces the ZIKV infectivity (Hamel et al. 2015; Savidis et al. 2016). AXL was demonstrated to be essential for ZIKV infection of human endothelial cells (Liu et al. 2016). Using engineered AXL receptor and AXL inhibitor R428, Meertens et al. demonstrated that AXL downregulates innate immune signaling and thereby facilitates viral entry into human glial cells (Meertens et al. 2017). All these results strongly suggest that AXL receptor is crucial for ZIKV entry. However, some other in vitro studies on human neuronal progenitor cells called into question the role of AXL in ZIKV infection since inhibition of AXL was found to be insufficient to inhibit ZIKV entry or cell death of neural cells (Wells et al. 2016). In addition to AXL, glycosaminoglycans (GAGs) present in human placenta and porcine brains can bind to ZIKV envelope proteins with high affinity and can act as viral attachment factors (Kim et al. 2017).

Zika virus NS4A and NS4B proteins were found to play a crucial role in ZIKV pathogenesis. NS4A and NS4B proteins deregulate Akt-mTOR signaling in human fetal neural stem cells to inhibit neurogenesis and induce autophagy (Liang et al. 2016). After entry into the host cells, ZIKV replicates in neuronal progenitor cells without triggering the host intrinsic immune responses. Recent studies by Hanners et al. showed that ZIKV survives and replicates in primary human neural progenitors (hNPs) while causing cytopathic effects including hNP cell rounding, pyknosis, and activation of caspase 3 (Hanners et al. 2016). Despite the cytopathic effects, ZIKV infection triggered no significant immune response as observed by the lack of cytokine response. The virus continued to replicate in hNP cells for 28 days while showing only subpar immune responses.

In vitro 3D human organoid models that recapitulate the complex neurological regions of early human embryogenesis are also being used to investigate the association of ZIKV to microcephaly. These cerebral organoid models showed that the virus moves towards neuronal progenitor cells and activates innate immune receptor TLR3 to trigger apoptosis and deplete neuronal progenitor cells (Dang et al. 2016). The proliferative neural cell zones decrease along with disruption of cortical layers (Cugola et al. 2016; Qian et al. 2016). Overall, brain organoid models are very promising in vitro models to understand the molecular mechanisms of ZIKV infection since they contain more than one cell type and probably model cell-to-cell contact-dependent brain development stages. However, the major caveats of this approach are uneven exposure of virus to neural cells and a lack of immune cells to mirror the real in vivo setting.

In vivo mouse models to understand ZIKV induced microcephaly In vivo models of ZIKV infection are essential to gain mechanistic insights on microcephaly. Many mice models including wild-type, engineered, and immunocompromised mice are being tested to establish links between maternal infection and microcephaly. Studies by Miner et al. suggested that mice fetal brain cells are vulnerable to viral infections irrespective of their maternal genotype. Zika viral infection of mice with baseline immune defects (lower circulating B cells but elevated circulating T cells) causes embryonic damage leading to cortical thinning, cortical malformations including neuronal cell apoptosis in newborns (Cugola et al. 2016). Mice lacking interferon alpha receptor ($Ifnar1^{-/-}$) or wild-type mice with antibodies against interferon alpha receptors showed placental damage, brain malformation, and fetal death (Miner et al. 2016a) due to ZIKV infection. Other mice models with defective interferon signaling (A129, AG129, and TKO) also showed placental damage, vertical transmission of the virus from placental to fetus, and neural damage in the fetus (Aliota et al. 2016; Dowall et al. 2016; Koppolu et al. 2013; Lazear et al. 2016; Li et al. 2016b; Miner et al. 2016a; Rossi et al. 2016). Interestingly, wild-type C57BL/6 mice with no immune defects did not show any fetal damage when injected at higher dose intravenously but showed microcephaly with vaginal and intraperitoneal exposure (Cugola et al. 2016; Yockey et al. 2016).

To further understand the mechanism underlying microcephaly, Wu et al. have developed a mice model that recapitulated the human ZIKV infections (Nguyen et al. 2016). Direct injection of virus into the lateral ventricle of fetal mouse brain during cortical neurogenesis (embryonic day 13.5) has resulted in viral replication and infection in cerebral cortex. ZIKV virus crossed placental and replicated in radial glial cells of the developing fetus brain when injected into the abdominal cavity of pregnant mice. It was postulated that the placental damage during ZIKV infection can facilitate the entry of the virus into fetal neural cells.

