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Therapeutic Multi-Epitope Vaccine Construction Using in Silico Strategies Against Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma is a chronic infection with progressing symptoms leading to an advanced stage with no effective therapy other than liver transplantation. Recent studies suggest that drugs such as sorafenib and lenvatinib however can provide some relief to the disease but have failed to provide complete protection. Thus considering all the facts, this study is conducted with an immunoinformatics approach targeting XIAP protein which is the key protein involved in the mechanism of Hepatocellular carcinoma (HCC) disease. The screening of the targeted genome sequence was performed to acquire CTL, HTL, and B-cell epitopes through different tools such as NetMHCPred, NetMHCPan, and BepiPred tool leading to the potential candidates for multi-epitope vaccine. Meanwhile, we also found the physiochemical, immunogenic and allergenicity scores of the filtered epitopes and these epitopes were found to be highly antigenic and immunogenic after validation of all the parameters. Meanwhile, the addition of adjuvant and linkers led to the formation of a strong vaccine construct and therefore the binding force between the final vaccine construct with protein through docking obtained was -905.7kcal/mol. Further in silico cloning and expression of the designed vaccine into the plasmid vector also depicted the good performance of designed vaccine thus making it a good choice for the novel vaccine. Henceforth, a potential therapeutic multi-epitope vaccine construct was found to be efficient and thus can be a promising vaccine that could induce strong immunity against Hepatocellular carcinoma.

Keywords: Immunoinformatics , Molecular docking, Simulation, in silico, epitope

Introduction

Hepatocellular Carcinoma (HCC) is the leading form of liver cancer that causes mortality globally constituting major tumor of the liver. The estimated number of death is more than 7 lakh annually and according to recent data 661000 cases of hepatocellular carcinoma were recorded in 2018 and the incidence of HCC cases is expected to increase between 2018 and 2030 according to World Health Organisation (Abbas et al. 2020). Rising HCC incidence cases occur in developing countries such as Sub-Sahara Africa, Southeast Asia, Asia, and China thus leading to a major concern in India (Tojjari et al. 2023). The Risk factors for HCC include chronic HBV (hepatitis B virus) and HCV (hepatitis C virus) infections, autoimmune hepatitis, chronic alcohol use, diabetes mellitus, and obesity. Thus, Hepatitis B and C infections primarily lead to progressive hepatic damage in patients and cause hepatocellular carcinoma (Tremosini et al. 2012). The HCC shows higher susceptibility in males and children compared to females and adults above 70 years of age. The major complications in patients of HCC indicate hepatic encephalopathy, portal vein thrombosis, worsening ascites, variceal bleeding, obstructive jaundice, abdominal pain, hypotension, and anemia and pyogenic liver abscess (Roayaie et al. 2015). At present, the treatment choices include liver transplantation and chemotherapy in the early stage of detection so there is an urgent and effective therapeutic therapy against hepatocellular carcinoma is required. In recent studies, peptide-based vaccines have emerged as functional cures in targeting the proteins that are involved in causing any particular disease thereby inhibiting their mode of action (Fariya et al. 2019).
Also, there is an urgent need to identify novel therapeutic candidates that can be used to inhibit the growth of HCC. X-linked inhibitor of apoptosis protein (XIAP) is a member of the inhibitor of apoptosis protein (IAP) family that is capable of neutralizing caspase-9 via the BIR3 domain that leads to the apoptosis deficiency in the cells which in turn causes cancer(Lencioni et al. 2016). Therefore, targeting inhibitors of XIAP would be an excellent treatment option for different cancer diseases including hepatocellular carcinoma (Wang et al. 2014). Despite the advances in this area, the molecular mechanism of hepatocellular carcinoma is still not completely understood (Harding et al. 2018). In silico approaches in designing the potential vaccine construct are widely used and specific targets against XIAP protein can be evaluated and designed. Recent literature depicts about the implementation of an epitope-based computational approach in designing vaccine constructs for diseases like Dengue (Gupta et al., 2020). Nowadays, immunoinformatics approaches like three-

dimensional modeling, virtual screening, population coverage analysis, molecular docking and dynamic simulation approaches are widely used to discover and develop, therapeutic candidates (Fariya et al., 2021; Dubey et al., 2024). *In silico* data will be helpful for drug and vaccine development for the treatment of HCC after wet lab experimentation.

Materials and Methods

A schematic representation of the steps involved in designing the epitope-based vaccine construct is reflected in the below flowchart in Fig.1

