Original Article

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Design of a new structure of immunogenic chimeric polytope against human various cancers using immunoinformatics and structural methods

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ABSTRACT

Cancer is one of the deadliest diseases in recent decades. Which has different types. Despite advances in the treatment of cancer, they are still the most critical threat to public health. Although conventional therapies have played a major role in the treatment or eradication of the disease, the emergence of emerging diseases requires new therapies such as vaccine design. Significant challenges in cancer drug treatment such as drug resistance and side effects of drug toxicity and high cost have made the treatment process more difficult. The aim of this study was to design a new and effective strategy for preparing a vaccine against cancer using some antigenic proteins in this disease. After preparing appropriate epitopes of antigenic protein compounds in cancers and examining their antigenic and immunogenic properties, the process of fusion vaccine composition was performed with the help of various bioinformatics tools to study the physicochemical properties and two-dimensional and three-dimensional structures and Their validation as well as immunological and simulation properties were investigated and finally the codons of vaccine constructs were optimized to increase the translation rate of its cloning process in the expression vector pET28a (+) to evaluate the expression of protein in prokaryotic cells in E. coli K12 system. Finally, the docking process was performed with some receptors that are effective in immunological processes in the body, such as TLRs, MHCI, and MHCII. Selected epitopes of physiologically important cancer proteins theoretically cover a high percentage of the world's population. The vaccine was designed with a stable, antigenic, and non-sensitizing composition. Structural analysis of the TRL5/vaccine binding complex and its simulation process reveal sufficiently stable critical with the prospect of receptor recognition. The dynamics of the immune response, having the potential to stimulate and produce active and memory B cells, and the production of CD8+T, and CD4+T cells show a favorable role in stimulating and creating effective immune responses by Th2 and Th1 cells. Computational results using bioinformatics tools showed that our designed immunogenic structure has the potential to stimulate cellular and humoral immune responses against cancer properly. Therefore, based on these data and after evaluating the effectiveness of the candidate vaccine through in vivo and in vitro immunological tests, it can be suggested as a candidate vaccine against cancer.

Keywords: cancer,Vaccine, Immunoinformatics, Antigenicity

INTRODUCTION:

Cancer is one of the most dangerous diseases of the modern age, which has different types. Despite advances in cancer treatment, they are still one of the most important public health threats. Although conventional therapies have played a major role in the treatment or eradication of many diseases, the emergence of emerging diseases requires new methods of treatment with fewer complications and is based on the stimulation and use of the body's own immune system(1). Exploring effective prevention and treatment strategies against cancer requires basic knowledge about the role of the immune system in controlling cancer, its origin and onset, its progression and heterogeneity (7). Today, advances in genome sequencing and the emergence of computer-related biotechnologies have opened up new methods for studying protective antigens, including vaccine design. In this method, probabilistic surface proteins will be identified by a method that starts from the genome and uses computational methods and pattern recognition (2). Therefore, in addition to the complete identification of antigens that can be detected by conventional methods, this method is able to identify new antigens that play an important role in the immunogenicity of new generation vaccines (3). Cancer vaccines are often accompanied by DNA peptides or their own proteins. In various studies, proteins such as NY-ESO-1 and MAGEA3 and other antigenic molecules have been observed to be associated with various cancers. NY-ESO-1 is an efficient target for cancer immunotherapy. This antigen is a member of cancer-testis antigens (CTAs) and is highly expressed in various cancers, including melanoma, ovarian, cervical cancer, etc. MAGE A3 is a tumor-specific shared antigen that is frequently expressed in lung cancer and melanoma, as well as in few other tumors. Its level is associated with disease burden and with prognosis, while normal tissues do not express it, except the testis and the placenta. The presence of antigens on tumor cells is usually associated with a poor prognosis for those tumors. In one study, the high level of MAGE-A3 expression in lung adenocarcinoma patients was associated with their mortality rate and shorter survival. MAGE-A3 is one of the tumor protein compounds that have been identified in some tumors such as melanoma, non-small cell lung cancer, blood malignancies by various studies. (38). The frequency of NY-ESO-1 expression in a large number of tumors, such as neuroblastoma, myeloma, metastatic melanoma, synovial sarcoma, bladder cancer, esophageal cancer, liver cancer, head and neck cancer, non-small cell lung cancer, and ovarian cancer, has been reported in various studies. has been (39). Of course, the amount and frequency of expression in these types of tumors are different from each other. This expression information has been confirmed to a large extent through several studies related to the analysis of RNA expression data and immunohistochemistry. (40). Due to the fact that the duration of specific immune response to self-genes is weak, the use of these vaccines along with radiation therapy, chemotherapy, and the use of other synthetic products prevents or delays tumor progression or growth delayed(4). With the help of today's technologies, one of the anti-tumor solutions is to use compounds based on peptides whose epitopes can be recognized by B-cells and MHCI, possible MHCII (5). The use of such structures to identify and stimulate the antitumor humoral immune response is preferable to other therapies, because the peptides are convenient in terms of synthesis and chemical stability, and lack adverse effects.(6). The development of new tools related to the design of new generation vaccines will help researchers to answer the important questions they face. Designing a cancer vaccine requires a lot of time and money, but using bioinformatics design tools can save a lot of time, energy and money. Therefore, in this research work, various bioinformatics tools were used in designing an immunogenic structure or vaccine based on predicted epitopes of two common proteins with antigenic properties in several different tumors called MAGEA3 and NY-ESO-1 to design a vaccine. It has immunological efficacy in various tumors.

2.Method

2.1. Preparation of the desired protein sequence from NCBI

The NCBI database was used to sequence the two proteins melanoma-associated antigen3 (MAGE3) and NY-ESO-1 protein and retrieved based on their access numbers.

2.2. Prediction of linear B cell epitopes

FASTA format protein sequencing was required to predict B cell epitopes. Here, different FBCPred, ABCPred and IEDB servers were used. The desired peptides from these two servers were then considered as the final epitope (8).

2.3. Predicting MHC-I and MHC-II epitopes and their antigens and analyzing

At this stage, MHC-I epitopes from ProPred 1 and IEDB servers and MHC-II epitopes from ProPred and IEDB servers and B CELL epitopes were retrieved and selected through IEDB and BCpreds.

2.4. Study of the population abundance of epitopes

The IEDB server (http://tools.iedb.org/mhcii) was used to study the prevalence of ali population among different global populations.

2.5. Design of vaccine safety structure.

We used selective MHC-I, MHC-II, and B CELL epitopes with some linker proteins to design the immunogenic construct of the multi-epitope vaccine. We also added adjuvants to the amino terminus of vaccines to increase vaccine antigen. Various adjuvants are used to target human extracellular Toll receptors (TLRs) such as TLR1, TLR2, TLR4, TLR5, and TLR6. Administration of TLR agonists as adjuvants to vaccine candidates elicits strong T cell responses and antibodies. Therefore, the inclusion of TLR agonists as adjuvants in vaccines can enhance vaccine protection by providing strong and long-lasting immune responses (9).

2.6. Predict the second structure of the vaccine

Investigation and analysis of the secondary structure of the vaccine structure were used by PRABI and Psipred servers. At this stage, the vaccine should be evaluated for alpha helix and beta plates and random screws for the second protein structure.

