Human Immunology 86 (2025) 111212

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Research article

Developing a multi-epitope vaccine against Helicobacter Pylori

Pedram Asadi Sarabi [a], Elham Rismani [b], Mahshid Shabanpouremam [c], Sara Talehahmad [d,**], Massoud Vosough [a,c,e,*]

[a] Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
[b] Molecular Medicine Department, Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran
[c] Department of Cellular and Molecular Biology, Faculty of Sciences and Advanced Technology in Biology, University of Science and Culture, Tehran, Iran
[d] Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
[e] Experimental Cancer Medicine, Institution for Laboratory Medicine, and Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden

ARTICLE INFO

Keywords:
Helicobacter pylori
Multi-epitope vaccine
Immunoinformatics
Therapeutic immunization

ABSTRACT

Helicobacter pylori, a significant factor in the development of gastric cancer and peptic ulcers, poses challenges for drug development due to its resilience. Computational approaches offer potential solutions for effective vaccine development targeting its antigens while ensuring stability and safety. The four critical antigenic proteins included in this study's innovative vaccine design are neuraminyllactose-binding hemagglutinin (HpaA), catalase (KatA), urease (UreB), and vacuolating toxin (VacA). Advanced immunoinformatics methods identified the possibility of triggering an immunological reaction. An adjuvant (50S ribosomal protein L7/L12) was fused to the vaccine sequence's N-terminus to improve immunogenicity. GROMACS molecular dynamics simulations with the OPLS-AA force field further improved the structure. The vaccine design and human Toll-like receptor 5 (TLR5) demonstrated a strong binding in docking tests. A model of simulating immune response confirmed the vaccine's efficacy and predicted how it would affect the immune system. Using the optimal restriction sites of the pET28b (+) expression vector, the vaccine candidate was cloned *in silico*. To validate the findings, this vaccine design will be synthesized in a bacterial system, and in experimental studies will be conducted in the following phase.

1. Introduction

Helicobacter pylori (*H. pylori*) is considered the primary etiologic factor for a variety of gastric disorders such as chronic gastritis, peptic ulcers, and gastric cancers (GC) [1,2]. Gastric cancer (GC) is a significant public health concern on a global scale. Although its incidence has declined over the previous fifty years, it remains the third most frequent cause of death from cancer. The incidence of GC varies geographically and is linked to several variables, including viral, environmental, and genetic traits. Most cases of GC are associated with *H. pylori* infections [3]. Even though the *H. pylori* infection causes several issues, including increasing antibiotic resistance and intestinal flora abnormalities, it is currently treated using an antibiotic-based triple regimen [4,5]. Hence, the development of vaccines could be potentially effective in the prevention and elimination of *H. pylori* infections. It has been proposed to use different target antigens to generate candidate vaccines [6].

Pathologic aspects of *H. pylori* include motility, adherence molecules, colonization, genotoxicity, and numerous virulence factors [7]. The primary factors that aid in the pathology of *H. pylori* are flagellar proteins and bacterial outer membrane proteins (OMPs). One of the well-defined OMPs of *H. pylori* is neuraminyllactose-binding hemagglutinin (HpaA) [8,9]. HpaA is lipoprotein a on the surface, and potential sialic acid binder [10,11]. Furthermore, the development of GC is supported by the significant impacts of the vacuolating toxin A (VacA) on epithelial cells. VacA is a pore-forming cytotoxin interacting with gastric epithelial cells [12,13]. The presence of the active form of this toxin elevates the risk of cancer. Karami et al. reported a significant

Abbreviations: MD, Molecular dynamics simulation; RMSD, Root Mean Square Deviation; RMSF, Root Mean Square Fluctuation; Rg, Radius of Gyration; SASA, Solvent Accessible Surface Area.

* Corresponding author at: Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
** Corresponding author.
E-mail addresses: s.taleahmad@royan-rc.ac.ir (S. Talehahmad), masvos@royaninstitute.org (M. Vosough).

https://doi.org/10.1016/j.humimm.2024.111212
Received 16 September 2024; Revised 17 November 2024; Accepted 27 November 2024
Available online xxxx

association between the presence of antibodies to the VacA (89 kDa) protein of *H. pylori* and an increased risk of gastric cancer. Specifically, seroreactivity to VacA was significantly higher among gastric cancer patients, with an odds ratio (OR) of 2.7, indicating an elevated cancer risk [14]. Additionally, the virulence factor catalase A (KatA) is essential for shielding *H. pylori* from oxidative stress and preserving an environment conducive to the bacteria's growth [15,16]. Another antigenic target could be urease, composed of two subunits of UreA and UreB, which is a functional protein essential for breaking down urea and neutralizing stomach acidity, and colonize the stomach with *H. pylori*. This process also promotes chemotaxis [17,18]. Recent studies have demonstrated that CD4+ T cells specific to subunit A of *H. pylori* urease (UreA) play a significant role in reducing stomach colonization by *H. pylori*. The adoptive transfer of these UreA-specific CD4+ T cells into naive hosts notably decreased bacterial load, suggesting potential for cellular immune-based strategies in vaccine development. Additionally, these UreA-specific T cells showed a mixed Th1-Th2 response, producing cytokines such as IFN-γ and IL-10, which may contribute to an effective local immune response against the pathogen [19]. UreB induces CD4+ T-cell responses and offers protection against *H. pylori* [20]. Prior research revealed that the B cell and CD4+ T cell epitopes of UreB are commonly employed as antigens [21–25]. In a recent study, Zhang et al. demonstrated that UreB also induces specific CD8 + T cell responses in infected individuals through the cytosolic pathway of cross-presentation [26]. On the other hand, toll-like receptors (TLRs) are essential to recognize pathogens, facilitate host-pathogen interaction, and regulate downstream signals [27,28]. In our study, human Toll-like receptor 5 (TLR5) was considered in the study of interactions for pathogen recognition.

2. Methodology

2.1. Candidate proteins

Using the UniProt database (https://www.uniprot.org), four proteins—HpaA, KatA, UreB, and VacA—were selected for inclusion in the design of a vaccine due to their significant involvement in the pathology of *H. pylori*.

2.2. Prediction of B-cell epitopes

The memory and antibody-producing cells produced by B lymphocytes initiate long-term immune responses against infections. The linear B cell epitope was predicted from the sequence of four proteins using BePipred Linear Epitope Prediction 2.0 algorithm at the Immune Epitope Database (IEDB). The input was the targeted proteins' FASTA sequences with the default parameters chosen [29,30].

