A Short Review on the Phenotypic, Genotypic and Immunological Developments for Zika Virus

A project submitted

by

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This work is dedicated to my parents to whom I owe my achievements.

Certification statement

This is to certify that, this project titled 'A Short Review on the Phenotypic, Genotypic and Immunological Developments for Zika Virus' submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Dr. Mohd. Raeed Jamiruddin, Assistant Professor, Department of Pharmacy, BRAC University and this project is the result of the author's original research and has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the project contains no material previously published or written by another person except where due reference is made in the project paper itself.

Signed,

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Countersigned by the Supervisor

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Marzana Monefa

Abstract

Zika Virus (ZIKV) has recently received much attention right after the outbreak in America. Initially ZIKV was not considered a dangerous flavivirus as the infections were asymptomatic with only mild complications. But, it became a grave public health concern, when a strong association of ZIKV was proven with various neurological disorders, like for children it was found to cause hereditary deformities and for adults it resulted in Guillain-Barre syndrome. Since then researches and developers are conducting various studies to gain knowledge about the virus for development of safe and effective therapeutic strategy. Considering all ZIKV transmission methods, the transfer of ZIKV from infected pregnant mother to fetus have positioned licensing of an effective vaccination against ZIKV at forefront. Even if there is no approved vaccination yet, various platforms are working with many types of vaccination strategies are progressing at a high rate. Currently, there are many possible candidates in the pipeline of ZIKV vaccines. DNA vaccine candidates has progressed to clinical phase II studies and many vaccine types including mRNA, PIV, LAV, vectored vaccines are exhibiting promising data in both preclinical and clinical studies. Nevertheless, there is still many knowledge gap and challenges which are needed to overcome in the coming years for the successful licensure of ZIKV vaccines. This review article highlights on the structural importance of the virus, defense mechanism of host towards the virus and the up-to-date condition of vaccination development and challenges in the context of ZIKV.

Table of Content

List of Tables

List of Figures

Abbreviation

Ad- Adenovirus

ADE- Antibody-Dependent Enhancement

BARDA- Biomedical Advanced Research & Development Authority

BIDMC- Beth Israel Deaconess Medical Center

CCL5- C-C Motif Chemokine Ligand 5

CNS-Central Nervous System

CZS- Congenital Zika Syndrome

DC-SIGN - Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin

DENV- Dengue Virus

DNA- Deoxyribonucleic Acid

EDE mAbs- Envelope Dimer Epitope Monoclonal Antibodies

ELISA- Enzyme-Linked Immunosorbent Assay

ENV-Envelope

ER- Endoplasmic Reticulum

FL-Fusion Loop

GBS- Guillain-Barré Syndrome

GMT- Geometric Mean Titer

HLA-Human Leukocyte Antigen

IFN-Interferon

IgG- Immunoglobulin G

IL- Interleukin

IP-10 - Interferon Gamma-Induced Protein 10

JEV- Japanese Encephalitis Virus

LAV- Lymphadenopathy-Associated Virus

LD- Lipid Droplets

MV- Measles Virus

MVA- Modified Vaccinia Virus Ankara

Nab- Neutralizing antibody

NIAID- National Institute of Allergy and Infectious Diseases

PIV- Purified Inactivated Virus

RANTES- Regulated on Activation, Normal T cell Expressed and Secreted

RNA- Ribonucleic Acid

RT-PCR- Reverse Transcription Polymerase Chain Reaction

RVP-Reporter Viral Particle

sfRNA- Sub-Genomic Flavivirus Ribonucleic Acid

TBEV- Tick-Borne Encephalitis Virus

TGN- Trans-Golgi Network

TIM- T-Cell Immunoglobulin

UTR- Un-Translated Region

VLP- Virus-Like Particles

VRC- Virus replication complex

WHO- World Health Organization

WNV- West Nile fever

WRAIR- Walter Reed Army Institute of Research

YFV- Yellow Fever Vaccine

ZIKV- Zika Virus

ZPIV- Zika Purified Inactivated Virus Vaccine

1. Introduction

Zika Virus is an emerging arbovirus of the family Flaviviridae and of genus Flavivirus (Thiel et al., 2005). ZIKV is predominantly transmitted by the same Aedes mosquito vectors that also transmit dengue fever, yellow fever, and chikungunya virus (CDC, 2015). In addition ZIKV transmission route includes sexual contact (Mansuy et al., 2016), blood transfusion (Musso et al., 2014) and placental transmission(Ventura et al., 2016).Before 2007, ZIKV had limited research and accessible data on it whereas, there were numerous research work on other mosquito born flaviviruses like dengue (DENV) viruses and yellow fever (YF) viruses. This is because ZIKV was reported to cause only mild-fever and the symptoms were not distinguishable to those of several other diseases like dengue (Filipe et al., 1973; Simpson et al., 1964; Olson et al., 1981; Heang et al. 2012; Duffy et al. 2009; Foy et al., 2011). Furthermore, the deficiency in known clinical assessment for the ZIKV until 2016 had caused many unidentified diagnosis with only few reports of the infection.

ZIKV strain was first discovered from serum of rhesus monkey during exploration of sylvatic yellow fever and was then consequently isolated from mosquitoes in 1947 and 1948 respectively. The first viral strain was isolated in the ZIKA forest situated in Uganda and was then named after it (Dick et al., 1952). The strain was also identified in humans after few years (MacNamara et al., 1954). At the beginning, ZIKV was prevalent only in certain portions of Africa and Asia with few human cases (Faye et al., 2014).But ZIKV caused enormous outbreak on Yap Island in 2007, where large population of that island were infected (Duffy et al., 2009) which also expanded geographically in North Pacific (Olson, et al., 1981; Haddow, et al., 2012), in area comprising of South Pacific zone (Cao-Lormeau et al., 2014) and French Polynesia. Furthermore, ZIKV travelled through the Pacific Ocean to conquer northeastern Brazil and rapidly invaded America in 2015 (Hennessey et al., 2016).Since then the total number of countries that had reported ZIKV spread has extended to 86, of which 49 were in the Americas (WHO 2018)

ZIKV grabbed most attention, when the most affected areas with the infection exhibited strong link with increasing number of infected pregnant women resulting with infants having microcephaly cases and birth defects (Brasil et al., 2016). Since December 1, 2015 to March 31, 2018 there were 116 infants with Zika-associated birth defects, 9 pregnancy losses with Zika-linked birth deformities and 2360 completed pregnancies

with or without Zika-linked birth deformities reports have been confirmed in US States and the District of Columbia alone (CDC 2018). Furthermore, severe neurological diseases like Guillain–Barré syndrome (Petersen et al. 2016; Oehler al., 2014), meningoencephalitis (Carteaux et al., 2016), and myelitis in adults (Petersen et al., 2016) has been linked with ZIKV infection. This has concerned World Health Organization to raise an alarm for Public Health Emergency of International Concern in 2016 against ZIKV (Roos et al., 2016). To address this crisis, many research groups are giving effort in improving understanding of pathogenesis and susceptibility of ZIKV, to discover the most effective and saferst immunization against it. Till date there is no approved ZIKV vaccine.

1.1 Transmission of ZIKV

The transmission of ZIKV can be categorized into non-vector-borne and vector-borne transmission. Numerous species belonging to the genus Aedes mosquitoes are the vectors for the transmission of ZIKV. On the other hand, sexual transmission, blood transfusion, perinatal or intra-uterine transmission, postnatal transmission, and animal bites are the modes of non-vector-borne transmission (Pati~no-Barbosa et al. 2015). ZIKV are largely carried to the human bodies by the means of Aedes aegypti mosquito and there are minimum 20 further species that have been proven for ZIKV transmission (Table 1.1). Moreover, ZIKV has been reported to circulate between non-human primates and Aedes mosquitoes and also between man and domestic mosquitoes

Table 1.1: List of mosquitoes vectors for ZIKV transmission (MacNamara 1952; Dick et al. 1952; Dick 1953; MacNamara 1954; Weinbren et al., 1958; Haddow et al. 1964; Simpson 1964; Fagbami 1979; Haddow et al. 2012; Petersen et al., 2016; Singh. et al. 2016; Zanluca et al., 2016; Li et al. 2016)

Vectors	Aedes aegypti	Aedes hensilii	Aedes taylori
	Aedes albopictus	Aedes apicoargenteus	Aedes unilineatus
	Aedes africanus	Aedes polynesiensis	Aedes apicoargenteus
	Aedes luteocephalus	Aedes dalzielik	Anopheles coustani
	Aedes vitattus	Aedes hirsutus	Culex perfuscus

	Aedes furcifer	Aedes metalicus	Mansonia uniformis

Mosquitoes consisting of Aedes Genus are prominent in several areas of the world and the vectors of ZIKV are same as that of Dengue and Chikungunya (Petersen et al. 2016) (Nishiura et al. 2016). The vector Aedes hensilli is found as common and most significant vector to transmit both Chikungunya and ZIKV (Ledermann et al. 2014).

Furthermore, ZIKV infection has also be observed be transmitted via person to- person through sexual contact as well as vertical transmission (Foy et al. 2011). High viral loads of ZIKV were detected from semen samples of infected men with ZIKV (Musso et al. 2018). There is threat if transmission of ZIKV through blood transfusion is real but diagnosing ZIKV RNA using polymerase chain reaction and post-donation surveillance together can minimize this risk (Mariana et al. 2018).Besides, cases of platelet transfusion transmission of ZIKV have been acknowledged in Brazil (CDC 2016)

Vertical transmission was observed when ZIKV RNA was identified by RT-PCR in the serum of both newborns and mothers within few days after the delivery. The infection of the newborns was most possibly due to transplacental transmission (Besnard et al. 2014). Additionally, RNA of ZIKV was being identified from some microcephaly samples and also from the amniotic fluid of pregnant women with microcephaly fetuses in Brazil (Mlakar et al. 2016; Rubin et al. 2016; Calvet, et al. 2016).

Additional mode of transmission includes monkey bite (Leung et al. 2015). Zika antibodies have been distinguished in sheep, goats, bats, and few species of rodents (Musso et al., 2016).

1.2 Pathology and Pathogenesis of ZIKV

In past reports, ZIKV has proven to exhibit its ability to traverse the blood brain barrier as it possessed a positive attraction towards brain cells (Dick et al., 1952). In addition, ZIKV also established their affinity towards dermal fibroblasts, human immature dendritic cells, and epidermal keratinocytes. Autophagy doesn’t occur in neurons, nonetheless glial cells and neurons are infected by virus generating viral factories (Bell et al. 1971). And, the latest studies reveal that ZIKV has the ability to infect neuroblast

cells in-vivo (Tang et al. 2016). There are reports where expansion of astrocytes is found and destruction of hippocampus particularly pyriform cells has been confirmed (Bell et al. 1971). Additionally, ZIKV have developed a defense mechanism against the trophoblasts and are suspected to be responsible for the fetal abnormalities which occur when the neural tissues are attacked (Sadovsky et al. 2016). In contrast, injury to the fetus is triggered by placenta's inflammatory responses to the ZIKV. (Mor 2016). Microcephaly has been suggested to be due to centrosomes abnormalities and has been linked to ZIKV infection but still validation is required by conduction more studies (Tetro et al., 2016).

ZIKV can pass into the cells through the skin and nerve cells' receptors like AXL, DC-SIGN, Tyro3 and Tim-1 (Hamel et al. 2015). Then, ZIKV utilize the host machinery inside the cells and later causes autophagy and apoptosis of the cells to invade other cells. ZIKV replication in the cells results the secretion of type I interferon (Hamel et al. 2015). Induction of autophagy enhances viral replication and inhibiting agents for autophagy can reduce virus load in the cells (Carneiro et al. 2016). Post viral replication in the neighboring sites, ZIKV acquires distribution to muscles, heart, CNS, and as well as to the fetus by passing through the placental barrier (Chan et al. 2016).

