An Immunoinformatics Approach to Design a Novel mRNA Vaccine Against West Nile Virus

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.

2. The thesis does not contain material previously published or written by a third party except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

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Abstract

The West Nile Virus (WNV) is a mosquito-transmitted flavivirus that causes a variety of ailments, from mild febrile illnesses to potentially lethal neuroinvasive encephalitis (Caren Chancey 1, 2015). The virus, which was initially discovered in Africa in 1937 , has spread widely over the world in recent years ,leading to recurrent outbreaks . There are currently no clinical vaccinations despite the virus's considerable economic burden , death and morbidity (Jaclyn A Kaiser 1, 2019). Major challenges for conventional vaccine development tactics include low efficacy , the need for numerous doses , reversion to pathogenic strain and high cost (Jaclyn A Kaiser 1, 2019). mRNA vaccine provides a feasible alternative to conventional vaccine techniques in such a scenario due to their high potency , rapid development , potential for low manufacture and promise for safe administration (Norbert Pardi 1, mRNA vaccines - a new era in vaccinology , 2018) (Rishi R Goel # 1 2, 2021) . With the discovery of immunoinformatics , a new age of vaccine development has begun where efficient Computational methods are being used to anticipate the proper antigens , epitopes , carriers and adjuvants (Mokhtar Nosrati 1, 2019) (Alba Grifoni 1, 2020) (Syed Faraz Ahmed 1, 2020). The epitopes were put through a filtering pipeline that included assessments of antigenicity , toxicity , allergenicity and cytokine inducibility with the aim choosing epitopes capable of choosing both T and B cell mediated immune responses. The epitopes and the associated MHC molecules underwent molecular docking modelling . To design the vaccine , epitopes, highly immunogenic adjuvant, components for optimum sub cellular trafficking , secretion booster and suitable linkers were combined . The vaccine was discovered to be antigenic , nearly neutral at physiological pH, non- toxic, non-allergenic , capable of eliciting a potent immune response and having a respectable level of global population coverage . This design can be regarded as a promising option for a WNV vaccine based on these parameters

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AI, Aliphatic index; ARCA, anti-reverse cap Analogs; WNV, West Nile Virus; CTL, Cytotoxic T Lymphocyte; HTL, Helper T lymphocyte; LBL, Linear B lymphocyte; dsRNA, double stranded RNA; GMP, Good Manufacturing process; GM-CSF, granulocyte macrophage colony Stimulating factor; GRAVY, Grand Average of hydropathicity; HLA, human leukocyte antigen; HPLC, High Performance Liquid Chromatography; IFN-lambda, interferon lambda; Ig, immunoglobulin; II , instability Index; IL-4, interleukin-4; IL-10, interleukin-10; LNP, Lipid nanoparticle; MHC, Major Histocompatibility Complex; MITD, MHC-I targeting domain; ORF, Open reading frame; PAMP, pathogen-associated molecular pattern; pAPC, professional antigen presenting cells; pDNA, plasmid DNA; PSSM, position-specific scoring matrix ; RBD, Receptor binding domain; RBM, Receptor binding motif; RTC, Replication/ Transcription complex , TAP, Transporter Associated with Antigen Processing; TCR, T cell receptor; TLR, Toll like receptors ; TMPRSS2, transmembrane protease serine S1 membrane 2 ; tPA, tissue plasminogen activator; UTR, Untranslated regions; WHO, World Health Organization.

Chapter 1

INTRODUCTION

As of 10 February 2022, European Union (EU) and European Economic Area (EEA) countries and EU neighboring countries reported 164 human cases of West Nile Virus infection in 2021 (European Centre for Disease Prevention and Control , n.d.). West Nile Virus (WNV) is a mosquito-borne arbovirus , a member of Flaviviridae family ,its name was derived from the west Nile district of Uganda. Now commonly found in Africa , Europe, the Middle East , North America and West Asia. The first case of WNV was diagnosed in a woman with having a symptom of febrile illness in 1937 in Uganda (World Health Organization , 2017). Large outbreaks occurred in Romania and Russia in 1990s. In 1999 , the first WNV cases identified in New York where 59 patients were hospitalized, among whom 12% died. In the twenty-first century, major WNV outbreaks occurred in Argentina, Canada, China and throughout the Europe. The European CDC reported a 7.2-fold increase in cases from 2017 to 2018 especially in Bulgaria , France and Italy. In the USA , WNV was the most common cause of neuroinvasive arboviral disease in 2018. In 2020, a major outbreak in Spain with 77 confirmed cases of WNV , of which 72 developed neuroinvasive disease and 7 died. In Asia very few human cases have been reported. The primary vector of WNV are the mosquitos of the Culex genus . The primary vertebrate host of WNV is birds. The infection of migratory species of birds through these mosquitos enable the virus to travel much further and introduce WNV to new locations. Other routes of WNV transmission, although rarely are organ transplantation , blood transfusions and breast milk. The transmission cycle begins when a female mosquito takes a blood meal from a viremic host . The ingested viral particles end up in the midgut of the insect . After infecting and replicating within the midgut epithelium , they are released into the mosquito hemolymph , the cavity containing the insect’s circulatory fluid . From there the virus disseminates throughout the insect's body, reaching and accumulating in the salivary glands . From here it can be transmitted to a new host during its next blood meal. Mammals are considered dead-end host as viremia levels in mammals are not sufficient for transmission which terminates the transmission cycle (Centers for Disease Control Prevention, n.d.). In humans the clinical courses of WNV infection ranges from asymptomatic or mild febrile illness to severe neurological manifestations and fatalities . The elderly and immunocompromised hosts are at increased risk for disseminated WNV infection . Most patients are present with acute onset of fever , chills , nausea, fatigue, headache, myalgia, arthralgia . These symptoms are mild and resolve in less than a week . The incubation period for WNV disease is typically 2-6 days but ranges from 2-14 days. In rare and severe cases , patients having neurological manifestations leads to WNND that includes syndromes of meningitis , encephalitis, movement disorders and Parkinson features have also been reported. WNV , an enveloped virion is an icosahedral particle with the capsid protein associating with the SS positive- sense RNA genome to form a nucleocapsid which is surrounded by a lipid bilayer . The genome consists of a single open reading frame of approximately 11 KB with no polyadenylation tail at 3'end. Both 5' and 3' form stem loop structures help in replication

, transcription , translation and packaging . The viral RNA is translated as single polyprotein cleaved by both host and viral proteases resulting in three structural (capsid, envelope and premembrane) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins. Structural proteins are necessary for virus entry , fusion and encapsidation of viral genome during assembly. During virus assembly , the Envelope protein embeds in the lipid bilayer of the virus and is exposed to the virion surface. This E protein binds the receptor on the cell surface for viral entry. Also , E protein's secondary structure helps in translation synthesis of RNA etc. The prM protein also embed in the lipid bilayer and is thought to protect E protein from undergoing premature fusion upon virus exocytosis to the cell surface . During infection , the virus population contains both mature and immature viral particles where immature prM particles are also found. Among Non- structural proteins , NS1 is highly immunogenic , play a role in replication. NS3 (viral protease) cleaves other non-structural proteins from viral polyprotein and encodes enzyme activities. NS5 is necessary for viral replication. Others inhibit one or more components of innate immune response against viral infection (Tonya M. Colpitts, 2012). WNV was not considered a major risk for human until the 1996 outbreak in Romania . However, WNV subsequently became a major veterinary and public health concern as it can cause lethal diseases . A vaccination prophylaxis of free- living and captive populations is desirable. To date , there is no approved vaccine for human in the market although some human vaccine candidates have been evaluated in phase I and phase II clinical trials. As E glycoprotein plays a crucial role viral entry to the host , it is considered as the prime target for the development of antibodies and vaccines in our study. We have decided to target this E protein for designing a mRNA vaccine . A wide number of vaccine approaches such as inactivated vaccine, subunit vaccine , DNA vaccine, recombinant vaccines have been tested in mice , hamsters , bird , horses and non-human primates. Unfortunately, probably due to lack of significant commercial interest there are no current FDA approved WNV treatments for human. Recent prevention approaches depend upon mosquito control programmes. These programmes are much affordable than vaccines , however they don't provide long-lasting effects. The main strategy is to reduce exposure to mosquitos via mosquito nets , insect repellents and by staying indoors. As WNV virus remains as an intimidating remark to human in so many regions of the world. This virus has its alarming ability to cause outbreaks in just recently endemic areas and has potential to obtain mutations leading to maximized virulence .Along with that it is flexible in using different mosquito species and birds as vectors and amplifying hosts that makes endemics very uncertain. Moreover , Global warming and ever-expanding human -animal traffics are acting as an additional factor to further-spread of WNV virus . So, to encounter this world-wide threat, we require effective vaccines for long-term protection if there are viable therapeutic interventions unavailable. The need of extant WNV vaccines induce us to assess the potential multi-epitope ensemble vaccine as an alternative exploiting our evolving technique to vaccine design. mRNA therapeutics can be a potent platform in terms of its safety , cost effective production compared to other vaccines , rapid development and higher efficacy (Ulbert, 2019). On top of that mRNA vaccine is a non- infectious agent with having natural degradation and adjustable half- life. We chose mRNA vaccine over other vaccines because these vaccines have higher efficacy that do not require passing through nuclear envelope for translation unlike DNA vaccines. The strategy to design vaccine using mRNA is that body cells can make targeted E protein from mRNA. After

