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MULTI-EPITOPE VACCINE DESIGN AGAINST THE MONKEYPOX VIRUS USING REVERSE VACCINOLOGY TECHNIQUES AND BIOINFORMATICS TOOLS



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**Abstract**

The emerging monkeypox virus (MPXV) is an orthopoxvirus that causes infections in humans and animals. Monkeypox (MPX) cases have been more frequently reported globally by the World Health Organization (WHO) since May 2022. There are currently no proven clinically effective vaccines for MPX infections. In this study, promising MPXV vaccine targets were found using a bioinformatics technique. Designing a specialized antigenic vaccination against diseases involved the use of computational techniques. The vaccine's T-cell lymphocyte, B-cell lymphocyte and interferon gamma (IFN-) inducing epitopes, along with other factors like antigenic and virulence profiles, were evaluated to determine the vaccine's safety after being subjected to a variety of bioinformatic tools and servers. By using dynamic simulation techniques, the vaccine's stability and interaction with Toll-like receptors (TLRs) were evaluated. These bioinformatic discoveries suggest that developed vaccine candidates may indeed be able to effectively stimulate an immune response against monkeypox virus infections. However, this study is an evaluation and prediction of an experimental vaccine. further experimentally conducted additional study and research are necessary to validate the results.

**Keywords:** Monkeypox, Vaccine Design, Bioinformatics, Multi-Epitopes

**INTRODUCTION**

Monkeypox Virus (MPXV) is a virus that belongs to the Orthopoxvirus genus and is known to be the causative agent for monkeypox (MPX). The MPXV is from the Poxviridae family and was originally discovered in vesicular lesions among captive cynomolgus monkeys (*Macaca fascicularis*) in Denmark in 1958 (Focosi et al., 2022). In 1970, this virus was recognized as a human pathogen when the first human case was documented (Mpox in the U.S., 2022). The MPXV is a lipoprotein-enveloped virus with a 196,858 base pair double stranded DNA genome that encodes 90 open reading frames (Aiman et al., 2022; Focosi et al., 2022). The virus normally measures between 200 and 250 nm in size, and the open reading frames of the MPXV genome encodes all transcription and replication enzymes needed for the viral genome (Hatmal et al., 2022; Kumar et al., 2023).

Currently, the MPXV is classified into two clades, the Central African (Congo Basin) clade and the West African clade. It was reported that the Central African (Congo Basin) clade is associated with more severe disease and has recorded more transmissibility as well as occurrences of human to human transmissions (Aiman et al., 2022; Focosi et al., 2022). The West African clade is more associated with milder presentations compared to the Central African clade (Focosi et al., 2022). Since the beginning of May 2022, cases of MPX (monkeypox) have been recorded by the World Health Organization (WHO) from non-endemic nations and geographically diverse countries (Aiman et al., 2022). Furthermore, the disease has continued to be reported in other endemic nations (Mpox (Formerly Named Monkeypox) Situation Update, as of 6 December 2022, 2022; Mpox (Monkeypox) Outbreak 2022 - Global, 2022).

The transmission of MPXV is found to be mainly through direct and strict contact with an infected organism or even contaminated materials (Focosi et al., 2022). It was also determined that the human-to-human transmission is driven by sexual contact, lesions in the oral and respiratory mucosae, or by respiratory droplets during face-to-face contacts (Aiman et al., 2022; Focosi et al., 2022). In terms of indirect contact, it involves surface contaminants such as fomites and more (Nörz et al., 2022). Consumption of undercooked meats and other infected animal products is found to be a potential risk factor that could transmit the virus from one organism to the other (Aiman et al., 2022).

Fatigue, headache, fever, myalgia, and lymphadenopathy are some of the early signs of MPX and are a significant way that it can be distinguished from smallpox since both have similar symptoms (Focosi et al., 2022). Mucosal lesions in the mouth begin to develop after one or two days, and then skin lesions on the hands, feet, and face become centrifugally focused (Aiman et al., 2022). The number of lesions can range from a few to thousands, and the rash might extend to other parts of the body. The incubation period for MPX is typically 6–13 days, although it can go up to 21 days (Grant et al., 2020).

According to the WHO and the Centre of Disease Control and Prevention (CDC) 2022, among the total cases recorded, homosexual men (men who have sex with men) comprise a large number of cases. Furthermore, based on the reports from the European Centre for Disease Prevention and Control (ECDC), as of November 22, 2022, 20 887 confirmed cases of MPX had been recorded from 29 EU/EEA nations since the outbreak began, and 62 cases had been reported from three Western Balkan nations and Turkey (Mpox (Monkeypox) Outbreak 2022 - Global, 2022).

As more MPX strains and clusters emerge, research is still being done on MPX therapies and prevention. To treat this virus, due to its structure's resemblance to the smallpox virus, immunity against smallpox vaccination may also protect against MPX infection. According to a study, the smallpox vaccine only prevents monkeypox around 85% of the time (Kumar et al., 2023). The vaccinations are reported to be effective when given within 4 days of exposure, but giving them after that time may only help with disease symptoms rather than disease prevention (Kumar et al., 2023). As of now, there are no clinically validated treatment methods for the MPX infections (Aiman et al., 2022). The discovery of novel treatment targets for a range of pathogenic strains has been made easier by improvements in immuno-informatics and bioinformatics tools. An innovative method for preventing harmful diseases is multi-epitope vaccination where it has been used for many other viruses and bacteria such as dengue virus, viral infections, tuberculosis and more (Aiman et al., 2022). The identification of the antigens in the MPX virus is required to design and develop effective vaccines. The immune system is essential in the battle against tumors and viral diseases (Zhang, 2018). An antigenic epitope is a fundamental component that causes an immune reaction, either cellular or humoral reactions. In order to prevent and treat tumors or viral infections, a multi-epitope vaccination made up of a number of or overlapping peptides is the best option (Zhang, 2018).

