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Construction of multi-epitopes vaccine against Ebola virus disease

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Abstract:
The Ebola virus causes Ebola virus disease, was discovered in two simultaneous outbreaks in Sudan and the Democratic Republic of Congo in 1976. While EVD is a rare condition, with substantial outbreaks and high mortality rates. Direct contact with infected individuals or animals; blood, secretions, organs, or other bodily fluid leads to the transmission of the virus. There is yet no cure for EVD, however hydration and symptom management can enhance life expectancies. This immunoinformatic approach which include both B cell and T cell epitopes has been used for candidate vaccine development against EVD. The prediction of B cell and T cell epitopes was done by targeting the VP24 and VP30 proteins of the Ebola virus and an antigenic multi-epitope vaccine construct was designed. The vaccine construct was then docked with human immunogenic TLR 4 and in silico immune simulation was done for prediction of immunogenic potential of constructedvaccine.

Keywords:
Ebola virus, Epitope-based vaccine, Molecular docking, Immunoinformatic, Reverse vaccinology

Introduction

The Ebola Virus causes the deadly, frequently fatal disease known as Ebola virus disease (EVD), usually referred to as Ebola haemorrhagic fever, in both humans and primates. The virus is transmitted to humans through wild animals including fruit bats, porcupines, and non-human primates (Rugarabamu et al., 2022). The Ebola virus has a significant fatality rate, with an average fatality rate that is approximately 50% (Singh et al., 2020). While there is no specific medications or vaccination for this illness, supportive care such as maintaining fluid balance, managing blood pressure, and treating other diseases can increase survival rates (Kyle et al., 2019). In parallel epidemics in the Democratic Republic of the Congo and Sudan in 1976, the first cases of the Ebola virus disease (EVD) were documented (then known as Zaire). The Ebola River in Zaire, where the first case was reported, inspired the name of the virus (Jacob et al., 2020). With a fatality rate as high as 90%, the epidemic in Zaire was especially bad. Since that time, there have been several EVD outbreaks throughout Africa, including ones in Uganda, Gabon, the Ivory Coast, and the Democratic Republic of the Congo. Between 2014 and 2016, West Africa saw its greatest and most current EVD outbreak, which mostly affected Guinea, Liberia, and Sierra Leone. In terms of its geographic reach, the volume of patients, and the difficulties encountered in managing the outbreak, this outbreak was unparalleled (Hasan et al., 2019).

Scientists have made great strides in understanding the virus and creating diagnostic tools, cures, and vaccinations throughout the years. Yet, EVD continues to pose a severe threat to the public health, particularly

in nations with constrained funding and shoddy healthcare systems. To better understand the illness and provide more efficient therapies to stop and manage epidemics, research must continue. There have been multiple Ebola outbreaks in Africa since the first case of the illness was recorded in 1976.

The following represent some of the most significant outbreaks (Wannier et al., 2019):

1976: The first reported outbreak occurred in Sudan and the Democratic Republic of Congo.

1995: Outbreak in Kiewit, Democratic Republic of Congo, with a total of 315 cases and a mortality rate of 81%.

2000: Outbreak in Uganda, with a total of 425 cases and a mortality rate of 53%.

2003: Outbreak in the Democratic Republic of Congo, with a total of 143 cases and a mortality rate of 83%.

2007: Outbreak in the Democratic Republic of Congo, with a total of 264 cases and a mortality rate of 71%.

2014-2016: The largest outbreak of EVD occurred in West Africa, primarily affecting Guinea, Liberia, and Sierra Leone, with a total of over 28,000 cases and more than 11,000 deaths.

2018-2020: Outbreak in the Democratic Republic of Congo, with a total of 3,481 cases and a mortality rate of 67%.

These outbreaks have highlighted the need for continued research and investment in the development of vaccines, treatments, and prevention strategies to help control and mitigate the impact of future outbreaks. There are six known species of Ebola virus, each named after the location where they were first identified. They are Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Bundibugyo ebolavirus (BDBV), Tai Forest ebolavirus (TAFV), Reston ebolavirus (RESTV), Bombali ebolavirus (BOMV) of these, Zaire ebolavirus (EBOV) is the most virulent and has caused the most widespread and deadliest outbreaks. Sudan ebolavirus (SUDV) is also highly virulent, while Bundibugyo ebolavirus (BDBV) causes less severe disease in humans. The Tai Forest ebolavirus (TAFV) has caused a single outbreak in Ivory Coast, and Reston ebolavirus (RESTV) has caused outbreaks in monkeys and pigs but has not been known to cause illness in humans (Whitfield et al., 2020). The death rate of Ebola Virus Disease (EVD) can vary depending on various factors, such as the virulence of the virus, the quality of medical care available, and the overall health of the affected individuals. The average case fatality rate for EVD is around 50%, with some outbreaks reporting mortality rates as high as 90% (Covés-Datson et al., 2019). The Zaire ebolavirus (EBOV) species, which has caused the most widespread and deadliest outbreaks, has a case fatality rate ranging from 60% to 90%, depending on the outbreak. Sudan ebolavirus (SUDV), another highly virulent species, has a case fatality rate ranging from 40% to 65% (Brémaud et al., 2022).