Tears of mouse have been detected with ZIKV RNA (Miner et al., 2016). It was also found in the cornea, optic nerve, and neurosensory retina of mouse. Then, the finding of viral RNA in human conjunctival fluid was validated when the virus replication in eye related tissue were observed (Sun et al., 2016).

Furthermore, after infection symptoms in human, the ZIKV RNA was observed to be evident in the cervical mucus and also when the virus had been removed from both urine and serum (Prisant et al., 2016). Importantly, ZIKV RNA is also observed in the vaginal secretions of human for several weeks (Murray et al., 2017).Correspondingly, impaired fetal development has been linked to uterine infection where human uterine fibroblasts has showed vulnerability towards infection caused by ZIKV (Chen et al., 2016).

Additionally, ZIKV RNA is identified in sperm and semen of human for a long period of time, which can even exceed as long as 6 months (Mansuy et al., 2016; Barzon et al., 2016). In mice, ZIKV has also infected spermatogonia and Sertoli cells and reduced sperm count resulting in lower fertility (Govero et al., 2016).This is also observed in humans.

1.3 Aim of the thesis

The aim of this review paper is to discuss the structural insights of the virus, adaptive immune response of virus in host and the current status of vaccination development and the challenges for the eradication of Zika virus.

2. Zika Virus structure

Zika Virus is a spherical flavivirus with icosahedral structure consisting of a positively charged single stranded RNA genome enclosed within it. The RNA genome is about 11 Kilobases in length. After transcription and translation, the genome is converted to an elongated polyprotein and is cleaved by proteases into three structural proteins, i) capsid (C) protein, ii) pre membrane (pmR) /membrane (M) protein, and iii) envelope (E) glycoprotein. Furthermore, seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) are also produced. All these proteins execute essential functions in various stages of the life cycle of ZIKV (Sirohi et al. 2016).

Recently, 3.8 Å resolution structure of infectious ZIKV has been determined using cryo-electron microscopy. ZIKV is structurally identical to other flaviviruses but only differs in the part which is related in virus-host binding state (Sirohi et al., 2016). The structure elucidates a basis for investigation of the antigenicity and pathogenesis of Zika virus.

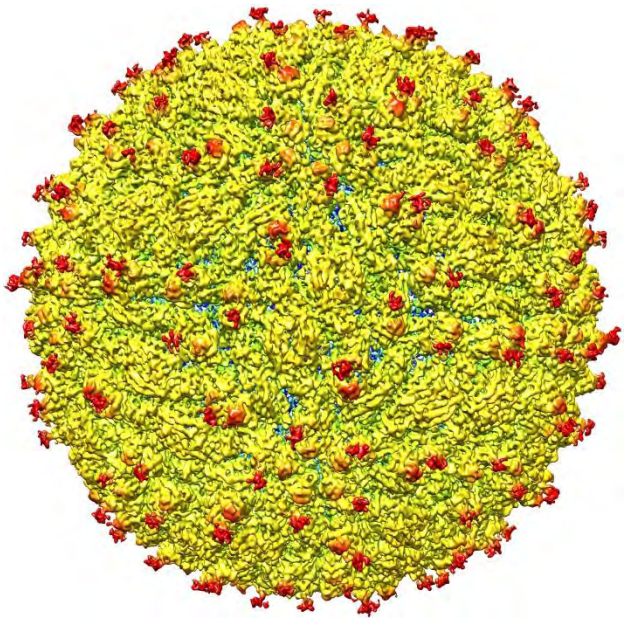

Figure 2.1: 3.8 Å resolution structure of ZIKV (Sirohi et al., 2016) [Reprint Permssion]

An illustration of the surface of ZIKV with projection of glycoproteins of Envelop (red) and is seen down the icosahedral twofold axis.

The following diagram illustrates the structure and genomic information of Zika virus in viral lifecycle.

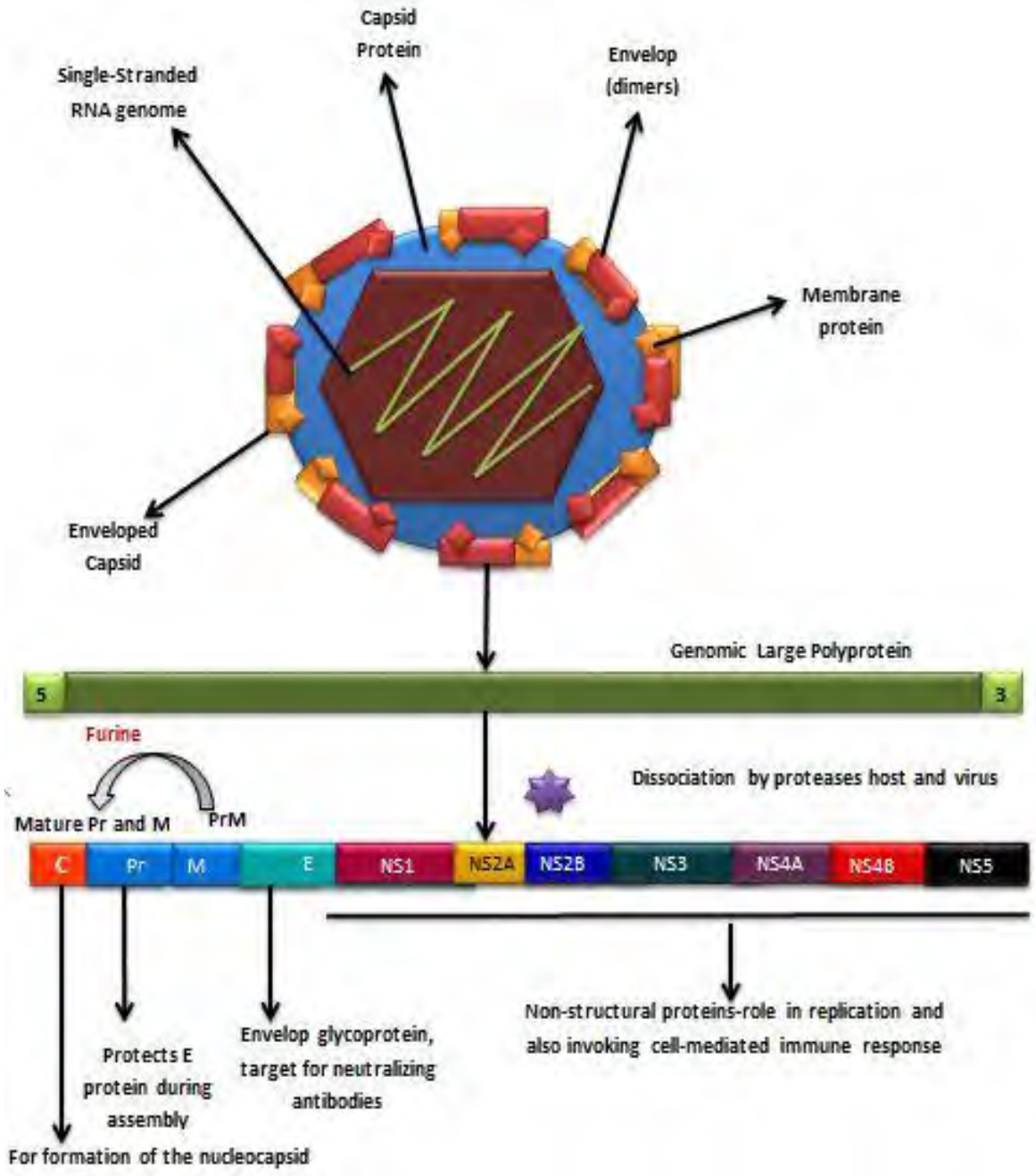

Figure 2.2: Genomic structure of Zika Virus

This is an illustration of Zika Virus along with its structural components and its genomic study. The ZIKV genome is a positive sense polypeptide with structural and non-structural genes. It also depicts the conversion of PrM to Pr and M with the action of furine enzyme.

2.1 Viral Components

2.1.1 Envelop

The Envelop of ZIKV consists of fluid lipid bilayer membrane comprising of 180 copies of the E glycoproteins and M proteins embedded on it. E glycoprotein dimers cover the viral surface compactly forming a 'herringbone' arrangement, exhibiting the icosahedral symmetry. The envelop surrounds the capsid enclosing the viral RNA genome. (Sirohi et al., 2016)

2.1.1.1 E glycoproteins of ZIKV

The ZIKV E proteins are inserted in the viral envelops as anti-parallel homodimers and it belongs to class-II fusion proteins (Mukhopadhyay et al., 2005). E monomer has large number of β-sheets structure and has three ectoderms. Domain I (DI) closely resembles barrel shape, domain II (DII) is long finger like projection with a FL tip as well as domain III (DIII) resembles C-terminal of Ig C (Dai et al., 2016). The region DII fusion loop (DII-FL) in E proteins has cross reactivity outcomes among all the flaviviruses. At E residue 154, glycan is situated on a loop that is next to the fusion peptide in the nearby E protein and is assumed to regulate the solvent entrance to the fusion loop (Nelson, et al., 2008). The main target and epitope for Nabs in contradiction of all the flaviviruses are found in the three structural domains of the E protein and as quaternary epitopes that span multiple E proteins (VanBlargan et al., 2016). Besides the epitope for various cross-reactive antibodies is the DII fusion loop (DII-FL) in E proteins and has cross reactivity outcome among all the flaviviruses (VanBlargan et al., 2016). The cross-reactive antibodies binding to DII-FL are not usually having much of neutralizing ability but there are two classes of quaternary epitope-specific (mAbs) targeting the E protein dimer strongly cross-neutralize all four DENV serotypes and also ZIKV(Barba-Spaeth et al., 2016; Dejnirattisai et al., 2015; Swanstrom et al., 2016). These cross-reactive antibodies vary significantly in potency and sensitivity according to the existence of uncleaved prM on the virion. These modifications control the sensitivity of ZIKV to antibodies that bind with the fusion loop epitopes. Furthermore, this region may also be vital for binding to cellular lectin receptors. The structural differences in the E protein of ZIKV and other flaviviruses are thought to direct cellular tropism and lead to consequences of diseases (Sirohi et al., 2016).

During infection ZIKV E-dimers are accountable for binding to cell of the host for infection. E-dimers bind to unknown cellular receptors of the host cell and are taken up to the target cells endosome. In the endosome, an alteration at the surface of ZIKV is caused as a result of the environment having acidic pH (Modis et al. 2004). The E-dimers dissociates into monomers with reorganization of the structure which allows the membrane of target cells to attach with the FL. Furthermore, the E-monomers reconnect inside the fusion facilitating trimers and then attract all the virion and cell membrane together for the termination of viral fusion. The E-protein of the mature virus is more reorganized into E homodimer creating an even exterior. However, virus maturing progression is frequently incomplete, and the formation of sharp prM-E projections upon the viral exterior will led by the uncleaved prM , which results into the formation of mosaic particles (Yu et al., 2008).

