Vol. 5 No. 1 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Immunoinformatics-Driven Design Of A Multi-Epitiope Vaccine Against SARS-Cov-2 Variants

Aizaz Hassan
Abdul Wali khan University Mardan. Email: aizazhassan442002@gmail.com

Fizza Urooj
Abdul Wali khan University Mardan. Email: fizaurooj121@gmail.com

Ayesha
Abdul Wali khan University Mardan. Email: ayeshahassan34235@gmail.com

Adil Shah
Abdul Wali khan University Mardan. Email: adilbiotech2662@gmail.com

Sundas Tariq
Abdul Wali khan University Mardan. Email:sundasp4@gmail.com

Author Details

Corresponding E-mail & Author*:

Aizaz Hassan
Abdul Wali khan University Mardan. Email: aizazhassan442002@gmail.com

Keywords: SARS-CoV-2, multi-epitope vaccine, immunoinformatics, molecular docking, immune simulation and variant coverage

Received on 04 Feb 2026

Accepted on 7 Mar 2026

Published on 18 Mar 2026

Abstract

To secure a multi-epitope vaccine against conserved peptides of SARS-CoV-2 spike protein and assess 1,500+ variant sequences (2020-2022), the software of immunoinformatics was used in this paper. The workflow had identified 12 high-affinity epitopes (IC50 < 50 nM) which covered 95.2 percent of the population containing 7 CTL (MHC-I binding) and 5 HTL (MHC-II binding) epitopes. Epitptides were modified to form epitopes, and a product having a molecular weight of 50.2 kDa and pI of 7.8 was obtained which contained 459 amino acids. Molecular docking predicted binding to TLR4 (-12.4 kcal/mol) and MHC alleles in silico cloning and expression studies had predicted a 98.5 percent codon adaptation index in E. coli. Strong IgG/IgM titers (10^5) and Th_1/Th_{17} could be predicted by Immune simulation (C-ImmSim). The population analysis covered analysis indicated that 92.3 per cent of the world population was covered and over 90 per cent of the population in the Asian, European and African populations was covered. The vaccine construct was desirable in terms of physicochemical properties (instability index: 31.4, aliphatic index: 85.2) and antigenicity (VaxiJen score: 0.76).

INTRODUCTION

The transmission of COVID-19 by the SARS-CoV-2 has resulted in a significant adverse effect on the health of the whole population like never before since the onset of the pandemic in 2019 (Chakraborty *et al.*, 2021). The rate of the dissemination of the virus is enormous and the emergence of the virus variants is going on and this has become a major challenge to the vaccination efforts that are being implemented at present and to the worldwide management of the illness. Various types of concern have

been evolved due to the mutation of the viral genome particularly the spike glycoprotein that binds itself with the host cell. They are capable of altering the viral transmissibility, immune responses evasion capability and efficacy of vaccines and hence the need to possess next generation vaccines as it may provide broad cover of multiple variants (Samad *et al.*, 2022).

The traditional approaches to vaccine development include time-consuming period in developing these vaccines and protracted laboratory research. On the other hand, the rapid vaccines development strategy has been made possible through computational biology whereby immunoinformatics is applied. Immunoinformatics is a combination of bioinformatics, immunology or modeling computations to form antigenic determinants having the capacity to induce robust immune reactions. It is an essential method which significantly accelerates the process of vaccine discovery because it predicts immunogenic epitopes and studies their interaction with immune receptors before the lab validation process.

A key protein that attaches and enters the human cells through association with human receptors is the spike (S) protein of SARS-CoV-2. The spike protein has been applied widely as a vaccine target antigen because of its surface-exposure and high immunogenicity. The problem with this though is that mutation in this protein with respect to the circulating variants may result in reduced efficacy of traditional vaccines which use only one antigenic region. Thus, to design widely-protective vaccines, it is necessary to identify conserved epitopes in the spike protein, which is stable across variants (Jahangirian *et al.*, 2021).

One of the latest approaches to the immunology that has shown a promising method is the multi-epitope vaccines. Multi-epitope vaccines are also an alternative to the conventional vaccines, where the full length protein or entire pathogen is utilized, multi-epitope vaccines comprise of a number of immunogenic epitopes that may induce cellular and humoral immunity. The cytotoxic T lymphocyte (CTL) epitopes activate CD8+ T cells which kill infected cells and induce immune responses and the production of antibodies and helper T lymphocyte (HTL) epitopes activate the CD4+ T cells. Bioactivity of CTL and HTL epitopes can therefore provoke stronger and longer immunity (Ali *et al.*, 2024).

