Journal of Biomedical Informatics 108 (2020) 103498

Contents lists available at ScienceDirect

Journal of Biomedical Informatics

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

Exploring HCV genome to construct multi-epitope based subunit vaccine to battle HCV infection: Immunoinformatics based approach

Hina Khalid, Usman Ali Ashfaq*

Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan

ARTICLE INFO

Keywords:
B cell
T cell
epitope
HCV
Immunoinformatics
Molecular docking

ABSTRACT

Hepatitis C Virus (HCV) infection is a major cause of chronic liver disease, hepatocellular carcinoma, and the single most common indication for liver transplantation. HCV vaccines eliciting specific T-cell responses, have been considered as potent method to prevent HCV infection. Despite several reports on progress of vaccine, these vaccine failed in mediating clinical relevance activity against HCV in humans. In this study we integrated both immunoinformatic and molecular docking approach to present a multiepitope vaccine against HCV by designating 17 conserved epitopes from eight viral proteins such as Core protein, E1, E2, NS2, NS34A, NS4B, NS5A, and NS5B. The epitopes were prioritized based on conservation among epitopes of T cell, B cell and IFN-γ that were then scanned for non-homologous to host and antigenicity. The prioritized epitopes were then linked together by AAY linker and adjuvant (β-defensin) were attached at N-terminal to enhance immunogenic potential. The construct thus formed were subjected to structural modeling and physiochemical characteristics. The modeled structure were successfully docked to antigenic receptor TLR-3 and *In-silico* cloning confers the authenticity of its expression efficiency. However, the proposed construct need to be validate experimentally to ensure its safety and immunogenic profile.

1. Introduction

HCV infection has become serious health issue worldwide as it can lead to chronic liver disease that results in cirrhosis and hepatocellular carcinoma. The current therapeutic regimen for treating HCV includes antiviral agents, sofosbuvir or simeprevir co-administration with ribavirin, and more often pegylated α-interferon (PEG-IFN) [1]. However, these therapies is associated with various limitations such as drug resistances, high cost and failure of protection from relapse [2]. Thus there is an intense need to develop efficacious and safe vaccine for preventative approach of global HCV pandemic.

HCV belongs to a family of Flaviviridae having positive orientation of RNA genome (single stranded) of about 9.6 kb flanked by 5′ untranslated region (UTR) that comprises of highly structured internal ribosomal entry site (IRES), a key element for translation that encodes for 3010 amino acids of single polyprotein precursor which subsequently cleave by proteases in virus and host to yield mature nonstructural (NS2, NS3, NS4A, NS4B, NS5A & NS5B) and structural (Core, E1, E2) proteins [3]. It is reported that 30% of HCV subjects can eliminate the virus during the acute phase which is ascribed to specific and strong host immune responses [4]. The immune response occurs partly due to release of neutralizing antibodies and CD8+, CD4+ T-cells activation against HCV proteins which subsequently release various proinflammatory cytokines (Th1-type) such as interferon-γ (IFN-γ) that activates antiviral mechanism against HCV and diminish viral load following acute infection [5]. In HCV chronic subjects, the delayed response of antibodies and T-cell activation has been noticed [6]. These observation has been noticed in model organism chimpanzees and human that mount an innate immunity subjected to viral clearance. This phenomenon fostered a study to enhance potency of immune signatures leading to development of comparatively effective vaccine against HCV [5]. However, certain factors including high genetic variation of HCV genome and potent associated with practicing killed or live-attenuated vaccine in clinical trial restrain successful development of HCV vaccine [7].

Several studies are available on discovery of vaccines against rapidly mutating infectious diseases via immunoinformatic approaches such as Chikungunya virus [8], Ebola Virus [9] have yielded promising results while maintaining a defensive approach and therapeutic potency of the developed vaccines candidate. Multiepitope vaccines targeting HIV-1 and influenza are in the process of clinical trials [10]. Furthermore, EMD640744, a multiepitope vaccine developed against advance grade solid tumor has entered phase I clinical trials [11].

Moreover, multiepitope vaccines is advantageous over conventional

* Corresponding author.
E-mail address: ashfaqua@gcuf.edu.pk (U.A. Ashfaq).

https://doi.org/10.1016/j.jbi.2020.103498
Received 16 July 2019; Received in revised form 3 May 2020; Accepted 25 June 2020
Available online 01 July 2020
1532-0464/

vaccines ascribed to safety profile and harboring immunogenic signature such as restricted epitopes of multiple major histocompatibility complex (MHC) I and II- targeted by clones of T-cells [12]. Intriguingly, the of HCV elimination during acute infection is ascribed to strong and sustained response of T cells such as CD4+ and CD8+ that recognizes various polyprotein HCV epitopes [13]. Several reports suggested the crucial role of both CD8+ and CD4+ T cells in spontaneous HCV elimination, particularly the association between alleles of class I (e.g., HLA-B27) with class II alleles (e.g., DRB1/1101) [14].

Thus HCV multiepitope vaccine should harbor immunogenic epitopes that are highly conserved that can evoke effective CD8+, CD4+ T and B-cell responses [15]. Activation of these immune responses represent an ideal phenomenon that can lead to development of ideal therapeutic vaccine that facilitate their induction in infected hepatoma cells that exhibit antiviral activity via secretion of various cytokines, specifically IFN-γ, or by direct killing of infected hepatoma cells [16]. Another molecule that contribute to innate immunity includes TLR3 that activates infection antiviral mechanism during infection and surveillance of infectious dead cells. Furthermore, induction of non-canonical NF-kβ cause production of pro -inflammatory cytokines CXCL10. Intriguingly, the macrophages termed Kupffer cells are present abundantly in liver and could release IFN-β in vitro [17]. The employment of conserved epitopes is significant in inducing HCV-specific immune responses with enhanced effectiveness. Aiming this goal, the study was carried out for identification of putative T-cell epitopes for design of multiepitope vaccine. For this purpose, the retrieval of structural and non-structural protein sequence was done from NCBI database for HCV major genotypes 1 to 7 and retrieved the followed by comprehensive conservational analysis of viral proteins in HCV major genotypes. The consensus sequence were then employed for prediction of MHC molecules (I and II) based on affinity prediction score. These predicted epitopes were subsequently scanned for their shared sequence with B-cell epitopes, conserved regions and IFN-γ-binding epitopes. The vaccine construct was subsequently joined with amino acid stretch of β-defensin at the N-terminal end that act as adjuvant [18]. An adjuvant was added to enhance the immunogenicity of the vaccine construct and for activation of various mediators related to innate and adaptive immunity [19]. Adjuvants tend to enhance vaccine performance by targeting antigen to antigen presenting cells (APCs), releasing cytokine that drive Th1 or Th2 immune responses, inducing cell-mediated immune response, and minimizing the amount of antigen needed for protective immunization [20]. It has been observed that the subunit vaccine is associated with poor immunogenicity which is an obstacle that must be improved to achieve an adequate immune response therefore, the addition of an adjuvant in these vaccines is a crucial element for them to drive appropriate immune responses [21].