injecting the vaccine , the cells will pick up the messenger RNA and make protein which is then displayed in the cell surface. In this way our immune system can recognize this protein later on whenever it gets affected by WNV virus , it can immediately induce an immune response against the virus. There are several advantages of using mRNA vaccine over other vaccines in terms of safety , efficacy and production . Unlike viral vector or live attenuated vaccines , this vaccine poses no safety concerns for integrating DNA as it is not able to penetrate the nucleus containing DNA. Moreover, protein-based vaccines or inactivated vaccines are produced using chemicals and cell cultures. On the other hand , mRNA vaccine strategy is a cell independent process which doesn't require inactivation . And it is safe from getting contaminated with toxic agents. In spite of its having few shortcomings such as instability , immunogenicity, delivery inefficiency where mRNA gets rapidly degraded in the body. Cells can't take up the mRNA as foreign molecule immediately. So, it is important for the mRNA to be stable until it is taken up by the cells. However, Via structural modifications of mRNA and recent advancements in synthesis and manipulation could solve these problems such as modification of mRNA molecules through recent technology make it more stable and packaged in the fat molecules which can help increase cell delivery efficiency. The more the increase in delivery efficiency , the more the amount of targeted protein can be produced by mRNA which can effectively stimulate immune response (Rishi R Goel # 1 2, 2021) (Saiz, 2020) (Norbert Pardi, 2018) . Immunoinformatics or Computational immunology , a special branch of bioinformatics is used for understanding and interpreting immunological data. Immunoinformatics tools comprise selecting , re-engineering or eliminating immunogenic and conserved epitopes and re- designing antigens to induce protection and immunogenicity. This approach facilitates time and cost- effective vaccine development against many infectious agents . For an advanced and effective vaccine design, it is important to have the best use of Immunoinformatics tools. Body's immune system and function related data's are stored and analyzed specifically and more precisely in Immunoinformatics using several tools and statistical, Computational, biological and mathematical knowledge. To develop vaccine against less predictable pathogens , Immunoinformatics provide predictive tools , creates databases. Human or animals have complex immune system , variability in pathogens and antigens nature , and their varieties of regulatory pathways produce data in huge quantities that can only be handled and organized through Immunoinformatics. This approach helps analyzing disease pathogenesis and recognizing vaccine candidates which requires methods like comparing the sequences , searching databases , modelling of structures etc. Tools in Immunoinformatics identifies necessary epitopes and make their 3D structures for vaccine design. Furthermore , they are used in protein screening , in identifying MHC binding aggregates are necessary for epitope-based vaccine production. Immunoinformatics are employed in docking to predict the physicochemical, antigenic , allergenic properties of vaccine . Immunoinformatics reduces the cost and time in vaccine construction as it poses a concern on mapping T cell and B cell epitopes (Saeed Khalili 1, 2014). Unlike these studies , In our study , we opted for more deep approach for designing multi-epitope mRNA vaccine consisting of Cytotoxic T Lymphocyte (CTL) , Helper T lymphocyte (HTL) , Linear B lymphocytes epitopes derived from WNV Envelope glycoprotein , a highly immunogenic adjuvant via advanced immunoinformatic tools , capable of obtaining very specific immune responses . Traditional approaches of designing vaccines use whole infectious agent or pathogen or different proteins which may cause inexcusable antigenic

loads , induces allergy . Peptide based vaccines with multi-epitope can yield very distinct immune response and targets antigens more precisely . These vaccines are made of several assembled epitopes collected from several target proteins or antigens of that an infection . As there are multiple HLA epitopes in the vaccine , multiple TCR's can recognize them so that immune response can easily be enhanced . Cell mediated and humoral both immune responses get activated due to the overlapping of CTL's , HTL's and LBL epitopes. As multi- epitopes are selectively filtered out for the vaccine construction, there is less chance of unwanted antigens to be introduced and reduces the unexpected immune response chances that may cause disease. In this way , This vaccine can fight the most infectious diseases.

Objectives :

- Our main goal in this analysis was to employ bioinformatics studies for the selection of potential epitopes against the target E protein of WNV virus, construct a mRNA vaccine and evaluating its properties and immune response through simulation.

Chapter 2

Materials and methods

2.1 Retrieval of WNV Envelop glycoprotein sequence

NCBI Database was used to search the complete WNV genome. From the results , the Fasta sequence of "Envelope protein E" from "West Nile Virus lineage 2" was downloaded. The accession number of the protein was NP_776014 (Eric W Sayers, 2022) .

2.2 Multiple Sequence Allignment of E protein sequences

WNV consensus sequence was created from WNV E glycoprotein sequence through multiple Sequence alignment in EMBOSS: Cons. This consensus sequence later on used for epitope predictions (Rice P., 2000) (F. Madeira, 2019).

2.3 Prediction and Assessment of CTL epitopes

CTL epitopes recognize viral antigens fragments bound to MHC class I epitopes on the infected cell surface and cause cell apoptosis to save nearby cells getting infected by pathogens (Alberts B, 2002). So it is essential in Immunoinformatics multi-epitope vaccine designing to predict CTL epitopes (SA Ali, 2019) . NetCTL – 1.2 server was used for the prediction of CTL epitopes. The server allows for prediction of CTL epitopes restricted to 12 MHC class I supertypes, namely A1, A2 , A3, A24 , A26, B7, B8 , B27, B39 , B44, B58 , B62 . Default values, 0.75 was used as the threshold for epitope identification , 0.15 weight on C terminal cleavage , 0.05 weight on TAP transport efficiency were used. NetCTL v1.2 delivers its final output for any given protein through integrating information about proteasomal cleavage , Transporter Associated with Antigen Processing (TAP) transport efficiency and MHC class I affinity (MV Larsen, 2007) . Later on , to predict the immunogenicity of CTL epitopes , Class I Immunogenicity tool of IEDB Analysis Resource was used (Jorg J. A. Calis, 2013). Merely epitopes that showed positive value for immunogenicity chosen for the next stage of evaluation. ToxinPred (S Gupta, 2013) and

AllerTop 2.0 (Dimitrov, 2014)servers were respectively used to check the toxicity and allergenicity of immunogenic epitopes . To evaluate the antigenicity , Non-toxic and Non-allergenic epitopes were subjected to VaxiJen server (IA Doytchinova, 2007). Epitopes that showed antigenicity prediction values less than or equal to 0.5 were considered antigenic . MHC class I allelic partners of those antigenic epitopes were predicted using the consensus algorithm in the IEDB server (M. Moutaftsi, 2006) where the percentile rank score less than or equal to 2 was considered for this study.

2.4 Prediction and Assessment of HTL epitopes

When viral antigens are proteolytically cleaved , epitopes bound to MHC class II make a complex form are presented to HTLs or CD4+ T cells through antigen presenting cells such B cells , Macrophages and Dendritic cells (T.M. Holling, 2004) . Prediction of HTL's is also a crucial part for vaccine construction in almost all immunoinformatic studies (H. Zahroh, 2016) (R. Kumar Pandey, 2018) . In this study , 15 Mer HTL epitopes and their corresponding MHC II alleles were predicted using the consensus algorithm of the IEDB MHC II binding tool (P. Wang, 2010). Host species was selected as Human, HLA-DR. A percentile rank less than or equal to 0.25 was considered for filtering the epitopes. VaxiJen server (IA Doytchinova, 2007) was used to calculate the antigenicity of the epitopes . ToxinPred and AllerTop 2.0 were subsequently used to filter out non-toxic and non- allergenic epitopes respectively. After that IFNepitope , IL4pred and IL10pred servers were respectively used to check whether the remaining epitopes are inducible to interferon -γ (IFN- γ) (S.K. Dhanda P. V., 2013), interferon -4 (IL- 4) (S.K. Dhanda S. G., 2013) and Interleukin -10 (IL-10) (G. Nagpal, 2017) . Depending upon these criteria , only the antigenic and all three Cytokine inducing epitopes were selected for vaccine construction .