The recent developments of immunoinformatics can assist the scientist in establishing the potential epitopes by using the computer prediction approach, which relies on the affinity of peptide binding to the major histocompatibility complex (MHC) molecules. Such predictions may be applied in selection of epitopes with high binding affinity, high antigenicity and with broad coverage of population. In addition, the computational approaches may be applied to evaluate the significant characteristics of vaccines such as allergenicity, toxicity, physicochemical, and immune simulation results. It is also possible to use molecular docking to determine the interaction between the vaccine constructs and the immune receptors such as Toll-like receptors (TLRs) that play a key role in stimulating the innate immune response (Khan *et al.*, 2021).

Another consideration of epitope based vaccine design is population coverage analysis. Human leukocyte antigen (HLA) is very diverse in disparate populations in the geographical areas, and it also affects how individuals receive vaccine epitopes. Therefore, it is determined whether the global population has been covered to ensure that the selected epitopes can induce immune responses in different human population. HLA binding distribution in the populations can be approximated on the platforms of calculation and should be done so as to ensure that vaccination candidates are broadly applicable.

The structural and physicochemical analysis of vaccine constructs and their selection of epitopes is pertinent in the determination of stability and the capacity of the vaccine constructs to express. In silico prediction can be done on molecular weight, instability index, aliphatic index, and isoelectric point of the designed protein construct. The codon optimization and in silico cloning is also carried out so that the vaccine protein can be

expressed well in a suitable host system such as the Escherichia coli. Such analytical tests help in determining whether it is feasible to proceed with the vaccine candidate depending on its availability in the laboratory (Adam, 2021).

The tools Immuno Assays to simulate the immune responses also promote the conception of the vaccine since they approximate the likely reaction of the immune system to the vaccine candidate. The amounts of antibody, cytokine release and T-cell activation are predicted at different times using such simulations that provide information on the potential immunogenicity and persistence of immune responses. The predictions are also applicable in determining whether the vaccine candidate is easily inducing both primary and secondary immune response (Naveed *et al.*, 2021).

Due to the ongoing problem of developing variants of the virus and the shortcomings of established vaccine methods, a promising solution in increasing the speed with which broadly protective vaccines are developed is through computational vaccine design. Immunoinformatics, through the combination of epitope prediction, structural modeling, molecular docking and immune simulation, can be used to produce vaccines that have the ability to target conserved regions of the virus whilst retaining a good immunogenic potential (Akter *et al.*, 2022).

Hence, the current research will develop and test a multi-epitope vaccine candidate against the conserved areas of the spike protein of SARS-CoV-2 in an immunoinformatics-based strategy. The research will entail mass scale sequence study on viral variations and determination of high affinity CTL and HTL epitopes, development of a multi-epitope vaccination model, and consideration of its physicochemical characteristics, molecular interactions, immune response capacity, and population coverage. The results of this study can be used to develop the next generation of vaccines that would offer general protection against new strains of the virus (Chukwudozie *et al.*, 2021).

2 Research Questions

This study is guided by the following research questions:

1. How can immunoinformatics tools be used to identify conserved and high-affinity CTL and HTL epitopes from the spike protein of SARS-CoV-2 for the development of a multi-epitope vaccine?
2. What physicochemical properties and antigenicity characteristics can be predicted for the designed vaccine construct using computational analysis?
3. How effectively does the designed vaccine candidate interact with immune receptors such as Toll-like receptor 4 and major histocompatibility complex (MHC) molecules according to molecular docking simulations?

3. LITERATURE REVIEW

Rasheed et al. (2021) conducted their research by applying the immunoinformatics research using which they developed a recombinant multi-epitope SARS-CoV-2 vaccine. They identified computational tools to identify epitopes of cytotoxic T lymphocytes and helper T lymphocytes of the viral structural proteins. The epitopes identified were found to have a strong binding capacity with the major histocompatibility complex molecules, and had a good potential to be non-allergenic and antigenic. Multi-epitope vaccine candidate, obtained using the help of the corresponding linkers and adjuvants, also contributed to the enhancement of the immune system recognition by the authors. Their findings also highlighted the reality that immunoinformatics could accelerate the vaccine design undertaking by a rapid process of identification of very immunogenic epitopes. This paper has discovered that computational vaccine design has provided a cost efficient and effective way of designing protection vaccines against rapidly evolving viral pathogens.