ZIKV varies structurally from other flaviviruses due to a loop of amino acids exposed on the surface of the virion. The attachment of a sugar molecule in E glycoprotein and E glygoprotein's sequence might be attributed in regulating Zika virus tropism and pathogenesis (Sirohi et al.2016). The ability of West Nile virus to enter the central nervous system of mice has been linked to glycosylation at a similar position, while cell receptors are thought to attach to sugars on the dengue virus capsid (Beasley et al., 2005)

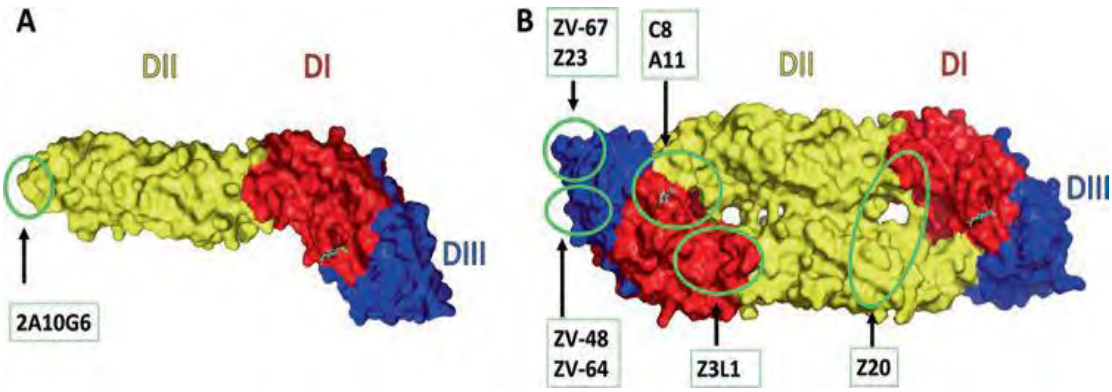

Figure 2.3: E glycoprotein of ZIKV (Shi et al. 2018). [Reprint Permission]

It is an Illustrative representation of ZIKV E proteins of ZIKV. Here A represents ZIKV E monomenr and B represents ZIKV E dimers displayed from the top view. Each E subunit contains DI, DII and DIII which are represented as yellow, red, and blue color. And, Cyan sticks here are depicting glycans which are located in the 150-loops. ZIKV E

proteins consist of the epitopes (marked by green circles) against neutralizing antibodies for ZIKV.

2.1.1.2 NS1 of ZIKA virus

NS1 is one of the non-structural proteins of ZIKV and it exists as homodimer where each monomer consists of three domains in all the flaviviruses. The domains are the β-ladder domain, the β-hairpin domain and the wing domain. In the case regarding ZIKV NS1, one β-roll dimerization part is formed because of small exchange in the N-terminal β-hairpin domain with the β-hairpin domain of another protomer. Moreover, wing domain comprises of three subdomains. It has an α/β subdomain, an elongated intertwined loop in the middle of the β5 and β6 strands and an intermittent connector subdomain linking wing domain to the β-ladder domains and to the central β-roll domains. Two NS1 monomers having the C-terminal β-ladder domains yield 20 β strands from a stretched β-sheet organized as that of rungs of a ladder. An uneven surface is formed with complex structure comprising a "spaghetti loop" in the reverse area of the surface that of ladder. There are two possible N-linked glycosylation sites in NS1 and they are greatly conserved for every flaviviruses. From the investigation of NS1 proteins among all the flaviviruses showed that the β-roll and the C-terminal tip of the central β-ladder to be the most conserved surfaces. Also, the exterior part of the wing domain is highly variable region compared to other parts. And, The ZIKV sNS1 model depicts a hydrophobic hole bounded by three NS1 dimers facing inwards, whereas the exterior part of the NS1 structure has been recommended to play a vital role in interactions of NS1 with host factors and protective antibodies (Shi et al., 2018).

In the life cycle process of ZIKV NS1 controls numerous functions. The function includes pathogenesis and replication and immune evasion of ZIKV (Amorim et al., 2014).The glycosylation of NS1 glycoprotein is vital for viral genome replication and it is found to be homodimer which is linked to the membrane once it passes the entrance in the lumen of endoplasmic reticulum. NS1 is secreted as sNS1 into the extracellular space when the host cell is infected. A sNS1 is hexameric lipoprotein gets involved with the host factors and different constituents of host immune system due to its exterior unique surface. This interaction plays key role in pathogenesis and immune evasion of ZIKV (2010; Chung et al., 2006; Avirutnan et al., 2010). Furthermore, NS1 indicates flavivirus infection during early stage due to increased level of secreted NS1. Hence, NS1 is

considered as the major biomarker in diagnosis of disease (Alcon et al., 2002). Moreover, on a recent research, isolation of MAB found in people who were infected with ZIKV resulted with antibodies against ZIKV and against NS1 proteins they are poorly cross-reactive memory T cells. Thus, ZIKV NS1 protein induces a strong immune response against ZIKV and NS1 against flavivirus infection is considered as probable candidate of immunization. Thus, ZIKV NS1 can be utilized in the progression for vaccines and also in the equipment for diagnosis aginst ZIKV diseases (Stettler et al., 2016).

Latest studies identifying crystal structure of ZIKV NS1 has revealed its significance and gained more information regarding ZIKV pathogenicity factor with its numerous aspects. ZIKV NS1 structure has exceptional surface features, and gained more information regarding ZIKV pathogenicity factor with its numerous aspects. The unique surface might be associated with the neurotropism of ZIKV and can even assist ZIKV to access the blood-placenta barrier, the blood-brain barrier, the blood-testis and the blood-eye barrier to cause possible neurological disorders (Stettler et al., 2016).

2.1.2 Capsid

The capsid is composed of capsid (C) proteins which is one of the essential structural proteins of ZIKV. The resolution is 1.9 Å of the crystalized form of protein C of ZIKV structure which illustrated that ZIKV C protein has overall structural similarity to that of DENV and WNV C structures. The crystal structure also exhibited that it exists as three dimers which are arranged in a hexameric orientation. The bottom layer of the structure comprises of four α helices facing inwards and the top layer is comprised of a unique pre-α1 loop. The four α helices at the bottom layer produces an equilateral triangle of increased positive interior surface. And the middle gap of the triangle formed helps the virus for RNA binding. Whereas, the pre-α1 loop at the top layer is essential in the formation of the dimeric assembly for membrane association. At the bilayer interface of lipid features like hydrophobic characteristics results in changed binding properties to lipid droplets of ZIKV and also to the biological membranes. Furthermore, ZIKV C protein has a wide-ranging binding capability with various nucleotide kinds, comprising one strand and two strands of Ribonucleic acids or Deoxyribonucleic acids. This structural ability has made (C) proteins to interact with viral RNA and assist in the course of assembly of the ZIKV nucleocapsid. This involves the C protein to bind with

immune response, numerous cellular proteins, apoptosis and modulating cellular metabolism and cause viral infection in the host (Zifang et al 2018).

By confocal microscopy analysis it has been observed that alteration or mutations of significant amino acid residues at the pre-α1 loop has the capability to totally inhibit buildup of C protein of ZIKV on lipid droplets in cells. Thus, it specifies the loop is crucial for membrane association and altering the loop containing the crucial amino acids which can help to inhibit interaction of lipid droplets and ZIKV C protein. Hence, this provides an opportunity for development of viral inhibitors or antiviral drugs designed against ZIKV (Zifang et al 2018).

2.1.3 Nucleic acid

The nucleic acid of ZIKV is a RNA genome consisting of a positive single strand and has a length estimating about 11 kilobases. The divergent hosts for successful replication of ZIKV are mosquitoes and primates. The nucleotide arrangement and RNA alterations of ZIKV has developed throughout the years to permit effective replication in both the hosts. Significantly, non-coding subgenomic flavivirus RNA (sfRNA) is yielded by the ZIKV RNA genome due to hindering of host5′-3′ ribonucleases on viral RNA structures in the 3′ untranslated region (UTR). The sfRNA has proviral functions including the provoking of the host innate interferon response and RNA interference. As ZIKV RNA and RNAs functions in viral assembly, replication and provokes immune response, comprehensive knowledge about viral genome will aid in the development of effective antiviral approaches and effective vaccines against ZIKV (Goertz et al., 2017).

2.2 Zika virus life cycle

Zika virions exists in three states throughout their life-

i) Immature and non-infectious,

ii) Mature, and infectious

iii) Fusogenic and host membrane–bindings states (Lindenbach et al., 2013)

The following diagram shows the detailed lifecycle of Zika Virus in a flow diagram.

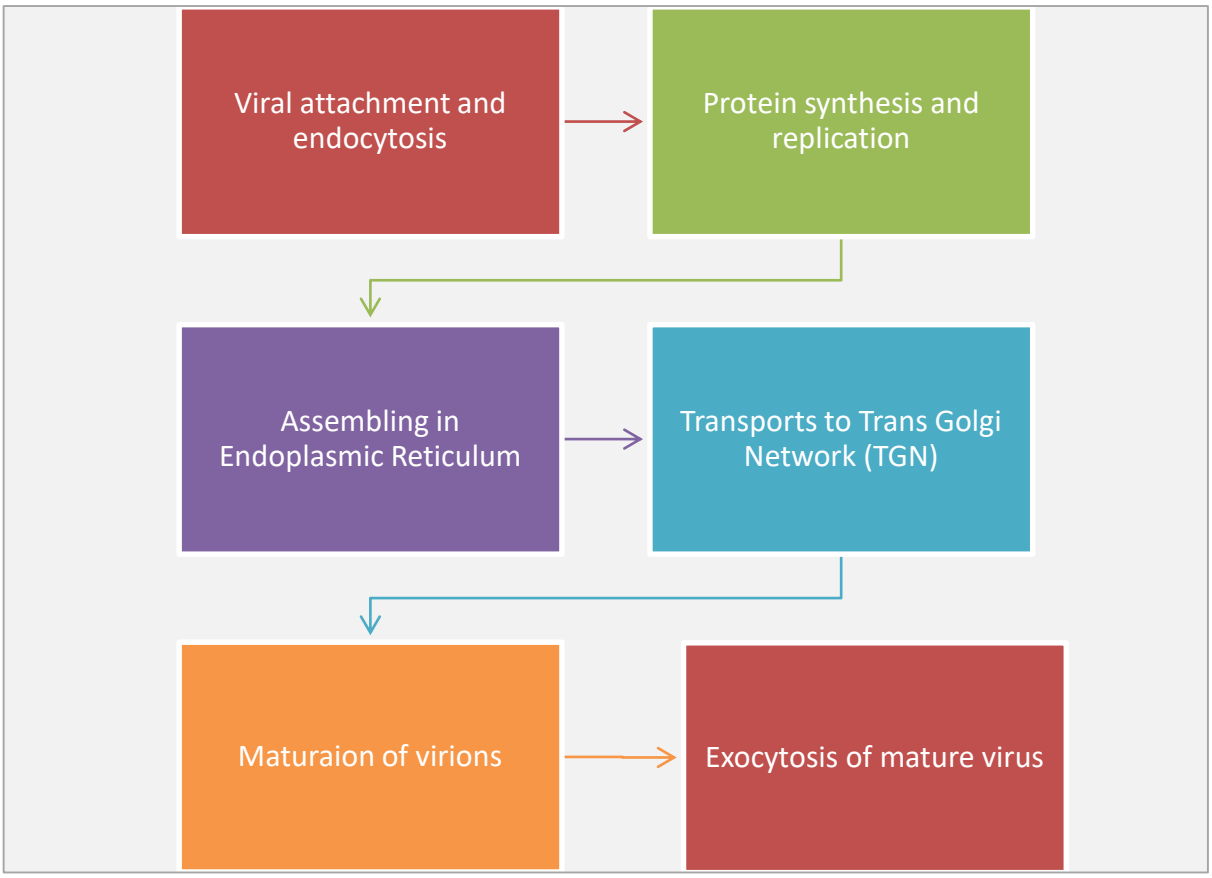

Figure 2.4: Lifecycle of Zika Virus

This is a schematic illustration of the important stages of the life cycle of ZIKV. Firstly, the attachment of virions to the host cell's surface and consequently entry of it to the cell through receptor-mediated endocytosis occurs .Then translation of the positive sense genome starts after it enters the cytoplasm of host cell, forming the only polyprotein and then its further dissociated with the aid of proteases found from virus and host . Hence, protein synthesis occurs and then on the intracellular membranes, replications take place. Furthermore, on the ER surface, the Virus assembly takes place. The resulting subviral particles and immature virions are then transported through the TGN. Then, the subviral particles and immature virion particles are converted to mature, infectious particles. Finally they are released to the bloodstream via exocytosis.