2.5 Prediction and Assessment of LBL epitopes

B cells are involved in humoral mode of immunity(adaptive immunity) where B cell is activated and differentiated into memory and plasma cells . Antibodies attached to plasma cells recognize and bind to specific foreign antigens and help pathogens to be degraded through phagocytosis and boost Long term immunity (T. Dörner, 2007) (R.K.G. Do, 2000) (M. Lechmann, 1996). Here the importance of B cell and T cell interaction to activate B cell is undeniable. Therefore, prediction of B cell epitopes with the aid of Computational tools play a significant role in epitope-based vaccine design. B lymphocyte epitopes are of two kinds – Linear and Conformational epitopes (T.A. Ahmad, 2016). Linear epitope is predicted in epitope-based vaccine design other than Conformational epitopes as Linear epitopes have simple structures,

easily can be synthesized compared to Conformational epitopes and are able to stimulate immune response without requiring 3D Conformation. In this study, LBL epitopes were predicted in ABCpred server (Sudipto Saha, 2006). Epitopes that were found to be positive were chosen for further analysis. Then the antigenicity , toxicity , allergenicity of LBL epitopes were checked through VaxiJen (IA Doytchinova, 2007), ToxinPred and AllerTop (Dimitrov, 2014) servers respectively. Epitopes which obtained antigenicity values more than or equal to 0.5 were selected for toxicity and allergenicity.

2.6 Molecular Docking between T Lymphocyte epitopes and MHC alleles

Molecular docking simulation was conducted in Hdock server (Yumeng Yan # 1, 2020)to evaluate the docking score between ligand and receptor. CTL and HTL epitopes were ligands and their corresponding MHC alleles were receptors . Firstly , MHC allele sequences were retrieved from IPD-IGMT HLA database (James Robinson 1 2, 2020). Then allele sequences to be predicted were run one by one in Alpha Fold.ipynb (Gabriele Pozzati, 2022). From here Pdb format of HLA structures which ranked 1 were downloaded. Ramachandran plot from the PROCHECK server was used to validate the MHC allele structures (R. Laskowski, 1996). On the other hand , the epitopes structures in three-dimensional form obtained from PEP-FOLD 3.5 server (S.K. Burley, 2018). When the epitope and alleles structures were ready finally docked in Hdock server (Yumeng Yan # 1, 2020) . Lowest docking scores (highly negative) were considered to have good binding affinity. Discovery studio visualizer was used for in-depth analyzation of the docking complexes evaluating their pose and various interactions, amino acid residues in 3D form (Umi Baroroh, 2023) .

2.7 Prediction of Population Coverage

Specific HLA alleles are expressed at different frequencies in different ethnicities . We need to find out targeting these different HLA alleles for our selected epitopes , how much population coverage can be achieved in different regions across the globe. In this study , IEDB population coverage tool has been used to analyze the population coverage of our selected 7 T Lymphocyte epitopes with their corresponding MHC alleles of different varieties (H.H. Bui, Predicting population coverage of T-cell epitope-based diagnostics and vaccines, 2006) .

2.8 Designing the vaccine construct

Five main elements are prerequisites for a highly immunogenic mRNA vaccine to be constructed in the open reading frame . These are Kozac Sequence , predicted epitopes , Adjuvants , linkers and stop codon. Kozac Sequence (Kozak, Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes, 1986) present in Eukaryotic mRNA provides the necessary information to the ribosome to recognize the start codon correctly and initiate translation initiation process. So that mRNA can be translated by the ribosome into target protein . Epitopes in this study have been predicted based on the criterias like antigenicity , non-toxicity ,non-allergenicity and cytokine inducing properties (only for HTLs) . When the epitopes are appropriately antigenic , a strong adjuvant can help boost the adaptive immune response (B. Pulendran, 2006) (A.S. McKee, 2017) . In this study , Co-stimulatory molecule CD40L is added which puts a significant impact in vaccine Construct by activating professional antigen presenting cells (pAPCs) . CD40L is found on the surface of Helper T cell binds with CD40 molecule of APC. Here B cell and Helper T cell interaction through T cell receptor-MHC ll and CD40L-CD40 bindings is very important for B cells to activate , get matured and produce plasma cells so that more antibodies specific to the target pathogen can be produced. To avoid any interaction and interference between protein domain while multi-epitope vaccine designing , selecting an appropriate linker which would be specific to the target epitope is an essential step (Norbert Pardi 1, 2018) (T. Schlake, Developing mRNA-vaccine technologies, 2012) (Sumera Zaib, 2022). These linkers must have ideal properties based on their length, flexibility and rigidity Epitopes and linkers or spacers must be in precise order and position with the help of experimental evidences in the following ways- 1.Linker (EAAK)2was used to link HTL and LBL epitopes. 2.Intra LBL epitopes were combined using (EAAAK)2 or (EAAAK)2E. 3.LBL and CTL epitopes were spaced by AAY. 4.Intra CTL epitopes were linked by the same AAY linker as before (B. Livingston, 2002) (N. Nezafat, 2016) (Y. Gu, 2017) (T. Farhadi, 2015) (L. Michel-Todó, 2019). For an increased antigen presentation, Signal peptide sequence and MITD sequence are reported to be useful. Signal peptide helps translated epitopes to move out of the cell and MHC-I targeting domain (MITD) directs CTL epitopes to MHC-I compartment of ER (endoplasmic reticulum) . In the study , in the 5'and 3' region of open reading frame , tPA (tissue plasminogen activator) as a signal peptide and MITD have been used (Y. Kou, 2017) (S. Kreiter, Increased antigen presentation efficiency by coupling antigens to MHC class I trafficking signals, 2007). Therefore, tPA and MITD sequences were retrieved from UniProt database . There is a major concern for any mRNA-based therapeutics is its instability . To resolve this issue , it is necessary to incorporate all the elements in mRNA vaccine generally found in Eukaryotic mRNAs. For that purpose , inclusion of 5'cap, poly A tail, 5' and 3' untranslated regions was crucial (Gallie, 1991). For better translation efficiency , optimum length of poly A tail has been proposed to be 120-150 bases long . Poly A tails and 5'm^7G cap sequences are found to work synergistically (M. Mockey, 1999). For increased stabilization of mRNAs , β globin 5′-UTR and α globin 3′-UTR were combined in this vaccine Construct (Z. Wang, 1999).

2.9 Prediction of antigenicity, allergenicity, toxicity and other physiochemical properties of the vaccine construct

To predict distinct characteristics of the vaccine Construct , we used translated mRNA peptide sequence removing off the tPA and MITD sequence portion in the ORF as the input as these portions gets cleaved while entering the secretory and MHC-I pathway after vaccine administration into the cytosol. Vaccine candidate must be highly antigenic and capable of evoking immune response and memory cell formation . For that purpose , antigenicity checking is important which was conducted in VaxiJen 2.0 (IA Doytchinova, 2007) server that works on different physicochemical properties of the protein . Next AllerTop2.0 (Dimitrov, 2014)server was used to predict the allergenicity of the mRNA peptide sequence using ACC(auto cross covariance) method that transforms protein sequences into uniform equal length vector. Then ToxIBTL (Lesong Wei, 2022)server was used for predicting its toxicity. Other physicochemical properties were estimated by online Web server ProParam including molecular weight ,Theoretical Isoelectric point, instability Index, Grand Average of Hydropathicity (GRAVY) , Aliphatic Index (AI) etc. (M.R. Wilkins, 2019).

2.10 *In silico* immune simulation

Using the C-ImmSim server, in silico immune simulation was used to estimate the multi-epitope mRNA vaccine's real-life immunogenic profile (N. Rapin, 2010). This simulator uses machine learning and Position-specific Scoring Matrix (PSSM) for immune interaction prediction and epitope prediction, respectively. The majority of vaccines now in use require a minimum of 4 weeks to elapse between the first and second dosage . Three injections, each comprising 1000 vaccine build units, were given four weeks apart for our immunological simulation (F. Castiglione, 2012). The "time-step" scale is used by the C-ImmSim server to determine how long simulations should last. Each time step in this scale corresponds to eight hours in the actual world. With the points of the three injections placed at time steps 1, 84, and 168, respectively, the total number of time steps for the simulation was set at 1050. The other settings were left at their default settings.

Chapter 3

Results

3.1 Multiple Sequence Allignment of E protein sequences

All the epitopes were found to be in conserved regions.