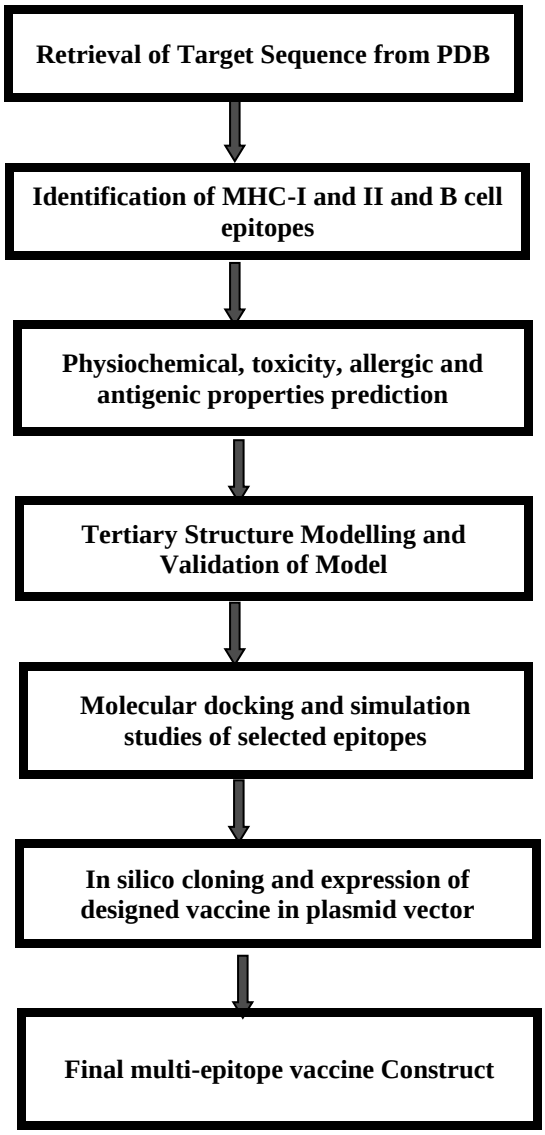

Figure 1. Schematic representation of flowchart of the steps towards the development of epitope based vaccine

Acquisition of Amino acid sequences of target protein

The amino acid sequences and the crystal structure of XIAP protein with PDB ID: 5OQW can be retrieved from the Protein Data Bank database (PDB). For the complex interaction analyses, the structure of protein can be visualized with UCSF Chimera (Petterson et al., 2004) and Discovery Studio Visualizer to understand the insights of the structure. Epitope-based vaccine designing using the protein structure of the target has been efficient massively in the past few years for other diseases also that have several serotypes such as Dengue virus (Nishat and Ajay 2023).

MHC Class I and II and B cell epitope prediction

This study involves the prediction of B cell epitope as B-cell prediction is very important step in order to design an efficient epitope based vaccine. B- cell epitopes can trigger humoral immunity therefore different tools such as ABCPred Saha and Raghava 2006), BepiPred 2.0 (Jespersen et al., 2017), and SVMTrip tools (B. Yao et al., 2012) were employed. The CTL epitopes binding to MHC-I alleles can be predicted through the NetMHCPred tool and binding score above a threshold value can be checked. The HTL epitope binding to their respective MHC class-II alleles can be identified through the NetMHCPan tool and the best epitopes with the binding energy can be noted.

Allergenicity, Toxicity, and Antigenicity prediction of the selected epitopes
Epitopes that scored the best predicted through rigorous methods were presented for allergenicity, and antigenicity check through AllergenFP and VaxiJen tool (Irini and Darren 2007), respectively. The best epitopes with the better prediction results can be finalized for vaccine construction.

Physiochemical properties prediction of the epitopes and construction of Multi-epitope vaccine construct
The physicochemical properties of all the selected CTL, HTL, and B-cell epitopes were checked through the ProtParam tool (Gasteiger et al., 2005) to fetch insight information about the potential candidate. All the selected epitopes will be linked together by using adjuvant and linkers to make it more immunogenic therefore in our study, four linkers EAAK, KK, AAY, and GPGPG have been used. The vaccine construct should be capable enough of fulfilling all the parameters in order to create the best-developed vaccine.

Tertiary Structure Modelling
The three-dimensional structure of protein plays an important role in checking the functionality of the protein therefore the 3-D structuring of the peptides was designed using trrosetta tool (Singh et al., 2015).. The quality of the model can be checked through the Ramachandran Plot using the RAMPAGE server (Laskowski et al. 2006). Ramachandran Plot can check the quality of the model in order to be use for docking studies and only the models above the threshold value can be selected for further analysis.

Molecular Docking Studies
The docking study is the prime approach to analyzing the binding affinity of the protein and peptide interactions. Here, in the present study we have used ClusPro tool to calculate the interactions between them and how well they form a complex. This docking tool generates the 10 protein-peptide complex with the best binding energies and the best complex with the highest score can be selected.

Normal Mode Analysis
After the docking studies of the multi-epitope vaccine construct, we performed Normal Mode Analysis (NMA) to check the motion in the internal coordinates of the complex generated using the iMODS server. This tool generates flexible transition pathways while maintaining the stereochemistry of the structure and calculates the backbone atoms of complex structures with a maximum execution time of 10 minutes. This is efficient in understanding the flexibility of each residue in the complex structure.

Molecular Dynamic simulation
To check the stability of the complex, the Molecular Dynamic simulation was performed for the multi-epitope construct using the CABS-flex 2.0 server. This server used the CABS model that generates dynamics of the complex structure at 10ns along with the detailed fluctuation plot of the residues. It takes the input file in PDB format along and gives the output of all the best conformations generated through trajectory analysis using the k-medoids algorithm and optimization.

Results:

Retrieval of Amino Acid sequences of target protein
The amino acid sequences and the crystal structure of XIAP target protein can be retrieved from Protein Data Bank with PDBID: 5OQW. It contains 127 numbers of amino acid sequences that can be downloaded in Pdb format in our present work. The structure can be visualized with the help of Discovery Studio visualise and the interaction between the residues can be observed.