The construct was then employed for allergenicity and antigenicity prediction and also evaluating physiochemical features ExPASy–ProtParam tool [22] was used. The secondary structure was also modeled using PSIPRED. The 3D structure was predicted through RaptorX [23] and the GalaxyRefine server was employed for refinement [24]. The model was then evaluated via SAVES server on the basis of ERRAT [25], WHATCHECK [26] and PROCHECK [27]. The structure was subsequently docked with Toll-like receptor (TLR-3) for evaluating ligand and receptor molecular interaction. *In silico* cloning approach was done for the assessment of translation and expression efficiency of vaccine construct

2. Materials and methods

2.1. Data collection

In the primary step of *in silico* vaccine development the protein sequences of both HCV non-structural protein including NS2 with accession numbers (YP_009272684.1, NP_751923.1, YP_009272639.1, YP_009272651.1, YP_009272663.1, YP_001491552.1), NS3/4A with accession numbers (NP_803144.1, YP_009272640.1, YP_009272664.1, YP_009272652.1, YP_001491553.1, YP_009272625.1, YP_009272685.1), NS5A with accession numbers (NP_751927.1, YP_001491556.1, YP_009272655.1, YP_009272667.1, YP_009272688.1, YP_009272643.1, YP_009272628.1), NS5B with accession numbers (NP_751928.1, YP_009272668.1, YP_009272644.1, YP_009272629.1, YP_009272656.1, YP_001491557.1, YP_009272689.1) and structural proteins including Core with accession numbers (YP_009272620.1, YP_009272680.1, NP_751919.1, AFY12176.1, AAB40039.1, YP_009272659.1, YP_009272647.1) envelope E1 with accession numbers (YP_009272621.1, YP_009272681.1, YP_001491550.1, YP_009272648.1, YP_009272660.1

NP_751920.1, ACZ27877.1) and E2 with accession numbers (NP_751921.1, YP_009272637.1, YP_009272649.1, YP_009272661.1, YP_009272622.1, YP_009272682.1, ADK94890.1) were retrieved in FASTA format from NCBI (http://www.ncbi.nlm.nih.gov/protein/) against all major HCV genotypes (1–7). The duplicated sequences were eliminated based on the annotation information available from the National Center for Biotechnology Information [28]. Then these proteins were subjected to the Basic Local Alignment Search Tool (BLAST) against the nonredundant protein database available at Uni-prot (www.uniprot.org/).

2.2. Sequence conservation analysis

In order to find the conserved region across protein sequence of all HCV genotypes, multiple sequence alignment (MSA) were performed for the retrieved sequences using ClustalW [29] and BioEdit v. 7.2.3 [30] were employed for visualization of generated consensus regions from each genotypes (1–7).

2.3. Prediction of T-cell epitope (MHC class I and class II)

The prediction of nine-mer epitopes of MHC class (I and II) for both structural and non-structural protein was achieved using the online server Propred I (http://crdd.osdd.net/raghava/propred1/) [31] and Propred II (http://crdd.osdd.net/raghava/propred/) [31] respectively. The epitopes were selected based on binding to most of alleles predominantly those alleles that participate in HCV elimination or protection. Both server generate a significant result as during calculation it employs a huge dataset of alleles of human leukocyte antigens (HLAs). The threshold value were set to 5% for Proteasome and immune-proteasome for class I allele's prediction.

2.4. Prediction of B-cell epitope

The cells B-lymphocyte, following its interaction with B-cell epitopes differentiate into memory cells and plasma cells specialized for antibody secretion [32]. B-cell epitopes are hydrophilic in nature and exhibit accessibility to flexible regions [33].The The cutoff score > -0.15 was selected for all predicted B-cell epitopes and 20-mer linear B-cell epitopes for the selected viral proteins were predicted via online analysis resources IEDB (http://tools.iedb.org/bcell/). It was predicted via hidden Markov model and a propensity scale method. The residues with score above the threshold (default value is 0.35) are predicted to be part of an epitope. There are two types of B cell epitopes, linear and conformational but linear epitopes were used in vaccine design.

2.5. Prediction of Interferon-γ inducing epitope.

IFN-γ is known to trigger innate immune responses and can directly inhibit viral replication. Moreover, they can directly induce the adaptive immune response by priming cytotoxic T cells and T helper cells. the epitopes of selected HCV protein against IFN-γ were predicted via IFNepitope server (http://crdd.osdd.net/raghava/ifnepitope/) [34].

2.6. Screening nonhuman homologues epitopes

The vaccine candidate should not exhibit homology to human proteome to overcome autoimmunity. To accomplish this, BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) [35] of epitopes was executed against human proteome and those exhibiting identity < 0% were selected for further analysis.

2.7. Antigenic epitopes screening

All T-cell epitopes predicted for all selected viral proteins were scanned and screened out for an overlapping with predicted B-cell and IFN-γ-inducing epitopes. Those screened epitopes were subjected to Antigenicity prediction using the online tool Vaxijen (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) with the cutoff of a score of ≥0.5. Epitopes having an antigenicity greater than this value were to be considered as antigenic in nature. This tool is alignment-free and prediction is entirely based on various physiochemical features of protein [36].

2.8. Allergenicity prediction

One of the most important feature about protein and peptide vaccines is that these molecules should not have to be allergenic. Therefore, the peptide was checked for allergenicity by AllerTOP (https://www.ddg-pharmfac.net/AllerTOP/index.html).

Prediction of various physicochemical properties. The analysis of the multi-epitope vaccine for several physicochemical characters were predicted using ProtParam tool [22]. The ProtParam tool assess various physicochemical properties based on pK values of amino acids involved [37]. The prediction of half-life (in vivo) is done on the basis of N-end rule' which depicts N-terminal amino acids specify the degradation of protein [38]. The protein stability is evaluated by Instability index that states value < 40 accounts for proteins stability and above this are termed unstable proteins. The aliphatic index predicts the occupied volume of the aliphatic side chains.