2.3. Recognition of MHC I and MHC II epitopes located within B cell epitopes

Using the prediction tool for T-cell epitope (NetMHCpan 4.1 BA, and NetMHCIIpan 4.1 BA), epitopes of the MHC-I and MHC-II classes were chosen from the predicted B-Cell epitopes. Using the T-cell epitope prediction tool on the IEDB website, the target proteins' B cell epitopes were estimated [31]. Due to the ability of these sharing epitopes to induce both humoral and cellular immunity, arrangement of epitopes in the construct holds potential for utilization in vaccination studies.

2.4. The development of the vaccination sequence

Common epitopes of B-cells and T-cells with low allergenicity and high antigenicity are selected for the final profile of candidate vaccine. The selected epitopes were joined with AAY and GPGPG linkers, respectively, to generate a multi-epitope vaccine. In order to enhance the immune response, the sequence of 50S ribosomal protein L7/L12 adjuvant is attached to the N-terminus of the construct through an EAAAK peptide linker.

2.5. Prediction the secondary and tertiary structures of the vaccine

The secondary structure of the vaccine construct was predict using PSIPRED 4.0 server [32]. The three-dimensional (3D) structure of the multi-epitope vaccine was obtained using the Iterative Threading Assembly Refinement (I-TASSER) server (The template library: 2024/04/09) [33]. Based on the modeling's confidence score (C-score), a forecast accuracy estimate is provided. A normal C-score falls between −5 and 2, and a higher number denotes a higher-quality model. Generally, models with a C-score higher than −1.5 have a valid fold.

2.6. Model validation

A critical stage in developing a model is validating the predicted tertiary structure of the amino acid chain. The protein model supplied by I-TASSER must next pass through a few validation servers, including (a) ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) (2024/06/09), which computes an energy plot and a Z score value to represent the target vaccine build model's overall quality score [34]. Using a Ramachandran plot, PROCHECK-server (https://saves.mbi.ucla.edu/) (saves 2024) (b) predicts the stereochemical characteristics of residues [35].

2.7. Assessment of the designed Vaccine's physiochemical properties, Allergenicity, and antigenicity

It is essential to analyze and assess the allergenic properties in order to modify any vaccine. The vaccine model's antigenic propensity was assessed using the VaxiJen v 2.0 server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) and verified using the AntigenPro server (https://scratch.proteomics.ics.uci.edu/) [36,37]. The AllergenFP v 1.0 server (https://ddg-pharmfac.net/AllergenFP/) was used to assess allergenicity in order to do further investigation. The Protein-Sol website (https://protein-sol.manchester.ac.uk/) was used to confirm the designed vaccine's solubility. The ExPASy ProtParam service (https://web.expasy.org/protparam/) was utilized to determine the diverse physicochemical characteristics of the vaccine construct [38].

2.8. Prediction of discontinuous B cell epitopes

The discontinuous B-cell epitopes on the 3D structure of the designed vaccine were evaluated using the Ellipro tool on the IEDB website (http://tools.iedb.org/ellipro/) [39].

2.9. Molecular dynamics simulation

The GROMACS version 2022.6 as utilized to conduct molecular dynamics simulations (MD) on a model protein [40]. The all-atom optimal potentials for liquid simulations (OPLS-AA) force field were used for the simulation. The SPC/E (extended simple point charge) water model was used in a cubic box to solvate the protein. It was possible to ionize and neutralize the system by adding Na + and Cl-ions. The steepest descent algorithm minimizes the system's energy and removes any steric conflicts. The isothermal-isobaric NPT ensemble (constant number of particles, pressure, and temperature) and the NVT ensemble (constant number of particles, volume, and temperature) were run for 500 ps to guarantee simulated equilibration. The simulation was then run at a temperature of 300 K for a period of 100 ns. Analyzing the root mean square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg) and the sec-

ondary structure was performed on the output trajectory. The graphs were visualized using GraphPad Prism v8. 4. 3.

2.10. The analysis of molecular docking and binding affinity

Molecular docking is the most promising *in silico* technique for determining how designed vaccines interact with and bind to TLR5. The human TLR5 crystal structure, obtained from the RCSB protein data bank (PDB ID: 3J0A), was used for molecular docking at the basic interface of the HADDOCK web server (v2.4) [41]. The binding affinity of the Vaccine/TLR5 complex as Gibbs free energy (ΔG) and dissociation constant (Kd) were evaluated by PRODIGY (https://rascar.science.uu.nl/prodigy/).

2.11. Simulating the immune system

The web-based simulation server of C-ImmSim (https://kraken.iac.rm.cnr.it/C-IMMSIM/index.php) was utilized to simulate the humoral and cellular immunological responses of the vaccine design. The pyrogen free candidate vaccine was administered through three injections at 4-week intervals, specifically at 1, 84, and 168 time intervals, using the default simulation parameters [42].

2.12. In-silico cloning after codon optimization

Codon optimization of the vaccine construct was conducted to achieve optimal expression in the *E. coli* K12 strain using the Java Codon Adaptation Tool (JCAT) (https://www.jcat.de/) [43]. The *in silico* cloning of vaccine sequence was performed in the pET28b (+) expression vector using SnapGene software (v7.2.0) [28,44].

3. Results

3.1. Collection of target proteins

Four *H. pylori* proteins were screened in this investigation in order to develop new candidate vaccine. The FASTA format of the proteins were obtained from the UniProt database and were identified as HpaA (UniProt ID: Q48244), katA (UniProt ID: P77872), ureB (UniProt ID: Q9AFB1), and VacA (UniProt ID: P55981).

3.2. B-cell epitopes of target proteins

The B-cell epitopes were predicted from the sequences of four target proteins. For every target protein, IEDB-predicted B-cell epitopes of different lengths with their start position, and sequence.

3.3. MHC-I and -II epitopes of target proteins within the B-cell epitopes

The same number of MHC-I and MHC-II binding epitopes are present in each of the four *H. pylori* target proteins, making common B-cell and T-cell epitopes more useful for developing vaccines. We used the 9mer epitope sequence to identify the MHC class I epitopes. In a similar vein, 15mer epitopic sequences were gathered in order to identify MHC II epitopes. Table S1 and S2 illustrate the selection of MHC-I and MHC-II Epitopes that were used in the vaccine development.