2.7. Evaluation of allergy and antigenicity of the designed vaccine

To evaluate the antigenicity of the entire vaccine structure, we used VaxiJen v2.0 servers and AntigenPro servers for validation. AllergenFP v.1.0 and AllerTOP v.2.0 servers were also used to evaluate the allergenicity of the vaccine structure.

2.8. Investigation of physicochemical properties and solubility and surface availability of vaccine

The physicochemical properties of the candidate vaccine were evaluated via ExPASy-ProtParam and epestfind servers. The solubility of the candidate vaccine was also evaluated and predicted through PEPCALC and Protein Sol servers, as well as the surface availability of amino acids from the IEDB server and the Emini Surface Accessibility section.

2.9. Examining the third structure and modifying and validating the vaccine

The third vaccine structure was evaluated via I-TASSER and SWISSMODEL servers (10). 3DREFINE and GalaxyWEB servers were used to modify the structure of the vaccine. From the five models created by the server, we selected a model based on MolProbity as the final structure. Also, the evaluation, evaluation and comparison of the vaccine model and the modified model were illustrated through PAYMOL and their RMSD was calculated. Then, in order to validate the model, the third structure was examined through the PROCHECK and PROSA servers in terms of the placement of bonds and amino acids that have been spatially examined in our proteins.

2.10. Docking

Molecular docking was performed to predict the affinity between the vaccine structure and some TLRs and human MHCI, MHCII (11). The structure of these receptors was retrieved from the RCSB protein database. The structure was then refined and validated by SPDBV, DISCOVERY STUDIO and MVD software and used for docking process. The protein-protein molecular docking process was performed by a ClusPro 2.0 server (12). As a result, several docking complexes with the lowest energy weight points are shown. Molecular docking helps predict the best ligand-receptor interaction from a group

of ligand-receptor complexes. The set was prepared and illustrated by Paymol, mvd and spbvd software and then the interactions between the complexes were identified by the PDBSUM server.

2.11. Normal analysis for structural stability analysis of ligand / receptor complex

Normal state analysis was performed to evaluate and analyze the structural stability of the selected docking complex through the iMODS web server. This NMA mobility server shows motion dynamics. Also, some special values indicate the stiffness of structures and deformation diagrams of non-rigid parts of the structure. The server also provides factor B, variance, covariance map, and matrix linkage of the resulting vaccine / recipient complex structure.

2.12. Immunological simulation

Insilico's safety simulation was performed using a C-ImmSim server to confirm immunization and immune response against the selected vaccine. This server uses a machine learning base to predict epitopes and related security interactions. The server automatically simulates three anatomical sections: (1) bone, where hematopoietic stem cells are stimulated, and myeloid cells are produced, (2) lymphatic organs, and (3) Thymus, in which simple T cells are selected to prevent autoimmunity. In this process, three injections containing the designed peptide vaccine were simulated at intervals of four weeks, ie days 0.28 and 56. The initial amplifier approach Our simulation was used at 4-week intervals to achieve a long-term preservation safety response based on the evaluation of safety readings. One of the default parameters is that each time step is set to 1, 84 and 168, meaning that each time step is 8 hours and each time step is 1 injection at time zero. Therefore, three injections were performed four weeks apart. However, eight injections were performed four weeks apart to stimulate repeated exposure to the antigen. In this scenario, T cell memory is continuously evaluated. And Simpson index was graphically interpreted from the analysis of this design (13).

2.13. Codon optimization and cloning preparation in Insilico

For maximum expression of the vaccine in the host, an optimization of the candidate vaccine codon was performed. In this process, reverse translation and codon optimization were performed to predict the expression of the vaccinated model in an expression vector using the Java Codon Compatibility Tool (JCat) to increase the vaccine translation rate in the E. coli K12 system. The server provides a codon matching index (CAI) that indicates the bias of codon usage and GC content. The CAI value is between 0.8 and 1, and the GC content between 30% and 70% is useful for translation activities. We evaluated the special. The obtained modified nucleotide sequences were then used in the cloning process using the SnapGene 4.2 tool and the pET28a (+) vector prototype.

3. Results

3.1. protein sequencing from NCBI

SSequences of two melanoma-associated antigen3 (MAGE3) and NY-ESO-1 proteins were retrieved from the NCBI database under access numbers NP_005353.1 and CAA05908.1, respectively.

3.2. Predictive results of linear B cell epitopes

At this stage, using the IEDB server, we examined and predicted the selected epitopes in the structure of the designed vaccine, which obtained better results. About 250 primary amino acids of the designed vaccine are the highest number of B CELL epitopes compared to other regions of the vaccine structure, which is completely consistent with the placement of epitopes in the structure of the vaccine structure. And hydrophobicity and antigenicity also have better conditions than other regions, all of which are designed because of the greater epitope sequences associated with B cells in the early regions of the vaccine structure(Fig1).

3.3. Predicting results of MHC-I and MHC-II epitopes and their antigens and their analysis

At this stage, Propred-I, MHC2pred and IEDB servers were used to predict epitopes by T cells. Propred-I was used as a basic matrix method to scan and predict peptides in front of the library of MHCI class alleles and MHC2pred alleles for MHCII class alleles. Was prepared for use in vaccine design, which was prepared in Table1Sequence specifications and their scores(table1).

Figure 1. The bipred diagram (A) indicates linear epitopes and Chou & Fasman (B) indicates areas with beta screws and Emini (C) indicates areas with optimal access levels and Karplus & schulz (D) indicates areas with high flexibility. Kolaskar & Tongaonkar (E) indicates areas with antigenicity. More desirable and Parker (E) also indicate areas with more suitable hydrophobicity in the structure of the vaccine designed using the IEDB server.

3.4. population coverage of vaccine epitopes

The tool calculates the number of people who are expected to respond to a set of epitopes with known MHC limitations. This calculation is based on HLA genotypic frequencies assuming a non-linking imbalance between HLA loci (14,15). Here PPC vaccine epitopes designed with the IEDB population coverage tool were calculated. CD8 + T cell PPCs and CD4 + T cell epitopes were examined for all populations of the world with "region-country-ethnicity" (16)(Fig2).

