Conserved B and T cell epitopes prediction of ebola virus glycoprotein for vaccine development: An immuno-informatics approach

Bilal Ahmad[a], Usman Ali Ashfaq[a],[*], Mahmood-ur Rahman[a], Muhammad Shareef Masoud[a], Muhammad Zubair Yousaf[b]

[a] *Department of Bioinformatics and Biotechnology, Government College University Faisalabad (GCUF), Pakistan*
[b] *Department of Biological Sciences, Forman Christian College University, Ferozpur Road, 54600, Lahore, Pakistan*

ABSTRACT

Ebola virus (EBOV), a non-segmented single-stranded RNA virus, is often-most transmitted through body fluids like sweat, tears, saliva, and nasal secretions. Till date, there is no licensed vaccine of EBOV is available in the market; however, the world is increasingly vulnerable to this emerging threat. Hence, it is the need of time to develop a vaccine for EBOV to hinder its dissemination. The current study has been designed for identification and characterization of the potential B and T-cell epitopes using the Immuno-informatics tools, and it helped in finding the potent vaccine candidates against EBOV. Prediction, antigenicity and allergenicity testing of predicted B and T cells' epitopes was done as well to identify their potential as a vaccine candidate and to measure their safety level respectively. Among B-cell epitopes "WIPAGIGVTGVIIA" showed a high antigenicity score and it would play an important role in evoking the immune response. In T-cell epitopes, peptides "AIGLAWIPY" and "IRGFPRCRY" presented high antigenicity score, which binds to MHC class-I and MHC class-II alleles respectively. All predicted epitopes were analyzed and compared with already reported peptides carefully. Comparatively, Peptides predicted in the present study showed more immunogenicity score than already reported peptides, used as positive control, and are more immunogenic as compared to them. Peptides reported in the present study do not target only Zaire EBOV (ZEBOV), as in previous studies, but also other species, i.e. Tai Forest EBOV (TAFV), Sudan EBOV (SUDV), Bundibugyo EBOV (BDBV), and Reston EBOV (RESTV) and would bring the promising results as potent vaccine candidates.

1. Introduction

EBOV, member of the Filoviridae virus family, an emerging zoonotic pathogen causes acute hemorrhagic fever (HF) along with coagulative necrosis of lungs, liver and other organs with a high mortality rate in humans up to 90% [1]. Symptoms of HF include coagulation abnormalities like intravascular coagulation and signs of shock and multiple organ failure, which eventually lead to death [2]. HF due to EBOV was first time reported in 1976, during its outburst in the Democratic Republic of the Congo (Zaire). The virus spreads among people due to direct contact with virus-containing fluids like stool, vomitus, breast milk, urine, sweat, saliva, tears, and respiratory secretions. The genome of EBOV, 19.1 kb long, contains seven genes code for different proteins. Transcriptional editing of the fourth gene results in the expression of two proteins, one is GP, 676 amino acid containing *trans*-membrane protein, and the other is 364-residue containing small glycoprotein (sGP) [3,4]. GP after translation is cleaved by Furin, a paired basic amino acid cleaving enzyme, into GP1 and GP2 subunits linked through a disulfide bond [5,6]. GP1 and GP2 in a respective manner are involved in attachment and fusion of the virus with the host cell [7–9]. Several studies showed GP dependent immune system response in non-human primates, i.e. a vaccine based on replication-competent vesicular stomatitis virus (VSV) containing the GP of Ebola virus instead of its own has been tested in phase III of ring vaccination which showed promising results in barring the transmission of EBOV among the vaccinated adults. As reported previously, vector-based vaccines, VSV and adenovirus, containing the GP of EBOV are also involved in evoking CD4 and CD8 T-cell specific immune response. One study reported that GP-directed antibody level was significantly high in animals containing virus-like particles (VLPs) lacking nucleoprotein (NP) rather than animals immunized with NP-containing VLPs or adenovirus-expressed GP [10]. In another study Wilson et al. reported that the GP-specific monoclonal antibodies protected the mice from lethal attack and infection of EBOV [11]. GP-specific murine monoclonal antibodies have been produced and tested in mice model which showed a positive response and prevented the mice from mortality [12]. Similar to these, several studies have been done to produce the GP specific monoclonal antibodies and to use them for protective and diagnostic measures [13,14]. Hence, owing to all reported studies, EBOV's GP is considered as a significant target to design a vaccine against Ebola virus to hinder its proliferation [15].

In conventional vaccines commonly attenuated or inactivated whole pathogens are used to induce the immunogenic response. The major problem of the conventional vaccine is related to their safety because the

---

> Glycoprotein Ebola Virus
MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQLRSVGLNL
EGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRYVHK
VSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPS
SGYYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPE
IDTTIGEWAFWETKKNLTRKIRSEELSFTVVSNGAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAM
VQVHSQGREAAVSHLTTLATISTSPQSLTTKPGPDNSTHNTPVYKLDISEATQVEQHHRRTDNDSTASDT
PSATTAAGPPKAENTNTSKSTDFLDPATTTSPQNHSETAGNNNTHHQDTGEESASSGKLGLITNTIAGVA
GLITGGRRTRREAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQ
LANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDF
VDKTLPDQGDNDNWWTGWRQWIPAGIGVTGVIIAV|IALFCICKFVF

Fig. 1. The amino acid sequence of Glycoprotein has been shown.

Table 1
Predicted disulfide bonds within the residues of Glycoprotein. The fifth one, indicated without any color, is a weak bond as it contains the lowest score.

Positions	Predicted bonds	Score
53–672	VDKLVCRDKLS - ALFCICKFVFX	0.99733
108–670	EWAENCYNLEI - VIALFCICKFV	0.73499
121–135	PDGSECLPAAP - RGFPRCRYVHK	0.88771
147–608	SGTGPCAGDFA - ILGPDCCIEPH	0.96172
511–601	NAQPKCNPNLH - RWGGTCHILGP	0.01039
556–609	QDGLICGLRQL - LGPDCCIEPHD	0.51169

attenuated pathogens can revert to their natural form and can cause harm instead of positive results. With the advancement in the field of genomics, proteomics and digital data analysis, new vistas of the specific vaccine (epitope-based vaccine) production have been opened [16]. Viral vaccine production is changing its way toward the novel vaccine production strategy known as the epitope-based vaccine or reverse vaccinology [17,18]. Epitope based vaccine strategy helped in minimizing the risk factors attached to the conventional vaccines [19]. Epitopes are part of pathogenic proteins which are responsible for evoking the immune response [20]. Immuno-informatics is the representation and analysis of immunogenic data with the help of a computer which helps in reducing the cost and time of vaccine development. It plays an important role in designing the algorithms, which play an important role in determining the B and T-cell epitopes. With Immuno-informatics tools, one can predict the highly immunogenic parts of an antigenic protein used for the synthesis of the peptide vaccine within a very short span of time and limited resources [18,20,21]. A number of B cell and T cell epitopes have been reported based on imuno-informatics approaches for adenovirus [22], chikungunya virus [23], dengue virus [24], zika virus [25], human coronaviruses [26] and rhinoviruses [27]. Dikhit et al. identified 28 epitopes from Ebola virus nucleoprtotein, RNA dependent RNA polymerase and GP which have wider population coverage and conservation in all virulent strains of EBOV [28].

Although a number of peptides of EBOV's GP, involved in evoking the immune response in primates, have been reported, yet there is no approved or licensed vaccine is available for humans [11,12,29,30]. Hence, there is a dire need to develop a vaccine against it to resist the devastating threat. Purpose of the present study is to find the most immunogenic peptides of EBOV's GP, which would be helpful in mediating the immune response and used as a vaccine in the future. A comparison of peptides, predicted in the present and already reported studies, was also made to compare their antigenicity and specificity.