2.9. Secondary structure prediction

Secondary structure prediction of the multi-epitope vaccine protein was performed using the PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/) [39] which yield prediction on the basis of two feed-forward neural networks. The prediction of first network is used as an input for the second network that executes analysis on output yielded from PSI-BLAST and result in structure refinement [40].

2.10. Tertiary structure prediction

The web based server RaptorX [23] was used for tertiary structure of final multi-epitope candidate. The prediction by RaptorX (http://raptorx.uchicago.edu/) relies on features such as single and multiple-template threading and alignment quality prediction. The tool use template for modeling the structure thereby generate the best aligned structure [41]. The prediction was followed by refinement via freely accessible GalaxyRefine server (http://galaxy.seoklab.org/) [42]. The method relies on potential for enhancing both the global and local structural quality. The refinement encompasses steps including rebuilding and repacking of side chains which are then subjected to molecular dynamics simulation that includes relaxation and repeated structural perturbation, the tri-axial loop closure method was applied to avoid structural perturbation [42].

The SAVES server (https://servicesn.mbi.ucla.edu/SAVES/) was employed to select the best developed model that assessed them using ERRAT [25], WHATCHECK [26] and PROCHECK [27].

2.11. Molecular docking of vaccine candidate with immune receptor

In order for appropriate elicitation of immune response, the interaction between the antigenic molecule and immune receptor molecule is necessary. The molecular docking was performed to analyze interaction between vaccine construct and immune receptor TLR-3(PDB ID: 2A0Z) using an online server GRAMM-X (http://vakser.compbio.ku.edu/resources/gramm/grammx/) that utilize smoothed potentials, refinement stage and knowledge-based scoring. For generation of best docked complex clustering is done by a 320 processor Linux [43]. The structure visualization tool Chimera was employed to analyze the structure of model and to generate illustrative figures [44].

2.12. In silico cloning

To evaluate translation efficiency of cloning and appropriate expression in vector, were analyzed from codon adaptation tool (http://www.jcat.de/Start.jsp). It generates output both in the form of codon adaptive index (CAI) values and graphical representation [45].

3. Results

3.1. Selection of conserved regions of HCV structural and non-structural protein

The exploration of conserved region is extensively employed for the prediction of functionally significant residues in protein sequences [46]. The conserved regions of HCV structural and nonstructural protein were identified which were 5 in core protein, 2 for NS2, 4 for HCV NS3/4A segments, 8 for in NS4B, 7 and 10 regions for NS5A and NS5B respectively, moreover, only 1 region were found to be conserved in E1 (Table 1). The regions in E2 were no more than two amino acid stretches, thus not selected. These regions were subsequently selected for B-cell, T-cell, and IFN-γ epitope prediction.

3.2. Epitopes prediction

The nine-mer epitopes of (MHC-I and II) T cells were predicted using the conserved regions. These were then screened based on high scores and binding to most of alleles such as more specifically, to the alleles involved in HCV clearance or protection including DRBl*ll04, DRB*5701, DRB*5703, DRB1*0701, DQBl*O301, HLA-A*03, DQA*0201, HLA-B*57, HLA-A*68, DRB1*0101, Cw*0102 and HLA-B*27 were screened DRBl*ll04, DQA*0201, DQBl*O301 and HLA-B*27 associated with HCV clearance or protection [47–49]. The purpose of B cell epitope prediction is identification of antigen for invoking humoral immunity. The features for epitope selection includes surface accessibility, hydrophobicity, flexibility, and antigenicity. The epitope had to be accessible for the humoral and the cellular immune systems [50]. Therefore, the epitopes constituting 20-mer and 15-mer B-cell and IFN-γ epitopes respectively were predicted for each viral proteins. Subsequently the epitopes of T-cell common to predicted epitopes of IFN-γ and B-cell were selected. Further, these epitopes analyzed by BLASTp so that none are homologous with human proteins. Those exhibiting homology with human protein were excluded. These epitopes then analyzed for antigenicity and highly antigenic epitopes were considered for further analysis (Table 2).

The epitopes finally selected for analysis exhibit features as T-cells with high score and potentially binding to most of alleles, moreover, sequences have to be overlapping with epitopes of IFN-γ and B-cell, should be non-human homologue and highly antigenic Table 2. Based on above mentioned criteria, only 17 epitopes were selected. Among selected epitopes, three were present in NS2 and NS5B, four were from NS3/4A, NS5B and core protein, 1 in NS5A (Table 2).

The multiepitope vaccine construct constitute total of 17 epitopes that were merged together with the help of a flexible linker AAY. The final

Table 1
Conserved regions among NS2, NS3/4A, NS5A and NS5B, E1 and Core protein.

Conserved regions	Positions
Core protein	
VKFPGGGQIVGGVY	22–35
LPRRGP	37–42
SERSQPRGRRQ	53–63
PGYPWPLY	79–86
GWAGWL	92–97
E1	
GHRMAWD	124–130
NS2 protein	
PYFV	181–183
LPVSAR	271–276
NS3/4A protein	
LHAPTGSGKSTKVP	202–215
VLVLNPSVAATLGFG	225–239
LGIGTVLDQAETAG	301–314
KGGRHLIFCHSKKKCDE	360–376
NS4B protein	
MWNF	54–57
SGIQYLAGLSTLPG	59–72
NILGGW	99–104
PGALVV	178–183
QWMNRLIAFASRGNH	203–217
NS5A protein	
GTFPIN	86–91
GSQLPC	185–190
HITAE	208–218
SSASQLS	229–235
SSMPPLEGE	421–429
GDPDL	431–435
SWST	459–462
NS5B protein	
KKVTFDR	50–56
TTIMAKNEVF	136–145
YGFQYSP	191–197
FSYDTRCFDST	217–227
GYRRCRA	275–281
LVCGDDLV	314–321
RYSAPPGD	345–352
SCSSNVSVA	365–373
YYLTRD	382–387
YLFNWAV	524–530

construct was found to be 213 amino acids in length. Moreover an adjuvant β-defensin, with 45 amino acids (GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK) was incorporated at N-terminal with the help of EAAAK linker. Following incorporation of adjuvant and linkers the final vaccine stretch was found to be 263 amino acids long (Fig. 1).