However, with improved medical care and early treatment, the death rate of EVD can be significantly reduced. The administration of supportive care, such as maintaining fluid levels and electrolyte balance, treating other infections, and managing complications, can help

improve the chances of survival. It is important to note that the death rate of EVD is not fixed, and there are many factors that can influence the severity of an outbreak. Rapid identification and containment of outbreaks, early diagnosis and treatment, and effective prevention and control measures can all contribute to reducing the death rate of EVD (Aruna et al., 2019). The infection rate of Ebola Virus Disease (EVD) can vary depending on various factors such as the virulence of the virus, the population density, and the level of public health preparedness. The virus is highly infectious and spreads through direct contact with bodily fluids of an infected person or an infected animal, such as blood, vomit, saliva, and faeces (Kamorudeen et al., 2020). The rate of infection during an EVD outbreak can vary depending on several factors, including the effectiveness of containment measures, the availability of personal protective equipment, and the ability to quickly identify and isolate infected individuals. In some outbreaks, the infection rate has been relatively low, with only a few hundred cases reported, while in others, such as the 2014-2016 West African outbreak, the infection rate was much higher, with over 28,000 cases reported (WHO, 2019). The reproductive number (R0), which represents the average number of people who will contract the virus from an infected person, varies depending on the strain of the virus and the conditions of the outbreak. For the Ebola virus, the estimated R0 can range from 1.5 to 2.5, which means that each person infected with the virus is likely to infect an average of 1.5 to 2.5 other people (Diallo et al., 2019). Prevention and control measures, such as isolation and quarantine, contact tracing, and rapid diagnosis, can help to reduce the spread of EVD during outbreaks. Additionally, promoting good hygiene practices, such as frequent handwashing and safe burial practices, can help to prevent the transmission of the virus. The symptoms of Ebola Virus Disease (EVD) can appear anywhere from 2 to 21 days after exposure to the virus, with an average incubation period of 8-10 days (Rojas et al., 2020). Symptoms of EVD are similar to other viral illnesses and can be initially mistaken for other diseases such as malaria, typhoid fever, or meningitis. The initial symptoms of EVD include fever, headache, muscle pain, weakness and fatigue. And these symptoms are often followed by diarrhea, vomiting, abdominal pain, bleeding or bruising, skin rash, red eyes, chest pain and sore throat. As the disease progresses, it can cause more severe symptoms such as difficulty breathing and swallowing, impaired kidney and liver function, internal and external bleeding, shock and coma (Cénat et al., 2020). The severity of symptoms can vary from person to person and depends on the individual immune system response and overall health. In severe cases, EVD can lead to death within a few days or weeks due to complications such as multiple organ failure and severe bleeding.

There are currently two vaccines and several drugs available for Ebola Virus Disease (EVD) treatment. Vaccines (Tomori et al., 2021):

VSV-EBOV vaccine: This is a live attenuated vaccine that contains a weakened form of the vesicular stomatitis virus (VSV) that has been genetically modified to express the Ebola virus glycoprotein. It was first tested during the 2014-2016 West African Ebola outbreak and showed a high level of protection against the virus. It has been approved for use by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

rVSVΔG-ZEBOV-GP vaccine: This is a recombinant, replication-competent vaccine that contains weakened form of the vesicular stomatitis virus (VSV) that has been genetically modified to express the Ebola virus glycoprotein. It was also tested during the 2014-2016 West African Ebola outbreak and has shown a high level of protection against the virus. It has been approved for use by the FDA and the EMA.

Drugs (Mirza et al., 2019):

ZMapp: This is a combination of three monoclonal antibodies that target the Ebola virus glycoprotein. It was used during the 2014-2016 West African Ebola outbreak and showed promising results in some patients. However, clinical trials have not been able to conclusively prove its efficacy.

Remdesivir: This is an antiviral drug that was originally developed to treat Ebola and other viral diseases. It has been shown to be effective against the Ebola virus in laboratory studies and was used during the 2018-2020 Ebola outbreak in the Democratic Republic of Congo. It has also been approved for emergency use by the FDA for the treatment of COVID-19.

Favipiravir: This is an antiviral drug that has been used to treat influenza and other viral diseases. It has been shown to be effective against the Ebola virus in laboratory studies and was used during the 2018-2020 Ebola outbreak in the Democratic Republic of Congo.

It is important to note that these vaccines and drugs are not universally effective and may have varying levels of efficacy depending on the strain of the virus and the individual patient's response to treatment. Ebola virus disease (EVD) is a severe and often deadly illness caused by the Ebola virus. Currently, there are several vaccines under development to prevent EVD. The most advanced vaccine is the rVSV-ZEBOV vaccine, which was used in the 2018-2020 Ebola outbreak in the Democratic Republic of Congo (Afolabi et al., 2022).

Clinical trials of the rVSV-ZEBOV vaccine have shown that it is highly effective in preventing EVD.

In a randomized controlled trial conducted during the 2014-2016 outbreak in West Africa, the vaccine demonstrated 100% effectiveness in individuals who received the vaccine immediately after being exposed to the virus (Schwartz et al., 2019). In the 2018-2020 outbreak in the Democratic Republic of Congo, the rVSV-ZEBOV vaccine was also highly effective, with an estimated effectiveness of 97.5%. The vaccine was given to people who were at high risk of contracting the disease, including healthcare workers, contacts of confirmed cases, and contacts of contacts (WHO,2019). Overall, the rVSV-ZEBOV vaccine has shown to be a highly effective tool for preventing EVD. However, it is important to note that the vaccine is still undergoing further research and development, and its long-term effectiveness and safety have not yet been fully established (Iversen et al., 2020). VP24 and VP30 are two important proteins of the Ebola virus and are potential targets for in-silico vaccine development for several reasons (Rojas et al., 2020):

Essential for viral replication: VP24 and VP30 are essential proteins for the replication of the Ebola virus.