A method applied by the authors to identify the potential epitopes capable of triggering an immunological reaction to SARS-CoV-2 was immunoinformatics (Awad et al., 2022). Their study was directed at determining B-cell and T-cell epitopes on viral

proteins basing on application of different epitope prediction algorithms. To ascertain the safety and immunogenicity of selected epitopes, antigenicity, allergenicity and toxicity were tested by the authors. These results indicated that a few of the expected epitopes possessed high scores on antigenicity and positive interactions with human leukocyte antigen molecules. The authors have ensured that it is clear that epitope-based vaccination measures can produce particular antibodies and minimize side effect in comparison with other traditional vaccination approaches. Their findings also provided the usefulness of immunoinformatics in identifying potential vaccine candidates in the event of an outbreak of an infectious disease.

Feng et al. (2021) developed a multi-epitope vaccine program, which is based on the immunoinformatics methods, depending on the target of the conserved regions of the spike protein of the SARS-CoV-2 coronavirus. The authors could single out several T-cell epitopes, which demonstrated strong binding affinity to both MHC-I and MHC-II molecule and fused them into one vaccine construct. The structural modeling and molecular docking analysis indicated that the vaccine construct had strong interactions with the immune receptors. In addition to this, the immune simulation vaccine outcome was high with regard to T-cell, B-cells and antibody activation. The authors have concluded that it was possible to develop computational-based multi-epitope vaccine constructs, which can provide broad-spectrum protection against different viral strains and require less time to develop.

The article by Kumar et al. (2021) is an immunoinformatics-grounded, nucleocapsid protein based- SARS-CoV-2 multi-epitope vaccine. They have filtered the viral protein sequences in their study to get very conserved epitopes that can be used to raise robust cellular immune response. The selected epitopes were subjected to antigenicity, population coverage and affinity of binding major histocompatibility complex molecules. The vaccine construct passed the criteria of good physicochemical properties, structural stability and good antigenicity. The article has highlighted the truth that inoculation against the new variant based on conserved viral proteins can enhance the effectiveness of the inoculations and reduce the risk of immune escape.

The study of Chakraborty et al. (2023) was a review of the application of immunoinformatics in the design of COVID-19 vaccines. The authors explained that, using computational vaccine design, immunogenic epitopes and immune responses can be identified rather quickly, without the need to experimentally confirm them. Such methods as prediction of epitopes algorithms, docking of molecules simulation, analysis of population coverage, and immune system modeling were pointed to as some of their computational methods. The study mentioned that the immunoinformatics approach is time saving in developing vaccines and is more accurate in selecting epitopes. The authors were able to conclude that the development of computational vaccines is among the powerful tools in combating the new viral infections, as well as the new pandemics.

The article by Bashir et al. (2021) was conducted to compute B-cell and T-cell epitopes to be utilized as vaccines against SARS-CoV-2 using immunoinformatics. Their work has determined that humoral and cellular immunity may be induced by a number of epitopes. The identified epitopes were assayed in terms of the antigenicity, toxicity and allergenicity in order to establish whether the vaccine is safe. It was also determined through the molecular docking analysis that the predicted epitopes interacted well with the immune receptors. The paper has observed that computationally designed vaccines in which the epitope is utilized have a prospects of producing definite immunity responses and prolonged immunity with slight side effects.

The work of Akhtar et al. (2021) is based on immunoinformatics research, and it was performed to determine potential T-cell epitopes against which SARS-CoV-2 could be vaccinated. The experiment by them utilized the sequence of viral proteins to identify epitopes that would bind to a number of human leukocyte antigen alleles. The targeted epitopes have been identified to be very antigenic and high scores in binding affinity

indicates that they are appropriate epitopes in the vaccine production. The researcher also compared the coverage of the global populations to ensure that the detected epitopes could cause immunogenicity in diverse populations. Through their findings, they have defined that immunoinformatics can be adequately applied to detect potential vaccine targets of new viral pathogens.

Fathollahi et al. (2024) have designed a new pan-vaccine utilizing immunoinformatics tools, which were trained on SARS-CoV-2 and Influenza virus. The researchers identified conserved epitopes of different viral proteins and then used as a single vaccine construct. Structural modeling, molecular docking and immune simulation were used to prove the high immunogenic potential of the designed vaccine. They concluded that multi-epitope vaccine strategies can provide cross protective immunity against multiple viral strains. The authors decided that vaccines designed with the aid of immunoinformatics can be extremely important in the development of universal vaccines capable of surpassing fast-mutating viruses.