This study focuses on four genes that are involved and are expressed independently in the virus. MPXV A33R is a gene that is crucial for efficient viral particle transfer from cell to cell. The protein serves as a potential target for the development of therapeutics, vaccines, and diagnostic tests. MPXV H3L gene codes for IMV heparin binding surface protein where it mediates cell adsorption and inhibits mammalian immune system (Sino Biological Inc., 2022). On the cell surface, heparan sulfate interacts with an envelope protein known as H3L. This could facilitate the virion's fusion with the host cell. It is a potential target for the development of a vaccine (News-Medical.net, n.d.). MPXV M1R It is an envelope protein that most likely contributes to viral entrance into the host cell. Additionally, it likely participates in the virus' attachment to the surface of the host cell and associates with the entry/fusion complex. The MPXV L1R protein is connected to the main membranes enclosing the virion core, although it is a component of both internal mature virus particles and extracellular enveloped virions that are expelled from the infected cell.

In this study, a multi-epitope vaccine against MPXV infection was created using reverse vaccinology techniques. Lead B- and T-cell epitopes among the chosen antigenic peptides were found using the four primary protein sequences of the most recent MPXV strain 2022. Utilizing bioinformatics tools and servers, the effectiveness of the developed vaccine constructs was assessed.

**METHODOLOGY**

**Retrieval of Protein Sequences**

All four proteins of the Monkeypox virus were retrieved from the UniProt database (https://www.uniprot.org). The UniProt ID for each of the proteins were retrieved and the proteins' complete amino acid sequences were obtained in FASTA format.

**Antigenicity and Virulence Analysis**

The protein antigenicity of the four proteins were predicted using the VaxiJen v 2.0 web server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). With the threshold set at 0.4 and the virus as the chosen target organism, the server helps with the prediction of protective antigens and subunit vaccinations. The Virulentpred web tool (http://203.92.44.117/virulent/submit.html) was used to investigate the virulence potentials of antigenic, non-toxic, and non-allergenic proteins. The Cascased SVM module was used as the prediction method, and the virulent sequences were found and subjected to additional analysis.

**B-cell and T-cell Epitopes Prediction**

The proteins that are found to be antigens and virulence were selected as the suitable vaccine candidates. The Immune Epitope Database (IEDB) was used to predict the B-cell epitopes, by utilizing the Bepipred Linear Epitope Prediction 2.0 and other default settings. By identifying linear B-cell epitopes, further filtration can be done by isolating and removing the epitopes that have a sequence length lesser than 14 amino acid bases. To verify the antigenicity of the B-cell epitopes, the remaining (filtered) epitopes will once more be run through VaxiJen. T cell epitopes are represented by class I (MHC-I) and class II (MHC-II) MHC molecules, which are each recognized by a different subset of T cells, CD8 and CD4, respectively (Ahmed & Maeurer, 2009). The IEDB was also used to determine the T-cell epitopes of the chosen protein sequences (Fleri et al., 2017). The MHC-I analysis used the HLA allele reference set with other default settings. The MHC-II was determined by utilizing the 7-allele HLA reference set. For MHC-I and MHC-II, the default IEDB recommended scoring method was used and the prediction was based on the percentile rank or MHC binding affinity.

**IFNepitope Prediction**

The interferon-inducing epitopes of MHC-II binding epitopes were separated using the IFNepitope online tool. The interferon-gamma (IFN-γ) induction area of antigenic protein sequences was predicted by the IFNepitope server. The SVM model was used to predict IFN-γ, and the IFN-gamma model was chosen over non-IFN-gamma models for prediction (Dhanda et al., 2013). To find IFN-producing peptides, a hybrid technique combining motif-based models from the pattern discovery software MERCI and Support Vector Machine (SVM) based models was used (Lim et al., 2021). Epitopes that were positive for IFN-γ inducers were chosen for further investigation.

**Epitope Selection and Vaccine Design**

The overlapping B-cell, MHC-I, MHC-II and IFN epitope data were selected and mutually compared based on cut-off values. This comparison between the overlapping B-cell and T-cell epitopes would ensure the developed vaccine would stimulate both humoral and cytotoxic immunological responses (Rahman et al., 2020).

**Molecular Docking against Immune Cell Receptor**

To successfully induce immunological responses, it is crucial to comprehend the pattern of interaction between proposed vaccines and the TLR4 immune cell receptor (Ismail et al., 2022). Using the PatchDock web service, the vaccine constructs were docked against the human TLR4 receptor (PDB ID: 4G8A) (Ismail et al., 2020) to analyze interactions between immune receptors and vaccines (Schneidman-Duhovny et al., 2005). The outcomes of the molecular docking were enhanced using the Fire Dock (Fast Interaction Refinement in Molecular Docking) service (Andrusier et al., 2007). The complex vaccine-TLR structure with the lowest docking energy score was shown to be the most effective (Abdi et al., 2022).