They play important roles in the transcription and replication of the viral genome. Therefore, targeting these proteins may inhibit the replication of the virus and prevent the spread of the disease.

Conservation across different strains: VP24 and VP30 are highly conserved proteins, meaning that they are similar across different strains of the Ebola virus. This makes them attractive targets for vaccine development because a vaccine that targets these proteins is likely to be effective against different strains of the virus.

Immunogenic: VP24 and VP30 are also known to be immunogenic, meaning that they can elicit an immune response in the body. Targeting these proteins in a vaccine can stimulate the production of antibodies and immune cells that can recognize and neutralize the virus.

Accessible: VP24 and VP30 are located on the surface of the Ebola virus and are accessible to the immune system. This makes them good targets for vaccines that aim to generate an immune response against the virus. Overall, the choice of VP24 and VP30 as targets for in-silico vaccine development for Ebola virus is based on their importance in viral replication,

conservation across different strains, immunogenicity, and accessibility to the immune system.

Materials and Methods

Polyprotein identification and analysis:

Identification of poly proteins available for EVD was done according to the literature survey and then the UniprotKB/Swissprot database (Boutet et al., 2007) was used to get the accession IDs of the polyproteins (UniprotKB ID Q05322 and Q05323). The BLAST

search (BLAST P) (Mahram et al., 2015) was performed to get the complete sequences with more than 97% sequence identity were considered for further analysis. As a good vaccine candidate should be antigenic in nature, non- allergic and non-toxic in nature, the identified poly proteins had undergone analysis by Vexijen 2.0 (Rahman et al., 2020), Allergen FP (Ratner et al., 1998) and ToxinPred (Kaushik et al., 2020) web server for antigenicity, allergenicity and toxicity analysis respectively and are mentioned in Table 1.

Prediction of B cell and T cell epitopes:

BepiPred linear epitope prediction tool of IEDB was used to predict the B cell epitopes (Jespersen et al., 2017), which uses Hidden Markov Model for the prediction of linear B cell epitopes. The NETMHC 4.0 web server was used to predict the T cytotoxic epitopes (Andreatta et al., 2016) that binds with MHC class I alleles, which uses Artificial neural network method.

Prediction of allergenic, antigenic, toxic nature of the epitopes:

To be a suitable and effective vaccine candidate, the predicted epitopes should not be toxic or allergenic and must be antigenic in nature. So, AllergenFP v.1.0 server which uses Tanimoto coefficient to predict the allergenicity, Vexijen 2.0 webserver which uses cross-covariance method to calculate the Vexijen score to predict the antigenicity, ToxinPred which uses SVM based method to predict epitope toxicity was used.

Vaccine construction and its physiochemical properties analysis:

For final vaccine construction, epitopes which were antigenic in nature, and were neither allergenic nor toxic in nature were taken into consideration. 50s ribosomal L7/L12 (Locus

RL7_MYCTU) sequence of Mycobacterium tuberculosis H37Rv with NCBI accession number P9WHE3 was used as adjuvants and as linkers EAAAK which connects adjuvant to epitopes from the N-terminal and GPGPG used for connecting epitopes with each other (Ismail et al., 2022) were used.

The analysis of physiochemical properties of the constructed vaccine was done by using ExPASy- ProtParam webserver (Gasteiger et al., 2005) which helps to determine the number of amino acid present, stability, molecular weight, GRAVY value, number of negatively charged residues, positively charged residues, aliphatic index and many other properties of the constructed vaccine. Vexijen webserver was used to predict the antigenicity of the vaccine.

Tertiary structure prediction of constructed vaccine and validation of the constructed tertiary structure:

I-TASSER webserver was used, which constructed the tertiary or 3D model of candidate vaccine (Yang et al., 2005). This server predicts the model by using multiple threading method and iterative template-based fragments assembly simulations.

Molecular docking of the constructed vaccine with toll- like receptor (TLR) molecule:

ClusPro 2.0, which is a protein-protein docking webserver was used for molecular docking (Zheng et al., 2021) of the constructed vaccine with the Toll-like receptor 4 molecule (PDB ID: 3FXI) (Rajpoot et al., 2021). This server provides 10 docked complex models with low energy structures in high population clusters.

Immune simulation:

Determination of the immunogenicity and immune response, C-IMMSIM webserver was used (Okumura et al., 2010, Castiglione et al., 2004).

Immune response is predicted by using scoring matrix on top of position specification obtained by machine learning. 8 weeks, 3 months or 6 months are the recommended interval between doses of the vaccine (Campo et al., 2021). Four weeks interval between three injections was the time period taken for studying immune response. The default parameters were used except for the time step of injection. For analysis time steps of 4 weeks and 8 weeks equivalent were considered.

Result and Discussion

Polyprotein identification and analysis:

As VP24 and VP30 proteins with UniprotKB ID Q05322 and Q05323 respectively helps EBOV for viral replication, conservation across different strains, immunogenic nature and accessibility to the immune system, these were considered as the target proteins and BLAST search was performed to get the identified proteins with 100% sequence identity. And also, these proteins were found to be potential vaccine candidates with high antigenicity and neither allergic nor non-toxic in nature (Table 1).