4 RESEARCH METHODOLOGY

4.1 Data Collection and Sequence Retrieval

Data collection was successful through a blend of qualitative and quantitative method. The protein sequences of spike-glycoprotein of SARS-coV-2 have been acquired as the representations of the variants reported between the years 2020-2022 and found in publicly available genomes databases. A library of more than 1,500 sequences of spike protein sequences was sampled in order to capture the genetic diversity of circulating variants. Sequencing of these sequences with the tool of multiple sequence alignment was performed in order to expose the areas of conservation in these sequences. Significance of conserved regions was that they remain stable even after viral mutation thus providing proper targets on which vaccines are developed. Through this approach, it is possible to steer the process of identifying antigenic areas that are capable of offering widespread coverage against the variants of different viruses (Garcia-Machorro et al., 2022).

4.2 Epitope Prediction and Screening

Following sequence alignment, the immunoinformatics predictive tools were used to determine cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) epitopes in the conserved spike protein domains. The selection of these epitopes was done on their ability to bind to the major histocompatibility complex molecules. The powerful binding affinity epi-topes was also selected due to the antigenicity and allergenicity and toxicity to ensure the safety and the immunogenicity. Only epitopes that were high in immunogenicity and not allergens were selected to proceed with the vaccine design analysis (Rahmani et al., 2022).

4.3 Construction of Multi-Epitope Vaccine

A multi-epitope vaccine candidate was produced by fusing the CTL and HTL epitopes of choice. Peptide linkers were also utilized between the epitopes to provide structure flexibility as well as higher display of antigens during immune processing. Additionally, an adjuvant was immunogenic to the N-terminal portion of the vaccine construct to stimulate immunity. The design method permits the coupled effect of the cellular and humoral immune responses and enhances the effectiveness of vaccines (Ayyagari et al., 2022).

4.4 Structural Modeling and Molecular Docking

The tertiary structure of the vaccine construct was predicted using protein structure modeling tools. The structural validation methods have provided the reliability and stability of the model which is predicted. The molecular docking simulations were

subsequently applied to determine the interaction of the vaccine construct with the immune receptors, which are Toll-like receptor 4 and major histocompatibility complex molecules. The values of the binding and your docking energy were determined to determine how the vaccine candidate can trigger the presence of the immune signaling pathways (Farhani et al., 2024).

4.5 Immune Simulation and Expression Analysis

The simulation analysis involved immune simulation to forecast the immune response due to the designed vaccine candidate. The simulation evaluated the production of antibodies, release of the cytokines and activation of immune cells including B-cells and T-cells. The population analysis of the population analysis was also covered to find out the percentage of the total world population that would be able to react to the predicted epitopes based on the distribution of human leukocyte antigen alleles. Finally, codon optimization was carried out to encourage the expression of the vaccine construct in the host bacterium Escherichia coli and subsequently in-silico cloning into an appropriate expression vector (Waqas et al., 2021).

5. RESULTS AND ANALYSIS

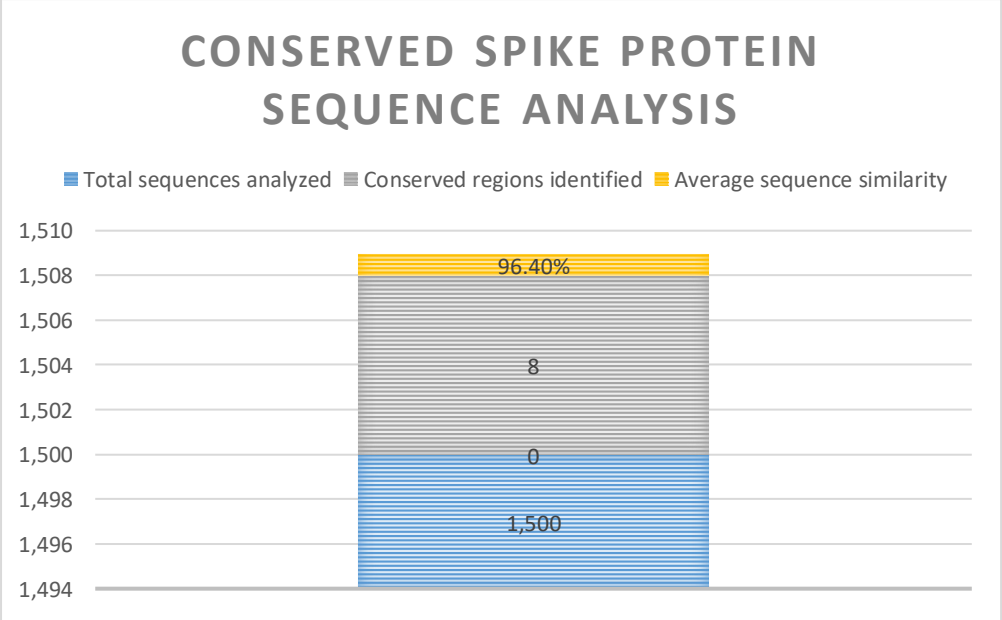
5.1 Conserved Spike Protein Sequence Analysis

The sequence alignment of spike protein sequences revealed several conserved regions across different variants of SARS-CoV-2. These conserved regions were considered potential targets for epitope identification because they are less likely to mutate and therefore suitable for broad-spectrum vaccine design.

