Zika virus: Relationship to fatty acid synthesis and the repurposing of developed drugs reveals novel therapeutic strategies

Thesis

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Abstract

Zika virus (ZIKV) is an emerging pathogen associated with a wide variety of adverse disease outcomes and a potential for worldwide transmission. Of particular concern is the correlation of ZIKV to an assortment of neurological disorders and its effects on developing fetuses. This document provides a concise overview of ZIKV epidemiology, the diseases caused by ZIKV, the molecular structure and tropism of the virus, and the mechanisms by which it may cause disease. Furthermore, the identification and development of therapeutics to treat ZIKV infection will be discussed, with a focus on pharmaceuticals that are in clinical trials or are already approved by the FDA for the treatment of other diseases. Finally, the relevance of lipid metabolism to ZIKV replication will be explored to highlight new discoveries in the field and their potential to be exploited as novel methods of treatment. While many articles use other flaviviruses such as Dengue virus (DENV) to make inferences on the structure and function of ZIKV, this review will primarily utilize publications that explicitly study ZIKV in an attempt to condense the current body of knowledge on its specific traits.

Dedication

I would like to dedicate this document to my family, who gave me confidence to pursue my passions with steadfast determination. The love and support I have received cannot be understated, and I hope to be able to share it every day.

To my cats, Checkers and Toast, as well as those no longer with us: Hemingway, Scout, Jack, and Chub Chub, I express my sincerest appreciation. Your company has comforted me through some of my longest nights.

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Chapter 1. Introduction

Zika virus (ZIKV) is an emerging pathogen associated with various epidemics across the world since its discovery in 1947. ZIKV is of particular concern due to its association with increased rates of Guillain–Barré Syndrome and increased rates of microcephaly in children born from ZIKV infected mothers.[1] Despite major outbreaks in 2015 and 2016,[2,3] there are no licensed therapeutics available to treat ZIKV infection. While many ongoing projects are exploring prospective vaccine candidates,[4–6] few projects are addressing the need for therapeutic interventions against ZIKV following infection. Most therapeutic interventions for ZIKV rely on repurposed drugs developed for other infections, though none have gained FDA clearance to specifically treat ZIKV.[7]

As such, it is important to identify potential therapeutic targets and to evaluate their effects on ZIKV infection and replication. ZIKV infection alters a diverse set of host cellular processes, but one notable change is to the host lipidome.[8,9] Previous studies have identified changes that resulted in upregulation of lipogenesis-associated transcription factors and decreased expression of lipolysis-associated proteins,[10] increased production of various lipid species in infected serum,[11,12] and increased biogenesis of lipids specifically related to lipid droplet formation.[13] Thus, modulating host fatty acid synthesis and lipid metabolism may warrant investigation as additional paths by which novel therapeutics for ZIKV infection could be developed.

Chapter 2. Epidemiology of ZIKV

ZIKV was discovered in 1947 in a sentinel rhesus monkey within the Zika Forest of Uganda during surveillance for yellow fever virus.[14] ZIKV, or *Orthoflavivirus zikaense* as of 2023,[15] belongs to the *Flaviviridae* family and the *Orthoflavivirus* genus, a relationship it shares with other emerging pathogens of concern such as dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), and Japanese encephalitis virus (JEV). Spondweni virus (SPOV) is the closest relative to ZIKV, and both viruses occupy their own clade when grouped under a phylogenetic tree.[16] Two distinct lineages, African and Asian, have been identified in ZIKV. The African lineage was the first to be identified and has been sporadically isolated since 1947 from various hosts.[16] The Asian lineage was first identified in Malaysia in 1966, and from it has evolved a distinct American sublineage that has caused epidemics in the Americas in 2015-2016.[17] Major outbreaks of ZIKV occurred in Indonesia in 1977-1978, Micronesia in 2007 (commonly known as the Yap outbreak, named after the island from which it originated), French Polynesia in 2013-2014, Brazil and Colombia in 2015, the United States and Puerto Rico in 2016, and India in 2021.[3,18] The Brazilian epidemic in 2015-2016 produced the largest number of recorded cases of ZIKV, as well as the largest number of recorded birth defects and other observable clinical outcomes.[19] ZIKV is primarily transmitted via *Aedes* species mosquitoes, with notable mention of *Aedes aegypti* and *Aedes albopictus* as common competent vectors.[20] ZIKV is thus commonly designated as an arbovirus (Arthropod borne virus). In regions where ZIKV is most prevalent, such as Africa, the virus participates in a sylvatic transmission cycle

between nonhuman primates and mosquitoes, with humans being considered an incidental host. As a result, regions with well-established populations of *Aedes* mosquitos pose the most risk for viral transmission, especially in areas where nonhuman primate reservoirs are readily available. ZIKV is also capable of being transmitted perinatally (during childbirth), in utero, sexually, or via blood during transfusions.[21] These methods of transmission combined with continuous mosquito-borne infections results in an urban cycle in human populations, as shown in Figure 1. Studies are ongoing to determine other potential animal reservoirs of ZIKV; many susceptible hosts have been indicated via serological tests and in vitro models, but no other definitive reservoirs have been identified.[3,22,23] As of 2023, the WHO has identified 92 countries in which ZIKV is circulating to some degree, but viral transmission has been difficult to monitor due to a lack of robust surveillance systems and a high level of cross-reactivity in laboratory tests with other *Orthoflaviviruses*.[24–26] An illustration of the spread of ZIKV from the World Health Organization is shown in Figure 2.