2. Methodology

2.1. Retrieval and analysis of primary structure of immunogenic proteins

Genbank, a repository of protein, DNA and RNA sequences, was taken into account while retrieving the sequence of GP using its
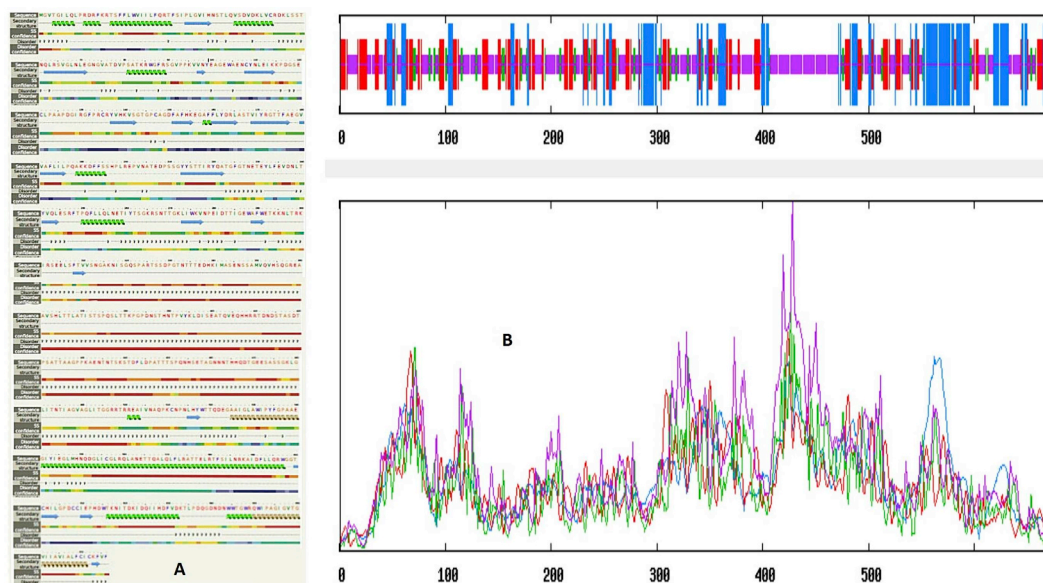

Fig. 2. (A) Secondary structure of GP, predicted using Phyre2, has been presented. **(B)** Secondary structure plot of GP developed through SPOMA has been shown. Here, blue color represents the helix while green and red represent beta turns and extended strands in a respective way.
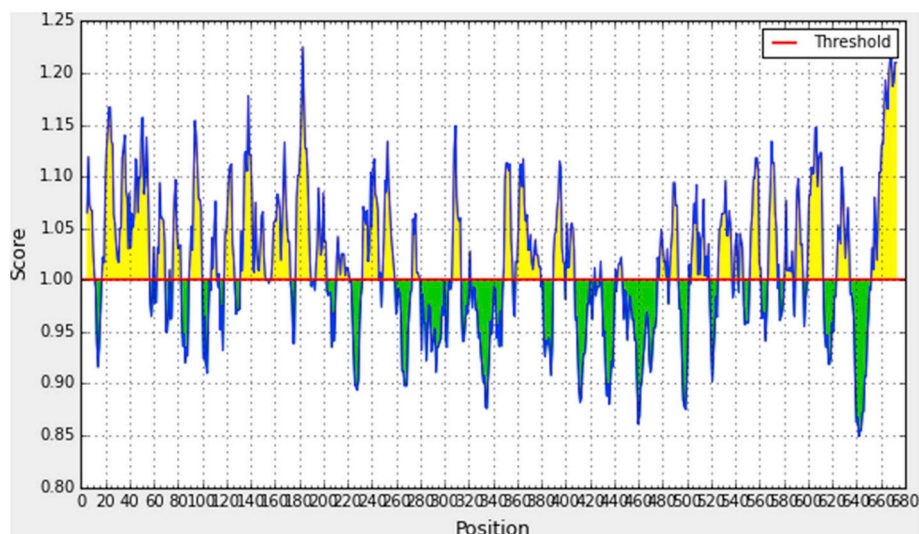
Fig. 3. Kolaskar&Tongaonkar Antigenicity Prediction: X-axis representing the position of residues, while the Y-axis indicating the propensity score. The red colored line in the diagram indicates the threshold value, while the Yellow region above the threshold line shows the antigenic part of the protein.
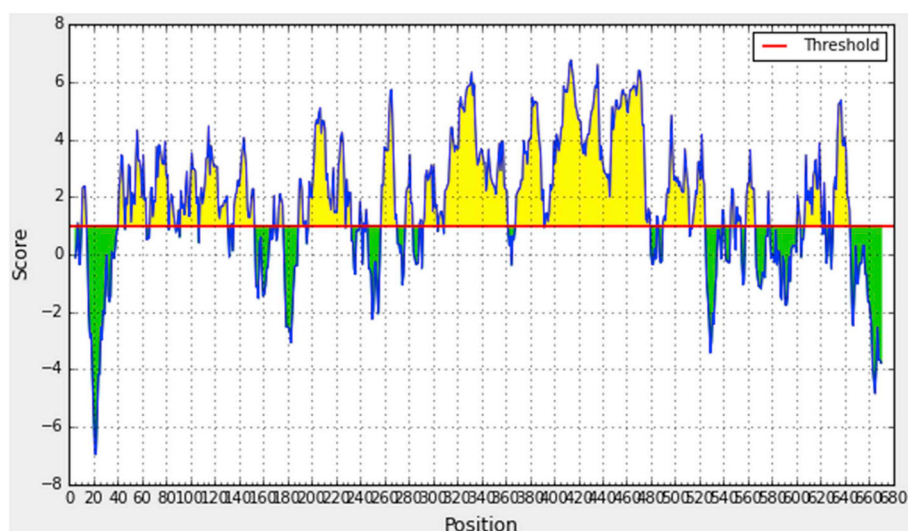

Fig. 4. Parker Hydrophilicity prediction's results have been shown here. X-axis representing the position of residues, while the y-axis indicating the propensity score. Line in red color indicates the threshold value and regions above the threshold value represent the beta turns in glycoprotein.

accession number, AAB81004.1. Antigenicity testing of protein was carried out using the Vaxijen 2.0 [31]. The sequence of the protein, in plain format, was given as an input in the text window. The threshold value was set at ≥0.5 to increase the specificity because values less than 0.5 are considered as non-antigenic, and the virus was chosen from the menu as an organism [32]. TMHMM, an online tool, was used to analyze the *trans*-membrane topology of GP [33]. Analysis of protein primary structure was performed via Protparam. Different parameters, like the composition of protein, the grand average of hydropathicity (GRAVY), atomic composition, aliphatic index, molecular weight, and stability index, were calculated. Presence of disulfide bonds was checked via a web tool DiANNA 1.1, which makes predictions on the basis of a trained neural network. Furthermore, different tools like SOPMA [34], Psipred [35], Phyre2 [36] and JPred [37] were used to evaluate the secondary structure of Protein.

2.2. B-cell epitope prediction

B-cell epitope prediction tools of IEDB [38] and BCPRED [39] were used to predict B-cell epitopes from the plain sequence of GP.