2.2.1 Viral attachment and endocytosis:

Firstly, the virus attaches to the host cell membrane by interaction of envelop (E) protein of the ZIKV and the host cell receptor found in its membrane (Chambers et al.1990). Subsequently, virus enters the host cell by receptor mediated endocytosis and clathrin protein in the acidic pH triggers this process of internalization of the virus into the host cell cytoplasm (Buathong et al., 2015).It has been found that, the lymphocyte antigen 6 locus E (LY6E) forms tubules during ZIKV infection and promotes internalization. Furthermore, the RNASEK (100-aa transmembrane protein) has also been observed facilitate viral endocytosis and helps in functioning of LY6E. Moreover, EB3 (end binding proteins) is crucial in promptly ZIKV entry uptake and LY6E tubularization. This provides a specified pathway vital for the entry of large clathrin-dependent endocytosis of ZIKV (Hackett et al., 2018).

2.2.2 Protein synthesis and replication:

After the endocytosis, the nucleocapsid is liberated into the cytoplasm. Then, the genome and capsid complex dissociates and the RNA genome gets separated. Replication of the RNA genome occurs which undergoes transcription to form mRNA. The mRNA is further translated to form seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) and a long polyprotein which gets dissociated by the action of host proteases and viral to form E glycoprotein, C and prM protein (Haddow, et al., 2012; Van et al., 2016; Lindenbach et al., 2003).

2.2.3 Assembling in Endoplasmic Reticulum (ER):

The new virions are formed and gathered inside the lumen of Endoplasmic Reticulum. The virions are immature and considered non-infectious as it consists of E-prM heterodimers which obstructs them to form fusion with the host cell. Furthermore, non-infectious Subviral particles consisting only glycoprotein and membrane are also synthesized in the ER (Yu et al., 2008).

2.2.4 Transports to trans-Golgi Network (TGN):

These immature virions and subvirals are then transported to the trans-Golgi network (Lindenbach et al., 2003).

2.2.5 Maturation of virions:

In the TGN**,** rearrangement of the E-prM hetrodimers into E homodimers and prM is produced. The prM is then cleaved by furine like protease of the host cell. Hence, the virion becomes mature and gains virulence composing of 180 copies of E and M proteins covering the viral envelop. (Li et al., 2008; Yu et al., 2008)

2.2.6 Exocytosis of mature virus:

Finally, the new mature virions are carried for exocytosis to the cell surfaces and are released outside the host cell. The infectious virions are then transferred by the bloodstream to infect more host cell in the same process (Lindenbach et al., 2003).

3. Immunology and immunity

ZIKV outbreak has been more outrageous and dreadful in the recent times compared to before, due to the occurrence of ZIKV in the same hyper-endemic areas as that of Dengue and Chikungunya virus. Diagnosing is challenging and hence the development of vaccination as well becomes more complex with it. Furthermore, amongst all kinds of erotypes found for DENV, Antibody-dependent enhancement (ADE) of infection has been evidently established. Moreover, this ADE has been suspected to boost the occurrence of ZIKV infection, because of its increased antibody production against Dengue virus (Lazear et al., 2016). For the re-occurrence of the infection, the time period of the immunity has not been confirmed yet (Tappe et al. 2015).

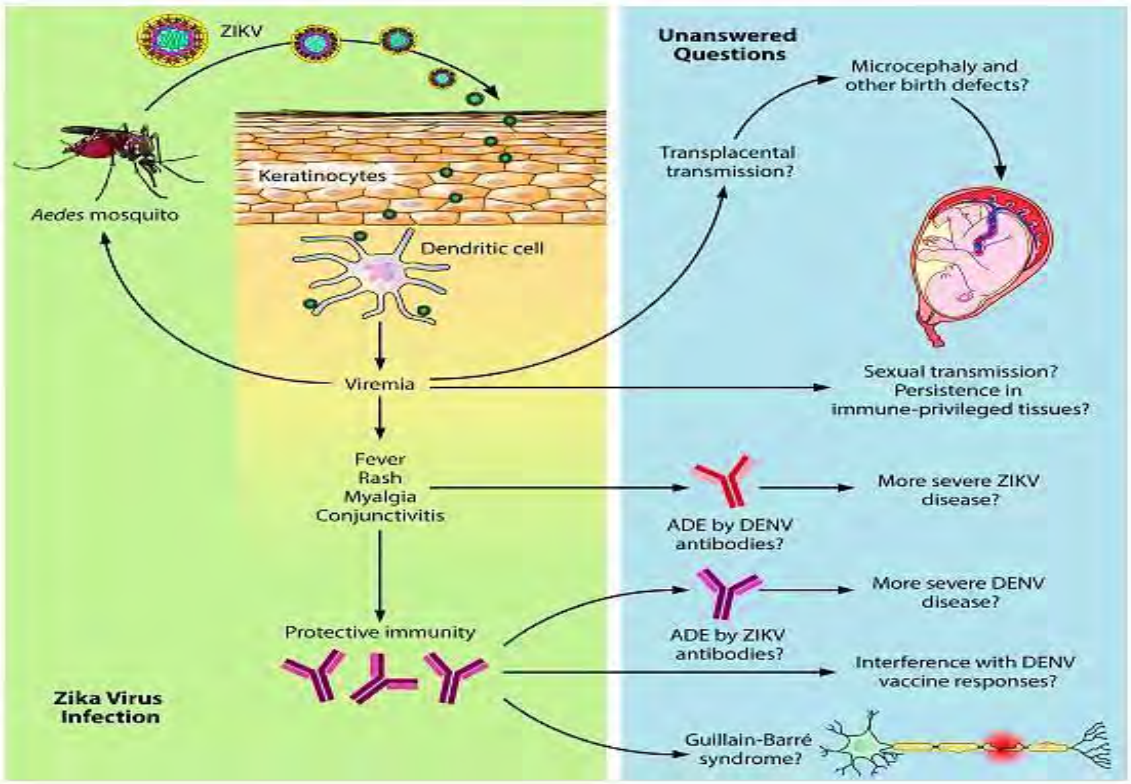

Figure 3.1: Immune response of host body towards Zika Virus (Lazear et al., 2016)

The left side of the diagram with green background shows how ZIKV is transmitted to cause viremia and resultant diseases. It also depicts the protective antibody production towards the ZIKV. The right part of the diagram with blue background with the challenges that needs to be investigated more to find out more about the immunity against ZIKV.

3.1 Changes in Immunobiology

The physiological changes during ZIKV include enhanced level of chemokines in comparison to cytokines. Also in acute phase of ZIKV infection, there is enhancement of CCL5 and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES) level. Furthermore, it has been confirmed that IL-2, IL-9, IL- 4, IL-13, IL-17 and, IFN-g has also been increased in case of acute ZIKV infection (Tappe et al. 2015).

However, during the recovery phase from the infection, the cytokines production is declined. And the IP-10 levels get inclined in the advanced recovery phase (Tappe et al. 2015). A study elucidated that human and ZIKV has a peptide in common, which has been suspected to be involved in GBS and microcephaly (Lucchese et al., 2016).More studies are required to clarify the host immune reaction to ZIKV infection.

3.2 Antibody dependent enhancement and ZIKV vaccines

In the cells exhibiting FcγR, the probability of having infection is higher when the antibodies are able to bind but are unsuccessful in neutralizing the DENV antigens. Moreover, there is then high level of viral load in the serum leading to high systemic viral titers due to ADE. The complexity of DENV infection has been associated to ADE, because of different DENV serotypes and secondary infections occurrence due to it (Katzelnick et al., 2017; Halstead et al. 2003). The ZIKV pathogenesis has been arguable when it is associated with ADE and prior Flavivirus immunity. In case of both heterologous flavivirus infection and vaccination, production of cross-reactive antibodies occurs and it synergies ZIKV infections in mice and cell culture (Stettler et al., 2016; Bardina et al. 2017; Priyamvada et al. 2016;Dejnirattisai et al. 2016), but it has not been observed in non-human primates. Rhesus macaques immunized with DENV did not show any signs of increased viral load or infection (Pantoja et al. 2017; McCracken et al., 2017). Furthermore, initial epidemiological indication in humans recommends that no boost of ZIKV disease has been seen in patients with previous DENV immunity, by comparing to other people who was not exposed to DENV disease or vaccination (Terzian et al. 2017). Exploration of vaccine efficacy concerning DENV-immunity involvement has not been carried out, but exposure of previous DENV-1 has been confirmed to have connection with higher neutralizing titers when humans are affected

by a secondary infection of ZIKV (Robbiani et al. 2017). The consequence of the corresponding sequence, where the effect of DENV pathogenesis due to prior ZIKV immunity also has been unclear, yet evidently has associations for ZIKV vaccine developments in hyper-endemic areas of both DENV and ZIKV. Both, in-vivo studies with mouse strains and cell cultures have confirmed to increase DENV infection when isolated antibodies from ZIKV infection or vaccination are used (Richner et al. 2017; Stettler et al., 2016). Current studies with non -human primates have elucidated that pre-existing ZIKV infection can cause elevated serum viral titers and increased level of cytokine and then finally causing DENV-2 infection. ZIKV vaccines which are under progress are designed against E proteins and it has the ability in generating the cross-reactive antibodies aiming for the domain II's fusion loop (Modjarrad et al. 2017; Tebas et al., 2017 ; Gaudinski et al., 2017). In the time of vaccination development program, considering the increment of DENV infection due to early ZIKV vaccination is important.

The description of the involvement of cross-reactive antibodies in the increased disease pathogenesis due to ADE and its vaccine efficacy will be difficult, which needs to carry out by analyzing and titration of early flavivirus antibody level in the studies including non-human primates. In Philippines, the vaccination using tetravalent Dengvaxia vaccine against DENV was terminated. Because it was observed to cause serious Dengue complications in children receiving that vaccine in compared to those children who were not immunized (Halstead et al., 2018). Hence it is crucial to confirm the safety of ZIKV vaccines by the researchers, in endemic regions having population who didn't receive this immunization before. Meantime, there has been various research and manufacturer groups working with ZIKV vaccines, have established many approaches to halt the cross-reactivity of flavivirus in the perspective of ZIKV vaccine. One research has observed reductions of ADE in both in vitro and in vivo of DENV, even if it had partial protection against ZIKV as comparable level of neutralizing antibodies were produced. In that study a vaccine was designed by discarding the conserved fusion loop epitope responsible for cross-reactivity, in a prM-E ZIKV vaccine and cause reduction in ADE (Richner et al. 2017). Moreover, there are other vaccines consisting of regions that are target of the viral proteome that does not confer ADE (Brault et al., 2017; Yang et al., 2017). For instance, vaccine utilizing vaccinia vector generates NS1 which is a non-structural protein of ZIKV, and it elicits anti-NS1 antibody reactions as well as CD8+ T

cells (Brault et al., 2017). Since NS1 is not displayed on the viral particle's surface, the antibodies which are generated, does not cause any increaser viral entry into the cells consisting of FcγR.