3.3. Physiochemical properties of the vaccine construct

The ProtParam server was used for prediction of physicochemical features of vaccine candidate. The molecular weight is 29191.87 g/mol and its PI was 9.26, depicting it as basic in nature. The estimated aliphatic index is 77.79 indicating its thermo stability. The estimated half-life of construct was computed to be 30 h in vitro (in mammalian reticulocytes), while it is determined to be > 20 h and > 10 h in *in vivo* (yeast and *Escherichia coli*) respectively. Grand average of hydropathicity (GRAVY) was calculated as −0.200, the value in negative designate its hydrophilic nature that render its interaction with neighboring water molecules.

3.4. Secondary structure prediction

For the prediction of secondary structure, online server PSIPRED was used which predicts secondary structure features based on the

Table 2
Selected 18 Epitopes for T-cell (MHCI and II) overlapping with epitopes of IFN-γ and B-cell among HCV NS2, NS3/4A,NS4B, NS5A, NS5B core and E1 proteins.

Epitopes (MHCI) CORE PROTEIN	Antigenicity score of ≥ 0.5	Epitopes (MHCII) CORE PROTEIN	Antigenicity score of ≥0.5
		WLSPRSRPW	1.4
		VYLPRRGPL	0.6
		LPRRGPLGV	1.3
		VRTRKSERS	1.1
Epitopes (MHCI) E1	**Antigenicity** score of ≥0.5	**Epitopes (MHCII) E1**	**Antigenicity** score of ≥0.5
		WPRDASYGC	1.4
Epitopes (MHCI) NS2		Epitopes (MHCII) NS2	
		WGLLVAEPF	0.6
		YYHLPWGLL	1.8
		YQTYYHLPW	1.0
Epitopes (MHCI) NS3/4A	**Antigenicity** score of ≥0.5	**Epitopes (MHCII) NS3/4A**	**Antigenicity** score of ≥0.5
KKCDETDAL	0.6	ICDECHLGI	0.7
GKSTKVPVL	0.6		
CHLGIGTVL	0.9		
Epitopes (MHCI) NS5A	**Antigenicity** score of ≥0.5	**Epitopes (MHCII) NS5A**	**Antigenicity** score of ≥0.5
ASSASQLSL	0.7		
Epitopes (MHCI) NS5B	**Antigenicity** score of ≥ 0.5	**Epitopes (MHCII) NS5B**	**Antigenicity** score of ≥ 0.5
GNTTCYKAA	0.7	YRRCRAGVT	0.7
		VYTSARKKV	0.2
		LPILSNRNV	1.3

amino acids of the protein sequence. Among 263 amino acids, 114 amino acids participate in formation of α-helix which accounts for 43.35%, 74 is β-strands which is 28.14%, and coils are formed by 75 amino acids which is 28.52% of whole vaccine construct (Fig. 2).

3.5. Tertiary structure prediction and refinement

The 3D structure of the final construct was predicted by use of the RaptorX server. The input given was amino acid sequence which yielded to have three domains and template with PDB ID 3ayfa was used as model. The P-value, calculated for the modeled structure was very low i-e 4.14 × 10–3 which reflects higher quality of the model. All 263 (100%) amino acids were included in modeling and merely 9 (3%) positions were predicted as disordered in the model (Fig. 3A). Furthermore, for the purpose of refinement of the predicted model, it was submitted to GalaxyRefine server. Ramachandran plot generated for vaccine construct, 87.8% of residues lie in the most favored region, 10.4% of amino acids reside in the additional allowed region, 1.3% of amino acids reside in the generously allowed region while 0.4% were displayed in disallowed regions. For further analysis, SAVES server was used that evaluated the constructed models by visualizing through ERRAT [25], WHATCHECK [26] and PROCHECK [27]

3.6. Allergenicity prediction

The result of allergenicity prediction of peptide revealed that these peptides are non-allergen.

3.7. Molecular docking of vaccine candidate with immunological receptor (Toll-like receptor 3)

The activation of immune response requires appropriate interaction between antigenic molecule and immune receptor molecule. The GRAMMX server enabled successful docking of the construct with TLR3. In docked complex, TLR3 is depicted in the blue color, while the construct is depicted in the green color, respectively, in Fig. 4. The intra

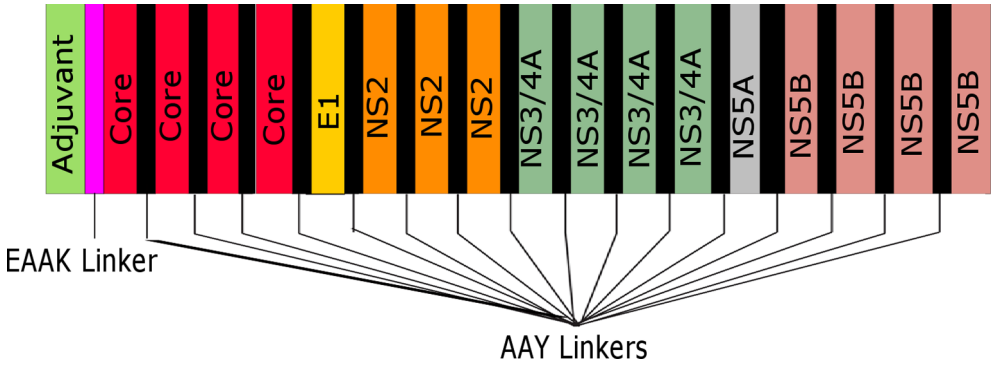

Fig. 1. Schematic illustration of 263 amino acid long multi-epitope vaccine construct combined together by linkers and an adjuvant.

model hydrogen bond includes GLN470 -TYR9, ASN620- TYR158. It was seen the distances of the hydrogen bonds between the construct and TLR3 molecule lie in the range of 2 Å to 3 Å, indicating strong interactions.

3.8. In silico cloning

The cDNA sequence was generated via online server codon adaptation tool followed by analysis for codon optimization that evaluates it on the basis of codon adaptive index (CAI) and GC content. The GC content of the construct was yielded as 69% which resides in the ideal range (30–70%), CAI as 1.00 which lies in the range (0.8–1.0) that depicts high expression of the protein thus potentiating its reliability.

4. Discussion

Vaccination is the most effective prophylactic approach for raising standard of public health as well as controlling spread of infection. The use of computation tools for antibody epitope prediction represent one of the significant phase of designing vaccine [51]. The current advancement aimed for enhancement of conventional assays for quantifying responses of T-cell against various vaccine candidates [52].