Antigenicity, hydrophilicity, surface accessibility, flexibility, linear and discontinuous epitope predictions are considered as most important properties for prediction of B-cell epitopes [40]. Analysis of hydrophilicity, surface accessibility, flexibility and linear epitope predictions have been done using Kolaskar and Tongaonkar antigenicity scale, Parker hydrophilicity prediction, Emini surface accessibility prediction [41], Karplus and Schulz flexibility prediction and Bepipred linear epitope prediction algorithms respectively. For prediction of beta turns in protein, Chou and Fasman beta turn prediction algorithm was used [42]. As the discontinuous epitopes are more specific and have more dominant features over linear epitopes, so, the prediction of discontinuous epitopes have also been carried out through DiscoTope 2.0 Server [30]. Threshold was set at ≥0.5 which showed 23% sensitivity and 90% specificity (as mentioned in instructions) [16]. In addition to that, Episearch was used to confirm the position of predicted B-cell epitopes. All predicted epitopes were given in plain format while the 3D structure of GP retrieved from Protein Data Bank (PDB) using PDB-ID "5JQ3" was given in PDB format. Pymol was used to observe the positions of predicted epitopes on the 3D structure of GP.
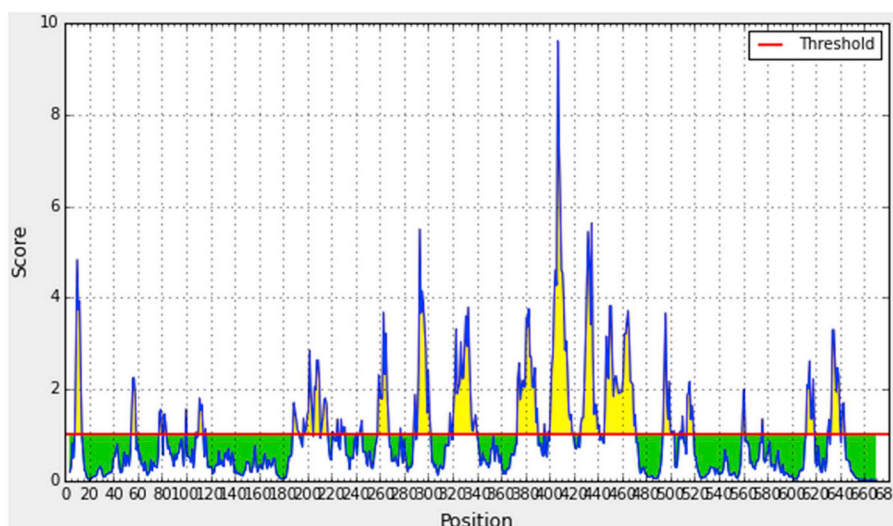
Fig. 5. Results of Emini Surface Accessibility Prediction tool are given here. X-axis contains the position of residues while the y-axis shows the propensity score. Red line indicates the threshold value. Regions above the threshold value, shown in yellow, are representing the surface accessibility.

2.3. T-cell epitope prediction

Prediction of cytotoxic T-lymphocyte (CTL) epitopes is an important milestone in rational vaccine design. Most important is that, it reduces the time and cost as compared to wet lab experiments to predict epitopes. Propred-I is a freely available web tool which was used for prediction of MHC class I allels [43]. This tool uses a matrix based method to predict peptides which bind to different 47 MHC class I alleles. It is also used to determine immuno-proteasome and proteasome cleavage sites. The sequence was given in plain format and all alleles were selected for prediction. Immuno-proteasome and proteasome filters were kept on with a threshold value of 5%. Prediction of MHC class II alleles was done using the Propred, a freely available tool [44]. It uses quantitative matrices based method to predict peptides from antigenic sequence which can bind to MHC class II alleles. The Sequence was given in fasta format, the threshold was set at 5% and all alleles were selected for prediction of potential peptides. Furthermore, antigenicity analysis of both MHC class I and MHC class II alleles were done with the help of Vaxijen 2.0.

2.4. Conservation analysis

Sequence of EBOV GP, belonging to 12 different countries, was taken from Genbank. Multiple sequence alignment of the retrieved sequences was done, to analyze the conservation of selected epitopes, by CLC workbench. Only highly conserved epitopes were included in final results. Besides this, sequences of all species of EBOV, i.e. ZEBOV, TAFV, SUDV, BDBV and RESTV were taken and aligned to identify the conserved epitopes among all of them. Alternative peptides for each species were also predicted using the IEDB conservation analysis sources. Glycosylation site analysis was also done by using the NXS formula (X representing any residue).

2.5. Evaluation of other eminent features of predicted epitopes

After the finalization of the selected epitopes, the other important features of these, i.e. digestion analysis, mass and PI values, were analyzed through Protein Digest server [13,45].

2.6. Interaction study of predicted peptides with HLA alleles

Molecular Docking is a best way to study the protein-protein or ligand –protein interactions to find out the mechanism how a protein interacts with other proteins or ligand molecules. It requires 3D structures to perform the interaction analysis.

2.7. 3D-structure prediction of predicted peptides

To study the interaction of predicted immunogenic peptides with HLA alleles, it is necessary to determine their 3D structures. For determination of 3D structures of peptides, PEPFOLD, based on a de-novo approach, was used. It works on the base of a structural alphabet (SA) letters to determine the confirmation of 4 consecutive residues. Plain sequences of predicted peptides with default parameters were given in PEPFOLD to determine their 3D structures.

2.8. Retrieval of 3D structure of HLA allele

Due to the availability of 3D structure and binding ability with most of the predicted MHC class-I peptides, the HLA-B7 was selected among the predicted alleles to perform the interaction analysis. The 3D

Table 2
Results of emini surface accessibility prediction.

Position	Residue	Start	End	Peptide	Score
407	H	405	414	EQHHRRTDND	9.615
408	H	406	415	QHHRRTDNDS	7.44
409	R	407	416	HHRRTDNDST	6.2
435	T	433	442	ENTNTSKSTD	5.631
293	T	291	300	WETKKNLTRK	5.495
432	A	430	439	PKAENTNTSK	5.446
10	P	8	17	QLPRDRFKRT	4.829
433	E	431	440	KAENTNTSKS	4.72
410	R	408	417	HRRTDNDSTA	4.603
405	E	403	412	QVEQHHRRTD	4.602
411	T	409	418	RRTDNDSTAS	4.534
406	Q	404	413	VEQHHRRTDN	4.273
431	K	429	438	PPKAENTNTS	4.211
295	K	293	302	TKKNLTRKIR	4.143
404	V	402	411	TQVEQHHRRT	3.977
12	D	10	19	PRDRFKRTSF	3.924
412	D	410	419	RTDNDSTASD	3.865
296	N	294	303	KKNLTRKIRS	3.847
450	T	448	457	TTTSPQNHSE	3.82
451	S	449	458	TTSPQNHSET	3.82
333	N	331	340	GTNTTTEDHK	3.789
383	G	381	390	KPGPDNSTHN	3.75
11	R	9	18	LPRDRFKRTS	3.737
465	H	463	472	NTHHQDTGEE	3.712

structure of HLA-B7 was taken from Protein Data Bank (PDB) using the PDB_ID "3vcl". Already bound peptide (RPHERNGFTVL) was removed prior to docking.

2.9. Molecular docking

To study the interaction of peptides with HLA-B7 allele, molecular docking was performed via the Molecular Operating Environment (MOE). MOE is an offline tool which can be used to study the protein-protein and protein-ligand interactions. 3D structures of predicted peptides were placed in a library, which was docked against HLA-B7. Before docking; removal of already bound peptide, energy minimization, protonation, and removal of water molecules were done. Docking scores were calculated by using the London-DG as scoring and restoring function. Furthermore, force field was applied for refinement.

2.10. Allergenicity analysis

Allergenicity assessment of predicted peptides was done with the

help of Aller Hunter [45], freely available web tool. It finds the allergens and non-allergens, very accurately, by comparing the query sequence of peptides with a database of reported allergens.