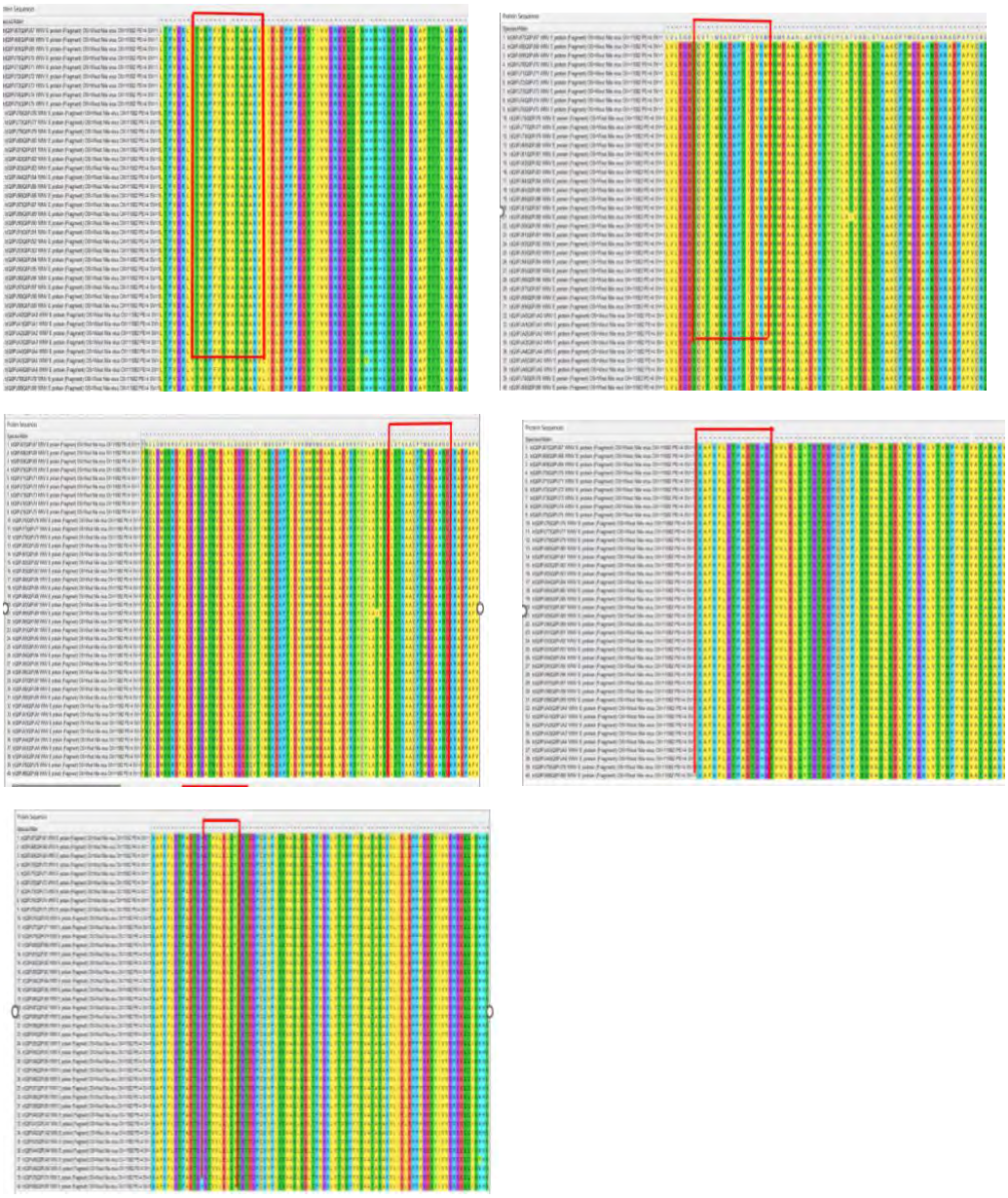

Figure 1 Multiple sequence Allignment of E glycoprotein sequences is shown. Boxes have been used to identify the epitope sequences used for the vaccination design. Apparently, there is no mutation in any of them.

3.2 Prediction and Assessment of CTL epitopes

NetCTL v 1.2 server identified a total of 130 MHC ligands for 12 MHC supertypes. Epitopes found positive in terms of immunogenicity by IEDB class I immunogenicity tool had been selected for checking toxicity , allergenicity and antigenicity in the next step by ToxinPred ,Allertop2.0 and VaxiJen server respectively . All of them were found to be non -toxic , 24 epitopes were non-allergen . Then depending upon the antigenic threshold value 0.5 , 2 . most antigenic 5 CTL epitopes (ALGDTAWDF, DTAWDFGSV, LLWMGINAR, NDKRADPAF, GTVVLELQY,) were selected for vaccine design. The IEDB allele binding prediction tool within percentile rank less than or equal to 2 resulted in 9 alleles for GTVVLELQY , 3 alleles for LLWMGINAR, 2 alleles for DTAWDFGSV , 2 alleles for NDKRADPAF and 4 alleles for ALGDTAWDF .

Table 1 List of selected epitopes for vaccine construction

Recognising cells	Epitope sequence
CD8+ Cytotoxic T Lymphocyte	GTVVLELQY LLWMGINAR DTAWDFGSV NDKRADPAF ALGDTAWDF
CD4+ Helper T lymphocyte	KAFKFLGTPADTGHG TVNPFVSVATANAKV
B lymphocyte	CVTIMSKDKPTIDVKM LSKAACPTMGEAHND

3.3 Prediction and Assessment of HTL epitopes

Within the percentile rank ≤0.25 , 30 unique epitopes were found with their corresponding alleles from IEDB MHC II allele binding prediction tool .Among them 19 epitopes met the VaxiJen threshold (≥0.5) for their antigenicity. All those antigenic epitopes were found to be non-toxic by ToxinPred server. Then among those antigenic , non-toxic epitopes , only 13 epitopes were non-Allergen by Allertop2.0 server. IFN-γ, IL-4 and IL-10 inducibility were checked for the HTL epitopes that resulted in only two epitopes (KAFKFLGTPADTGHG), (TVNPFVSVATANAKV) fulfilling all the criteria by the IFNepitope, IL4pred, and IL10pred servers respectively. (Table 1)

3.4 Prediction and Assessment of LBL epitopes

ABCpred server predicted 17 possible LBL epitopes within threshold 0.8 Among them 10 epitopes met the VaxiJen criteria(more than or equal to 0.5) for being antigenic , non-toxic and among them only 2 epitopes(LSTKAACPTMGEEAHND, CVTIMSKDKPTIDVKM) resulted in as non-allergen have been selected for vaccine construction.

3.5 Molecular Docking between T lymphocyte epitopes and MHC alleles

7 T Lymphocyte epitopes recognized 20 MHC alleles in total . With the exception of only two epitopes (KAFKFLGTPADTGHG,TVNPFVSVATANAKV) the rest of them had more than one binding allele . One had even as high as 9(GTVVLELQY) allele. Out of these 7 epitopes and their corresponding MHC allelic partners ,molecular docking was performed between each epitope and one of their corresponding alleles for an illustrative docking analysis. Table shows which of the T Lymphocyte epitopes and their allelic partners chosen finally for docking . Those alleles structures that were retrieved from Alpha Fold.ipynb server are – HLA-A*30:02, HLA-A*33:01, HLA-A*68:02, HLA-B*44:02, HLA-B*15:01,HLA-DRB1*04:05, HLA-DRB1*08:02. From the validation data of these allele models , it is observable that in case of all alleles over 80% residues belong to the most Favorite regions meaning most of the amino acids fell in the desired zones in the Ramachandran plot (Table) . Table Shows molecular docking results obtained from Hdock server in terms of docking scores . The epitope (DTAWDFGSV) with lowest docking score(-210.04) showed strongest binding affinity for its corresponding MHC allele. Docking analysis shows that the epitope DTAWDFGSV nicely fits into the blinding cleft of HLA-A*68:02 molecule (Fig) . It has been revealed that this epitope had 4 types(3 favorable, 1 unfavorable) of interactions with various residues of the molecule. The favorable interactions include H bonds(Classical and non-classical), Electrostatic interactions (Pi-charge) and Hydrophobic interactions (pi Hydrophobic, Alkyl Hydrophobic, Mixed pi/Alkyl Hydrophobic). On the contrary , the unfavorable interaction includes charge repulsion. In terms of number of interactions there were 7 conventional H bonds, , 9 pi-charge interactions , 1 Negative -Negative repulsion .

Table 2 Selected T lymphocyte epitopes (CTL+HTL) and their corresponding MHC alleles

CTL epitopes	MHC I binding alleles
GTVVLELQY	HLA-A*30:02, HLA-A*01:01, HLA-B*58:01, HLA-A*11:01, HLA-B*15:01, HLA-A*26:01, HLA-A*32:01, HLA-B*35:01
LLWMGINAR	HLA-A*33:01, HLA-A*31:01, HLA-A*03:01
DTAWDFGSV	HLA-A*68:02, HLA-A*26:01
NDKRADPAF	HLA-B*44:02 , HLA-B*44:03
ALGDTAWDF	HLA-A*32:01, HLA-A*24:02, HLA-A*23:01, HLA-B*15:01
HTL epitopes	MHC II binding alleles
KAFKFLGTPADTGHG	HLA-DRB1*04:05
TVNPFVSVATANAKV	HLA-DRB1*08:02

Table 3 CTL and HTL epitopes and their corresponding MHC alleles chosen for docking analysis.