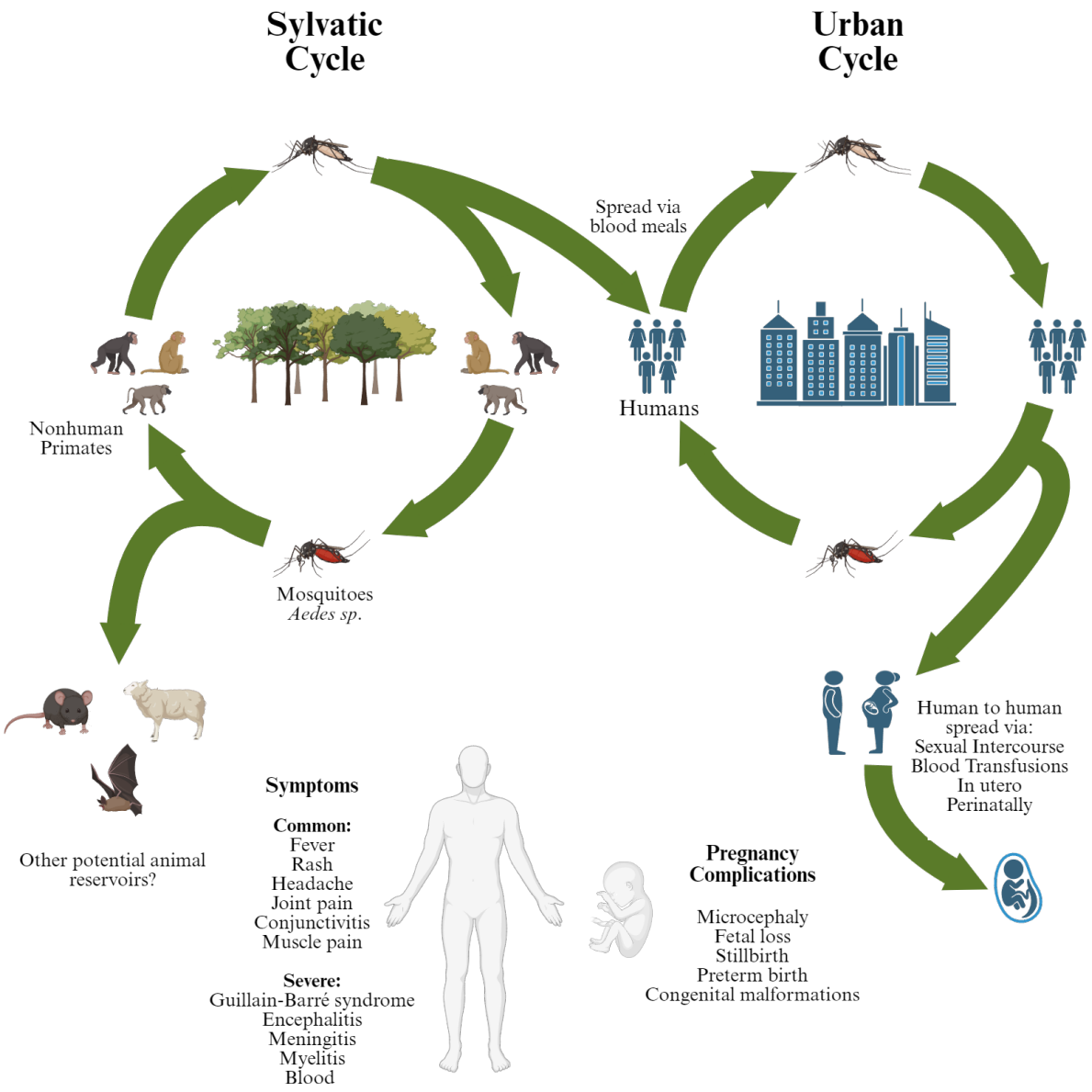

Figure 1: Lifecycle of ZIKV and effects of infection

ZIKV participates in a sylvatic life cycle with nonhuman primates such as monkeys, chimpanzees, and baboons. *Aedes* species mosquitos are the most common vector for transmission, with *Aedes aegypti and Aedes albopictus* playing a notable role. Studies have shown the potential for ZIKV to infect other animals, although no definitive reservoir has been determined. Once passed to humans, ZIKV can be continuously spread from infected persons, to mosquitos, and back to humans. Spread can also occur from sexual intercourse, blood transfusions, exposure perinatally, or in utero. Many symptoms of ZIKV infection are similar to the common cold, but ZIKV has been correlated with many severe adverse outcomes. The most significant adverse outcomes typically occur during pregnancy and affect the fetus. Figure created in BioRender.

