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Regular paper

A reverse vaccinology approach to design an mRNA-based vaccine to provoke a robust immune response against HIV-1

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There have been substantial advances in HIV research over the past three decades, but we are still far from our goal of eliminating HIV-1 infection entirely. Numerous ever-evolving antigens are produced as a result of HIV-1's genetic variability. Developing an effective vaccination is challenging because of the structural properties of the viral envelope glycoprotein that obscure conserved receptor-binding sites and the presence of carbohydrate moieties that prevent antibodies from reaching potential epitopes. To work on an HIV-specific vaccine, this study identified 5 HIV-surface proteins, from the literature, to screen potential epitopes and construct an mRNA vaccine. A wide range of immunological-informatics techniques were utilized to develop a construct that efficiently stimulated cellular and humoral immune responses. The vaccine was produced with 31 epitopes, a TLR4 agonist termed RpfE that acts as an adjuvant, secretion boosters, subcellular trafficking structures, and linkers. It was determined that this suggested vaccine would cover 98.9 percent of the population, making it widely available. We, furthermore, carried out an immunological simulation of the vaccine illustrating the active and stable responses from innate and adaptive immune cells, the memory cells remained active for up to 350 days after vaccine injection, whereas the antigen was excreted from the body within 24 hours. Docking performed with TLR-4 and TLR-3 showed significant interaction with –11.9 kcal/mol and –18.2 kcal/mol^{-1} respectively. Molecular dynamics simulations further validated the vaccine's stability, with a dissociation constant of 1.7E-11 for the TLR3-vaccine complex and 5.8E-11 for the TLR4-vaccine complex. Lastly, codon optimization was carried out to guarantee that the designed mRNA construct would be translated into the host successfully. This vaccine adaptation, if tested *in-vitro*, would be efficacious and potent as predicted.

Keywords: HIV-1, clinical trials, mutagenesis, membrane proteins, immune stimulation.

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Abbreviations: ACC, Auto cross covariance; AUC, Area under the ROC curve; GRAVY, Grand average of hydropathy; iMODS, Internal Coordinates Normal Mode Analysis Server; RMSD, Root Mean square density; RpfE, Resuscitation-promoting factor SARS-Cov-2, severe acute respiratory syndrome coronavirus 2; Theoretical pI, Theoretical Isolelectric Point

INTRODUCTION

AIDS or acquired immunodeficiency syndrome is caused by a very notorious virus called HIV or human immunodeficiency virus. It directly attacks and affects the immune system of the host and makes it vulnerable and susceptible to many diseases (Del Amo *et al.*, 2020). The HIV consists of two strains namely HIV-1 and HIV-2 both having two RNA strands, 15 different viral proteins and could also contain a few proteins from the last host from which it is transferred (Jewell *et al.*, 2020). All these components are encapsulated by a lipid bilayer membrane. It can be transferred via blood or saliva but not by mere contact. In general, it is transferred through sexual contact, sharing needles or blood transfusion. It can also be transferred to the baby from the mother through breastfeeding (Cooper *et al.*, 2020). HIV-1 is transferred into the host more rapidly and is found all over the world. This infectious virus was first reported on June 5, 1981 in Los Angeles (Finlayson *et al.*, 2019). By the second month of 1983, the Centers for Disease Control in the United States reported that approximately 1000 people were HIV positive (Kanny *et al.*, 2019; Sullivan *et al.*, 2021). Although it was first reported in the United States, it had a greater impact in Africa. Nine countries in Africa had one-third of the world's HIV infection (Lancet HIV, 2020; Ssentongo *et al.*, 2021).

For a long time now, Antiretroviral therapy or ART has been considered the best possible measure for controlling HIV (Jiang *et al.*, 2020). It basically consists of a combination of drugs which are prescribed to infected patients to be taken on daily. This therapy does not cure the viral infection but helps the infected persons to live a comparatively longer and healthier life (Chen, 2019). The virus basically destroys the CD4$^+$ T cells which are responsible for fighting off different infections and diseases. The medication basically works by reducing the amount of HIV in the body (Suryawanshi *et al.*, 2018). The virus is not completely eliminated by them but the reduced number gives the body a chance to regrow CD4$^+$ T cells and fight off the infections and keep the body healthy (Nami *et al.*, 2019). Another approach to cure HIV is to produce a suitable vaccine. To date, many vaccines have been developed by different research institutions but none of them have been successful enough to be prescribed for mass treatment (Yoshida *et al.*, 2021). All of them had notable demerits due to which they failed the early trial stages. Also, the new approaches to HIV vaccine production were recently leveraged by SARS-Cov 2 response so, the dire need for successful vaccine production remains (Karim *et al.*, 2021).

The sequence variability of HIV is one of the primary underlying reasons why an HIV vaccine is not yet commercially available on a worldwide basis (Vasavi *et al.*, 2019; Fenwick *et al.*, 2019). There are up to 20% differ-

ences between the strains of HIV in terms of proteins that are largely conserved, and there are up to 35% differences between the strains in terms of the outer envelope. Additionally, there is a wide variety of ways in which the virus might spread (Akbari *et al.*, 2021). Due to the fact that it can be introduced into the body by sexual contact or the intravenous route, the virus can spread fast throughout the body; nevertheless, it typically takes between one and two weeks for the virus to colonize healthy cell populations (Ng'uni *et al.*, 2020). The first form of vaccination, also known as the conventional method, involves the use of complete organisms, which might provoke allergic reactions due to the presence of big antigens in the body (Abdulla *et al.*, 2019). Any new epidemic could emerge at any time in the future, just like SARS COV-2 did. The development of an mRNA vaccination that incorporates epitopes from a variety of conserved and membrane proteins is one strategy that can be implemented to reduce the severity of allergic reactions (Andrabi *et al.*, 2018; Naveed *et al.*, 2023a; Naveed *et al.*, 2023b).

The fundamental objective of this study was to design an in-silico technique for the construction of an mRNA-based peptide-based immunization that was based on antigenic proteins derived from HIV-1 (Liang *et al.*, 2023). This was illustrated in Figure 1. (A). (Larijani *et al.*, 2018; Muttaqin *et al.*, 2021). Using a number of approaches, it was possible to make predictions about autoimmune disease as well as B cell, CTL, and HTL epitopes and then confirm these predictions. The construct of the vaccine was made by combining each epitope with several linkers and various adjuvants. RpfE was used as an adjuvant, and other aspects of the vaccine, including its population coverage, antigenicity, allergenicity, and toxicity, were investigated. Both the secondary and tertiary structures of the vaccine had been predicted. In addition, the TLR-3 and TLR-4 molecular docking systems were included in the vaccine construct. Simulations using molecular dynamics confirmed that the vaccine does not lose its effectiveness over time.

MATERIALS AND METHODS

The pipeline of the methodology is described in Fig. 1A.