T-cell and B-cell epitope prediction
A total of 15 CTL epitopes were identified using the NetMHCPred tool and were further scrutinized with their antigenic parameters(Table 1). Further, HTL epitopes were noted with their binding score and their antigenic properties were checked (Table 2). We found a total number of 45 B-cell epitopes were identified from the target protein sequence and were displayed in Table 3. Another BepiPred tool was used to identify more epitopes of varying lengths on the basis of cut-off score of 0.51(Table 4). Therefore, in order to obtain the best epitopes that can have a robust role in providing better immunity the common epitopes identified through the SVMTrip tool were finalized. Hence, we obtained a total of 10 epitopes for further analysis in designing towards epitope vaccine construct (Table 5).

Table 1: Prediction of CTL epitopes binding to MHC-I Allele through NetMHCPred tool

Peptide	Allele	Position	Predicted binding Score	Antigenic score	Grand average of hydropathicity (GRAVY) value (+/-)	Alipatic Index	Instability Index
VVDISDTIY	HLA-A*01:01	145	0.660	1.5664	0.844	151.11	-24.51
SMADYEARI	HLA-A*02:01	260	0.657	1.0827	-0.400	65.56	4.79
AIRMGFSFK	HLA-A*03:01	378	0.712	- 0.389	0.467	54.44	-7.81
DYEARIFTF	HLA-A*24:02	263	0.679	1.2236	-0.178	54.44	14.22
ELASAGLYY	HLA-A*26:01	182	0.633	0.2337	0.433	108.89	22.60
LARAGFYAL	HLA-B*07:02	283	0.656	0.1712	1.067	120.00	71.42
CRFINGFYL	HLA- B*27:05	89	0.639	-0.2838	0.744	86.67	28.41
NNIHLTHSL	HLA-B*39:01	339	0.574	0.8286	-0.311	130.00	71.42
SETHADYLL	HLA-B*400:1	132	0.768	0.5607	-0.400	97.78	20.86
HAAVDRWQY	HLA-B*58:01	66	0.589	0.4999	-1.011	54.44	39.08
ELASAGLYY	HLA-B*15:01	182	0.601	0.2337	0.433	108.89	22.60
AVDKCPMCY	HLA-A*01:01	476	0.630	-0.2811	0.289	43.33	-4.22
WYPGCKYLL	HLA-A*24:02	322	0.636	-0.2468	0.078	86.67	22.60
QEYINNIHL	HLA-B*40:01	335	0.741	0.6576	-0.633	130.00	92.82
KEISTEEQL	HLA-B*40:01	432	0.728	0.8020	-1.233	86.67	109.31

Table: 2. Prediction of HTL epitopes binding to MHC-II Allele through IEDB NetMHCPan tool

Peptide	Allele	Position	Predicted binding Score	Antigenic score	Grand average of hydropathicity (GRAVY) value (+/-)	Alipatic Index	Instability Index
IRSESDAVS	HLA-DRB1*04:04	236	0.1432	1.5071	-0.378	86.67	100.51
LKTFANFPS	HLA-DRB1*15:01	29	0.2112	0.4448	0.078	54.44	39.27
YAHLTPREL	HLA-DRB1*15:01	28	0.2152	0.1909	-0.600	97.78	17.39
FYLENSATQ	HLA-DRB1*04:05	94	0.1319	0.4009	-0.544	54.44	36.73
LRRLQEEKL	HLA-DRB1*12:01	439	0.0971	0.7530	-1.333	130.00	178.34
VITFKQKIF	HLA-DRB1*12:01	485	0.0979	-0.0560	0.756	118.89	22.78
IKKIMEEKI	HLA-DRB1*12:01	387	0.0893	0.1432	-0.367	130.00	16.81
FNRLKTFAN	HLA-DRB1*04:05	25	0.0825	1.3777	-0.544	54.44	13.17
FKDIKKIME	HLA-DRB1*11:01	385	0.0628	0.8977	-0.556	86.67	-26.68
WIYSVNKEQ	HLA-DRB1*09:01	273	0.1109	0.9330	-0.967	75.56	56.60

ISTEEQLRR	HLA-DRB1*03:01	433	0.1376	1.2758	-1.411	86.67	172.96
IHLTHSLEE	HLA-DRB1*07:01	340	0.0795	1.2048	-0.311	58.82	-0.311
YLGSRDHFA	HLA-DRB1*04:05	118	0.0390	1.1738	-0.589	54.44	18.77

Table 3: Identified B-cell epitopes on envelope protein using ABCPred tool.