T Lymphocyte	Epitope Sequence	MHC alleles
CTL	GTVVLELQY	HLA-A*30:02
	LLWMGINAR	HLA-A*33:01
	DTAWDFGSV	HLA-A*68:02
	NDKRADPAF	HLA-B*44:02
	ALGDTAWDF	HLA-B*15:01
HTL	KAFKFLGTPADTGHG	HLA-DRB1*04:05
	TVNPFVSVATANAK	HLA-DRB1*08:02

Table 4 Validation of the docked models

Ramachandran plot	Quality Parameters	MHC Alleles						
		HLA A*30:02	HLA A*33:01	HLA A*68:02	HLA B*44:02	HLA B*15:01	HLA DRB1*04:05	HLA DRB1*08:02
	Residues in Most favourite regions (%)	84.0	84.3	83.4	83.5	83.8	87.3	86.3
	Residues in Additional allowed regions (%)	8.5	10.0	11.6	10.2	8.3	8.3	9.3
	Residues in Generously allowed regions (%)	5.6	3.8	2.5	4.8	5.1	2.6	2.2
	Residues in disallowed regions (%)	1.9	1.9	2.5	1.6	2.9	1.8	2.2

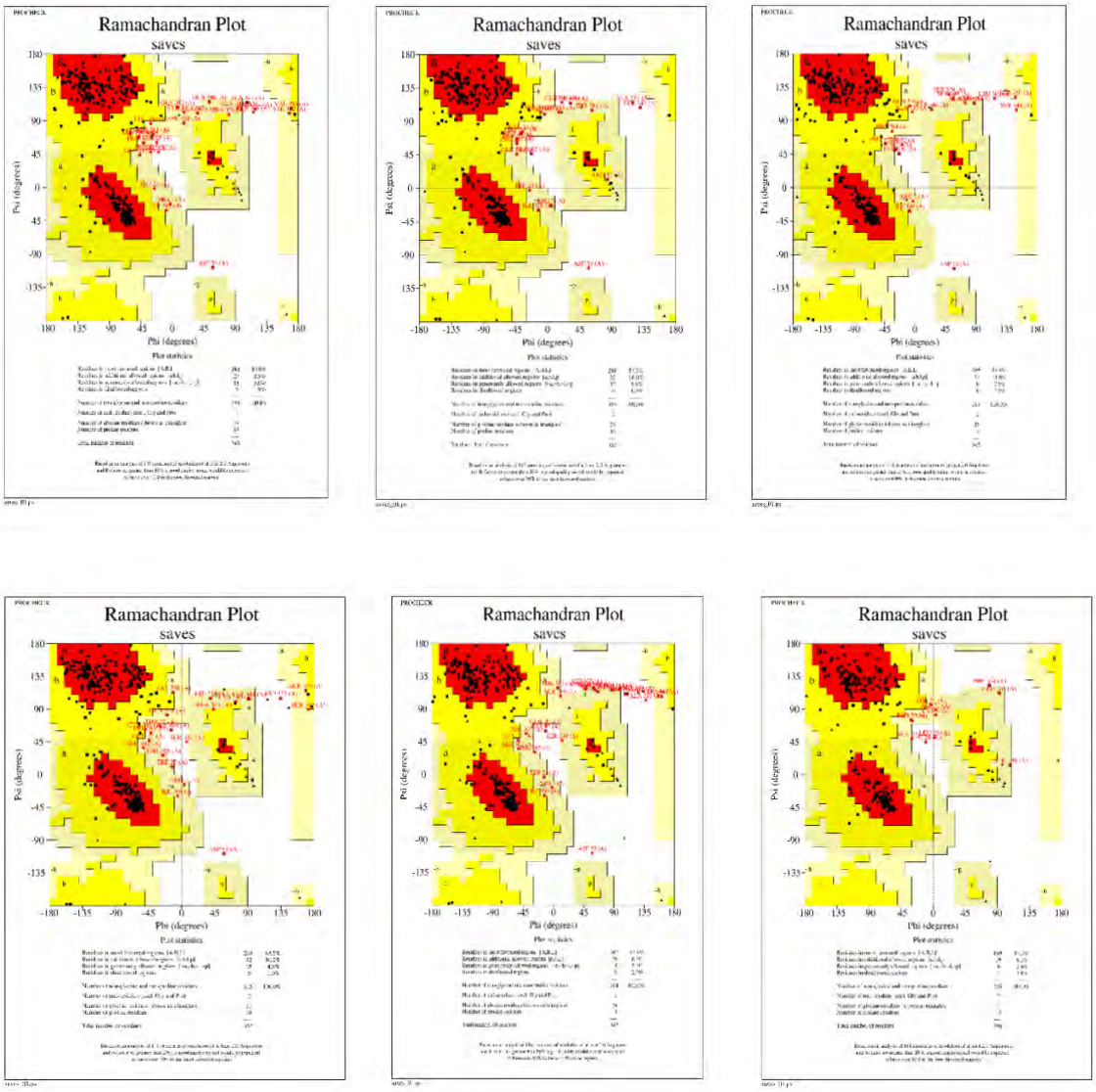

Figure 2 Ramachandran plots of the 3D models of the MHC alleles

Table 5 Binding affinity (kcal/mol) between the epitopes and their corresponding MHC alleles

Ligand	Allele	Docking score
GTVVLELQY	HLA-A*30:02	-166.90
LLWMGINAR	HLA-A*33:01	-199.77
DTAWDFGSV	HLA-A*68:02	-210.04
NDKRADPAF	HLA-B*44:02	-183.77
ALGDTAWDF	HLA-B*15:10	-208.88
KAFKFLGTPADTGHG	HLA-DRB1*04:05	-203.44
TVNPFVSVATANAKV	HLA-DRB1*08:02	-195.79

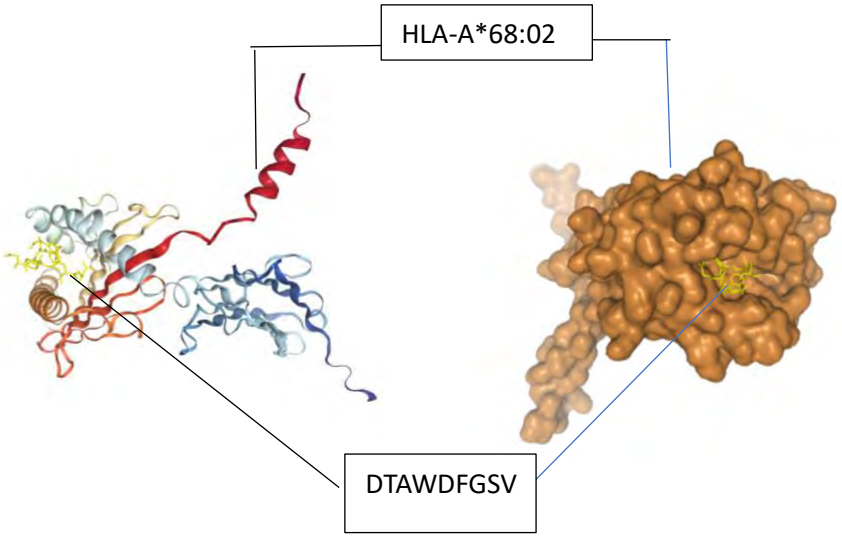

Figure 3 Docking between the epitope DTAWDFGSV and its corresponding MHC alleles, HLA-A*68:02 (A) Cartoon representation of HLA-A*68:02 and Licorice model of DTAWDFGSV (Left) (B) Surface view of HLA-A*68:02 around Licorice model of DTAWDFGSV (Right)

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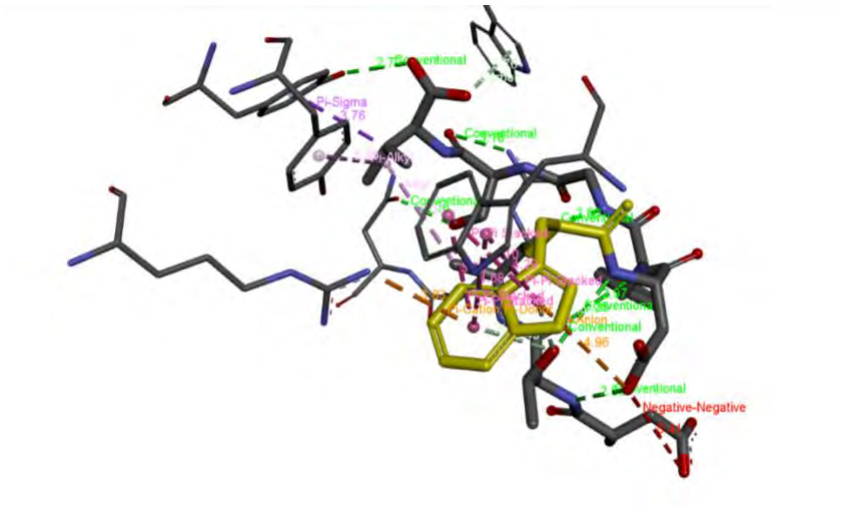

Figure 4 Various interactions and bond length (in angstrom) between the epitope DTAWDFGSV and the residues of its corresponding MHC allele, HLA-A*68:02.