3. Results

3.1. Retrieval and analysis of primary structure of candidate protein

Protein sequence of GP was taken from NCBI Genbank repository using the accession number AAB81004.1 (Fig. 1). After that, antigenicity of protein was assessed through Vaxijen 2.0 by adjusting threshold at ≥0.5, for the sake of more specificity. Results of Vaxijen 2.0 indicated that GP is an antigenic protein with score 0.5. TMHMM was used to analyze the *trans*-membrane topology of GP indicated that residues ranging from 1 to 650 are exposed on the surface, while the residues from 651 to 669 are part of the *trans*-membrane helix, and residues from 670 to 676 are buried inside. Furthermore, the primary structure analysis of protein, GP, through the Protparam indicated that molecular weight (MW) of protein is 74464.4 kDa and its theoretical
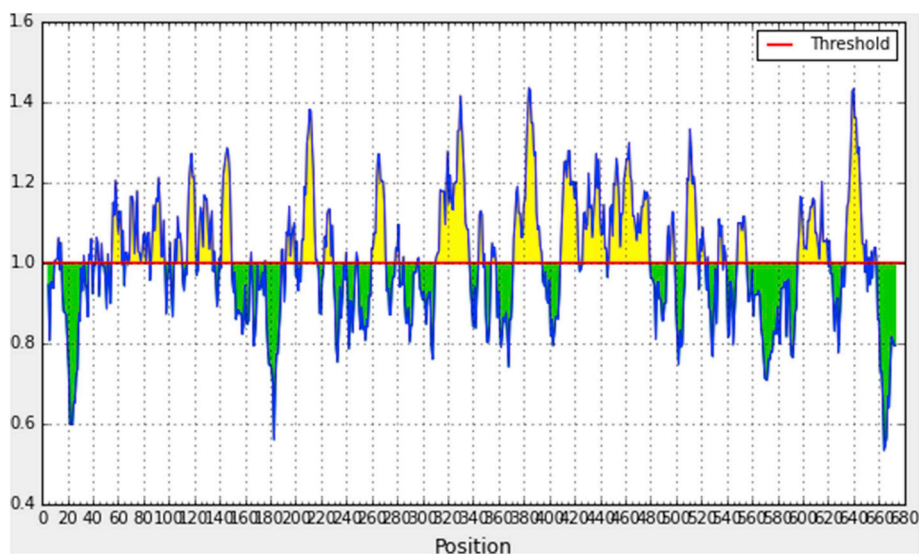
Fig. 6. Chou and Fasman beta-turn prediction's results have been presented. X-axis representing the position of residues, while the y-axis contains the propensity score. Red line indicates the threshold value and the regions above the threshold line contain beta turns which are shown in yellow.
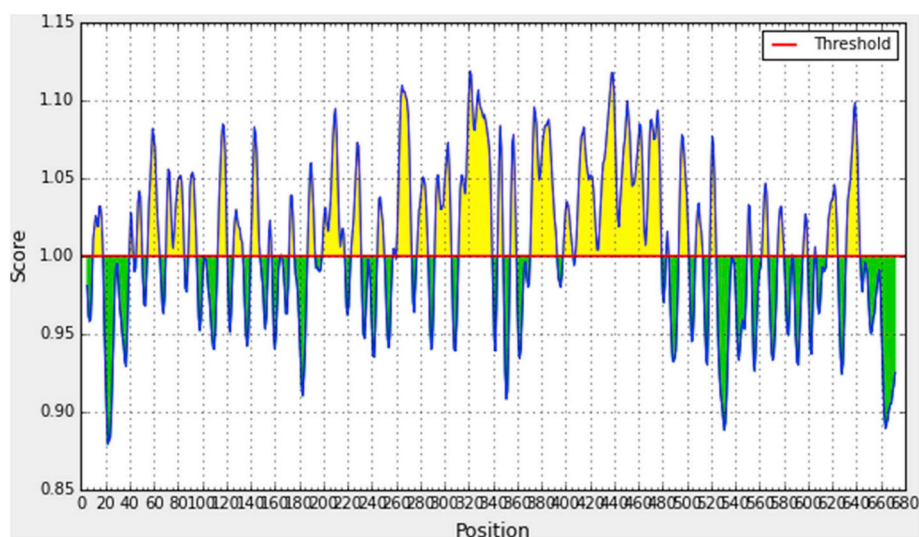
Fig. 7. Karplus& Schulz Flexibility Prediction: X-axis representing the position of residues while y-axis indicating the propensity score. Red line indicates the threshold value and the yellow color above the threshold line is indicating the flexible regions of proteins.

isoelectric point (pI) is 6.16. The protein, composed of 676 residues, contains 71% negatively charged residues while the remaining as positively charged. Calculated instability value was 38.36 which indicated that protein is stable. The total number of carbons (C), Hydrogen (H), Nitrogen (N), Oxygen (O) and sulfur (S) were represented by molecular formula which is "$C_{3298}H_{5123}N_{919}O_{1019}S_{16}$". A grand average of hydropathicity (GRAVY) of GP was −0.380. In addition to that, Diana 1.1 analysis indicated that there are six disulfide bonds present in GP (Table 1). In addition to that, secondary structure analysis indicated that there are 155 (22.93%) residues which are part of the alpha helix, 167 (24.70%) residues make an extended strand, 58 (8.58%) residues are part of the beta-turn, and 296 (43.79%) make a random coil (Fig. 2A–B).

3.2. B-cell epitope prediction

B-cell epitopes are recognized by B-cells on the base of several features including hydrophilicity, beta turn prediction and surface accessibility. Plain amino acid sequence of the target protein was scanned through BCPRED and different tools of the IEDB to predict the potent B cell epitopes. Kolaskar and Tongaonkar antigenicity measuring tools analyzed the protein sequence for prediction of B-cell epitopes by assessing the physicochemical properties of the amino acids and their abundance in known B cell epitopes. Results of Kolaskar and Tongaonkar analysis have been given in Fig. 3. The threshold value of the tool was set at 1.00 and window size was kept 7. It predicted the antigenic propensity values of protein as: 1.015 (average), 0.894 (minimum) and 1.225 (maximum).

Hydrophilic regions of proteins are mostly exposed on the surface and play an immense role in evoking the immune response. Therefore, to find the surface accessibility of potential B-cell epitopes, the Parker hydrophilicity, and Emini surface accessibility prediction tools were used. The graphical representation of the output of both tools is shown

Table 3
Predicted B-cell epitopes with starting positions. FBCPRED model of prediction has been used to identify the vigorous immune response initiating B-cell epitopes (14 residues long).

Sr#	Position	Epitope	Score	Vaxijen
1	87	GFRSGVPPKVVNYE	0.997	0.9284
2	155	KEGAFFLYDRLAST	0.800	0.7879
3	515	LHYWTTQDEGAAIG	0.886	0.3980
4	595	QRWGGTCHILGPDC	0.990	0.3660
5	651	WIPAGIGVTGVIIA	0.88	1.3276

Table 4
Discontinuous epitopes predicted via DISCO TOP 2.0 Server. The residues are shown in the form of three-letter codes, and the number of contacts indicates the possible connections, which an amino acid can establish with others.

Sr#	Residue-position	Residue	Number of contacts	Propensity score	DiscoTope score
1	618	ASN	3	1.420	0.682
2	620	THR	6	2.494	1.518
3	621	ASP	7	3.400	2.204
4	622	LYS	6	3.049	2.009
5	623	ILE	3	2.487	1.856
6	624	ASP	2	2.754	2.207
7	625	GLN	8	2.696	1.466
8	626	ILE	2	1.585	1.173

in Figs. 4 and 5 respectively. Results of Emini surface accessibility analyzing tool are shown in Table 2. Beta turns are generally surface exposed, hydrophilic in nature and play a critical role in initiating the immune response, so the Chou and Fasman Beta turn analyzing algorithm was used to predict the beta turns in GP. Threshold of the tool was set at 1.00. It resulted the values as: 0.553 (minimum), 1.007 (average) and 1.437 (maximum). Output of the Chou and Fasman algorithm has been presented in Fig. 6. Results indicated that the amino acids from 320 to 340 and from 420 to 480 are part of the B-turns.

It has been reported by experimental data that the parts of epitope which interact with alleles or antibodies are mostly elastic in nature. Karplus and Schulz flexibility analyzing tool was used to detect the flexible regions of the target protein. The region ranging from 380 to 480 is most favorable in flexibility prediction (Fig. 7).