Table 1. Proteins and their respective UniprotKB IDs

Protein name	UniprotKB ID	Vexijen score	Allergen/ Non-allergen	Antigen /Non-antigen	Toxic/Non-toxic
Membrane-associated structural protein	AAB81006.1	0.4735	Non-allergen	Antigen	Non- toxic
VP24	AAD14588.1	0.4735	Non-allergen	Antigen	Non-toxic
VP24	AAG40170.1	0.4795	Non-allergen	Antigen	Non-toxic
Membrane-associated VP24	AAN37510.1	0.4735	Non-allergen	Antigen	Non-toxic
VP24	AAM76037.1	0.4502	Non-allergen	Antigen	Non-toxic
Membrane-associated protein	NP_066250.1	0.4735	Non-allergen	Antigen	Non- toxic
VP24	AAG40170.1	0.4795	Non-allergen	Antigen	Non-toxic

VP24	AAM76037.1	0.4502	Non-allergen	Antigen	Non-toxic
Membrane-associated protein VP24	Q05322.2	0.4735	Non-allergen	Antigen	Non-toxic
VP30	AAM76036.1	0.5199	Non-allergen	Antigen	Non-toxic
VP30	AAG40169.1	0.5221	Non-allergen	Antigen	Non-toxic

Prediction of B cell and T cell epitopes:

The linear B cell epitopes, T helper cell epitopes were selected with good binding affinities.

Prediction of the allergenic, antigenic, toxic nature of the epitopes:

For all predicted linear B cell, T helper cell and T cytotoxic cell epitopes, the allergenicity, antigenicity and toxicity analysis were done and out of which two epitopes were found be antigenic in nature and also neither allergenic nor toxic in nature and are mentioned in Table 2.

Table 2. Epitopes that are non-allergen, non-toxic and antigenic in nature

Epitope Name	Epitope Sequence	Vexijen score	Allergen/ Non-Allergen	Antigen/ Non-antien	Toxic /Non-toxic
B cell	KTNDFAPAWSM	1.2175	Non-Allergen	Antigen	Non-toxic
T cytotoxic	RVKEQLSLK	1.1974	Non-Allergen	Antigen	Non-toxic

Vaccine construction and its physiochemical properties analysis:

Screened epitopes were then further used to construct the vaccine using adjuvant, 50S ribosomal L7/L12 (Locus RL7_MYCTU) sequence with NCBI accession no. P9WHE3. These epitopes and adjuvants were then linked by linkers, EAAAK which was used to connect adjuvant to epitopes from the N-terminal and GPGPG were used to connect epitopes with each other.

Final Vaccine Construct:

MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVE AAEEQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKE AADEAKAKLEAAGATVTVKEAAAKRV KEQLSLKGPGPGKTNDFAPAWSM

Physiochemical properties of constructed vaccine are mentioned in Table 3. The vaccine was found to be stable in nature and also the instability index score and GRAVY score using ExPASy ProtParam tool and antigenicity by Vexijen webserver, and are mentioned in Table 3.

Table 3. Physicochemical Properties of the constructed vaccine

Molecular weight	16608.12
Instability index	20.52(STABLE)
Aliphatic index	94.12
Theoretical Pi	4.82
Extinction coefficient	5500
Total number of negatively charged residues (Asp + Glu)	28
Total number of positively charged residues (Arg + Lys)	21
Estimated half-life	The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo).

	>10 hours (Escherichia coli, in vivo).
Total number of atoms	160
Grand average of hydropathicity (GRAVY)	0.013
Antigenicity using Vexijen sever	0.4003

Tertiary structure prediction of the constructed vaccine and validation of the constructed tertiary structure: Predicted secondary structure of the vaccine construct under gone analysis and reported having all the three, alpha helix, beta-sheets and coils in it. The tertiary structure predicted by the I- TASSER webserver had a C-score of -0.71.

Molecular docking of the constructed vaccine with toll- like receptor (TLR) molecule:

The docking was performed using ClusPro 2.0 webserver. Here the human immunogenic TLR- 4 (PDB ID: 3FXI) was used as the receptor and the constructed vaccine as the ligand molecule (Fig.1,2). It resulted in generating 30 models and the lowest binding energy generated from the models, was found to be - 823.4kcal/mol.