3.4. The vaccine construct was designed

To create vaccine constructions, 15 MHC class I and 16 MHC class II epitopes were screened and connected using an AAY and GPGPG linker, respectively. As an adjuvant, 50S ribosomal protein L7/L12 with NCBI accession number P0A7K2 was attached to the vaccine construct's amino terminus using linker EAAAK (Fig. 1.a). Subsequently, the combined molecular weight of the adjuvant, MHC-I, and MHC-II coupled as the final vaccine design was approximately 64,609.60 kDa, consisting of 621 amino acids.

3.5. The secondary and tertiary structures of the designed vaccine

Analysis of the secondary structure of the vaccine component indicated the number of amino acids involved in creating the alpha helix, beta-sheet, and coil in the vaccine model. The vaccine model had 363 (48.79 %), 62 (10.31 %), and 207 (40.90 %) residues in the coil, beta-sheet, and alpha-helix structures, respectively, as indicated in Table 1. The cartoon structure produced by PSIPRED is displayed in Fig. S1.

The I-TASSER method is a critical step in computational structural biology that allows predicting 3D protein architecture from the sequence. PyMol (v2.6) was used to visualize the vaccine construct's three-dimensional structure. Fig. 1.b shows the expected three-dimensional structure of the vaccine design. The adjuvant, the MHC-I and MHC-II epitopes are shown in orange, green and gray, respectively.

3.6. Quality and structural validation of predicted model

Stereochemical analysis of 3D model in the Ramachandran plot revealed that 87.1 % of amino acid residues are located in the most favored region (Fig. 1c). The ProSA and energy plot are shown in Fig. 1d and e.

3.7. The designed Vaccine's immunogenicity, Allergenicity and physiochemical properties

The antigenic probability score of the candidate vaccine is estimated by the VaxiJen v2.0 and ANTIGENPro servers to be 1.0509 and 0.907464, respectively (Table 2). Both AllerTOP v.2.0 and AllergenFP v.1.0 server determined that the vaccine construct contains a non-allergenic sequence. The vaccine construct showed solubility of 0.675 using the Protein-Sol server. The ProtParam results provide additional information about the physiochemical characteristics of the designed vaccine (Table 2).

3.8. Discontinuous B-cell epitope was predicted

The discontinuous B-cell epitopes were predicted from the tertiary structure of vaccine construct using ElliPro at IEDB. The discontinuous epitope was identified as the epitope with the maximum prediction score of 0.779 out of five predicted epitopes obtained from the ElliPro server. Fig. 2 displays the expected discontinuous epitopes' 3D structure.

3.9. The dynamics of candidate vaccine

The protein model was subjected to MD simulation using the GROMACS package. The RMSD plot showed fluctuations in the initial steps of simulation that reached to the equilibrium state in the range from 0.9 to 1.4 nm. RMSD plot is depicted in Fig. 3a. Furthermore, the average fluctuation of each residue during simulation was calculated as the root mean square fluctuation (RMSF) of the carbon alpha (Cá) atoms. RMSF values ranged from 0.1 to 0.4 nm (Fig. 3b). Folding and compactness of the protein model was evaluated by the radius of gyration (Rg). The Rg plot displayed a decline after the initial 10 ns that could be due to the fluctuations leading to the conformational changes of the unfold regions (Fig. 3c). The secondary structure prediction of the protein model indicated the presence of alpha helices, coil, and beta sheet. DSSP analysis after simulation showed that approximately 280 residues of protein model were contributed in A-helix, B-sheet, B-bridge, and turn and more than 179 residues displayed the coil structure (Fig. 3d).