3.3 Adaptive immunity to ZIKV and vaccination

Generation of long lasting adaptive B and T cells is essential for protection against virus by inhibiting the viral replication and spreading of that virus in the host. For successfully eliminating the viral infection, an effective viral vaccination is required to induce adaptive immunity. This idea is supported by the observation, where rhesus macaques infected with primary ZIKV suppressed the secondary infection with both heterologous and homologous virus (Aliota et al., 2016; Dudley et al., 2016; Osuna et al., 2016) by its adaptive immunity mechanism and provided protection. Numerous groups of vaccines against ZIKV has shown to evoke CD4+ and CD8+ T cells immune response and also produced high amount of neutralizing antibody. Besides, it has been confirmed that just antibodies itself has the capability for providing protection against ZIKV to both unimmunized mice and rhesus monkey, when IgG from rhesus moneky which are immunized with PIV are passively transferred to those unimmunized recipients (Abbink et al., 2016).Correspondingly, immuno-deficient mice (Tebas et al., 2017) were protected against ZIKV, when sera isolated from humans immunized with DNA or PIV vaccines encoding for the prM-E viral epitopes by passive transfer of the sera has successfully inhibited ZIKV viremia (Modjarrad et al. 2017).

To identify the association of protection, it is essential to estimate the threshold neutralization titers required for immunity provided by vaccine. In case of immunization of non-human primates, a neutralizing titer threshold in the systemic circulation needs to be around 1/100 (Abbink et al., 2016; Abbink et al. 2017; Pardi et al., 2017) to 1/1000 (Dowd et al., 2016) to suppress ZIKV challenges after the immunization. This idea was extended in human clinical trials, where two doses of PIV immunization or three doses of PrM-E generated by a DNA vaccine was able to attain average neutralizing antibodies titers exceeding 1/100 threshold (Modjarrad et al., 2017; Gaudinski et al., 2017). Apart from these outcomes, to achieve sterilizing immunity, greater level of neutralizing antibody titers is needed. Additionally, the neutralizing antibody titer was required to be

more than 1/10 000 to inhibit presence of ZIKV in blood, tissues which are prone to viral load and for the secondary immune response against ZIKV, in immuno-deficient mice (Richner et al., 2017). But it is yet to be clarified if sterilizing immunity is an important consideration in hindering of vertical transmission of ZIKV.

The epitope targets displayed on the E protein surface of protective and strong neutralizing monoclonal antibodies were demonstrated from depiction of memory B cells and plasmablasts, which were circulating in the serum of ZIKV-infected patients (Stettler et al., 2016; Sapparapu et al., 2016; Robbiani et al., 2017; Wang et al.,2016;Rogers et al., 2017). The antibodies (EDE mAbs) were generated against the quaternary epitopes spanning domains I, II, and III throughout the E protein. Furthermore, antibodies were also produced against the ridge epitope that is laterally situated in domain III and the vastly neutralizing sites were domains I or II (Sapparapu et al., 2016; Robbiani et al., 2017; Wang et al.,2016;Rogers et al., 2017; Dai et al., 2016; Swanstrom et al., 2016). On the other hand, weak neutralizing sites were the conserved fusion loop which is locate in domain II, and are targets for strong cross-reactive antibodies and this result in ADE infection (Stettler et al., 2016; Zhao et al., 2016).

Perhaps, there are various vaccines under development can elicit memory B cells as well as antibodies with distinctive specificity of epitope, which maybe are critical for cross-reactivity and protection against ZIKV. Outlining the epitopes of the polyclonal antibodies induced from immunization is a significant objective where structural analysis can confer long term protection and efficacy.

Furthermore, not only B cells are able to facilitate immunity but also T cells can confer protection against ZIKV. In studies it has been observed that CD4+ and CD8+ T cells which has multifunctional roles and which had many epitopes to confer immunity against ZIKV (Grifoni et al., 2017), was determined in infected human, non-human primates and mice [Dudley et al., 2016; Grifoni et al., 2017; Huang et al., 2017; Elong et al., 2017; Muthumani et al., 2016). Additionally, when mice prone to ZIKV infections were subjected to a strategy of removal of their CD8+ T-cells, it was seen that the viral load and death rate increased. However, with passive transfer of T cells in the CD8+ T cell deficient mice were shown to inhibit ZIKV infection (Huang et al., 2017; Elong et al., 2017). T cell immunity is stimulated by immunization. In supporting this idea, induction of antiviral CD4+ and CD8+ T cells were observed when mice were

immunized with mRNA or DNA vaccines producing prM-E or when non-human primates were immunized with DNA, PIV or adenovirus-vectored vaccines (Abbink et al., 2016; Pardi et al., 2017; Muthumani et al., 2016; Larocca et al., 2016). Nevertheless, there is no impact in the protective immunity when in mice with absence of CD4+ and/or CD8+ T cells, when they are immunized with DNA vaccine (Larocca et al., 2016). This signifies that even if T cells are involved in mediating adaptive immunity, but they are not significant in providing protection in significantly.

4. Vaccination Development for ZIKA Virus

4.1 Significance of ZIKV vaccination

Notably, the recent epidemics of ZIKV has led to studies which has displayed a strong connection of ZIKV infections with destructive Congenital Zika Syndromes (CZS), involving various neurological problems like inherited deformity, fetal death and microcephaly from woman who are pregnant and who has been diagnosed with ZIKV (Hoen et al., 2018). Moreover, studies have confirmed that ZIKV results in an auto immune disorder named Guillain-Barre´ syndrome which is caused by the failure of self-recognition of immune system destructing own peripheral nerves, resulting in rapid initiation of lack of muscle strength and even loss of sensation in adults (Dos Santos et al., 2016). This revealed that ZIKV was not an infection associated with mild diseases but was able to cause CZS and GBS and was life threatening. Because of such outburst and developmental malformations, the World Health Organization announced vaccine development for ZIKV as an international and the topmost concern in February 2016. From that point onwards, serious worldwide endeavor has been achieved towards comprehension of ZIKV biological analysis and development of vaccines against ZIKV eradication, with an extraordinary high rate, prompting strong foundation of a hopeful immunization pipeline.

4.2 Approved Vaccines against Flavivirus

Globally, several infections caused by flaviviruses have been minimized due to the development of effective vaccination programs against those microbes. The possibility of ZIKV vaccines to be safe and efficacious to obtain license is assisted by other flaviviruses to get approval and license. This includes YFV to gain live-attenuated immunization, TBEV to obtain inactivated immunization. Also, JEV is utilized to obtain live-attenuated as well as inactivated immunization, and chimeric live-attenuated immunization programs were obtained using DENV. A guideline for the development of ZIKV vaccines can be perceived from the important insights of these approved flavivirus immunizations. It was a challenging job to develop a tetravalent DENV vaccine because of four different serotypes of DENV consisting of only 30% to 35 % amino acid dissimilarity among serotypes. For DENV vaccine to be effective it needs to generate

lifelong immunity in contradiction to all types of serotypes, because a partial immunization upon subsequent DENV diagnosis can emerge to be sensitized to dangerous dengue shock syndrome or hemorrhagic fever. This idea is supported by the outcomes of the overall efficacy of the trials of 2b/3 phase which has proven these problems when using Dengvaxia. Also, the children whose age were below 9 and whose blood didn't have DENV during immunization, showed intensification in the risk of hospitalization of those who were given Dengvaxia. In comparison with DENV strain, Asia and Africa originated genomic strains are found in case of ZIKV with just less than 5 % variation in amino acid among them. ZIKV has been proven to be a single serotype, as many of the strains of ZIKV from both Asian and African lineages have demonstrated that they are equally neutralized by sera from animals or human who have been diagnosed with ZIKV (Dowd et al., 2016). On this basis, it is seen that using DNA vaccines, adenovirus vectored vaccine or purified inactivated vaccine causes cross neutralization of ZIKV strains from Uganda, Thailand, Puerto Rico, Philippines and Brazil when non-human serum is used (Abbink et al., 2017).

4.3 Ongoing clinical trials of ZIKV vaccines

Currently, there is no ZIKV vaccine which gained approval or licensing. Nevertheless, due to WHO's concern in 2016, many researchers and companies have initiated and are still developing numerous vaccine candidates which have been confirmed to have potential results in both non-clinical(table 4.3) and clinical trials (table 4.2). These research works from multiple laboratories have headed to the exceptional leap for the ZIKV vaccine development.

Presently, the researchers are working with various kinds of vaccine candidates with animal models and who has advanced to also working on human volunteers (table 4.1).

Table 4.1: List of type of vaccine candidate for ZIKV (WHO, 2017)

Vaccine candidates	**DNA vaccines**	**Recombinant E or E+ NS1 proteins**	**Virus-like particles (VLPs)**
	Purified inactivated viruses (PIVs)	**mRNA vaccines**	**Measles virus (MV)**
	Live attenuated viruses (LAVs)	**Viral vectored vaccines**	**Adenovirus (Ad) vectors**

Among these candidates, majority have undertaken an effective preclinical development. There is successful induction of neutralization antibodies where they conferred protection and inhibit the ZIKV infection for short term and long term duration in the test animals like mice and monkeys as well. Moreover, a notable quick progression has taken place where these candidates leaped towards the clinical trials of phase I (Barrett et al. 2016). So far, there are 14 ongoing researches with various ZIKV immunizations, for it to get licensure for treating pregnant woman along with their fetuses and adults who are very much prompt or susceptible towards ZIKV, living in the ZIKV endemic regions.

Table 4.2: List of vaccines against ZIKV in the clinical development

Vaccine	Type	Antigen	Developer	Clinical Trial	Stage	Reference
VRC5283	DNA	prM-E (ZIKV)	NIAID	NCT02996461	Phase I	Gaudinski et al. 2017
				NCT03110770	Phase II	Dowd et al. 2016
VRC5288	DNA	prM-E (Chimeric ZIKV-JEV)	NIAID	NCT02840487	Phase I	Gaudinski et al. 2017; Dowd et al. 2016
GLS-5700	DNA	prM-E	GeneOne Life Science Inovio Pharmaceuticals	NCT02809443	Phase I	Tebas et al. 2017 Muthumani et al. 2016
mRNA-1325	mRNA	prM-E	Moderna Therapeutics BARDA	NCT03014089	Phase I/II	Richner et al.,2017 Richner et al.,2017
ZPIV	Purified inactivated virus	Whole virus	WRAIR	NCT02963909	Phase I	Modjarrad et al., 2018
			Saint Louis University	NCT02952833	Phase I	

			BIDMC	NCT02937233	Phase I	
TAK-426	Purified inactivated virus	Whole virus	TAKEDA BARDA	NCT03343626	Phase I	TAKEDA 2017
VLA1601	Purified inactivated virus	Whole virus	ValNeva Emergent BioSolutions	NCT03425149	Phase I	Clinical Trial.Gov 2018
MV-ZIKA	Measles-vectored	prM-E	Themis Biosciences	NCT02996890	Phase I	Drug Development Technology 2017
Ad26. ZIKV.001	Adeno-vectored	Ad26-M-Env	Janssen Vaccines Prevention B.V	NCT03356561	Phase I	Clinical Trial.Gov 2018
AGS-v	Peptide	prM-E	NIH	NCT03055000	Phase I	NIH 2017

Table 4.2 summarizes the vaccine candidates which has being analyzed successfully in clinical I and clinical II phases. They will be reviewed in details in the following section with their efficacy as well.