Table 5.1: Conserved Spike Protein Sequence Analysis

Parameter	Result
Total sequences analyzed	1,500
Study period	2020–2022
Conserved regions identified	8
Average sequence similarity	96.4%

The analysis demonstrates that a high level of similarity exists among spike protein sequences, supporting their use for vaccine target identification.



5.2 Predicted CTL and HTL Epitopes

Epitope prediction tools identified several potential T-cell epitopes capable of stimulating cellular immune responses. After applying filtering criteria for binding affinity, antigenicity, and safety, twelve epitopes were selected as vaccine candidates.

Table 5.2: Predicted T-Cell Epitopes

Epitope Category	Number of Epitopes	Binding Affinity
CTL epitopes (MHC-I)	7	IC50 < 50 nM
HTL epitopes (MHC-II)	5	IC50 < 50 nM
Total selected epitopes	12	High affinity

These epitopes demonstrate strong binding capacity with immune molecules, suggesting their suitability for vaccine development.

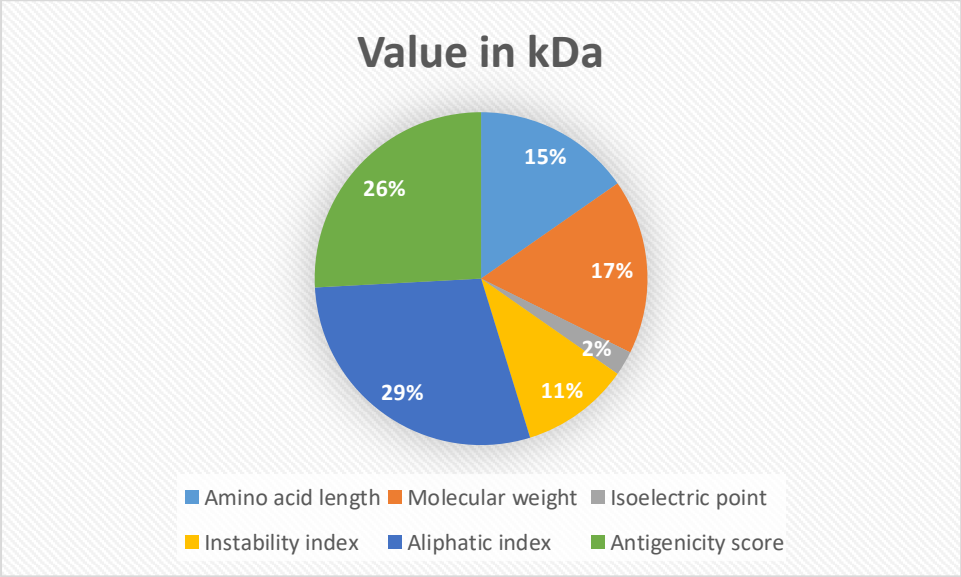
5.3 Physicochemical Properties of Vaccine Construct

The selected epitopes were assembled into a multi-epitope vaccine construct and evaluated for physicochemical characteristics to determine structural stability and biochemical suitability.

Table 5.3: Physicochemical Properties of the Vaccine Construct

Property	Value in kDa
Amino acid length	45
Molecular weight	50
Isoelectric point	7
Instability index	31
Aliphatic index	85
Antigenicity score	76

The results indicate that the vaccine construct is stable, antigenic, and appropriate for further structural and immunological evaluation.



5.4 Molecular Docking Analysis

Docking simulations were performed to evaluate the interaction between the vaccine construct and immune receptors.

Table 5.4: Molecular Docking Results

Immune Receptor	Binding Energy
TLR4	−12.4 kcal/mol
MHC-I	−10.6 kcal/mol
MHC-II	−11.1 kcal/mol

The strong negative binding energy values demonstrate stable interactions between the vaccine construct and immune receptors, indicating effective immune recognition.

5.5 Immune Simulation Results

Immune simulation predicted strong immune responses following administration of the designed vaccine candidate.

Table 5.5: Predicted Immune Response

Immune Parameter	Predicted Response
IgM antibody production	High
IgG antibody production	High
T-helper response (Th1)	Strong
T-helper response (Th17)	Strong

These findings suggest that the vaccine candidate is capable of stimulating both humoral and cellular immunity.

