Abstract

Monkeypox is an uncommon disease brought on by the monkeypox pathogen. The monkeypox virus is associated with the iconic variola (smallpox) virus which causes a febrile rash condition in people that is comparable to but mild than smallpox. Human monkeypox was largely an uncommon zoonotic disease isolated to West and Central African forests in the twentieth century. However, the number of cases as well as geographic distribution have expanded dramatically in this century, coinciding with the worldwide population's declining immunity to smallpox vaccination. The epidemic of human monkeypox in various nations since May 2022 has been exceptional in terms of the high number of cases and the lack of obvious ties to endemic countries, prompting worries about a possible shift in monkeypox transmission patterns that might represent a bigger worldwide danger. (1) In this paper, viable MPXV vaccine targets were identified using an immunoinformatic paradigm.

Keywords: vaccine design, multi-epitope vaccine, molecular docking, immunization

Introduction

In 1958, the first MPV strain was discovered when ill laboratory monkeys from Singapore were transported to a Danish research facility.(2) In Central and West Africa, rodents and monkeys are frequently infected with the virus, where monkeypox has proven to be especially harmful in youngsters, with fatality rates as high as 10% in certain epidemics. (3) The United States endured the first monkeypox epidemic far outside Africa in the year 2003, when a caged prairie dog in Milwaukee, Wisconsin, transmitted the virus to a kid. Eventually, 71 people became ill as a result of the outbreak, which was linked to Ghanaian-imported Gambian pouched rats. Between 2018 and 2021, there were a few isolated instances reported in the UK, the US, and Singapore; in each of these, the underlying index case was a person who had just left Nigeria.

In 2022, a more serious outbreak broke out, including widespread human-to-human transmission in regions other than Africa. The outbreak started in the UK in May 2022 and spread quickly over the next months, touching down in countries throughout Australia, Asia, Africa, and the Americas. The epidemic was classified as a Public Health Emergency of International Concern by the WHO in late July after more than 18,600 cases had been documented. (4)

There is no conventional MPV vaccination available. Consequently, no next-generation MPV vaccination is anticipated any time soon. However, it has been observed that the smallpox vaccination is 85% cross-protective against the monkeypox virus.(5) If the vaccine is given within four days after a smallpox infection, it may prevent the onset of clinical illness. (6)Smallpox was successfully eradicated thanks to the vaccination. Its restrictions, however, include the need that users be at least 40 years old and have questionable background immunity. (5)

Throughout this timeframe, MPXV appeared more often in human populations who had not received vaccinations. Vaccinating individuals against MPXV is currently a critical necessity.

Materials and Methods

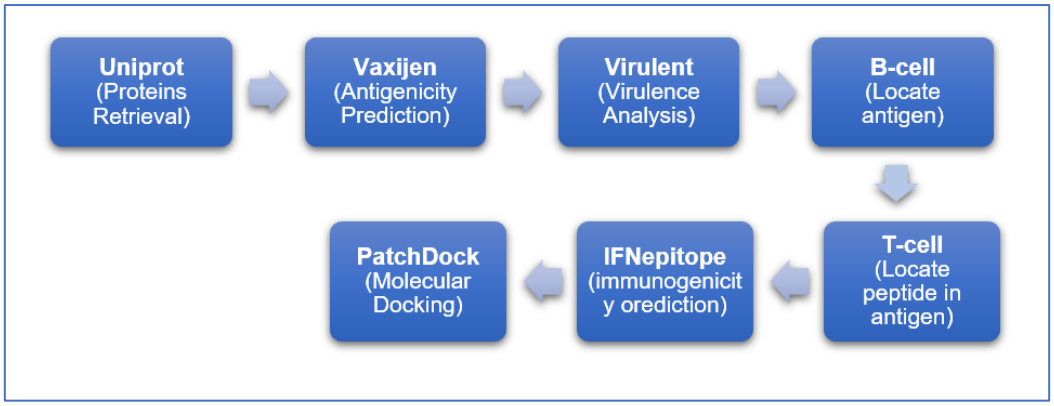

Figure 1.0: *The current paper's methodical workflow to design a vaccine for the human monkeypox virus (MPXV).*

UniProt

The Universal Protein Resource (UniProt) is a central, freely available database that offers accurate and thorough information on protein sequences and functional annotation. From Uniprot, the cell surface-binding of four monkeypox protein sequences was obtained. With humans serving as the virus' host, the proteins' complete amino acid sequences were obtained in FASTA format.