BCPRED, a freely available web server, was used to predict the B-cell epitopes using the protein sequence of GP as a query. The sequence was given in plain format, and length of the epitope was kept 14 residues long. Several B-cell epitopes were predicted and further screened
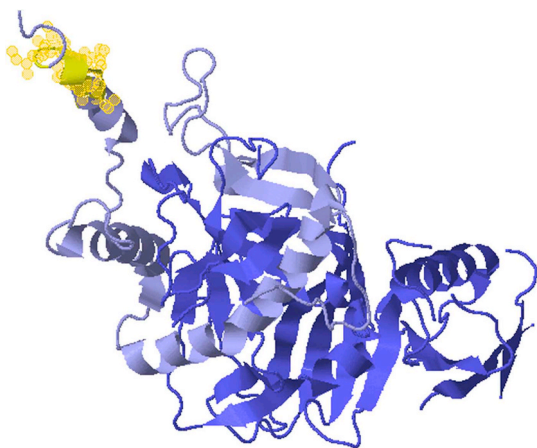

Fig. 8. Results of Disco tope server have been presented. Discontinuous epitopes were predicted through Disco tope server using the 3D structure of glycoprotein as an input. Yellow color indicates the B-cell epitopic region.
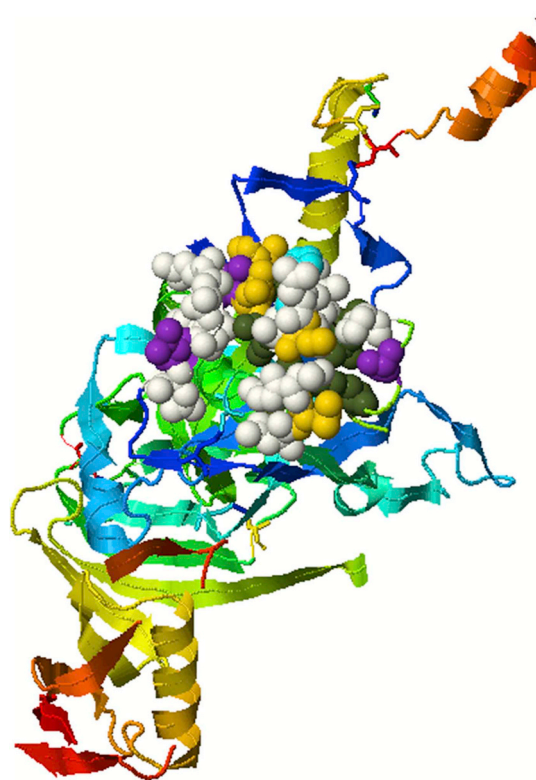

Fig. 9. Epitope position confirmation on the 3D structure of the protein, done with the help of Epi-search, has been shown. Different colors of balls are representing the different epitopes.

by antigenicity testing through Vaxijen 2.0, and only five top scoring epitopes were selected (Table 3). Among them, the peptide 'WIPAGI-GVTGVIIA' showed the highest immunogenicity score, 1.3276, and higher conservation in all species of EBOV. Antigenicity scores of other epitopes, GFRSGVPPKVVNYE and KEGAFFLYDRLAST, are 0.9284 and 0.7879 which indicate these are probable antigens. The scores of the other two epitopes are less than 0.50 indicating as non-antigen; hence, these two will not be able to act as potential vaccine candidates.

Disco Tope 2.0 Server an online available tool calculates the surface accessibility on the base of residue contact numbers and novel pro-pensity score. It took the 3D structure of the protein as input and pre-dicted the discontinuous B-cell epitopes with 90% specificity. Results of Disco Tope 2.0 Server have been shown in Fig. 8 and Table 4. Episearch, another freely available web resource for positional confirmation of predicted B-cell epitopes, was used to confirm the positions of B-cell epitopes as shown in Fig. 9.

3.3. T-cell epitope prediction

Propred and Propred-I, predict the MHC-class II and MHC-class I binding peptides respectively, were used for the prediction of T-cell epitopes. Propred-I uses a matrix-based approach to predict and scan the peptides against the library of 47 MHC class-I alleles. Several pep-tides having the ability to bind with MHC class-I alleles were predicted, initially, with Propred-I. Further screening of peptides, antigenicity testing, was done with the help of Vaxijen 2.0. Only five potential peptides were selected for further processing on the base of the anti-genicity score (Table 5). A peptide which has the ability to bind with

large number of alleles is considered as most significant peptide be-cause it has potential to evoke a strong immune response. Among MHC-class I predicted epitopes, the peptide "AIGLAWIPY" showed a highest antigenicity score '1.0672' but binding with a little number of alleles, while, "GREAAVSHLTTL" was considered as most potential peptide due to its binding ability with 27 different alleles like HLA-A*0205, HLA-A2.1, HLA-B14, HLA-B*2702, HLA-B*2705, HLA-B*3501, HLA-B*3701, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B40, HLA-B*4403, HLA-B*5101, HLA-B*5102, HLA-B*5103, HLA-B*5201, HLA-B*5301, HLA-B*5401, HLA-B*51, HLA-B*5801, HLA-B60, HLA-B7, HLA-B8, HLA-Cw*0301, HLA-Cw*0602, MHC-Db and MHC-Kb.

Propred (51 MHC Class-II alleles), a quantitative matrix based method and interacts with MHC class-II alleles, was used for prediction of T-cell peptides. Several peptides, containing the binding ability with different MHC class-II alleles, were predicted. Vaxijen was used to screen the predicted epitopes, and only five (top score containing) of them were selected for further processing (Table 6). The peptide "IRGFPRCRY" was, computationally, considered as the most antigenic for its high antigenicity score '0.7901'. While, another peptide 'WVII-LFQRTFSIPL' with antigenicity score 0.6682 showed virtual binding with a large number of alleles (almost 41), i.e. HLA-DRB1_0101, HLA-DRB1_0102, HLA-DRB1_0301, HLA-DRB1_0305, HLA-DRB1_0306, HLA-DRB1_0307, HLA-DRB1_0308, HLA-DRB1_0309, HLA-DRB1_0311, HLA-DRB1_0401, HLA-DRB1_0402, HLA-DRB1_0404, HLA-DRB1_0405, HLA-DRB1_0408, HLA-DRB1_0410, HLA-DRB1_0421, HLA-DRB1_0423, HLA-DRB1_0426, HLA-DRB1_0701, HLA-DRB1_0703, HLA-DRB1_1107, HLA-DRB1_1114, HLA-DRB1_1120, HLA-DRB1_1121, HLA-DRB1_1128, HLA-DRB1_1301, HLA-DRB1_1302, HLA-DRB1_1304, HLA-DRB1_1305,

Table 5
MHC class-I allele binding epitopes predicted through Propred. Vaxijen score indicates their putative antigenicity scores.

Sr#	Peptide	Allels	Vaxijen score
1	FHKEGAFFLYDR	HLA-A*0205, HLA-A2.1, HLA-B14, HLA-B*2702, HLA-B*2705, HLA-B*3501, HLA-B*3701, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B40, HLA-B*4403, HLA-B*5101, HLA-B*5102, HLA-B*5103, HLA-B*5201, HLA-B*5301, HLA-B*5401, HLA-B*51, HLA-B*5801, HLA-B60, HLA-B7, HLA-B8, HLA-Cw*0301, HLA-Cw*0602, MHC-Db, MHC-Kb	0.7355
2	YWTTQDEGAAIGL	HLA-B*3701, HLA-B40, HLA-B*4403, HLA-B*5301, HLA-B*5401, HLA-B*51, HLA-B7	0.8685
3	MHNQDGLICGL	HLA-A*0205, HLA-B*2702, HLA-B*2705, HLA-B*3801, HLA-B*3901, HLA-B*3902, HLA-B*5201, HLA-Cw*0401, HLA-Cw*0602,	0.6003
4	AIGLAWIPY	HLA-B40, HLA-B*4403, HLA-B*5301, HLA-B*5401, HLA-B*51, HLA-B60, HLA-B61	1.0672
5	FLRATTELRTFS	HLA-A24, HLA-B*3501, HLA-B*3701, HLA-B*3801, HLA-B*3902, HLA-B*4403, HLA-B*51, HLA-B*5801, HLA-B60, HLA-B62, HLA-B*0702, HLA-B7, HLA-B8, HLA-Cw*0301,	0.3413

Table 6
MHC class-II allele binding epitopes predicted using Propred-I.