Although most in vitro studies proclaimed that AXL as a major receptor for ZIKV entry and infection, in vivo mice studies contradicted those observations. Wang et al. showed that ZIKV is capable of successfully infecting AXL-deficient mice (Wang et al. 2017). In another study by Hasting et al., TAM family candidate receptors including AXL, Tyro3, and Mertk were found to be not essential for ZIKV infection, mother-to-fetus transmission, and ZIKV tropism in placenta and brain (Hastings et al. 2017). However, the same study showed that AXL could mediate ZIKV infection in vitro. In a similar study, Zika viral RNA was observed in the brain of AXL-deficient mice (Miner et al. 2016b). High level of ZIKV infection was also found in testis and epididymis of ZIKV-infected AXL-deficient mice (Govero et al. 2016). These observations imply that AXL may not be very important for infection in vivo. It is likely that ZIKV utilizes multiple attachment factors or receptors, and further work is required to elucidate the molecular mechanism of entry.

Guillain-Barré syndrome

GBS is an autoimmune demyelinating disorder that causes reduced signal transmissions, progressive muscle weakness, and paralysis. A significant number of GBS cases are found to have ZIKV infections in both recent outbreak in Americas and the previous outbreak in French Polynesia, suspecting a possible association (Araujo et al. 2016; Cao-Lormeau et al. 2016). GBS cases are increased by 20 to 40-fold during the ZIKV outbreak in French Polynesia () (Araujo et al. 2016; Bahia and Bahia 2015). Data released by WHO also showed a substantial increase in GBS cases in South America in 2016 (WHO 2016b, d).

In addition to ZIKV, infections by DENV and CHIKV can lead to GBS. Therefore, an accurate diagnosis of GBS causal agent is critical to choose an adequate treatment. GBD diagnosis is complicated due to overlapping clinical features of GBS with myelitis resulting from direct flaviviral infection of the spinal cord. Recently, a ZIKV-infected immunodeficient mice TKO (triple knockout of interferon regulatory factors 3, 5, 7) and $Ifnar1^{-/-}$ (interferon alpha receptor knockout) showed evidence of direct infection of the spinal cord and live virus in both brain and spinal cord, consistent with myelitis (Lazear et al. 2016). So, a correct distinction of GBS-induced paralysis from myelitis and proper diagnosis of the type of flavivirus causing the GBS are essential factors for choosing an effective treatment strategy (Araujo et al. 2016; Cao-Lormeau et al. 2016).

Other complications associated with ZIKV infections

Eye diseases ZIKV causes mild eye infections in adults with symptoms such as conjunctivitis and uveitis in adults (Benzekri et al. 2017; Duffy et al. 2009; Furtado et al. 2016). However, ZIKV-related eye infections in infants are much severe and include blindness, optic neuritis, chorioretinal atrophy, bilateral iris coloboma, and intraretinal hemorrhages. ZIKV-related microcephaly case studies revealed ocular abnormalities in 10 out of 29 infants (de Paula Freitas et al. 2016). ZIKV is found to cause panuveitis in mice and sheds from tears (Miner et al. 2016b). The presence of ZIKV in eyes suggests that eyes may act as one of the reservoir hosts for ZIKV following the acute phase of infection.

Meningoencephalitis ZIKV-related meningoencephalitis can occur rarely. To date, it was observed only in a single case of 81-year-old male returned from ZIKV endemic area (Mecharles et al. 2016). Brain infection resembling meningoencephalitis was seen in immunodeficient mice subjected to intravenous ZIKV injections (Fernandes et al. 2016). These shreds of evidence suggest a possible association of ZIKV infections to meningoencephalitis and thus merit further studies in this direction.

Possible reasons for emergence of ZIKV as neuropathogen

Despite being discovered over 50 years ago, ZIKV was not considered as a potential human neuropathogen until 2015. The possible reasons for the emergence of ZIKV as neuropathogen can be (a) emergence of more virulent strains, (b) adaptation of new routes of viral transmission, (c) genetic differences in populations making some groups more susceptible, and (d) under-diagnosis of ZIKV-associated neurological defects in previous outbreaks in Africa due to poor epidemiological data. Sequence analysis of all 41 known ZIKV RNA open reading frames belonging to different African, Asian, and Americas ZIKV strains indicated that ZIKV has undergone significant changes in both the nucleotide and protein sequences in the past 50 years (Wang et al. 2016). These changes might have made the virus more virulent in neural cells causing severe neurological complications. While emergence of new virulent strains remains a possibility, it is contradicted by the lack of conclusive evidence for differences between strains isolated from Africa and Americas regarding neural tropism or cell death, as shown in existing models (Bayer et al. 2016; Cugola et al. 2016; Lazear et al. 2016; Liang et al. 2016; Qian et al. 2016).