Viral Proteins Sequence Retrieval

The complete Genome of the Human Immunodeficiency Virus (HIV) was obtained from National Centre for Biotechnology Information Database (NCBI) under Accession number: MN692147.1. The proteins of HIV that were selected based on the previously reported data from UniProt with accession numbers: K0J9R5, Q01804, Q9QBZ6, P04585, 0.5886.

Evaluation and Analysis of Proteins

In order to determine the antigenicity of viral proteins, we utilized the Vaxijen 2.0 internet server. The threshold value was set at 0.4 so that it would be more specific. We utilized a technique called AllerTop to determine whether or not proteins were allergenic (https://www.ddgpharmfac.net/Vaxijen/Vaxijen/Vaxijen.html) and AllerTop v2 is used for determining allergenicity (https://www.ddgpharm.net/AllerTOP/method.html).

Physiochemical Analysis

Using the ExPASy Protpram tool (https://www.expasy.org/resources/protpram), the physiochemical properties of a chosen protein, including its theoretical PI, amino acids composition, instability index, *in-vitro* and *in-vivo* half-life, aliphatic index, molecular weight, and most important grand average of hydropathicity (GRAVY), were investigated.

B-Cell Epitope Prediction

For the prediction of B-cell epitopes, the IEDB Linear Epitope Prediction Tool version 2.0 was utilized, and its default settings were left alone. It makes use of complicated algorithms that are based on the architecture of antigen-antibody proteins. It was put to use in an accessibility examination of the surface.

CTL and HTL Epitope Prediction

IEDB was utilized in order to make a prediction regarding the T-cell epitopes. When a sequence is supplied in FASTA format, various build-in IEDB tools are used to create MHC-I and MHC-II epitopes. It was decided that humans would serve as the host species. All alleles with length 9 were chosen for further study. As the output format, an XHTML table was utilized, and all of the other parameters and choices were left in their default states. (http://tools.iedb.org/mhcii/).

Evaluation of Epitopes

The antigenicity, allergenicity, and toxicity of each single epitope were assessed. We used the VaxiJen antigen server (http://www.ddg-pharmfac.net/Vaxijen/VaxiJen/VaxiJen.html) to assess antigenicity. In an alignment-free

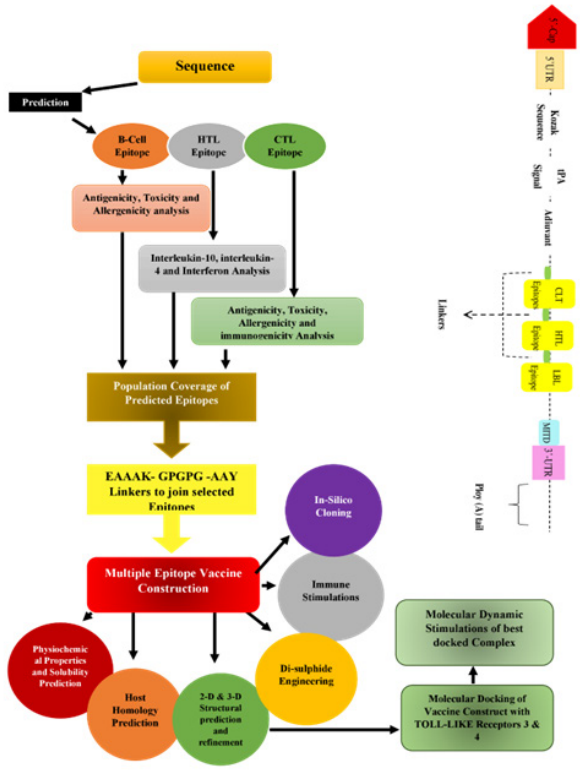

Figure 1. (A) Flow Chart of Diagram of Vaccine Design, (B) Flow Diagram of Vaccine Construct from N-terminal to C-terminal.

method, the physicochemical characteristics of epitopes form the basis for the prediction. For this experiment, bacteria with a cutoff of 0.5 were used. AllerTop V.2.0 (http://www.ddg-pharmfac.net/AllerTOP) was used for epitope analysis due to its user-friendly interface and comprehensive database. The parameters were left in their default states (Naveed *et al*., 2022b). Finally, epitope toxicity was quantified using the ToxinPred (https://webs.iiitd.edu.in/raghava/txnpred/multisubmit.php) site for toxicity analysis. For further study, only antigenic, non-toxic, and allergy-free epitopes have been selected.

Resemblance with Human Proteins

Using the BLASTp program (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins), all of the predicted peptides were analyzed in relation to the protein database for Homo sapiens (Taxid:9606). If the E-value is more than 0.05, then all of the peptides in the vaccine are evaluated as potential non-homologous peptides.

Population Coverage Calculation

Population Coverage for vaccine design aimed at targeting T-lymphocyte epitopes and associated MHCI and MHCII alleles can be calculated using the IEDB database (http://tools.iedb.org/population/). This number is based on how many different MHC alleles the construct's epitopes are able to identify. This is because there is a wide range in MHC-allele distribution across different regions and peoples.

Construct of the proposed vaccine

The construct of the vaccine is shown in Fig. 1B. Each anticipated epitope was joined together using one of three linkers: AAY, KK, or GPGPG. These linkers are necessary for separating and isolating different functional domains. They are tough, bendable, and easy to cut. Resuscitation-promoting factor (RpfE) was utilized as an adjuvant in order to boost the adaptive immune response. The mRNA vaccine needs to have a Kozak sequence, which consists of an ORF start codon and a stop codon. In addition, two structures were added to the construct: first, the tissue plasminogen activator (tPA) secretory signal sequence (UniProt ID: P00750) in the 5' region of the construct. Once translated, epitopes can be secreted from cells with the help of this signal sequence. Second, the mRNA vaccine's 3' locus end contains an MHC I-targeting domain (MITD; UniProt ID: Q8WV92).

Evaluation of Vaccine Protein

Antigenicity was analyzed by the Vaxigen v2.0 and for allergenicity we used AllerTop. ToxinPred was used for toxicity analysis. Solubility analysis with the help of SoLpro was done to determine the purity of a substance. ExPasy-Protparam Tool was used for physiochemical properties analysis.

Peptide Secondary and Tertiary Structure Prediction

Secondary and tertiary structures were analyzed by the PsiPred and TsRosseta respectively to explore multi-epitope vaccine. The tertiary structure was assessed using Tr Rosetta (https://yanglab.nankaledu.en/trRosetta/).

mRNA Vaccine Secondary Structure Prediction

Predicting the secondary structure of the mRNA vaccine with the help of the RNAfold tool (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). It makes use of McCaskill's technique to determine the secondary structure with the lowest free energy (MFE). This method was used to determine the minimum free energy (MFE) of a structure, as well as the MFE of a centroid secondary structure.