Vaxigen Prediction

The first service for alignment-independent prediction of tumors, viruses, and bacterial protective antigens is VaxiJen (VaxiJen (ddg-pharmfac.net). The server has models created by pre-processing the characteristics of amino acids using ACC. Protective antigens, tumor antigens, and subunit vaccinations are all predicted by VaxiJen (7). VaxiJen was created to enable users to evaluate a protein's capacity to trigger protection. Vaxijen server processes FASTA-formatted submissions of proteomes in their whole as well as single proteins. The Vaxijen models can predict if a protein sequence will function as a therapeutic antigen which serves as the foundation for subunit vaccinations or otherwise. In this paper, VaxiJen outlines the primary physicochemical characteristics of the amino acids that build the examined MPXV sequence, then transforms the generated strings into uniform vectors by auto cross-covariance (ACC) (8), chooses the pertinent variables by genetic algorithm (GA) (9), and lastly categorized the proteins as protective antigens or non-antigens along with the antigen score. Vaxijen for the MPXV protein sequence was run at a threshold of 0.4 according to the criteria toll and with virus as the target organism.

Virulent Prediction

A pathogen's production of virulence factors is fundamental for inflicting illness on the host. In certain cases, they also serve as the basis for the evasion of host defensive mechanisms. They let the pathogen embed itself within the host, increasing its capacity to cause disease. It is appealing to identify such molecules, especially those with immunological significance, and use them to develop vaccines (10). With that, VirulentPred (Prediction of bacterial virulent proteins) was employed as one of the methods for MPV vaccine design, a method for predicting the bacterial virulent proteins relying a on bi-layer cascade Support Vector Machine (SVM). The VirulentPred web server was employed because, with such a precision of 81.8%, it distinguishes between bacterial proteins that are virulent and non-virulent. The predictions were made for the MPXV proteome runs using a threshold, of 0.0, for an effective prediction (11)

B-Cell Epitope Prediction

A B-cell epitope and perhaps an antigen determinant is the part of an antigen that binds. A patch of atoms on the protein surface in three dimensions, or a specific peptide from the query protein, may serve as an epitope if the antigen is a protein. (12)A pathologic antigen is identified by antibodies or B-cell receptors (BCR) during humoral immunization through certain areas of the antigen's surface, sometimes referred to by the B-cell epitope. In order to create a successful vaccination, it is crucial to identify the epitopes on the antigen as these are what trigger humoral responses rather than the antigen as a whole. X-ray crystallographic and NMR approaches are the most trustworthy ways for identifying an epitope, but they require time and resources. (13) Thus, in this report, the Immune Epitope Database (IEDB) is employed (http://tools.iedb.org/bcell/), which is an extensive tool designed for the analysis and prediction of immunological epitopes. It detects autoantigens, allergens, developing and resurging pathogens, category A–C pathogens, and their corresponding epitopes. Additionally, IEDB provided options such as amino acid scales and Paratome, PIGS, ElliPro, Paratome, HMMs, and DiscoTope for the identification of linear B-cell epitopes from protein sequences. It was utilized to analyze the length of the peptides in each MPXV sequence (14)and the sequence with a length of more than 14 was recorded and tabulated.

T-Cell Epitope Prediction

The objective of T-cell epitope prediction is to locate the smallest peptides in an antigen that can activate CD4 or CD8 T cells (15). Immunogenicity, the ability to activate T cells, is demonstrated in tests that ask for synthetic peptide generated by antigen (16). Antigens contain a variety of unique peptides, and T-cell prediction techniques seek to find peptides that are immunogenic. A peptide can only activate effector T cells when it is provided by an MHC I molecule. MHC I molecules can only attach to peptides that are 8 to 14 amino acids long in sequence, as opposed to MHC II molecules, which could adhere to longer and more varied peptides(17) Thus, the IEDB's epitope analysis tool was employed to estimate the binding epitopes for MHC class I (http://tools.iedb.org/mhcI) and class II (http://tools.iedb.org/mhcII)(18) Determining these epitopes makes it possible to monitor, phenotype, and activate T cells that are important in immunological responses to the monkeypox virus. (19) The peptides with a percentile rank of 0.05 and below only were taken into consideration to cover most of the immune responses.