Another possibility could be viral adaptation of new routes of transmission. The only reported transmission route for the virus until a few years ago was vector transmission. Now potential new routes such as sexual and blood transmission have been identified and may have contributed to wider spread of the virus. However, the majority of the ZIKV infections even in the recent outbreak in North and South America are still through mosquitos and thus considered as a less distinct possibility. Susceptibility to ZIKV may be population

specific, and genetic differences or prior exposure to other flaviviruses could contribute to increased susceptibility to neurological complications in one population over others. It has been evident that prior infection with one DENV serotype makes the individual more susceptible to other DENV serotypes. Thus, ZIKV may be more effective in causing microcephaly in persons that have been previously exposed to dengue or other flaviviruses. Experimental evidence for this phenomenon was found in *in vitro* experiments where exposure of DENV immune plasma and monoclonal antibodies caused a 12-fold increase in ZIKV infection (Dejnirattisai et al. 2016). Nonetheless, further understanding of cross-reactivity of ZIKV and other flaviviruses is needed to determine whether specific populations are prone to severe neurological consequences over others.

ZIKV diagnosis

An accurate and timely diagnosis of ZIKV infection is essential to choose correct treatment strategies. Clinical diagnosis of ZIKV is difficult because of its symptoms overlap with other arboviruses and cross-reactivity to other faviviral antibodies present in the serum at the time of infection. Current diagnosis of ZIKV infection includes reverse transcription polymerase chain reaction (RT-PCR) approach that detects virus-specific nucleic acids in the blood or urine samples. Additionally, serological approaches such as ELISA or immunofluorescence assay (IFA) that detects viral antigen-specific antibodies (IgG or IgM) in the patient's serum are also being considered. However, diagnosis by serological approaches must be confirmed through virus neutralization assays and virus isolation from cell cultures. According to European Center for Disease Control and Prevention (ECDC), a Zika-confirmed case should require a positive identification of Zika viral RNA in RT-PCR, Zika antibodies in serum, confirmation of ZIKV in neutralization test, and isolation of ZIKV virus in cell cultures. A Zika case is considered as probable if only Zika-specific antibodies are found in serum with no further confirmatory tests for the presence of other distinguishing factors.

Nucleic acid screening

Molecular methods to detect ZIKV-specific nucleic acids by RT-PCR are preferred during the acute phase of ZIKV infection because of its high specificity and sensitivity. ZIKV DNA by RT-PCR can be detected up to 2 weeks of symptom onset in urine and saliva samples, and up to 2 months in blood samples. RT-PCR methods that detect either conserved regions of the viral genome or multiplex assays that allows simultaneous detection of ZIKV, DENV, and CHIKV have been developed (Corman et al. 2016; Faye et al. 2013; Faye et al. 2008; Lanciotti et al. 2008; Nicolini et al. 2017; Pyke et al. 2014;

Tappe et al. 2014; Waggoner et al. 2016). Recently, a high-throughput and rapid "NASBA-CRISPR Cleavage (NASBACC) RT-PCR method" was developed. NASBA-CRISPR Cleavage (NASBACC) RT-PCR method leverages the sequence-specific nuclease activity of CRISPR/Cas9 and allows for strain differentiation at a single-base resolution in 4 h (Pardee et al. 2016). Other methods such as reverse transcription loop-mediated isothermal amplification (RT-LAMP) and EBOV POC test have allowed the detection of ZIKV rapidly in 40–90 min without the need for RNA extraction (Song et al. 2016; Zhang et al. 2017). A list of RT-PCR methods for diagnosis of ZIKV is shown in Table 1.

In addition to RT-PCR-based molecular assays, next-generation sequencing is also gaining popularity for ZIKV diagnosis (Calvet et al. 2016a; Faria et al. 2016; Mlakar et al. 2016; Sardi et al. 2016; Skredenske et al. 2013). With next-generation sequencing, full genome of the virus can be sequence for proper identification and characterization. Given the small genome size of ZIKV, complete genome sequencing can be done quickly. With the technical advances and reduction in the cost, sequencing can become a routine technology in the laboratories for the detection of ZIKV and other infectious diseases.

Serological approaches

At present, the diagnosis of ZIKV by serological techniques can be quite challenging because of cross-reactivity of ZIKV to sera of other flaviviruses especially when vaccinated or previously infected with other flaviviruses. Immunoassays such as ELISA and IFA detect IgG and IgM antibodies from serum and cerebrospinal fluid. ZIKV-specific antibodies are detectable in serum after 4 days of symptom onset and stay up to 2–12 weeks. Immunoassays detecting ZIKV NS1 antigen are preferred over whole virus or E protein antigens due to the high diversity and less cross-reactivity of NS-1 antigen (Huzly et al. 2016). A positive result in immunoassay is considered as a probable ZIKV case and needs confirmation by neutralization tests, seroconversion, or fourfold increase in ZIKV-specific antibodies in paired serum samples (Rabe et al. 2016).