Refinement and Validation of tertiary structure

The vaccine's structure was refined using the Galaxy-Refine server. This involved, first, the rebuilding and repacking of sidechains, and second, the relaxation of the structure using molecular dynamics simulation processes. (http://galaxy.seoklab.org/). Multiple servers were used to verify the accuracy of the 3D structure based on references. To begin, RAMPAGE (Ramachandran Plot Assessment) was utilized. After that, PROCHECK was utilized to verify the building's integrity. (https://bip.weizmann.ac.il/toolhox/structure/validation.htm).

Analysis of Conformation B-Cells Epitope Prediction

New conformational B-cell epitopes can be stimulated by the protein's tertiary structure. Estimates of discontinuous B-cell epitopes in the protein structure were generated using ElliPro, a web-based service (http://tools.iedb.org/ellipro/). Ellipro benefits from the 3D model's geometric properties. When compared to other known strategies for predicting discontinuous B-cell epitopes, ElliPro has the greatest AUC value of 0.732 for the protein model.

Docking Analysis

Peptide vaccines with either toll-like receptor 4 (TLR-4) (PDB ID: 3FXI) or TLR-3 (PDB ID: 3FXI) (PDB ID: 1ZIW) To dock 3D structures on the ClusPro server, we employed the PIPER docking method. Both TLR4 and TLR3 were blocked by a RpfE adjuvant. It is possible that this server will produce numerous models using various scoring mechanisms. Free binding energy (G), dissociation constant (Kd), and percentages of charged and polar amino acids on the non-interacting surface were calculated by receptor-ligand 3D interaction using the PRODIGYY tool on the HADDOCK website (https://haddock.science.uu.nl/).

Evaluation of Binding Energies

T cells and MHC alleles underwent a molecular docking simulation to determine their binding affinities. RCSB's PDB database was queried for the tertiary structure of MHC alleles. PyMol was used to visualize the structures, and the Swiss-PDB viewer was employed to reduce power consumption. Epitopes were folded into 3D structures using the PEP-FOLD 3.5 server, and energy was conserved by viewing the structures in the Swiss-PDB viewer. The docking and binding affinity computations were performed on the Cluspro 2.0 server. Additional visualization and analysis were performed using PyMol and Discovery Studio.

Molecular Dynamics Simulations

iMods online was used for molecular dynamics simulations. It studies the molecule and interaction on the basis of torsion angles. It was utilized for the analysis of RMSD values, co-variance among residues, eigenvalue of interacting residues and distortion of structure. It was utilized to discover the stability of the complex.

Table 1. List of Proteins selected Previous Literature

No.	Protein	Uniprot ID	Antigenicity	Function	Location
1	Envelope glycoprotein gp160	K0J9R5	0.5767	Host-Virus Interaction	Surface Protein
2	OTU domain-containing protein 4	Q01804	0.5	Pathogenesis and Innate Immune Response	Cytoplasmic Protein
3	Gag polyprotein	Q9QBZ6	0.58	Virion Assembly	Cell Membrane
4	Gag-Pol polyprotein	P04585	0.6778	Virion Assembly and Lipid Anchor to Host	cell membrane
5	Tat- Protein	Q76PP9	0.5886	Receptor Binding to Host	Cytoplasmic Protein

Codon Optimization and Expression Analysis of Proposed Vaccine

The codon of the vaccine was optimized to ensure that the peptide vaccine construct was produced efficiently in human cells. This was achieved with the help of the GenSmart Codon Optimization software (http://www.genscript.com/). In order to determine whether or not a product is of high quality, GenScript uses Rare Codon Analysis (http://www.genscript.com/) (GS). How well mRNA is expressed and translated is quantified by the Codon Adaptation Index (CAI). The sequence was back-translated using the backtranseq software EMBOSS 6.0.1 so that the mRNA protein could be expressed in the expression vector. after then, the Java Codon Adaptation Tool was used for codon optimization (JCAT). As an expression vector, we employed the pub18 gene from E. coli. SnapGene used in-silico PCR for construct amplification.

Immune Simulation

C-ImmSim was used to verify the immune response. It uses an immune epitope and interaction with the help of a position-specific scoring matrix (PSSM). All the procedure was done at default parameters. (https://kraken.iac.rm.cnr.it/C-IMMSIM)

RESULTS

Protein sequence Retrieval

The FASTA format of amino acids of Human immunodeficiency virus 1 proteins that are present on the surface or on transmembrane having accession number CCA61241.1, QEE91975.1, Q9QBZ6.2, NP_057849.4, UED13371.1 respectively, were obtained from the NCBI database. The function and cellular localization of the shortlisted proteins are given in Table 1.

B-cell Epitope Predictions

IEDB Linear Epitope Prediction v2.0 was used to predict the B cell epitopes. At first, 25 epitopes were predicted which were then filtered out on the basis of the antigenicity and allergenicity. At the end, only 8 epitopes were selected for the vaccine construct as shown in Table 2.

CTL or HTL Epitopes and their Evaluation

T-cell Epitopes are of two types: MHC-I and MHC-II. These epitopes are predicted or selected on the basis of the value of IC50. Those epitopes with values lower than 200 are selected.. The lower the ic50, the high the binding affinity of the epitope is. For this whole dataset of the HLA alleles are selected. A total of 40,419 MHC Class I Epitopes were predicted and out of them, only 8 were selected on the basis of their antigenicity and their allergenicity. A total of 18,279 MHC Class II Epitopes were predicted and out of them, only 15 were selected on the basis of their antigenicity and their allergenicity. The shortlisted MHC I and II epitopes are shown in Table 2.

Interaction between Epitopes and MHC-alleles

In total, there are 23 T-lymphocyte epitopes and 120 MHC-alleles. As can be seen in Table 4, whereas some epitopes only recognize a single MHC allele, others can detect as many as seventeen different alleles. We selected 5 epitopes that matched up with certain MHC alleles for further molecular docking study. The ClusPro 2.0 server docking results are shown in Table 4 as energy affinities. With an MHC allele (HLA-A*32:01) and its associated epitope, the YALFYKLDIV region displays the highest binding affinity (–9.2 kcal/mol). As can be seen in Figs 2A and 2B, as a result, epitopes bind competently to the MHC-allele binding fork. As can be seen in Fig. 3, we also assessed every potential interaction between the targeted epitope and allele residues.