**RESULTS**

**Retrieval of Sequence**

The four protein sequences that are associated with monkeypox were retrieved from the UniProt database. The sequences obtained from the database in FASTA format and the UniProt ID for the sequences are as follows; Q5IXN2 (A33R), Q5IXT2 (H3L), Q5IXU5 (M1R), and Q3I7N2 (L1R). It was identified in the database that the subcellular localization of Q5IXT2 (H3L) was found and is an integral component of the membrane and Q5IXU5 (M1R) is a membrane protein.

**Antigenicity and Virulence Analysis**

The four proteins were subjected to antigenicity analysis in VaxiJen (Table 1). The predictions from VaxiJen suggest that all four proteins were antigenic where their antigenicity scores are 0.4775 (A33R), 0.4684 (H3L), 0.6173 (M1R) and 0.4085 (L1R) with the threshold value of >0.4. Since all four protein sequences were antigenic, they were submitted to pathogenicity testing (Table 2). The virulence analysis resulted similar to the antigenicity analysis where all four protein sequences were virulent with virulence score of 1.0645 (A33R), 0.4979 (H3L), 0.9963 (M1R) and 1.0501 (L1R). The fact that the prioritized vaccine candidate proteins are virulent and antigenic suggests that host cell-induced immune responses solely target the virus and not the host (Gupta et al., 2013).

**B-cell and T-cell Epitopes Prediction**

The four proteins targeted in this work were subsequently investigated to determine the lead epitopes for building chimeric vaccine constructs against MPXV. The B-cell epitopes were determined by utilizing the Bepipred Linear Epitope Prediction 2.0 method. Among the results (Table 3) obtained only the epitopes that have more than 13 amino acid bases were selected for further investigations. The selected peptides were subjected to the antigenicity analysis again via VaxiJen v 0.2 to identify the peptides that have antigenicity properties. For the MPXV A33R protein sequence, only “KVNNNYNNYNNYNCYNNYNCYNYDDTFF” was found to be an antigen with a score of 0.8273. Among the 3 peptides from the MPXV H3L sequence, both “PPSETFPNVHEHINDQKFDDVKDNEVMQEKRDVVIVNDDPDHY” and “ALWDSKFFTELENKN” are antigens with scores of 0.4473 and 0.6294 respectively. In the MPXV M1R, the “KATTQIAPRQVAGT” peptide with a score of 0.6607 is an antigen and there were no antigens found in the peptides of the MPXV L1R protein sequence. The IEDB server was used to determine T-cell (MHC-I and MHC-II) epitopes for the chosen proteins based on specific criteria. According to the selection of predicted binders that was done based on the recommended IEDB percentile rank of % for each MHC allele and length combination to cover most of the immunological responses, the MHC class I T cell epitope predictions were conducted for all four protein sequences (Table 4.1, Table 4.2, Table 4.3, Table 4.4). In a manner similar to that of the MHC class I, the MHC class II T cell epitope binders’ predictions are likewise chosen based on the percentile rank or MHC binding affinity (Table 5.1, Table 5.2, Table 5.3, Table 5.4). According to IEDB recommendations, the selection of the predicted binders was based on a consensus adjusted percentile rank of 0.01. The top 10 results based on the adjusted percentile rank of 0.01 were selected for each sample. However, due to errors that occurred during the subsequent analysis, the next 10 samples (11-20) were recorded for both MPVX M1R and MPVX L1R protein sequences (Table 5.3, Table 5.4).

**IFNepitope Prediction and Epitope Selection and Vaccine Design**

The IFNepitope prediction predicts the regions of the antigenic peptides in the protein sequences. The MHC-II was used for the prediction and among the 10 peptides for all four protein sequences, only the peptides that contain antigenic properties were isolated together with their IFN scores (Table 6.1, Table 6.2, Table 6.3 and Table 6.4). The results of the B-cell epitopes, MHC-I, MHC-II and IFN results were compared together to identify any overlapping residues. The ultimate aim was to locate key epitopes that could activate host interferons as well as humoral and cell-mediated immune responses. In multiple strains of MPXV, the conservation of the chosen epitopes was validated. The multi-epitope vaccine design would offer broader protection against various MPXV strains if conserved epitopes were used (Bui et al., 2007). The results of the overlapping residue were only available for the MPXV A33R, MPXV H3L and MPXV M1R only (Table 7.1, Table 7.2 and Table 7.3). Because the MPXV L1R B-cell epitope's prior results showed no antigenic characteristics, the MPXV L1R results were not available. As a result, the subsequent investigations into the overlapping residues of the MPXV L1R protein sequence were discontinued. The remaining three protein sequences were subjected to further investigations.

**Molecular Docking against Immune Cell Receptor**

Molecular docking is an effective technique for determining the best interaction between vaccine designs and receptor molecules. The vaccination designs were docked with the surface human TLR4 immune receptor using the PatchDock service, a blind docking technique. The FireDock web server was used to further improve the docked complexes of the top 10 results (Table 8). High-throughput complex refinement is made possible by FireDock, which addresses potential protein flexibility problems that can arise during protein-peptide docking. The results show the MPXV A33R (Figure 1) with -10.65kcal/mol binding energy, MPXV H3L (Figure 2) with -7.58kcal/mol binding energy and MPXV M1R (Figure 3) with -23.79kcal/mol binding energy with the TLR4 receptor during this study. Strong binding affinities between the vaccine constructs and the TLR4 receptor for MPXV A33R and MPVX H3L were discovered by docking studies whereas the MPVX M1R showed the lowest binding energy with the TLR4 receptor.