The current serological diagnostic tests take 2–3 days for getting the results and are authorized to be conducted only in labs that are designated by Center for Disease Control and Prevention (CDC). To rapidly diagnose ZIKV infections within 4 h and to significantly advance the ZIKV testing to commercial and healthcare facility laboratories, a rapid diagnostic test "ZIKV DetectTM IgM Capture ELISA" is being developed (Abbasi 2016). The results from the test are designated as either presumptive Zika positive or possible Zika positive or presumptive another flavivirus positive. In case of patients with previous flavivirus infections, CDC recommends additional orthogonal testing for further confirmation. In addition, Food and Drug Administration (FDA) has issued Emergency

Table 1 Emergency use kits authorized by FDA for in vitro diagnosis of ZIKV

Detection method	Purpose	Samples	Organization
Zika MAC-ELISA	IgM antibody	Serum, cerebrospinal fluid	Center for Disease Control and Prevention (CDC)
Trioplex Real-Time RT-PCR Assay	Qualitative detection and differentiation of RNA from Zika virus, dengue virus, and chikungunya virus	Serum, cerebrospinal fluid	Center for Disease Control and Prevention (CDC)
Zika virus detection by RT-PCR	Qualitative detection of RNA	Serum, plasma, urine	ARUP Laboratories
Sentosa® SA ZIKV RT-PCR	Qualitative detection of RNA	Serum, plasma, urine	Vela Diagnostics USA, Inc.
LightMix® Zika rRT-PCR	Qualitative detection of RNA	Serum, plasma	Roche Molecular Systems, Inc.
ZIKV Detect™ IgM Capture ELISA	ZIKV IgM antibodies	Serum	InBiosInternational, Inc.
xMAP® MultiFLEX™ Zika RNA Assay	Qualitative detection of RNA	Serum, plasma, urine	Luminex Corporation
VERSANT® Zika RNA 1.0 Assay (kPCR) Kit	Qualitative detection of RNA	Serum, plasma, urine	Siemens Healthcare Diagnostics Inc.
("Viracor-IBT") Zika Virus Real-Time RT-PCR	Qualitative detection of RNA	Serum, plasma, urine	Viracor-IBT Laboratories, Inc.
Aptima® Zika Virus assay	Qualitative detection of RNA	Serum, plasma	Hologic, Inc.
RealStar® Zika Virus RT-PCR Kit	Qualitative detection of RNA	Serum, urine	Altona Diagnostics
Zika Virus RT-PCR test	Qualitative detection of RNA	Serum	Focus Diagnostics, Inc.
ZikaELITe MGB® RT-PCR Kit	Qualitative detection of RNA	Serum, plasma	ELITechGroup Inc. Molecular Diagnostics
RealTime Zika assay	Qualitative detection of RNA	Serum, plasma, urine	Abbott Molecular Inc.

Use Authorization (EUA) for different diagnostic methods for presumptive detection of anti-ZIKV IgM antibodies in human sera. A list of these FDA-recommended serological approaches is detailed in Table 1. Based on the current guidelines for ZIKV diagnosis, algorithms that detail the diagnosis approaches were also being developed to assist in proper laboratory diagnosis (Barzon et al. 2016).

Treatment options including vaccines and antiviral drug candidates

Vaccines and antiviral therapeutics to treat Zika infections are currently under trials and may take at least few more years to enter the market. Under this scenario, the best approach to control Zika infections relies on preventing the contact between the vector *A. aegypti* and humans (especially pregnant women). Avoiding travel to Zika endemic regions, utilizing insect repellents DEET, treat clothing with permethrin, and eliminating standing water to prevent mosquito egg laying and larval development are recommended choices to prevent the infections. Using genetically modified male mosquitoes that express a lethal dominant gene in larva leading to larval death, releasing *A. aegypti* infected with endosymbiotic bacteria such as *Wolbachia* that interfere with viral replication in mosquitoes, and use of lethal mosquito traps such as autocidal gravid ovitrap are also being implemented in smaller scale

with moderate success (Barrera et al. 2014; Ritchie et al. 2015; Weaver et al. 2016; Wise de Valdez et al. 2011).

Vaccine candidates

The severity of current Zika outbreak and its possible link to microcephaly and other neurological disorders necessitated the urgent development of treatment strategies. Vaccines are currently available for other arthropod-borne flaviviruses such as West Nile virus (WNV), dengue virus (DENV), tick-borne encephalitis virus (TBEV), YFV, and JEV. Attempts to make a ZIKV vaccine took a rapid pace with the recent outbreak. Platforms exploited for ZIKV vaccine development include purified DNA vaccine, purified inactivated viral vaccines, live vectored vaccines, chimeric vaccine, and recombinant protein vaccines (see Table 2 for a list of ZIKV vaccine candidates).