Proposal for a Vaccine Construct

The proposed design of the vaccine construct is like the following:

5' m7GCap- 5' UTR–Kozak sequence-EAAAKMKNARTTLIAAAIAGTLVTTSPAGIANADD AGLDPNAAAGPDAVGFDPNLPPAPDAAPVDTPPAPEDAGFDPNLPPPLAPDFLSPPAEEAPPVPVAYSVNWDAIAQCESGGNWSINTGNGYYGGLRFTAGTWRANGGSGSAANASREEQIRVAENVLRSQGIRAWPVCGRRG (adjuvant) -YVPPIRGEIGPGPGYALFYKLDIVGPG PGWSNKSYDDIWGPGPGVGRKKRAAVGPGPGRAAVGLGAVGPGPGGLGAVLLGFLGPGPGAIAVANWTDRGPGPGGLIGLRIVFAGPGPGAARAVELLGRSSLKGKKAAVGLGAVLLGFLSTKKALAWDDLRSKKALFYKLDIVKKAPAGFAILKCRDKEFKKARAVELLGRSSLKGLKKAVGLGAVLLGFLSTAKKCAPAGFAILKKCAPAGFAILKCRDKEKKFYKLDIVPIDDNGKNKKG-

Table 2. List of epitopes candidates to design the vaccine

Cell Type	Sequence of Epitope	Antigenicity
MHC I Cells	YVPPIRGEI	0.6365
	YALFYKLDIV	0.7604
	WSNKSYDDIW	0.8368
	VGRKKRAAV	1.5567
	RAAVGLGAV	1.1827
	GLGAVLLGFL	0.8696
	AIAVANWTDR	1.2624
	GLIGLRIVFA	1.8903
MHC II	AARAVELLGRSSLKG	0.9438
	AAVGLGAVLLGFLST	1.1029
	ALAWDDLRS	0.8966
	ALFYKLDIV	0.8914
	APAGFAILKCRDKEF	1.3346
	ARAVELLGRSSLKGL	0.926
	AVGLGAVLLGFLSTA	1.083
	CAPAGFAIL	1.1739
	CAPAGFAILKCRDKE	1.3168
	FYKLDIVPIDDNGKN	0.8964
	GDLEITTHSFNCRGE	1.1009
	YDDIWNNLTWVEWER	0.858
	YKLDIVPIDDNGKNS	1.0139
	YSPLSFQTL	1.4447
	YSPLSFQTLTHHQRE	1.1407
B Lymphocytes	NVNSTKNATTPTVTPTPTSLMKDTGEL	0.769
	IRDKKKQEYALF	1.3047
	TIKQACPKMSFDPIP	1.0224
	CRDKEFNGT	0.7076
	ITFNSSAGGDL	0.5866
	NSSSLASNNSNEN	0.5
	GKNNTNETFRPAGGDMRDNWRSELYKY KVVKIKPLGIAPTKARRRVVGRKKR	0.6997
	QEKNEQDLLALDKWASL	0.752

DLEITTHSFNCRGEKKYDDIWNNLTWVEWERKKYKLDIVPIDDNGKNSKKYSPLSFQTLKKYSPLSFQTLTHHQREKKNVNSTKNATTPTVTPTPTSLMKDTGELAAYIRDKKKQEYALFAAYTIKQACPKMSFDPIPAAYCRDKEFNGTAAYITFNSSAGGDLAAYNSSSLASNNSNENAAYGKNNTNETFRPAGGDMRDNWRSELYKYAAYKVVKIKPLGIAPTKARRRVVGRKKRAAYQEKNEQDLLALDKWASLAAY

The antigenicity score of the constructed vaccine is 0.7056 at a threshold of 0.5. The overall allergenicity of the vaccine is predicted as non-allergen. This shows that the construct is a good vaccine candidate.

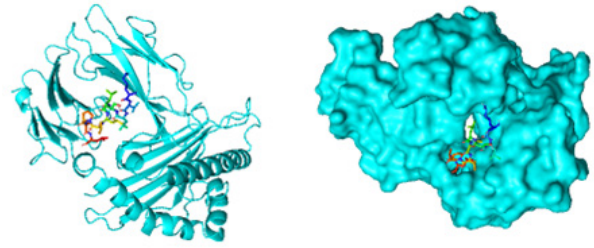

Figure 2. Docking visualization between the epitope AGFRQYRAASIQVGN and its corresponding MHC allele (HLA-DRB1*01:01) using the PyMol software: (A) Surface View (B) Cartoon View.

Physiochemical Properties of Vaccine

To determine the antigenicity, allergenicity, toxicity, and soluble nature of a construct, scientists can use programs like VaxiJen, ANTIGENpro, AllerTOP, ToxinPred, and SolPro. The results showed that the vaccine m*et all* of the criteria for an effective vaccine: it was antigenic, non-allergenic, non-toxic, and water-soluble. Table 5 displays the results of using the ExPasy ProtParam service to establish the physiochemical profile of the construct. Based on the vaccine's known physiochemical properties, it was projected that the construct would be thermally stable. Due to the GRAVY value being -0.357, it may be concluded that the vaccine is hydrophilic. These results indicate that the mRNA vaccine design is a promising potential vaccination candidate.

Prediction of Population Coverage

Combining all 120 alleles of the matching 23 epitopes on MHC-I and MHC-II, the IEDB population coverage technique was able to estimate that the human population would be protected against all known pathogens. Eventually, global vaccination rates would reach about 98.9 percent.

Immune Simulation of Vaccine

We used three vaccinations to kick off the immune response (see Fig. 4). Both the second and third options performed better than the first. After antigen suppression, immunoglobulin levels were high, and it was discovered that more IgM than IgG was being produced. This increase may be indicative of the formation of immunological memory following antigen exposure. B-cell isotype persistence across time reveals the existence of memory cells within the B-cell population. It's also true that more CTL and HTL cells were produced as a direct consequence of memory formation. Furthermore, macrophage activity rose while dendritic cell activity remained unchanged. IFN- and IL-2 levels also went up. There is a rise in both innate immunity and the number of epithelial cells, which play a role in innate immunity.

mRNA Vaccine Prediction for secondary structure

The mRNA vaccine's structure was inferred using the RNAfold website. The free energy of the structures was also computed using this site. As a starting point, we took the codons that were optimized for usage in the vaccine. As shown in Fig. 5F, the mRNA vaccine had a minimum free energy of -930.20 kcal/mol during pro-