IFNepitope Prediction

IFN-gamma is the distinguishing cytokine of the adaptive and innate immune systems, inducing antiviral, immuno-regulatory, and anti-tumor actions. The main component of the Th1 response that is essential for the management of intracellular infections is the production of IFN-gamma. Subsequently, IFNepitope is an online tool for predicting and creating the epitope that could cause the release of interferon-gamma. The website was created using a dataset that includes MHC class II binders that induce and do not induce IFN-gamma. The IFN-gamma-inducing peptide or epitope in a collection of peptides for all four monkeypox sequences was predicted using this website. The pathogenic protein sequence region that induces interferon-gamma (IFN-γ) is predicted. The SVM model was used to predict IFN-γ, and the IFN-gamma model was chosen over non-IFN-gamma models. The projected ability of the submitted peptides to trigger IFN-gamma was used to rank and order them. (20)

Docking with PatchDock Webserver

PatchDock is an approach for molecular docking that is geometry-based. Its goal is to identify docking modifications that provide favorable complementarity of molecule shape. The PatchDock algorithm begins making predictions after receiving the docking request, and the findings are communicated through email with a link to a webpage where the predictions are shown. The user can read particular predictions on the page and download a zipped file with the highest-scoring answers. The top 1 result was tallied after the results were seethed to the FireDock website for refinement. (21)

Results

Protein Sequence Retrieval

The Uniprot database search returned 909 available protein sequences the most recent variants of MPXV. Only four cell surface-binding proteins from all monkeypox virus types were given the highest priority for this study as possible vaccine candidates. These possible MPXV strains have the UniProtIDs of Q51XN2 (A33R), Q51XT2 (H3L), Q51XU5 (M1R), and Q317NU (L1R) respectively.

Antigenicity and Virulence Prediction

At a threshold of 0.4, the final proteins had significantly antigenic predicted scores of 0.4775, 0.4683, 0.6173, and 0.4085, respectively according to VaxiJen's prediction. Moreover, the four MPXV virulent sequences are further distinguished from nonvirulent proteins by VirulentPred, which yields a precision of 81.8% with scores of 1.06, 0.4979, 0.9963, and 1.0501.

T-Cell and B-Cell Epitope Prediction

The lead epitopes for creating the chimeric vaccine constructions that are against the MPVX had to be predicted, so MPXV A33R, H3L, M1R, and L1R needed to go through the additional analysis. The obtained b-cell epitopes were filtered based on their peptide length, which needed to be 14 or above. Two sequences for the MPVX A33R protein were chosen as candidates. Three protein sequences for MPXV H3L were next shortlisted, then two sequences for MPXV M1R, and finally two sequences for MPXV L1R. The Vaxigen was used once more to assess the antigenicity of the filtered peptide sequences. Inferred from this were MPXV A33R's single antigenic peptide sequence, MPXV H3L's dual antigenic sequence, and MPXV M1R's single antigenic sequence. A33R, H3L, and M1R may be present in the three protein sequences that were further examined because the MPXV LIR lacked an antigenic sequence. The peptide with a percentile rank of 0.05 and below was chosen for the MHC 1 binding data and it was filtered according to the peptides with similar alleles. Meanwhile, for MCH-II-related peptides, each peptide was correlated to the alleles it was associated with where selections were based on a consensus percentile rank of 99% and above.

IFNepitope Prediction

17 positive epitopes were prioritized using the IFNepitope server given their high antigenicity, IFN-positivity, nontoxicity, and low allergic reactions. (Table 3.14 – 3.17). The final goal was to locate key epitopes that could activate host interferons as well as humoral and cell-mediated immune responses. In multiple MPXV variants, the stability of the chosen epitopes was validated. The highest score was for Epitope10_M1R for the sequence of AALFMYYAKRMLFTS at 0.61842338.