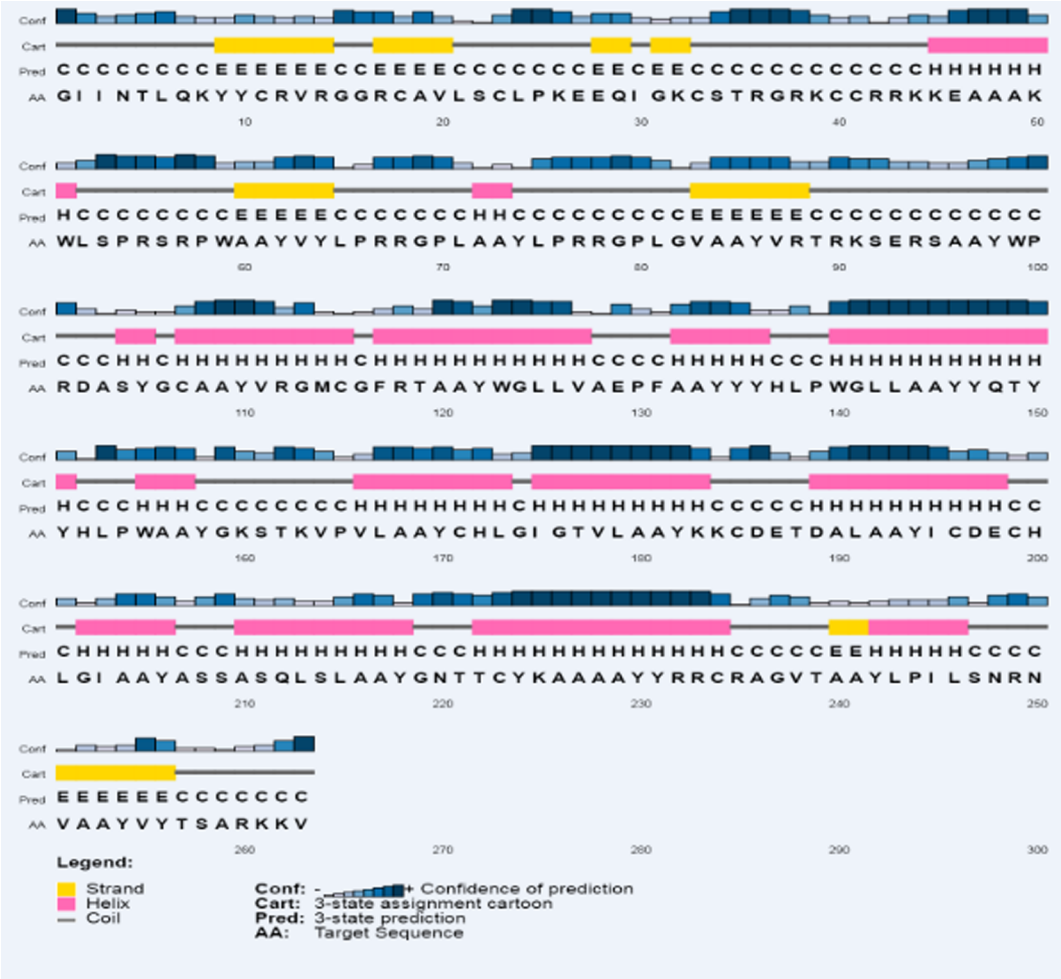

Fig. 2. Schematic illustration of secondary structure of the final vaccine candidate. It depicts the arrangement of α-helix (43.35%), β-strands (28.14%) and coils (28.52%).

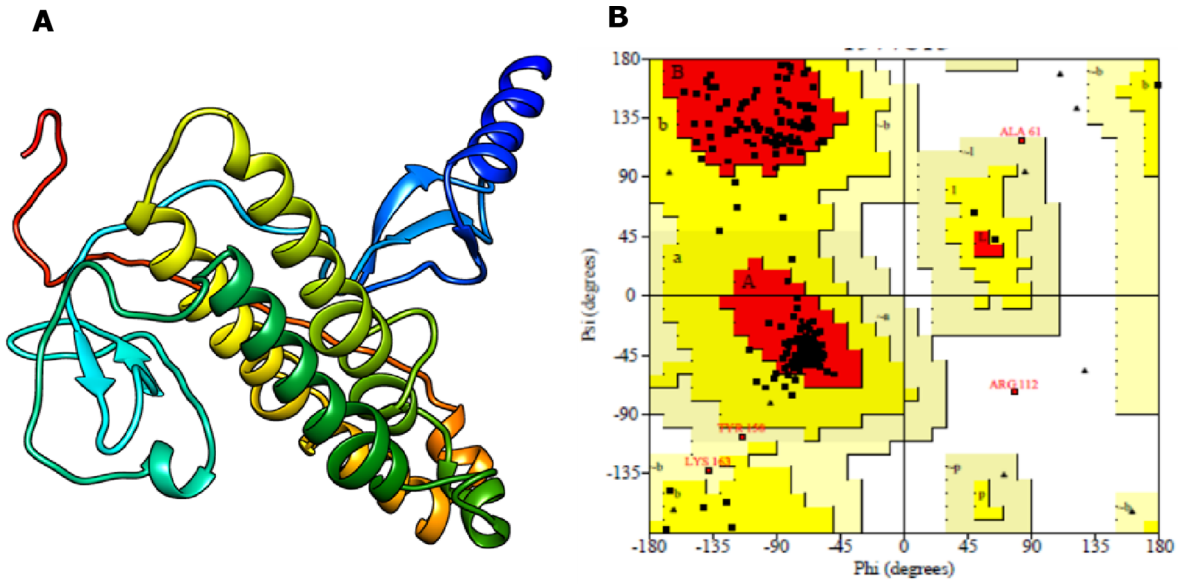

Fig. 3. 3D structure prediction and validation of Multi-epitope vaccine (A) 3D structure of final multi-epitope vaccine candidate. (B) The validation via Ramachandran plot of multi-epitope vaccine 3D structure where 87.8% residues reside in most favored region, 10.4% residues were present in additional allowed region, 1.3% and 0.4% in generously allowed and outlier region respectively.