Table 3. T-Lymphocytes and their corresponding alleles

MHC I Epitopes	MHC I Binding Alleles	MHC II Epitopes	MHC II Binding Alleles
YVPPIRGEI	HLA-B*40:01, HLA-B*44:03, HLA--B*44:02, HLA-A*02:06, HLA-B*08:01, HLA-A*32:01, HLA-B*51:01	AARAVELLGRSSLKG	HLA-DRB1*12:01, HLA-DRB1*01:01, HLA-DQA1*01:02/DQB1*06:02, HLA--DQA1*05:01/DQB1*03:01
YALFYKLDIV	HLA-B*40:01, HLA-B*44:03, HLA--B*44:02, HLA-A*02:06, HLA-B*08:01, HLA-A*32:01, HLA-B*51:01	AAVGLGAVLLGFLST	HLA-DQA1*05:01/DQB1*03:01
WSNKSYDDIW	HLA-B*57:01, HLA-B*58:01, HLA--B*53:01, HLA-A*32:01	ALAWDDLRS	HLA-DQA1*05:01/DQB1*03:01
VGRKKRAAV	HLA-A*68:01, HLA-A*31:01, HLA--A*33:01, HLA-A*11:01, HLA-A*03:01, HLA-A*30:01, HLA-A*26:01	ALFYKLDIV	HLA-DRB1*12:01, HLA-DRB1*04:01, HLA-DQA1*05:01/DQB1*03:01, HLA--DRB1*15:01
RAAVGLGAV	HLA-A*02:06, HLA-B*39:01, HLA--B*15:01, HLA-B*35:01, HLA-C*12:03, HLA-B*15:02, HLA-C*14:02	APAGFAILKCRDKEF	HLA-DRB3*02:02, HLA-DRB1*04:01, HLA-DRB1*09:01, HLA-DRB1*01:01, HLA-DRB1*07:01, HLA-DRB1*04:05, HLA--DRB5*01:01
GLGAVLLGFL	HLA-A*68:01	ARAVELLGRSSLKGL	HLA-DRB1*15:01, HLA-DRB1*04:05, HLA-DRB1*07:01, HLA-DQA1*05:01/ DQB1*03:01, HLA-DRB1*09:01, HLA--DQA1*01:02/DQB1*06:02, HLA--DRB3*02:02, HLA-DRB1*04:01, HLA--DRB1*08:02, HLA-DPA1*02:01/DPB1*14:01, HLA-DRB1*01:01, HLA-DRB3*01:01, HLA--DQA1*01:01/DQB1*05:01
AIAVANWTDR	HLA-C*12:03, HLA-A*68:02, HLA--A*02:06, HLA-A*68:01, HLA-C*03:03	AVGLGAVLLGFLSTA	HLA-DRB1*09:01, HLA-DRB1*01:01, HLA--DRB1*07:01, HLA-DRB5*01:01, HLA--DRB1*04:05, HLA-DRB3*01:01
GLIGLRIVFA	HLA-A*68:02, HLA-A*30:01, HLA--A*30:02, HLA-A*26:01, HLA-A*01:01, HLA-A*68:01, HLA-B*58:01	CAPAGFAIL	HLA-DRB1*04:03, HLA-DRB1*04:05, HLA--DRB1*04:01, HLA-DRB1*04:04
	HLA-A*02:01, HLA-A*02:03, HLA--A*02:06, HLA-A*68:02, HLA-B*51:01	CAPAGFAILKCRDKE	HLA-DRB1*04:01, HLA-DRB3*03:01, HLA--DRB1*13:02, HLA-DRB1*04:04, HLA--DRB1*04:05
	HLA-A*68:02, HLA-A*30:01, HLA--A*30:02, HLA-A*26:01, HLA-A*01:01, HLA-A*68:01, HLA-B*58:01	FYKLDIVPIDDNGKN	HLA-DRB1*04:01, HLA-DRB4*01:01, HLA--DPA1*01:03/DPB1*02:01, HLA-DRB1*13:02, HLA-DRB3*02:02
		GDLEITTHSFNCRGE	HLA-DRB5*01:01, HLA-DQA1*05:01/ DQB1*03:01, HLA-DRB3*02:02, HLA--DRB1*09:01, HLA-DRB1*04:01
		YDDIWNNLTWVE-WER	HLA-DRB3*02:02, HLA-DRB5*01:01, HLA--DRB1*04:01, HLA-DRB1*07:01, HLA--DRB1*01:01, HLA-DRB1*09:01
		YKLDIVPIDDNGKNS	HLA-DRB3*02:02, HLA-DRB3*01:01, HLA--DRB1*09:01, HLA-DRB1*13:02, HLA--DRB5*01:01, HLA-DRB1*15:01
		YSPLSFQTL	HLA-DRB1*01:01, HLA-DRB1*15:01, HLA--DRB1*04:05
		YSPLSFQTLTHHQRE	HLA-DRB5*01:01, HLA-DRB1*04:01, HLA--DRB1*07:01

duction, and the energy of its secondary centroid structure was -700.67 kcal/mol. The results indicate that the mRNA structure will be stable.

Secondary and tertiary structures of the mRNA vaccine

We used the PSIPRED server to try and guess the secondary structure of the vaccine. Figure 5A depicts the structure, which is composed mostly of alpha helices. To learn about the vaccine's tertiary structure, we used the Tr Rosetta server (see Fig. 5B). The PROCHECK service was then used to confirm the structure's stereochemical accuracy. Figure 5C's Ramachandran plot shows that 98.3% of residues were located in the recommended zones, 1.6% in the additional allowance zone, and 0.2% in the prohibited allowance zone. The vaccine has an overall quality factor of 92.64211, which is

Table 4. Molecular Docking of T-Lymphocytes epitopes with corresponding MHC alleles and their Binding affinity

Type of Epitope	Epitope	MHC Allele	PDB ID	Binding Affinity
MHC I	YALFYKLDIV	HLA-A*32:01	4PR5	-9.2
	VGRKKRAAV	HLA-A*03:01	3RL1	-6.0
	RAAVGLGAV	HLA-B*15:01	1XR8	-4.9
MHC II	APAGFAILKCRDKEF	HLA-DRB1*01:01	2FSE	-5.1
	ARAVELLGRSSLKGL	HLA-DRB1*15:01	1BX2	-5.3

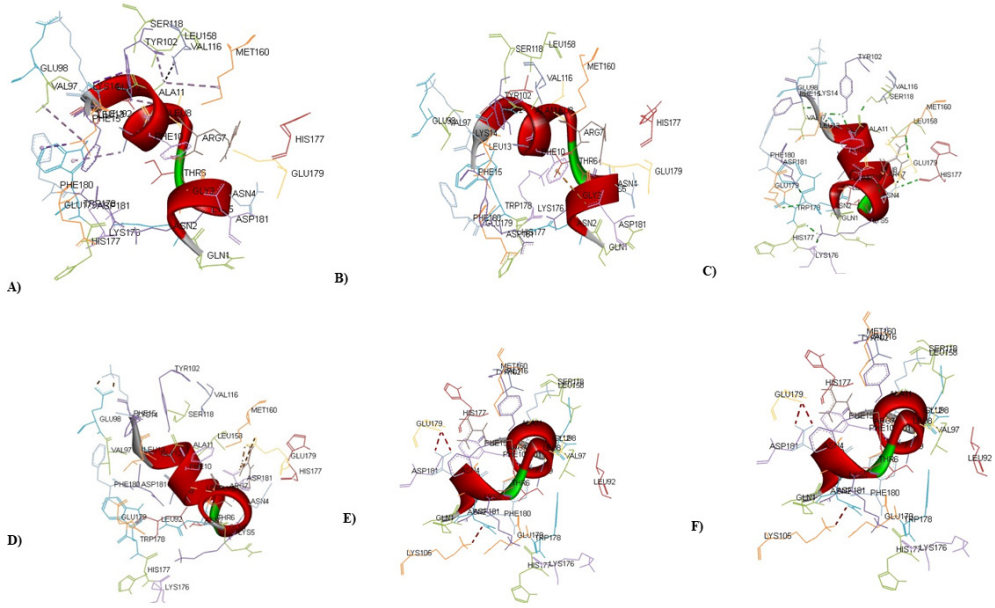

Figure 3. Different Interactions between the epitope and its corresponding MHC allele visualized using the discovery studio. (A) Conventional Hydrogen Bonds (B) Salt Bridge, attractive Charge interactions (C) Hydrophobic Interactions (D) Cation-Pi interactions (E) Donor-Donor Clash (F) Pi Donor Hydrogen Bond