Rank	Sequence	Start position	Score	Toxicity
1	NWPDYAHLTPRELASA	172	0.91	Non-Toxic
2	TRRIDDTIFQNPMVQE	363	0.90	Non-Toxic
3	TGEGDTVRCFSCHAAV	55	0.89	Non-Toxic
4	AMYSEEARLKSFQNWP	159	0.89	Non-Toxic
5	CAEAVDKCPMCYTVIT	474	0.88	Non-Toxic
6	EARIFTFGTWIYSVNK	266	0.88	Non-Toxic
7	TTEKTPSLTRRIDDTI	355	0.87	Non-Toxic
8	FHCGGGLTDWKPSEDP	301	0.87	Non-Toxic
9	THSLEECLVRTTEKTP	345	0.86	Non-Toxic
10	WKPSEDPWEQHAKWYP	310	0.86	Non-Toxic
11	WQYGDSAVGRHRKVSP	73	0.84	Non-Toxic
12	CFSCHAAVDRWQYGDS	63	0.84	Non-Toxic
13	VVDISDTIYPRNPAMY	146	0.83	Non-Toxic
14	PWEQHAKWYPGCKYLL	316	0.82	Non-Toxic
15	EGDKVKCFHCGGGLTD	294	0.82	Non-Toxic
16	SSDRNFPNSTNLPRNP	245	0.82	Non-Toxic
17	CRFINGFYLENSATQS	90	0.81	Non-Toxic
18	GDQVQCFCCGGKLKNW	195	0.80	Non-Toxic
19	CPMCYTVITFKQKIFM	481	0.79	Non-Toxic
20	EEKIQISGSNYKSLEV	394	0.79	Non-Toxic
21	NPMVQEAIRMGFSFKD	373	0.79	Non-Toxic
22	SGIQNGQYKVENYLGS	108	0.79	Non-Toxic
23	CKICMDRNIAIVFVPC	450	0.78	Non-Toxic
24	HRRHFPNCFFVLGRNL	220	0.78	Non-Toxic
25	NWEPCDRAWSEHRRHF	209	0.77	Non-Toxic
26	NKEEEFVEEFNRLKTF	18	0.77	Non-Toxic
27	DYLLRTGQVVDISDTI	138	0.77	Non-Toxic
28	SFEGSKTCVPADINKE	5	0.76	Non-Toxic
29	TNLPRNPSMADYEARI	254	0.76	Non-Toxic
30	CGGKLKNWEPCDRAWS	203	0.76	Non-Toxic
31	AGLYYTGIGDQVQCFC	187	0.76	Non-Toxic
32	KVENYLGSRDHFALDR	116	0.76	Non-Toxic
33	YLLEQKGQEYINNIHL	329	0.75	Non-Toxic
34	RAGFYALGEGDKVKCF	286	0.75	Non-Toxic
35	ARLKSFQNWPDYAHLT	165	0.74	Non-Toxic
36	TEEQLRRLQEEKLCKI	437	0.73	Non-Toxic
37	HRKVSPNCRFINGFYL	83	0.72	Non-Toxic
38	YLENSATQSTNSGIQN	97	0.71	Non-Toxic
39	VFVPCGHLVTCKQCAE	461	0.70	Non-Toxic
40	TWIYSVNKEQLARAGF	274	0.68	Non-Toxic
41	DRPSETHADYLLRTGQ	130	0.67	Non-Toxic
42	GSPVSASTLARAGFLY	39	0.65	Non-Toxic
43	TFANFPSGSPVSASTL	32	0.64	Non-Toxic
44	DSMQDESSQTSLQKEI	420	0.60	Non-Toxic
45	FSFKDIKKIMEEKIQI	384	0.55	Non-Toxic

Table 4: Identified B-cell epitopes on envelope protein using Bepipred tool.

Sl. No.	Start	End	Bepipred predicted B cell epitope	Antigenicity score by VaxiJen	Toxicity
1	5	52	SFEGSKTCVPADINKEEEFVEEFNRLKTFANFPSGSPV	0.7012	Non-Toxic
2	71	88	DRWQYGDSAVGRHRKVSP	0.4942	Non-Toxic
3	92	113	FINGFYLENSATQSTNSGIQNG	0.8459	Non-Toxic
4	114	135	QYKVENYLGSRDHFALDRPSET	0.4350	Non-Toxic
5	136	160	HADYLLRTGQVVDISDTIYPRNPAM	1.0429	Non-Toxic
6	161	180	YSEEARLKSFQNWPDYAHLTP	0.8996	Non-Toxic
7	208	227	KNWEPCDRAWSEHRRHFPNC	-0.6994	Non-Toxic
8	229	259	FVLGRNLNIRSESDAVSSDRNFPNSTNLPRN	0.5700	Non-Toxic
9	260	281	PSMADYEARIFTFGTWIYSVNKEQ	0.6194	Non-Toxic
10	308	337	TDWKPSEDPWEQHAKWYPGCKYLLEQKGQ	-0.1043	Non-Toxic
11	337	370	EYINNIHLTHSLEECLVRTTEKTPSLTRRIDDTI	0.9427	Non-Toxic
12	371	391	FQNPMVQEAIRMGFSFKDIKK	0.1417	Non-Toxic
13	392	404	IMEEKIQISGSNY	0.5896	Non-Toxic
14	405	422	KSLEVLVADLVNAQKDSM	0.3142	Non-Toxic
15	423	448	QDESSQTSLQKEISTEEQLRRLQEEK	0.934	Non-Toxic

Table 5: Physiochemical properties and antigenic score prediction of B- cell epitopes

Location	Peptide	Score	Antigenic Score	Grand average of hydropathicity (GRAVY) value (+/-)	Alipatic Index	Instability Index
361 - 370	SLTRRIDDTI	1.000	1.1596	-0.540	117.00	51.25
228 - 237	FFVLGRNLNI	0.941	0.8895	1.000	146.00	65.28
71 - 80	DRWQYGDSAV	0.872	-0.0554	-1.240	39.00	61.97
145 - 154	QVVDISDTIY	0.719	1.5861	0.410	136.00	-28.60
454 - 463	MDRNIAIVFV	0.665	-0.1396	1.240	146.00	49.25
411 - 420	VADLVNAQKD	0.633	0.9765	-0.210	117.00	0.51
296 - 305	DKVKCFHCGG	0.598	1.7265	-0.330	29.00	1.48
278 - 287	SVNKEQLARA	0.579	0.6749	-0.810	88.00	51.94
341 - 350	NIHLTHSLEE	0.567	0.8885	-0.630	117.00	97.88
178 - 187	HLTPRELASA	0.563	0.1109	-0.310	98.00	1.46

Allergenicity, Toxicity, and Antigenicity prediction of the selected epitopes

All the identified epitopes were scrutinized with the toxicity, allergy and antigenic test. The potential epitopes were found to be highly antigenic on the basis of high VaxiJen scores, they were found to be non-toxic and non-allergen. On the basis of the calculated antigenic scores out of 15 CTL epitopes, the top 2 epitopes VVDISDTIY and SMADYEAR were selected, out of 13 HTL epitopes, two epitopes with the best antigenic scores IRSESDAVS and FNRLKTFAN were shortlisted and the best top two B-cell epitopes QVVDISDTIY and DKVKCFHCGG has been selected Multi-epitope vaccine construct.