A plasmid DNA vaccine and a purified inactivated virus (PIV) vaccine are being developed by Dan Borouch and his associates (Larocca et al. 2016). Plasmid DNA vaccine was constructed from Zika strain BeH815744 with gene encoding precursor transmembrane (prM) and envelope (E) proteins with the intent that these genes, when transferred to host, will produce proteins that are recognized by the host to develop antibodies against them. A single dose of this prM-E DNA vaccine produced a complete protection to Balb/c mice from subsequent ZIKA-BR (Brazil/ZKV2015) and ZIKA-PR (PR: Puerto Rico PRVABC59) virus challenge, as measured by the

Table 2 Zika virus vaccine candidates in pre-clinical and clinical trials

Type of vaccine	Zika antigens targeted	Preclinical/clinical	Reference
Plasmid DNA vaccine	1. VRC-ZKADNA085-00-VP vaccine targeting precursor transmembrane M (prM) and envelope (E) proteins from the H/PF/2013 strain of ZIKV 2. DNA vaccine targeting the pre-membrane and envelope proteins of original ZIKV-MR766 isolate or a recently circulating ZIKV strain from Brazil 3. DNA vaccine targeting the pre-membrane and envelope proteins of Zika strain BeH815744	Phase 2/2b Pre-clinical Pre-clinical	Abbasi (2016) Muthumani et al. (2016) Larocca et al. (2016)
Purified inactivated virus (PIV)	A purified inactivated virus (PIV) vaccine prepared from ZIKV strain PRVABC59	Pre-clinical	Abbink et al. (2016)
Purified immunoglobulins	Adoptive transfer of purified immunoglobulins from PIV vaccinated monkeys	Pre-clinical	Abbink et al. (2016)
Adenovirus serotype vector 52 recombinant vaccine	ZIKV premembrane protein Envelope protein	Pre-clinical	Abbink et al. (2016)
Adenovirus serotype-5 vector vaccine (Ad5.ZIKV-Efl)	Envelope protein fused to T4 fibritin fold on trimerization domain (Efl)	Pre-clinical	Kim et al. (2016)
subunit recombinant envelope Efl vaccine (MNA-ZIKV-rEfl)	Envelope protein	Pre-clinical	Kim et al. (2016)

absence of detectable viremia, increased Zika-specific neutralizing antibody titers, and increased envelope-specific CD4 and CD8 responses. Passive protection was also observed due to adoptive transfer of IgG antibodies purified from the vaccinated mice even after depletion of CD4 and CD8 lymphocytes. A chimeric vaccine with DENV-2 prM-E genes also generated a robust antibody response and protected mice from subsequent ZIKV challenge (Xie et al. 2017). A purified inactivated virus (PIV) vaccine prepared from ZIKV strain PRVABC59 also demonstrated complete protection against ZIKV infection in mice. In another study, both plasmid prM-E DNA vaccine and PIV vaccine elicited neutralizing antibodies and provided complete protection to rhesus monkeys against ZIKV challenge (Abbink et al. 2016). A recombinant adenovirus serotype 52 vector vaccine expressing ZIKV premembrane and envelope proteins also elicited a similar response in rhesus monkeys (Abbink et al. 2016). Observations of robust protection with these vaccine candidates (PIV vaccine, DNA vaccine, and adenovirus serotype 52 vector vaccine) suggest a clear path forward for clinical testing of their efficacy in humans.

Group of researchers led by Dr. David Weiner (Wistar Institute, Philadelphia, USA) have also developed a DNA vaccine candidate expressing the precursor transmembrane (prM) and envelope (E) surface proteins and tested its efficacy in mice and in non-human primate models (Muthumani et al. 2016). The animals when introduced with the vaccine through electroporation, produced antigen-specific cellular and humoral responses, and neutralization activity. A single dose of vaccine elicited protection in immune-deficient mice (lacking interferon alpha

and beta receptors) and prevented the viral pathology in brain tissues.