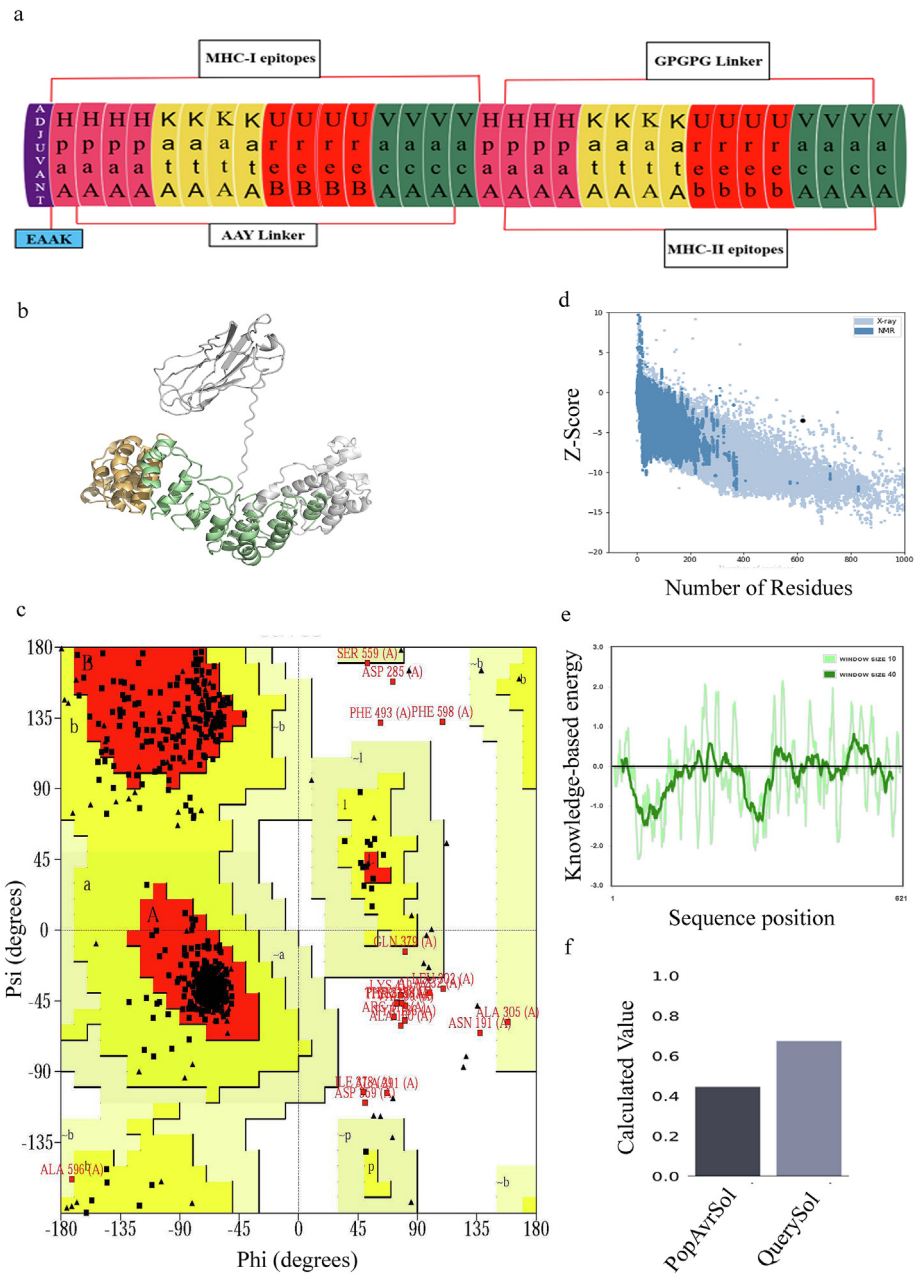

Fig. 1. Overall view of the vaccine construct and 3D structure validations. **a**. Schematic diagram of final multi epitopic peptide vaccine construct. **b**. The 3D structure of the vaccine candidate: (adjuvant in orange, MHCI fragments in green, and MHCII fragments in gray). **c**. Ramachandran plot. **d**. ProSA validation of predicted structure by Z score. **e**. ProSA energy plot for structure validation. **f.** The solubility plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Analysis of the secondary structure of vaccine construct.

Amino acid count, the secondary structure features	Alpha helix (%)	Beta sheet (%)	Coil shape (%)
Designed vaccine (621 aa)	254 aa, 40.90 %	64 aa, 10.31 %	48.79 aa, or 303

3.10. Analysis of molecular dockin

The top cluster of HADDOCK results includes the complex that contains the proper interaction of the ligand (vaccine construct) with receptor (TLR5). The profile energy of top cluster of HADDOCK including electrostatic, desolvation, and Van der Waals are summarized in the Table 3. The binding affinity of the Vaccine/TLR5 complex as Gibbs free energy (ΔG) and dissociation constant (Kd), which were − 11.5 kcal mol − 1 and 3.4e-09 M, respectively, were evaluated by PRODIGY (Table 3). Molecular docking of the human TLR5 with the vaccine construct is depicted in Fig. 4a. Additionally, a zoom-in view of the TLR5 residues is shown in black italics, and the vaccine candidate is shown in red in Fig. 4b. The residues involved in the hydrogen bonds of the two protein molecules are listed in Table S3.

3.11. Simulation of immunity

The immunological simulation performed on the C-ImmSim server yielded significant results. A prominent primary immune response is

Table 2
Characterization of the multi-epitope-based vaccine construct.

Instability index	Antigenicity		Allergenicity		Solubility	Grand average of hydropathicity (GRAVY)
	VaxiJen	ANTIGENPro	AllerTOP	AllergenFP		
25.76	1.0509 (Probable ANTIGEN)	0.907464 (Probable ANTIGEN)	Probable non-allergen		0.675 (Soluble)	− 0.637

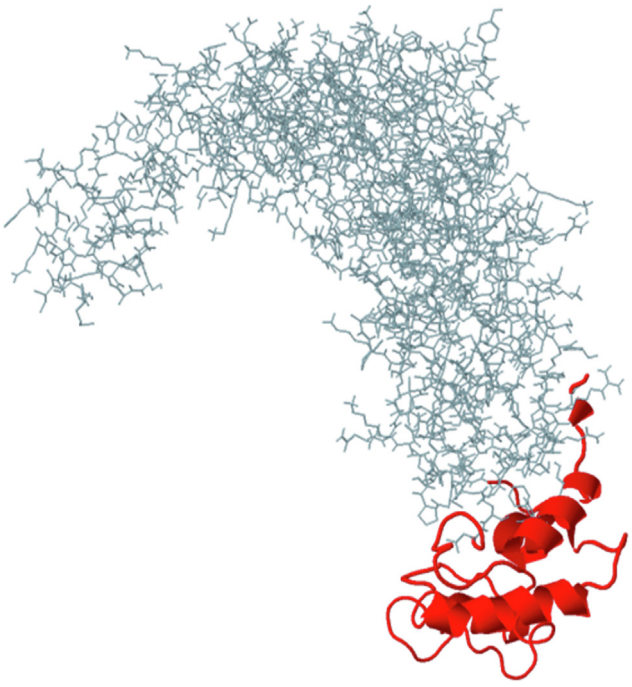

Fig. 2. Discontinuous B-cell epitopes in the 3D model of multi-epitope vaccine.

detected which indicates high levels of IgM (Fig.S2). Additionally, rising expression of immunoglobulins such as IgG1 + IgG2, IgM, and IgG + IgM was noted along with an increase in the population of B-cells that results in decreasing the concentration of antigen. Interestingly, there was a discernible increase in TH (helper) and TC (cytotoxic) cells during the memory expansion phase. Furthermore, it was found that there was an increase in IFN-γ production after immunization. The T cell population grew more accessible as the memory response developed, while the populations of the other immune cells were stable. A comprehensive analysis of the immunological simulation over an extended period (~ one year) revealed that three doses of the candidate vaccine could develop long-lasting immunity (Fig.S3).

3.12. In silico cloning

The protein sequence of the designed vaccine was codon optimized in *E. coli* (K12 strain) using the Java Codon Adaptation (JCat) program. Two filter parameters were selected in the program to avoid the prokaryotic ribosome binding sites and rho-independent transcription terminators. The GC content and the CAI value of the optimized vaccine is 52.7106 and 1.0, where an ideal range of GC content is from 30 to 70 % and from 0.8 to 1.0 for the CAI value. The binding site for two specific restricted endonucleases (*Xho*I and *Xba*I) was incorporated at the ends of vaccine sequence. Finally, using SnapGene software, the vaccine design was cloned into the pET28b (+) vector (Fig. S4). The clone's size was 7.053 kb.