Physiochemical properties prediction of the designed Multi-epitope vaccine constructs

The physicochemical properties of all screened epitopes were checked to ensure the stability, Grand average of hydropathicity (GRAVY), and instability index for invoking a strong immune response against hepatocellular carcinoma. All the high antigenic score peptides were found to be stable therefore making it stable in nature. Thus, all six epitopes qualified all the parameters that are required for the development of efficient vaccine construct and they were linked together by using linkers. HTL epitopes were linked together by using AAY linker, CTL epitopes were linked with each other using GPGPG and B-cell epitopes were merge together with the help of KK linker(Figure 2).

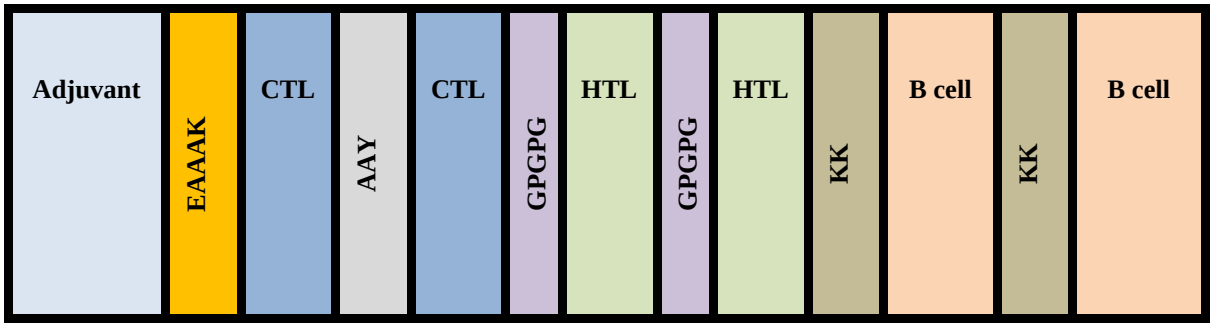

Figure. 2 Multi-Epitope Vaccine construct designed using Linkers and adjuvant

Three-dimensional modeling and Molecular Docking Studies of the target protein and vaccine construct

The 3-D structure of the designed vaccine construct was prepared and the prepared model was checked for quality with the help of the RAMPAGE tool. The structure prepared showed above 90% score making it suitable for docking analysis (Figure 3). The interaction between the protein and the multi-epitope vaccine construct was studied through the molecular docking process. The Cluspro tool calculates the interaction on the basis of the lowest binding energy formed by the protein and epitope complex. Therefore, the designed vaccine constructs binding energy at which they bind forming a strong hydrogen bond represented in below table 6. Here, we obtained -905.7kcal/mol binding energy for the best model and the hydrophobic force of interactions was -1183.4 making it to be used as a potential vaccine construct. The final Multi-vaccine construct can be visualized in figure 4.

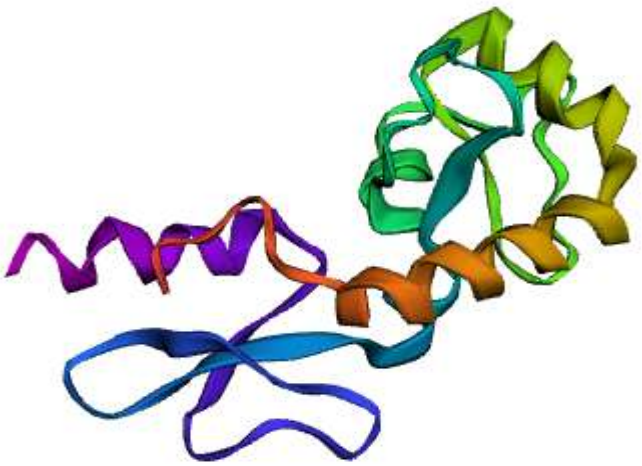

Figure 3. Three-dimensional structure of Multi-epitope vaccine construct

Table 6: Binding energy score of the Multi-epitope construct performed through Cluspro tool

Peptide	Binding energy	Hydrophobic force	Electrostatic force	VdW+Elec
Muti-epitope construct	-905.7kcal/mol	-1183.4	-899.3	-236.0