**DISCUSSION**

The recent increase in Monkeypox cases and clusters worldwide is making it more difficult to curb the MPXV epidemic. Unfortunately, the vaccines that are available are only able to offer limited and temporary protection against the virus, especially for adults and children with underlying health conditions (Ladnyj et al., 1972). There is no effective course of action for monkeypox (Abdi et al., 2022). For greater protection or eradication of the monkeypox virus infection, a specialized vaccination capable of targeting particular proteins of the virus is necessary. Therefore, for newly emerging MPXV infections, novel therapeutic approaches are needed. The development of vaccines has benefited from developments in reverse vaccination as well as the accessibility of both proteomic and genomic data (Aiman et al., 2022). Furthermore, using cutting-edge bioinformatics tools is faster and more efficient than using conventional methods (Lim et al., 2021). Epitope-based vaccines are a cutting-edge therapeutic strategy for creating effective vaccines with high potency, logistical feasibility, and enhanced safety. Multi-epitope vaccines have the ability to produce particular immune responses depending on conserved epitopes in full antigenic sequences. Hence, it avoids reactions against unfavorable epitopes that could trigger immunological-pathogenic or immune-modulating reactions against the host (Vartak & Sucheck, 2016; Zhou et al., 2009).This study aims to use bioinformatics tools and techniques to design a multi-epitope MPX vaccine which is capable of including immune responses in humans.

The four proteins that were used in the research were filtered based on their antigenicity and virulence capabilities to identify suitable B-cell and T-cell epitopes for the vaccine design. Utilizing this technique, vaccine developers can assess vaccine candidates' suitability for experimental validation (Naz et al., 2019). The goal of T-cell epitope prediction is to locate the shortest peptides in an antigen that can activate CD4 or CD8 T cells (Ahmed & Maeurer, 2009). T-cells are attracted to peptide antigens attached to MHC molecules. The binding affinity of the peptides towards the MHC-I and MHC-II class molecules can be predicted using a variety of bioinformatics methods (Lim et al., 2021). The MHC-I and MHC-II epitopes are crucial for adaptive immunity because they are able to promote long-lasting immunity to get rid of viruses and infected cells from the host and produce both cellular and humoral immune responses respectively (Sunita et al., 2020). The alleles that are associated with the MHC-I and MHC-II epitopes were also discovered to be the group of genetic loci encoding many of the proteins involved in presenting antigen to T cells (Janeway et al., 2001).

B-cell epitope prediction was also conducted because the peptides include peptides that can serve as antigens for vaccinations and the development of antibodies. The study utilized the Bepipred server to identify the linear B-cell epitopes (Aiman et al., 2022; Lim et al., 2021). Numerous continuous anticipated epitopes of the MPXV A33R, MPXV H3L, MPXV M1R, and MPXV L1R were found by Bepipred server analysis which are represented (Table 3). Among all the B-cell epitopes that were determined, only the epitopes that are found to be antigens because all the designs vaccine constructs are to be antigens and virulent. The VaxiJen v 2.0 tool was used to identify the antigenicity score for the epitopes and filter them (Tables 4.1, 4.2, 4.3 and 4.4). The epitopes' immunological characteristics will increase their potential as vaccine candidates (Aiman et al., 2022). Due to the MPXV L1R protein sequence's B-cell epitope not being an antigen, further research into it was halted during this procedure. With the epitope not being an antigen, creating a vaccine construct using that epitope would be inefficient as it would not be able to induce or trigger an immune response. Using the IFNepitope server, interferon-gamma (IFN-) inducing epitopes were predicted. The IFNepitope results were compared together with the B-cell epitope, MHC-I and MHC-II to identify any overlapping peptides. These overlapping peptides were isolated to be used as the vaccine constructs.

Molecular docking analysis was conducted to determine the ability of the proposed vaccines candidates to attach to the human immune cell receptor. The Toll-like receptor 4 (TLR4) immune cell receptor was used because it represents a key receptor on which both infectious and noninfectious stimuli converge to induce a proinflammatory response (Molteni, 2016). TLR receptors play a vital role in innate immunity because they are crucial for immune cell activation that results in adaptive immunological responses (Aiman et al., 2022). The vaccine designs and the receptor protein's active site have substantial binding affinities, based on the analysis of molecular docking between the vaccine candidates and the immune receptor. This evaluates the vaccine's ability to induce consistent immunogenic reactions (Otuokere et al., n.d.). Based on docking configurations, interacting atoms, and binding free energies, the best, most reliable vaccination candidate was chosen. The refined docking structure of the vaccine candidates were produced by FireDock. It aids in rearranging the side chains at the contact and modifying the molecules' relative orientation. The binding energy from the results shows the interaction between the TLR4 and the vaccine candidates. Based on the global energy, MPXV M1R (-23.79kcal/mol) has the lowest energy value whereas the MPXV H3L (-7.58kcal/mol) has the highest global energy value. The attractive Vander Waals, repulsive Vander Waals and atomic contact energy (ACE) were also predicted. Since the lowest global energy is considered to be the best vaccine construct, the MPXV M1R was chosen as the best vaccine construct (Figure 3).