3.5. Design of vaccine immunogenic structures

At this stage, the chimer vaccine protein had to be created from the epitopes we screened. To do this, the B cell epitopes were first put together and linked together by linker structures called EAAAK which is used to connect adjuvants with vaccines and KK. Then, with the help of

Table 1. Sequence table of selected epitopes of two proteins NY-ESO 1 and MAGE 3 used in vaccine design

CANCER PROTEIN	VAXIJEN SCORE	B CELL EPITOPE	VAXIJEN SCORE	MHCI EPITOPE	VAXIJEN SCORE	MHCII EPITOPE
MAGE3	0.8536	RAREPVTKAE	1.7175	GNWQYFFPVIFSKAS	1.2449	EVDPIGHL
	0.5759	LLKYRAREPV	1.1244	NWQYFFPVIFSKASS	1.3475	MEVDPIGHLY
	0.5687	EQRSQHCKPE	0.6495	PDLESEFQAALSRKV	0.5101	ESEFQAALSR
	0.6143	ASSSSTLVEV	0.9397	WQYFFPVIFSKASSS	1.2013	STFPDLESEF
	0.8004	LKYRAREPVTKA	-	-	0.5560	AELVHFLLLKYRARE
	1.4848	SVVGNWQYFFPV	-	-	-	-
NY-ESO1	1.5120	ARASGPGGGA	0.6981	MPFATPMEA	0.5991	GPESRLLEFYLAMPF
	1.3804	CCRCGARGPE	0.8179	SLAQDAPPL	0.5041	ITQCFLPVFLAQPPS
	1.8778	RGTGGSTGDA	0.8179	SLAQDAPPL	0.6510	SRLLEFYLAMP
	0.5260	TPMEAELARR	0.9028	GPRGAGAARA	0.6713	RGPESRLLEFYLAMP
	0.7216	GPGGGAPRGPHG	0.6827	ITQCFLPVF	-	-
	1.9259	AEGRGTGGSTGD	-	-	-	-

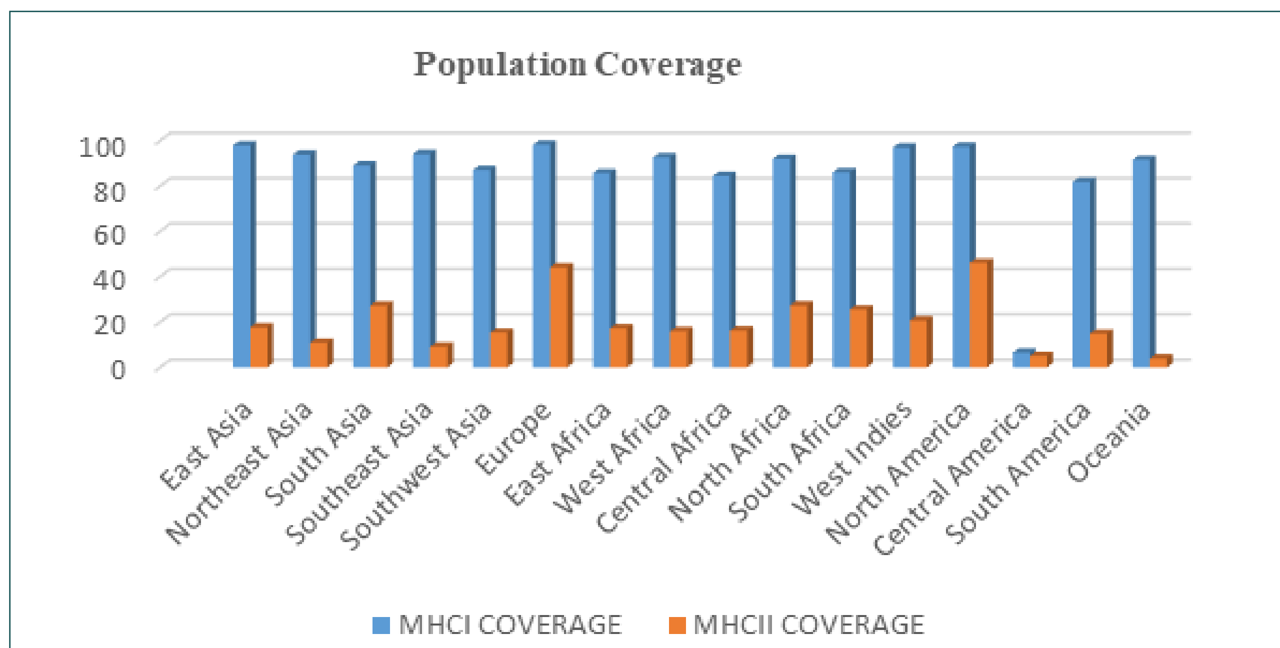


Figure 2. Percentage of population coverage of epitopes used in vaccine design

GPGPG linker, they were connected to MHCI-derived epitopes, which are also linked by KK linker, and finally, by AAY linker, a connection was established between the section containing MHCI and MHCII epitopes. After completing the structural sequences, adjuvant composition was added to the end of the generated chimer sequence(Fig3).

3.6. second structure prediction

At this stage of the evaluation of the chimer composition produced in terms of two-dimensional and three-dimensional structure was examined that if undesirable folds are created in its structure, at the very beginning of the work, the disruptive epitope parts of the chimer structure are removed from its original structure. With the

Figure 3. Schematic illustration of the structure of the designed vaccine

help of software, psipred prabi, the second structure of the chimer was investigated and the results of this study were as follows(Fig4)

3.7. allergenicity and antigenicity and physicochemical properties of the designed vaccine

By examining the antigenicity of the selected epitopes by the VAXIJEN server and ensuring that all selected epitopes used in the design of the vaccine were antigenic, it is time to investigate their antigenicity, which at this stage with the help of ALLerTPv.2.0 and ALLERTOP software. Evaluated and those with allergy risk were removed and those without allergy were isolated and used in the construction of the vaccine structure (17).

3.8. physicochemical properties and solubility

In evaluating the vaccine, we evaluated a number of physical and chemical properties in it by software such as protparam, pepcalc, PROSOL and epestfind. It was present in the body of the host and has a suitable half-life of one hour in the body of mammals. Also, the case composition had a molecular weight of 46887.84 and a gravity of -0.761 and an aliphatic coefficient of 57.16 and also in terms of hydrophopicity and extinction coefficient is in good condition. According to the results of pepcalc and PROSOL servers with a score of 0.563, it had good solubility in water, which allows it to be introduced in laboratory phase processes (37). PEST motifs are sequences rich in the amino acids proline, glutamic acid, serine, threonine, and to a lesser extent aspartic acid, which dramatically reduce the intracellular half-life of proteins. Hence, they target the protein for proteolytic cleavage. The epestfind server detects PEST motifs in both poor and potential proteins based on low and high threshold scores, respectively. The higher the score, the greater the chance that the protein will be broken down by the PEST motif in the eukaryotic cell. In the studied vaccine, three weak PEST motifs were identified, which can be seen in the image below(Fig5).

3.9. third structure of vaccine modification and validation

To obtain our three-dimensional structure, modeling methods were used and SWISSMODEL and I-TASSER servers were used. RMSD was selected as the best model among the models prepared from I-TASSER server in terms of these three indicators, which were selected with a score of 1.83, 0.49 ± 0.15 and 11.2 ± 4.6Å, respectively (19,20). This structure scored -85 in the prosa server, which indicates that the model obtained is in the range of combinations obtained from X-RAY technology. The model obtained after modification by 3DRefine server in terms of placement of its amino acids in the desired structural position according to the results of the prochek server and through the Ramachandran diagram was about 98.8% in an acceptable and desirable position, which indicates the suitability of the model. The quality and comparison of the initial model of the vaccine obtained from the I-TASSER server and the modified model obtained from the 3DREFINE server were performed between the two models through PAYMOL Alinmet software and RMSD = 0.342 was obtained from it, which indicates that the model improved after modification.(Fig6)