Sr#	Peptide	Allels	Vaxijen score
1	WVIILFQRTFSIPL	HLA-DRB1_0101, HLA-DRB1_0102, HLA-DRB1_0301, HLA-DRB1_0305, HLA-DRB1_0306, HLA-DRB1_0307, HLA-DRB1_0308, HLA-DRB1_0309, HLA-DRB1_0311, HLA-DRB1_0401, HLA-DRB1_0402, HLA-DRB1_0404, HLA-DRB1_0405, HLA-DRB1_0408, HLA-DRB1_0410, HLA-DRB1_0421, HLA-DRB1_0423, HLA-DRB1_0426, HLA-DRB1_0701, HLA-DRB1_0703, HLA-DRB1_1107, HLA-DRB1_1114, HLA-DRB1_1120, HLA-DRB1_1121, HLA-DRB1_1128, HLA-DRB1_1301, HLA-DRB1_1302, HLA-DRB1_1304, HLA-DRB1_1305, HLA-DRB1_1307, HLA-DRB1_1311, HLA-DRB1_1321, HLA-DRB1_1323, HLA-DRB1_1327, HLA-DRB1_1328, HLA-DRB1_1501, HLA-DRB1_1502, HLA-DRB1_1506, HLA-DRB5_0101, HLA-DRB5_0105	**0.6682**
2	IRGFPRCRY	HLA-DRB1_0801, HLA-DRB1_0802, HLA- DRB1_0804, HLA-DRB1_0806, HLA-DRB1_0813, HLA-DRB1_0817, HLA-DRB1_1101, HLA-DRB1_1102, HLA-DRB1_1104, HLA-DRB1_1106, HLA-DRB1_1114, HLA-DRB1_1120, HLA-DRB1_1121, HLA-DRB1_1128, HLA-DRB1_1301, HLA-DRB1_1302, HLA-DRB1_1304, HLA-DRB1_1305, HLA-DRB1_1307, HLA-DRB1_1311, HLA-DRB1_1321, HLA-DRB1_1322, HLA-DRB1_1323, HLA-DRB1_1327, HLA-DRB1_1328, HLA-DRB1_1501, HLA-DRB1_1502, HLA-DRB1_1506	**0.7901**
3	VVAFLILPQ	HLA-DRB1_0306, HLA-DRB1_0307, HLA- DRB1_0308, HLA-DRB1_0311, HLA-DRB1_0402, HLA-DRB1_0404, HLA-DRB1_0405, HLA-DRB1_0408, HLA-DRB1_0410, HLA-DRB1_0423, HLA-DRB1_0801, HLA-DRB1_0802, HLA-DRB1_0804, HLA-DRB1_0806, HLA-DRB1_0813, HLA-DRB1_0817, HLA-DRB1_1101, HLA-DRB1_1102, HLA-DRB1_1104, HLA-DRB1_1106, HLA- DRB1_1107, HLA-DRB1_1114, HLA-DRB1_1120, HLA-DRB1_1121, HLA-DRB1_1128, HLA-DRB1_1301, HLA-DRB1_1302, HLA-DRB1_1304, HLA-DRB1_1305, HLA-DRB1_1307, HLA-DRB1_1311, HLA-DRB1_1321, HLA-DRB1_1322, HLA-DRB1_1323, HLA-DRB1_1327, HLA-DRB1_1328, HLA-DRB1_1501, HLA-DRB1_1502, HLA-DRB1_1506	**0.6924**
4	DKLVCRDKLSS	HLA-DRB1_0301, HLA-DRB1_0305, HLA-DRB1_0306, HLA-DRB1_0307, HLA-DRB1_0308, HLA-DRB1_0309, HLA-DRB1_0311, HLA-DRB1_0402, HLA-DRB1_0801, HLA-DRB1_0802, HLA-DRB1_0804, HLA-DRB1_0806, HLA-DRB1_0813, HLA-DRB1_0817, HLA-DRB1_1101, HLA- DRB1_1102, HLA- DRB1_1104, HLA- DRB1_1106, HLA- DRB1_1107, HLA- DRB1_1121, HLA- DRB1_1311, HLA-DRB1_1321, HLA- DRB1_1322, HLA- DRB1_1501, HLA- DRB1_1502	**0.7714**
5	LQLFLRATTELR	HLA- DRB1_0309, HLA- DRB1_0401, HLA- DRB1_0402, HLA- DRB1_0408, HLA- DRB1_0421, HLA- DRB1_0426, HLA- DRB1_0801, HLA-DRB1_0802, HLA- DRB1_0804, HLA- DRB1_0806, HLA- DRB1_0813, HLA- DRB1_1101, HLA- DRB1_1102, HLA- DRB1_1104, HLA- DRB1_1106, HLA- DRB1_1121, HLA- DRB1_1304, HLA- DRB1_1321, HLA- DRB1_1322, HLA- DRB1_1501, HLA- DRB1_1506	**0.6327**

Table 7
Alternative B T-cell (MHC Class-I and II) peptides for all five species of EBOV have been shown. The peptides were predicted using the IEDB Analysis Resource. Residues in boldface are indicating the variations.

Peptide	MHC Class-I Peptides				
	Zaire	Sudan	Tai Forest	Bundibugyo	Reston
P1	FHKEGAFFLYDRL	FHK**D**GAFFLYDRL	FHKEGAFFLYDRL	FHKEGAFFLYDRL	FHK**N**GAFFLYDRL
P2	YWTTQDEGAAIGL	NA	YWT**A**LDEGAAIG	YWTTQDEGAAIGL	YWT**AV**DEGAA**V**GL
P3	MHNQDGLICGL	MHNQ**NALV**CGL	ME**N**Q**N**GLICGL	MHNQ**N**GLICGL	MHNQ**N**GLICGL
P4	FLRATTELRTFS	FLRATTELR**TYT**	FLRATTELRTFS	FLRATTELRTFS	FLRATTELRT**YS**
P5	AIGLAWIPY	A**A**GIAWIPY	AIGLAWIPY	AIGLAWIPY	A**V**GLAWIPY
	MHC Class-II Peptides				
P1	WVIILFQRTFSIPL	WVIILFQ**KAFSM**PL	WVIILF**HKV**FSIPL	WVIILF**HKV**F**P**IPL	WVIILFQR**AISM**PL
P2	IRGFPRCRY	**V**RGFPRCRY	NA	**V**RGFPRCRY	**V**RGFPRCRY
P3	VVAFLILPQ	NA	V**IA**FLILP**K**	VVAFLILP**K**	VVAFLIL**SE**
P4	DKLVCRDKLSS	NA	DK**F**VCRDKLSS	DKLVCRDKLSS	D**Q**LVCRDKLSS
P5	LQLFLRATTELR	LQLFLRATTELR	LQLFLRATTELR	LQLFLRATTELR	LQLFLRATTELR
	B-Cell Peptides				
P1	GFRSGVPPKVVNYE	GFRSGVPPKVV**S**YE	GFR**A**GVPPKVVNYE	GFR**A**GVPPKVVNYE	GFRSGVPPKVV**S**YE
P2	QRWGGTCHILGPDC	**R**RWGGTC**R**ILGPDC	QRWGGTCHILGPDC	QRWGGTCHILGPDC	QRWGGTC**R**ILGP**SC**
P3	LHYWTTQDEGAAIG	NA	LHYWT**A**LDEGAAIG	LHYWTTQDEGAAIG	LHYWT**AV**DEGAA**V**G
P4	WIPAGIGVTGVIIA	WIPAGIG**ITGIII**A	W**V**PAGIG**IT**GVIIA	W**V**PAGIG**IT**GVIIA	WIPAGIG**IIG**VIIA
P5	KEGAFFLYDRLAST	K**D**GAFFLYDRLAST	KEGAFFLYDRLAST	KEGAFFLYDRLAST	K**N**GAFFLYDRLAST

HLA-DRB1_1307, HLA-DRB1_1311, HLA-DRB1_1321, HLA-DRB1_1322, HLA-DRB1_1323, HLA-DRB1_1327, HLA-DRB1_1328, HLA-DRB1_1501, HLA-DRB1_1502, HLA-DRB1_1506, HLA-DRB5_0101, HLA-DRB5_0105.

