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Design of a novel and potent multivalent epitope based human cytomegalovirus peptide vaccine: An immunoinformatics approach

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ABSTRACT

Human Cytomegalovirus (HCMV) is one of the most common causes of congenital infections globally. They infect the fetus and cause severe birth defects such as hearing loss, complications in the eyes, epilepsy, autism, and various other neurological disorders. They are also the leading cause of death in patients with compromised immunity. Due to the prevalence and severity of the virus, the United States National Academy of Medicine has announced the development of the HCMV vaccine as a matter of high priority for public health. Hence, this study is focused on designing a novel potent multi-epitope HCMV vaccine. The envelope glycoproteins B, H, L and M of HCMV were targeted to predict T cell (both Helper and Cytotoxic) epitopes that were non-toxic, highly antigenic, and non-allergenic and could induce interferon-γ production. Further, the selected epitopes were subjected to molecular docking and population coverage analysis. Eventually, using the two best epitopes and adjuvants like RS09, flagellin protein and PADRE sequence a 283 amino acid long vaccine was designed. The vaccine is antigenic, non-allergenic, stable, and soluble. The 3D model of the vaccine was developed and validated using the Ramachandran plot. Docking with Toll-like receptor 5 (TLR5) molecule and molecular dynamics simulation confirmed the stability of the vaccine. *In silico* cloning simulation using pET28a (+) vector showed that the vaccine can be successfully cloned and expressed in *E. coli* K12 strain. However, the efficacy and the safety of the vaccine need to be further validated by *in vivo* studies.

1. Introduction

One of the other major concerns of public health is Human Cytomegalovirus (HCMV) infections. HCMV is one of the most common causes of congenital infections [22]. In developed countries, there are about 5–7 cases of congenital cytomegalovirus infections in every 1,000 live births whereas in underdeveloped and poor parts of the world there are 10–30 cases per 1,000 live births [20]. The rate of HCMV infections in infants is higher than the infection caused by Zika virus [18,36]. They infect the fetus and cause severe birth defects such as hearing loss, complications in eye, epilepsy, autism, and various other neurological disorders [39,64]. They can also cause jaundice and swelling of liver and spleen in infants [56]. Apart from infants they also infect immunocompromised patients suffering from AIDS, and organ and bone-marrow transplant recipients [22]. About 40% patients suffering from AIDS also suffer from HCMV infections and these infections help in AIDS progression eventually leading to death [14]. Another problem is the resistance to antiviral drugs in the cases of HCMV infections. Resistance to ganciclovir, cidofovir and foscarnet in HCMV infections has been reported [17]. The use of hyperimmune globulin transfusion method for the treatment of HCMV infection has been reported to be ineffective [18]. So, it is imperative to look for novel avenues to combat these infections. One such avenue can be the design and development of vaccines against HCMV. Previously, vaccines for HCMV have been developed but in clinical trials, they have shown only modest efficacy [9,18]. One advanced strategy to develop vaccines is to design multi-epitope vaccines. This strategy has already been used to design vaccines against Human Papillomavirus, *Candida auris*, Dengue virus, SARS-CoV-2, and Type-1 and Type-2 Herpes Simplex Virus *in silico* [3,4,27,32,38,63]. But so far no study has been reported regarding the *in silico* design of vaccine against Human Cytomegalovirus. The world is currently suffering from one of the most serious public health concerns in recent decades due to infections caused by SARS-CoV-2. This pandemic has highlighted the importance of being prepared for future outbreaks of various infectious diseases as the probability of future pandemics has also increased [43].

Even the United States National Academy of Medicine has considered the development of effective vaccine to control HCMV infections as top most priority [18]. Hence, in this study, a multi

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epitope vaccine has been designed against the Human Cytomegalovirus (HCMV) by using Immunoinformatics approach. The *in silico* Immunoinformatics approach can be a quick and cost effective method to develop vaccines that can generate strong immunity [31,49,61]. This approach can also help to overcome the genetic variations which are a major limitation in the process of vaccine development [49]. The epitope-based vaccine can also be particularly effective against viruses which exhibit antigenic shift and drift [10]. For the identification of potential antigenic epitopes, the envelope glycoproteins of HCMV were targeted. There are 20 envelope glycoproteins in HCMV [14]. Out of these total 20 proteins, only four protein coding for envelope glycoprotein B (GB), envelope glycoprotein H (GH), envelope glycoprotein L (GL) and envelope glycoprotein M (GM) were selected for the identification of vaccine candidate. The protein GB is selected because it is required for the membrane fusion, entry and cell to cell spread of the HCMV [18,28]. Moreover, this protein is currently being explored widely for vaccine research and so far it has also been used to develop recombinant vaccines and DNA vaccines against HCMV infections [7]. Anderholm *et al.* have extensively reviewed the various vaccines that are being developed using GB protein [7]. But so far GB protein has not been reported to be used for the development of multi epitope based peptide vaccine for HCMV. Other proteins GL, GH and GM are also important for HCMV. GM is required for virus tethering and it is one of the most conserved proteins in HCMV. It has shown 99% mean identity among various strains of HCMV [18]. Hence, it is also an important candidate for vaccine development. Foglierini *et al.*, reported that GH and GL are important for virus entry and suggested that they can be potential targets for identifying a vaccine candidate against HCMV infections [18].

So, in this study epitopes that can bind with MHCI and MHCII molecules were identified from GB, GH, GL and GM envelope glycoproteins of HCMV. Then based on the antigenicity, allergen property, toxicity and interferon-γ inducing ability the epitopes were further screened. The best epitopes were selected and analyzed for their ability to bind with MHC molecules by molecular docking. Then, using the best epitopes, a vaccine was engineered which consisted of adjuvants N- terminal and C-terminal sequence of *Salmonella typhimurium* flagellin protein [42], PADRE sequence, RS09 sequence. Linker GGS was used to link the epitopes and adjuvants. The role of these different adjuvants is explained in the discussion section of this article. Then, the various properties like antigenicity, allergenic potential, solubility, stability, hydrophobicity etc. were determined for the final vaccine construct. The vaccine was docked with a Toll-like receptor 5 molecule to determine the protein-protein interaction and their stability was confirmed by 100 ns molecular dynamics simulation study. Then, the codon usage optimization of the vaccine was done for expression in *Escherichia coli K12* strain and cloned *in silico* in pET28a (+) vector. Fig. 1 indicates the basic workflow used in this study. This investigation can be beneficial for the development of new vaccine candidates and also help to improve the existing subunit vaccines developed against HCMV.

2. Material and methods

2.1. Sequence retrieval and allergenic prediction of proteins

The protein sequences for Envelope Glycoprotein (EG): B, H, L and M with NCBI Protein IDs: YP_081514.1, YP_081523.1, YP_081555.1 and YP_081547.1 respectively were retrieved from NCBI. The allergenic property was determined through a support vector machine based AlgPred server [52]. It helps in predicting allergens based on amino acid or dipeptide composition. Further, conservancy and homology of proteins were determined by using NCBI BLASTP program [5].

2.2. T Cell epitope prediction

The proteins which were found to be non-allergenic and had a high degree of conservancy across various strains were further analyzed for the prediction of T cell epitopes. For the prediction of T cell epitopes two servers NETMHC2 version 2.3 and NETMHC 4.0 were used [8,29]. NETMHC2 2.3 server predict epitopes which interact with MHC class II molecules and generate T helper cells mediated immunity [29]. Similarly, NETMHC 4.0 server predicts epitopes which interact with MHC class I molecules and elicit immediate immune response by activating cytotoxic T cells [8].

2.3. Analysis of Antigenicity, Allergenicity, toxicity and Interferon-γ activating potential of the epitopes

For the epitope to be selected as a candidate for further analysis, it needs to be antigenic, non-toxic, and induce interferon-γ production [63]. The antigenic potential of the epitopes was analyzed by using the Vaxijen server [16]. This server predicts if the peptides are antigen or non-antigen by analyzing the physiochemical characteristics of the proteins. To analyze the toxicity of the epitopes ToxinPred server was used by applying the SVM (Swiss-Prot) method [25]. This server helps to determine the toxicity of peptides and modulate the toxicity of peptides by using machine learning method [25]. Also, the allergenic potential of the epitopes was determined by using the AllergenFP version 1.0 server (http://ddg-pharmfac.net/AllergenFP/index.html). This server helps to determine whether a protein is allergen or non-allergen using the Tanimoto coefficient. Interferon-γ plays an important role in the activation of adaptive and innate immunity [34]. Interferon-γ also interferes with viral replication [34]. Hence, to predict the interferon-γ production ability of the epitopes IFN epitope server was used [15]. This server uses machine learning techniques and allows to predict and design peptides and epitopes that can induce interferon-γ production [15].

2.4. Molecular Modeling, binding affinity prediction and population coverage analysis of the epitopes

The 3D structures of the predicted epitopes were modeled using the PEPstrMOD server [58]. This server predicts the 3D structure of epitopes by using molecular dynamics simulation on the basis of information about secondary structure and beta turns [58]. Binding affinity predictions of epitopes with alleles were predicted by MHCPred server [24]. Immune Epitope Database (IEDB) Population Coverage analysis tool was used to predict the percentage of global population that will respond to the epitopes [13].

2.5. Molecular docking of epitopes with HLA allele

Then, the 3D structures of the epitopes were docked with human leukocyte antigen (HLA) alleles by using PatchDock server [55]. This server predicts the interaction between proteins by finding transformations that generate the best molecular shape complementarity [55]. Proteins with PDB ID: 3C5J, 4AH2 and 6CQL were used as a template for HLA allele: DRB1_0701, DRB1_0101 and DRB1_1301 respectively.