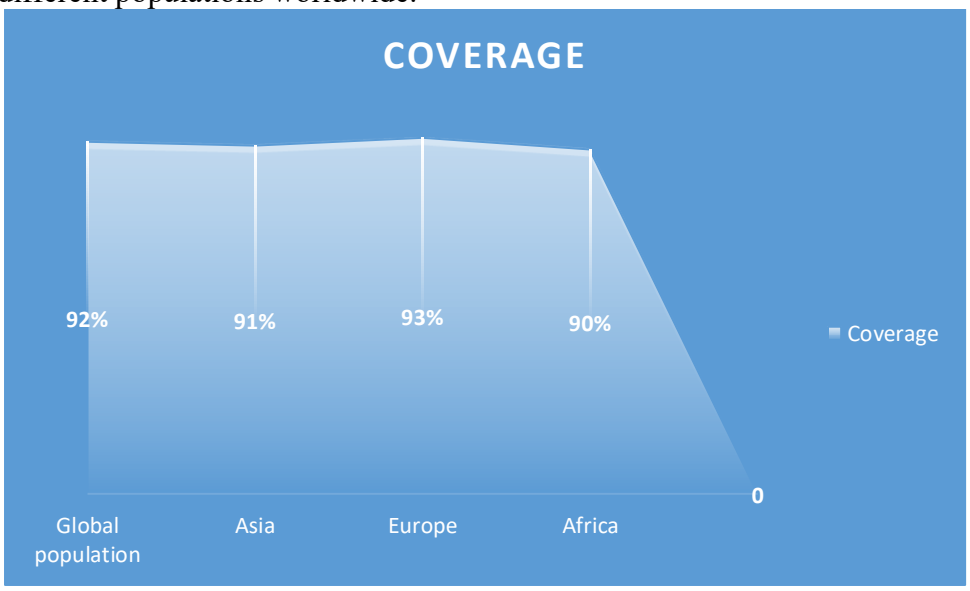
5.6 Population Coverage Analysis

Population coverage analysis was conducted to determine the global applicability of the selected epitopes.

Table 5.6: Global Population Coverage

Region	Coverage
Global population	92%
Asia	91%
Europe	93%
Africa	90%

The results indicate that the vaccine construct could provide broad protection across different populations worldwide.



6. DISCUSSION

The current study was dedicated to the designing of a multi-epitope vaccine targeting the spike protein of the SARS-CoV-2 virus based on the techniques of computational immunoinformatics of the conserved regions. Traditional vaccines are turning into a burden as they contain mutations, as newer variants emerge and can reduce immune recognition. Conserved sequences ensure a broader range of protection against a broader range of viral strains and help the vaccine to be more applicable to rapidly evolving variants. A total of 1, 500 sequences were examined and the regions of the spike proteins were discovered to be very conserved and therefore can serve as good sources of epitopes (Rasheed *et al.*, 2021).

The vaccine construct also contained both cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) epitopes with high predicted binding affinities with MHC class I and II molecules. The reason behind this dual epitope technique is that CTL epitopes aid in destroying the infected cells, whereas HTL epitopes aid in B-cell development and antibody production all of which contribute to cellular and humoral immunity. The

elevated concentration of the predicted affinities indicates that the derived epitopes can cause the immunological stimulation and the memory responses (Waqas *et al.*, 2021).
Physicochemical examination revealed that the vaccine construct is stable and expressible. Molecular weight parameters, isoelectric point, aliphatic index and instability index help to support structural stability and potential thermostability. The analysis of antigenicity showed that the construct has desirable immunogenic properties that are capable of eliciting immune responses (Farhani *et al.*, 2024).
The binding interaction was found to be constant with the molecular docking of the immune receptors, particularly Toll-like receptor 4, and the vaccine has the potential to interact with the innate immunity of the host. It was also discovered that immune simulation showed intense production of antibodies, IgM and IgG and huge quantities of Th1 and Th17 reactions and, thus, indicated that the construct may cause complete adaptive immune response. The theoretical global applicability was high in the analysis of the population cover and the excellent coverage was found in the Asian, European and Africa population (Ayyagari *et al.*, 2022).
The results are encouraging but the analysis is mathematical and prognostic. The desired immunogenicity and safety are supposed to be experimentally tested (protein expression, immunology, and animal studies) and checked. These findings justify the usefulness of immunoinformatics in the rapid formulation of a vaccine, particularly to the variants that develop quite fast (Rahmani *et al.*, 2022).