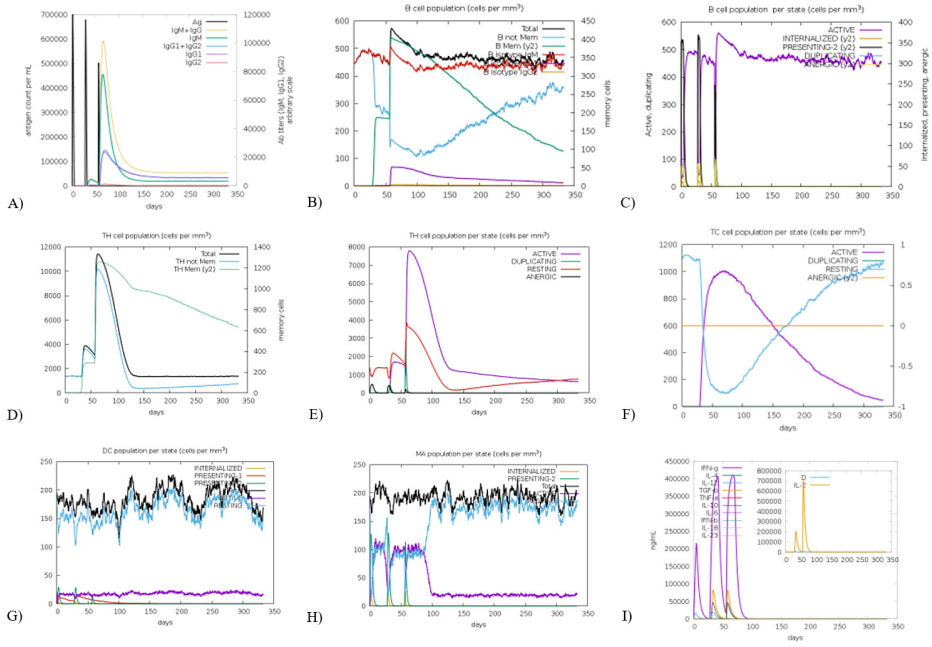

Figure 4. Immune simulation response against Vaccine construct from C-ImmSim server.
(**A**) The immunoglobulin production after antigen injection. (**B**) The B cell population after three injections. (**C**) The B Cell Population per state (**D**) The Helper T Cell Population (**E**) The Helper T Cell Population per state (**F**) The Cytotoxic T Cell Population per state (**G**) Macrophage Population per state (**H**) Dendritic Cell Population per state (**I**) Cytokines and Interleukins Production with Simpson Index of the immune response.

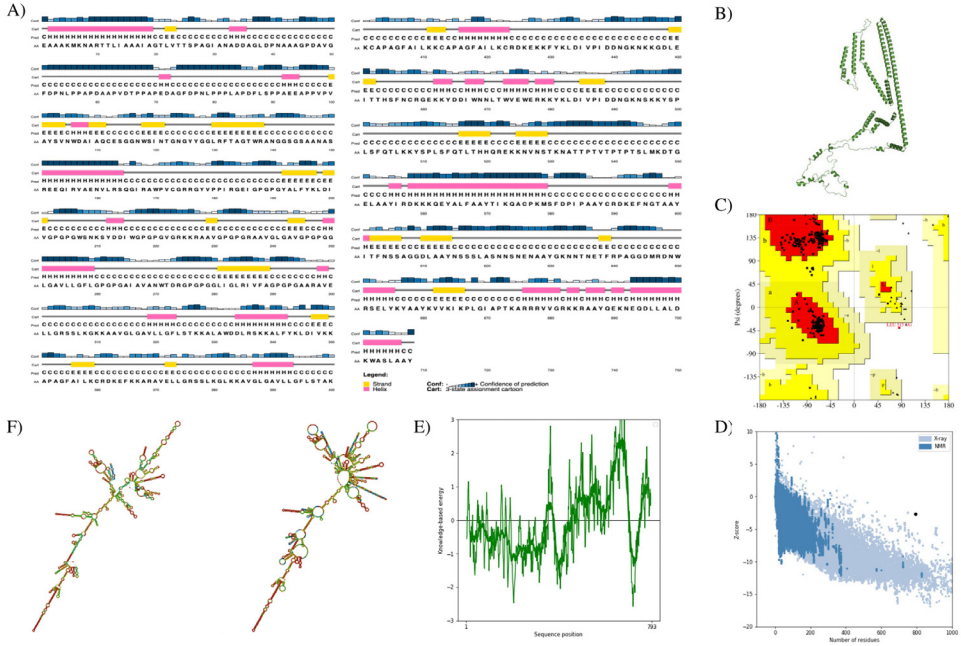

Figure 5. Structure prediction and validation of Vaccine Construct:
(**A**) PSIPRED server results of secondary structure of vaccine (**B**) The Robetta server used to predict the Tertiay structure of vaccine (**C**) The PROCHECK server used to analyze the Ramachandran Plot (**D**) Z-score analysed by the Pro-SA webserver (**E**) Local Quality Factor (**F**) mRNA secondary structure

quite high. The tertiary protein model is very consistent, as predicted by the ProSA-web server, which yielded a negative Z-score of -6.01.

Conformational B-cell epitopes Prediction

For the purpose of locating B-cell conformational epitopes, a server named ElliPro was used to execute vaccination model folding. Eleven discontinuous conformational B-cell epitopes were forecast using this method. Figure 6 reveals that the anticipated score for the 285 residues for secondary and tertiary models of conformational cell Epitopes ranges from 0.512 to 0.883.