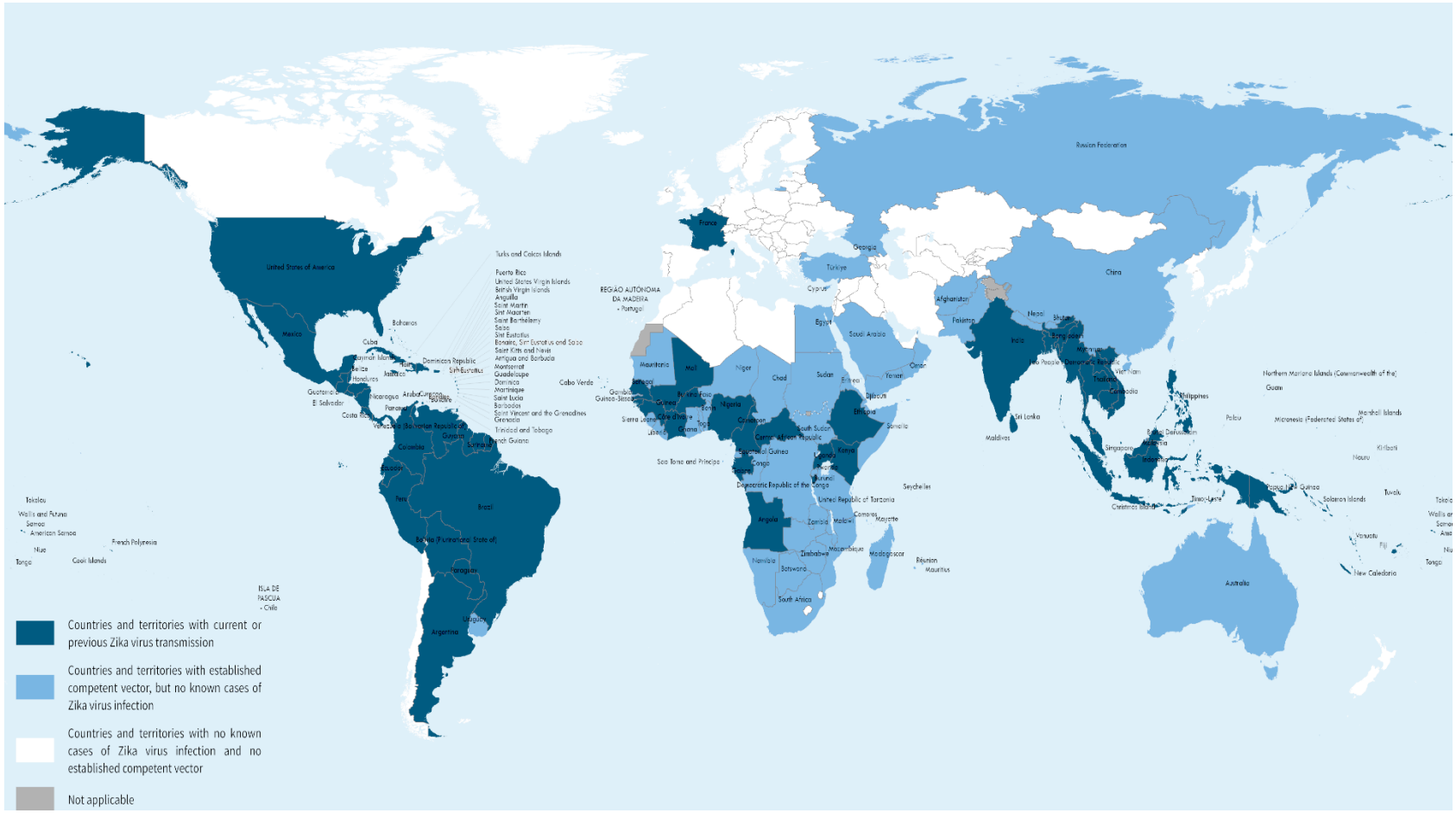

Figure 2: Global spread of ZIKV

World map showing the spread of ZIKV. Dark blue regions are those with current or previous ZIKV transmission. Light blue regions have a well-established competent vector but no known cases of ZIKV transmission. White regions have no known vectors or transmissions to date. Gray regions are those with no applicable data available. Data and figure courtesy of the WHO *Zika epidemiology update*, May 2024.

Effects of ZIKV infection

ZIKV is typically asymptomatic, but it can cause mild flu-like symptoms such as fever, rash, and migraines in around 20-25% of cases[14]; however, of utmost concern is the association of ZIKV with increased rates of Guillain-Barré syndrome (GBS)[27,28] and microcephaly in infants born to infected mothers.[29] Following infection, ZIKV primarily disseminates throughout the body via the blood and then by crossing the blood-tissue barrier.[30]

Guillain-Barré syndrome is an autoimmune disease caused by antibodies targeting gangliosides, causing acute neuromuscular paralysis.[31] The approximate mortality rate of Guillain-Barré syndrome is 5%, but 20% of patients experience lifelong disability. While the mechanism by which ZIKV could trigger GBS is unknown, electrophysiological findings have suggested demyelinating neuropathy, acute inflammatory demyelinating polyradiculoneuropathy, and axonal neuropathy as key drivers of disease. The heterogeneity of these findings may suggest multiple mechanisms and immune interactions whereby ZIKV can trigger GBS.[32–35]