**CONCLUSION**

The emerging MPXV was targeted in the current investigation using bioinformatics methods to find possible therapeutic vaccine proteins. The suitable vaccine candidates were selected based on the protein’s antigenicity and virulence to determine the most suitable epitopes that would induce a human response. The multi-epitope-based vaccination constructs were created using overlapping B-cell and T-cell epitopes from the chosen proteins. The MPXV M1R was determined to be the best vaccine construct by molecular docking studies because it had the lowest global energy for the TLR4 immunological receptor. Since this research only provides computational predictions of the prospective vaccination candidates against MPX, the precise efficacy of the vaccine design can only be verified through experimental analysis. To validate the acquired results, experimentally conducted additional study and research are necessary.

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**APPENDICES**



Figure 1 – Docking between MPXV A33R with TLR4 human receptor



Figure 2 – Docking between MPXV H3L with TLR4 human receptor.



**Figure 3** – Docking between MPXV M1R with TLR4 human receptor.

**Table 1** – Antigenicity Prediction Scores for the MPXV Protein Sequences

|  |  |  |
| --- | --- | --- |
| **MPX Protein Sequence** | **Antigen Score** | **Status** |
| MPXV A33R | 0.4775 | Antigen |
| MPXV H3L | 0.4684 | Antigen |
| MPXV M1R | 0.6173 | Antigen |
| MPXV L1R | 0.4085 | Antigen |

**Table 2** – Virulence Prediction Scores for the MPXV Protein Sequences

|  |  |  |
| --- | --- | --- |
| **MPX Protein Sequence** | **Virulence Score** | **Status** |
| MPXV A33R | 1.0645 | Virulent |
| MPXV H3L | 0.4979 | Virulent |
| MPXV M1R | 0.9963 | Virulent |
| MPXV L1R | 1.0501 | Virulent |

**Table 3** – B-cell Epitope Prediction and Antigenicity Analysis of the Selected Peptides

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **MPX Protein Sequence** | **Peptide Sequences** | **Peptide Length** | **Antigen Score** | **Status** |
| MPXV A33R | FLEKTSFYNCNDSITKEKIKI | 21 | 0.2106 | Not Antigen |
| KVNNNYNNYNNYNCYNNYNCYNYDDTFF | 28 | 0.8273 | Antigen |
| MPXV H3L | PPSETFPNVHEHINDQKFDDVKDNEVMQEKRDVVIVNDDPDHY | 43 | 0.4473 | Antigen |
| ALWDSKFFTELENKN | 15 | 0.6294 | Antigen |
| ITGNKVKTELVIDKDH | 16 | 0.2125 | Not Antigen |
| MPXV M1R | AALNIQTSVNTVVRDFENYVKQTCN | 25 | 0.2174 | Not Antigen |
| KATTQIAPRQVAGT | 14 | 0.6607 | Antigen |
| MPXV L1R | KLEAVGHCYESLSEEYRQLTKFTDSQDFKKLFNKVPIVTD | 40 | 0.0919 | Not Antigen |

**Table 4.1** –T-cell Epitopes, MHC-I prediction of MPXV A33R

|  |  |  |
| --- | --- | --- |
| **Peptide** | **Alleles Associated** | **Percentile Rank** |
| SLNRTIVTK | HLA-A\*03:01 | 0.01 |
| HLA-A\*11:01 | 0.03 |
| VTSSGAIYK | HLA-A\*11:01 | 0.01 |
| HLA-A\*03:01 | 0.05 |
| MTSILNTLR | HLA-A\*68:01 | 0.04 |
| KVNNNYNNY | HLA-A\*30:02 | 0.01 |
| GVTSSGAIYK | HLA-A\*11:01 | 0.03 |
| RFLEKTSFY | HLA-A\*30:02 | 0.02 |
| FYKPKHSTV | HLA-B\*08:01 | 0.03 |
| IYHDDLVVL | HLA-A\*24:02 | 0.05 |

**Table 4.2** – T-cell Epitopes, MHC-I prediction of MPXV H3L

|  |  |  |
| --- | --- | --- |
| **Peptide** | **Alleles Associated** | **Percentile Rank** |
| RYPGVMYTF | HLA-A\*24:02 | 0.01 |
| HLA-A\*23:01 | 0.01 |
| NVIEDITFLR | HLA-A\*68:01 | 0.01 |
| ETMKPNFWSR | HLA-A\*68:01 | 0.01 |
| HLA-A\*33:01 | 0.01 |
| KRYPGVMYTF | HLA-A\*24:02 | 0.01 |
| HLA-A\*23:01 | 0.01 |
| RQIMDNSAKY | HLA-B\*15:01 | 0.01 |
| HLA-B\*15:01 | 0.04 |
| SLSAYIIRV | HLA-A\*02:03 | 0.01 |
| HLA-A\*02:01 | 0.02 |
| TMKPNFWSR | HLA-A\*31:01 | 0.01 |
| HLA-A\*33:01 | 0.01 |
| TPVIVVPVI | HLA-B\*51:01 | 0.01 |
| ITFLRPVLK | HLA-A\*11:01 | 0.01 |
| HLA-A\*03:01 | 0.02 |
| HLA-A\*30:01 | 0.02 |
| ETFPNVHEHI | HLA-A\*68:02 | 0.02 |
| KYTHFFSGF | HLA-A\*24:02 | 0.02 |
| HLA-A\*23:01 | 0.03 |
| QIMDNSAKY | HLA-A\*26:01 | 0.02 |
| NEMKINRQI | HLA-B\*44:02 | 0.03 |
| HLA-B\*44:03 | 0.04 |
| ETFPNVHEH | HLA-A\*26:01 | 0.03 |
| TELENKNVEY | HLA-B\*44:03 | 0.04 |
| HLA-B\*44:02 | 0.05 |
| NVIEDITFL | HLA-A\*02:06 | 0.04 |
| HLA-A\*68:02 | 0.04 |
| HEHINDQKF | HLA-B\*44:03 | 0.05 |
| HLA-B\*44:02 | 0.05 |
| FLRPVLKAI | HLA-A\*02:03 | 0.05 |
| TFTTPLISF | HLA-A\*23:01 | 0.05 |
| FTTPLISFF | HLA-A\*26:01 | 0.05 |