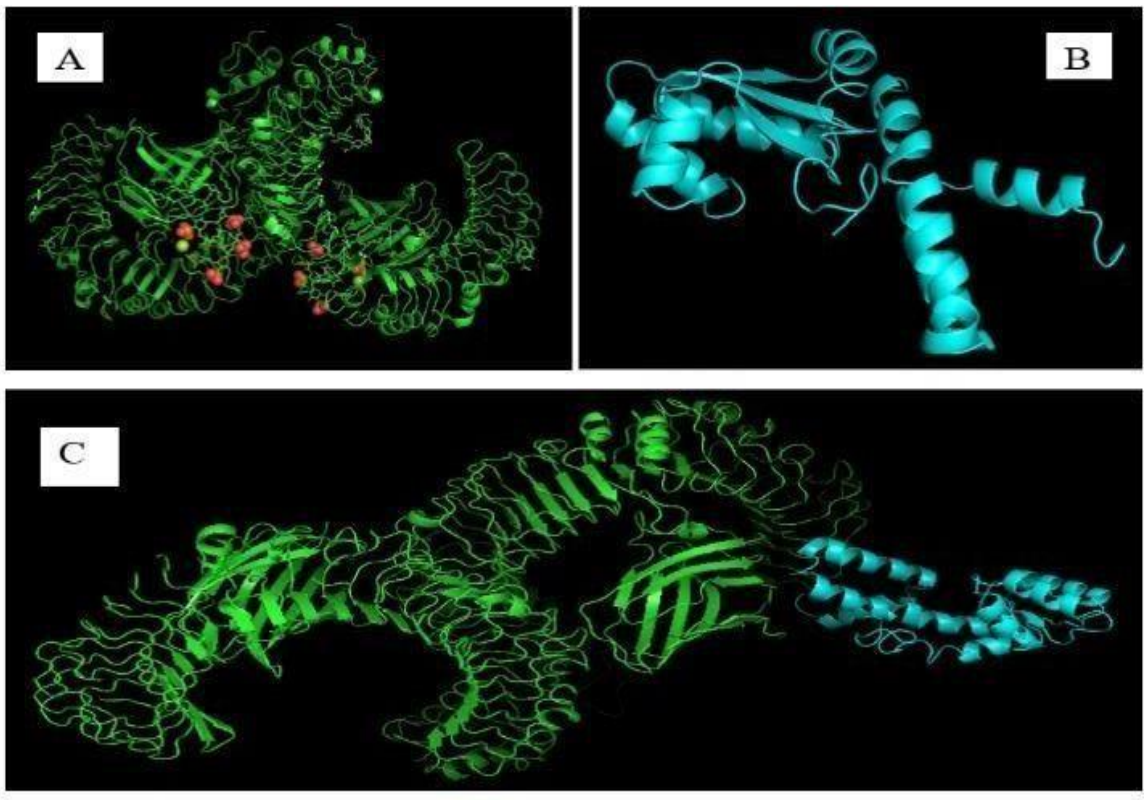

Figure 1. A. TLR4(PDB ID: 3FXI), B. constructed

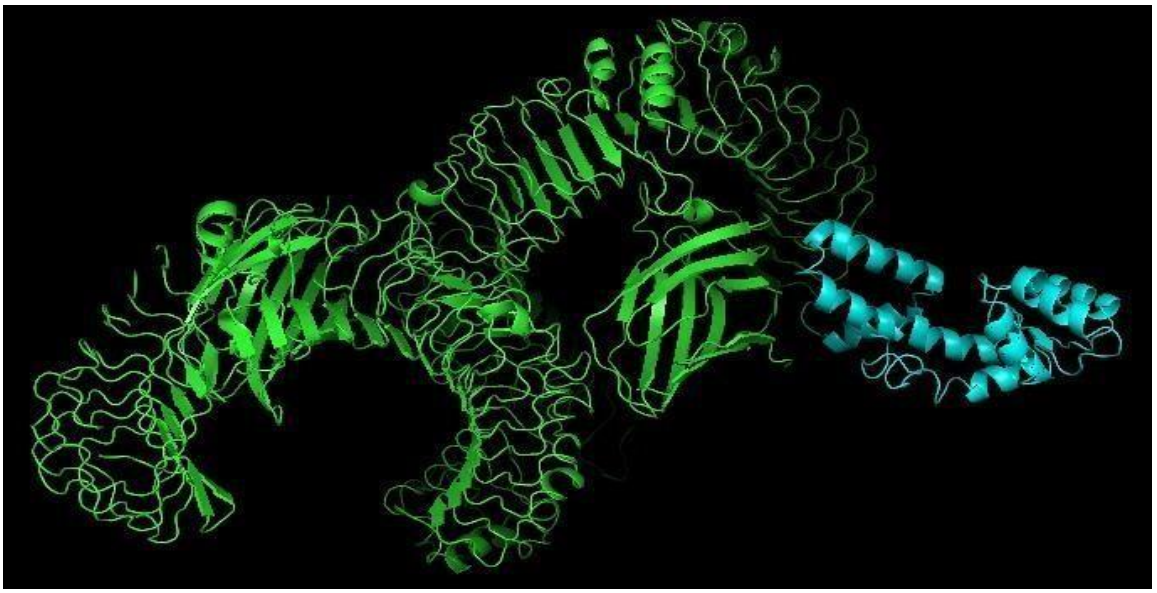

Figure 2. Docked complex (constructed vaccine and TLR4)

Immune simulation:

C-IMMSIM webserver predicted the graphical results of the immune simulation (Fig.3). The B-cell population graph interprets that for the first 30 days there is a significant event happening where in there is a significant decline in B Memory cell which is the period aligned with the first dose. With subsequent second and third dose there is an increase of B isotype IG1 and B isotype IG2 where the isotype IG1 has a significant growth. Isotype IG2 has a growth but it's very minimal. The TH cell population graph interprets that after the first dose there is a spike in TH not memory cell and TH memory Y2 cell thus having a spike in total as well. After second dose, there is a significant rise in TH not memory cell and TH memory Y2 cell as well. But after the third dose we see some difference i.e., TH not memory cell gradually reduces, TH memory Y2 cell increases more and in total the peak has been touched with a steady slump following it. The TH population per state graph interprets that after each dose there is a significant spike in the cell population with the second dose having a major spike. The concentration of cytokines and interleukins graph interprets that after the first dose we see a significant spike in IFN-g where in the spike is in form of a plateau, which is reached after 5 days. Similarly for second and third dose as well we see spikes more significant than the first spike and in a reducing pattern wherein after third spike there is a slump. For both the second and third spike we see the peak being reached at a mid-time period with respect to the dose cycle. The total results include B-cell population, PLB cell population, T-cell population, B-cell population per state, T-cell population per state and TH

cell population per state. This interprets the response of these epitopes when administered with first second and third dose by following specific time routine.