Microcephaly is a clinical presentation in which the head of an infant is significantly smaller than the heads of most other infants (2-3 standard deviations below the mean for sex, age, and ethnicity), resulting in deleterious effects to brain development and cognitive function. Microcephaly is correlated to an imbalance between progenitor cell production and death that causes a decrease in neuronal and glial cells within the brain.[36,37] Microcephaly is not necessarily fatal, but it is associated with many life-threatening conditions and lifelong developmental challenges. It has been demonstrated that ZIKV can directly infect placental cells such as Hofbauer cells (placental macrophages) and trophoblasts[38] to gain access to the fetus. Further experiments have suggested that ZIKV likely spreads from basal and parietal decidua to chorionic villi and amniochorionic membranes,[39] and that the decidua acts as a reservoir for

trimester dependent transmission.[40] It has been recorded that disease and adverse outcomes for the fetus are most prevalent when infection occurs in the first trimester of pregnancy, although infection can occur over the entire course of gestation.[40,41]

ZIKV infection could cause microcephaly by directly infecting neural progenitor cells and other cells essential to central nervous system development, causing an immune response which may result in dysregulation of developmental genes and apoptosis.[42–44] Alternatively, inflammation of the placenta caused by the proliferation of macrophages in response to infection could also lead to non-autonomous effects on developing fetal cells, resulting in reduced neurogenesis and microcephaly.[43] Accordingly, much of ZIKV research has been directed toward treating perinatal and in utero transmission, which introduces an additional challenge: any therapeutics targeting ZIKV must be safe and efficacious for both the mother and the fetus.

Chapter 3. Structure and molecular mechanisms of ZIKV

Like other *Orthoflaviviruses*, ZIKV is an enveloped icosahedral virus with a 10.8 kb single-stranded positive-sense RNA (+ssRNA) genome; however, unlike most *Orthoflaviviruses* ZIKV seems to demonstrate a high level of homologous recombination activity, allowing for its adaptation to and proliferation in various mosquito vectors.[45,46] The primary building blocks that comprise a mature ZIKV virion are its structural proteins, nonstructural proteins, and the RNA genome. ZIKV has three structural proteins: the capsid (C), precursor membrane (prM, which matures into the membrane, M), and envelope (E). The seven nonstructural proteins include NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Nonstructural proteins play vital roles in replicating the viral genome, packing the genome, assembling the maturing virion, and subverting host defenses. The viral genome, once inside a cell and replicating, produces a single polyprotein that is then cleaved into the individual genes for its aforementioned proteins.[47] ZIKV heavily utilizes and rearranges the host endoplasmic reticulum to act as an anchor, replication site, and source for the viral envelop before proceeding to the Golgi apparatus for further maturation.[48–52] A basic overview of the structure and genome of ZIKV is demonstrated in Figure 3.

The predominant targets of ZIKV are primary human placental cells such as human trophoblast stem cells, fibroblasts, and Hofbauer cells[39,53,54]; however, ZIKV can establish infection in a broad range of human cells, ranging from spermatogonia, fibroblasts, neural progenitor cells,[55] macrophages,[56] and fetal endothelial cells,[21] among many others. The ability

of ZIKV to vertically infect cortical progenitor cells is notable and also unique amongst *Orthoflaviviruses*.[57] The wide range of susceptible host cells is a proposed to be a result of the ZIKV envelope protein and the prM-E heterodimer complex being able to interact with a variety of receptors, most notably C-type lectin receptors like DC-SIGN, TIM (T cell immunoglobulin mucin), and TAM (Tyrosine-protein kinase receptors: TYRO3, AXL, and MER).[58–60] The virus is typically taken up via clathrin-mediated endocytosis, after which the viral particle changes conformation, fuses with the endosomal membrane, and releases its genome into the cytoplasm following signaling from low pH.[47,55,61] Similarly, mature viral particles bud from infected cells via exocytosis.[62] In addition to inhibiting apoptosis by blocking at least one key inflammatory pathway,[63] exocytosis avoids immediately lysing infected cells,[64] thereby allowing more continuous replication and spread. The basic mechanism by which ZIKV infects a cell and replicates is shown in Figure 4.

ZIKV is known to produce an interferon (IFN) response in infected cells. While dependent on cell type, ZIKV infection can result in the production of type I (α, β), type II (γ), and type III (λ) interferons alongside the activation of IFN-stimulated genes (ISGs). It has been noted that ZIKV seems to target the STAT1 and STAT2 (signal transducer and activator of transcription) molecules to limit type I IFN signaling, thus allowing for more efficient viral replication. Following the innate immune response, the adaptive immune response to ZIKV infection typically produces Th1 T-cells, CD8+ T-cells, and B cells to fully clear the infection. The E, prM, and NS1 proteins present as the most common targets for the antibody response. Alterations to the immune system in pregnant women may explain both the ability of ZIKV to preferentially infect placental cells as well as the effects of ZIKV on the developing fetus.[60,65]