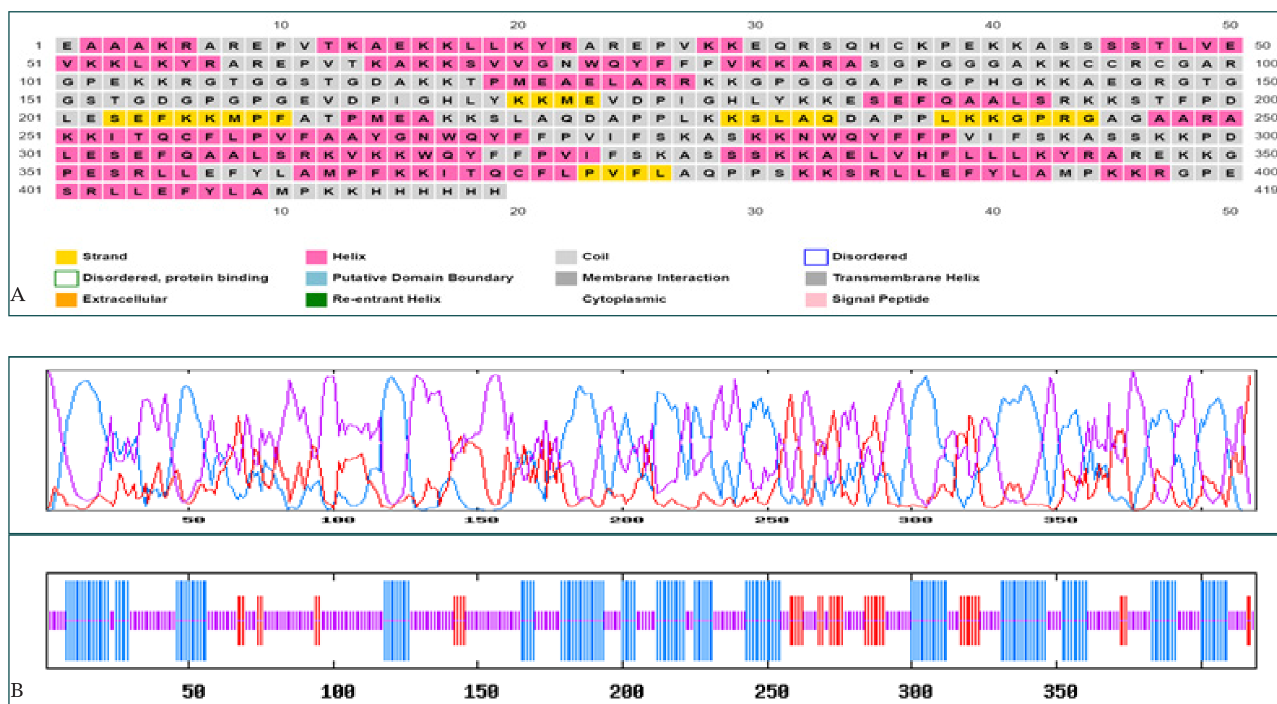


Figure 4. Image of the second structure of the designed vaccine and determination of alpha helix and beta plate regions by servers psipred(A) and prabi(B)

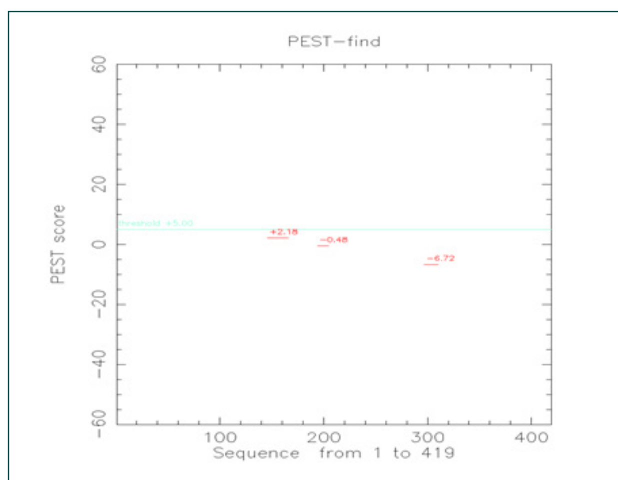


Figure 5. Epestfind server results and positioning of poor PEST motifs in the diagram

3.10. Docking

In this study, the receptors used for the docking process, TLR5, were selected due to its immunomodulatory ability to stimulate IFN-g as well as activation of type I IFN responses [38]. In the study of epitopes selected as CD4 + epitopes, they were able to stimulate both the cytokines Th1 and Th2. Docking studies between VACCINE and the human TLR5 complex were performed using a ClusPro server. The server reflects about 25 to 30 possible docking structures with their corresponding energy values in each case. Among these, complexes with the lowest energy scores were selected. Complex between vaccines with different types of TLR1,2,3,4,5,7,8 receptors and two receptors of human HLA related to MHCI and MHCII classes, which are named HLA, A0201, HLA. DRB1.0201 in terms of energy and Analyzed (table) that among TRLs, TRL5 established the lowest energy level of 1649.8 - and the most stable state of connection with the vaccine(table2).

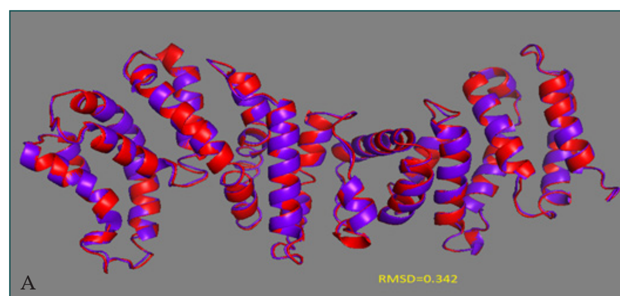
Then some of the docking structures were illustrated using PyMOL software. Interactions between vaccines designed with different receptors and residues and the type of links involved in these interactions were analyzed and obtained by the PDBsum server(Fig7).

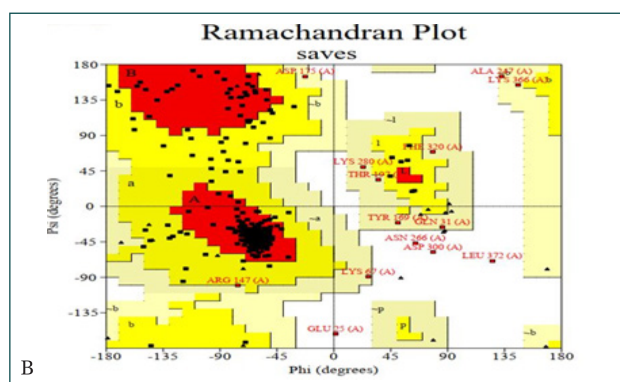
3.11. Normal state analysis for structural stability analysis of ligand / receptor complex

Subsequently, to analyze the biophysical stability and changes of the VACCINE -TLR5 complex, molecular dynamic simulations were performed via the iMODs server

Table 2. loest energy vaccine docking with some TLRS

RECEPTOR	TLR1	HLA.A 0201	HLA.DR B1.0101	TLR2	TLR3	TLR4	TLR5	TLR7	TLR8
Lowest Energy	-1051.9	-1117.4	-1609.0	-1042.8	-1358.7	-1253.1	-1649.8	-1257.3	-1198.70