**Table 4.3** – T-cell Epitopes, MHC-I prediction of MPXV M1R

|  |  |  |
| --- | --- | --- |
| **Peptide** | **Alleles Associated** | **Percentile Rank** |
| LANKENVHW | HLA-B\*58:01 | 0.01 |
| HLA-B\*57:01 | 0.01 |
| HLA-B\*53:01 | 0.03 |
| ILANKENVHW | HLA-B\*57:01 | 0.05 |
| HLA-B\*58:01 | 0.04 |
| MYYAKRMLF | HLA-A\*23:01 | 0.01 |
| HLA-A\*24:02 | 0.01 |
| KENVHWTTY | HLA-B\*44:03 | 0.02 |
| HLA-B\*44:02 | 0.02 |
| ALMQLTTKA | HLA-A\*30:01 | 0.02 |
| HLA-A\*02:03 | 0.05 |
| TTVNTLSER | HLA-A\*68:01 | 0.01 |
| VPAMFTAAL | HLA-B\*07:02 | 0.04 |
| VLSAATETY | HLA-B\*15:01 | 0.01 |
| TLSERISSK | HLA-A\*03:01 | 0.03 |
| AQLDAVLSA | HLA-A\*02:06 | 0.04 |
| QTSVNTVVR | HLA-A\*68:01 | 0.09 |
| APRQVAGTGV | HLA-B\*07:02 | 0.05 |
| KIKLILANK | HLA-A\*03:01 | 0.04 |
| TTYMDTFFR | HLA-A\*68:01 | 0.02 |
| RQVAGTGVQF | HLA-B\*15:01 | 0.01 |

**Table 4.4** – T-cell Epitopes, MHC-I prediction of MPXV L1R

|  |  |  |
| --- | --- | --- |
| **Peptide** | **Alleles Associated** | **Percentile Rank** |
| YLFDFVISL | HLA-A\*02:01 | 0.01 |
| HLA-A\*02:06 |
| HLA-A\*02:03 |
| EEYRQLTKF | HLA-B\*44:03 | 0.01 |
| HLA-B\*44:02 |
| KLFNKVPIV | HLA-A\*02:01 | 0.01 |
| HLA-A\*02:06 |
| HLA-A\*02:03 |
| QYLDFLLLL | HLA-A\*23:01 | 0.02 |
| HLA-A\*24:02 | 0.03 |
| TAIDPVRYI | HLA-B\*51:01 | 0.04 |
| HLA-A\*68:02 | 0.05 |
| FADDDSFFKY | HLA-A\*01:01 | 0.01 |
| LSDILQITQY | HLA-A\*01:01 | 0.01 |
| NVMDILKSNK | HLA-A\*68:01 | 0.1 |
| SLSEEYRQL | HLA-A\*02:03 | 0.05 |

**Table 5.1** – T-cell Epitopes, MHC-II prediction of MPXV A33R

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Allele** | **Method Used** | **Peptide** | **Percentile Rank** | **Adjusted Rank** |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | YNCYNYDDTFFDDDD | 0.52 | 0.52 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | NYNCYNYDDTFFDDD | 0.59 | 0.59 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | NNYNCYNYDDTFFDD | 0.76 | 0.76 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | GKVTINDLKMMLFYM | 0.86 | 0.86 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | KVTINDLKMMLFYMD | 0.86 | 0.86 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | LGKVTINDLKMMLFY | 0.88 | 0.88 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | VLGKVTINDLKMMLF | 0.91 | 0.91 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | VVLGKVTINDLKMML | 0.94 | 0.94 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | YNNYNCYNYDDTFFD | 1.30 | 1.30 |
| HLA-DRB1\*15:01 | Consensus (smm/nn/sturniolo) | KGMLFVFYKPKHSTV | 1.40 | 1.40 |