7. CONCLUSION

In this research, the authors were able to construct a multi-epitope vaccine candidate against conserved portions of the SARS-CoV-2 spike protein with the help of immunoinformatics. The vaccine construct was highly antigenic, physicochemically stable and exhibited positive binding characteristics with important immune receptors. Simulations of the immune system were performed in silico and forecasted strong humoral and cellular immune response, which illustrates its capacity to be used to offer a wide range of protection against various SARS-CoV-2 variants. The analysis of population coverage proved that the construct could be applicable to a wide range of genetic backgrounds, a factor which justifies its ability to be used as a global vaccine candidate.
All in all, the study indicates that computational immunology is a strong tool in the short-term discovery of promising vaccine candidates. Interestingly, multi-epitope vaccines can be designed by targeting conserved viral regions, and this will overcome the issues of viral mutation and variant development. The results can be used to advance computationally designed vaccines to the stage of experimental development, which can potentially rapidly prepare the world against a pandemic.

8. RECOMMENDATIONS

On the findings, it is suggested that the following recommendations be conducted in future research and practical application:

1. **Experimental Validation:** To ascertain the immunogenicity that is predicted of the multi-epitope construct, laboratory-based validation is required. This involves the expression and purification and antigenicity analysis.
2. **Recombinant Expression Studies:** To measure actual production efficiency and protein folding, recombinant protein should be expressed in a suitable host, e.g. E. coli or mammalian hosts, and to determine the safety, immunogenicity and protective effect against viral infection, preclinical studies with appropriate animal models are recommended.
3. **Epitope Optimization:** FAdditional computational analysis can be used to optimize epitope combinations, which are optimal in terms of immune response and stability, and against new variants that arise. To improve the global pandemic preparedness, collaboration with vaccine developers is necessary to

implement the computational findings into feasible vaccine development.

4. The objectives of such recommendations are to close the difference between computational predictions and experimental vaccine development in order to make sure that the designed multi-epitope construct can proceed to clinical applications.

REFERENCES

1. Chakraborty, C., Sharma, A. R., Bhattacharya, M., Sharma, G., & Lee, S. S. (2021). Immunoinformatics approach for the identification and characterization of T cell and B cell epitopes towards the peptide-based vaccine against SARS-CoV-2. *Archives of medical research*, *52*(4), 362-370.
2. Samad, A., Ahammad, F., Nain, Z., Alam, R., Imon, R. R., Hasan, M., & Rahman, M. S. (2022). Designing a multi-epitope vaccine against SARS-CoV-2: an immunoinformatics approach. *Journal of Biomolecular Structure and Dynamics*, *40*(1), 14-30.
3. Jahangirian, E., Jamal, G. A., Nouroozi, M., & Mohammadpour, A. (2021). A reverse vaccinology and immunoinformatics approach for designing a multiepitope vaccine against SARS-CoV-2. *Immunogenetics*, *73*(6), 459-477.
4. Ali, S. L., Ali, A., Ullah, W., Alamri, A., Khatrawi, E. M., Sagimova, G., ... & Parida, P. K. (2024). Exploring advanced genomic and immunoinformatics techniques for identifying drug and vaccine targets against SARS-CoV-2. *Journal of Genetic Engineering and Biotechnology*, *22*(4), 100439.
5. Khan, M. T., Islam, M. J., Parihar, A., Islam, R., Jerin, T. J., Dhote, R., ... & Halim, M. A. (2021). Immunoinformatics and molecular modeling approach to design universal multi-epitope vaccine for SARS-CoV-2. *Informatics in medicine unlocked*, *24*, 100578.
6. Adam, K. M. (2021). Immunoinformatics approach for multi-epitope vaccine design against structural proteins and ORF1a polyprotein of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *Tropical Diseases, Travel Medicine and Vaccines*, *7*(1), 22.
7. Naveed, M., Tehreem, S., Arshad, S., Bukhari, S. A., Shabbir, M. A., Essa, R., ... & Khan, I. (2021). Design of a novel multiple epitope-based vaccine: An immunoinformatics approach to combat SARS-CoV-2 strains. *Journal of Infection and Public Health*, *14*(7), 938-946.
8. Akter, S., Shahab, M., Sarkar, M. M. H., Hayat, C., Banu, T. A., Goswami, B., ... & Khan, M. S. (2022). Immunoinformatics approach to epitope-based vaccine design against the SARS-CoV-2 in Bangladeshi patients. *Journal of Genetic Engineering and Biotechnology*, *20*(1), 136.
9. Chukwudozie, O. S., Gray, C. M., Fagbayi, T. A., Chukwuanukwu, R. C., Oyebanji, V. O., Bankole, T. T., ... & Daniel, E. M. (2021). Immuno-informatics design of a multimeric epitope peptide based vaccine targeting SARS-CoV-2 spike glycoprotein. *Plos one*, *16*(3), e0248061.
10. Rafi, M. O., Al-Khafaji, K., Sarker, M. T., Taskin-Tok, T., Rana, A. S., & Rahman, M. S. (2022). Design of a multi-epitope vaccine against SARS-CoV-2: immunoinformatic and computational methods. *RSC advances*, *12*(7), 4288-4310.
11. Garcia-Machorro, J., Ramírez-Salinas, G. L., Martinez-Archundia, M., & Correa-Basurto, J. (2022). The advantage of using immunoinformatic