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Table 6 Docking output between the epitope DTAWDFGSV and HLA-A*68:02 in terms of interactions

Conventional H bonds	Charge-Charge interactions	Hydrophobic Interactions	Charge Repulsion	
2.86	3.83	3.76	5.41	
3.25	4.96	5.46		
2.97	3.49	4.37		
2.80	3.04	4.10		
3.18		3.99		
2.36		4.68		
2.72		4.29		

3.6 Prediction of population coverage

IEDB population coverage tool analyses the coverage of our selected 7 T lymphocytes including (CTL &HTL) in terms of their 20 different varieties of MHC allelic partners. The distribution of their MHC alleles in 16 geographical regions in the IEDB database has been distributed. Fig represents the region wise coverage of the alleles. The global coverage of our vaccine stands at 25.49% . East Africa , East Asia, North Africa , South America, , West Africa , West Indies showed some of the highest regional coverage while , South-East Asia, South-West Asia , South-Asia regions showed some of the least . Coming to country-specific vaccine coverage , we looked into the countries that have been hardest hit through WNV . From the IEDB population coverage analysis , Uganda and United States showed highest vaccine coverage .Vaccine coverage in Israel , Turkey , Uganda and Greece would be 14.9% 5.52%, 32.02% , 4.41%.

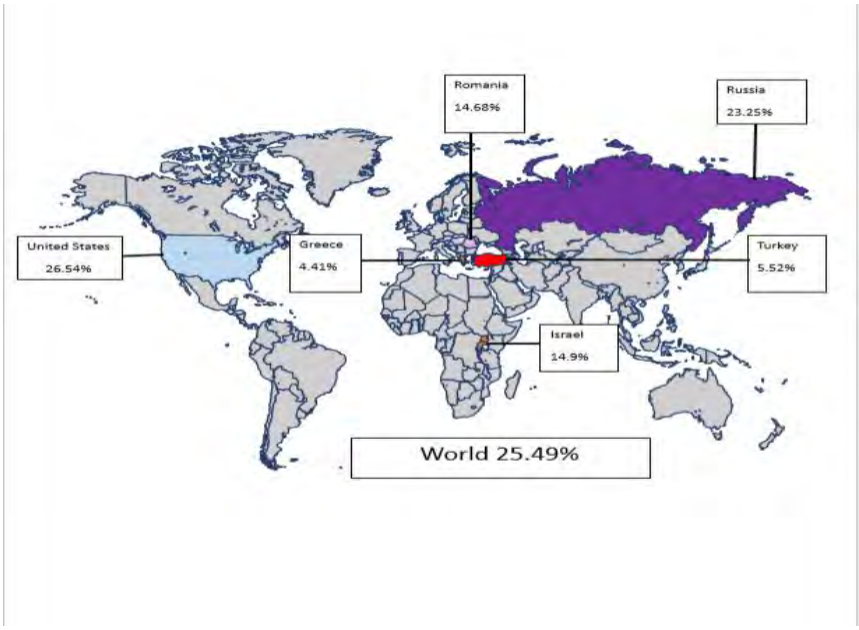

Figure 5 Population coverage of the selected T lymphocyte epitopes. Globally it covers 25.49% of the World's population

3.7 . Vaccine Construct Design

The final whole vaccine construct elements are ordered following direction from N terminal to C terminal.

5′m7G Cap – 5′UTR – Kozak sequence – tPA (Signal peptide) – CD40L (Adjuvant) – GPGPG linker – HTL epitope – GPGPG– HTL epitope – EAAAKEAAAK linker – LBL epitope – EAAAKEAAAK linker – LBL epitope – AAY linker – CTL epitope – AAY linker – CTL epitope – AAY linker – CTL epitope – AAY linker – CTL epitope – AAY linker – CTL epitope – AAY linker – MITD sequence – Stop codon – 3′UTR – Poly (A) tail.

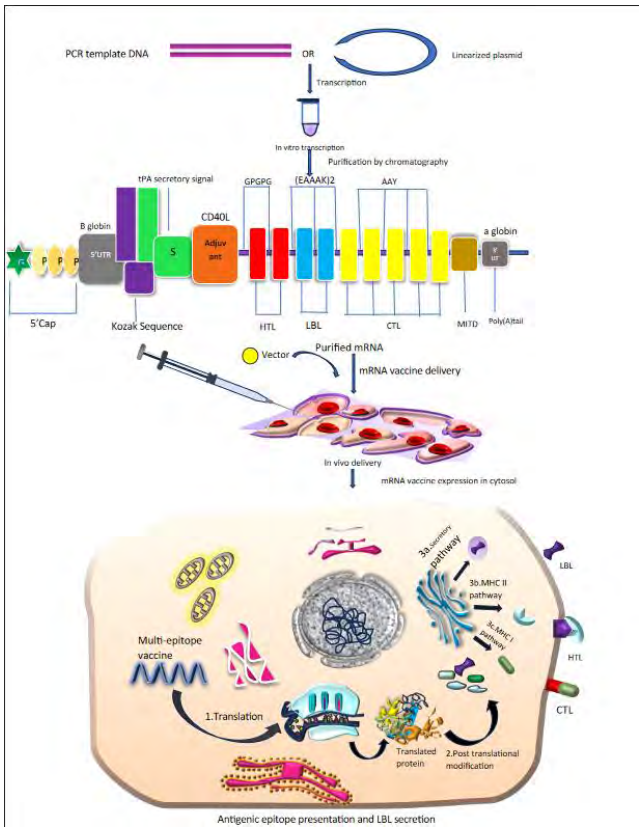

Figure 6 Synthesis, delivery and mechanism of action of the mRNA vaccine against WNV as proposed. RNA polymerase and nucleotide phosphates are added to a medium to facilitate the

transcription of the PCR template DNA or linearized plasmid DNA containing the intended vaccination sequences. As a result, several aberrant products including double stranded RNAs are produced. To acquire the necessary length and quantity of mRNA, chromatographic purification (using FPLC , for example) is used. The mRNA transits to the cytosol following vector-mediated distribution into the body. The cellular machinery responsible for translation synthesizes proteins in the cytoplasm. These proteins then go through post-translational modifications to become correctly folded and fully functional proteins. Peptides are directed by the secretory signals and MITD sequences to particular Golgi body and endoplasmic reticulum compartments for effective secretion (LBL) and presentation by MHC -I (HTL) and MHC-II(CTL)

3.8 Antigenicity, allergenicity, toxicity and physicochemical evaluation of the vaccine construct

Results of the antigenicity, allergenicity, toxicity and physicochemical analyses have been listed in Table

Table 7 Antigenic, allergenic, toxicity and physiochemical assessment of the translated protein form of mRNA vaccine translated peptide.

Features	Assessment	Remark
Number of Amino acids	413	Suitable
Molecular weight	44890.18	Average
Chemical formula	$C_{2000}H_{3125}N_{535}O_{601}S_{19}$	
Theoretical PI	7.59	Slightly basic
Total number of negatively charged residues (Asp+Glu)	40	
Total number of positively charged residues (Arg+Lys)	41	
Total number of atoms	6280	
Instability index	33.60	
Aliphatic Index	76.90	Thermostable
Grand Average of Hydropathicity (GRAVY)	-0.156	Hydrophilic
Antigenicity	0.6274(VaxiJen)	Antigenic
Allergenicity	Non-Allergen(Allertop2.0)	Non-Allergen
Toxicity	Non-toxin (ToxIBTL)	Non-toxic

The vaccine construct was predicted to be antigenic with scores 0.6274 VaxiJen. ALLERTOP2.0 server predicted the construct as non-allergen, and the vaccine was non-toxic according to ToxIBTL. The ProParam server calculated different features like molecular weight of the vaccine construct was 44890.18 kDa. Theoretical pi 7.59 indicates vaccine construct to be close to neutral in nature . Total amino acid numbers found 413 which was suitable. The number of positively and negatively charged residues were close to each other 41 & 40 respectively. Instability index computed to be 33.60 revealed that the vaccine construct would be Stable. The Aliphatic Index computed to be 76.90 indicating vaccine to be Thermostable. The GRAVY (Grand Average of Hydropathicity) resulted in negative (-0.156) Showing vaccine's hydrophilic nature . Taking these criterias into account , this multi-epitope vaccine can be predicted as a potential vaccine construct .

3.9 . Evaluation of the simulated immune response against the vaccine

The secondary responses were higher than the primary response as would be predicted. Firstly, elevated levels of immunoglobulin (Ig) M relative to IgG were found. Typical high levels of immunoglobulin activity IgG1 + IgG2, IgM, and IgG + IgM antibodies) were visible with concurrent antigen decrease in secondary immune response. This means that when the antigen is exposed again, immune memory will develop, leading to competent immunity. Long-term existence of a number of B-cell isotypes was also noted, which raises the possibility of isotype switching and memory formation. With respect to their individual memory development, the CTL and HTL groups showed a correspondingly higher reaction. Furthermore, a steady level of dendritic cell activity was detected alongside an increase in macrophage activity. There was also clear evidence of elevated IFN-γ and IL-2 levels. In addition, elements of the innate immune system—such as epithelial cells—were functioning. Additionally, a lower Simpson index (D) suggested that different immunological responses might be possible.