2.6. Vaccine engineering and determination of its physiochemical properties

The vaccine was engineered for the selected epitopes by using the previously reported method used to develop vaccines against

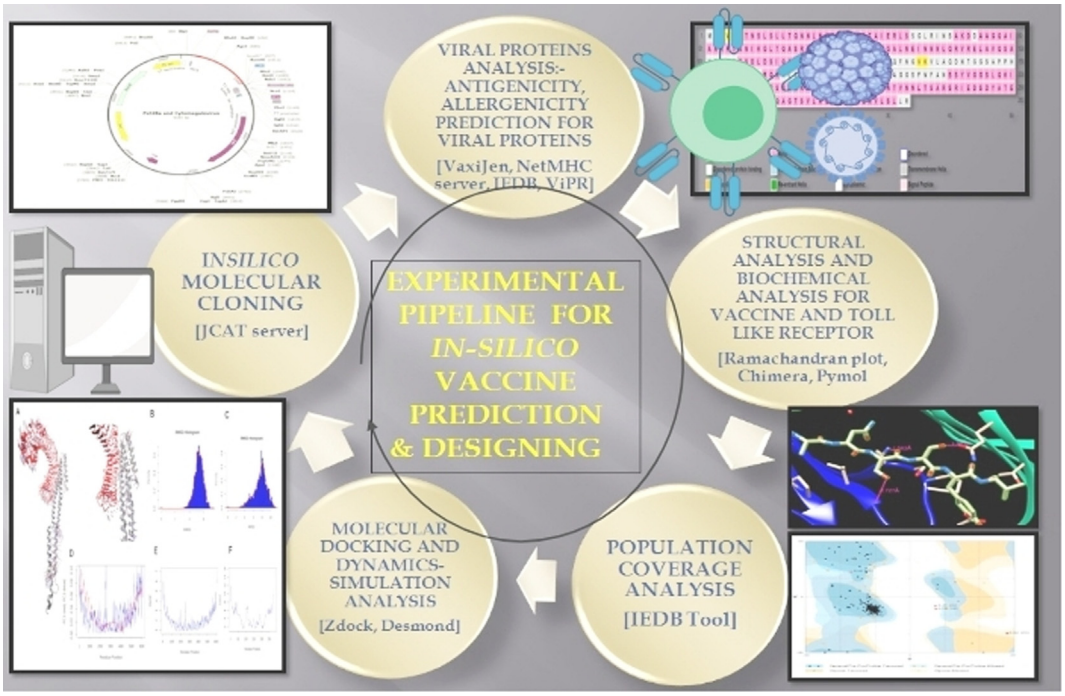

Fig. 1. Experimental Workflow for prediction of epitope based vaccine.

Human Papilloma Virus, *C. auris*, and Herpes Simplex Virus type 1 and 2 [27,47,3,63]. In brief N and C terminal of flagellin protein, RS09 peptide were used as adjuvants [47,63]. PADRE sequence was used to counter the problems caused by polymorphic HLA alleles and to improve the stability of the vaccine [27]. To link the epitopes and adjuvants linker sequence GGS was used [63]. After the construction of the vaccine, its physicochemical parameters were evaluated by using ExPASy ProtParam tool [21]. This server helps to predict the molecular weight, instability index, GRAVY score, isoelectric point, number of amino acids, aliphatic index, total number of atoms, number of positively and negatively charged residues [21]. The solubility of the vaccine was determined by using Solpro server [44] and cc SOL omics tool [2]. Both these servers can predict the solubility of the proteins upon overexpression in *E. coli* with 74% accuracy. Then, the antigenicity of the final vaccine was confirmed by Vaxijen server (threshold value of 0.4) [16] and ANTIGENpro server (http://scratch.proteomics.ics.uci.edu/) simultaneously.

2.7. Determination of secondary and tertiary structure of the vaccine construct

The PSIPRED server was used to predict the alpha helix, beta sheets and coil in the designed vaccine construct [12]. I-Tasser server was used to predict the 3D structure of the vaccine construct [62]. The server uses a multiple threading approach and iterative template based fragment assembly simulations to predict the high quality 3D model of proteins. The modeled 3D structure of the vaccine was validated by generating a Ramachandran plot using Rampage server [41].

2.8. Molecular docking of the vaccine construct with Toll like receptor molecule and its molecular dynamics simulation

Molecular dynamics simulation (MDS) was conducted using the Desmond MDS suite that is freely available to the academia. The Toll like receptor 5 with PDB ID: 3J0A.pdb was downloaded from the Protein Data Bank (https://www.rcsb.org/) and its Chain A was selected for MD simulation with the conjugated vaccine. Before MDS, the TLR-vaccine conjugate complex was generated through docking using the online ZDOCK docking tool [51]. The top protein-protein interaction pose was selected based on the best binding energy score. The bond order, bond lengths, missing hydrogens were added, missing atoms and side chains in the complex were corrected, selenomethionine was converted to methionine, disulphide bonds were generated using the Protein Preparation Wizard tool of the Schrodinger Maestro which is provided freely along with the Academic Desmond MDS suite [11]. The side chain protonation state of amino acids in the TLR and vaccine conjugate were assigned to the pH 7.0 through the pKa prediction by the propka tool. The resulting structure was optimized and then energy minimized. The resulting complex was then solvated in the TIP3P water model with orthorhombic system shape and 10 Ã... periodic boundary conditions (PBC). The overall charge of the system was set to neutral through the addition of appropriate Na + and Cl- counter ions. The physiological salt concentration of 0.15 Molar was achieved through the addition of NaCl. The resulting solvated system was once again energy minimized using the Desmond Minimization. The default relaxation protocol was used for preparatory phase MDS. The extent of the production MD was 100 ns and the conformations were recorded every 100 picoseconds. While the root mean square deviation/fluctuation (RMSD/F) and principal component analysis were done through R based BIO3D module. Structural artworks were created through pyMOL and VMD.

2.9. Codon adaptation and in silico cloning

The vaccine construct needs to be codon optimized so that it can be cloned and expressed in suitable expression vectors. Hence, for the codon optimization of the vaccine sequence, JCatserver was used [23]. While optimizing the codon for expression in *E. coli* strain K12 rho-independent transcription terminators, prokaryotic ribosome binding sites and cleavage sites of several restriction

enzymes were avoided as explained by Hasan et al. [27]. For *in silico* cloning of the vaccine construct SnapGene restriction cloning module in SnapGene software developed by Insightful Science was used. This software has been previously used for the *in silico* cloning of multivalent chimeric vaccine against Herpes Simplex Virus type I and II [27]. The codon adapted vaccine sequence was inserted between XhoI (158) and EcoRI (192) pET28a (+) vector for cloning.

3. Results

3.1. Retrieval and analysis of the protein sequences

The protein sequence of the envelope glycoprotein B, envelope glycoprotein H, envelope glycoprotein L, and envelope glycoprotein M were retrieved. The corresponding Protein ID and their length of these envelope glycoproteins have been listed in Table 1. All the selected proteins were predicted as non-allergen after the analysis by the AlgPred server. It has been reported in literature that for consideration of a protein to identify epitopes, the proteins must have more than 90% conservancy [27]. Upon analysis of the proteins using the BLASTP tool, all the proteins showed more than 90% identity with their corresponding proteins in the other strains of HCMV. The conservancy and allergenicity of the proteins are listed in Table 1.

3.2. T Cell epitope prediction

All the four selected proteins were found to be non-allergen and showed a high degree of conservancy. Hence, they were considered for further analysis and prediction of T cell epitopes to generate a robust cell mediated immunity. Both Helper T cell epitopes and Cytotoxic T cell epitopes were predicted. For the prediction of Helper T cell epitopes, HLA alleles DRB1_0101, DRB1_0701 and DRB1301 were selected. Altogether, 167 high binding Helper T cell epitopes were predicted. The high binding Cytotoxic T cell epitopes were predicted against 10 different MHC class I alleles in NetMHC 4.0 server. Out of all the different epitopes that were predicted, 19 epitopes were found to be common among Helper T cell epitopes and Cytotoxic T cell epitopes that have a Vaxijen score above 1.1. These common Helper T cell and Cytotoxic T cell epitopes and the MHC allele that they bind with are listed in Table 2.

3.3. Analysis of Antigenicity, Allergenicity, toxicity and Interferon-γ activating potential of the epitopes

Epitopes that have Vaxijen score above 1 are considered to be highly antigenic [1,53]. In the present study, all the selected epitopes were found to be antigenic and had the Vaxijen score $\geq$ 1.1. Further, all of these epitopes were also found to be non-toxic. Out of the twenty selected epitopes, only 9 were found to be having the ability to induce the production of interferon-γ and were non-allergen. Finally, after the overall analysis of the epitopes only five epitopes (*i.e.* FNFANSSYV, YLIYTRQRR, LIYTRQRRL, IYTRQRRLC, and FFCLTIFNV) were found to be non-toxic, non-allergenic, and highly antigenic and could induce the production of interferon-γ (Table 2).