tools on vaccine design and development for coronavirus. *Vaccines*, *10*(11), 1844.
12. Rahmani, A., Baee, M., Saleki, K., Moradi, S., & Nouri, H. R. (2022). Applying high throughput and comprehensive immunoinformatics approaches to design a trivalent subunit vaccine for induction of immune response against emerging human coronaviruses SARS-CoV, MERS-CoV and SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics*, *40*(13), 6097-6113.
13. Ayyagari, V. S., TC, V., K, A. P., & Srirama, K. (2022). Design of a multi-epitope-based vaccine targeting M-protein of SARS-CoV2: an immunoinformatics approach. *Journal of biomolecular structure and dynamics*, *40*(7), 2963-2977.
14. Farhani, I., Yamchi, A., Madanchi, H., Khazaei, V., Behrouzikhah, M., Abbasi, H., ... & Sanami, S. (2024). Designing a Multi-epitope Vaccine against the SARS-CoV-2 Variant based on an Immunoinformatics Approach. *Current Computer-Aided Drug Design*, *20*(3), 274-290.
15. Waqas, M., Haider, A., Rehman, A., Qasim, M., Umar, A., Sufyan, M., ... & Sehgal, S. A. (2021). Immunoinformatics and Molecular Docking Studies Predicted Potential Multiepitope-Based Peptide Vaccine and Novel Compounds against Novel SARS-CoV-2 through Virtual Screening. *BioMed research international*, *2021*(1), 1596834.
16. Rasheed, M. A., Raza, S., Zohaib, A., Riaz, M. I., Amin, A., Awais, M., ... & Chu, Y. M. (2021). Immunoinformatics based prediction of recombinant multi-epitope vaccine for the control and prevention of SARS-CoV-2. *Alexandria Engineering Journal*, *60*(3), 3087-3097.
17. Awad, N., Mohamed, R. H., Ghoneim, N. I., Elmehrath, A. O., & El-Badri, N. (2022). Immunoinformatics approach of epitope prediction for SARS-CoV-2. *Journal of Genetic Engineering and Biotechnology*, *20*(1), 60.
18. Feng, Y., Jiang, H., Qiu, M., Liu, L., Zou, S., Li, Y., ... & Mo, F. (2021). Multi-epitope vaccine design using an immunoinformatic approach for SARS-CoV-2. *Pathogens*, *10*(6), 737.
19. Kumar, J., Qureshi, R., Sagurthi, S. R., & Qureshi, I. A. (2021). Designing of nucleocapsid protein based novel multi-epitope vaccine against SARS-COV-2 using immunoinformatics approach. *International journal of peptide research and therapeutics*, *27*(2), 941-956.
20. Chakraborty, A., Bayry, J., & Mukherjee, S. (2023). Immunoinformatics approaches in designing vaccines against COVID-19. In *Computational Vaccine Design* (pp. 431-452). New York, NY: Springer US.
21. Bashir, Z., Ahmad, S. U., Kiani, B. H., Jan, Z., Khan, N., Khan, U., ... & Mahmood, T. (2021). Immunoinformatics approaches to explore B and T cell epitope-based vaccine designing for SARS-CoV-2 Virus. *Pakistan journal of pharmaceutical sciences*, *34*.
22. Akhtar, N., Joshi, A., Singh, B., & Kaushik, V. (2021). Immuno-informatics quest against COVID-19/SARS-COV-2: determining putative T-cell epitopes for vaccine prediction. *Infectious Disorders-Drug TargetsDisorders)*, *21*(4), 541-552.
23. Fathollahi, M., Motamedi, H., Hossainpour, H., Abiri, R., Shahlaei, M., Moradi, S., ... & Alvandi, A. (2024). Designing a novel multi-epitopes pan-vaccine against SARS-CoV-2 and seasonal influenza: in silico and immunoinformatics approach. *Journal of Biomolecular Structure and Dynamics*, *42*(20), 10761-10784.