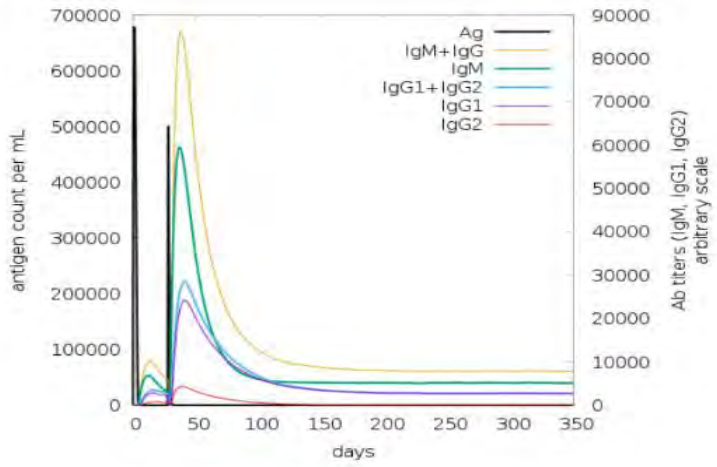

Figure 7 The virus, the immunoglobulins and the immunocomplexes

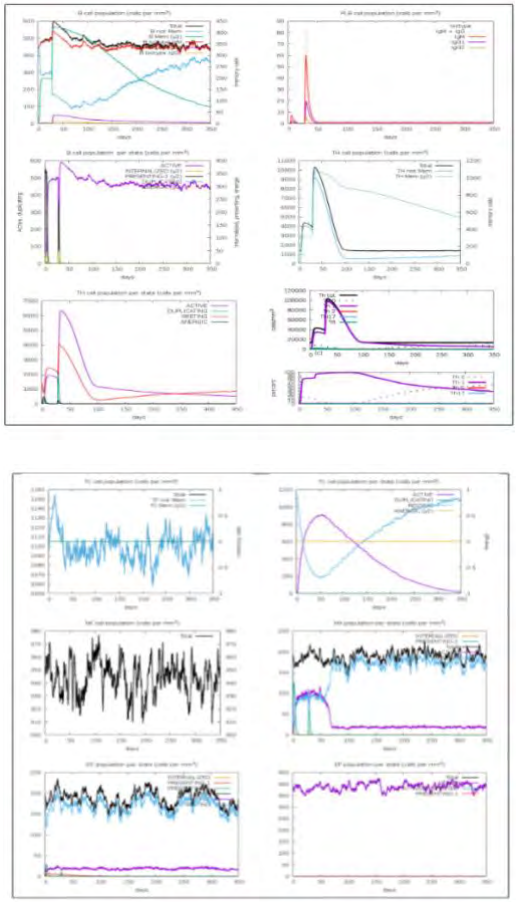

Figure 8 Cell counts shown. Legend: ACT= active; Intern=Internalized the Ag; Press II= presenting on MHC II ; Dup= in the mitotic cycle ; Anergic= anergic; Resting= not active.

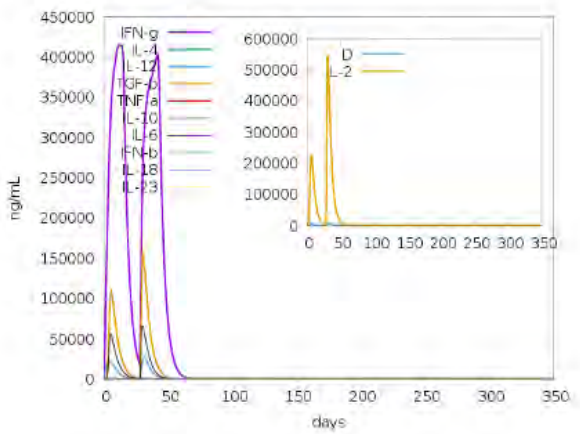

Figure 9 Concentration of cytokines and interleukins. Inset plot shows danger signal together with leukocyte growth factor IL-2

Chapter 4

Discussion

mRNA vaccines for many viruses have represented a flexible and highly effective subgroup of vaccine candidates from the first successful example of mRNA treatments in 1990. The development of mRNA vaccine has advanced significantly despite challenges such as mRNA instability caused by ubiquitous RNases' degradation of it and innate immunogenicity recognized by innate immune sensors.

Optimizing factors like mRNA structure , screening excipients , LMP delivery systems , manufacturing processes could help solve this issue. Immunological memory from vaccination can last from several years to several decades (I.J. Amanna, 2007) (B.W. Kareko, 2019). Any successful vaccination method is thought to require the stimulation of both B and T Lymphocyte mediated immune responses because it can result in a quicker and more effective immune response when the host comes into contact with the target pathogen in the future (V. Kalia, 2006).

The purpose of vaccination is to deceive the body into believing that it has been attacked by a pathogen so the body can trigger an immune response that produces memory T and B lymphocytes (V. Vetter, 2017) (T. Hamaoka, 1988) . Successful recognition of particular target antigens or more precisely particular fragments of antigens known as epitopes are necessary for effector and memory B and T cell development. Predicting B and T cell epitopes on target antigens is therefore extremely important for vaccine development .

The immune response to viral infections must include CTL- mediated Cytotoxic activity. Virus infected cells break down a few viral proteins and then deliver them to CTL's together with MHC class I molecules . Through the release of Cytotoxic granules (S. Rosendahl Huber, 2014) (J.M. Rojas, IL-10: a multifunctional cytokine in viral infections, 2017) , CTL's that recognize degraded viral protein regions known as epitopes kill infected cells. Five CTL epitopes have been filtered out checking various parameters in this study for WNV vaccine development .

Viral particles are presented to Antigen presenting cells by MHC II molecules activating HTL's in the process. The HTL's release a variety of cytokines and chemokines upon epitope identification including IFN-gamma , IL-4, IL-10 etc. which have different functions in the immune response to invaders (U. Dittmer, 2001) (D.A. Chesler, 2002) (J.M. Rojas, 2017) (R.V. Luckheeram, 2012). A small percentage of effector T Lymphocytes (CTL& HTL) survive this phase and makeup the reservoir of memory T cells after the majority of them die once the antigens have been eliminated (S.M. Kaech E. W., 2007) (S.M. Kaech R. A., 2001) . Two HTL epitopes have been chosen for the vaccine development based on our findings.

B lymphocytes bind to antigenic epitopes present on the surface of target cells using B cell receptors which are essentially membrane bound immunoglobulins . After internalization, processing and presentation to T cells (F.D. Batista, 2001) , these antigenic epitopes are then

internalized by the target cells. The processed epitopes are displayed alongside MHCII on the surface of B cells where they are recognized by HTL's with a corresponding T cell receptor . As a result, B lymphocyte becomes plasma cells that secrete antibodies (P.D. Hodgkin, 1994) (N.J. Kräutler, 2017) (Mitchison, 1992). In order to neutralize infections , these antibodies are crucial. In contrast , the activated B lymphocytes may start germline center responses which result in the production of memory B cells and long-lived plasma cells (B.P. O'Connor, 2017) . Two LBL epitopes were found to be suitable for inclusion in this investigation .

For epitope prediction , we primarily employed the WNV virus lineage 2 , complete genome. However, because WNV virus is a RNA virus , more mutations are inevitable (Duffy, 2018). In order to overcome this issue, we performed multiple Sequence alignment creating consensus sequence resulting in zero mutation in the region of our chosen epitopes that indicates the vaccine's design is effective. In the event of potential future mutation inside the epitope regions , the mRNA vaccines can be updated using the filtering techniques we have used.

To anticipate the binding affinity and pose between a ligand and corresponding receptor , molecular docking is a vital bioinformatics technique (I. Ahammad, 2019) (T.K. George, 2019) (S. Yadav, 2017) (M. Aamir, 2018). Molecular docking has not only proven crucial to Computational drug design but also to studies on vaccine design. The simulation of binding between T Lymphocyte epitopes and their corresponding MHC alleles is a significant application of molecular docking in Immunoinformatics (M. Tahir ul Qamar, 2019) (R.A. Tahir, 2018) . The energy generated during the spontaneous bond formation between a receptor and its ligand can be used to determine the binding affinity between the two , and the lower the energy , the more closely linked the receptor to its ligand is (Y. Fu, 2018). In this study docking score was taken into consideration while molecular docking Although it shouldn't be treated as true binding affinity . In Hdock , a more negative docking score refers to a more possible binding model . 22 MHC alleles corresponded to the 7 T cell epitopes we chose . Each of these CTL and HTL epitopes together with one of their corresponding alleles were docked in molecular docking. The docked compounds were three dimentially visualized in discovery studio visualizer.