3.4. Molecular Modeling, binding affinity prediction and population coverage analysis of the epitopes

Further experiments were conducted with the five epitopes that were predicted to be antigenic, non-toxic, and non-allergic and could induce interferon-γ production. The 3D structures of epitopes and their corresponding HLA alleles were modeled. Then, the epitopes were docked with their corresponding HLA alleles. All the epitopes were found to interact with their corresponding HLA alleles by the formation of hydrogen bonds and hydrophobic interactions. The free binding energy between epitopes and the HLA alleles was strong with the least entropy. The binding affinity prediction analysis showed that these epitopes had the ability to interact with other HLA alleles also, listed in Table 3. The population coverage of these epitopes is also predicted ~~in~~ and the results are listed in Table 3.

3.5. Molecular docking of epitopes with HLA allele

The docked images of epitopes, which had higher population coverage, better binding affinity the HLA allele and also were non-toxic, non-allergen, antigenic and had the ability to elicit interferon-γ production shown in Fig. 2. Hydrogen bonds between the epitope and HLA alleles are shown in pink color with the distance in Angstrom units.

3.6. Vaccine engineering and determination of its physiochemical properties

After the analysis of the epitopes in the previous sections, two epitopes IYTRQRRLC and FNFANSSYV were selected for the design of the vaccine. Three adjuvants RS09 (APPHALS), PADRE sequence (AKFVAAWTLKAAA) and N-terminal and C-terminal sequence of *Salmonella typhimurium* flagellin protein were linked to the epitopes with the help of GGS linker. RS09 adjuvant is asynthetic Toll like receptor-4 agonist and flagellin is a Toll like receptor-5 agonist and also helps in activation of innate immunity and adaptive immunity [19,26,57,63]. Recently, these Toll like receptor agonists flagellin and RS09 have been used as adjuvants while designing vaccines [47,63]. Toll like receptors are involved in the activation of innate immunity and also improve antigen presentation by Antigen Presenting Cells (APCs) [63]. PADRE sequence provides stability to the vaccine and it has also been used as adjuvant in vaccine design [27]. The complete sequence of vaccine is "MAQ VINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSAKDDAAGQAIANRF TANIKGLTQASRNANDGISIAQTTEGALNEINNNLQRVRELAVQ SANSTNSQSDLDSIQAEITQRLNEIDRVSGQTQFNGVKVLAQDNTGGSAP PHALSGGSIYTRQRRLCGGSAKFVAAWTLKAAAGGSFNFANS

Table 1
Analysis of the allergenicity and conservancy of the selected proteins.

Serial Number	Protein ID	Protein Product	Protein Length	AlgPred Prediction	Percentage Identity with proteins of other strains
1	YP_081514.1	Envelope glycoprotein B	907 AA	Non-allergen	93.29% identity with envelope glycoprotein B of other strains of HCMV
2	YP_081523.1	Envelope glycoprotein H	742 AA	Non-allergen	95.69% identity with envelope glycoprotein H of other strains of HCMV
3	YP_081555.1	Envelope glycoprotein L	278 AA	Non-allergen	97.84% identity with envelope glycoprotein L of other strains of HCMV
4	YP_081547.1	Envelope glycoprotein M	371 AA	Non-allergen	97.05% identity with envelope glycoprotein M of other strains of HCMV

Table 2
T cell epitopes and the analysis of their antigenicity, allergenicity, toxicity and interferon-γ production ability.

Protein ID	Peptide	MHC II Allele	MHC I Allele	Vaxijen Score	Antigen/Non-antigen	Toxicity	Interferon-γ Production Ability	Allergenic Potential
YP_081514.1	FNFANSSYV	DRB1_0701	HLA-A0301	1.3513	Antigen	Non-toxin	Positive	Non-allergen
YP_081514.1	YLIYTRQRR	DRB1_1301	HLA-A0301	1.3687	Antigen	Non-toxin	Positive	Non-allergen
YP_081514.1	LIYTRQRRL	DRB1_1301	HLA-B0801	1.2988	Antigen	Non-toxin	Positive	Non-allergen
YP_081514.1	IYTRQRRLC	DRB1_1301	HLA-A2402	1.7618	Antigen	Non-toxin	Positive	Non-allergen
YP_081514.1	VTITTARSK	DRB1_1301	HLA-A0301	1.1381	Antigen	Non-toxin	Negative	Non-allergen
YP_081514.1	RLRHRKNGY	DRB1_1301	HLA-A0301	1.3737	Antigen	Non-toxin	Negative	Non-allergen
YP_081523.1	LRYVKITLT	DRB1_0101	HLA-B2705	1.6678	Antigen	Non-toxin	Negative	Allergen
YP_081523.1	FMITCLSQT	DRB1_0701	HLA-A0201	1.4104	Antigen	Non-toxin	Negative	Allergen
YP_081555.1	FNVVVAIRN	DRB1_0101	HLA-B5801	1.3435	Antigen	Non-toxin	Negative	Allergen
YP_081555.1	FSFSPGPVI	DRB1_0701	HLA-B5801	1.4205	Antigen	Non-toxin	Positive	Allergen
YP_081547.1	FFCLTIFNV	DRB1_0101	HLA-A0201	1.2691	Antigen	Non-toxin	Positive	Non-allergen
YP_081547.1	FTLTMSFRL	DRB1_0701	HLA-A0201	1.4739	Antigen	Non-toxin	Positive	Allergen
YP_081547.1	ISWSFGMLF	DRB1_0701	HLA-A2402	1.5169	Antigen	Non-toxin	Positive	Allergen
YP_081547.1	YRTGISWSF	DRB1_0701	HLA-B2705	1.3546	Antigen	Non-toxin	Positive	Allergen
YP_081547.1	FVRTGHMVL	DRB1_0701	HLA-B0702	1.1752	Antigen	Non-toxin	Negative	Non-allergen
YP_081547.1	FFFSRLHPK	DRB1_0701	HLA-A0301	1.3587	Antigen	Non-toxin	Negative	Allergen
YP_081547.1	FFVRTGHMV	DRB1_1301	HLA-A2402	1.3101	Antigen	Non-toxin	Negative	Allergen
YP_081547.1	LVCWIKISM	DRB1_1301	HLA-A0201	1.2582	Antigen	Non-toxin	Negative	Allergen
YP_081547.1	LFTLTMSFR	DRB1_1301	HLA-A0301	1.1488	Antigen	Non-toxin	Negative	Non-allergen

Table 3
Binding Affinity Prediction and Population Coverage Analysis of the Epitopes.

Epitope	Number of HLA binders	HLA with predicted IC50 value (nM)	Percentage Population Coverage
FNFANSSYV	3	HLA-DRB1*01:01 (7.64), HLA-DRB1*04:01 (46.67), HLA-DRB1*07:01 (123.31)	38.05%
YLIYTRQRR	3	HLA-DRB1*01:01 (4.54) HLA-DRB1*04:01 (1774.19) HLA-DRB1*07:01 (185.35)	44.91%
LIYTRQRRL	2	HLA-DRB1*01:01 (5.50), HLA DRB1*04:01 (non-binder), HLA-DRB1*07:01 (1221.80)	36.01%
IYTRQRRLC	3	HLA-DRB1*01:01 (2.53), HLA-DRB1*04:01 (1489.36), HLA-DRB1*07:01 (1096.48)	44.91%
FFCLTIFNV	3	HLA-DRB1*01:01 (1.32), HLA-DRB1*04:01 (2128.14), HLA-DRB1*07:01 (68.71)	38.05%

SYVGGSLQKIDAALAQVDTLRSDLGAVQNRFNSAITNLGNTVNNLT
SARSRIEDSDYATEVSNMSRAQILQQAGTSVLAQANQVPQNVLSLLR".
The design of vaccine made using Illustrator for Biological Sequences is illustrated in Fig. 3 [40].

The ProtParam server was used to analyze the physicochemical properties of the engineered vaccine. The length of the vaccine construct was 283 amino acids and it was predicted to be stable (instability index: 39.72). The estimated half-life of the vaccine was 30 h in mammalian reticulocytes, >20 h in yeast and >10 h in *E. coli*. The vaccine construct had a molecular weight of 29.83 KDa and the aliphatic index was 89.08. The protein insolubility after expression causes limitations during various experiments, hence, the solubility of the vaccine was determined. Both the servers cc Sol omics and Solpro predicted the vaccine construct to be soluble after expression in *E. coli*. Similarly, both the tools Vaxijen and AntigenPro predicted the vaccine to be antigenic. Finally, the vaccine construct was predicted as non-allergen. The physiochemical properties of the vaccine have been listed in Table 4.

3.7. Determination of the secondary and tertiary structure of the vaccine construct

The PSIPRED server predicted the presence of coil structures, beta sheet and alpha helix in the vaccine construct (Fig. 4). Ramachandran plot showed that 89.7 percent residues were in the favored region, 8.9% residues were in the allowed region and only 1.4% residues were in the outlier region. The Ramachandran plot of the 3D model of the vaccine is shown in Fig. 5.