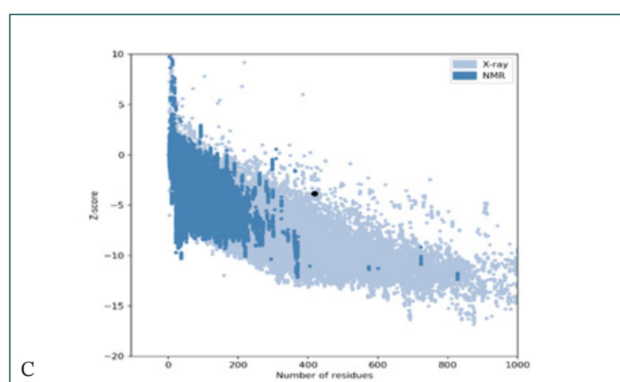


Figure 6. (A) Comparison the original model and the modified model of the vaccine designed by Paymol and calculated RMSD.(B) validation of the third structure of the vaccine designed by the prochek, (C)prosa servers

(32). The resulting iMOD original chain deformation is shown in Figure A. The area where the hinges are located has a strong tendency to deform. The values of factor B calculated by normal state analysis are proportional to the square root of the mean, and factor B shows the fluctuations of the atomic position and measures the unpredictability of each atom, as shown in Figure B. Figure C shows the eigenvalues that are closely correlated with the energy required to smooth the structure, and in general this figure reflects the stability of the dock complex by the eigenvalue shown. The eigenvalue of the VACCINE-TLR5 complex is 1.023391e-05, which indicates less energy required for deformation of the structure and indicates the stability of the complex. Activates immune cascades to kill antigens. The variance is inversely related to the eigenvalue. In Figure D, the individual variance with red and green represents cumulative variance. The covariance map shows the relationship between the pairs of residues, because the colors red, blue, and white indicate the correlated, anti-correlated, and unrelated pairs of residues shown in Figure E. The elastic lattice diagram shows the pair of springs attached to the springs, and each point on the diagram represents a springs spring between the corresponding pair of atoms. In the diagram, the darker gray color indicates the stiffer springs shown in Figure F.

3.12. Immunological simulation

The results provided by the C-ImmSim server were consistent with the actual safety responses, as evidenced by the increased production of secondary responses. The initial response to the vaccine was demonstrated by increasing high IgM levels. In the secondary and tertiary responses, the increase in B cell population as an increase in IgG1 + IgG2, IgM, and IgG + IgM levels is shown in Figures 7A and B. In addition, we found that there was an increase in cytotoxic T cell populations (Figure 7C) and cytotoxic T cell populations with memory cell proliferation (Figure 7D). We also noticed higher levels of cytokines such as IFN-γ, IL-10, and TGF. -B, IL-12 as shown in Figure E 7. These observations show that the designed vaccine elicits promising anti-cancer immunogenic reactions(Fig9).

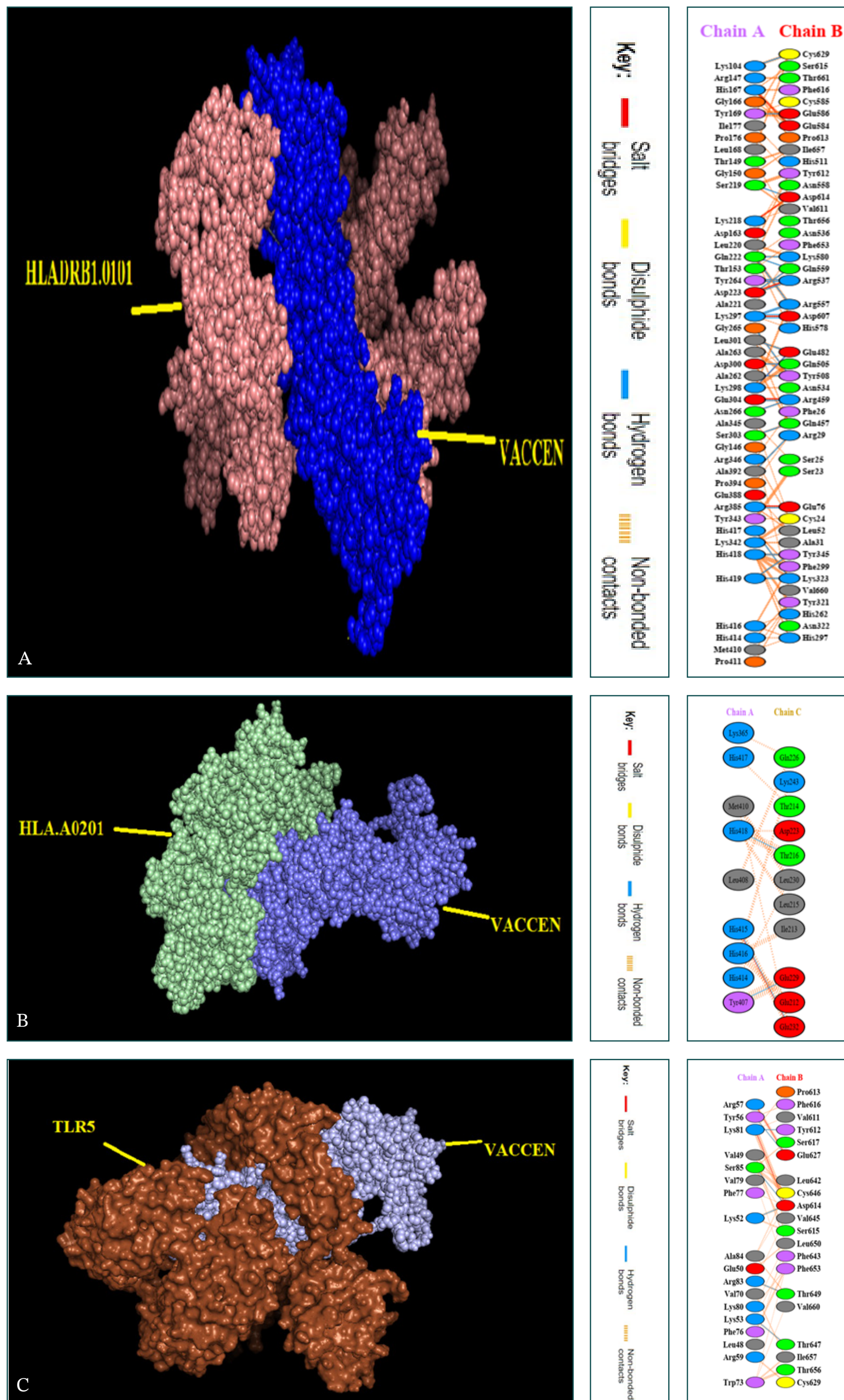


Figure 7. Docking between designed (A),DRB1-0101(B),HLA-A0201(C) vaccine and TLR5 observation of amino acids involved and effective bonds in the binding of vaccine / receptor complexes.