**Table 5.2** – T-cell Epitopes, MHC-II prediction of MPXV H3L

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Allele** | **Method Used** | **Peptide** | **Percentile Rank** | **Adjusted Rank** |
| HLA-DRB1\*15:01 | Consensus (smm/nn/sturniolo) | LIVILFIMFMLIFNV | 0.26 | 0.26 |
| HLA-DRB1\*15:01 | Consensus (smm/nn/sturniolo) | VILFIMFMLIFNVKS | 0.29 | 0.29 |
| HLA-DRB1\*15:01 | Consensus (smm/nn/sturniolo) | IVILFIMFMLIFNVK | 0.33 | 0.33 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | KTELVIDKDHAIFTY | 0.34 | 0.34 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | DVVIVNDDPDHYKDY | 0.36 | 0.36 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | NDNVIEDITFLRPVL | 0.36 | 0.36 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | VVIVNDDPDHYKDYV | 0.36 | 0.36 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | RDVVIVNDDPDHYKD | 0.37 | 0.37 |
| HLA-DRB1\*07:01 | Consensus (comb.lib./smm/nn) | LSAYIIRVTTALNIV | 0.4 | 0.4 |
| HLA-DRB1\*03:01 | Consensus (smm/nn/sturniolo) | DNVIEDITFLRPVLK | 0.42 | 0.42 |

**Table 5.3** – T-cell Epitopes, MHC-II prediction of MPXV M1R [11-20]

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Allele** | **Method** | **Peptide** | **Percentile Rank** | **Adjusted Rank** |
| HLA-DRB1\*15:01 | Consensus (smm/nn/sturniolo) | ILAALFMYYAKRMLF | 1.50 | 1.50 |
| HLA-DRB1\*15:01 | Consensus (smm/nn/sturniolo) | AALFMYYAKRMLFTS | 1.70 | 1.70 |
| HLA-DRB4\*01:01 | Consensus (comb.lib./smm/nn) | SAVVDNKLKIQNVII | 1.70 | 1.70 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | TTYMDTFFRTSPMII | 1.80 | 1.80 |
| HLA-DRB1\*07:01 | Consensus (comb.lib./smm/nn) | GTGVQFYMIVIGVII | 1.90 | 1.90 |
| HLA-DRB1\*07:01 | Consensus (comb.lib./smm/nn) | GVQFYMIVIGVIILA | 1.90 | 1.90 |
| HLA-DRB1\*07:01 | Consensus (comb.lib./smm/nn) | QFYMIVIGVIILAAL | 1.90 | 1.90 |
| HLA-DRB1\*07:01 | Consensus (comb.lib./smm/nn) | TGVQFYMIVIGVIIL | 1.90 | 1.90 |
| HLA-DRB1\*07:01 | Consensus (comb.lib./smm/nn) | VQFYMIVIGVIILAA | 1.90 | 1.90 |
| HLA-DRB1\*15:01 | Consensus (smm/nn/sturniolo) | IILAALFMYYAKRML | 2.00 | 2.00 |

**Table 5.4** – T-cell Epitopes, MHC-II prediction of MPXV L1R [11-20]

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Allele** | **Method** | **Peptide** | **Percentile**  **Rank** | **Adjusted**  **Rank** |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | LTMFFADDDSFFKYF | 0.06 | 0.06 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | QYLLTMFFADDDSFF | 0.07 | 0.07 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | YLLTMFFADDDSFFK | 0.07 | 0.07 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | LLTMFFADDDSFFKY | 0.09 | 0.09 |
| HLA-DRB3\*01:01 | Consensus (comb.lib./smm/nn) | TMFFADDDSFFKYFA | 0.09 | 0.09 |
| HLA-DRB5\*01:01 | Consensus (smm/nn/sturniolo) | FDFVISLMRFKKESA | 0.42 | 0.42 |
| HLA-DRB5\*01:01 | Consensus (smm/nn/sturniolo) | GYLFDFVISLMRFKK | 0.42 | 0.42 |
| HLA-DRB5\*01:01 | Consensus (smm/nn/sturniolo) | KGYLFDFVISLMRFK | 0.42 | 0.42 |
| HLA-DRB5\*01:01 | Consensus (smm/nn/sturniolo) | LFDFVISLMRFKKES | 0.42 | 0.42 |
| HLA-DRB5\*01:01 | Consensus (smm/nn/sturniolo) | YLFDFVISLMRFKKE | 0.42 | 0.42 |

**Table 6.1** – IFN Epitope Prediction of MPXV A33R

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Epitope Name** | **Sequence** | **Method** | **Results** | **Score** |
| Epitope1\_A33R\_ | [YNCYNYDDTFFDDDD](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=YNCYNYDDTFFDDDD&method=svm&model=main) | SVM based | POSITIVE | 0.57336052 |
| Epitope2\_A33R\_ | [NYNCYNYDDTFFDDD](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=NYNCYNYDDTFFDDD&method=svm&model=main) | SVM based | POSITIVE | 0.36477612 |
| Epitope3\_A33R\_ | [NNYNCYNYDDTFFDD](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=NNYNCYNYDDTFFDD&method=svm&model=main) | SVM based | POSITIVE | 0.15087603 |
| Epitope9\_A33R\_ | [YNNYNCYNYDDTFFD](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=YNNYNCYNYDDTFFD&method=svm&model=main) | SVM based | POSITIVE | 0.34938794 |