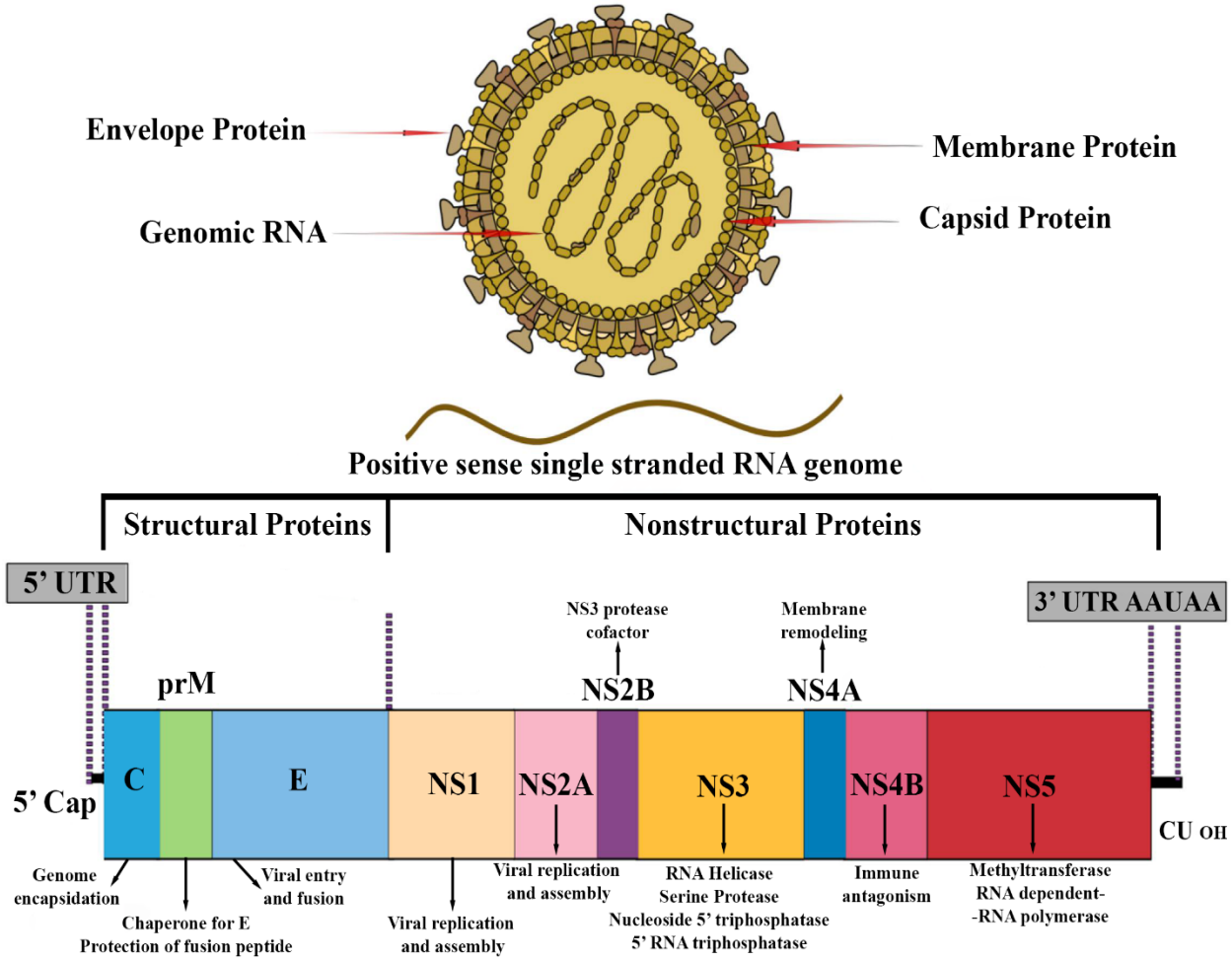

Figure 3: Basic structure and genome of ZIKV

ZIKV is an enveloped icosahedral virus with a positive sense single stranded RNA genome. Translation of its genome produces a single polyprotein that is then cleaved with both host and viral proteases and translated to produce ZIKV components. Nonstructural proteins play critical roles in mediating the replication and assembly of the virus along with antagonizing host defenses. The structural proteins make up the mature ZIKV virion. Figure courtesy of and adapted from[113] with additional information from [47].