3.8. Molecular docking of the vaccine with Toll like receptor molecule and its molecular dynamics simulation

TLR-vaccine conjugate complex was generated through docking using the online ZDOCK docking tool. The top protein-protein interaction pose was selected based on the best binding energy score. To study the binding and dynamics of the TLR5 with the

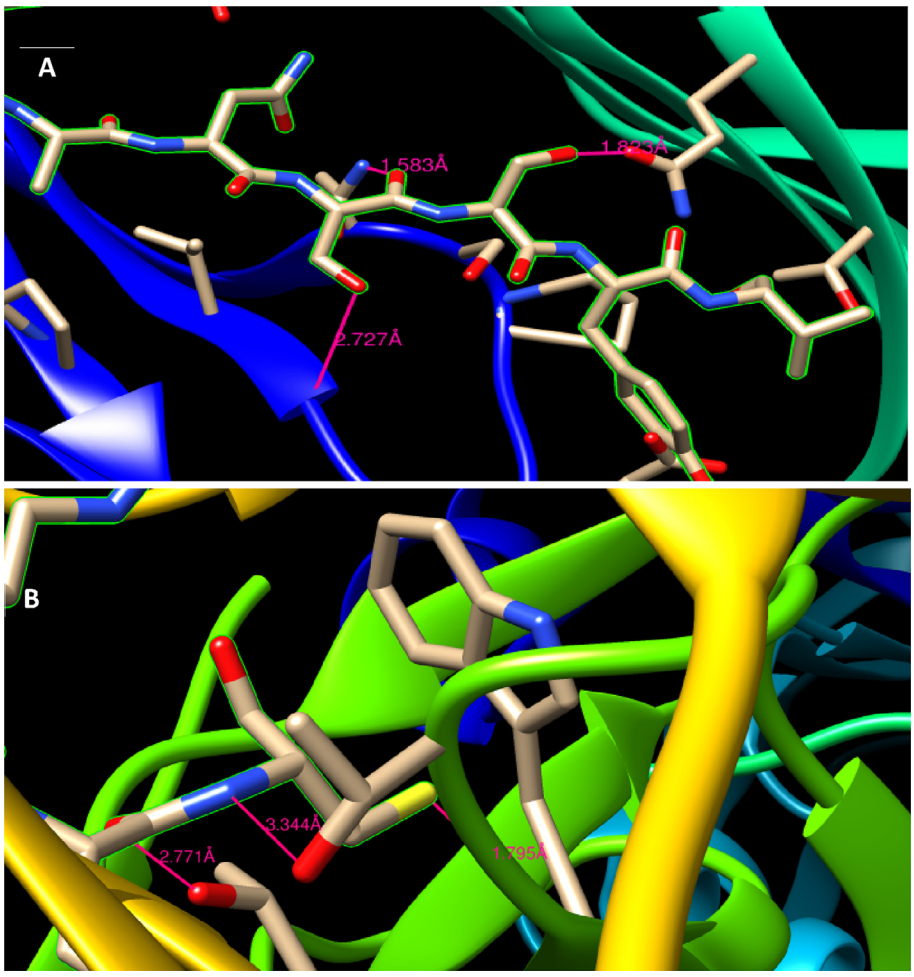

Fig. 2. A. Docking of epitope FNFANSSYV with DRB1_0701 **B**. Docking of epitope IYTRQRRLC with DRB1_1301.

Fig. 3. Design of the engineered vaccine: adjuvant attachment with considered epitope E1 and E2.

Table 4
Physiochemical properties of the vaccine construct.

Number of amino acids	283
Molecular weight	29836.98
Instability index	39.72 (Stable)
Aliphatic index	89.08
Theoretical pI	9.45
Total number of negatively charged residues (Asp + Glu)	20
Total number of positively charged residues (Arg + Lys)	24
Total number of atoms	4175
Grand average of hydropathicity (GRAVY)	−0.324
Solubility determined using Solpro server	Soluble (0.780652 probability)
Allergenicity	Non-allergen
Antigenicity using Vaxijen server	Antigen (0.4721)
Antigenicity using AntigenPro server	Antigen (0.934890)

designed vaccine adjuvant complex, a 100 ns long molecular dynamics simulation was conducted. The overall solvated complex consisted ~180,000 atoms. The cartoon representation of the complex is shown in Fig. 6 A. Through the RMSD density distribution analysis, a bell shaped plot was observed for both TLR5 and vaccine construct. The RMSD remained around 5.5 and ~8 Ã. . . for TLR5 and vaccine construct respectively. The higher RMSD values can be expected considering the extended geometry of both these proteins and their high number of solvent exposed residues (Fig. 6 B&C). Following this, the RMSF analysis reported the lowest fluctuations for residues from both TLR5 and designed vaccine adjuvant which was involved in interaction with each other (Fig. 6 E&F). To visualize the essential dynamics and direction of motion of overall TLR 5 and vaccine adjuvant vis-a-vis the region involved in the interaction between the proteins, the PCA analysis was also conducted (Fig. 7). The contribution of each amino acid from both TLR5 and vaccine adjuvant on the PC 1 and were plotted and the resulting essential motion along with the direction was superimposed on the TLR5 – Vaccine adjuvant complex as CPKs with Blue – White-Red coloring scheme to show the time evolved magnitude of motion along with its directionality (Fig. 6 D). Through the results, it was observed that the residues positioned at the

Fig. 4. Predicted residues of the vaccine involved in the formation of various secondary structures.

interface underwent the least essential dynamics throughout the 100 ns MDS and along with no away ward motion of these amino acids from the partner protein was observed suggesting stable complex. The total energy (E), potential energy (Ep), temperature (T), Pressure (P) and Volume (V) throughout the 100 ns long production molecular dynamics simulation is shown through the plots (Fig. 8) it can be observed that all these simulation quality parameters remained constant throughout the length of the simulation thus suggesting the good quality of the MD simulation.

The noncovalent interactions between the TLR5 and the designed vaccine were also recorded. The consistent hydrogen bonds between the interacting pairs were calculated using the definition drawn and reported in literature [46,33,60]. Glutamine at position 80 in TLR5 of the modelled structure was found to be interacting through its side chain Oxygen and Nitrogen with the backbone and side chain oxygen of Asparagine 219 and Serine 223 respectively. Glutamine 156 of TLR5 was also observed to form hydrogen bond through its side chain Nitrogen with the backbone

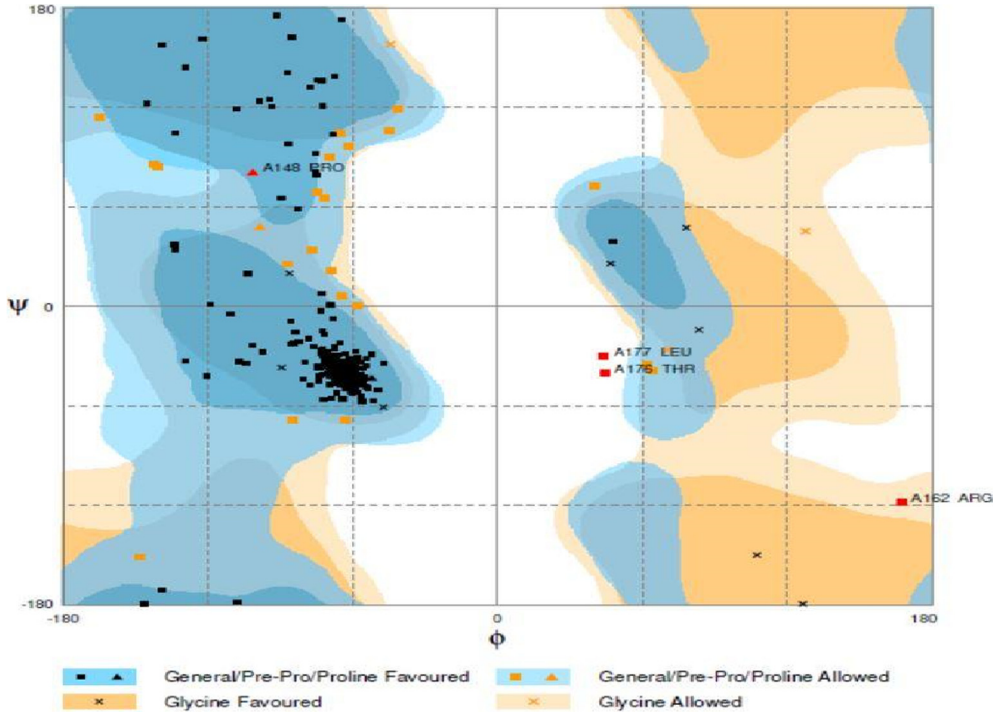

Fig. 5. Ramachandran Plot of the 3D model of the vaccine.