Another investigational vaccine VRC-ZKADNA085-00-VP is under development by National Institute of Allergy and Infectious Diseases (NIAID) at the National Institute of Health (NIH) (Abbasi 2016). The vaccine is also a DNA vaccine encoding the precursor transmembrane M (prM) and envelope (E) proteins from the H/PF/2013 strain of ZIKV. Based on the highly encouraging animal results (as mentioned by NIAID Director Anthony S. Fauci), NIAID is expeditiously performing phase I clinical trial (NCT02840487) in healthy human adults. The initial results of this phase I study intended to examine vaccine safety and ability to stimulate immune responses are encouraging. To further build on the promising phase I results, NIAID initiated a phase 2/2b study in March 2017. The phase 2/2b study consists of two parts. In the first part, 90 healthy men and non-pregnant women from ages 18–35 years will be enrolled from three sites in Houston, Miami, and San Juan (Puerto Rico) and injected with vaccine three times intramuscularly and followed for 32 weeks. In the second part, at least 2400 healthy men and non-pregnant women from ages 18–35 years will be enrolled from USA (Houston, Miami, and San Juan), Costa Rica, Peru, Peru, Brazil, Panama, and Mexico. Participants will be randomly assigned to the vaccine or placebo in three visits in a double-blind study and evaluated for 2 years. NIAID developed a similar DNA vaccine against West Nile virus NY99 strain which demonstrated both safety and efficacy of this type of vaccine and is optimistic of success of ZIKV clinical trials (Ledgerwood et al. 2011).

Other vaccine candidates that are in the early stages of development include an adenovirus serotype-5 vector vaccine

(Ad5.ZIKV-Efl) that expresses envelope protein fused to T4 fibritin fold on trimerization domain (Efl) and a subunit recombinant envelope Efl vaccine delivered by carboxymethyl cellulose microneedle array (MNA-ZIKV-rEfl) (Kim et al. 2016). Both vaccine candidates induced ZIKV-specific antibodies and protected C57BL/6 mice. Evaluation of 7-day-old pups born to these mice challenged with lethal dose of intraperitoneal ZIKV injections showed a 100% protection from Ad5.ZIKV-Efl, and 50% protection from MNA-ZIKV-rEfl. Overall, the current anti-ZIKV vaccine strategies utilize a diverse range of molecular approaches targeting pre-membrane and envelope proteins. The success of these approaches would be critical for the fight against ZIKV.

Challenges in developing ZIKV vaccine

Developing a vaccine to treat ZIKV infection is the most effective public health strategy. However, the development of ZIKV vaccine can be challenging. A vaccine developed against one ZIKV serotype could worsen the infections of other serotypes or closely related viruses. This phenomenon is called antibody-dependent enhancement (ADE) and may happen when antibodies generated during a primary infection will not be of enough concentration or avidity to neutralize another serotype, instead causing enhanced infectivity of target cells through virus opsonization and Fc-receptor-mediated endocytosis. In previous studies with DENV (a close relative to ZIKV), antibody-dependent enhancement phenomenon was observed. Individuals who have antibodies against one DENV serotype from prior infection presented severe infections when infected with another DENV serotype than people who do not have antibodies. Recent observations suggested a similar phenomenon for ZIKV (Castanha et al. 2016; Dejnirattisai et al. 2016; Priyamvada et al. 2016; Singh et al. 2017; Stettler et al. 2016). For example, human anti-DENV immunosera and monoclonal antibodies with broad neutralizing activity showed cross-reactivity to ZIKV antigens with varying degrees of neutralization activity but enhanced ZIKV infection (Castanha et al. 2016). The presence of DENV immunosera and monoclonal antibodies enhanced the ZIKV infection by 12-fold in cultured cells (Dejnirattisai et al. 2016). This suggests that pre-existing antibodies to other flavivirus infections can increase the ZIKV infection severity. Nonetheless, antibodies to DENV EDE1 (envelope dimer epitope1) neutralized ZIKV showing that DENV antibodies can be protective in some ways. This implies that a thorough understanding of the interplay between ZIKV strains and other flaviviruses is essential for the design of vaccines for ZIKV.

Human serum antibodies for ZIKV infection

Monoclonal antibodies that can neutralize ZIKV can be used to treat ZIKV infections. In search of candidate therapeutic agents, a group of researchers led by Gopal Sapparapu isolated a panel of 29 antibodies from B cells of patients recovered from Zika infection and tested their immune specificity to envelope protein (ELISA binding assay), neutralizing activity (IC50), and envelope protein epitope recognition patterns (epitope mapping) (Sapparapu et al. 2016). Among the 29 antibodies, two inhibitory antibodies ZIKV-116 and ZIKV-117 showed strong neutralization activity and bound to ZIKV envelope protein at a lateral ridge of DIII (DIII-LR) and DII epitopes, respectively. Both ZIKV-116 and ZIKV-117 antibodies neutralized diverse ZIKV strains encompassing African and Asian-American lineages. ZIKV-117 antibody neutralized MR766 and Dakar 41519 (African), Malaysia P6740 and H/PF/2013 (Asian), Brazil Paraiba 2015 (American) strains and did not cross-react with other DENV and WNV infected cells. ZIKV-116 neutralized four of the five strains (except the original African strain MR766) but was reactive to some dengue-infected cells. Immunization of pregnant mice deficient in type-I interferon signaling with a single dose of ZIKV-117 antibody protected mice against lethal infection, decreased placental damage, and improved fetal recovery in both prophylactic and therapeutic settings.