Molecular Docking

In structural biology and computer-aided drug development, molecular docking is a crucial tool. Predicting the dominant interaction mode(s) of ligands with a protein having a known 3D structure is the aim of ligand-protein docking. Effective docking methods use a scoring system that accurately evaluates candidate dockings and efficiently explores high-dimensional spaces. (22) The surface human TLR4 immune cell receptor was docked against the MPXV protein structures using the PatchDock server that automatically generated the best 20 solutions. With one row for each solution, the solutions were displayed in a table. The solution's real rigid transformation as well as the geometric score, desolvation energy, interface area size, and geometric score were all displayed. (21) However, to enhance the docked complexes, the top 10 solutions were then submitted to FireDock website for refinement.

Discussion

The observed increase in human MPXV cases and scattered clusters throughout the world make eradication of the MPXV outbreak tough. The MPXV vaccinations on the market provide only a little protection, especially for infants and individuals with prior medical disorders (23). Therefore, for newly developing MPXV infections, innovative treatment approaches are needed. Epitope-based vaccinations are a cutting-edge therapeutic strategy for creating effective vaccinations with improved efficacy, logistical feasibility, and improved reliability. It has the ability to trigger certain antibody responses based on common epitopes in whole viral sequences, eliminating reactions to unfavorable epitopes that might trigger immunopathogenic reactions against the host. As of right now, there is no known cure for MPVX, hence the only preventive measure is immunization. The goal of this project was to develop an innovative epitope based MPXV vaccine that's competent in eliciting antibody responses in infected patients. (24)

The VaxiJen server was used to evaluate the antigenicity of the monkeypox proteins, with a score threshold for viruses set at 0.4. This implies that proteins with such a score of more than 0.4 are classified as antigenic, whilst proteins with a score below 0.4 are classified as non-antigenic. All four proteins examined for antigenicity against the monkeypox virus had VaxiJen scores greater than 0.4; evaluation of those conserved portions reveals that all of the antigenic regions satisfied VaxiJen's standard criteria of 0.4. (Table 1.0) (25)

The Virulentpred web tool was used to investigate the virulence potentials of antigen. All four monkeypox proteins were shown to be non-toxic, non-allergenic, and involved in virulence, indicating that recipient cells-induced antibody responses only target the virus and not the host. (26). (Table 2.0)

B-lymphocytes are a crucial component of the host immune system because it generates a variety of novel pathogen responses that can aid in neutralizing antigens and reducing viral loads. The antigenic proteomics was evaluated using the IEDB tool in order to incorporate potential B-cell activating epitopes further into vaccine design. (25) Eight conserved peptides with a length of more than 14 were selected from the server's results to execute the antigenicity test again. (Table 3.5)

Adaptive immunity is greatly aided by T-cell epitopes. MHC-II epitopes are in charge of producing simultaneous humoral and cellular immune responses, whilst MHC-I epitopes stimulate resistant antibodies to remove pathogens and virulence factors from the host (27). These epitopes trigger a CD4+ helper T-cell response, which produces CD8+ T-cell recognition and activates B cells (28,29). (Table 3.6,3.8.3.10 and 3.12) summarizes the MHC class-I T cell epitope predictions based on the percentile rank of <= 0.05% for each MHC allele

and length combination to cover most of the immune responses. Meanwhile, for MCH-II-related peptides, each peptide was correlated to the alleles it was associated with where selections were based on a consensus percentile rank of 99% and above. (Table 3.7,3.9,3.11 and 3.13)

Immunogenic B-cell and T-cell epitopes that may elicit certain antibodies serve as the biochemical building blocks for peptide vaccines. A B-cell epitope and a T-cell epitope can indeed be merged to create a target molecule immunogenically (30,31) Manual comparison and cut-off values were used to choose overlapped B-cell, T-cell, and IFN epitopes to verify that the developed vaccine might elicit both host immune and cytotoxic immunogenicity (32). In light of their significant antigenicity, IFN-Gamma positive score, low cytotoxicity, and mild immune reactions, eight epitopes were identified and tabulated. (Table 3.15 – Table 3.18)

The IFNepitope prediction server predicted the region of antigenic protein sequences that causes interferon-gamma (IFN-γ) induction. The SVM based model was utilized to identify the IFN-gamma-inducing MHC II binder peptides in the required monkeypox protein sequences. The projected ability of the submitted monkeypox peptides to trigger IFN-gamma was used to rank and order them and only positive results were selected for further analysis (Table 3.14 – Table 3.17). (20)