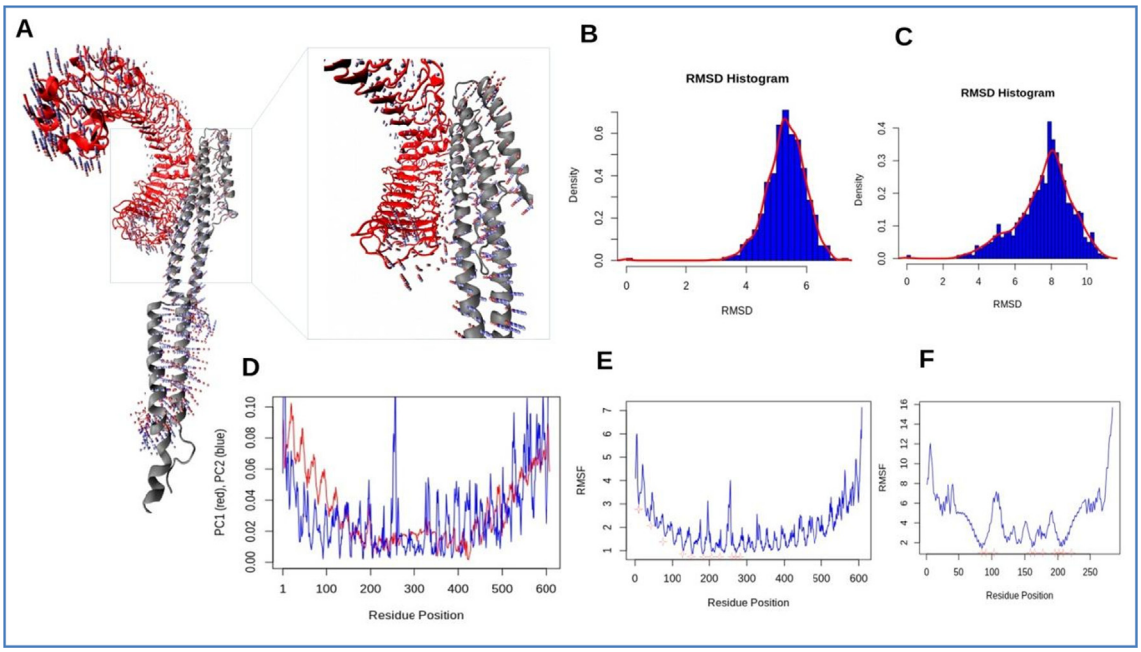

Fig. 6. The cartoon representation of the TLR5 and vaccine adjuvant complex shown as red and steel color respectively. The Blue-White-Red CPKs generated from the PCA analysis based contribution to motion along with direction of all amino acids for PC1 are also shown (A). The RMSD density distribution histograms for the TLR5 and vaccine adjuvant are shown separately (B & C). Contribution of each amino acid of the TLR5 to first two PCs *i.e.* PC1 and PC2 are shown (D). The RMSF plots of TLR5 and vaccine adjuvant are shown separately along with the regions involved in interaction with the partner protein highlighted as red stars. Least RMS fluctuation can be seen in these interface residues (E & F).

Oxygen of Serine 168 of designed Vaccine. Hydrophobic interactions between them were calculated based on the less than 7 Ã... proximity of beta carbon of hydrophobic residues. Valine and Phenylalanine at positions 240 and 299 in TLR5 paired in hydrophobic interactions with the Tyrosine and Leucine residues at position 192 and 197 in the designed Vaccine. The electrostatic interactions between the amino acids of both the proteins were also observed between Lysine 106, Histidine 262 and Histidine 263 of TLR5 and Aspartates at position 201 and 213 of the designed Vaccine. Numerous Potential short contacts were also observed between the interacting TLR5 and designed Vaccine and have been shown in detail in Table 5.

3.9. Codon adaptation and in silico cloning

Codon usage adaptation is necessary for accelerating the expression of the vaccine construct in prokaryotic hosts. After the codon adaptation of the vaccine constructs, the Codon Adaptation Index was predicted to be 0.941 which ensures a high degree of the sequence expression and the GC content of the improved

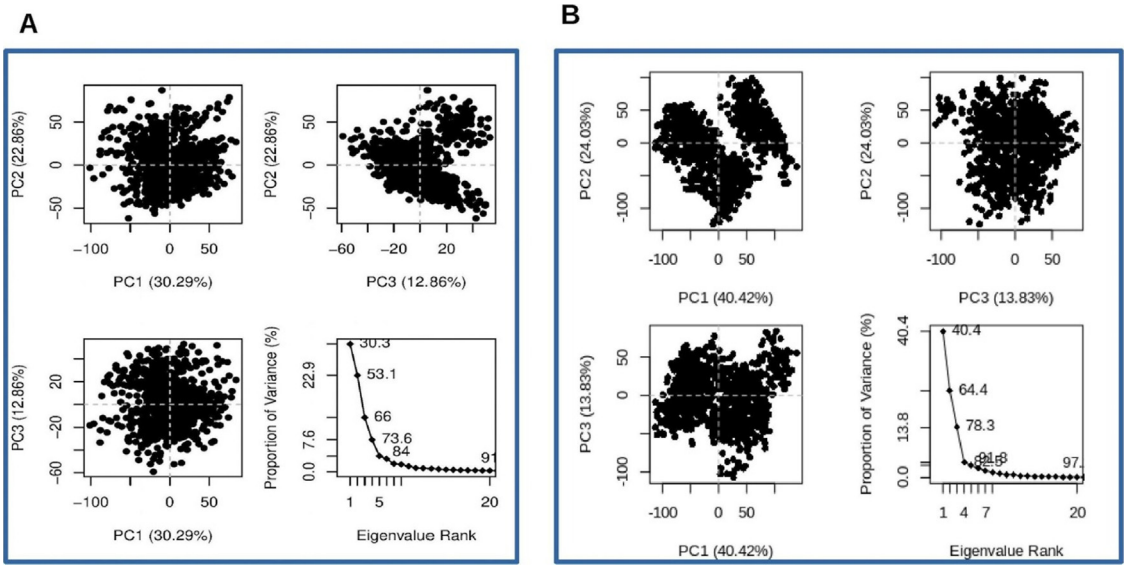

Fig. 7. The alpha carbon PCA analysis was conducted for the TLR5 and vaccine adjuvant separately to study the essential dynamics (A & B). The PCA plots of PC1 vs PC2, PC1 vs PC3 and PC2 vs PC3 for both the partners of the simulated complex are shown along with the scree plots to report the contribution of individual PCs to overall essential dynamics.

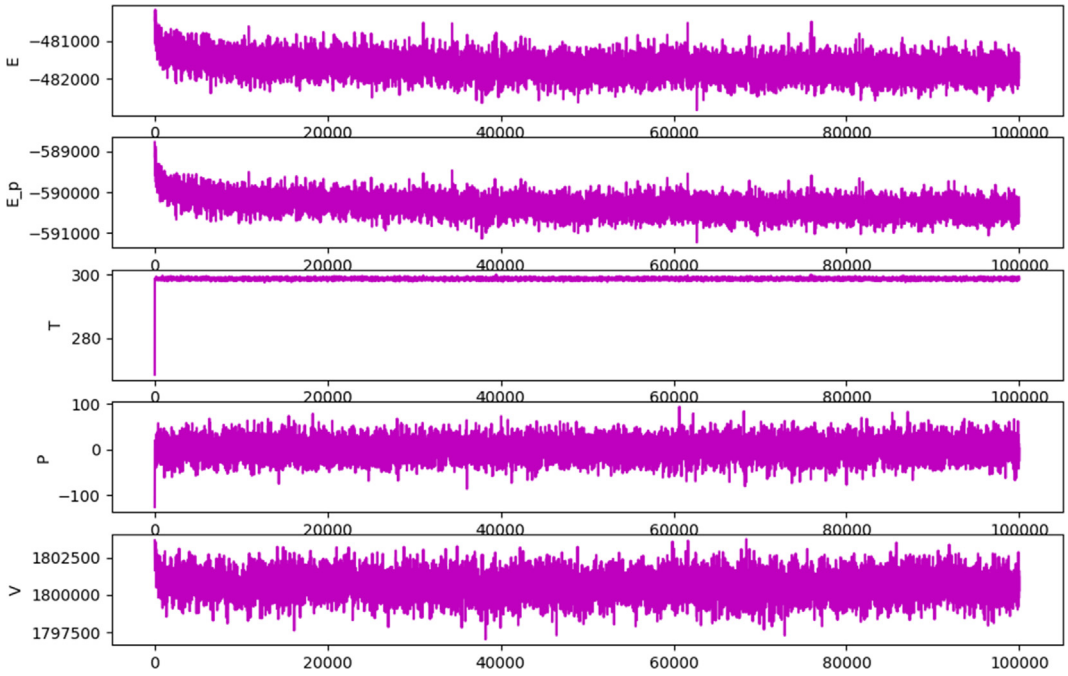

Fig. 8. Simulation quality analysis for putative vaccine and TLR5 complex.

Table 5
Non-covalent interactions between TLR5 and designed vaccine.

Potential Hydrogen Bonds									
RESIDUE-1				**RESIDUE-2**				**Type of H-Bond**	**Distance (D-A)**
Res Num	**Res Name**	**TLR5(Chain A)**	**Atom Name**	**Res Num**	**Res Name**	**Vaccine(Chain B)**	**Atom Name**		
80	GLN	A	NE2	219	ASN	B	O	SB	3.21
80	GLN	A	OE1	223	SER	B	OG	SS	3.18
156	GLN	A	NE2	168	SER	B	O	SB	3.05
Potential Hydrophobic Interactions									
RESIDUE-1				**RESIDUE-2**				**Distance**	
Res Num	**Res Name**	**TLR5(Chain A)**	**Atom Name**	**Res Num**	**Res Name**	**Vaccine(Chain B)**	**Atom Name**		
240	VAL	A	CB	192	TYR	B	CB	5.81	
299	PHE	A	CB	197	LEU	B	CB	6.51	
Potential Favorable Electrostatic Interactions									
RESIDUE-1				**RESIDUE-2**				**Distance**	
Res Num	**Res NameTLR5**	**TLR5(Chain A)**	**Atom Name**	**Res Num**	**Res Name**	**Vaccine(Chain B)**	**Atom Name**		
106	LYS	A	CB	213	ASP	B	CB	9.55	
262	HIS	A	CB	201	ASP	B	CB	7.39	
263	HIS	A	CB	201	ASP	B	CB	9.32	
Potential Short Contacts									
RESIDUE-1				**RESIDUE-2**				**Distance**	
Res Num	**Res Name**	**TLR5(Chain A)**	**Atom Name**	**Res Num**	**Res Name**	**Vaccine(Chain B)**	**Atom Name**		
55	PHE	A	HZ	226	THR	B	HG1	1.75	
79	SER	A	HB2	219	ASN	B	HD22	1.98	
80	GLN	A	HE21	219	ASN	B	C	2.40	
80	GLN	A	HE21	220	ARG	B	HA	1.75	
156	GLN	A	HB2	168	SER	B	HA	1.97	
183	PHE	A	CZ	168	SER	B	HG	2.39	
183	PHE	A	HZ	168	SER	B	HG	1.79	
273	ASN	A	CB	191	SER	B	HG	2.44	
273	ASN	A	ND2	191	SER	B	HG	2.11	
273	ASN	A	HB3	191	SER	B	HG	1.64	

sequence was 52.17%. Finally, the vaccine was inserted between XhoI (158) and EcoRI (192) of pET28a (+) vector by using a restriction cloning module in SnapGene. The size of the cloned product was 6183 bp. The final cloned vaccine construct is shown in Fig. 9, where the insert (vaccine) is highlighted in red.