ZIKV-117 represents the first medical intervention that prevented infections of diverse Zika virus strains in both adult mice and fetuses. ZIKV-117 was also the first antibody for which the epitopic interactions with envelope proteins are identified. The extent to which these results will translate into human protection needs further evaluation. Nonetheless, this gives us a possibility of protecting fetus by administering a natural serum-derived antibody to pregnant women. Regardless of ZIKV infection, women in ZIKV endemic areas can receive antibodies just before pregnancy and during pregnancy to protect the fetus from developing neurological abnormalities.

Antiviral drug candidates

Given the lack of specific knowledge on viral and host proteins essential for ZIKV infections, chemotherapeutic approaches for ZIKV antiviral drug development depend mostly on cell-based screening of pharmacologically active small molecules, and FDA-approved drugs (Table 3). These high-throughput drug repurposing screens were previously explored to develop antiviral drugs for DENV, CHIKV, and other infections (He et al. 2015; Johansen et al. 2015; Kouznetsova et al. 2014; Sun et al. 2014).

Xu et al. have screened ∼ 6000 small molecules (including pharmacologically active compounds, approved drugs, and clinical trial active candidates) against ZIKV and identified candidates that inhibit ZIKV replication (antiviral) or suppress infection induced caspase-3 activity (neuroprotective) in many neural cell types (Xu et al. 2016). The identified candidates include a pan-caspase inhibitor emricasan, 10 cyclin-

Table 3 Promising antiviral candidates currently under different stages

Antiviral candidate	Effective against ZIKV strain	Type of cell line	Reference
Emricasan	MR766 (1947 Ugandan strain), FSS13025 (2010 Cambodian strain)	SNB-19 Forebrain-specific human neural progenitor cells	Xu et al. (2016)
PHA-690509	PRVABC59 (2015 PuertoRican strain)	SNB-19 Forebrain-specific human neural progenitor cells Astrocytes	
Niclosamide		SNB-19 Forebrain-specific human neural progenitor cells Astrocytes	
Bortezomib	ZIKV MEX_1_7	Human hepatoma cell (HuH-7), neural stem cells, HeLa, JEG3, primary human amnion epithelial cells	Barrows et al. (2016)
Mycophenolic acid			
Daptomycin			
Lovastatin, 5-fluorouracil, 6-azauridine, kitasamycin, and palonosetron	KX197192.1 (isolated from a patient in Brazil 2015)	Human hepatoma cell Huh7 (JCRB0403)	Pascoalino et al. (2016)
Sofosbuvir	PRVABC59 (Puerto Rico, 2015), ZIKV Dakar 41519 (Senegal, 1984), Paraiba (Brazil, 2015)	Huh-7 Jar (human placental choriocarcinoma) Mice	Bullard-Feibelman et al. (2017)
NITD008	MR766 (ZIKV)	Vero-76	Adcock et al. (2017)
SaliPhe	PRVABC59 (ZIKV)		
Brequinar	NY-99 (West Nile Virus)		
CID 91632869	B956 (West Nile Virus)		
6-Azauridine finasteride	S27 (Chikungunya)		
Mevastatin			
2′-C-methylated nucleosides	MR766	Vero	Eyer et al. (2016)

dependent kinase inhibitors (CDKis), and anthelmintic drug niclosamide that are previously approved by the FDA for worm infections. Emricasan has inhibited caspase-3 activity and protected human cortical progenitor cells in both monolayer and 3D organoid cultures. Cyclin-dependent kinase inhibitors were found to inhibit ZIKV replication indicating that ZIKV utilizes CDKs for replication. Combination treatments involving neuroprotective emricasan and antiviral CDK inhibitors showed an additive effect in inhibiting caspase-3 activity and preserving astrocyte viability. Niclosamide showed antiviral properties and inhibited ZIKV replication post-entry into neural cells. Studies prior to FDA approval of niclosamide against worm infections have shown that the drug niclosamide is very well tolerated and poses no risk to the fetus. Niclosamide is not mutagenic, teratogenic, or embryotoxic and can be potentially given to pregnant women.