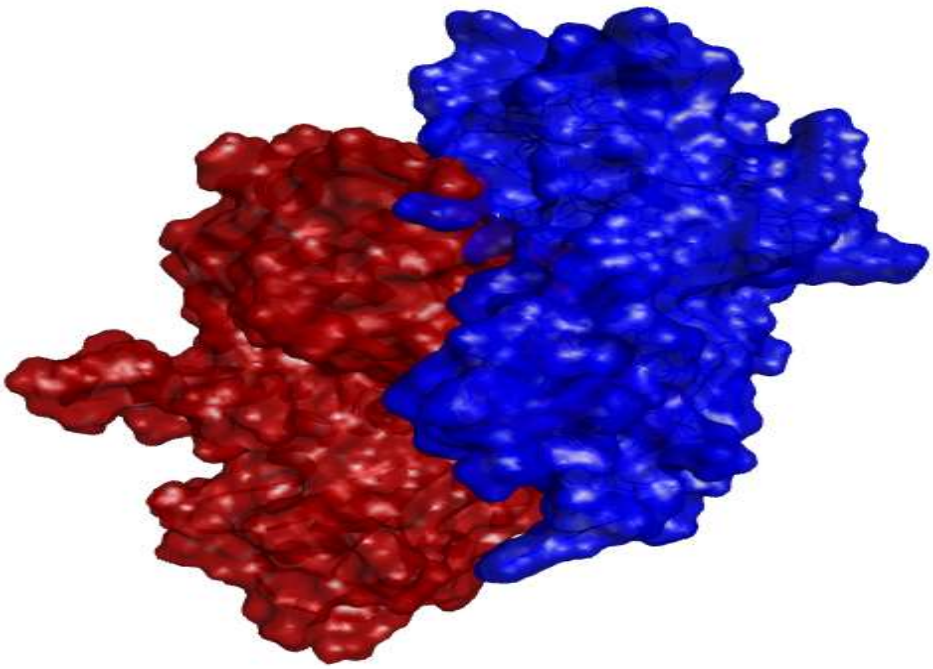

Figure 4. Visualization of docked complex of Multi-epitope vaccine construct (blue color) with target protein (red color).

Normal Mode Analysis

Normal Mode Analysis (NMA) was performed to predict the variations and deformability among residues in the multi-epitope complex through the iMODS server. In Figure 5, we can observe that we have measured the mobility measurement of the complex structure to understand the potential of each residue to deform to get an idea about the stability of the complex. So here, plot 5A shows deformability ranges of most of the residues are below 0.6 value, and Plot 5B shows the Bfactor that gives a piece of information on the average RMS fluctuation using the calculation 8pi^2. In plot 5D we can see the eigenvalue is 5.092236e- 05 which means lower the eigenvalue deformation is easier and therefore the structure will be more stable. In Plot 5 E, we can analyze the covariance matrix that shows correlated residues in red color while uncorrelated residues in white color and anti-correlated in blue color. The elastic network model is generated in Figure 5F, which indicates the interaction of atoms with spring and each dot colour indicates the stiffness of the complex. Thus, darker grey dots means complex are more stiff during the trajectory analysis process.