Molecular Docking

The probable connections between the construction and TLR-4 and TLR-3 receptors were verified using ClusPro software for molecular docking. Additionally, we used the PRODIGY website to analyze the binding affinities and dissociation constants at 37°C for the more

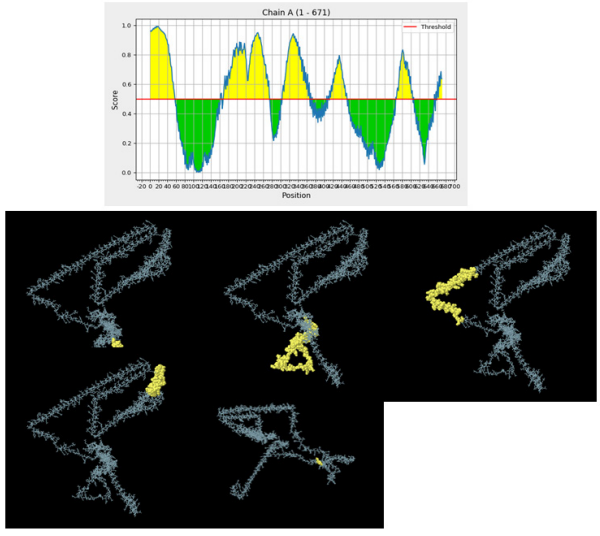

Figure 6. The ElliPro server of IEBD database for the prediction of eleven conformational B-cell epitopes:
(**I**) Position of Conformational B-cell epitopes 2D illustration. (**II**) 3D models of B-cells epitopes where yellow spheres present the conformational B-cell epitopes.

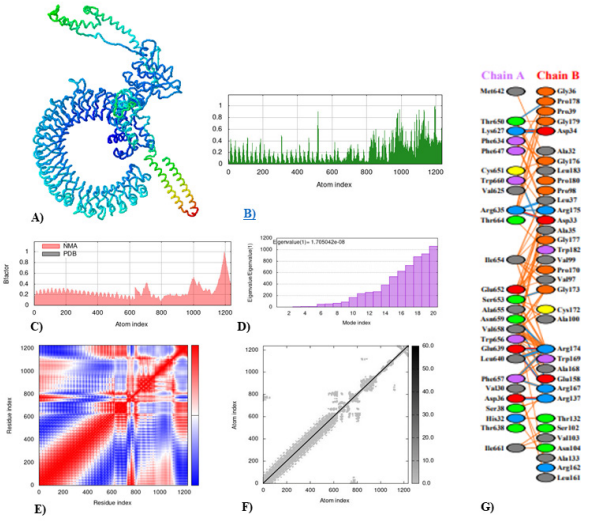

Figure 7. Molecular dynamics simulation, Normal Mode Analysis, and receptor-ligand interactions:
(**A**) Vaccine-TLR4 docked complex using the Cluspro server (**B**) Deformability graph (**C**) Eigenvalue of vaccine-TLR4 complex (**D**) B-factor graph (**E**) Covariance matrix (**F**) Elastic network model using the iMODS server (**G**) Receptor-ligand interaction using the PDBsum webserver.

Table 5. Physiochemical Properties, Antigenicity and Allergenicity of the Vaccine

Property	Measurement	Indication
Total Number of Amino Acid	708	Appropriate
Molecular Weight	75209.01 KDa	Appropriate
Formula	$C_{3380}H_{5329}N_{943}O_{976}S_{13}$	-
Theoretical pI	9.64	Basic
Total number of positively charged residues (Arg + Lys)	64	-
Total number of negatively charged residues (Asp + Glu	100	-
Total Number of Atoms	10641	-
Instability index (II)	31.84	Stable
Aliphatic Index	76.51	Thermostable
Grand Average of Hydropathicity (GRAVY)	-0.357	Hydrophilic
Antigenicity VaxiJen	0.7596	Antigenic
Antigenicity AntigenPro	0.821798	Antigenic
Allergenicity	Non-allergenic	Non-allergenic
Toxicity	Non-toxic	Non-toxic

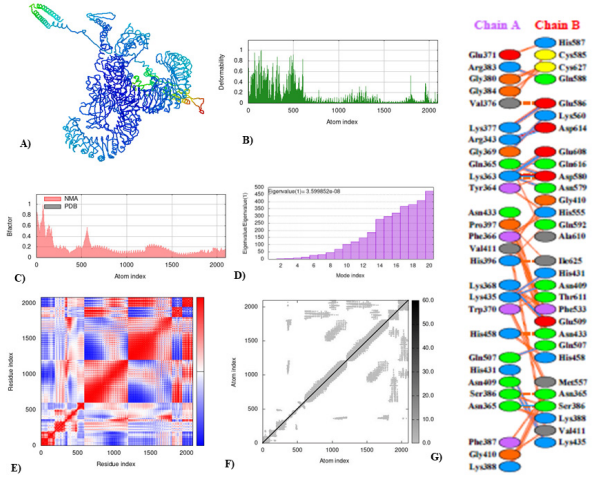

Figure 8. Molecular dynamics simulation, Normal Mode Analysis, and receptor-ligand interactions:
(**A**) Vaccine-TLR4 docked complex using the Cluspro server (**B**) Deformability graph (**C**) Eigenvalue of vaccine-TLR4 complex (**D**) B-factor graph (**E**) Covariance matrix (**F**) Elastic network model using the iMODS server (**G**) Receptor-ligand interaction using the PDBsum webserver.

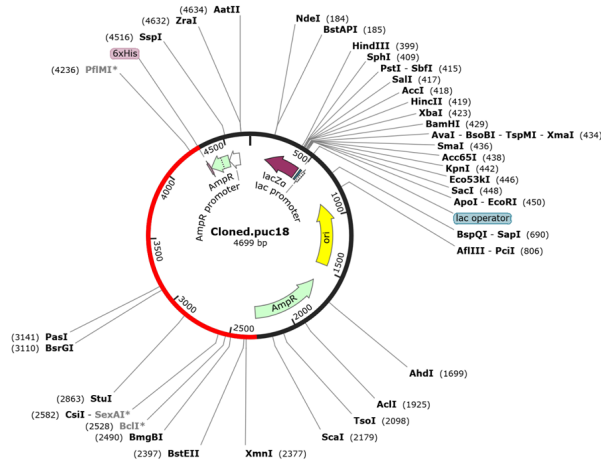

Figure 9. Optimized Vaccine Cloned in PUC18 Vector

clustered component of each complex. As a benchmark, we relied on the adjuvant docked complex.

There was a difference in binding affinity of –18.2 kcal/mol^{-1} between the TLR3-Vaccine complex and the control. At 25 degrees Celsius, the complex dissociation constant for Vaccine-TLR3 was 1.7E-11, while the value for the control was 1.1E-06. The binding affinity for the TLR4-Vaccine complex was –11.9 kcal/mol^{-1}, while it was only –9.0 kcal/mol^{-1} for the control. Vaccine-TLR4 had a complex dissoiation constant at 25°C of 5.8E-11, significantly higher than the control's value of 2.5E-07. PDBsum was used to analyze the vaccine's enhanced interaction with TLR-3 and TLR-4 receptors.