4. Discussion

The complexity of the immune response of the host to *H. pylori* and the genetic variability of the bacterium present challenges for vaccine development. One approach to advance vaccine development is identification of effective antigen combinations [45,46]. Due to recent research, “subunit vaccines” have been developed that efficiently stimulate the immune system. These vaccines are made up of specific protein sequences from harmful bacteria. One of this vaccine's main advantages is its ability to elicit specific immune responses against particular epitopes, and improving the binding affinity with target receptor molecules [28,47]. The current research focuses on the developing a multi-epitope vaccine against *H. pylori* by utilizing epitopes from antigenic proteins including HpaA, KatA, UreB, and VacA.

The MHC-I and -II epitopes that are also recognized within the B-cell epitopes were arranged in a construct to generate a multi-epitope vaccine. The epitopes were selected based on their VaxiJen scores, which were all greater than 0.8, indicating a high probability of antigenicity. The selection process of epitopes is crucial as it ensures that the chosen epitopes are likely to elicit a strong immune response. The inclusion of both MHC-I and MHC-II epitopes that are recognized within the B-cell epitopes allows for the activation of CD8+ cytotoxic T cells and CD4+ helper T cells, as well as the stimulation of antibody production against H.*pylori*, which are essential for generating a comprehensive immune response. Furthermore, the strategic choice of linkers, specifically AAY and GPGPG, was made to facilitate proper folding and presentation of these epitopes, enhancing their immunogenic potential.

Multiple studies have demonstrated that the production of subunit vaccines made of specific pathogenic protein sequences from bacteria has been made more accessible by the availability of extensive knowledge on the genomes and proteomes of microorganisms [48]. In order to identify potential vaccine candidates, researchers can utilize epitope databases that store experimentally known T-cell epitopes [49]. By employing immunoinformatics, it is possible to select epitopes that provide optimal protection to the entire population. However, the prediction of B-cell epitopes is currently less advanced in comparison [50].

Gosh et al. described using four pathogenic proteins (HpaA, FlaA, FlaB, and Omp18) in multi-epitope vaccine candidates against *H. pylori* [51]. They conclude that their vaccine construct could be a promising candidate against *H. pylori* infection, but further *in-vitro* and *in-vivo* validation is required. Furthermore, Ma et al. (2021) reported using key virulence factors of *H. pylori* (UreB, PLA1, and Omp6) to trigger robust B and T cell responses [52]. These studies also employed stability assessments using molecular dynamics simulations to improve structural integrity. However, while Ma et al.'s construct targets TLR2, TLR4, and TLR9, the focus in Gosh et al. and our study was on TLR5 that may better engage immune pathways specific to *H. pylori*. Overall, this shared emphasis on computational design, and planned experimental validations advances vaccine development for more targeted immune engagement.

The findings of our study indicate that vaccinations might improve humoral and cellular immunity. Following three repeated exposures to the antigen, a general rise in immune responses was observed. It was

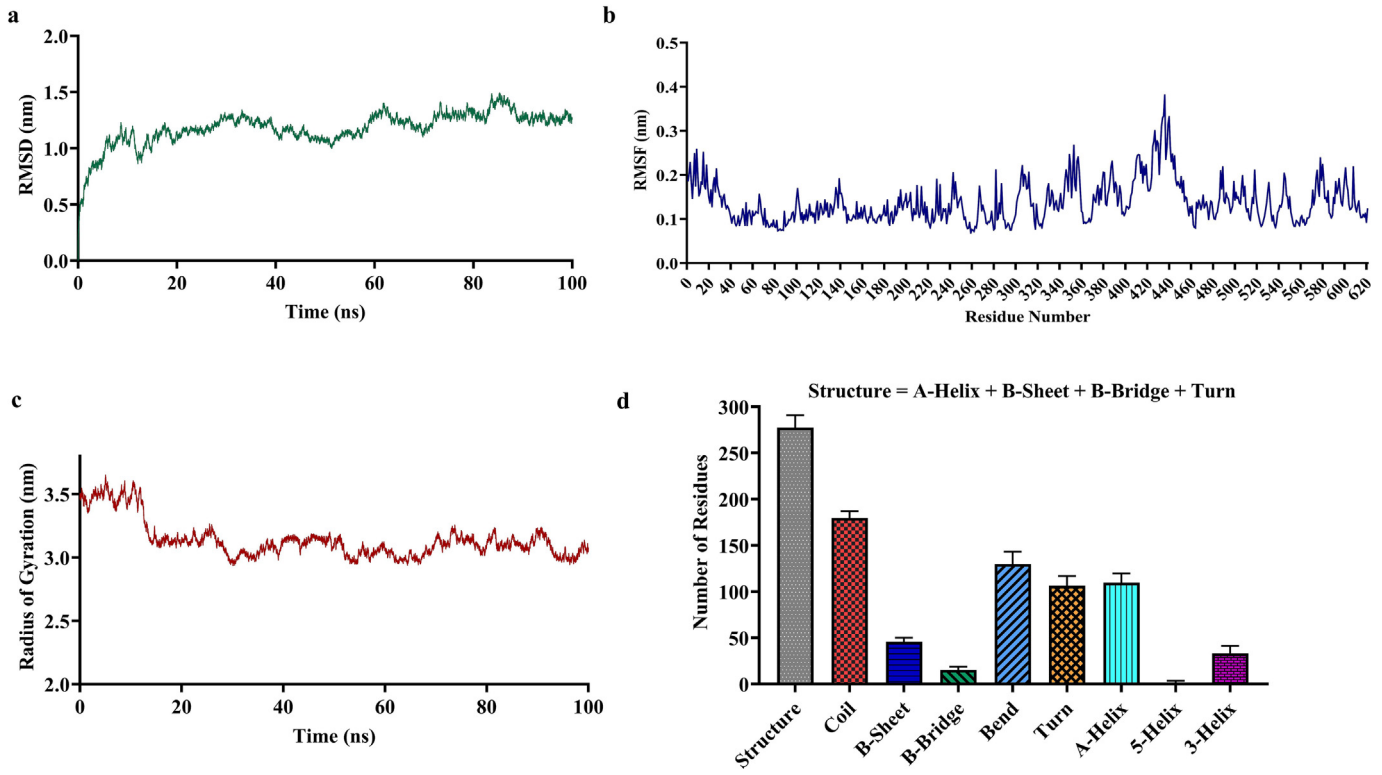

Fig. 3. Molecular dynamics simulation of the designed vaccine: **a**. RMSD plot (backbone fluctuations); **b**. RMSF plot (Cα fluctuations); **c**. Radius of gyration plot; and **c**. The secondary structure analysis by DSSP.