The effectiveness of the developed vaccines in binding to the TLR4 immune cell receptor was evaluated by molecular docking using the PatchDock server. TLR receptors play a vital role in innate immune system because they are crucial for T-cell stimulation that results in adapt immune activation (33,34). MPXV L1R was not suitable for further analysis of docking the B-cell prediction of MPXV L1R appeared to be non-antigen. (Table 3.15) The ability of the potentially developed vaccine to produce a durable antibody response was determined by the strength of the binding energy between TLR4 and the active site of the three monkeypox receptor proteins, which were evaluated by molecular docking tests. (24) Based on its minimum global energy, MPXV M1R was chosen as the most suitable vaccine design and was taken into consideration for underlying molecular dynamic modeling analyses and immunological computation.

Conclusion

Using cell surface-binding monkeypox receptors as a target, an epitope vaccine that can elicit an immune response against MPXV was designed to provide a potential vaccine that is harmless and potent. Following a meticulous process, the most viable peptides from the cell surface receptor were chosen and used in the development of the vaccine. The MPXV M1R vaccine design was shown to have the minimum global energy and ligand binding for the TLR4 receptor in all of the analyses carried out in this paper. Additionally, it is understood that the MPXV M1R vaccination has the capacity to elicit immune cellular and humoral reactions against the MPXV. However, to verify the findings of the current investigation, further clinical and laboratory testing are needed.

REFERENCES

1. Xiang Y, White A. Monkeypox virus emerges from the shadow of its more infamous cousin: family biology matters. https://doi.org/101080/2222175120222095309 [Internet]. 2022 [cited 2022 Nov 9];11(1):1768–77. Available from: https://www.tandfonline.com/doi/abs/10.1080/22221751.2022.2095309

2. Cho CT, Wenner HA. Monkeypox virus. Bacteriol Rev [Internet]. 1973 Mar [cited 2022 Nov 10];37(1):1. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC413801/

3. Kmiec D, Kirchhoff F. Monkeypox: A New Threat? International Journal of Molecular Sciences 2022, Vol 23, Page 7866 [Internet]. 2022 Jul 17 [cited 2022 Nov 9];23(14):7866. Available from: https://www.mdpi.com/1422-0067/23/14/7866/htm

4. monkeypox | Description, Cause, Symptoms, Treatment, & Prevention | Britannica [Internet]. [cited 2022 Nov 9]. Available from: https://www.britannica.com/science/monkeypox

5. Immunological responses against monkeypox virus concerning the 2022 outbreak [Internet]. [cited 2022 Nov 10]. Available from: https://www.news-medical.net/news/20221109/Immunological-responses-against-monkeypox-virus-concerning-the-2022-outbreak.aspx

6. Simpson K, Heymann D, Brown CS, Edmunds WJ, Elsgaard J, Fine P, et al. Human monkeypox – After 40 years, an unintended consequence of smallpox eradication. Vaccine [Internet]. 2020 Jul 7 [cited 2022 Nov 10];38(33):5077. Available from: /pmc/articles/PMC9533855/

7. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics [Internet]. 2007 Jan 5 [cited 2022 Nov 25];8:4. Available from: /pmc/articles/PMC1780059/

8. Wold S, Jonsson J, Sjörström M, Sandberg M, Rännar S. DNA and peptide sequences and chemical processes multivariately modelled by principal component analysis and partial least-squares projections to latent structures. Anal Chim Acta. 1993 May 28;277(2):239–53.

9. Leardi R, Boggia R, Terrile M. Genetic algorithms as a strategy for feature selection. J Chemom [Internet]. 1992 Sep 1 [cited 2022 Nov 25];6(5):267–81. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cem.1180060506

10. Chaudhuri R, Ramachandran S. Prediction of Virulence Factors Using Bioinformatics Approaches. Immunoinformatics [Internet]. 2014 [cited 2022 Nov 25];1184:389. Available from: /pmc/articles/PMC7123293/

11. Garg A, Gupta D. VirulentPred: A SVM based prediction method for virulent proteins in bacterial pathogens. BMC Bioinformatics [Internet]. 2008 Jan 28 [cited 2022 Nov 25];9(1):1–12. Available from: https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-9-62

12. (PDF) B cell epitope prediction [Internet]. [cited 2022 Nov 25]. Available from: https://www.researchgate.net/publication/265745548_B_cell_epitope_prediction