4. Discussion

There are antiviral drugs to treat these HCMV infections. However, reports of antiviral drug resistance in HCMV and the hyperimmune globulin transfusion for the treatment of HCMV have

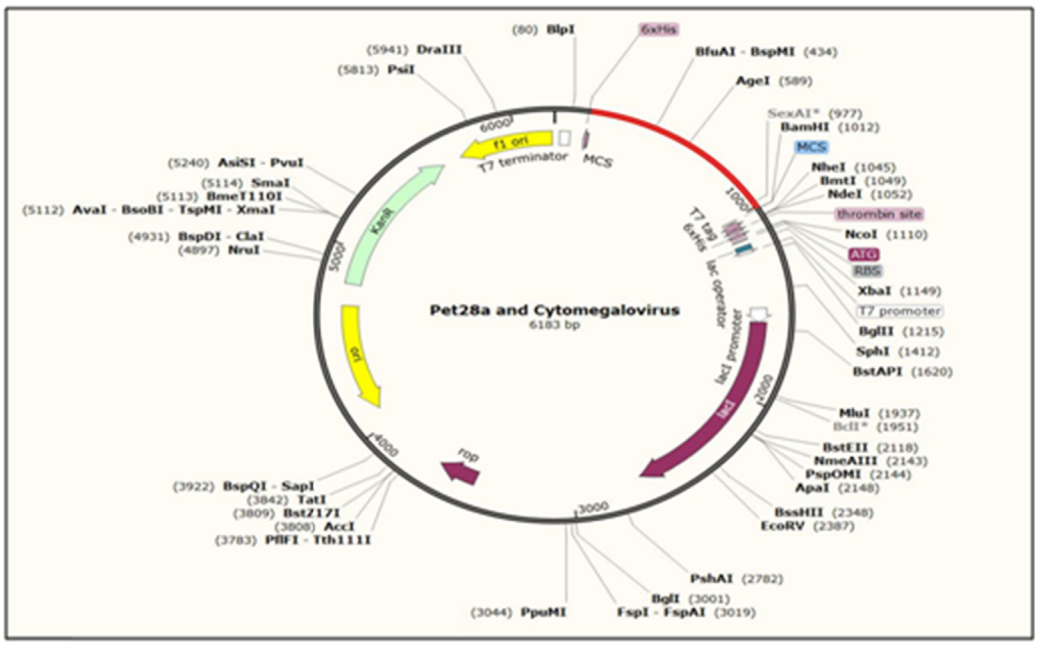

Fig. 9. *In silico* cloning of the designed vaccine in pET28a (+) vector.

also been reported to be ineffective [17,18]. There is no authorized vaccine for the treatment of HCMV. A subunit vaccine using glycoprotein B and MF59 adjuvant has shown 50% efficacy in preventing maternal acquisition of HCMV in Phase II clinical trials [48,54]. Even the United States National Academy of Medicine has considered the development of effective vaccines to control HCMV infections as top most priority [18]. Hence, in this study Immunoinformatics has been used to design a multivalent T cell epitope based peptide vaccine. This approach has been previously used to design vaccine for Human Papillomavirus and Herpes Simplex Virus type I and II, and Zika virus [27,50,47,63]. The *in silico* Immunoinformatics approach can be a quick and cost effective method to develop vaccines that can generate strong immunity; and this approach can also help to overcome the limitations like genetic variations, antigenic shift and antigenic drift [10,49,61].

To design the multivalent T epitope based vaccine, four envelope glycoprotein: glycoprotein B (GB), glycoprotein H (GH), glycoprotein L (GL) and glycoprotein M (GM) of HCMV were targeted because of their importance as already discussed in the introduction part. On initial analysis, all of the four envelope proteins selected for this study were found to be non-allergen and had more than 90% identity with their corresponding proteins in other strains of HCMV. For a protein to be considered for the development of vaccine, it must be non-allergen and have high degree of conservancy among various strains of the virus [27,35]. As all of the selected proteins were found to be non-allergen and had a high degree of conservancy, they were considered for the prediction of T cell epitopes. Both Helper T cell epitopes and Cytotoxic T cell epitopes were predicted from these proteins. Helper T cells and Cytotoxic T cells interact with MHC-II and MHC-I molecules respectively and they play an important role in the generation of robust cell mediated immunity which is necessary for the complete removal of the infections. Altogether 19 epitopes were predicted that were commonly found to interact with both MHC-I and MHC-II molecules and had Vaxijen score $\geq$ 1.1. If the epitope has a Vaxijen score $\geq$ 1.1, it is considered highly antigenic [1]. Hence, these 19 epitopes were selected for further analysis. For an epitope to be selected for vaccine engineering, it must also be non-toxic, non-allergen and can induce the production of interferon-γ. Interferon-γ plays an important role in the activation of adaptive and innate immunity [34]. Interferon-γ also interferes with viral replication [34]. Out of the19 epitopes, only 5 were found to be non-toxic, non-allergenic and having the ability to induce interferon-γ production. These five epitopes FNFANSSYV, YLIYTRQRR, LIYTRQRRL, IYTRQRRLC, and FFCLTIFNV were analyzed further for their binding affinity and global population coverage. It has been suggested that the epitopes which are selected for vaccine should have binding affinity with more than one HLA allele and cover a major population [59]. The five selected epitopes were predicted to bind with more than one HLA allele and had moderate population coverage. Further, these epitopes were also found to bind with HLA alleles by forming hydrogen bonds and hydrophobic interactions after the molecular docking analysis. Finally, after the evaluation of all the data generated in the analysis of the previous sections, two epitopes that are namely FNFANSSYV and IYTRQRRLC were selected for the vaccine engineering.

For a vaccine to be effective it must be able to elicit a prolonged and high level of antibody secretion [6]. The adjuvants enhance the immune response and induce the prolonged synthesis of antibodies [45]. Toll-like receptors play an important role in the maturation of antigen presenting cells (APC), activation of innate immunity and self/non-self-differentiation [30,37,63]. Hence, for the vaccine design, in this study two Toll-like receptor agonists RS09 (APPHALS) and N-terminal and C-terminal sequence of *Salmonella typhimurium* flagellin protein were linked to the epitopes with the help of GGS linker. RS09 adjuvant is a synthetic Toll like receptor-4 agonist and flagellin is a Toll like receptor-5 agonist and also helps in activation of innate immunity and adaptive immunity [19,26,57,63]. PADRE sequence (AKFVAAWTLKAAA) improves the stability of the vaccine [27]. So, PADRE sequence was also added to the vaccine construct. Linkers help to facilitate antigen processing and help to avoid junctional epitope formation in multi-epitope vaccines. These adjuvants have been previously used for the *in silico* design of vaccines against Human Papilloma virus and Herpes Simplex Virus type I and type II [27,47,63]. After linking the epitopes and adjuvants, a vaccine construct of 283 amino acids was formed. The designed vaccine was further tested for its solubility, stability, antigenicity and allergenicity. The

vaccine was predicted to be non-allergenic, stable and antigenic. The vaccine was also predicted to be soluble after expression in *E. coli*. The analysis of the secondary structure of the vaccine showed the presence of alpha helix, beta sheets and coil structure. The quality of the 3D model of the vaccine was validated by Ramachandran plot assessment. The plot revealed that a significant number of residues were in allowed regions, while only 1.4% residues were in outlier regions, suggesting that the model is of good quality. The vaccine was docked with a TLR5 receptor. The vaccine was able to bind with stability to the TLR5 receptor which was further confirmed by molecular dynamics simulation analysis. Finally, *in silico* cloning was done after codon adaptation to ensure the proper expression of the vaccine. The *in silico* cloning suggests that the vaccine can be expressed in *E. coli*. On further analysis using NCBI protein-protein BLAST, no human proteins (taxid: 9606; *Homo sapiens*) were found to be similar to the vaccine which implies the safety of the vaccine in clinical trials. As all these studies were performed by using various bioinformatics tools, the validation of the findings needs to be done by performing *in vivo* experiments.

5. Conclusion

After the diligent and meticulous analysis of the 4 envelope glycoproteins of HCMV by using several bioinformatics tools various safe and antigenic epitopes were identified. These epitopes which could elicit humoral, as well as cell mediated immunity was linked together with different adjuvants to design a multivalent T cell epitope based peptide vaccine against HCMV. The vaccine construct was predicted to hold appropriate physicochemical characteristics such as solubility and stability. The final full-fledged vaccine construct was found to be antigenic, non-allergenic and had the ability to be cloned in an expression vector for large scale production. Furthermore, molecular docking and molecular dynamics simulation studies suggest that the vaccine construct can stably interact with Toll like receptor molecule to activate the immune response. The *in silico* experiments performed in this study suggest that the current vaccine construct can be a safe and potent therapy against HCMV infections. However, the claims made in this study needs to be validated by further *in vivo* studies.