Table 4.3: List of ZIKV vaccine candidates which are in non-clinical and preclinical stage (Poland et al. 2018)

Type	Stages	Candidates / Developers	Vaccine
Inactivated	Preclinical	Bharat Biotech	Purified inactivated virus
	Preclinical	NewLink Genetics	Purified inactivated virus
	Preclinical	PaxVax	Purified inactivated virus
	Nonclinical	Bio-Manguinhos	Purified inactivated virus
	Nonclinical	Valneva	Purified inactivated virus
	Nonclinical	GlaxoSmithKline	Inactivated, whole virus
	Nonclinical	Emergent BioSolutions	Alum adjuvanted, inactivated whole virus
	Nonclinical	Takeda	Alum adjuvanted, Inactivated whole virus
Live	Non clinical	Geovax	Live modified vaccinia ankara recombinant
	Nonclinical	US Centers for Disease Control and Prevention	Live adenovirus recombinant
	Nonclinical	Oxford University	Live adenovirus recombinant
	Nonclinical	Sementis	Live poxvirus recombinant

	Nonclinical	Themis Bioscience	Live measles recombinant
DNA	Preclinical	Pharos Biologicals	DNA vaccine
	Non-clinical	Bio-Manguinhos	DNA vaccine
RNA	Non-clinical	CureVac	Thermostable mRNA-based vaccine
	Non-clinical	GlaxoSmithKline	Self-amplifying mRNA platform
	Non-clinical	Morderna	Lipid nanoparticle-delivered mRNA
Peptide/ Protein nanoparticle	Preclinical	Novavax	Protein nanoparticle vaccine
	Preclinical	Replikins	Synthetic peptide vaccine
	Non clinical	Hawaii Biotech	Alhydrogel and recombinant protein
	Nonclinical	Protein Sciences	Recombinant envelope protein
	Nonclinical	Mayo Clinic Vaccine Research Group	Naturally processed and HLA presented ZIKV peptides packaged with biodegradable nanoparticles

	Non clinical	Sanofi Pasteur	Yellow fever 17D chimera
	Nonclinical	Jenner Institute	Simian adenovirus vector
	Nonclinical	Bio-Manguinhos	Yellow fever 17DD chimera
	Nonclinical	Vaxtart	Recombinant oral vaccine
Virus like particles	Preclinical	Bharat	Virus-like particle expressing polyprotein
	Non clinical	Bio-Manguinhos	Virus-like particle
	Non Clinical	US Centers for Disease Control and Prevention	Virus-like particle expressing Zika virus DNA
	Non Clinical	Institute Pasteur of Shanghai	Recombinant subunit virus-like particle
	Non Clinical	VBI Vaccine	VLP containing envelope and NS1

Table 4.3 reviews the total situation of ZIKV vaccine candidates in the pipeline of their development progress. They have been only tested in test animals but are soon believed to advance to clinical phase 1 trial. The subsequent part focuses on the summarization of the published outcomes of the trials involving both animal and human models of notable vaccine research institutions.

4.3.1 Subunit vaccines against ZIKV

Subunit vaccines consist of two important ZIKV epitopes, prM and E. These epitopes are supported utilizing various vectors, which involves mRNA, DNA, and even vectors which are viruses including, adenovirus, modified vaccinia virus, measles virus and vesicular stomatitis virus. On the other hand, subunit vaccine can be directly expressed using E protein produced by recombinant technology, or by using purified particles similar to viruses, consisting prM and E proteins. Efficacy is confirmed in mice and non-human primate in several of these subunit vaccine against ZIKV infection (Larocca et al., 2016; Richner et al., 2017; Abbink et al., 2016; Pardi et al., 2017; Dowd et al., 2016).

4.3.2 DNA vaccines against ZIKV

DNA vaccines has a prevailing strategy where the host cells easily take it and employ the endogenic translation and transcription mechanism of the host cell for formation of target viral proteins, which works as antigens. DNA vaccination is a process by using genetically engineered DNA contains the gene of interest to encode the wanted antigen for immunological response against ZIKV. Developing of and production of DNA vaccines are rapid, and have the ability to induce both humoral and cellular immune responses (Ferraro et al., 2011) Administration route of the vaccines are intradermal electroporation, which allows optimal cellular entry and following protein production. ZIKV DNA vaccine studies has shown data designating alteration in sequence antigen in anti-ZIKV immunity through the full-length prME gene, full-length E gene, and C-terminal shortened E ectodomain gene (Larocca et al., 2016).

Vaccine Research Center (VRC) of the US National Institute of Allergy and Infectious Diseases (NIAID) has also led two phase I clinical trials. The first clinical trial started in August 2016 (clinical trial NCT02840487) with the first ever DNA subunit vaccines (VRC5288 and study VRC319). It was constructed to produce the ZIKV prM-ENV utilizing a JEV envelope stem region, JEV region is added to enhance the discharge level of hollow particles similar to virus (Gaudinski er al., 2018). In this study, the vaccines were administered by intramuscular injection to the participants who received three 4mg doses of it. The doses were given at an interval of 0 and 8 weeks, 0 and 12 weeks, 0, 4 and 8 weeks, or 0, 4, and 20 weeks. The study showed 89% of the participants with identifiable neutralizing antibodies and Neutralization antibody GMT titers were 120. The same center led another clinical trial (clinical trial NCT02996461)

assessing the second DNA subunit vaccine (vaccine VRC5283 and study VRC320). The DNA vaccine was designed for expression of a wild-type ZIKV prM-ENV and the viral genome (H/HF/2013) found from French Polynesia was used for the sequence of the prM-E (Gaudinski er al., 2018).In this study, schedules of administration of four kinds were studied: injection with single-dose needle, injection with split-dose needle, injection with single-dose needle-free using the Stratis device, or injection of split-dose needle-free. The vaccine was administered to the participants who received 4mg doses of it at 0, 4 and 8 weeks (Yousafzai et al., 2017). In this study, 100% of the participants of the split dose, needle free administration group generated with the neutralizing antibody responses and at the week 4 the utmost reactions were recorded with the last immunization. The measurement made using a reporter replicon particle assay, observed that the neutralization antibody titers of R1:100 with GMT was 304. The DNA subunit vaccine also showed a substantial T cell response (Gaudinski et al., 2018). Two of the clinical studies ensured the vaccine caused mild to moderate adverse reactions with local pain and tenderness (46%–80%) at the injection site, malaise (27%–38%), and headache (22%–33%). Moreover, it also reported that both the DNA vaccines were immunogenic and well tolerated. But the VRC5283 vaccine was more immunogenic than that of the vaccine VRC5288 of the first clinical trial. Recently, VRC5283 has promoted to phase II efficacy trial of vaccination at 0, 4, and 8 weeks using needle-free delivery, in endemic regions for ZIKV transmission which includes South and Central America, the Caribbean and the United States (NCT03110770).

GeneOne Life Science and Inovio Pharmaceuticals (clinical trial NCT02809443) conducted another clinical evaluation of the safety and immunogenicity of a ZIKV DNA subunit vaccine (GLS-5700 DNA) and indicated the ZIKV precursor membrane and envelope (prM-ENV) genes, derived from pre-2016 human ZIKV strains.Dose of either 1mg or 2mg of the GLS-5700 DNA vaccine was given to two groups consisting of 40 participants. The vaccine along with boosts at week 4 and week 12 was administered to the participants by intradermal injection with electroporation at weeks 0, 4 and 12..No severe adverse reactions were observed because of the vaccine, but around 50% individuals reported injection site pain, redness, swelling, and itching. By means of enzyme-linked immunosorbent assay (ELISA), antibody levels against ZIKV were evaluated at the 14th week, and it was complete seroconversion for binding antibodies in both dose groups. The outcomes specified that the antibodies produced due to

vaccination were dose dependent. In 60 % of the 1 mg dose groups and 63% of the 2 mg dose groups had the Neutralizing antibody titers above the detection limit. The DNA vaccine elicited both humoral response and also T cell activation. Study shows that the adoptive transmission of vaccinated human serum for 14 weeks into interferon (alpha and beta) receptor causes (A129) mice to die and hence it is a fatal challenge of ZIKV (Aliota et al., 2016). But in this study, it led to 92% of survival of the mice with such lethal dose ensuring that clinical phase I clinical trial of this DNA vaccine was safe and with no serious adverse effects. . Surprisingly, five vaccinated human serum with no neutralizing titer managed to confer protection of those mice in this experiment with lethal dose (Tebas et al., 2017). Hence, these data recommends that the survival of the mice was not dependent on the neutralizing titer resultant from vaccinated humans. More studies are needed for determining the molecular mechanism that contributed to this independency of the neutralization titers to improve the understanding of this vaccine. Though in the clinical phase I trials, the DNA subunit vaccines displayed promising immunogenicity, the long life of protective immunity has to be displayed in the humans too. It has been seen that in 1 year period, monkey recipients administered with two vaccinations of a DNA subunit vaccine caused decrease in protective efficacy and declination in neutralizing antibody titers to minimal protective level (Abbink et al., 2017).

To date, the DNA-based vaccines results have been confirmed to be safer than the live attenuated viral vaccines. Because live attenuated viral vaccines are not capable to revert and replicate, which is a significant safety concern mostly in pregnant women.

4.3.3 mRNA vaccines against ZIKV

The mRNA vaccines are an innovation (Pardi et al. 2018) which has been designed contrary to ZIKV. The mRNA vaccines works by encoding of gene of interest under the influence of a promoter. The mRNA vaccine does not require crossing the nuclear envelope for expression because proteins are directly translated by mRNA right after the entrance into the cell cytoplasm. This passageway of mRNA could effectively reduce the doses which are essential for the safety and immunogenicity as compared to DNA vaccines. There is possibility of vaccines being economically effective due to huge amount of doses can be produced efficiently.

Moderna Therapeutics and Biomedical Advanced Research & Development Authority (BARDA) are the first one to conduct phase I/II clinical trial (NCT03014089) using human volunteers. It is presently continuing to evaluate the safety and immunogenicity of escalating doses of mRNA (mRNA-1325) expressing prM-ENV. The lipid nanoparticle encapsulates ZIKV prM-ENV mRNA for delivery and stability (Reichmuth et al., 2016). Vaccination employing mRNA vaccines produced high levels of neutralizing antibodies that conferred protection of monkeys and mice against ZIKV infection (Richner et al. 2017; Pardi et al., 2017). In an animal model study using pregnant mice, it was observed that immunization with mRNA vaccines stopped the fetal demise, while fetal resorption was found in infected pregnant mice which did not receive the vaccine. Though, the maternal spleen, maternal brain, placenta and fetal head had detectable levels of ZIKV RNA in mice which were vaccinated (Richner et al., 2017). As these outcomes were attained in an immunocompromised mouse model, which assisted in the enhancement of viral replication, it is unclear if viral replication would also be observed in animal models with immune-competency. Yet, it is to find if these pre-clinical data are successfully translated in human volunteer as well. Furthermore, there is also a concern in the stability of the mRNA vaccines. Moderna Therapeutics is presently funding a phase 1 clinical trial of mRNA-1235 ZIKV vaccine to evaluate its safety, acceptability, and immunogenicity in immunocompetent human.

4.3.4 Purified inactivated virus vaccines against ZIKV

The ZIKV purified inactivated virus vaccine (ZPIV) is a vaccine which consists of ZIKV strain (PRVABC59), derived from Puerto Rico and that have been grown in Vero cells culture. The strain was then inactivated by 0.05% formalin and was subsequently purified by chromatographic column and formulated with alum adjuvant (Abbink et al., 2016).

ZIKV PIV vaccines (a test led by US Army) after 4 weeks of a single dose administration, elicited protection against viremia in the mice (BALB/c) against ZIKV-BR strain. This vaccine was further analyzed in rhesus monkeys for immunogenicity and protective efficacy. Constant with the study with mice, neutralizing antibodies against ZIKV were also induced in monkey recipients, and it conferred protection contrary to of following challenges of both ZIKV-BR and ZIKV-PR strains. No viral load was

identified in serum, urine, or cerebrospinal fluid of the immunized monkeys (Abbink et al., 2016; Barouch et al., 2017).