13. Barlow DJ, Edwards MS, Thornton JM. Continuous and discontinuous protein antigenic determinants. Nature [Internet]. 1986 [cited 2022 Nov 25];322(6081):747–8. Available from: https://pubmed.ncbi.nlm.nih.gov/2427953/

14. Potocnakova L, Bhide M, Pulzova LB. An Introduction to B-Cell Epitope Mapping and In Silico Epitope Prediction. J Immunol Res [Internet]. 2016 [cited 2022 Nov 25];2016. Available from: /pmc/articles/PMC5227168/

15. Ahmed RKS, Maeurer MJ. T-cell epitope mapping. Methods Mol Biol [Internet]. 2009 [cited 2022 Nov 25];524:427–38. Available from: https://pubmed.ncbi.nlm.nih.gov/19377963/

16. Malherbe L. T-cell epitope mapping. Ann Allergy Asthma Immunol [Internet]. 2009 [cited 2022 Nov 25];103(1):76–9. Available from: https://pubmed.ncbi.nlm.nih.gov/19663131/

17. Schaap-Johansen AL, Vujović M, Borch A, Hadrup SR, Marcatili P. T Cell Epitope Prediction and Its Application to Immunotherapy. Front Immunol. 2021 Sep 15;12:2994.

18. Shantier SW, Mustafa MI, Abdelmoneim AH, Fadl HA, Elbager SG, Makhawi AM. Novel multi epitope-based vaccine against monkeypox virus: vaccinomic approach. Scientific Reports 2022 12:1 [Internet]. 2022 Sep 25 [cited 2022 Nov 25];12(1):1–17. Available from: https://www.nature.com/articles/s41598-022-20397-z

19. Peters B, Nielsen M, Sette A. T Cell Epitope Predictions. https://doi.org/101146/annurev-immunol-082119-124838 [Internet]. 2020 Apr 27 [cited 2022 Nov 25];38:123–45. Available from: https://www.annualreviews.org/doi/abs/10.1146/annurev-immunol-082119-124838

20. IFNepitope: A server for predicting and designing IFN-gamma inducing epitopes [Internet]. [cited 2022 Dec 4]. Available from: https://webs.iiitd.edu.in/raghava/ifnepitope/index.php#thumb

21. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res [Internet]. 2005 Jul 7 [cited 2022 Dec 7];33(Web Server issue):W363. Available from: /pmc/articles/PMC1160241/

22. Morris GM, Lim-Wilby M. Molecular docking. Methods Mol Biol [Internet]. 2008 [cited 2022 Dec 18];443:365–82. Available from: https://pubmed.ncbi.nlm.nih.gov/18446297/

23. Ladnyj ID, Ziegler P, Kima E. A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. Bull World Health Organ [Internet]. 1972 [cited 2022 Dec 11];46(5):593. Available from: /pmc/articles/PMC2480792/?report=abstract

24. Aiman S, Alhamhoom Y, Ali F, Rahman N, Rastrelli L, Khan A, et al. Multi-epitope chimeric vaccine design against emerging Monkeypox virus via reverse vaccinology techniques- a bioinformatics and immunoinformatics approach. Front Immunol [Internet]. 2022 Aug 25 [cited 2022 Dec 11];13. Available from: /pmc/articles/PMC9452969/

25. Shantier SW, Mustafa MI, Abdelmoneim AH, Fadl HA, Elbager SG, Makhawi AM. Novel multi epitope-based vaccine against monkeypox virus: vaccinomic approach. Scientific Reports 2022 12:1 [Internet]. 2022 Sep 25 [cited 2022 Dec 13];12(1):1–17. Available from: https://www.nature.com/articles/s41598-022-20397-z

26. Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Raghava GPS. In Silico Approach for Predicting Toxicity of Peptides and Proteins. PLoS One [Internet]. 2013 Sep 13 [cited 2022 Dec 14];8(9). Available from: /pmc/articles/PMC3772798/

27. Sunita, Singhvi N, Singh Y, Shukla P. Computational approaches in epitope design using DNA binding proteins as vaccine candidate in Mycobacterium tuberculosis. Infect Genet Evol [Internet]. 2020 Sep 1 [cited 2022 Dec 13];83. Available from: https://pubmed.ncbi.nlm.nih.gov/32438080/