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.

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Ethical standards & approval

The authors did not perform any experiments on human or animals.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Author's contribution

NA, JS and VK conceived the study. NA, AJ and VK performed the computational work. NA and JS wrote the manuscript. All the authors read and approved the final manuscript.

References

[1] U.K. Adhikari, M. Tayebi, M.M. Rahman, Immunoinformatics Approach for Epitope-Based Peptide Vaccine Design and Active Site Prediction against Polyprotein of Emerging Oropouche Virus, J. Immunol. Res. 2018 (2018) 6718083, https://doi.org/10.1155/2018/6718083.
[2] F. Agostini, D. Cirillo, C.M. Livi, R. Delli Ponti, G.G. Tartaglia, cc SOL omics : a webserver for solubility prediction of endogenous and heterologous expression inEscherichia coli, Bioinformatics 30 (2014) 2975–2977, https://doi.org/10.1093/bioinformatics/btu420.
[3] N. Akhtar, A. Joshi, V. Kaushik, M. Kumar, M.A. Mannan, In-silico design of a multivalent epitope-based vaccine against Candida auris, Microb. Pathog. 155 (2021), https://doi.org/10.1016/j.micpath.2021.104879.
[4] N. Akhtar, A. Joshi, B. Singh, V. Kaushik, Immuno-Informatics Quest against COVID-19/SARS-COV-2: Determining Putative T-Cell Epitopes for Vaccine Prediction, Infect Disord. Drug Targets (2020), https://doi.org/10.2174/1871526520666200921154149.
[5] S.F. Altschul, J.C. Wootton, E.M. Gertz, R. Agarwala, A. Morgulis, A.A. Schäffer, Y. K. Yu, Protein database searches using compositionally adjusted substitution matrices, FEBS J. 272 (2005) 5101–5109, https://doi.org/10.1111/j.1742-4658.2005.04945.x.
[6] C.R. Alving, G.R. Matyas, O. Torres, R. Jalah, Z. Beck, Adjuvants for vaccines to drugs of abuse and addiction, Vaccine. 32 (2014) 5382–5389, https://doi.org/10.1016/j.vaccine.2014.07.085.
[7] K.M. Anderholm, C.J. Bierle, M.R. Schleiss, Cytomegalovirus Vaccines: Current Status and Future Prospects, Drugs 76 (2016) 1625–1645, https://doi.org/10.1007/s40265-016-0653-5.
[8] M. Andreatta, M. Nielsen, Gapped sequence alignment using artificial neural networks: application to the MHC class I system, Bioinformatics 32 (2015) 511–517, https://doi.org/10.1093/bioinformatics/btv639.
[9] I. Baraniak, B. Kropff, L. Ambrose, M. McIntosh, G.R. McLean, S. Pichon, C. Atkinson, R.S.B. Milne, M. Mach, P.D. Griffiths, M.B. Reeves, Protection from cytomegalovirus viremia following glycoprotein B vaccination is not dependent on neutralizing antibodies, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 6273–6278, https://doi.org/10.1073/pnas.1800224115.
[10] T. Ben-Yedidia, R. Arnon, Epitope-based vaccine against influenza, Expert Rev. Vaccines 6 (2007) 939–948, https://doi.org/10.1586/14760584.6.6.939.
[11] K.J. Bowers, E. Chow, H. Xu, R.O. Dror, M.P. Eastwood, B.A. Gregersen, J.L. Klepeis, I. Kolossvary, M.A. Moraes, F.D. Sacerdoti, J.K. Salmon, Y. Shan, D.E. Shaw, Scalable Algorithms for Molecular Dynamics Simulations on Commodity Clusters, Proceedings of the ACM/IEEE Conference on Supercomputing (SC06), Tampa, Florida, 2006, https://doi.org/10.1109/SC.2006.54
[12] D.W.A. Buchan, D.T. Jones, The PSIPRED Protein Analysis Workbench: 20 years on, Nucleic Acids Res. 47 (2019) W402–W407, https://doi.org/10.1093/nar/gkz297.
[13] H.H. Bui, J. Sidney, K. Dinh, S. Southwood, M.J. Newman, A. Sette, Predicting population coverage of T-cell epitope-based diagnostics and vaccines, BMC Bioinformat. 7 (2006) 153, https://doi.org/10.1186/1471-2105-7-153.
[14] T. Crough, R. Khanna, Immunobiology of Human Cytomegalovirus: from Bench to Bedside, Clin. Microbiol. Rev. 22 (2009) 76–98, https://doi.org/10.1128/CMR.00034-08.
[15] S.K. Dhanda, P. Vir, G.P.S. Raghava, Designing of interferon-gamma inducing MHC class-II binders, Biol. Direct. 8 (2013) 30, https://doi.org/10.1186/1745-6150-8-30.
[16] I.A. Doytchinova, D.R. Flower, VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines, BMC Bioinformat. 8 (2007) 4, https://doi.org/10.1186/1471-2105-8-4.
[17] W.L. Drew, Cytomegalovirus Resistance Testing: Pitfalls and Problems for the Clinician, Clin. Infect. Dis. 50 (2010) 733–736, https://doi.org/10.1086/650463.
[18] M. Foglierini, J. Marcandalli, L. Perez, HCMV envelope glycoprotein diversity demystified, Front. Microbiol. 10 (2019) 1005, https://doi.org/10.3389/fmicb.2019.01005.
[19] V. Forstnerič, K. Ivičak-Kocjan, T. Plaper, R. Jerala, M. Benčina, The role of the C-terminal D0 domain of flagellin in activation of Toll like receptor 5, PLOS Pathog. 13 (2017), https://doi.org/10.1371/journal.ppat.1006574 e1006574.
[20] K.B. Fowler, S.B. Boppana, Congenital cytomegalovirus infection, Semin Perinatol. 42 (2018) 149–154, https://doi.org/10.1053/j.semperi.2018.02.002.
[21] E. Gasteiger, C. Hoogland, A. Gattiker, S. Duvaud, M.R. Wilkins, R.D. Appel, A. Bairoch, Protein Identification and Analysis Tools on the ExPASy Server, in: The Proteomics Protocols Handbook, Humana Press, 2005, pp. 571–607, https://doi.org/10.1385/1-59259-890-0:571.
[22] P. Griffiths, I. Baraniak, M. Reeves, The pathogenesis of human cytomegalovirus, J. Pathol. 235 (2015) 288–297, https://doi.org/10.1002/path.4437.
[23] A. Grote, K. Hiller, M. Scheer, R. Münch, B. Nörtemann, D.C. Hempel, D. Jahn, JCat: a novel tool to adapt codon usage of a target gene to its potential expression host, Nucleic Acids Res. 33 (2005) W526–W531, https://doi.org/10.1093/nar/gki376.