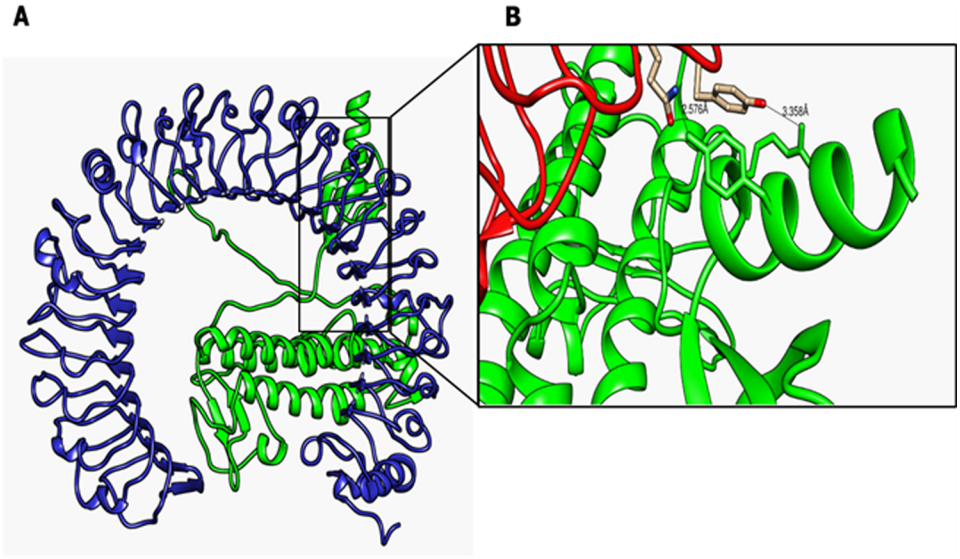

Fig. 4. (A) Vaccine construct-TLR3 docked complex: the receptor TLR3 is shown in blue color while green color is representing final vaccine construct. (B) Illustration highlighting Interacting residues of docked vaccine (chain B) with TLR3 (chain A). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Immunoinformatic approaches act as a milestone in the pace for subunit vaccines regarding prediction of epitopes of B-cell and T-cell [53]. Identification of cytotoxic T-lymphocyte (CTL) and epitopes of B-cell epitopes are crucial steps for subunit vaccine development [54].The design of epitope vaccine construct against tuberculosis, HIV and malaria has yielded promising results while maintaining defensive approach and therapeutic potency of the developed vaccines candidate [55]. Although the massive progress has been occurred in HCV therapy with the advent of DAA, but for the purpose to halt the unstoppable spread of HCV infection fostered intensive need to design epitope based vaccine which is however a unprecedented scientific challenge [16]. So far, the modern strategy of T-cell epitopes based vaccinology yielded successful results against diseases like malaria and cancer and has exhibited the potent immunogenicity regarding activation of T-cell responses [10].

Currently various immunoinformatic approaches have been employed for prediction of $CD4^+$ and $CD8^+$ T-cell epitopes that possesses high potential for binding to different MHC-I and MHC-II molecules. A study by de Freitas e Silva et al. encompasses identification of 10 top-ranked epitopes from the proteome of Leishmania braziliensis via immunoinformatic approach followed by synthesis and assessment in vitro to enable peripheral blood mononuclear cells (PBMC) stimulation in cutaneous leishmaniasis post-treated patients and 50% of these epitopes were revealed to be immunogenic [56]. Another study by Khan et al. involved identification of T-cell epitopes from proteome of Mycobacterium tuberculosis via in silico methods ane proposed the effective response of T-cell during experimental evaluation of the epitopes being identified [57]. These analysis clearly strengthen the potential of immunoinformatic approach in vaccine design or in vitro study.

The current objective of multiepitope T-cell vaccine development includes curbing HCV chronicity and inhibition of reinfection. In this study, we aimed to design multiple-epitope vaccine that may imparts protective immunity against HCV. Multiepitope vaccines are advantageous over monovalent vaccine as it can elicit both cellular and humoral immunities [58]. In the acute course of HCV infection, specific T-cell responses are elicited against nonstructural proteins of HCV, while

the response tends toward HCV structural proteins during chronic infection [59]. Therefore the current study, encompasses both structural and non-structural HCV proteins for multiepitope vaccine design to consider T-cell reactivity relevant to resolution of both acute as well as chronic HCV infection. Briefly, conserved T-cell epitopes were predicted via multiple sequence analysis followed B and IFN-γ epitopes prediction. Once prediction was done T-cell epitopes were screened for overlapping with B and IFN-γ epitopes. The selected epitopes therefore were then analyzed for antigenicity prediction and essentially non-homologous to hosts. The screening of overlapped regions offers advantage of being concomitantly inducing both T-cell and B-cell response. Moreover the vaccine construct capable to invoke IFN-γ response, inducing T-helper cells, render them highly effective in invoking strong immune responses [60]. It is proposed that epitopes that are conserved and non-homologous host specific harbors the potential to circumvent barriers for vaccines that are epitope-based [5]. The addition of adjuvant in the designed multiepitope vaccines was meant to enhance immunogenicity activate various mediators of adaptive and innate immunity [19].

The analysis of physiochemical parameters of the construct depicted it to be basic, stable and hydrophilic, and basic in nature. The validation via Ramachandran plot exhibited the stability of the predicted model. The final construct thus generated was then docked against TLR3 to analyze enough binding to prompt immune response. It have been proposed that TLR3 invoke antiviral mechanism during infection and contribute to surveillance of infectious dead cells [17]. Another study reported that in HCV subjects, agonists for TLR8 and TLR3 downregulated the level of HCV RNA [61,62]. Some of the epitope predicted in our study is the part of specified regions predicted in analysis by Abraham et al., such as HSKKKCDEL and TIMAKNEVF in NS3/4A and NS5B respectively [63].

The study undertaken provides a milestone for development of multiple epitope-based vaccines against HCV infection. The study incorporated both non-structural and structural proteins of HCV for epitope prediction for the purpose of developing epitope-based vaccine for HCV which is novel strategy so far. Furthermore, successful docking of the construct with antigenic receptor significantly enhanced the precision and scope of this study.

5. Conclusion

This study employs multiple approaches including immunoinformatic to design multiepitope vaccine that could be safe and harbors immunogenic potential which may consequently elicit both sort of responses: innate and adaptive thus contribute for control and prevention of HCV. Despite the computational prediction and validation of epitopes, this study warrants further experimental validation. This approach grant a template for the research of other emerging viruses and their subtype.

CRediT authorship contribution statement

Hina Khalid: Investigation, Methodology, First draft Writing. **Usman Ali Ashfaq:** Supervision, conceptualization, Data curation, Methodology, Formal analysis, Project administration, Writing - review & editing.

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.

Acknowledgement

The author Usman Ali Ashfaq acknowledge financial support from Higher Education Commission of Pakistan through 20-5004/NRPU/R&D/HEC/14/264.