Table 3
Molecular docking analysis of Vaccine construct-TLR5 complex.*

Vaccine construct-TLR5 complex	Cluster 1
HADDOCK score	−111.5 +/− 6.3
Cluster size	86
RMSD (from the overall lowest-energy structure)	1.5 +/− 0.7
Van der Waals energy	−60.5 +/− 3.3*
Electrostatic energy	−174.0 +/− 12.2*
Desolvation energy	−32.0 +/− 6.1*
PRODIGY binding score	ΔG: − 11.5* Kd: 3.4e-09 M

* Energy (kcal mol − 1).

particularly evident that there was a growth in memory B-cells and T-cells, with the memory of B-cells persisting for several months. Important immunological response markers, IFN-γ and IL-2, increased following the first injection and continued to rise even after additional antigen injections. These findings are consistent with the immune responses documented by Lucas et al., which demonstrated that CD4 + T cells specific for urease subunit A (UreA) significantly reduced *H. pylori* colonization in mouse models through mechanisms involving the production of protective cytokines, including IFN-γ and IL-10. The activation of these T cells was shown to be crucial for mediating protection against *H. pylori*, indicating that similar CD4+ T cell responses may play a role in our observed immune profile [19].

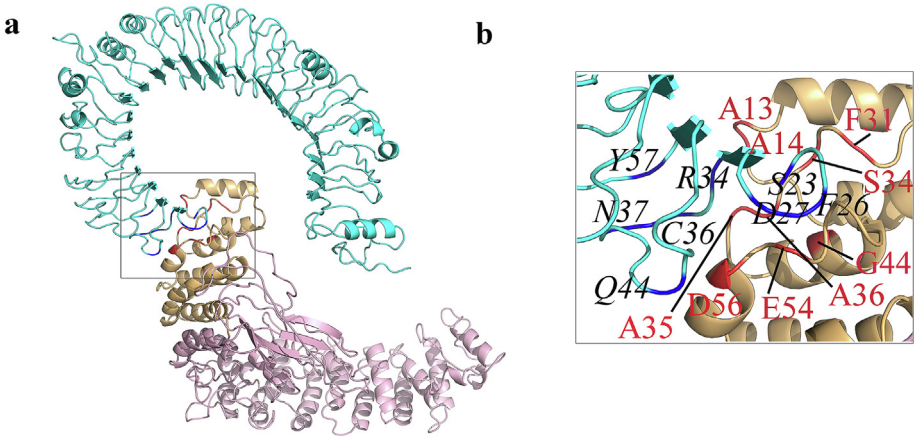

Fig. 4. The interaction pattern of the designed vaccine with human TLR5: **a.** TLR5 in cyan, adjuvant in orange. **b**. Zoom-in view of the interaction including residues of TLR5 in black italic and vaccine candidate in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The presence of a substantial count of bloodstream's TH cells in our findings supports an effective humoral response, suggesting that the mechanisms we observed may similarly contribute to protection against *H. pylori in vivo*. Specifically, studies indicate that a Th2-type response, characterized by IL-4 production, is correlated with successful elimination of *H. pylori* following immunization [19]. Liu et al. also emphasized the importance of Th1 and Th2 responses in their model, where a balanced cytokine profile was associated with effective bacterial clearance [53]. Our findings indicate a strong activation of TH cells, which not only supports humoral immunity but also suggests a potential for a mixed Th1/Th2 response that may enhance protection against diverse *H. pylori* strains. The presence of significant levels of memory B-cells in our results may provide an additional layer of long-term immunity, which is crucial for sustained protection. Furthermore, the analysis of clonal specificity through Simpson index D also indicates a heterogeneous immune response characterized by heterogeneity that may enhance the adaptability of the immune system to effectively combat *H. pylori* infections, as seen in successful vaccination strategies in animal models. In our study, the 50S ribosomal protein L7/L12 was utilized as an adjuvant to stimulate a more robust activation of T helper (Th) cells and promote a balanced Th1/Th2 response [54]. Indeed, we aim to not only boost the immunogenicity of our vaccine but also ensure a more durable and adaptable immune response, which is crucial for effective long-term protection against *H. pylori* infections.

Finally, the SnapGene tool was used to clone the vaccine sequence into the pET28b (+) expression vector. The subsequent step would be synthesizing this vaccine construct within a bacterial expression system and performing a set of immunological studies to validate the outcomes of the immunoinformatics analysis. Future work will focus on *in vivo* efficacy testing of the synthesized vaccine in appropriate animal models, particularly BALB/c mice, and characterization of immune responses elicited by our vaccine, specifically focusing on T-cell proliferation, cytokine production, and antibody responses. In addition, conducting longitudinal studies to monitor the duration of immunity conferred by the vaccine, including memory B-cell persistence and T-cell memory formation over time could be beneficial.

5. Conclusion

In summary, a reverse vaccinology strategy was employed to design and develop a multi-epitope vaccine that may cause humans to experience both cellular and humoral immune responses. The assessment of vaccine candidate revealed a high level of antigenicity and non-allergenic properties. The vaccine construct showed effective binding affinity with human TLR5 receptor. Our study identified an immunogenic response through in-silico approach. However, further experimental studies are needed to verify the effectiveness, immunogenicity, and safety of the vaccine candidate before it can be administered to humans.

Author contribution

PAS and ER performed literature review, analysis, and drafted the manuscript. MS prepared figs. MV and ST developed the idea, reviewed and approved the final manuscript.

Funding information

This work was supported by no funding.

Ethics statement

N/A.

CRediT authorship contribution statement

Pedram Asadi Sarabi: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Elham Rismani:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Mahshid Shabanpouremam:** Writing – original draft, Investigation. **Sara Talehahmad:** Writing – review & editing, Validation, Conceptualization. **Massoud Vosough:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Data availability statement

The data that support the findings of this study are available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors would like to express their sincere gratitude to their colleagues in Regenerative Medicine department in Royan Institute.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2024.111212.