To date, Walter Reed Army Institute of Research (WRAIR), Saint Louis University, and Beth Israel Deaconess Medical Center (BIDMC) are all situated in United states and are conducting three phase I clinical trials(NCT02963909, NCT02952833 and NCT02937233). They are collaborating for a short term investigation of the initial outcomes for the same group for each separate study. (Modjarrad et al., 2018) This combined short-term investigation comprised of the human volunteers from individual study they had been administered intramuscularly with 5μg ZPIV along with added aluminum hydroxide adjuvant. They were given two-dose regimen on day 1 and day 29. ZPIV triggered only mild to moderate adverse reactions with reports of local pain or tenderness at the injection site, fatigue, headache, and malaise, with no reports of any severe adverse reactions. By usage of microneutralization (MN50) assays at WRAIR, Neutralizing antibody titres of all three clinical trials were identified. After administration of second dose, 95% of the volunteers' peak neutralizing antibody titers was observed with a GMT of 286. On day 57, 92% of the participants had neutralizing antibody titers of R1:10 and 77% of the participants had neutralizing antibody titers of R1:100. The studies of adoptive transfer of purified immunoglobulin G from immunized volunteers to mice was carried out. It was observed that, when the neutralizing titers reached >1:100 in receiver mice, it conferred complete protection against ZIKV in 41 out of 50 BALB/c mice. Moreover, it also minimized viraemia of the infected mice (Modjarrad et al., 2018).Conclusion of these trials confirmed that the inactivated ZIKV vaccine was well tolerated and immunogenic and also the antibodies produced caused protection in adoptive transfer studies in mice. More follow-up comprehensive studies are needed to define the effect of different doses and immunization plans.

Takeda Pharmaceutical Company Ltd is also conducting phase I clinical trial with a ZIKV inactivated vaccine (TAK-426) and is still an ongoing process (NCT03343626). The safety and immunogenicity of this candidate will be evaluated by a dose increment study in 240 healthy human volunteers. (TAKEDA, 2017)

Contrasting to the live-attenuated viral vaccines, an inactivated vaccine does not possess the possibility of reactivation and viral replication, thus can be effectively used in pregnant women or in individuals with compromised immunity. An adjuvant is usually

required which can complicate the usage of a purified inactivated virus (PIV) in case of pregnancy.

4.3.5 Viral-vector-based vaccines against ZIKV

Viral-vector based vaccines are another favorable method to immunize against ZIKV. Moreover, these vaccines have displayed promising outcomes for ZIKV in preclinical studies, and was successful vectors for other vaccination development against various disease causing microorganism (Baden et al., 2016, Ledgerwood et al. 2015) .The Protection is conferred against ZIKV infection in preclinical models by vaccine induced high humoral and cellular immunity (Ura et al., 2014; Lauer et al., 2017).

Themis Bioscience GmbH is leading the development process of an MV ZIKV vaccine which is now in a phase I clinical trial (NCT02996890). The MV Schwarz vaccine strain (Combredet et al, 2003) was constructed to produce ZIKV prM-ENV (MV-ZIKA) and mice and monkey recipients are used for assessing the immunogenicity (ZIKAVAX et al., 2018). The phase I clinical trial which is still proceeding for analyzing the safety and immunogenicity, both high and low dose are administered as single or two-dose schedules.

Adenovirus vectors have been extensively used in many vaccine developments for viral infections, and have successfully offered numerous benefits. They are capable of inducing a wide-range and robust immunity against virus and are also easy to produce. Janssen Vaccines and Prevention B.V. are sponsors for development of an Ad serotype 26 (Ad26) ZIKV-based vaccine (Ad26. ZIKV.001). It is engineered to produce same antigen as that of rhesus Ad serotype 52 (RhAd52) preclinical vaccine candidate. The phase I clinical trial (NCT03356561) of this vaccine is an ongoing study at two sites of United States (Kansas and Massachusetts). The study comprises of using vaccine where two kind of doses will be administered in a double-blind, placebo-controlled clinical trial. This will provide result if it is a safe vaccine and if it can provide immunogenicity as well. There is a possibility of enablement the advancement of this vaccine candidate is there due to enhancement of knowledge regarding Ad26 vaccine vector as it was also used in many in clinical trials for other disease causing microbes (Barouch et al. 2013; Baden et al., 2016; Winslow et al., 2017; Milligan et al., 2016).

Another recombinant adenoviral vector (pAd.ZIKV-Efl) was developed (Kim et al. 2016) and that produced a codon-optimized E antigen derived from ZIKV strain (BeH815744) (Kim et al. 2016). The sequence of E was connected to the trimerization domain of T4 fibritin, a Tobacco Etch Virus protease, and a six-histidine tag, to enhance folding, cleavage, and purification of the vaccine. On day 14, mice (C57BL/6) were administered with the vaccine and booster via subcutaneous pathway and there was elicitation of humoral immunity in the mice. Moreover, antibodies against ZIKV were identified after 2 weeks from the booster shot (Kim et al. 2016). Additionally, passive protection was confirmed in the infants born to the pregnant immunized mice, as the infants did not undergo any weight loss and death after inoculation with ZIKV strain (DAKAR41542) [22]. Furthermore, more vectors have been in a process of development with negligible to no history of immunity (Abbink et al. 2018).

4.3.6 Live-Attenuated Vaccines against ZIKV

An attenuated vaccine is a vaccine formed by decreasing the virulence of a pathogen, but still keeping it alive. Attenuation converts the harmful pathogen harmless or less virulent by altering it [Halstead et al., 2017; Capeding et al., 2014). Nevertheless, the attenuation may require more boost to confirm long-term protection. The engineering of live-attenuated ZIKV vaccines has followed a similar approach as that of DENV live attenuated tetravalent chimeric vaccine (Dengvaxia). Generally, the development of live attenuated viruses involves stability between immunogenicity and safety (Capeding et al., 2014). Stimulation of a robust immune response is seen with low level of attenuation but it produces viremia and neuro-virulence and enhances the likelihood of viral relapse, shedding, and spread. In Contrast, high level of attenuated viruses are safer but they stimulate immune responses weakly. In April 2017, Themis Bioscience initiated the first global research of a live attenuated ZIKV vaccine utilizing a MV backbone (NCT03014089)

Two methods have been used to create live-attenuated ZIKV vaccines. The first method includes constructing attenuating mutations in a genuine ZIKV isolate. In this method two live-attenuated vaccine candidates is engineered by deletion from a pre epidemic Cambodian strain (FSS13025) of a 10-nt (ZIKV-30 UTR-D10) or 20-nt (ZIKV-30 UTR-D20) in the 30 UTR of the ZIKV genome (Shan et al., 2017b). The choice of Cambodian strain (FSS13025) was because it is weakened in neurovirulence, mosquito

infectivity and immune antagonism, in comparison to that of the epidemic American strain (Xia et al., 2018). Efficacy is exhibited in both mice and non-human recipients after a single-dose immunization. Using mCherry ZIKV assay, the neutralizing titers antibody was measured and found to be >1:1,000 in 14 days by the 20-nt deletion candidate and it caused sterilizing immunity in monkey recipients. On the other hand, the 30 UTR deletion candidates inhibited the viral transmission from mother to fetus in pregnant mice and also prevented the infection in male reproductive tract in infected mice. Significantly, outstanding safety profile was ensured by both the vaccine candidates which were above 1,000 fold less neurovirulence in comparison to the approved live-attenuated vaccines JEV SA14-14-2 and YFV 17D. The study validated the immunogenicity and safety in immunocompromised (AG129 mice), as well as immunocompetent (CD-1) mice. Furthermore, pups from CD-1 who was only 1 day of age, lived after the administration of lethal dose of ZIKV. The compromised virulence of the vaccine can be a result of enhanced sensitivity to the effects of type I IFN and reduced synthesis formation of viral RNA [28]. This vaccine candidate merits further development and investigation. (Shan et al., 2017a, 2017b).

The second method includes creating a chimeric flavivirus by means of DENV, JEV, or YFV to form ZIKV prM-E genes. In this method, protection of mice was confirmed after a single dose administration of chimeric DENV-2 and JEV SA14-14-2 vaccines consisting of ZIKV prM-E genes (Li et al., 2018; Xie et al., 2017). Furthermore, non-human primates were protected from ZIKV infection and transmission of ZIKV from mother to fetus was inhibited by JEV SA14-14-2 chimeric ZIKV vaccine (Li et al., 2018).

NIH is following the similar pathway to develop a chimeric ZIKV vaccine utilizing a live-attenuated DENV-4 vaccine backbone (rDEN4D30). In spite of the potential of the live-attenuated vaccines, they hold many safety issues, especially in pregnant women.

4.3.7 In-Silico Approaches for vaccines against ZIKV

Addition to the convention strategies of vaccines development, researchers are attempting in discovering of immune-informatics methods to design of epitope-based vaccines for optimal immunization and safety. Nine HLA class I-restricted epitopes of ZIKV that stimulated reactions from CD8+ T cells of human, by means of in-silico

algorithms has been determined. The sequences were drawn from epitopes preserved throughout all known strains of ZIKV strains within five proteins (Dikhit et al. 2016).

Table 4.4: List of the sequences of nine peptides for designing of ZIKV vaccine (Dikhit et al. 2016)

Peptide	Sequence
Capsid	MVLAILAFL
Envelope	RLITANPVI; RLKGVSYSL
NS2A	AILAALTPL
NS4B	LVAHYMYLI; LLVAHYMYL
NS5	ALNTFTNLV; SLINGVVRL; YLSTQVRYL

It has been established that 99% of the worldwide population, irrespective of their ethnicity, if provided with all the nine peptides, it enhances the ability of their binding to minimum one HLA molecule. Although the in-silico approach to design for ZIKV immunization evidently holds boundless potential, the specific immunological implication and suitability of the identified epitopes needs subsequent analysis and confirmation in all phases of the clinical studies.

4.4 Protective Efficacy against ZIKV

4.4.1 Protective efficacy in preclinical models

Due to high incidence rate of ZIKV in Brazil, numerous research labs initiated the vaccine candidate development and testing with animals for vaccine efficacy evaluation. For instance, rhesus monkeys and wild-type BALB/c mice with ZIKV infection mainly reviewed the ZIKV viraemea extent and duration in human. Then it resulted with viraemia of 7 to 10 days and slight clinical indications. On the other hand, A129 or AG129 mice were utilized to learn the neurological problems in both fetal and adult mice. These mice have compromised immunity as they have type I or I/II interferon and showed extended viraemia. Firstly, mice were also used to prove efficacy of DNA and ZPIV vaccine (Larocca et al., 2016). In addition, Rhesus monkey showed prohibition of