[24] P. Guan, I.A. Doytchinova, C. Zygouri, D.R. Flower, MHCPred: a server for quantitative prediction of peptide–MHC binding, Nucleic Acids Res. 31 (2003) 3621–3624, https://doi.org/10.1093/nar/gkg510.
[25] S. Gupta, P. Kapoor, K. Chaudhary, A. Gautam, R. Kumar, G.P.S. Raghava, In Silico Approach for Predicting Toxicity of Peptides and Proteins, PLoS One 8 (2013), https://doi.org/10.1371/journal.pone.0073957 e73957.
[26] S.K. Gupta, P. Bajwa, R. Deb, M.M. Chellappa, S. Dey, Flagellin a toll-like receptor 5 agonist as an adjuvant in chicken vaccines, Clin Vaccine Immunol. 21 (2014) 261–270, https://doi.org/10.1128/CVI.00669-13.
[27] M. Hasan, S. Islam, S. Chakraborty, A.H. Mustafa, K.F. Azim, Z.F. Joy, M.N. Hossain, S.H. Foysal, M.N. Hasan, Contriving a chimeric polyvalent vaccine to prevent infections caused by herpes simplex virus (type-1 and type-2): an exploratory immunoinformatic approach, J. Biomol. Struct. Dyn., 38, 2019, pp. 2898–2915, https://doi.org/10.1080/07391102.2019.1647286.
[28] M.K. Isaacson, T. Compton, Human Cytomegalovirus Glycoprotein B Is Required for Virus Entry and Cell-to-Cell Spread but Not for Virion Attachment, Assembly, or Egress, J. Virol. 83 (2009) 3891–3903, https://doi.org/10.1128/jvi.01251-08.
[29] K.K. Jensen, M. Andreatta, P. Marcatili, S. Buus, J.A. Greenbaum, Z. Yan, A. Sette, B. Peters, M. Nielsen, Improved methods for predicting peptide binding affinity to MHC class II molecules, Immunology 154 (2018) 394–406, https://doi.org/10.1111/imm.12889.
[30] M.J. Jiménez-Dalmaroni, M.E. Gerswhin, I.E. Adamopoulos, The critical role of toll-like receptors - From microbial recognition to autoimmunity: A comprehensive review, Autoimmun. Rev. 15 (2016) 1–8, https://doi.org/10.1016/j.autrev.2015.08.009.
[31] A. Joshi, V. Kaushik, In-Silico Proteomic Exploratory Quest: Crafting T-Cell Epitope Vaccine against Whipple's Disease Online publication ahead of print, Int. J. Peptide Res. Therap., 1–11, 2020, https://doi.org/10.1007/s10989-020-10077-9.
[32] A. Joshi, B.C. Joshi, M.A. Mannan, V. Kaushik, A). Epitope based vaccine prediction for SARS-COV-2 by deploying Immuno-informatics approach [published online ahead of print, 2020 Apr 29], Inform. Med. Unlocked. 2020 (19) (2020) 100338, https://doi.org/10.1016/j.imu.2020.100338.
[33] W. Kabsch, C. Sander, Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features, Biopolym.: Original Res. Biomol. 22 (1983) 2577–2637.
[34] S. Kang, H.M. Brown, S. Hwang, Direct antiviral mechanisms of interferon-gamma, Immune Netw. 18 (2018), https://doi.org/10.4110/in.2018.18.e33 e33.
[35] V. Kaushik, In Silico Identification of Epitope-Based Peptide Vaccine for Nipah Virus, Int J Pept Res Ther. 26 (2020) 1147–1153, https://doi.org/10.1007/s10989-019-09917-0.
[36] T. Kirby, Congenital cytomegalovirus-a neglected health problem, Lancet Infect Dis. 16 (2016) 900–901, https://doi.org/10.1016/S1473-3099(16)30226-2.
[37] S. Kumar, R. Sunagar, E. Gosselin, Bacterial protein toll-like-receptor agonists: A novel perspective on vaccine adjuvants, Front Immunol. 10 (2019) 1144, https://doi.org/10.3389/fimmu.2019.01144.
[38] S. Krishnan, A. Joshi, N. Akhtar, V. Kaushik, Immunoinformatics designed T cell multi epitope dengue peptide vaccine derived from non structural proteome, Microb Pathog. 150 (2021), https://doi.org/10.1016/j.micpath.2020.104728 104728.
[39] S. Landolfo, M. Gariglio, G. Gribaudo, D. Lembo, The human cytomegalovirus, Pharmacol Ther. 98 (2003) 269–297, https://doi.org/10.1016/S0163-7258(03)00034-2.
[40] W. Liu, Y. Xie, J. Ma, X. Luo, P. Nie, Z. Zuo, U. Lahrmann, Q. Zhao, Y. Zheng, Y. Zhao, Y. Xue, J. Ren, IBS: an illustrator for the presentation and visualization of biological sequences, Bioinformatics 31 (2015) 3359–3361, https://doi.org/10.1093/bioinformatics/btv362.
[41] S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson, D.C. Richardson, Structure Validation by Calpha Geometry: Phi, psi and Cbeta Deviation, Proteins 50 (2003) 437–450, https://doi.org/10.1002/PROT.10286.
[42] Y. Lu, J.R. Swartz, Functional properties of flagellin as a stimulator of innate immunity, Sci. Rep. 6 (2016) 18379, https://doi.org/10.1038/srep18379.
[43] N. Madhav, B. Oppenheim, M. Gallivan, P. Mulembakani, E. Rubin, N. Wolfe, Pandemics: Risks, Impacts, and Mitigation, in: D.T. Jamison (Ed.), Disease Control Priorities, Third Edition (Volume 9): Improving Health and Reducing Poverty, The World Bank, Washington (DC), 2017.
[44] C.N. Magnan, A. Randall, P. Baldi, SOLpro: accurate sequence-based prediction of protein solubility, Bioinformatics 25 (2009) 2200–2207, https://doi.org/10.1093/bioinformatics/btp386.
[45] D.J. Marciani, Vaccine adjuvants: Role and mechanisms of action in vaccine immunogenicity, Drug Discov. Today. 8 (2003) 934–943, https://doi.org/10.1016/S1359-6446(03)02864-2.
[46] M. Nardelli, Parst: A system of fortran routines for calculating molecular structure parameters from results of crystal structure analyses, Comput. Chem. 7 (1983) 95–98, https://doi.org/10.1016/0097-8485(83)85001-3.
[47] M. Negahdaripour, M. Eslami, N. Nezafat, N. Hajighahramani, M.B. Ghoshoon, E. Shoolian, A. Dehshahri, N. Erfani, M.H. Morowvat, Y. Ghasemi, A novel HPV prophylactic peptide vaccine, designed by immunoinformatics and structural vaccinology approaches, Infect. Genet. Evol. 54 (2017) 402–416, https://doi.org/10.1016/j.meegid.2017.08.002.
[48] C.S. Nelson, T. Huffman, J.A. Jenks, E.C. De La Rosa, G. Xie, N. Vandergrift, R.F. Pass, J. Pollara, S.R. Permar, HCMV glycoprotein B subunit vaccine efficacy mediated by nonneutralizing antibody effector functions, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 6267–6272, https://doi.org/10.1073/pnas.1800177115.
[49] P. Oyarzún, B. Kobe, Recombinant and epitope-based vaccines on the road to the market and implications for vaccine design and production, Hum. Vaccines Immunother. 12 (2016) 763–767, https://doi.org/10.1080/21645515.2015.1094595.
[50] R.K. Pandey, R. Ojha, A. Mishra, V. Kumar Prajapati, Designing B- and T-cell multi-epitope based subunit vaccine using immunoinformatics approach to control Zika virus infection, J. Cell. Biochem. 119 (2018) 7631–7642, https://doi.org/10.1002/jcb.27110.
[51] B.G. Pierce, K. Wiehe, H. Hwang, B.H. Kim, T. Vreven, Z. Weng, ZDOCK server: interactive docking prediction of protein–protein complexes and symmetric multimers, Bioinformatics 30 (2014) 1771–1773, https://doi.org/10.1093/bioinformatics/btu097.
[52] S. Saha, G.P.S. Raghava, AlgPred: prediction of allergenic proteins and mapping of IgE epitopes, Nucleic Acids Res. 34 (2006) W202–W209, https://doi.org/10.1093/nar/gkl343.
[53] S.B. Sayed, Z. Nain, M.S.A. Khan, F. Abdulla, R. Tasmin, U.K. Adhikari, Exploring Lassa Virus Proteome to Design a Multi-epitope Vaccine Through Immunoinformatics and Immune Simulation Analyses, Int J Pept Res Ther. 26 (2020) 2089–2107, https://doi.org/10.1007/s10989-019-10003-8.
[54] M.R. Schleiss, Recombinant cytomegalovirus glycoprotein B vaccine: Rethinking the immunological basis of protection, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 6110–6112, https://doi.org/10.1073/pnas.1806420115.
[55] D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, H.J. Wolfson, PatchDock and SymmDock: servers for rigid and symmetric docking, Nucleic Acids Res. 33 (2005) W363–W367, https://doi.org/10.1093/nar/gki481.
[56] V. Schottstedt, J. Blumel, R. Burger, C. Drosten, A. Groner, L. Gurtler, M. Heiden, M. Hilderbrandt, B. Jansen, T. Montag-Lessing, R. Offergeld, G. Pauli, R. Seitz, U. Schlenkrich, J. Strobel, H. Willkommen, C.W. von Konig, Human Cytomegalovirus (HCMV)-Revised, Transfus. Med. Hemotherap. 37 (2010) 365–375, https://doi.org/10.1159/000322141.
[57] A. Shanmugam, S. Rajoria, A.L. George, A. Mittelman, R. Suriano, R.K. Tiwari, Synthetic Toll Like Receptor-4 (TLR-4) Agonist Peptides as a Novel Class of Adjuvants, PLoS One 7 (2012), https://doi.org/10.1371/journal.pone.0030839 e30839.
[58] S. Singh, H. Singh, A. Tuknait, K. Chaudhary, B. Singh, S. Kumaran, G.P.S. Raghava, PEPstrMOD: Structure prediction of peptides containing natural, non-natural and modified residues, Biol. Direct 10 (2015) 73, https://doi.org/10.1186/s13062-015-0103-4.
[59] R.E. Soria-Guerra, R. Nieto-Gomez, D.O. Govea-Alonso, S. Rosales-Mendoza, An overview of bioinformatics tools for epitope prediction: Implications on vaccine development, J. Biomed. Inform. 53 (2015) 405–414, https://doi.org/10.1016/j.jbi.2014.11.003.
[60] A. Sukhwal, R. Sowdhamini, Oligomerisation status and evolutionary conservation of interfaces of protein structural domain superfamilies, Mol. Biosyst. 9 (2013) 1652–1661.
[61] M. Tahir Ul Qamar, S. Saleem, U.A. Ashfaq, A. Bari, F. Anwar, S. Alqahtani, Epitope-based peptide vaccine design and target site depiction against Middle East Respiratory Syndrome Coronavirus: An immune-informatics study, J. Transl. Med. 17 (2019) 362, https://doi.org/10.1186/s12967-019-2116-8.
[62] J. Yang, R. Yan, A. Roy, D. Xu, J. Poisson, Y. Zhang, The I-TASSER suite: Protein structure and function prediction, Nat. Methods 12 (2014) 7–8, https://doi.org/10.1038/nmeth.3213.
[63] Z. Yazdani, A. Rafiei, R. Valadan, H. Ashrafi, M.S. Pasandi, M. Kardan, Designing a potent L1 protein-based HPV peptide vaccine: A bioinformatics approach, Comput. Biol. Chem. 85 (2020), https://doi.org/10.1016/j.compbiolchem.2020.107209 107209.
[64] X.Y. Zhang, F. Fang, Congenital human cytomegalovirus infection and neurologic diseases in newborns, Chin. Med. J. (Engl). 132 (2019) 2109–2118, https://doi.org/10.1097/CM9. 0000000000000404.