References

[1] Y. Wei, J. Li, J. Qing, M. Huang, M. Wu, F. Gao, et al., Discovery of novel hepatitis C virus NS5B polymerase inhibitors by combining random forest, multiple e-pharmacophore modeling and docking, PLoS ONE 11 (2016) e0148181.
[2] C. Sarrazin, S. Zeuzem, Resistance to direct antiviral agents in patients with hepatitis C virus infection, Gastroenterology 138 (2010) 447–462.
[3] R. Bartenschlager, L. Ahlborn-Laake, J. Mous, H. Jacobsen, Kinetic and structural analyses of hepatitis C virus polyprotein processing, J. Virol. 68 (1994) 5045–5055.
[4] C.M. Walker, A. Grakoui, Hepatitis C virus: why do we need a vaccine to prevent a curable persistent infection? Curr. Opin. Immunol. 35 (2015) 137–143.
[5] M. Houghton, S. Abrignani, Prospects for a vaccine against the hepatitis C virus, Nature 436 (2005) 961.
[6] E. Scarselli, H. Ansuini, R. Cerino, R.M. Roccasecca, S. Acali, G. Filocamo, et al., The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus, EMBO J. 21 (2002) 5017–5025.
[7] G. Serviddio, Practical management of chronic viral hepatitis, 2013.
[8] M. Tahir ul Qamar, A. Bari, M.M. Adeel, A. Maryam, U.A. Ashfaq, X. Du, et al., Peptide vaccine against chikungunya virus: immuno-informatics combined with molecular docking approach, J. Transl. Med. 16 (2018) 298.
[9] B. Ahmad, U.A. Ashfaq, M.-u. Rahman, M.S. Masoud, M.Z. Yousaf, Conserved B and T cell epitopes prediction of ebola virus glycoprotein for vaccine development: an immuno-informatics approach, Microbial Pathogenesis 132 (2019) 243–253.
[10] P. Oyarzún, B. Kobe, Recombinant and epitope-based vaccines on the road to the market and implications for vaccine design and production, Hum. Vacc. Immunotherap. 12 (2016) 763–767.
[11] L. Zhang, Multi-epitope vaccines: a promising strategy against tumors and viral infections, Cell. Mol. Immunol. 15 (2018) 182.
[12] W. Li, M. Joshi, S. Singhania, K. Ramsey, A. Murthy, Peptide vaccine: progress and challenges, Vaccines 2 (2014) 515–536.
[13] J. Schmidt, H.E. Blum, R. Thimme, T-cell responses in hepatitis B and C virus infection: similarities and differences, Emerg. Microbes Infect. 2 (2013) 1–8.
[14] D. Miki, H. Ochi, A. Takahashi, C.N. Hayes, Y. Urabe, H. Abe, et al., HLA-DQB1* 03 confers susceptibility to chronic hepatitis C in Japanese: a genome-wide association study, PLoS ONE 8 (2013) e84226.
[15] L.P. Sabet, T. Taheri, A. Memarnejadian, T.M. Azad, F. Asgari, R. Rahimnia, et al., Immunogenicity of multi-epitope DNA and peptide vaccine candidates based on core, E2, NS3 and NS5B HCV epitopes in BALB/c mice, Hepatitis Monthly 14 (2014).
[16] L.M.J. Law, A. Landi, W.C. Magee, D.L. Tyrrell, M. Houghton, Progress towards a hepatitis C virus vaccine, Emerg. Microbes Infect. 2 (2013) e79.
[17] A. Boltjes, D. Movita, A. Boonstra, A.M. Woltman, The role of Kupffer cells in hepatitis B and hepatitis C virus infections, J. Hepatol. 61 (2014) 660–671.
[18] T. Mohan, C. Sharma, A.A. Bhat, D. Rao, Modulation of HIV peptide antigen specific cellular immune response by synthetic α-and β-defensin peptides, Vaccine 31 (2013) 1707–1716.
[19] Y. Perrie, A.R. Mohammed, D.J. Kirby, S.E. McNeil, V.W. Bramwell, Vaccine adjuvant systems: enhancing the efficacy of sub-unit protein antigens, Int. J. Pharm. 364 (2008) 272–280.
[20] F.R. Vogel, Improving vaccine performance with adjuvants, Clin. Infect. Dis. 30 (2000) S266–S270.
[21] D. Christensen, Vaccine adjuvants: why and how, Hum. Vacc. Immunotherap. 12 (2016) 2709–2711.
[22] E. Gasteiger, C. Hoogland, A. Gattiker, M.R. Wilkins, R.D. Appel, A. Bairoch, Protein identification and analysis tools on the ExPASy server. The proteomics protocols handbook, Springer, 2005, pp. 571–607.
[23] J. Peng, J. Xu, RaptorX: exploiting structure information for protein alignment by statistical inference, Proteins Struct. Funct. Bioinf. 79 (2011) 161–171.
[24] J. Ko, H. Park, L. Heo, C. Seok, GalaxyWEB server for protein structure prediction and refinement, Nucl. Acids Res. 40 (2012) W294–W297.
[25] C. Colovos, T.O. Yeates, Verification of protein structures: patterns of nonbonded atomic interactions, Protein Sci. 2 (1993) 1511–1519.
[26] R.W. Hooft, Errors in protein structures, Nature 381 (1996) 272.
[27] R.A. Laskowski, J.A.C. Rullmann, M.W. MacArthur, R. Kaptein, J.M. Thornton, AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR, J. Biomol. NMR 8 (1996) 477–486.
[28] N.R. Coordinators, Database resources of the national center for biotechnology information, Nucl. Acids Res. 41 (2013) D8.
[29] J.D. Thompson, T.J. Gibson, D.G. Higgins, Multiple sequence alignment using ClustalW and ClustalX, Curr. Protocols Bioinformatics (2003) 2.3.1–2.3.22.
[30] T. Hall, BioEdit v 7.2. 3. Biological sequence alignment editor for Win 95/98/NT/2K/XP7. Ibis Biosciences Carlsbad, California, 2013.
[31] H. Singh, G. Raghava, ProPred1: prediction of promiscuous MHC Class-I binding sites, Bioinformatics 19 (2003) 1009–1014.
[32] D.T. Nair, K. Singh, Z. Siddiqui, B.P. Nayak, K.V. Rao, D.M. Salunke, Epitope recognition by diverse antibodies suggests conformational convergence in an antibody response, J. Immunol. 168 (2002) 2371–2382.