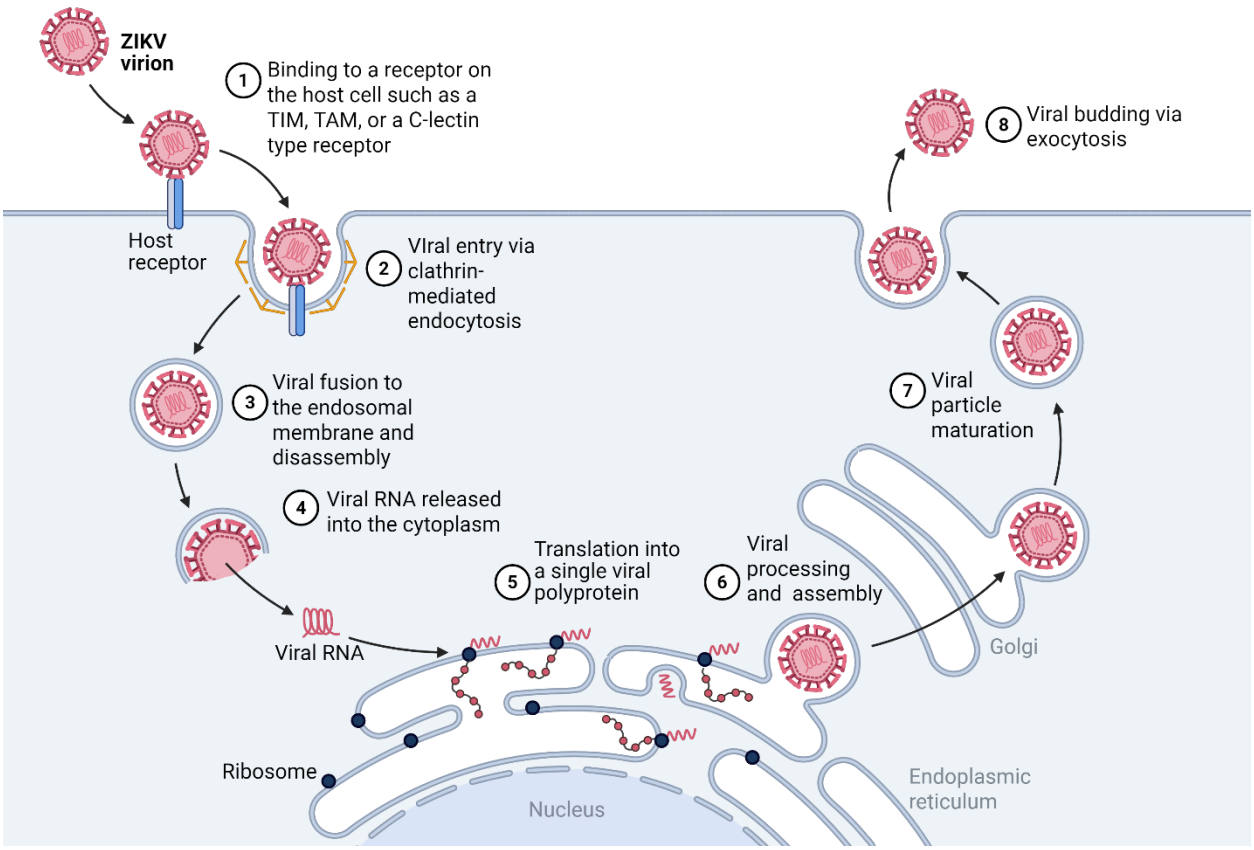

Figure 4: ZIKV infectious lifecycle

ZIKV binds to host cells via a variety of proposed host cell receptors (C-lectin type, TIM, TAM) and is taken up via clathrin-mediated endocytosis. The viral particle then fuses to the endosomal membrane and disassembles to release its RNA into the cytoplasm. The viral genome is translated by host ribosomes to produce a single polyprotein which is then cleaved by a variety of host and viral factors to produce the individual components of ZIKV. The maturing virus acquires its envelope from the endoplasmic reticulum and proceeds to the Golgi apparatus for further processing. Assembly of the mature virion allows for subsequent budding from the host cell via exocytosis. Figure courtesy of and created in BioRender.

Chapter 4. Condensed overview of ongoing clinical trials related to ZIKV and studies on potential therapeutic targets

As of yet, there are no approved vaccines for ZIKV, and there are few options for treatment following infection. While primary prevention remains the ultimate goal, it is essential to recognize that infections will still occur and that treatment options are necessary in such circumstances. No therapies have thus far been approved to treat ZIKV directly, but some pharmaceuticals exhibit an effect on the progression of ZIKV infection, as covered in some reviews.[66–68] While many compounds show some degree of antiviral activity in vitro or in vivo, typically in mouse models, few have progressed to clinical trials. Of particular note are the following, detailed below and in summary in Table 1:

Polyanion suramin is an antiparasitic drug that demonstrated some antiviral properties by targeting the NS2B/NS3 proteinase complex of ZIKV.[69–71] NS2B acts as a cofactor to stabilize proper protein folding and joins with the NS3 proteinase to mediate post-translational processing of the viral polyprotein alongside host proteases.[72] While not approved by the FDA in the United States, suramin is a traditional medicine typically used to treat trypanosome infections in Africa. Similarly, the FDA approved antimalarial drug atovaquone seemingly blocked ZIKV infection in C6/36 and Vero cells; experiments suggested that atovaquone likely blocked the envelope protein from fusing to host cells and blocked pyrimidine synthesis.[73,74] Chloroquine, another FDA approved antimalarial drug, also demonstrated an ability to reduce ZIKV infection in mice and cell models by blocking viral uptake and disassembly in the endosome.[75–77]

Both asunaprevir and simeprevir demonstrated significant effects in Vero 76 cells by targeting NS3, theoretically neutralizing its activity by binding to the active site of NS3 and preventing cleavage of the viral polyprotein.[78] Sofosbuvir demonstrated significant effects against ZIKV in mice by decreasing mortality, increasing survival time, and preventing acute neuromotor impairments.[79] Sofosbuvir targets NS5, the viral RNA-dependent RNA polymerase (RdRp), by acting as a uridine analog that causes chain termination and prevents further genetic replication. Merimepodib (MMPD) inhibited ZIKV replication in Huh7 cells by inhibiting inosine-5'-monophosphate dehydrogenase (IMPDH).[80] Interestingly, all four of these antivirals were designed to treat hepatitis C virus (HCV), another member of the *Flaviviridae* family. Of the four, only asunaprevir is not FDA approved for the treatment of chronic hepatitis C infection. The molecular basis by which approved HCV therapeutics can target ZIKV warrants further exploration in clinical trials.