28. Kar T, Narsaria U, Basak S, Deb D, Castiglione F, Mueller DM, et al. A candidate multi-epitope vaccine against SARS-CoV-2. Sci Rep [Internet]. 2020 Dec 1 [cited 2022 Dec 13];10(1). Available from: https://pubmed.ncbi.nlm.nih.gov/32616763/

29. Sauer K, Harris T. An Effective COVID-19 Vaccine Needs to Engage T Cells. Front Immunol [Internet]. 2020 Sep 28 [cited 2022 Dec 13];11:581807. Available from: /pmc/articles/PMC7549399/

30. Meloen RH, Langeveld JPM, Schaaper WMM, Slootstra JW. Synthetic peptide vaccines: unexpected fulfillment of discarded hope? Biologicals [Internet]. 2001 [cited 2022 Dec 11];29(3–4):233–6. Available from: https://pubmed.ncbi.nlm.nih.gov/11851321/

31. Dermime S, Gilham DE, Shaw DM, Davidson EJ, Meziane EK, Armstrong A, et al. Vaccine and antibody-directed T cell tumour immunotherapy. Biochim Biophys Acta Rev Cancer [Internet]. 2004 Jul 6 [cited 2022 Dec 11];1704(1):11–35. Available from: https://pubmed.ncbi.nlm.nih.gov/15238242/

32. Rahman N, Ali F, Basharat Z, Shehroz M, Khan MK, Jeandet P, et al. Vaccine Design from the Ensemble of Surface Glycoprotein Epitopes of SARS-CoV-2: An Immunoinformatics Approach. Vaccines (Basel) [Internet]. 2020 Sep 1 [cited 2022 Dec 13];8(3):1–17. Available from: /pmc/articles/PMC7565012/

33. Compton T, Kurt-Jones EA, Boehme KW, Belko J, Latz E, Golenbock DT, et al. Human Cytomegalovirus Activates Inflammatory Cytokine Responses via CD14 and Toll-Like Receptor 2. J Virol [Internet]. 2003 Apr 15 [cited 2022 Dec 13];77(8):4588. Available from: /pmc/articles/PMC152130/

34. Vaure C, Liu Y. A Comparative Review of Toll-Like Receptor 4 Expression and Functionality in Different Animal Species. Front Immunol [Internet]. 2014 [cited 2022 Dec 13];5(JUL). Available from: /pmc/articles/PMC4090903/

Final Project Rubric (Written Report)

EXCEEDS STANDARDS (10-8 M)	MEETS STANDARDS (7-5 M)	NEARLY MEETS STANDARDS (4-2 M)	DOES NOT MEET STANDARD (1-0 M)	E1	E2
THESIS STATEMENT Clearly and concisely states the paper's purpose in a single sentence, which is engaging, and thoughts provoking	**THESIS STATEMENT** clearly states the paper's purpose in a single sentence	**THESIS STATEMENT** States the paper's in a single sentence	**THESIS STATEMENT** Incomplete and/or unfocused		
IDEAS & CONTENT Purpose and main ideas: clear, focused and interesting Supporting details: • Relevant, carefully selected details • Makes connections and shares insights	**IDEAS & CONTENT** Purpose and main ideas: clear and focused Supporting details: • General or limited in places • Some connections and insights are present	**IDEAS & CONTENT** Purpose and main ideas: overly broad or simplistic Supporting details: • Limited, off-topic, predictable or too general • Connections and insights are missing	**IDEAS & CONTENT** Purpose and main ideas: unclear and require inferences by reader Supporting details: • Minimal development; insufficient details • Irrelevant details • Extensive repetition		
ORGANIZATION Order and structure are strong and move the reader through the text. • Effective sequencing and paragraph breaks • Introduction: inviting beginning that draws the reader in • Conclusion: Satisfying sense of resolution or closure	**ORGANIZATION** Organization is clear; order and structure are present. • Clear sequencing and paragraph breaks; organization is predictable. • Introduction: recognizable, developed • Conclusion: developed	**ORGANIZATION** Overall structure is inconsistent or skeletal. • Some sequencing and paragraphs breaks; order of ideas may be unclear. • Introduction: too short, obvious or ineffective (e.g., "My topic is..."). • Conclusion: too short, obvious or ineffective.	**ORGANIZATION** Organizational structure is unclear and difficult to follow, or too short to demonstrate organization. • Paragraph breaks are missing. • Introduction: missing or underdeveloped • Conclusion: missing or underdeveloped		
WORD CHOICE Employs a broad range of words, which have been carefully chosen and thoughtfully placed for impact. • Accurate, specific words; word choices energize the writing. • Fresh, vivid expression; slang, if used, seems purposeful and is effective.	**WORD CHOICE** Employs a variety of words that are functional and appropriate to audience and purpose. • Expression that is accurate and effective. • Words and phrases are natural.	**WORD CHOICE** Does not employ a variety of words, producing a "generic" paper filled with familiar words and phrases. Language lacks precision and variety, or is inappropriate to audience and purpose. • Expression is ordinary or general; slang, if used, is not purposeful or effective.	**WORD CHOICE** Language is repetitive and/or misused, taking away from the meaning and impact. • General, vague words. • Extremely limited range of words.		
SENTENCE FLUENCY Writing has an easy flow and rhythm. Sentences are carefully crafted, with strong and varied structure.	**SENTENCE FLUENCY** Writing is easy to read aloud; sounds natural; variety of sentence beginnings, lengths and patterns.	**SENTENCE FLUENCY** Some parts are easy to read aloud; occasional awkward constructions force the reader to slow down. • Sentence beginnings: many sentences begin the same way.	**SENTENCE FLUENCY** Writing tends to either be choppy, rambling or incomplete. Awkward constructions force the reader to slow down or reread. • Sentence beginnings: begin the same way.		