**Table 6.2** – IFN Epitope Prediction of MPXV H3L

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Epitope Name** | **Sequence** | **Method** | **Results** | **Score** |
| Epitope\_1\_H3L | [LIVILFIMFMLIFNV](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=LIVILFIMFMLIFNV&method=svm&model=main) | SVM based | POSITIVE | 0.21168838 |
| Epitope\_6\_H3L | [NDNVIEDITFLRPVL](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=NDNVIEDITFLRPVL&method=svm&model=main) | SVM based | POSITIVE | 0.59020904 |
| Eptiope\_9\_H3L | [LSAYIIRVTTALNIV](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=LSAYIIRVTTALNIV&method=svm&model=main) | SVM based | POSITIVE | 0.17927901 |
| Epitope\_10\_H3L | [DNVIEDITFLRPVLK](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=DNVIEDITFLRPVLK&method=svm&model=main) | SVM based | POSITIVE | 0.63518805 |

**Table 6.3** – IFN Epitope Prediction of MPXV M1R

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Epitope Name** | **Sequence** | **Method** | **Results** | **Score** |
| Epitope1\_M1R\_ | [ILAALFMYYAKRMLF](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=ILAALFMYYAKRMLF&method=svm&model=main) | SVM based | POSITIVE | 0.054070519 |
| Epitope2\_M1R\_ | [AALFMYYAKRMLFTS](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=AALFMYYAKRMLFTS&method=svm&model=main) | SVM based | POSITIVE | 0.66307256 |
| Epitope3\_M1R\_ | [SAVVDNKLKIQNVII](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=SAVVDNKLKIQNVII&method=svm&model=main) | SVM based | POSITIVE | 0.36986168 |
| Epitope5\_M1R\_ | [GTGVQFYMIVIGVII](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=GTGVQFYMIVIGVII&method=svm&model=main) | SVM based | POSITIVE | 0.34573237 |
| Epitope6\_M1R\_ | [GVQFYMIVIGVIILA](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=GVQFYMIVIGVIILA&method=svm&model=main) | SVM based | POSITIVE | 0.63652388 |
| Epitope7\_M1R\_ | [QFYMIVIGVIILAAL](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=QFYMIVIGVIILAAL&method=svm&model=main) | SVM based | POSITIVE | 0.69101888 |
| Epitope8\_M1R\_ | [TGVQFYMIVIGVIIL](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=TGVQFYMIVIGVIIL&method=svm&model=main) | SVM based | POSITIVE | 0.53425968 |
| Epitope9\_M1R\_ | [VQFYMIVIGVIILAA](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=VQFYMIVIGVIILAA&method=svm&model=main) | SVM based | POSITIVE | 0.4923535 |
| Epitope10\_M1R\_ | [IILAALFMYYAKRML](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=IILAALFMYYAKRML&method=svm&model=main) | SVM based | POSITIVE | 0.83197596 |

**Table 6.4** – IFN Epitope Prediction of MPXV L1R

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Epitope Name** | **Sequence** | **Method** | **Results** | **Score** |
| Epitope\_1\_L1R | [MFFADDDSFFKYFAS](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=MFFADDDSFFKYFAS&method=svm&model=main) | SVM based | POSITIVE | 0.036560014 |
| Epitope\_3\_L1R | [FLLLLLIQSKNKLEA](https://webs.iiitd.edu.in/raghava/ifnepitope/pep_design.php?sequence=FLLLLLIQSKNKLEA&method=svm&model=main) | SVM based | POSITIVE | 0.18321976 |

**Table 7.1** – Overlapping Sequence between the B-cell, T-cell and IFN Epitopes of MPXV A33R

|  |  |  |  |
| --- | --- | --- | --- |
| **B – cell Epitope** | **MHC I Epitopes** | **MHC II Epitopes** | **IFN Gamma Positive Score** |
| KVNNNYNNYNNYNCYNNYNCYNYDDTFF | KVNNNYNNY | NNYNCYNYDDTFFDD | 0.36477612 |

**Table 7.2** – Overlapping Sequence between the B-cell, T-cell and IFN Epitopes of MPXV H3L

|  |  |  |  |
| --- | --- | --- | --- |
| **B – cell Epitope** | **MHC I Epitopes** | **MHC II Epitopes** | **IFN Gamma Positive Score** |
| PPSETFPNVHEHINDQKFDDVKDNEVMQEKRDVVIVNDDPDHY | ETFPNVHEHI | LIVILFIMFMLIFNV | 0.21168838 |
| ALWDSKFFTELENKN | SLSAYIIRV | LSAYIIRVTTALNIV | 0.17927901 |

**Table 7.3** – Overlapping Sequence between the B-cell, T-cell and IFN Epitopes of MPXV M1R

|  |  |  |  |
| --- | --- | --- | --- |
| **B – cell Epitope** | **MHC I Epitopes** | **MHC II Epitopes** | **IFN Gamma Positive Score** |
| AALNIQTSVNTVVRDFENYVKQTCN | QTSVNTVVR | QFYMIVIGVIILAAL | 0.69101888 |

**Table 8** – Molecular Docking Analysis of Vaccine Constructs using FireDock

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Ligand** | **Receptor** | **Solution Number** | **Global Energy** | **Attractive VdW** | **Repulsive VdW** | **ACE** | **HB** |
| MPXV A33R | TLR4 | 6 | -10.65 | -10.67 | 1.40 | 8.02 | -1.72 |
| MPXV H3L | TLR4 | 7 | -7.58 | -17.97 | 5.87 | 7.69 | -2.08 |
| MPXV M1R | TLR4 | 7 | -23.79 | -32.18 | 11.34 | -1.24 | -1.66 |