The novel monoclonal antibody tyzivumab was developed specifically to treat ZIKV infection by targeting the viral envelope protein. The treatment partially completed a phase I trial; enough volunteers finished the study to evaluate the safety and dosage of tyzivumab in healthy human adults, but not enough ZIKV infected volunteers were enrolled to complete the trial. As of yet, no results or publications have been made available regarding the progress of the treatment.[68] A phase I clinical trial was also conducted with human anti-ZIKV immunoglobulin (ZIKV-Ig) created from purified IgG fractions of human plasma containing anti-ZIKV antibodies. The trial demonstrated that ZIKV-Ig was well tolerated in volunteers and demonstrated potential as a prophylactic treatment option; however, no updates in results or publications on ZIKV-Ig have emerged since 2021.[66,68,81]

As mentioned previously, the ability to safely administer treatment to pregnant women without harming the fetus is one of the key concerns in drug development for ZIKV. A screening of various FDA approved drugs for effects on ZIKV identified daptomycin as being able to lower ZIKV infection rates in various cell types. Daptomycin falls under the FDA Pregnancy Category B, meaning animal reproduction studies have thus far failed to demonstrate a risk to the fetus, but no adequate and well-controlled studies in pregnant human women have been conducted. None of the screened candidates fall under Category A which applies to drugs with no demonstrated risk to human fetuses. Daptomycin, an antibiotic, had not been previously shown to have antiviral activity, but it may affect phosphatidylglycerol (PG) rich endosomal membranes which are critical for ZIKV entry.[82]

Another study demonstrated that alpha-linoleic acid (ALA), a polyunsaturated ω-3 fatty acid, was able to inhibit ZIKV infection in some cell lines by potentially destabilizing the viral envelope.[83] Treatment with ALA did not demonstrate any adverse effects for mothers or their fetuses in previous studies aimed at evaluating the effects of ALA supplementation in pregnant women.[84,85]

As the some of the studies indicate, many fatty acid synthesis pathways and products seem to be related to ZIKV infection. This raises interesting questions regarding the role of fatty acid synthesis in relation to ZIKV viral infection.

Drug	**Target**	**Stage**	**Reference(s)**
Polyanion Suramin	Viral NS2B/NS3 complex	Various clinical trials, not FDA approved	[69–71]
Atovaquone	Viral envelope protein	FDA approved for treatment of *P. jirovecii* pneumonia	[73,74]
Chloroquine	Viral envelope protein, endosome pH	FDA approved for treatment of malaria	[75–77]
Asunaprevir and simeprevir	Viral NS3 protein	Only simeprevir FDA approved for treatment of chronic hepatitis C virus	[78]

Table 1: Notable drugs and therapies with effects on ZIKV infection Continued

Merimepodib	Host inosine-5'-monophosphate dehydrogenase (IMPDH)	Stalled following phase 2b trials for hepatitis C virus treatment	[80]
Tyzivumab	Viral envelope protein	Stalled following phase I trials for ZIKV treatment	[68]
ZIKV-Ig	Induces antibody immune response to ZIKV envelope protein	Stalled following phase I trials for ZIKV treatment	[81]
Daptomycin	Host phosphatidylglycerol-rich endosomal membranes	FDA approved for the treatment of *E. faecalis* and *S. aureus* infections	[82]
Alpha-linoleic acid	Viral envelope protein(?)	In vitro studies	[83]

Table 1 continued

Chapter 5. A brief overview of fatty acid synthesis and its relationship to ZIKV

Experiments have shown that ZIKV infection induces increased expression of genes related to lipid production and metabolism[8]; accordingly, serum samples from ZIKV infected individuals demonstrate a notably altered lipidome with observable increases in various fatty acid species.[11,12] Fatty acid synthase (FASN, encoded by the gene *FAS*) is a large dimeric enzyme that plays a key role in fatty acid synthesis. The enzyme forms palmitate from acetyl-CoA and malonyl-CoA, constituting one of the first steps in lipid metabolism.[86] FASN has garnered interest as more studies have identified it as an oncogene and a potential target for therapy[87]; additionally, multiple studies have shown that viral infections specifically increase intracellular FASN levels, and that FASN activity may be essential to viral replication.[88,89] The upregulation of FASN and the production of fatty acids may serve multiple roles for viruses, including producing energy by breaking down lipids via beta-oxidation,[89,90] making post-translational protein modifications via fatty acylation,[91] and forming lipid droplets to anchor viral replication close to molecular building blocks.[92,93] Accordingly, more studies have focused on evaluating the utilization of fatty acids during viral replication and targeting host factors to reduce viral viability.[94]

Lipid droplets are of particular interest in exploring the interactions of ZIKV with fatty acids. Lipid droplets are storage organelles that are created from the endoplasmic reticulum and reside in the cytoplasm; they contain a hydrophobic core of neutral lipids, such as triacylglycerols and sterol esters, surrounded by a monolayer of phospholipids and proteins.[95]