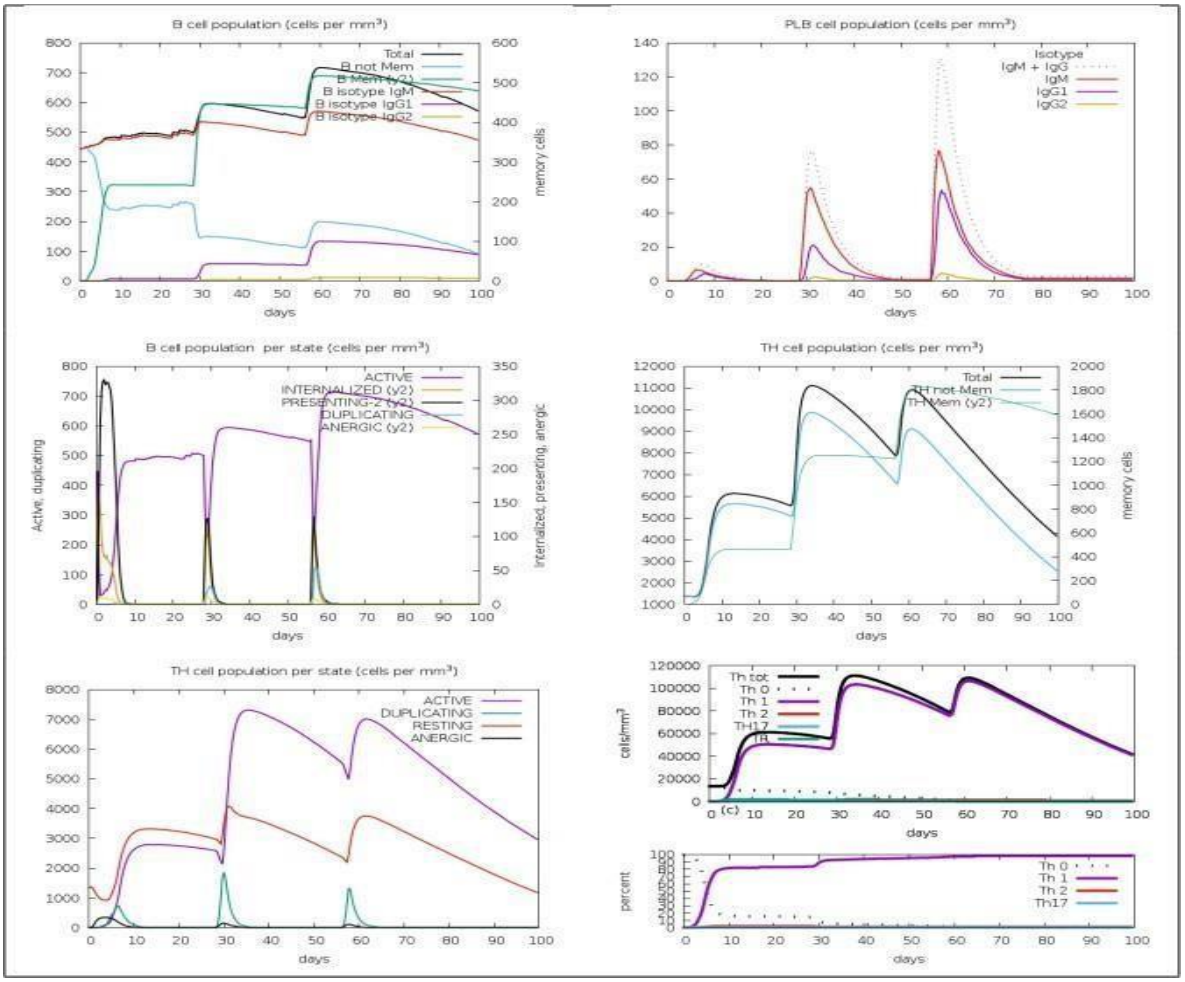

Figure 3. Graphs representing immune stimulation of the B cell and T cell epitopes

EVD outbreak has created devastation and it was declared a World Public Health Emergency by WHO. This study includes in silico methods to develop the multi-epitope vaccine against EBOV. Any engineered multiepitope vaccine against EBOV is not present till date (Alizadeh et al., 2022). In silico peptide based multi-epitope vaccine has been acceptable as it is time relevant and cost efficient (Lee et al., 2020). Immunoinformatic methods are easy to implement and is very useful (De Groot et al., 2002). Conventional vaccines are either inactivated form of pathogens or live attenuated but in this new era of proteome analysis the multi epitope vaccine designing is time saving and also this was previously proved useful for analysing other proteins of different structural and non- structural proteins of zika virus, hepatitis c virus and many more (Jaydari et al., 2020). This also develops a strong immune response with high antigenicity and low allergenicity and toxicity. The vaccine is considered