References

[1] T. Rokkas, J.P. Gisbert, Y. Niv, O'morain C: the association between Helicobacter pylori infection and inflammatory bowel disease based on meta-analysis, United European Gastroenterol J 3 (2015) 539.
[2] K. Sugano, Effect of Helicobacter pylori eradication on the incidence of gastric cancer: a systematic review and meta-analysis, Gastric Cancer 22 (2019) 435.
[3] C.D. Iwu, C.J. Iwu-Jaja, Gastric Cancer epidemiology: current trend and future direction, Hygiene 3 (2023) 256.
[4] S. Suzuki, M. Esaki, C. Kusano, H. Ikehara, T. Gotoda, Development of Helicobacter pylori treatment: how do we manage antimicrobial resistance?, World J Gastroenterol. 25 (2019) 1907.
[5] Y.-H. Wang, Huang Y: effect of lactobacillus acidophilus and Bifidobacterium bifidum supplementation to standard triple therapy on Helicobacter pylori eradication and dynamic changes in intestinal flora, World J. Microbiol. Biotechnol. 30 (2014) 847.
[6] M. Keikha, M. Eslami, B. Yousefi, A. Ghasemian, M. Karbalaei, Potential antigen candidates for subunit vaccine development against Helicobacter pylori infection, J. Cell. Physiol. 234 (2019) 21460.
[7] M. Koeppel, F. Garcia-Alcalde, F. Glowinski, P. Schlaermann, T.F. Meyer, Helicobacter pylori infection causes characteristic DNA damage patterns in human cells, Cell Rep. 11 (2015) 1703.
[8] A.M. Lundström, K. Blom, V. Sundaeus, I. Bölin, HpaA shows variable surface localization but the gene expression is similar in different Helicobacter pylori strains, Microb. Pathog. 31 (2001) 243.
[9] P. Voland, N. Hafsi, M. Zeitner, S. Laforsch, H. Wagner, C. Prinz, Antigenic properties of HpaA and Omp18, two outer membrane proteins of Helicobacter pylori, Infect. Immun. 71 (2003) 3837.
[10] K. Blom, B.S. Lundin, I. Bölin, A.-M. Svennerholm, Flow cytometric analysis of the localization of Helicobacter pylori antigens during different growth phases, FEMS Immunology & Medical Microbiology 30 (2001) 173.
[11] D.G. Evans, D.J. Evans Jr., J.J. Moulds, D.Y. Graham, N-acetylneuraminyllactose-binding fibrillar hemagglutinin of campylobacter pylori: a putative colonization factor antigen, Infect. Immun. 56 (1988) 2896.
[12] H. Tsugawa, H. Suzuki, H. Saya, M. Hatakeyama, T. Hirayama, K. Hirata, et al, Reactive oxygen species-induced autophagic degradation of Helicobacter pylori CagA is specifically suppressed in cancer stem-like cells, Cell Host Microbe 12 (2012) 764.
[13] M.R. Terebiznik, D. Raju, C.L. Vázquez, K. Torbricki, R. Kulkarni, S.R. Blanke, et al, Effect of Helicobacter pylori's vacuolating cytotoxin on the autophagy pathway in gastric epithelial cells, Autophagy 5 (2009) 370.
[14] N. Karami, Y. Talebkhan, S. Saberi, M. Esmaeili, A. Oghalaie, A. Abdirad, et al, Seroreactivity to Helicobacter pylori antigens as a risk indicator of gastric cancer, Asian Pac. J. Cancer Prev. 14 (2013) 1813.