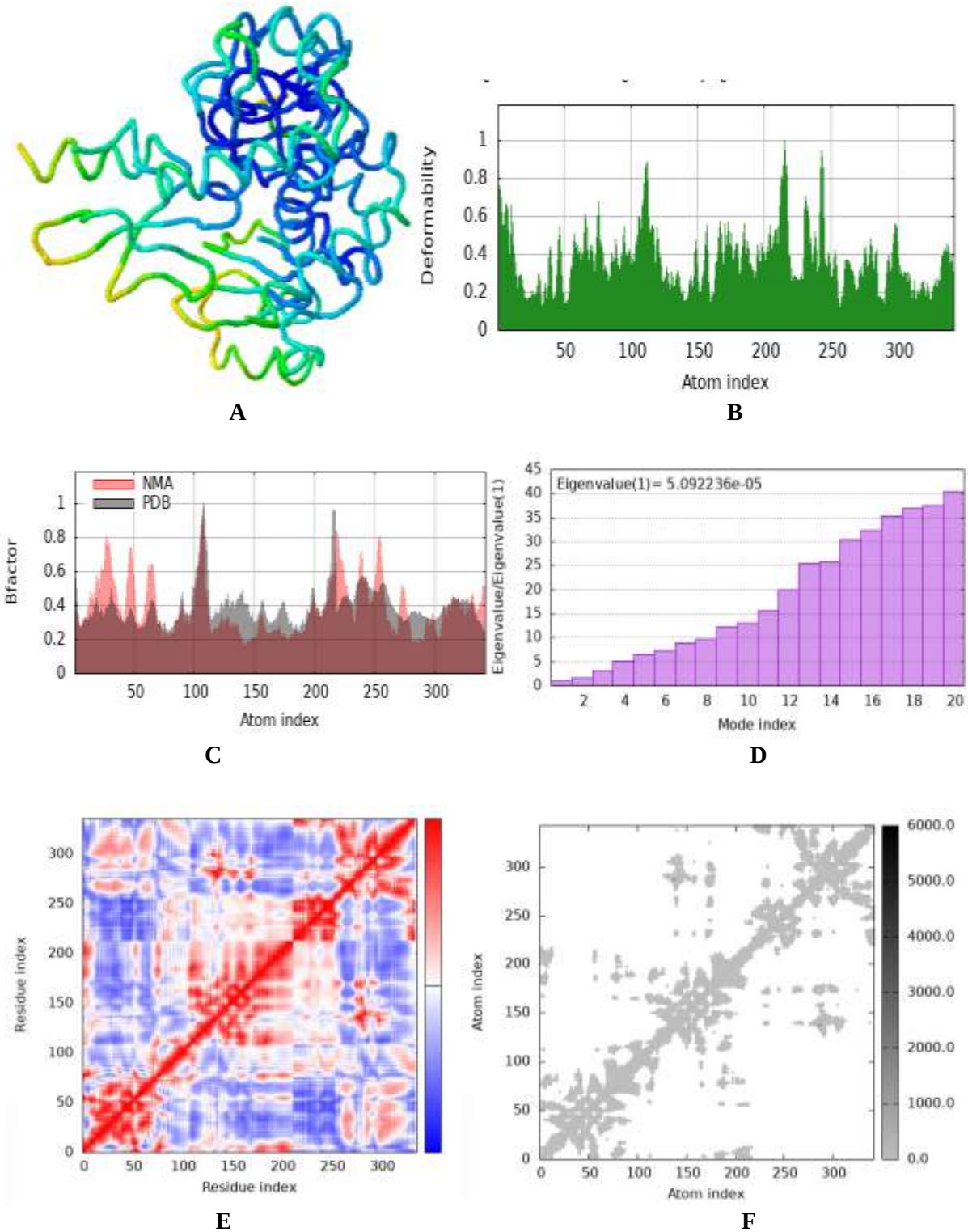

Figure 5: Normal Mode Analysis Study to analyze the stability of multi-epitopes construct (A) Visual cartoon representation (B) Deformability of complex at each residue (C) B factor showing average RMS graph (D)Eigenvalues showing motion stiffness of complex to deform (E) Covariance map of each residue (F) Elastic network plot showing interactions between atoms.

Molecular Dynamic Simulation Study

The strongest docking complex based on their binding affinity and the stability of the complex were calculated over the MD simulation, therefore the root mean square deviation (RMSD) of C-alpha atom for each trajectory frame concerning their initial conformation was analyzed and time series can be depicted in Fig.6. So, we can observe that Root Mean square fluctuation (RMSF) of each residues in the complex are below 3Å and shows minimal fluctuations of the residues. The highest RMSF value can be observed at 3.89Å, as the residues present at the N and C terminals are not taken in consideration during the simulation process. Therefore, after analyzing the overall RMSF plot, we can state that the mult-epitope construct was found to be stable during the simulation process to check the flexibility of the overall conformation.

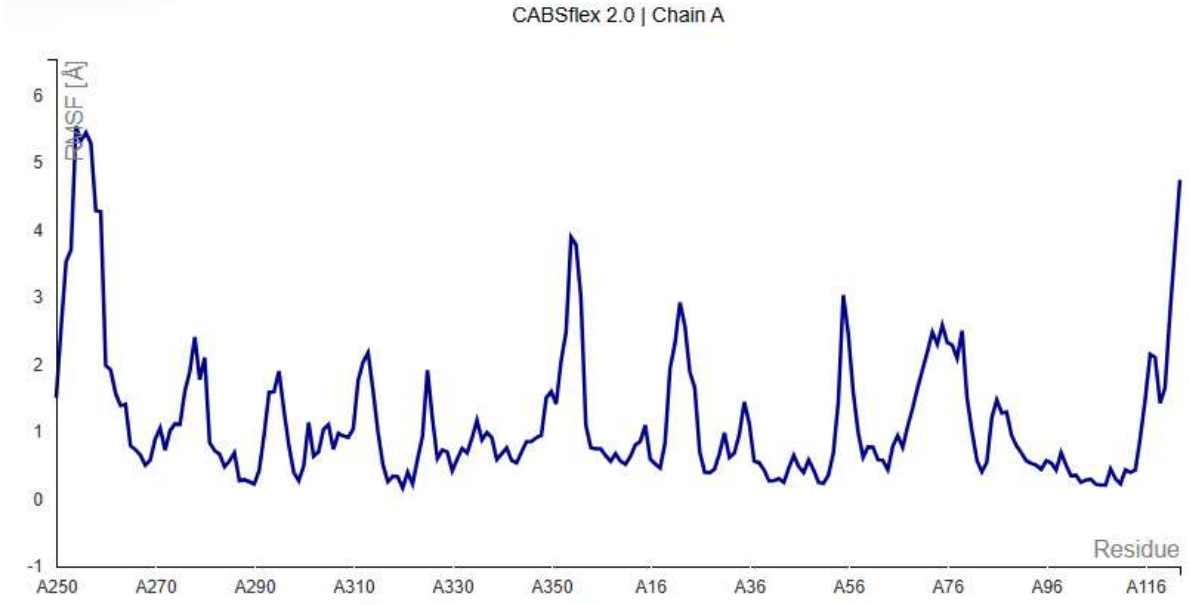

Figure 6- Molecular Dynamic Simulation Study showing the RMSF values of residues for Multi-epitope construct

In Silico cloning and expression of Multi-Epitope vaccine construct

Further, the final epitopes were joined together by using adjuvant and linker and designed construct as depicted in the above figure. For codon optimization of designed vaccine construct, Java Codon Adaptation tool was used. To perform in silico cloning, the pET28a-EgC plasmid vector was used and cloned with the help of restriction enzymes XboI and XhoI using the SnapGene tool (Figure 7).