Molecular Simulation of Vaccine

When exposing the Vaccine-TLR3 and Vaccine-TLR4 complexes to the iMOD server, molecular dynamic simulation analysis was performed. Vaccine deformable loci and amino acids with coiled forms are both shown by peaks in the deformability graph. NMA (normal mode analysis) is a computational tool for studying protein adaptability. As can be seen in Figs 7C and 8B, the B-factor graph represents the complex's connection to the Normal Mode Analysis and PDB areas. The eigenvalues of the docked complexes are displayed in Figures 7D and 7D, respectively. Figures 7E and 8E demonstrate the

interactions between amino acid duplets in the dynamical area as a covariance matrix, where the red part denotes correlated residues, the white part for anti-correlated residues, and the blue part for non-correlated residues.

Codon Adaptation and In-silico Cloning

JCat which is a codon adaptation tool was used to optimize the vaccine construct codon to get maximum protein expression. The optimized codon (Fig. 9) sequence has 70% GC Content. The optimized codon sequence was inserted into PUC18 Vector to make a recombinant plasmid which is then amplified through In-silico PCR using SnapGene software.

DISCUSSION

Vaccination against a particular disease not only protects individuals from contracting the illness but also results in an overall improvement in the health of the population, cuts down on the spread of the disease, and is very cost-efficient. However, the process of immunization calls for a significant investment of time and money while it is still in the developmental stage. In this context, the contribution of specialists is absolutely essential. Because of advances in technology and the accessibility of various bioinformatics tools, it is now possible to save costs while simultaneously reducing the amount of time spent. Analyses of the pathogen on the genomic and proteomic levels are being carried out in order to create vaccinations (Pandey *et al.*, 2018). The goal of this research is to decrease the amount of viral material that is bound to virus particles and transferred to human cells by focusing on the outer-membrane and transmembrane proteins. The investigation of the secondary and tertiary structure has been demonstrated to offer a highly antigenic potential for the development of vaccines. Based on the allergenicity and antigenicity report of the vaccine, this study demonstrates, in general, the potential of this in silico produced vaccine targeting the gp160 in membrane and trans-membrane.

As a result of advancements in the clinical research field, we are now able to choose and target histocompatibility complex (MHC) T-cells that have the most interaction with human leukocyte antigen (HLA) scheme using computational biology. Alternatively, we can choose to use B-cells as the source of vaccine design. This option is available to us. In the beginning, it is necessary for us to g*et al*l of the databases and software that are available for all of the nucleocapsid and trans-membrane epitopes that are feasible on the protein. The IEDB database was selected for the epitope prediction of B-cells because it can predict antigenicity in two different methods. The cut-off score for antigenicity has been set to 5.0. On the other hand, we identified T-cell epitopes by screening them first to find those with an IC50 value of less than 200, which ensures that they are highly active against the targeted protein. After that, the epitopes are combined with MHC Class-I and MHC Class-II Class, and then the two classes are separated for screening (Naveed *et al.* 2023c).

Vaxijen v2.0 may be used to calculate the antigenic characteristics of B and T cells, as well as the ACC of peptides based on their physiochemical parameters. Those with levels more than the limit are considered antigens in nature. This antigenic epitope should be safe in its natural condition, so that it does not cause problems when employed as an immune response (Cong *et al.*, 2023). We also assess vaccine allergenicity since vaccine allergenicity is the greatest impediment to advancement in the field of vaccine development. Both classes' MHC have shown a high degree of conservation over the world, resulting in an increase in the proportion of the HIV patient population protected by peptide vaccines (Meron-Sudai *et al.*, 2023).

Following the acquisition of all necessary criteria, the process of vaccine manufacturing will involve the selection of 8 epitopes from MHC Class-I T-cells, 8 epitopes from B-cells, and 15 epitopes from MHC Class-I T-cells as the subunits (Naveed *et al.*, 2022c). The vaccine demonstrates a growing response of the vaccination in the identification of pathogens and activation of the immune system, immune-reactivity is one of the features that adjuvants like 50S ribosomal protein L7/LI2 are used to boost. In the first stages of the production of multiple epitope vaccines, linkers are utilized for the purpose of fusing B-cell and T-cell epitopes together. Linkers such as EAAAK, CPGPG, KK and AAY were incorporated between the critical predicted epitopes in order to produce a viable vaccine with the highest antigenicity possible.

The generated protein sequence has been subjected to bioinformatic analysis as well as immunologic testing, both of which have concluded that it does not include any allergenic or poisonous components. GalaxyRefine was utilized in order to refine the 3D structure of the chimeric vaccine, which resulted in the acquisition of the desired features (Al Tbeishat, 2022). RAMPAGE, also known as the Ramachandran Plot, demonstrates that a possible vaccination candidate possesses suitable characteristics. According to the findings, the majority of the residues are located in favorable areas, while just a few residues were found in regions that were considered to be outliers (Naveed *et al.*, 2022e).

This demonstrates that the quality of the desired model can be considered acceptable. Clustpro2.0 is utilized in the Docking process, which displays the most promising models of the vaccine-receptor complex by bringing them into close proximity to the reference structure. A simulation using molecular dynamics was carried out, with the goal of emulating the natural behavior of our dynamic system (Naveed *et al.*, 2022d). The docked complex of the vaccine construct of viral gp160 protein and CD4 receptor of the host should that the computational vaccination model of human immunodeficiency virus has the potential to combat Autoimmune Deficiency Syndrome.

The primary emphasis of this research was on the development of an in-silico strategy for the design of an mRNA peptide-based vaccination based on HIV-1 antigenic proteins (Shabani *et al.*, 2022). Using immunoinformatic methods, the advised mRNA vaccine was tested for stability, thermostability, antigenicity, lack of allergenicity, and hydrophilicity. The ability of the vaccine to establish memory cells upon exposure, as well as to produce chemokines that increase B-cell response and humoral response, was verified using molecular simulation after three doses were administered (Naveed *et al.*, 2022a). Macrophages, dendritic cells, and the Simson index were markers for the development of memory cells. Finally, it was determined that the manufactured vaccine shows promise as a potential candidate for use in the prevention of Human Immunodeficiency syndrome infections.

CONCLUSION

Humanity has faced significant scientific, medical, and moral challenges due to HIV/AIDS since its discovery in 1983. The results of this investigation indicate that the vaccine design has both desirable physicochemical properties and immune responses against HIV-1. Using a variety of immune-informatics techniques or methodologies, it was discovered that this immunization will activate an immunological response against HIV-1 in the host. Our results were supported by the immunological response that the vaccine elicited, as measured by Immune Stimulation. Accordingly, this construct is suggested for use as a potential subject for in vitro and in vivo studies against *S. marcescens*, employing a wide variety of serological assays to elicit a reaction. By developing a reliable vaccine, this study aids the global effort to wipe out HIV.

Declarations

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Conflicts of Interest. The authors declare no conflict of interest.

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