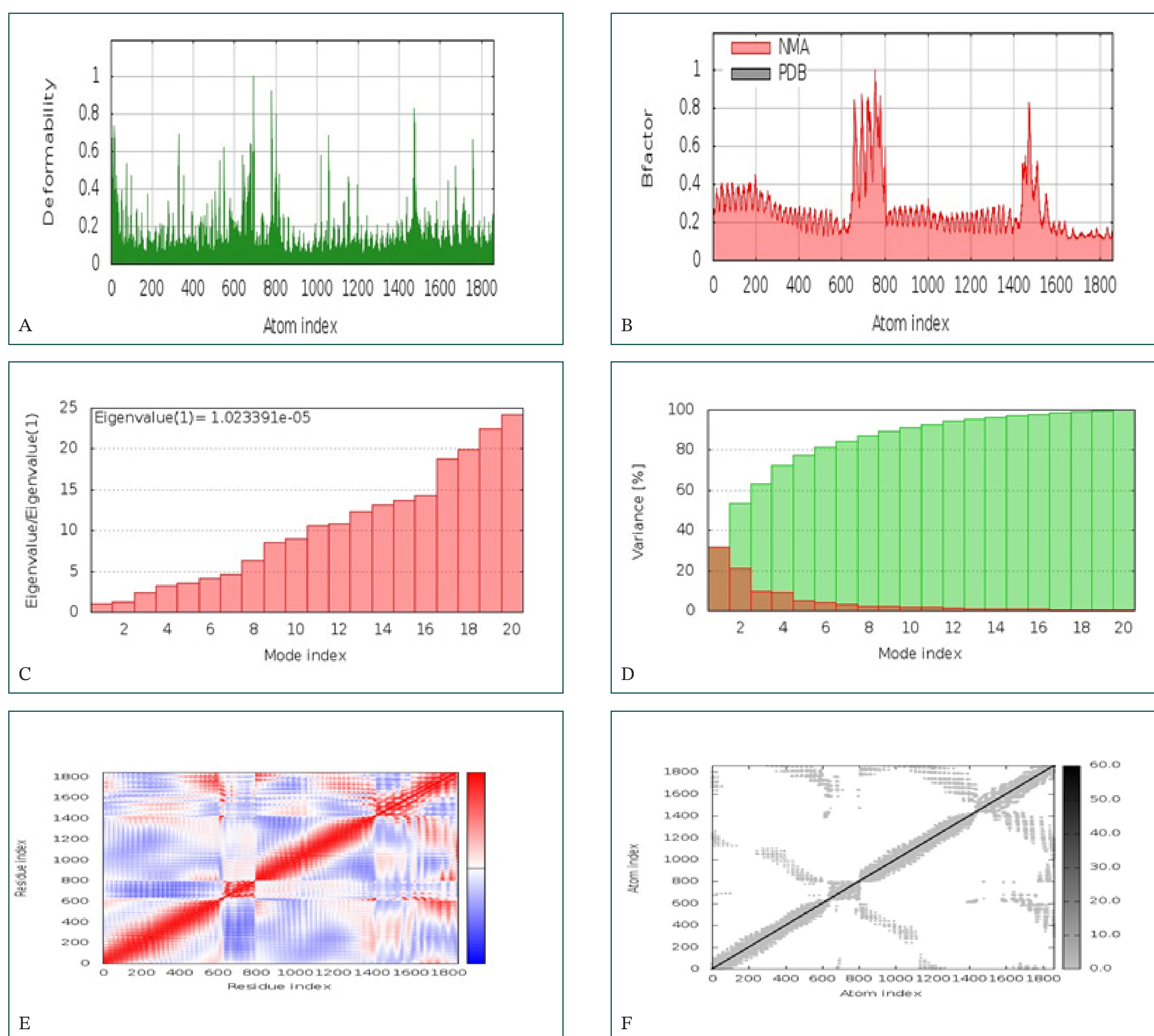


Figure 8. Molecular dynamics simulation of the vaccine-TLR5 complex, showing(A) deformability;(B) B-factor; (C) eigenvalue; (E) Covariance matrix indicates coupling between pairs of residues (red), uncorrelated (white) or anti-correlated (blue) motions and (F) elastic network analysis which defines which pairs of atoms are connected by springs.

We received a 629 bp nucleotide sequence for VACCINE after reverse translation and codon optimization on the JCat server. The CAI values of the optimized sequences were 0.997 and the GC content was 50.734%, indicating good expression of vaccines in E. coli. Then, by creating a recombinant plasmid by inserting the designed vaccine sequence into the pET28a (+) vector after adding the two restriction sites of EcoRI and BamHI restriction enzymes at the N and C terminals of the nucleotide sequence, the vaccine cloning process using software SnapGene was performed. Also, the accuracy of the cloning process was performed with the help of SnapGene software. The gene sequence structure of the vaccine was designed and the vector and recombinant structure of the clone resulting from the vector and the gene sequence of the vaccine were designed after digestion by EcoRI and BamHI through electrophoresis(Fig10).