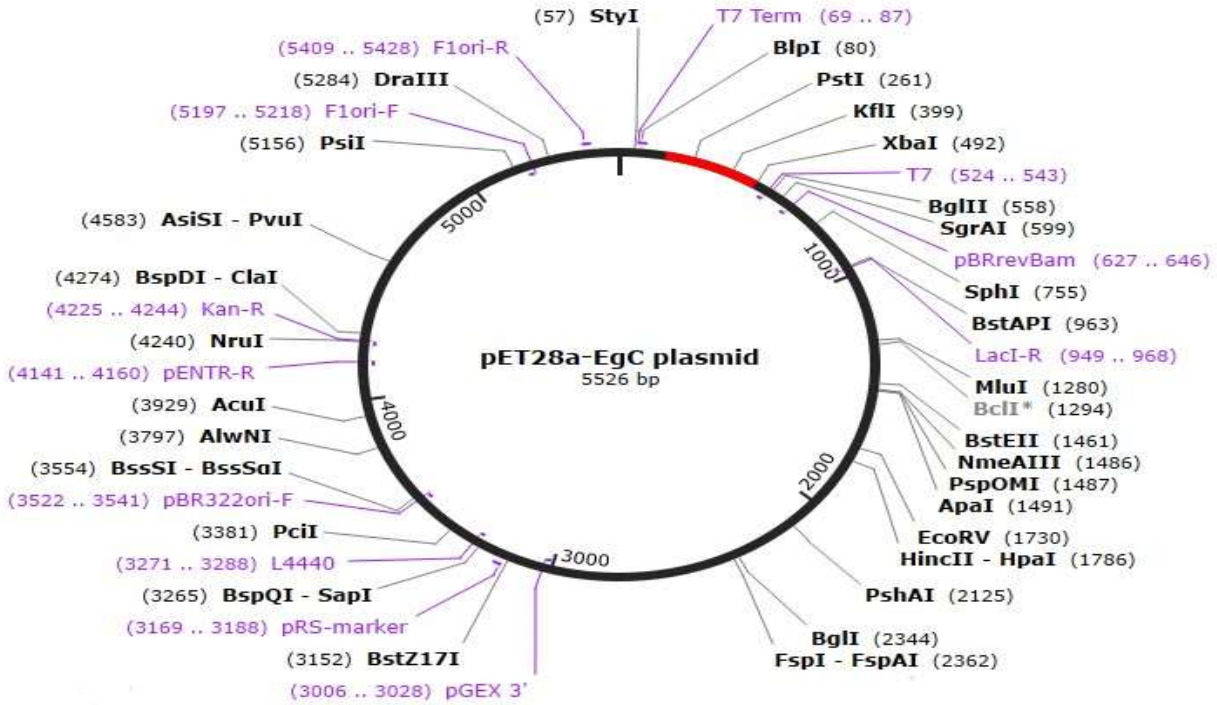

Figure 7. In silico cloning of Multi-epitope construct highlighted in red color inserted in pET28a-EgC plasmid vector

Discussion

Hepatocellular carcinoma (HCC) has become a major cause of death with cancer worldwide but limited medications against this type of liver cancer are a global burden and therefore it is high time to develop an effective approach to control this. In this study, we deployed an immunoinformatics approach to design a multi-epitope vaccine using novel tools. Motamedi et al. 2022 have targeted on proteins causing HCC by using different invitro strategies including western blotting and purification of protein. The concept of linking of CTL, HTL and B-cell epitopes with known linkers is inducing strong adaptive and innate immune response and thus reducing the risk of liver transplantation at extreme stage (Dehbarez et al. 2020). Recent studies showed the success rate of the use of bioinformatics tools in the identification of toxicity, antigenicity, and binding capacity of multi-epitopes against Hepatitis C Virus (HCV) infection dengue virus having different serotypes (Alsaiari et al. 2023). Screening of B and T cell epitopes was performed with the help of identifying the research gap and highlighting the demand for newer technology to develop novel epitopes that can be incorporated into a potential vaccine construct to robust efficient results. This approach is cost-effective, time-saving, and generation of strong multiple epitopes together but it has some limitations in terms of validation of results as the accuracy of these tools is not appropriate so experimental validation is mandatory. At present, there are no drugs and vaccines available to cure this progressive disorder and therefore this study opens the latest research strategies against hepatocellular carcinoma leading to a multi-epitope vaccine construct.

Conclusion

Until now, there is no efficient proven vaccine against hepatocellular carcinoma and therefore transplantation of the liver is the only option left that is merely impossible for every individual. So, considering this fact this work is aimed at on immunoinformatics approach to developing a multi-epitope novel vaccine that could lead to potential epitopes that can induce a better immunogenic response. The vaccine candidates identified in the present work are safe, non-toxic, antigenic in nature, and stable therefore it can be strongly targeted to the disease. Here, in our analysis the screening of CTL epitopes led to the identification of two epitopes VVDISDTIY and SMADYEAR that were found to be immunogenic, the two best HTL epitopes IRSESDAVS and FNRLKTFAN binding to MHC class-II alleles were shortlisted and the top antigenic scorer B-cell epitopes QVVDISDTIY and DKVKCFHCGG were selected and combined together with the help of linkers. The docking of Multi-epitope vaccine construct shown good binding energy scores and in silico cloning of designed vaccine into plasmid vector also showed the compatibility of the construct as well as how well it can be expressed in vector. Therefore more strong multi-epitope vaccine construct can be highly suggested for invitro and invivo analysis.

List of Abbreviations
HCC: Hepatocellular carcinoma
CTL: Cytotoxic T-lymphocyte
HTL: HelperT-lymphocyte
XIAP: X-linked inhibitor of apoptosis protein
PDB: Protein Database bank
RMSD: Root Mean Square
RMSF: Root Mean Standard Fluctuation

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