ZIKV infection and provided efficacy for RhAd52-vector-based vaccine, DNA vaccine and ZPIV vaccine (Abbink et al. 2016). Even after a year later of immunization of RhAd52-based vaccine, complete protection against ZIKV and stable neutralization antibody titers were found in Rhesus monkeys (Abbink et al., 2017). The protective efficacy has been established by many researches with DNA vaccines, mRNA vaccines, inactivated virus vaccines and viral-vector-based vaccines (Abbink et al. 2016; Richner et al., 2017a; Richner et al, 2017b; Pardi et al., 2017; Kwek et al., 2018; Dowd et al., 2016; Li et al., 2018; Shan et al., 2017; Brault et al., 2017; Larocca et al., 2016). DNA vaccines exposing deviations of the prM-ENV antigen were rapidly produced and verified effectively for confirming efficacy in both recipient mice and monkeys (Dowd et al., 2016).To provide protective efficacy, one-regimen dose of 10µg (Richner et al., 2017b) for mice and dose of 50µg (Pardi et al., 2017) was required for monkeys when they are immunized with mRNA vaccines with both modified and wild-type antigens expressions. All live attenuated ZIKV vaccines have proved immunogenic and protective in mice and monkeys. Furthermore, live attenuated vaccines have been produced against ZIKV using the model of Yellow fever virus or backbone of JEV vaccine or omissions in 3′ UTR region of the genome for attenuation (Kwek et al., 2018; Li et al., 2018; Shan et al., 2017). All of these vaccines have proved to have efficacy in safety in both recipient mice and monkeys. Lastly, the vector based vaccines like MV and MVA vaccines had been manufactured against ZIKV, as they express the antigens prM-ENV and NS1 correspondingly. These vaccine candidates have also proved to provide protection efficacy in the preclinical studies (Brault et al., 2017). The protective efficacy and safety by all the vaccine candidates are because of the production of neutralization antibody production against ZIKV as per all the studies have proven. Vaccine that stimulates antibody production against ZIKV has been measured using numerous assays. The deduction that the vaccine mediated antibody is responsible to confer protection for ZIKV is same as that of the protection witnessed for WNV, JEV and DENV using the antibody induced by these vaccines (Larena et al., 2013; Austin et al., 2014; Xu et al., 2017). The results for titers of neutralizing antibodies (using MN50 assays) recommend that the titers have to be approximately 100 to observe protection against ZIKV (Abbink, et al., 2017). Additionally, to measure the neutralization titers, the most common assays include ZIKV reporter viral particle (RVP) assay and the plaque-reduction neutralization test (PRNT). RVP assay have been proven for being more sensitive and producing about ten times higher titers than other assays (Dowd et al., 2016). If neutralizing antibodies

surpass this protective limit, then the activity and immunity provided by CD4+ and CD8+ T cell are not needed (Larocca et al., 2016).Nevertheless, the viral load in mice has been declined by CD8+ T cells produced in response to both ZIKV or DENV (Elong et al. 2017; Wen et al., 2017). Moreover, more research is necessary to define the effect on short and long period of protection against ZIKV.

4.4.2 Protective efficacy in animal pregnancy models

The infection caused by ZIKV in pregnant woman is different from that of the infection in woman who aren't pregnant or that of men (Osuna et al., 2017; Driggers et al., 2016; El et al., 2016). Longer period of viraemia is found in fetuses and pregnant women than others. Also there is detection of ZIKV RNA in both fetus and their mothers in all the animal models which were studied (Nguyen et al., 2017; Miner et al. 2016; Li et al., 2016). But the immune reactions displayed by the ZIKV infected pregnant monkeys and mice was observed to be same as the ZIKV infected, non-pregnant mice and monkeys (Vermillion et al. 2017; Nguyen et al., 2017). The vaccine is of highest priority for pregnant women as ZIKV has the capability to transverse the fetal-placental barrier and harms the fetus when infected. Hence, vaccination for protection is significant to prevent CZS. Consequently, measuring of the efficacy of vaccine candidates to avoid fetal malformations and demise is essential. Many considerations are still required in conducting a proper research on efficacy. But important knowledge has been gained regarding enhanced viral replication and the effect of ZIKV on the central nervous system using immune-deficient pregnant mouse models against ZIKV (Mysorekar et al. 2016; Vermillion et al. 2017). The first ever vaccines to gain protection for fetal malformations and fetal demise were achieved by usage of live attenuated and mRNA vaccines for ZIKV infection (Li et al. 2018 ; Richner et al., 2017b; Shan et. al 2017). In spite of the vaccines having a greater effect on the fetal demise, there was lack of sterilizing protection. There was identification of viral RNA in the brain, spleen and placenta of mother mice and fetal heads in maximum of the mice. Importantly, the immunized mother mice with a Live-attenuated vaccine against ZIKV gave birth to its child who survived the lethal central nervous system challenge of ZIKV (Li et al. 2018). Additional studies with both mother et al., and child is required to analyze the effect of little level of viraemia. Moreover, with the improving knowledge on the prolonged effect on infants born with ZIKV infection and does not acquire microcephaly during pregnancy in humans , there is a need for more research to confirm if sterilizing

immunity can inhibit the long term consequences of ZIKV in those infants (Van et al., 2016). Because primates and rodents have major differences (Malassine et al., 2003), hence a pregnancy model of monkey is more desirable for accuracy (Grigsby et al., 2016). To date, there is initiation of research with the monkey pregnancy model and it is still in progress (Adams et al., 2016; Nguyen et al., 2017).The research has elucidated that both species of monkeys(rhesus and pigtail monkeys) when pregnant, the ZIKV can effectively transverse from mother to its child. There was detection of viraemia caused by ZIKV in many parts of the mother's body as well as in the fetuses and placenta (Adams et al., 2016; Nguyen et al., 2017). Furthermore, irregularities of the fetal brain were identified, which included anomalies in white matter hypoplasia, pathology of central nervous system and also eyes and its optic nerve. Hazardous effects were marked in the cases of early pregnancy of monkeys which has been seen in the cases of human early pregnancy too (Rather et al., 2017; Lissauer et al., 2016). Significantly, deliberation for the development of vaccine to prevent CZS should be the prime goal. For this goal to be achieved, vaccine candidates selected for clinical development must be analyzed in preclinical pregnancy models for their protective efficacy.

5. Challenges and future aspects of ZIKV

Regardless of hopeful progression of vaccine development against ZIKV, there is still many research required to fill our knowledge gap regarding it.

5.1 Challenges in GBS

Studies have shown ZIKV infection to account for GBS at an occurrence rate of approximately 1 out of 4,000 to 5,000 adults who have been reported with ZIKV infection (Dos Santos et al., 2016).Identification of the antigen(s) of ZIKV that are suspected to be the origin of GBS is essential as they stimulate the production of antibodies against peripheral nerves of the infected person and results in compromised central nervous system. If the viral antigens for provoking antibodies for causing GBS are confirmed, then vaccine candidates can be constructed eliminate cross-reactive epitope(s). Moreover, finding the appropriate animal models to analyze GBS and relate with human physiology isn't available to research about etiology of the disease.

5.2 Challenge in cross-reactive antibodies

There is a doubt concerning that the antibodies produced due to ZIKV vaccines are contributing to the susceptibility of other flavivirus infections or not. One of the biggest challenges in the progression of vaccine development against ZIKV is the cross reactions because of closely related antibodies that increases the chance of other infections, where the flaviviruses like DENV, ZIKV and others are commonly found in their endemic regions. To date, there is report of complex interaction between DENV and ZIKV infections. Rise in ZIKV replication has been confirmed in mice and cell culture when induced with antibodies of DENV or WNV (Bardina et al., 2017; Dejnirattisai et al., 2016).However, in non-human primates model, there was no rise of ZIKV replication in it (Pantoja et al., 2017). On the other hand, it has been observed that ZIKV infection if occurred in a patient earlier can lead to noteworthy increase of DENV-2 infections. This was proven when rhesus macaques with early ZIKV infection steered to a major increment of DENV-2 viremia along with all the symptoms and signs of DENV-2. (George et al., 2017)

Cross-serotype increment in human due to successive DENV infections has been proven in a pediatric unit research. It was found that, the susceptibility of critical dengue disease was the maximum within a limited range of previous anti-DENV antibody titer, although high antibody titers inhibited all dengue diseases, within all the four DENV serotypes (Katzelnick et al., 2017).

The antibodies displaying sub-neutralization against ZIKV caused increased viral replication in successive ZIKV infection when studied in cell culture, but did not exhibit it in the non-human primate (Abbink et al., 2017). In addition to the complexity and interaction found among DENV and ZIKV infections, pre-existing DENV infection generating cross reactive T cell immune response can also enhance the possibility of ZIKV infection in both mice and human, which signifies how T cell of pre-existing DENV can play a role in triggering ZIKV infection (Grifoni et al., 2017; Wen et al., 2017).Moreover, the coordination of T-cells and antibody immunity are significant because the signaling and memory of antibodies are reliant on CD4+ T cells. All these information supports the idea that, the immunity for ZIKV vaccination and its probable effect on following DENV infection will not be same among individuals exposed with or without a pre-existing flavivirus disease. To combat tis challenge, a vaccine should be engineered to confer long-term neutralizing antibodies without or negligible cross-reactivity to inhibit enhancement of other flavivirus infections. Hence, studies for recognizing specific epitopes are vital.

5.3 Challenge in human volunteer model

Due to the decrease in cases of ZIKV infection in human, a challenge has arisen for the efficacy testing using non-pregnant and pregnant women participants. This has been hindering the research that could find valuable information of vaccine development for neurological disorders, as there is deficient in formation of controlled ZIKV infection human model. Moreover, more studies regarding ZIKV vaccine-induced epitopes, efficacy and the details of the ZIKV complication are not being able to study because of the lack of proper model. For successful development of vaccination against DENV-2, such controlled human model played a great contribution (Kirkpatrick et al., 2016). Furthermore, getting approval from regulatory bodies are important for researching with

controlled human models and the regulator bodies approves after strong supervision for ethics regarding safety issues in human volunteers and social welfare of the study.

5.4 Challenge of ZIKV symptoms

A well-established association between protection and infection mechanism in uterus are necessary for quick and precise evaluation of efficacy of ZIKV vaccine. Because most patients infected with ZIKV does not show in clinical symptoms or display a mild disease. As mentioned earlier, link between protections found as a result of current clinical trials and a controlled human model to study ZIKV infection, will accelerate the development of vaccination against ZIKV and earn license for it.

5.5 Challenge of diagnostic assay for ZIKV

For clinical trials a proper and dependable diagnostic assay is necessary (Balmaseda et al., 2017). Viral RNA can be easily determined and measured using well-defined RT-PCR assays, throughout the viremia. Post-viremia session, another assay is carried out for identification of ZIKV infection. Because there is cross reactivity of antibodies between closely related flavivirus infections, hence it is difficult to develop an accurate diagnostic assay to distinguish among early flavivirus induced, ZIKV vaccine induced and ZIKV infection induces antibodies. Development of such diagnostic assay which is virus specific will accelerate the efficacy testing.

5.6 Challenge in pregnancy

Administration of ZIKV vaccines prior to pregnancy has proven to stop crossing of ZIKV from mother to fetus in mice (Richner et al., 2017a; Shan et al., 2017a). But it is yet to confirm that if immunization after pregnancy can stop the ZIKV transmission from mother to fetus in animal models or not. It is evident that immune responses differ among pregnant and non-pregnant woman. But investigations needed to find the accurate dose of vaccination to inhibit utero transmission of ZIKV during pregnancy and prior pregnancy as well. Furthermore, it is not defined if both humoral and cellular immune

responses are required in pregnant woman to prevent CZS. Extensive research is prerequisite to establish the detailed information of ZIKV in humans and by engineering and investigating vaccine clinical trials. After the WHO announcement 2016, academic and research institutions, industries, and governmental collaboration have proven a hopeful pipeline of ZIKV vaccine development. Even though there is decline in number of human cases significantly ever since 2017, a vaccine that can counteract CZS remains compulsory. We need to keep the drive to develop safe and efficacious ZIKV vaccines for obtaining license.

6. Conclusion

In conclusion, several ZIKV vaccine candidates in the pipeline have been advancing to clinical efficacy tests and have been proven safe, immunogenic and generated neutralizing antibody titers in human which has proven to have protection in preclinical test models. With the astonishing leap of ZIKV vaccines that been developed has headed to a prompt enhance in our knowledge of ZIKV virus. Even though, there are many knowledge gap and significant challenges to overcome for gaining licensure for ZIKV vaccine. Because of the neurological danger ZIKV possesses in both infants and adults, it's of a top priority to develop the most effective as well as safe approved immunization. The success of rubella immunization and its inhibition of inborn defects have enlightened us with a hope that success with ZIKV vaccination can come with extensive research work.

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