On the basis of Ramachandran plot , the docked models' quality was assessed. Ramachandran plots are regarded as one of the most important ideas in structural biology. It divides the amino acids of any given three-dimensional protein structure into four groups: amino acids in the most favored regions, amino acids in additional allowed regions, amino acids in generously allowed regions and amino acids in disallowed regions . Over 80% of the amino acids in the protein model must be located at the most favored regions for it to be regarded as a good model (B.K. Ho, 2009). In this study all the models satisfied the condition and were considered reliable.

According to molecular docking of our chosen epitopes with their respective alleles , The epitope DTAWDFGSV had the highest binding affinity in terms of lowest docking score for its associated MHC allele, HLA-A*68:02. It displayed a diverse range of interactions in discovery studio visualizer where it participated in the bond forming process with nearby residues.

The population coverage of T cell epitopes must be carefully considered when developing vaccines . Because only people with a certain MHC allele capable of recognizing the epitopes in

our vaccine construct would elicit an immunological response when exposed to the vaccination, the existence of more than a thousand human MHC alleles must be taken into consideration (H.H. Bui, 2006). In order to forecast the efficacy of our vaccine throughout various geographical locations of the world, the combined coverage of the CTL and HTL epitopes was examined. Although IEDB population coverage method anticipated a respectable level of coverage globally with a higher degree of coverage predicted for countries that experienced the most devastation from the WNV epidemic .

The production of mRNA , quality control, formulation, immunological and physicochemical characteristics of the vaccine as well as the translated form of the peptide , are crucial considerations when creating an mRNA vaccine . The need of creating the vaccine construct in a way that avoids interaction with RNA sensors should be stressed in order to achieve successful cellular uptake , immunological activation , induction of long-term memory . Some viewpoints, including cost-effectiveness, are significant problems for establishing Good Manufacturing Practice (GMP). RNA polymerase attachment site in DNA (such as those found in linearized plasmid DNA or PCR templates are used to produce mRNA in industrial settings (P.A. Krieg, 1987) . The presence of double stranded RNA impurities in this mRNA preparation leads to strong type 1 interferon production because dsRNA is a potent PAMP (pathogen associated molecular pattern) (K. Karikó, Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA, 2011) (C. De Haro, 1996). As a result , translation is inhibited, and cellular mRNA and ribosomal RNA is degraded. High Performance Liquid Chromatography (HPLC) mRNA purification has increased translation by up to 1000 times to address this issue (K. Karikó, 2011) . By removing contaminated shorter or longer transcripts , the PURE messenger chromatographic purification method has produced very pure mRNA transcripts . The HPLC-purified and nucleoside modified mRNA however has been associated with the highest level of protein synthesis . Evidently, mRNA vaccine production is more expensive than DNA vaccine production because of the transcription step . However, because of the increased rate of transfection it is less expensive over time .

Because there is often a risk of excessive host immune system activation and inflammatory responses , creating an adjuvant that is both effective and safe involves a delicate balance between immunogenicity and safety. Several strategies , including those utilizing protamine and the cytokine granulocyte macrophage colony stimulatory factor showed promise in this area (A. Isaacs, 1963) (M. Absher, 1969) (B. Scheel, 2005) . Additionally , mRNA itself has been shown to act as a self-adjuvant when delivered naked (M. Fotin-Mleczek, 2011) . However, it is a subpar strategy because of in vivo deterioration . We included the co-stimulatory molecule CD40L since it has been shown to activate pAPCs and subsequently cause the production of immune response molecules (T. Schlake, 2012) (C. Caux, 1994). However , leaving out the adjuvant would undoubtedly save time and money . Therefore, additional research can determine whether the inclusion was absolutely required . By avoiding inter-domain interactions , ideal spacers that strike a balance between flexibility and rigidity also help the mRNA vaccination work well.

The enzymatic action of the vaccinia virus capping complex or " Anti-reverse" Cap Analogs (ARCAs) can be used to co transcribely cap mRNA during in vitro transcription (J. Stepinski, 2001) (E. Grudzien, 2005) (F.T. Zohra, 2007) (E. Grudzien-Nogalska, 2007). It has been discovered that poly A tails and 5'm7G Cap sequences interact favorably with the ideal length falling between 120 and 150 base pairs . The mRNA ORF must be flanked by the 5' and 3' UTRs for improved translation and stability (Kozak, 1989) (G.S. Wilkie, 2003). Globin UTRs are frequently employed for mRNA transcription in vitro . The 5' and 3' UTRs of the Xenopus globin have been reported to boost translational efficiency by a factor of roughly 1000 (R.W. Malone, 1989). As 5' UTR and globin 3' UTR were known to stabilize mRNAs , we chose to combine them. The sequences near the stop codon may be optimized and the Kozac Sequence also needs to be taken into consideration .

Higher efficacy of mRNA and DNA based vaccines has been linked to the inclusion of secretory signal sequences as well as those that provide directional information regarding particular endoplasmic reticulum compartments such as MHC1 (Y. Kou, Tissue plasminogen activator (tPA) signal sequence enhances immunogenicity of MVA-based vaccine against tuberculosis, 2017) (S. Kreiter, Increased antigen presentation efficiency by coupling antigens to MHC class I trafficking signals, 2007). Protein expression in vivo and steady-state mRNA levels in vitro have both been found to increase with G:C content enrichment . Codon bias or substitution of uncommon codons with regularly Occurring synonymous codons , has been linked to both efficiency and a possible negative effect in some species (G. Kudla, 2006) (F. Buhr, 2016). However, in humans , it does not correlate with the tRNA levels or gene expression . As a result , when constructing our mRNA vaccine, it was avoided. In vitro translation has been shown to be improved by the incorporation of naturally Occurring chemically modified nucleosides such 1-methylepseudouridine and pseudo uridine. Its ability to evade toll like receptor and innate immune sensors subsequently lessen type 1 interferon signaling is the basis for its effectiveness . It still needs to be studied to see if it can affect in vivo translation .

Additionally essential elements for antigen expression are mRNA synthesis and administration . A promising delivery system for mRNA vaccines has been identified as lipid nanoparticles (LNP) Another role in this is the route of administration . It has been demonstrated that mRNA-LNP delivery via intramuscular and intradermal routes causes three times more sustained protein expression than intravenous delivery. Stronger half-life can increase the potency of the vaccine because continuous antigen availability after vaccination promotes stronger antibody titers and more robust immune responses (N. Pardi, Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination, 2017) (J.M. Richner, 2017) (N. Pardi, 2015). Since the virus has been known to infiltrate the central nervous system , intranasal injection of our vaccine is most likely a viable approach . mRNA interacts with the cytosolic translation complex after being incorporated into the cytoplasm. The vaccine prepares for an immunological response after translation and post- translational modifications . Our mRNA construct' s translated form was projected by a variety of in sillico methods to be nearly neutral , stable , highly antigenic , non-allergenic and hydrophilic, thermostable in nature making it a promising candidate for a vaccine .

Following repeated exposure to the antigen, an overall increase in immune responses was observed in the in silico immune response simulation. Higher B and T-cell activity as well as B cell memory that persisted for several months were indicators of humoral immunity, which is necessary to support the immunological response. High production of IL-2 and IFN-γ after repeated exposure suggested a cell-mediated immune response. In addition to its involvement in Ig isotype switching and B-cell proliferation (D. Fang, 2018) , the cytokine IFN-γ can also facilitate a humoral response. Since the vaccine build was designed to include IL-10 and IL-4 positive HTL epitopes, moderate levels of IL-10 and IL-4 activity were noted. The Simson index, dendritic cell, and macrophage activity, and other parameters were found to be satisfactory. According to the vaccine's profile, the body will naturally establish immunological memory and become resistant to WNV.

Immunoinformatics offer the benefit of quick and low-cost identification , screening and vaccine design instead of starting with expensive and time-consuming laboratory- based procedures . Rapid and high yield technologies like mRNA vaccines are the answer to the WNV virus broadly distributed across the world causing frequent outbreaks . This innovative vaccine candidate has shown promise as a weapon in the fight against the deadly virus . This is only possible after additional testing of its effectiveness in vivo and in vitro through coordinated action between pertinent quarters.

Conclusion

This study was done for designing West Nile Virus mRNA vaccine through *In Sillico* approaches. The study was initiated through the retrieval of West Nile Virus E glycoprotein sequence as a target protein to facilitate immune responses inside our body as LBL, HTL,CTL development and activation to fight against the virus. Certain epitopes that selectively bind with MHC alleles , adjuvants, linkers and other crucial elements such as tPA and MITD , 5'-3' poly(A) tail were used to make this vaccine construct. Using the bioinformatic tools, vaccines' properties and qualities were checked. The immune simulation of a vaccine construct proves its potential to fight WNV virus and help prevent future outbreaks.

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