to be effective, when the level of antibody secretion is high. In this study, we selected the targeted VP24 and VP30 proteins with UniprotKB ID Q05322 and Q05323 respectively as these are responsible for viral replication, conservation across different strains, immunogenic nature and its accessibility to the immune system for the designed vaccine. Also, it was used to predict the B linear cell T and cytotoxic cell epitopes. Then the antigenicity, allergenicity and toxicity analysis was done. Then the screened epitopes were selected for construction of the vaccines. After these criteria, the epitopes were linked by using linker sequences and adjuvant. These linkers have been used previously in construction of vaccine design of the many viruses and 50s ribosomal L7/L12 of M. tuberculosis was used as adjuvants used to construct multi epitope vaccine against many viruses. Based on the analyses, 114 amino acid long vaccine was projected. The constructed vaccine was analysed as stable and soluble in nature and with high antigenicity. The tertiary structure of the vaccine was constructed and C score of the constructed vaccine was found to be -0.71, where the C-score value should range between - 5 to 2 and higher the C-score, higher is significance of the model (Hevener et al., 2009). Then the analysis of molecular docking was carried out to find binding efficacy of constructed vaccine with Toll-like receptor molecule (TLR 4). TLR4 was the adjuvant used, binds with the TLR4 molecule to activate immune response resulting in binding energy -823.4kcal/mol; as the lower the binding energy, the higher is the binding affinity (Wu, Z. et al., 2020). For the stability analysis immune simulation was performed which resulted in production of good number of antibodies after the third dose of vaccine and was found to be stable. This study could result in safe and potential therapy measure against EVD.

Conclusion

After all the in-silico analysis, a vaccine candidate was designed by targeting the VP24 and VP30 proteins of EBOV. The designed vaccine was predicted as stable and could interact with TLR4 molecule. Furthermore, the immune simulations analysis showed the vaccine construct could elicit robust immune response after second and third vaccine doses. However, to further establish the safety and efficiency of vaccine construct designed in this study several in vitro studies as well as in vivo studies are required.

References

Rugarabamu, S., George, J., Mbanzulu, K. M., Mwanyika, G. O., Misinzo, G., & Mboera, L. E., Epidemiologia,3, 68-80 (2022), DOI: N/A

Singh, C.Chems.', Solitons & Fractals, 138, 109992 (2020), DOI: N/A

Kyle, J. E., Burnum-Johnson, K. E., Wendler, J. P., Eisfeld, A. J., Halfmann, P. J., Watanabe, T., ... & Metz, T. O., Proceedings of the National Academy of Sciences,116, 3919-3928 (2019), DOI: 10.1073/pnas.1815356116

Jacob, S. T., Crozier, I., Fischer, W. A., Hewlett, A., Kraft, C. S., Vega, M. A. D. L., ... & Kuhn, J.H., Nature reviews Disease primers,6, 13 (2020), DOI: 10.1038/s41572-020-0147-3

Hasan, S., Ahmad, S. A., Masood, R., & Saeed, S., Journal of family medicine and primary care,8, 2189 (2019), DOI: N/A

Wannier, S. R., Worden, L., Hoff, N. A., Amezcua, E.,Selo, B., Sinai, C., ...&Kelly, J. D.,Epidemics,28,100353 (2019), DOI: N/A

Whitfield, Z. J., Prasad, A. N., Ronk, A. J., Kuzmin, I. V., Ilinykh, P. A., Andino, R., & Bukreyev, A., Cell reports,32(7),108028 (2020), DOI: https://doi.org/10.1016/j.celrep.2020.108028

Covés-Datson, E. M., Dyall, J., DeWald, L. E., King, S. R., Dube, D., Legendre, M., ... & Markovitz, D. M., PLoS neglected tropical diseases,13, e0007595 (2019), DOI: https://doi.org/10.1371/journal.pntd.0007595

Brémaud, E., Favard, C., & Muriaux, D., Membranes,12, 441(2022), DOI:10.3390/membranes12050441

Aruna, A., Mbala, P., Minikulu, L., Mukadi, D., Bulemfu, D., Edidi, F., ... & Worrell, M. C., Morbidity and Mortality Weekly Report,68,1162(2019), DOI: N/A

Kamorudeen, R. T., Adedokun, K. A., & Olarinmoye, A. O., Journal of infection and public health,13,956- 962(2020), DOI: https://doi.org/10.1016/j.jiph.2020.03.029

Ebola Virus Disease Democratic Republic of Congo: External situation report 72, World Health Organization (2019), DOI: N/A

Diallo, M. S. K., Rabilloud, M., Ayouba, A., Touré, A., Thaurignac, G., Butel, C., ... & YAZDANPANAH, Y., The Lancet Infectious Diseases, 19(3),308-316(2019), DOI: https://doi.org/10.1016/S1473-3099(18)30725-3

Rojas, M., Monsalve, D. M., Pacheco, Y., Acosta- Ampudia, Y., Ramírez-Santana, C., Ansari, A. A., ... & Anaya, J. M., Journal of autoimmunity,106,102375(2020), DOI: https://doi.org/10.1016/j.jaut.2019.102375

Cénat, J. M., Felix, N., Blais-Rochette, C., Rousseau, C., Bukaka, J., Derivois, D., ... & Birangui, J. P., Psychiatry research,289,113033(2020), DOI: https://doi.org/10.1016/j.psychres.2020.113033