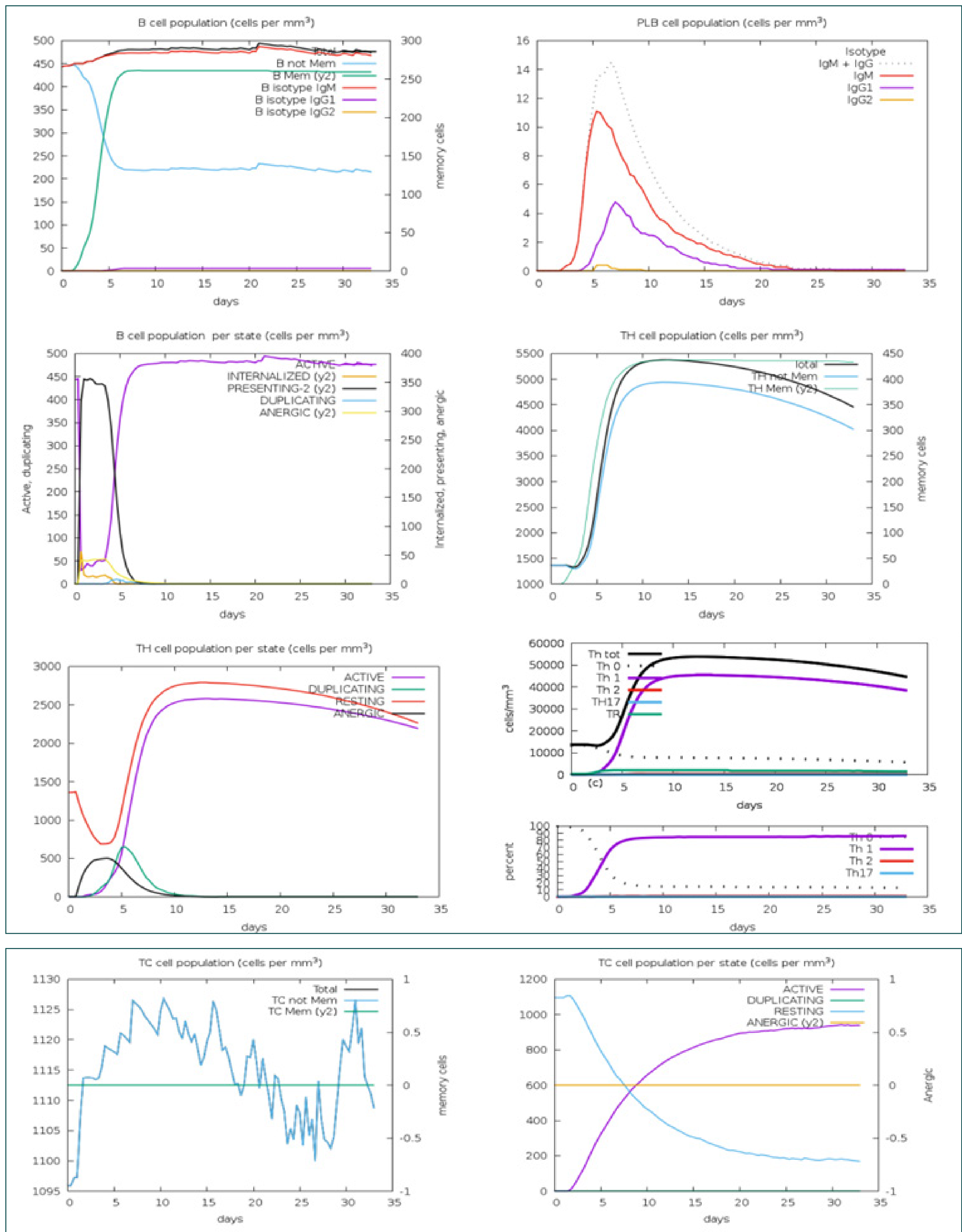

B cell population (cells per mm³)
Total
B not Mem
B Mem (y2)
B isotype IgM
B isotype IgG1
B isotype IgG2
memory cells
days
PLB cell population (cells per mm³)
Isotype
IgM + IgG
IgM
IgG1
IgG2
days
B cell population per state (cells per mm³)
ACTIVE
INTERNALIZED (y2)
PRESENTING-2 (y2)
DUPLICATING
ANERGIC (y2)
Active, duplicating
Internalized, presenting, anergic
days
TH cell population (cells per mm³)
Total
TH not Mem
TH Mem (y2)
memory cells
days
TH cell population per state (cells per mm³)
ACTIVE
DUPLICATING
RESTING
ANERGIC
days
Th tot
Th 0
Th 1
Th 2
TH17
TR
cells/mm³
(c)
days
percent
Th 0
Th 1
Th 2
Th17
days
TC cell population (cells per mm³)
Total
TC not Mem
TC Mem (y2)
memory cells
days
TC cell population per state (cells per mm³)
ACTIVE
DUPLICATING
RESTING
ANERGIC (y2)
Anergic
days

Figure 9. The induced immune cells by the peptide vaccines.(A) B CELL Population and CD4 T-helper lymphocytes count, CD4 T-helper lymphocytes count. (B) T CELL Population and CD8 T-cytotoxic lymphocytes count, CD8 T-cytotoxic lymphocytes count per entity-state,Natural Killer cells (total count),Dendritic cells. (C) B lymphocytes: total count, memory cells, and sub-divided in isotypes IgM, IgG1 and IgG2 (D) Induced array of cytokines during prime boost

4. Discussion

The in-depth insights gained in recent years from clinical studies and molecular mechanisms of cancer have led many researchers and pharmaceutical and biotechnology factories to conduct more extensive research and reviews on the role of the immune system in tumors and the role of immunotherapy in cancer. After a long period of clinical failure in cancer treatment, the idea of immunotherapy emerged as an attractive treatment for cancer. The immune system against tumor antigens is able to

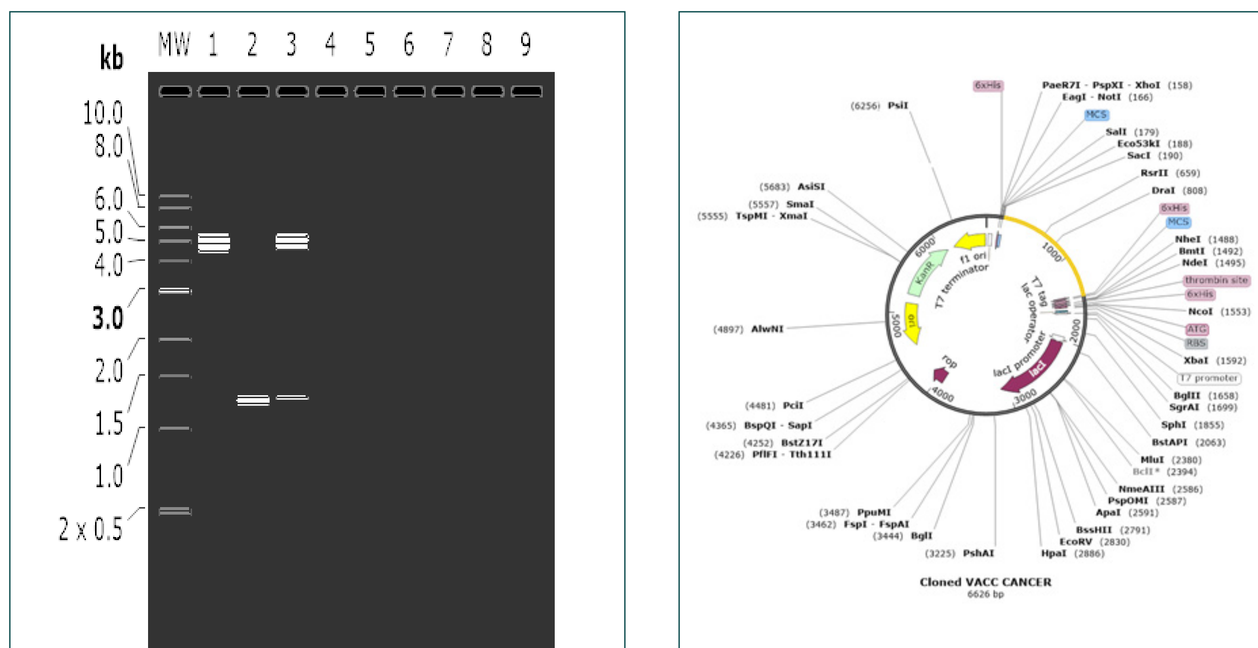


Figure 10. In-silico cloning of the gene sequence of VACCINE into pET28a(+) expression vector. The vaccine region is represented by yelwe color

activate the humoral and cellular immune systems. Antigens that are delivered on the surface of cancer cells are detected by T cells and antibodies, which eventually kill the tumor or reduce the proliferation of cancer cells. However, it is clear that the design of our vaccine as a multi-epitope combination has several advantages, so that it can induce protective cellular and humoral immunity in the long run, provided that 3 doses of the vaccine are administered. Therefore, the design and development of appropriate and safe vaccines for the treatment of various cancers, microbial diseases and parasites seems necessary. In this regard, peptide-based vaccines are one of the attractive methods in this field (18,21). Cancer vaccines are generally able to stimulate immune responses against tumor cells (28). In the field of vaccine design, bioinformatics software is useful for evaluating the important features of the candidate vaccine as well as improving its properties, which have been used in various studies (23). In a study of breast cancer vaccines, Fooladi et al. Designed a chimeric protein containing tumor antigen, enterotoxin B (ROR-1-Enterotoxin B), and a suitable linker against breast cancer using bioinformatics approaches. Based on the results in silico, Fooladi et al. Showed that chimeric protein is able to induce immune responses with acceptable antigen and solubility without any allergenicity, so it can be suggested as a potent vaccine for breast cancer. In another study, the in silico technique was used to design the TG-FαL3-SEB fusion protein, as a new anti-tumor candidate agent that can provide strong immunity against tumors (24). Due to the low immunogenicity of protein vaccines, it is necessary to overcome this barrier. Therefore, various adjuvants such as monophosphoryl lipid A (MPL), aluminum salt, IFA agonists, Montanide, ISA-51 and TLR (LPS) CpG DNA moif (dsRNA, ssRNA and flagellin) have been used in various studies (25). In our study, in order to induce a strong immune response, glycoprotein 96 heat shock protein (HSP GP96) was used as an adjuvant with strong immune properties to defeat the low immunogenicity of the vaccine. GP96 plays an important role in various processes such as antigen delivery, tumor immunity, and innate immune activation. The interaction between Gp96 and TLR4 in APCs leads to the activation of some molecules such as the secretion of proinflammatory cytokines and Th1 (TNF-α, IL-1β and IL-12) (26,27). In the present study, a hybrid struc-