Similar drug-repurposing screens on 774 FDA-approved drugs in human hepatoma cell line (HuH-7) have found ~ 20 drugs that reduce ZIKV infection (Barrows et al. 2016). The anti-ZIKV activity of these compounds was further validated in human cervical, placental, and neural stem cell lines, as well as primary human amnion cells. These drugs include established antiviral flavivirus drugs such as bortezomib and mycophenolic acid and drugs with previously unknown antiviral activity (e.g., daptomycin). Another screen of ~ 725 FDA-approved drugs in human hepatoma cells (HuH-7) has identified ~ 29 candidates with anti-ZIKV activity. Among

which, five candidates lovastatin, 5-fluorouracil, 6-azauridine, kitasamycin, and palonosetron were found to have high anti-ZIKV activity (Pascoalino et al. 2016). All the five candidates belong to different classes, have different structures, and thus possibly have different mechanisms of blocking ZIKV infections. Other screens have identified several broad-spectrum antiviral compounds and pyrimidine synthesis inhibitors that suppress the ZIKV-induced cytopathic effects (Adcock et al. 2017). Another candidate, bromocriptine, had shown inhibitory effects on viral replication by interfering ZIKV NS2B-NS3 protein proteolytic activity (Chan et al. 2017). Overall, different screening studies provided a resource of small molecules that could be tested in animal and clinical models.

Studies have also identified candidates that target specific viral proteins such as viral RNA polymerases (De Clercq and Neyts 2009). Nucleoside analogs target viral RNA polymerases of hepatitis B, hepatitis C, herpes simplex virus, cytomegalovirus, and AIDS. Nucleoside analogs are well tolerated and target viral but not cellular polymerases to cause premature termination of viral replication. Nucleoside analogs, 2′-C-methylated nucleosides, have inhibited ZIKV replication in Vero cells and RNA-dependent RNA polymerase activity in vitro (Eyer et al. 2016; Lu et al. 2016). Sofosbuvir, an FDA-approved ribonucleotide analog (2′-F-2′-C-Me-UTP) targeting NS5 polymerase of hepatitis C virus, have inhibited replication of several ZIKV strains in the human tumor, fetal-derived neural cell lines, and mice models (Bullard-Feibelman

et al. 2017). Sofosbuvir inhibited ZIKV RNA-dependent RNA polymerase activity with an IC50 as low as 0.38 μM (Sacramento et al. 2016). In vitro experiments using purified ZIKV NS5 polymerase suggested that sofosbuvir (2′-F-2′-C-Me-UTP) serves as a substrate for ZIKV NS5 polymerase and causes chain termination upon incorporation. Another ribonucleoside analog 2′-C-ethynyl-UTP was found to inhibit ZIKV NS5 polymerase activity (Lu et al. 2016), providing further proof of concept for using ribonucleotide analogs as an effective antiviral treatment strategy against ZIKV. In addition to the NS5 polymerase, Zika proteins NS4A and NS4B can be potential target candidates (Liang et al. 2016).

Very recently in July 2017, Cao et al. showed that an autophagy inhibitor hydrochloroquinone (HCQ) approved by FDA for treating patients with malaria during pregnancy was effective in limiting the maternal-fetal transmission of ZIKV in mice. HCQ-protected mice from placental and fetal ZIKV infections and ameliorated placental and fetal outcomes (Cao et al. 2017). Although the mice placenta is anatomically different from human placenta, the in vivo evidence of promising anti-Zika activity in a mouse model of pregnancy would provide the foundation for experiments in non-human primates and human clinical trials.

Conclusions and outstanding questions

Since the outbreak in Americas in early 2015 and subsequent WHO declaration of ZIKV as public health emergency of international concern in February 2016, a huge amount of data has been generated on ZIKV infections pertinent to ZIKV pathogenesis, transmission routes, diagnosis, and vaccines and antiviral development. In vivo and in vitro models now show clear links between ZIKV and neurological complications. ZIKV is found to be unique as no other mosquito-borne virus is ever found to be associated with congenital disabilities and transmit sexually. Although vast information was gained through clinical observation and basic research, significant knowledge gaps remain in the field. Our immediate focus should be on filling these gaps including (a) detailed molecular mechanism of ZIKV associated neuronal loss, (b) degree of population-specific vulnerability to ZIKV infection, (c) effects of antibody-mediated enhancement on vaccine development, (d) impact of ZIKV on growth and development of children born to infected Zika mothers but with no signs of neurologic defects at birth, and (e) effect of ZIKV on infants and toddlers with relatively plastic brains when a mosquito carrying ZIKV bites them. Our growing knowledge in understanding these challenges and experiences gained from research on similar flaviviruses would tremendously help in our effort to fight the disease. At this moment, it is profoundly uplifting to see neurobiology researchers, clinicians, vaccine, and drug development scientists working collaboratively to respond to this time-sensitive health emergency.

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