Tomori, O. and Kolawole, M. O., Ebola virus disease: Current vaccine solutions, Current Opinion in Immunology,71,27-33 (2021),DOI: 10.1016/j.coi.2021.03.006

Mirza, M. U., Vanmeert, M., Ali, A., Iman, K., Froeyen, M., & Idrees, M., Journal of medical virology,91(12),2029-2048(2019), DOI: 10.1002/jmv.25598

Afolabi, M. O., Ishola, D., Manno, D., Keshinro, B., Bockstal, V., Rogers, B., ... & Ishola, D., The Lancet Infectious Diseases,22(1), 110-122 (2022), DOI: https://doi.org/10.1016/S1473-3099(21)00291-2

Schwartz, D.A., Maternal and infant death and the rVSV-ZEBOV vaccine through three recent Ebola virus epidemics-West Africa, DRC Équateur and DRC Kivu: 4 years of excluding pregnant and lactating women and their infants from immunization, Current Tropical Medicine Reports, 6,213-222 (2019), DOI: N/A

World Health Organization (2019), DOI: N/A

Iversen, P. L., Kane, C. D., Zeng, X., Panchal, R. G., Warren, T. K., Radoshitzky, S. R., ... & Saunders, D. L., The Lancet Infectious diseases,20, e231-e237 (2020), DOI: 10.1016/S1473-3099(20)30098-2

Boutet, E., Lieberherr, D., Tognolli, M., Schneider, M., & Bairoch, A., Methods in molecular biology (Clifton, N.J.),406,89-112 (2007),DOI: https://doi.org/10.1007/978-1-59745-535-0_4

Mahram, A., & Herbordt, M. C., ACM Transactions on Reconfigurable Technology and Systems (TRETS),7(4), 1-20 (2015), DOI: 10.1145/2818344

Rahman, N., Ajmal, A., Ali, F., & Rastrelli, L., Genomics, 112(5),3473-3483 (2020), DOI: 10.1016/j.ygeno.2020.06.021

Ratner, P. H., Van Bavel, J. H., Martin, B. G., Hampel,F. C., Howland, W. C., Rogenes, P. R., ... & Cook, C.K., Journal of family practice,47,118-126(1998), DOI: N/A

Kaushik, V., International Journal of Peptide Research and Therapeutics,26,1147-1153 (2020), DOI: https://doi.org/10.1007/s10989-019-09956-3

Jespersen, M. C., Peters, B., Nielsen, M., & Marcatili, P., Nucleic Acids Research, 45(W1), 24-W29 (2017), DOI: https://doi.org/10.1093/nar/gkx346

Andreatta, M., & Nielsen, M., Bioinformatics,32(4),511-517(2016), DOI: https://doi.org/10.1093/bioinformatics/btv639

Ismail, S., Abbasi, S. W., Yousaf, M., Ahmad, S., Muhammad, K., & Waheed, Y., Vaccines,10,378 (2022), DOI: https://doi.org/10.3390/vaccines10030378

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S. E., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server (pp. 571-607). Humana press. DOI: N/A

Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., & Zhang, Y. (2015). The I-TASSER Suite: protein structure and function prediction. Nature methods, 12(1), 7-8. DOI: N/A

Zheng, W., Zhang, C., Li, Y., Pearce, R., Bell, E. W., & Zhang, Y., Cell reports methods,1,100014 (2021), DOI: https://doi.org/10.1016/j.crmeth.2021.100014

Rajpoot, S., Ohishi, T., Kumar, A., Pan, Q., Banerjee, S., Zhang, K. Y., & Baig, M. S., Drugs in R&D,21,273- 283(2021), DOI: N/A

Okumura, A., Pitha, P. M., Yoshimura, A., & Harty, R. N., Journal of virology,84,27-33 (2010), DOI: 10.1128/JVI.01275-09

Castiglione, F., & Bernaschi, M., Proceedings of the sixteenth international symposium on mathematical theory of networks and systems (MTNS2004), (2004), DOI: N/A

Campo, F., Venuti, A., Pimpinelli, F., Abril, E., Blandino, G., Conti, L., Pellini, R., Vaccines,9,1125 (2021), DOI: 10.3390/vaccines9101125

Alizadeh, M., Amini-Khoei, H., Tahmasebian, S., Ghatrehsamani, M., Ghatreh Samani, K., Edalatpanah, Y., ... & Bagheri, N., Scientific Reports,12,7757 (2022), DOI: 10.1038/s41598-022-11620-5 Lee, C. H., & Koohy, H., F1000Research, 9, 145 (2020), DOI: 10.12688/f1000research.22457.1

De Groot, A. S., Sbai, H., Aubin, C. S., McMurry, J., & Martin, W., Immunology and cell biology,80,255- 269(2002), DOI: 10.1046/j.1440-1711.2002. 01077.x

Jaydari, A., Nazifi, N., & Forouharmehr, A., Human Immunology,81,596-605 (2020),DOI: 10.1016/j.humimm.2020.07.005

Hevener, K. E., Zhao, W., Ball, D. M., Babaoglu, K., Qi, J., White, S. W., & Lee, R. E., Journal of Chemical Information and Modeling,49,444-460 (2009), DOI: 10.1021/ci800337e

Wu, Z., Jiang, M., Liu, Z., Jamshaid, A., Ono, L. K., & Qi, Y., Advanced Energy Materials,10, 1903696 (2020), DOI: 10.1002/aenm.201903696