• Sentence beginnings: sentences begin in different ways, adding interest. • Sentence lengths: a variety of lengths that add interest.	• Sentence beginnings: most sentences begin in different ways. Some repetition detracts from overall impact. • Sentence lengths: some sentences are shorter; some are longer. Some repetition detracts from overall impact.	• Sentence lengths: many sentences are the same length.	• Sentence lengths: same lengths-either short and choppy or long and rambling.		
CONVENTIONS Strong control of conventions; uses conventions effectively to enhance readability. Errors are few and minor. • Correct grammar and usage that contribute to clarity and style. • Skill in using a wide range of conventions. • Little need for editing.	**CONVENTIONS** Control of conventions. Minor errors do not impede readability. • Control over conventions used, although a wide range is not demonstrated. • Correct end-of-sentence punctuation; internal punctuation is sometimes incorrect. • Moderate need for editing.	**CONVENTIONS** Limited control of conventions. Errors begin to impede readability. • Some control over basic conventions; text is too simple or too short to reveal proficiency. • End-of-sentence punctuation is usually correct; however, internal punctuation contains frequent errors.	**CONVENTIONS** Little control of conventions. Frequent errors impede readability. • Many end-of-sentence punctuation errors; internal punctuation contains frequent errors. • Spelling errors frequently distract the reader; misspelling of common words often occurs.		
MECHANICS No errors in punctuation capitalization and spelling	**MECHANICS** Almost no errors in punctuation, capitalization and spelling	**MECHANICS** Many errors in punctuation, capitalization and spelling	**MECHANICS** Numerous and distracting errors in sentence structure and word usage		
USAGE No errors sentence structure and word usage	**USAGE** Almost no errors in sentence structure and word usage	**USAGE** Many errors in sentence structure and word usage	**USAGE** Numerous and distracting errors in sentence structure and word usage		
CITATION All cited works, both text and visual are done in the correct format with no errors	**CITATION** Some cited works, both the text and visual are done in correct format. Inconsistence evident	**CITATION** Few cited works, both text and visual are done in the correct format	**CITATION** Absent		
BIBILOGRAPHY Done in the correct format with no errors. Include major reference from journal articles with less than two internet sites	**BIBILOGRAPHY** Done in the correct format with few errors. Include major reference from journal articles with less than two internet sites	**BIBILOGRAPHY** Done in the correct format with some errors. Include major reference from journal articles with less than two internet sites	**BIBILOGRAPHY** Done in the correct format with more errors. Include major reference from journal articles with less than two internet sites		

	Name	Signature	Marks
Evaluator1:			
Evaluator2:			
		Total Marks	/100