3.4. Conservation analysis

Sequences of EBOV's GP belonging to 12 different countries were subjected to multiple sequence alignment to analyze the conservation of selected epitopes by CLC workbench. Besides this, sequences of all five species of EBOV were aligned, through multiple sequence alignment tool (ClustalW), and most conserved epitopes were tried to pick. In addition to that, IEDB conservation analysis tool was used to analyze the conservancy of selected peptides. Alternative peptides, predicted by IEDB, of each species for B and T-cells have been presented in Table 7.

3.5. Evaluation of other eminent features of predicted epitopes

Other vital features of predicted peptides were assessed through Protein Digestion Server. All B and T-cell epitopes along with their non-digesting enzymes, PI and mass have been presented in Table 8. The peptides digested by fewer enzymes are more stable and potent vaccine candidates. Among the B-cell epitopes, the peptide 'WIPAGIGVTGVIIA'

showed, apparently, highest stability and in MHC class-I and class-II peptides, the peptides 'MHNQDGLICGL' and 'VVAFLILPQ' were most stable.

Table 9
Glycoprotein peptides of EBOV reportedly involved in initiating the immune response in primates have been presented. Antigenicity score was calculated via Vaxijen 2.0.

Peptides	Antigenicity score	References
LYDRLASTVI	0.4157	(43)
VSTGTGPGAGDFAFHK	0.7059	(43)
EYLFEVDNL	0.3974	(43)
TELRTFSI	0.6579	(44)
ATQVEQHHRRTDNDSTA	0.7406	(11)
HNTPWKLDISEATQVE	0.6490	(11)
CKLCLITNTIAGVACL	0.6767	(11)
GPCAGDFAF	0.7327	(45)
LYDRLASTV	0.4157	(45)
VSTGTGPGAGDFAFHK	0.7059	(46)
WIPYFGPAAEGIYTE	0.4157	(46)
EYLFEVDNL	0.3974	(46)

Table 8
Digestion analysis' results have been presented. Non-digesting enzymes are indicating those enzymes which do not digest the peptides into fragments.

MHC Class-I binding peptides			
Peptide	Non-digesting Enzymes	Mass	PI
FHKEGAFFLYDR	Clostripain, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Trypsin_R,	1642.88	6.75
YWTTQDEGAAIGL	Trypsin, Clostripain, Cyanogen_Bromide, Proline_Endopept, Trypsin_K, Trypsin_R,	1424.53	3.67
MHNQDGLICGL	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease, Trypsin_K, Trypsin_R,	1200.39	5.60
FLRATTELRTFS	Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Trypsin_K, AspN,	1441.65	9.60
AIGLAWIPY	Trypsin, Clostripain, Cyanogen_Bromide, Proline_Endopept, Staph_Protease, Trypsin_K, Trypsin_R, AspN	1003.21	5.57
MHC Class-II binding peptides			
WVIILFQRTFSIPL	Cyanogen_Bromide, Staph_Protease, Trypsin_K, AspN,	1733.13	9.75
IRGFPRCRY	Chymotrypsin, Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, Trypsin_K, AspN, Chymotrypsin (modified),	1167.40	10.76
VVAFLILPQ	Trypsin, Clostripain, Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, Trypsin_K, Trypsin_R, AspN,	999.26	5.49
DKLVCRDKLSS	Chymotrypsin, Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Staph_Protease,	1263.47	8.20
LQLFLRATTELR	Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Trypsin_K, AspN,	1460.74	9.60
B-Cell Peptides			
GFRSGVPPKVVNYE	Cyanogen_Bromide, IodosoBenzoate, Staph_Protease, AspN,	1548.76	8.59
QRWGGTCHILGPDC	Cyanogen_Bromide, Staph_Protease, Trypsin_K,	1542.75	6.73
LHYWTTQDEGAAIG	Trypsin, Clostripain, Cyanogen_Bromide, Proline_Endopept, Trypsin_K, Trypsin_R,	1561.67	4.35
WIPAGIGVTGVIIA	Trypsin, Chymotrypsin, Clostripain, Cyanogen_Bromide, Staph_Protease, Trypsin_K, Trypsin_R, AspN,	1366.67	5.52
KEGAFFLYDRLAST	Cyanogen_Bromide, IodosoBenzoate, Proline_Endopept, Trypsin_K,	1617.82	6.07

Table 10
Docking score and interacting residues of HLA-B7 with MHC class-I binding peptides have been given. The interacting residues were determined using the MOE.

Sr#	Peptide	Docking Score	Interacting residues
1	AIGLAWIPY	−13.15	Ser B28, Glu A232, GlnA115
2	FHKEGAFFLYDR	−18.38	Gln B2, Glu B36, Val B85, Thr B86, Leu B87,
3	FLRATTELRTFS	−25.90	Asp B34, Glu B50, His B51,
4	MHNQDGLICGL	−20.36	Ile B35, Glu B50, His B51
5	YWTTQDEGAAIGL	−24.97	Arg A14, Arg 35, Tyr B66

3.6. Interaction study of predicted peptides with HLA alleles

3D structures of MHC classes-I binding peptides were predicted through PEPFOLD. It produces five models of each peptide; one best model for each epitope was selected and placed in the database after energy minimization with the help of MOE. The 3D structure of HLA-B7 was taken from PDB which was bound with "RPHERNGFTVL". Peptide removal and energy minimization were done before docking. A library of five potential peptides was docked against HLA-B7 to study the interaction of peptides with the allele. 10 confirmations for each epitope were allowed and only the best one was selected. Results of the Interaction analysis provided the information about the interacting residues and docking scores as given in Table 10. The peptide "FLRA-TTELRTFS" hold a highest score (−25.90) and showed a strong interaction with Asp B34, Glu B50 and His B51. Interactions of all predicted peptides with HLA-B7 have been presented in Fig. 10.

3.7. Allergenicity analysis

Allergenicity assessment of query protein was performed via Aller Hunter. Query sequence did not fulfill the criteria set by FAO/WHO for allergen prediction; therefore, it was declared as non-allergenic with a score of 0.0 (SE = 91.6%, SP = 89.3%).