Cells use lipid droplets to store excess lipids, maintain homeostasis, and act as a buffer against cellular stress.[96] Previously, ZIKV capsid protein has been shown to accumulate around lipid droplets.[93,97] Studies have also shown ZIKV to have various effects on lipid droplet formation and accumulation, both increasing and decreasing lipid droplet regulation depending on the cell type.[92]

In one study,[10] both SH-SY5Y cells and neural stem cells infected with ZIKV demonstrated a significant increase in mRNA expression of transcripts related to lipid metabolism when compared to uninfected cells. Upregulated genes included *PLIN2*, *DGAT1*, and *FAS*. PLIN2 (perilipin 2, also called adipose differentiation-related protein, ADRP, or adipophilin) plays a key role in lipid droplet formation and intracellular triglyceride accumulation.[98] Similarly, DGAT1 (diacylglycerol acyltransferase) is a crucial enzyme that catalyzes the formation of triglycerides.[99] In the study, pharmacological inhibition of DGAT1 using A922500 significantly decreased lipid droplet accumulation and ZIKV replication.

In another study,[13] primary placental cells acquired during the first trimester were infected with ZIKV and analyzed with quantitative shotgun lipidomics. The study provided evidence that neutral lipids were increased in the infected cohort as compared to mock infected cohort, and it demonstrated a significant increase in *FAS, DGAT1,* and *FAT/CD36* (fatty acid translocase, an enzyme that facilitates fatty acid transport). Under immunostaining, it was noted that lipid droplets accumulated in greater numbers near infected focal sites and that lipid droplets experienced a significant increase in global distribution. As in the previously mentioned study, inhibition of DGAT1 using A922500 substantially reduced lipid droplet quantity and viral infection rates.

Confoundingly, a different study[100] observed a significant decrease in lipid droplets within Huh-7 cells following ZIKV infection. These results are not necessarily contradictory, however, as one suggested hypothesis for this decrease is an overall consumption or exhaustion of lipid droplets as the viral infection grows. Another proposed hypothesis is that viral infection induces lipophagy which could release free fatty acids from lipid droplets to be used in viral replication.[51,100,101]

Based on the above studies, it can be inferred that fatty acid biogenesis plays a significant role in ZIKV replication. As such, targeting the pathways and enzymes related to fatty acid synthesis may yield important information on the precise mechanisms by which ZIKV replicates intracellularly. One caveat, however, is that alterations in lipid metabolism may have deleterious effects on the fetus due to the disruption of cellular membranes. Perhaps of most consequence is that FASN knockouts in mice suggested that lack of proper FASN expression may be lethal in fetal development,[102,103] may be affiliated with preterm deliveries,[104] or may cause an array of gastrointestinal issues.[105]

Chapter 6. Discussion

The highly adaptable nature of ZIKV and its potential to cause significant disease across the world highlights the importance of developing novel vaccines and treatments for infection. Understanding the molecular structure of ZIKV and the mechanisms by which it infects and alters host cells is paramount in combating its spread.

As noted previously, many inferences about ZIKV have been drawn from other flaviviruses,[106,107] but the unique qualities of ZIKV, such as its ability to infect neural cells with alarming ease, deserve dedicated study. Moreover, there are many aspects of ZIKV that warrant further studies to rectify competing data. One such example is the debate on which host receptors are essential to viral entry. While some studies demonstrate that a receptor such as Axl is important to ZIKV infection in human cells,[108] others have demonstrated that Axl may not be important in mice cells.[109–111] It should be considered pertinent to explore whether this is a matter of cell tropism, or if the receptor is used in other aspects of pathogenesis as suggested by further articles.[112] Additionally, experiments to explore the differences in lipid droplet regulation between cell types[92] should be prioritized to elucidate the precise molecular basis for the changes observed. It would be intriguing to explore if ZIKV relies on various types of protein acylation, as well as if beta oxidation and lipid droplet formation are crucial processes in productive infections. Such changes to cellular functions could be explored with gene knockouts, transient knockdowns, or via pharmacological inhibitions.

Finally, while progress has been made in identifying compounds that may be able to inhibit or alleviate ZIKV infection, no vaccines or pharmaceuticals that precisely target ZIKV exist. No clinical trials for ZIKV specific therapies have progressed past phase II, highlighting an

urgent need for investment into research and development. While there is still much work to be done in creating more robust antivirals in general, some drugs currently under development or in use for the treatment of other conditions demonstrate the potential to be repurposed for antiviral therapy. For example, one drug that has not specifically been associated with effects on ZIKV but may be intriguing for future pharmacological tests is denifanstat, previously referred to as TVB-2640. Denifanstat is a FASN inhibitor that acts by inhibiting the β-ketoacyl reductase domain of the FASN enzyme complex.[113] It is primarily used to treat metabolic dysfunction-associated steatohepatitis (MASH)[114,115] and has been investigated as a treatment for certain cancers such as glioblastomas.[116] As a result of its positive effects in MASH patients, the FDA has granted denifanstat breakthrough therapy designation to accelerate development, and it is currently proceeding to phase III clinical trials. Additionally, denifanstat was among a variety of FASN inhibitors tested for effects on SARS-CoV-2 replication, and it demonstrated a significant impact on viral replication.[117] Therefore, denifanstat, along with the other FASN inhibitors of significance, may merit further study for potential antiviral applications.

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