[33] L. Potocnakova, M. Bhide, L.B. Pulzova, An introduction to B-cell epitope mapping and in silico epitope prediction, J. Immunol. Res. 2016 (2016).
[34] S.K. Dhanda, P. Vir, G.P. Raghava, Designing of interferon-gamma inducing MHC class-II binders, Biol. Direct. 8 (2013) 30.
[35] R. Lavigne, D. Seto, P. Mahadevan, H.-W. Ackermann, A.M. Kropinski, Unifying classical and molecular taxonomic classification: analysis of the Podoviridae using BLASTP-based tools, Res. Microbiol. 159 (2008) 406–414.
[36] I.A. Doytchinova, D.R. Flower, VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines, BMC Bioinf. 8 (2007) 4.
[37] B. Bjellqvist, G.J. Hughes, C. Pasquali, N. Paquet, F. Ravier, J.C. Sanchez, et al., The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences, Electrophoresis 14 (1993) 1023–1031.
[38] A. Varshavsky, The N-end rule pathway of protein degradation, Genes Cells 2 (1997) 13–28.
[39] L.J. McGuffin, K. Bryson, D.T. Jones, The PSIPRED protein structure prediction server, Bioinformatics 16 (2000) 404–405.
[40] H. Kaur, G.P.S. Raghava, Prediction of β-turns in proteins from multiple alignment using neural network, Protein Sci. 12 (2003) 627–634.
[41] M. Källberg, H. Wang, S. Wang, J. Peng, Z. Wang, H. Lu, et al., Template-based protein structure modeling using the RaptorX web server, Nat. Protoc. 7 (2012) 1511.
[42] L. Heo, H. Park, C. Seok, GalaxyRefine: protein structure refinement driven by side-chain repacking, Nucl. Acids Res. 41 (2013) W384–W388.
[43] A. Tovchigrechko, I.A. Vakser, GRAMM-X public web server for protein–protein docking, Nucl. Acids Res. 34 (2006) W310–W314.
[44] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, et al., UCSF Chimera—a visualization system for exploratory research and analysis, J. Comput. Chem. 25 (2004) 1605–1612.
[45] 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, Nucl. Acids Res. 33 (2005) W526–W531.
[46] J.A. Capra, M. Singh, Predicting functionally important residues from sequence conservation, Bioinformatics 23 (2007) 1875–1882.
[47] M.H. Kuniholm, A. Kovacs, X. Gao, X. Xue, D. Marti, C.L. Thio, et al., Specific human leukocyte antigen class I and II alleles associated with hepatitis C virus viremia, Hepatology 51 (2010) 1514–1522.
[48] A. Mangia, R. Gentile, I. Cascavilla, M. Margaglione, M.R. Villani, F. Stella, et al., HLA class II favors clearance of HCV infection and progression of the chronic liver damage, J. Hepatol. 30 (1999) 984–989.
[49] C.L. Thio, X. Gao, J.J. Goedert, D. Vlahov, K.E. Nelson, M.W. Hilgartner, et al., HLA-Cw* 04 and hepatitis C virus persistence, J. Virol. 76 (2002) 4792–4797.
[50] A. Ikram, T. Zaheer, F.M. Awan, A. Obaid, A. Naz, R. Hanif, et al., Exploring NS3/4A, NS5A and NS5B proteins to design conserved subunit multi-epitope vaccine against HCV utilizing immunoinformatics approaches, Sci. Rep. 8 (2018) 1–14.
[51] K.K. Dubey, G.A. Luke, C. Knox, P. Kumar, B.I. Pletschke, P.K. Singh, et al., Vaccine and antibody production in plants: developments and computational tools, Briefings Funct. Genomics 17 (2018) 295–307.
[52] P. Ip, H. Nijman, T. Daemen, Epitope prediction assays combined with validation assays strongly narrows down putative cytotoxic T lymphocyte epitopes, Vaccines 3 (2015) 203–220.
[53] G.M. Gillespie, M.R. Wills, V. Appay, C. O'Callaghan, M. Murphy, N. Smith, et al., Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8+ T lymphocytes in healthy seropositive donors, J. Virol. 74 (2000) 8140–8150.
[54] M.H. Van Regenmortel, Mapping epitope structure and activity: from one-dimensional prediction to four-dimensional description of antigenic specificity, Methods 9 (1996) 465–472.
[55] A.M. Khan, O. Miotto, A. Heiny, J. Salmon, K. Srinivasan, E.J. Nascimento, et al., A systematic bioinformatics approach for selection of epitope-based vaccine targets, Cell. Immunol. 244 (2006) 141–147.
[56] L.F.G.R. Ferreira, M.Z. Hernandes, M.E.F. de Brito, B.C. de Oliveira, A.A. da Silva, O.P. de-Melo-Neto, et al., Combination of in silico methods in the search for potential CD4+ and CD8+ T cell epitopes in the proteome of Leishmania braziliensis, Front. Immunol. 7 (2016) 327.
[57] M.K. Khan, S. Zaman, S. Chakraborty, R. Chakravorty, M.M. Alam, T.R. Bhuiyan, et al., In silico predicted mycobacterial epitope elicits in vitro T-cell responses, Mol. Immunol. 61 (2014) 16–22.
[58] I.J. Amanna, M.K. Slifka, Contributions of humoral and cellular immunity to vaccine-induced protection in humans, Virology 411 (2011) 206–215.
[59] J.T. Blackard, M.T. Shata, N.J. Shire, K.E. Sherman, Acute hepatitis C virus infection: a chronic problem, Hepatology 47 (2008) 321–331.
[60] B. Latimer, R. Toporovski, J. Yan, P. Pankhong, M.P. Morrow, A.S. Khan, et al., Strong HCV NS3/4a, NS4b, NS5a, NS5b-specific cellular immune responses induced in Rhesus macaques by a novel HCV genotype 1a/1b consensus DNA vaccine, Hum. Vacc. Immunotherap. 10 (2014) 2357–2365.
[61] N. Wang, Y. Liang, S. Devaraj, J. Wang, S.M. Lemon, K. Li, Toll-like receptor 3 mediates establishment of an antiviral state against hepatitis C virus in hepatoma cells, J. Virol. 83 (2009) 9824–9834.
[62] C.-H. Wang, H.-L. Eng, K.-H. Lin, C.-H. Chang, C.-A. Hsieh, Y.-L. Lin, et al., TLR7 and TLR8 gene variations and susceptibility to hepatitis C virus infection, PLoS ONE 6 (2011) e26235.
[63] M. Molero-Abraham, E.M. Lafuente, D.R. Flower, P.A. Reche, Selection of conserved epitopes from hepatitis C virus for pan-populational stimulation of T-cell responses, Clin. Develop. Immunol. 2013 (2013).