ture consisting of the ECD of HER2 antigen, and the N-terminal region of GP96 (NTGP96), was designed and characterized by various bioinformatics software. Choosing the right linker to integrate different parts of the vaccine is very important, especially in terms of preserving the biological activity of the domains. Based on this, a flexible binder was used to separate the two parts of the chimeric protein. . These types of linkers are flexible and provide the potential for folding T and B cell epitopes independently (28). The physicochemical parameters of our chimeric structure were evaluated by ProtParam (29). Showed that the chimeric structure of our vaccine has a positive charge. The extinction index shows how fast a species or chemical absorbs light at a given wavelength. The extinction coefficient of molecules such as peptides or proteins is related to its amino acid composition, especially the amino acids tryptophan (W), tyrosine (Y) and cysteine (C). The ProtParam Expasy tool calculates the extinction coefficient for the 276, 278, 279, 280 and 282 nm ranges. The results showed that our chimeric protein had a molecular weight of about 85 kDa and an extinction coefficient of 280 nm M-1cm-141745. This value of extinction coefficient can be a sign of high concentration of Cys amino acids and shows that UV spectral analysis methods can be used to evaluate this fusion protein. At the isoelectric point, the net charge of the protein is zero, because the positive and negative charges are equal. The calculated pI index of chimeric protein was 10.20, which showed that this chimeric protein has a play property. This index can be used to develop buffer systems for protein purification by isoelectric concentration method (30). Provides an estimated instability index of protein stability in a test tube. A protein with an instability index less than 40 is predicted to be stable; a value above 40 predicts that the protein may be unstable (29). The chimeric protein instability index was 36.77, indicating that the protein is stable. Aliphatic index indicates that our chimeric protein is stable at a range of temperatures. In general, the aliphatic index indicates how much of a protein is occupied by the aliphatic side chains of valine (V), alanine (A), isoleucine (I), and leucine (L). In addition, it is used to evaluate the thermal conductivity of spherical proteins (22). Due to the low GRAVY index (-0.761), our chimeric protein is classified as a hydrophilic protein and appears to interact better with water. Predicting secondary protein structure (PSSP) plays an important role in the performance and three-dimensional model of protein structure. Analysis of secondary protein structure with GOR IV server showed that they have alpha helix (36.04) and random coil structures (53.94%) compared to others, which may be due to the high content of amino acids proline and glycine. Examination of the second structure of the vaccine structure showed that a major percentage of polyepitopic structures are random coil structures, and since these regions have more flexibility than other secondary structures, the results showed that epitopes are relatively flexible (31). The three-dimensional structure of the protein affects the biological function of the recombinant protein (Ashokan and Pillay 2008; IKA 1980). Therefore, the details of the three-dimensional structure of our protein are useful in this regard. In this study, VACCEN 3D structure modeling was performed by three different servers (SWISS-MODEL, Galaxy web and I-TASSER). All models obtained by ProSA-web servers were validated prochecked to detect possible errors and improve the quality of the projected 3D model. Based on the validation data, the superior model produced by the I-TASSER server was selected as the final model. Validation results showed that the selected model is of high quality and after modification by 3DREFINE server, its quality also increased. As mentioned earlier. In this study, after preparing the receptors and vaccine model designed by MVD software, SPDBV_4.10_PC. DISCOVERY STUDIO Molecular docking analysis between chimeric vaccine protein and TLR5, MHCI, MHCII was performed by CLUSPRO.02 software. . Software 29 provided the best model. Among the models based on hydrophobicity, the electrostatic complement of VACCEN surfaces after their analysis by PyMOL programs, the best model of protein complex, TLR5 / VACCEN, MHCI, MHCII was prepared and illustrated. Analysis of B cell epitopes is one of the requirements for the design of effective vaccines. B cell epitopes were retrieved and selected based

on surface accessibility, hydrophobicity, antigenicity, and other characteristics through BCPreds and IEDB. CTL epitopes are also very important in subunit vaccine design (32). CTLPred and IEDB servers were used to predict CTL epitopes in our structure. One of the important steps in the design and development of a vaccine candidate is the prediction of antigenic epitopes that have a strong affinity for different HLA alleles (33,34). In this regard, effective interaction between epitopes and MHC I molecules and MHCII can stimulate high levels of immune responses. In this study, different HLA alleles, including HLA-A0201 from MHCI complex and HLA-DQB1 * 0101 alleles from MHC II complex were selected. They are important in protecting against tumors (50). In another study, Baloria et al. Developed high-affinity T and B cell epitopes for a candidate vaccine in silicon DNA against breast cancer. They also matched population-scattered HLAs (HLA-HLA.A * 0201, HLA.A * 0301, HLA.B * 0702, DRB1 * 0101) with antigen to form a suitable peptide epitope to cover the majority of breast cancer patients. Provide. According to the obtained computational results, the designed vaccine was suggested as a suitable candidate for strong induction of multitopic responses of B and T cells (35,36). In addition, bioinformatics analysis of the chimeric peptide composition showed that the structure of the designed vaccine is a strong non-allergenic structure. The results also showed that various indices obtained from the vaccine designed such as CAI and GC content to achieve high level protein expression in the E. coli host after optimization had appropriate values for cloning and all these indices mentioned in The above showed that our designed chimeric structure could be well cloned and expressed in E. coli. Finally, based on immunoinformatics evaluations of the above vaccine, it was determined that this chimeric structure is a safe, soluble, stable antigen at different temperatures and hydrophilic. Therefore, this peptide compound could be a new candidate for anti-cancer immunity. However, further confirmation of the efficacy of the above vaccine requires evaluation in vitro and in vivo studies.

Conclusions

This is a new approach to predicting the epitope peptide-based vaccine of some cancer antigenic proteins that targets the spike protein, using safety informatics tools and safety simulation measures. These antigen-anticipated antigens accelerate the production of protective vaccines for patients around the world whose immune systems are compromised. The last second injection of the amplifier is computationally stimulated.This corresponding decrease in antigen level with each simulated vaccination suggests that the immune response may be able to clear rapidly during in vivo testing. Our selected epitopes (cells B and T) will be a suitable vaccine against spike protein. In the future, other stimuli or adjuvants that can facilitate the cells' rapid response to antigens will be considered and evaluated.

Authors' Contribution

The authors contributed equally to this study

Conflict of Interest

The authors declare that they have no conflict of interest.

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