[15] S.L. Hazell, D.J. Evans Jr., D.Y. Graham, Helicobacter pylori catalase, Microbiology 137 (1991) 57.
[16] A.A. O'Riordan, V.A. Morales, L. Mulligan, N. Faheem, H.J. Windle, D.P. Kelleher, Alkyl hydroperoxide reductase: a candidate Helicobacter pylori vaccine, Vaccine 30 (2012) 3876.
[17] J.Y. Huang, E.G. Sweeney, M. Sigal, H.C. Zhang, S.J. Remington, M.A. Cantrell, et al, Chemodetection and destruction of host urea allows Helicobacter pylori to locate the epithelium, Cell Host Microbe 18 (2015) 147.
[18] S. Ansari, Y. Yamaoka, Survival of Helicobacter pylori in gastric acidic territory, Helicobacter 22 (2017) 12.
[19] B. Lucas, D. Bumann, A. Walduck, J. Koesling, L. Develioglu, T.F. Meyer, et al, Adoptive transfer of CD4+ T cells specific for subunit a of Helicobacter pylori urease reduces H. Pylori stomach colonization in mice in the absence of interleukin-4 (IL-4)/IL-13 receptor signaling, Infect. Immun. 69 (2001) 1714.
[20] B. Li, L. Chen, H. Sun, W. Yang, J. Hu, Y. He, et al, Immunodominant epitope-specific Th1 but not Th17 responses mediate protection against Helicobacter pylori infection following UreB vaccination of BALB/c mice, Sci. Rep. 5 (2015) 14793.
[21] K. Hirota, K. Nagata, Y. Norose, S. Futagami, Y. Nakagawa, H. Senpuku, et al, Identification of an antigenic epitope in Helicobacter pylori urease that induces neutralizing antibody production, Infect. Immun. 69 (2001) 6597.
[22] H.-X. Li, X.-H. Mao, Y. Shi, Y. Ma, Y.-N. Wu, W.-J. Zhang, et al, Screening and identification of a novel B-cell neutralizing epitope from Helicobacter pylori UreB, Vaccine 26 (2008) 6945.
[23] Y. Qiu, Y.-C. Wang, H.-X. Tao, D.-W. Zhan, S.-L. Yuan, P. Wang, et al, Identification of B-cell epitopes in urease B subunit of Helicobacter pylori bound by neutralizing antibodies, Vaccine 28 (2010) 5220.
[24] Y. Shi, C. Wu, W.-Y. Zhou, X.-H. Mao, G. Guo, Q.-M. Zou, Identification of H-2d restricted Th epitopes in urease B subunit of Helicobacter pylori, Vaccine 25 (2007) 2583.
[25] W.-C. Yang, L. Chen, H.-B. Li, B. Li, J. Hu, J.-Y. Zhang, et al, Identification of two novel immunodominant UreB CD4+ T cell epitopes in Helicobacter pylori infected subjects, Vaccine 31 (2013) 1204.
[26] Z. Zhang, X. Chen, B. Li, T. Xia, X. Wu, C. Wu, Helicobacter pylori induces urease subunit B-specific CD8(+) T cell responses in infected individuals via cytosolic pathway of cross-presentation, Helicobacter 28 (2023) e13005.
[27] M.G. Netea, C. Van der Graaf, J.W. Van der Meer, B.J. Kullberg, Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system, Journal of Leucocyte Biology 75 (2004) 749.
[28] M. Bhattacharya, A.R. Sharma, B. Mallick, G. Sharma, S.-S. Lee, C. Chakraborty, Immunoinformatics approach to understand molecular interaction between multi-epitopic regions of SARS-CoV-2 spike-protein with TLR4/MD-2 complex, Infect. Genet. Evol. 85 (2020) 104.
[29] M.C. Jespersen, B. Peters, M. Nielsen, P. Marcatili, BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes, Nucleic Acids Res. 45 (2017) W24.
[30] Y. Kim, J. Ponomarenko, Z. Zhu, D. Tamang, P. Wang, J. Greenbaum, et al, Immune epitope database analysis resource, Nucleic Acids Res. 40 (2012) W525.
[31] P. Patra, M. Bhattacharya, A.R. Sharma, P. Ghosh, G. Sharma, B.C. Patra, et al, Identification and design of a next-generation multi epitopes bases peptide vaccine candidate against prostate cancer: an in silico approach, Cell Biochem. Biophys. 78 (2020) 495.
[32] D.W. Buchan, D.T. Jones, The PSIPRED protein analysis workbench: 20 years on, Nucleic Acids Res. 47 (2019) W402.
[33] Y. Zhang, I-TASSER server for protein 3D structure prediction, BMC bioinformatics 9 (2008) 1.
[34] M. Wiederstein, M.J. Sippl, ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins, Nucleic Acids Res. 35 (2007) W407.
[35] R.A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, PROCHECK: a program to check the stereochemical quality of protein structures, J. Appl. Crystallogr. 26 (1993) 283.
[36] I.A. Doytchinova, D.R. Flower, VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines, BMC bioinformatics 8 (2007) 1.
[37] C.N. Magnan, M. Zeller, M.A. Kayala, A. Vigil, A. Randall, P.L. Felgner, et al, High-throughput prediction of protein antigenicity using protein microarray data, Bioinformatics 26 (2010) 2936.
[38] E. Gasteiger, C. Hoogland, A. Gattiker, Duvaud Se, M.R. Wilkins, R.D. Appel, et al, Protein Identification and Analysis Tools on the ExPASy Server, Springer, 2005.
[39] J. Ponomarenko, H.-H. Bui, W. Li, N. Fusseder, P.E. Bourne, A. Sette, et al, ElliPro: a new structure-based tool for the prediction of antibody epitopes, BMC Bioinformatics 9 (2008) 1.
[40] M.J. Abraham, T. Murtola, R. Schulz, S. Páll, J.C. Smith, B. Hess, et al, GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers, SoftwareX 1-2 (2015) 19.
[41] S.J. de Vries, A.M. Bonvin, CPORT: a consensus interface predictor and its performance in prediction-driven docking with HADDOCK, PLoS One 6 (2011) 176.
[42] K.A. Peele, C.P. Durthi, T. Srihansa, S. Krupanidhi, V.S. Ayyagari, D.J. Babu, et al, Molecular docking and dynamic simulations for antiviral compounds against SARS-CoV-2: a computational study, Informatics in medicine unlocked 19 (2020) 100345.
[43] A. Grote, K. Hiller, M. Scheer, R. Münch, B. Nörtemann, D.C. Hempel, et al, JCat: a novel tool to adapt codon usage of a target gene to its potential expression host, Nucleic Acids Res. 33 (2005) W526.
[44] LLC GB, GSL Biotech LLC, 2015.
[45] D. Stubljar, T. Jukic, A. Ihan, How far are we from vaccination against Helicobacter pylori infection?, Expert Rev Vaccines 17 (2018) 935.
[46] P. Sutton, J.M. Boag, Status of vaccine research and development for Helicobacter pylori, Vaccine 37 (2019) 7295.
[47] F.E. Terry, L. Moise, R.F. Martin, M. Torres, N. Pilotte, S.A. Williams, et al, Time for T? Immunoinformatics addresses vaccine design for neglected tropical and emerging infectious diseases, Expert Rev. Vaccines 14 (2015) 21.
[48] I. Jęśkowiak, B. Wiatrak, P. Grosman-Dziewiszek, A. Szeląg, The incidence and severity of post-vaccination reactions after vaccination against COVID-19, Vaccines 9 (2021) 502.
[49] F. Rampoldi, L. Ullrich, I. Prinz, Revisiting the interaction of γδ T-cells and B-cells, Cells 9 (2020) 743.
[50] M. Yu, Y. Zhu, Y. Li, Z. Chen, T. Sha, Z. Li, et al, Design of a novel multi-epitope vaccine against Echinococcus granulosus in immunoinformatics, Front. Immunol. 12 (2021) 668492.
[51] P. Ghosh, S. Bhakta, M. Bhattacharya, A.R. Sharma, G. Sharma, S.-S. Lee, et al, A novel multi-epitopic peptide vaccine candidate against Helicobacter pylori: in-silico identification, design, cloning and validation through molecular dynamics, Int. J. Pept. Res. Ther. 27 (2021) 1149.
[52] J. Ma, J. Qiu, S. Wang, Q. Ji, D. Xu, H. Wang, et al, A novel design of multi-epitope vaccine against Helicobacter pylori by immunoinformatics approach, Int. J. Pept. Res. Ther. 27 (2021) 1027.
[53] M. Liu, Y. Zhong, J. Chen, Y. Liu, C. Tang, X. Wang, et al, Oral immunization of mice with a multivalent therapeutic subunit vaccine protects against Helicobacter pylori infection, Vaccine 38 (2020) 3031.
[54] S.J. Lee, S.J. Shin, M.H. Lee, M.-G. Lee, T.H. Kang, W.S. Park, et al, A potential protein adjuvant derived from mycobacterium tuberculosis Rv0652 enhances dendritic cells-based tumor immunotherapy, PLoS One 9 (2014) e104351.