4. Discussion

Having surpassed 7 billion, the population of the world has been facing serious health threats due to viruses. The rate is much higher in developing countries as compared to the developed ones. So there is an urgent need to combat this devastating threat. Medical biotechnology is playing an impressive role through the preparation of powerful vaccines to hamper the disaster caused by viruses. Identification of epitopes on the basis of experimental approach is time consuming and expensive. Rapid development in immuno-informatics approaches are playing a critical role in decreasing the cost and time, while, increasing the specificity of the epitope-based vaccine designing. It helps in the identification of antigenic parts of the surface exposed protein that could be used to evoke the strong immune response. Therefore, the current study incorporates various immuno-informatics tools and algorithms for the prediction of B cell and T cell epitopes. In recent decades, the vaccine developers have been focusing and relying on GPs, as they are involved in entry and attachment of pathogen with host cell; therefore it plays the role of the disease-causing agent. EBOV's GP, a surface exposed and highly immunogenic protein as having been described by previous studies plays an impressive role in attachment and entry of viral particle into host [46–51]. Due to the surface exposition and reported antigenicity, GP has been chosen as the target protein to develop the epitope-based vaccine in the current study. Numerous GP peptides have been reported and preliminarily checked in primates, and they provided positive feedback; though, there is no approved or licensed vaccine available till the date [11,12,29,30]. Reportedly, three peptides: LYDRLASTV (with antigenicity score 0.4157), VSTGTGPGA-GDFAFHK (0.7059) and EYLFEVDNL (0.3974), of GP have been used to immunize the mice. Two of them were involved in evoking the immune response, while, the peptide "LYDRLASTV" was failed to activate the T cells [52]. In another study, three peptides of GP have been used to test their immunogenicity in mice, and all of those were successful in evoking the immune response and described as immunogenic. Further, two peptides, LYDRLASTV and GPCAGDFAF, of the EBOV GP were screened via immuno-informatics tools and later on used to immunize the mice. Both of these showed a positive response and were involved in the stimulation of the T cells [45]. Despite all these reported studies, there is no licensed vaccine of Ebola virus is present in the market till the day. Therefore, the present study was designed to find out the potential B and T cell epitopes, which could be used as vaccine candidates to resist the spread of EBOV. For prediction of B and T cell epitopes, several analyses were carried out at the primary, secondary and the tertiary structure of the protein. Most of the B cell epitopes are discontinuous and nearly 10% epitopes are linear [53]. Different B cell
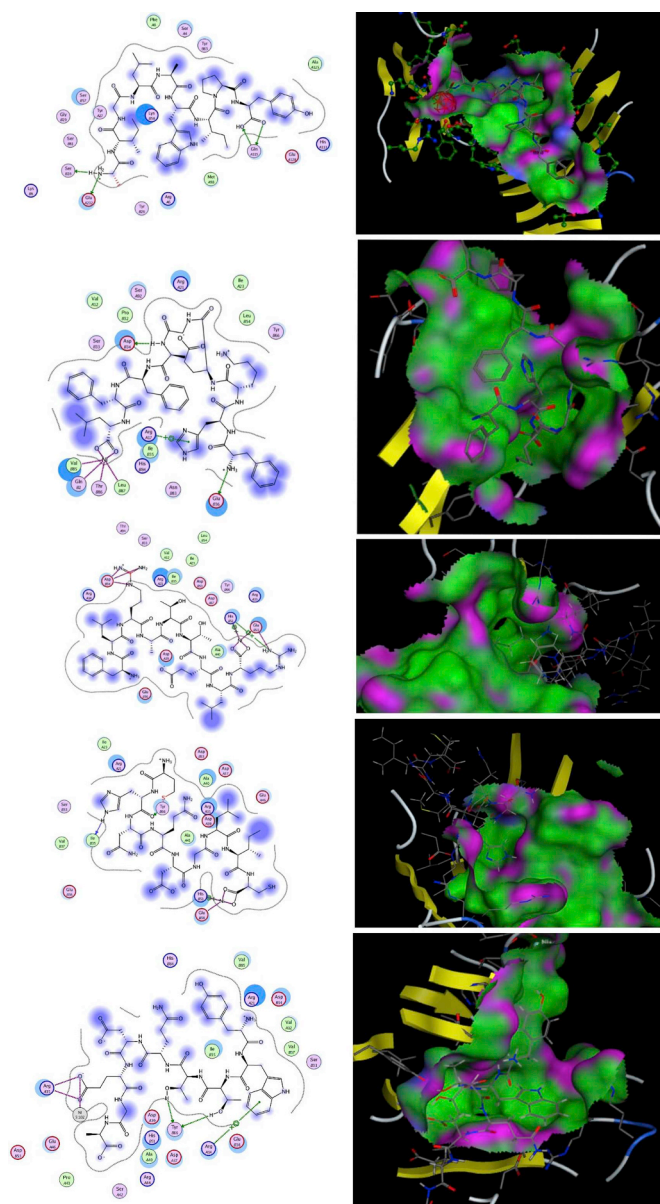

Fig. 10. Interaction analysis of MHC class-I alleles binding peptides has been given. The peptides are in symmetry as provided in Table 10 showing the docking scores and interacting residues. Figures on the left side are showing the interacting residues, and on the right side showing the binding of the peptides in the binding pocket of HLA-B7.

epitopes were predicted with the help of BCPRED and other B-Cell epitope finding tools of IEDB. All the predicted B-cell epitopes are 14 residues long and are highly conserved among all the five species: ZEBOV, TAFV, SUDV, BDBV and RESTV, of EBOV except the 'LHYW-TTQDEGAAIG' which is missing only in the SUDV. Among these, the peptide 'WIPAGIGVTGVIIA' showed the highest immunogenicity score (1.3276) and would act as a robust B-cell epitope and vaccine candidate. Additionally, the position of all predicted B-cell epitopes was confirmed with the help of Episearch. Discontinuous epitopes were also predicted because > 90% of B cell epitopes are distantly separated segments in the pathogen protein sequence and functionally folded 3D structure brings the separated segments into close proximity [54]. Disco Tope 2.0 server was also used to predict the discontinuous epitopes, which are more specific as compared to the linear epitopes as has been reported earlier [26].

Currently mostly vaccines are designed on the basis of B cell immunity. But recently, vaccine based on T cell epitope can generate a strong immune response by CD8 + T cell against the infected cell [55]. 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 [56]. In this study, several T-cell epitopes have been predicted, which have the binding ability with MHC class-I and class-II alleles. Varying in length, the T-cell epitopes (MHC class-I) are highly conserved among the five species of EBOV except for the peptides 'YWT-TQDEGAAIGL' and 'TTGKLIWKV' which are not applicable to SUDV, and SUDV and RESTV respectively. Among the MHC class-II epitopes, all the peptides are mostly conserved in the all five species of EBOV except for: "IRGFPRCRY", "VVAFLILPQ" and 'DKLVCRDKLSS' which are not only conserved in TAFV and SUDV respectively. If compared, the peptides already reported (Table 9) are not conserved among all EBOV species, and their antigenicity score is low in contrast to the reported in the current study. The digestion analysis and allergenicity testing vividly presented that the peptides predicted in the current study are stable and safe to use. Moreover, we did the interaction analysis of predicted MHC class-I binding peptides with HLA-B7 allele to check the binding and immune response evoking capability of the predicted peptides. Flexible docking results, score, interacting residues and root mean square deviation, showed that all the predicted peptides have a strong interaction with MHC class-I alleles and can play a positive role in the initiation of immune response and prevention of risks caused by EBOV. On the basis of immunogenicity score, conservation and interaction analysis, it would not be wrong to say that the peptides purposed in the present study would be more immunogenic and conserved 'among all species of EBOV' as compared to the already reported peptides.

5. Conclusion

Reverse vaccinology, eminently, focuses on the surface exposed proteins instead of the whole pathogen. It played its role in increasing the specificity while reducing the time and economic cost simultaneously. Only immunogenic parts of the pathogen are screened to find out the potential vaccine candidates. Hence, the present study was designed to find out the most immunogenic protein in EBOV and to use it for further screening to identify powerful vaccine candidates. GP, already reported as an antigenic protein, was screened to find out the potential B and T-cell epitopes, which would act as vaccine candidates. Peptides predicted in the present study are, virtually, more immunogenic, stable and conserved as compared to the already reported ones. Thus, it is concluded that the predicted peptides would play an eminent role in vaccine designing to save humanity from this devastating menace.

References

[1] A. Marzi, H. Feldmann, T.W. Geisbert, D. Falzarano, Vesicular stomatitis virus-based vaccines for prophylaxis and treatment of filovirus infections, J. Bioterrorism Biodefense 1 (4) (2011) 2157-2526-S1-004.

[2] B. Bannister, V. Puro, F.M. Fusco, J. Heptonstall, G. Ippolito, E.W. Group, Framework for the design and operation of high-level isolation units: consensus of the European Network of Infectious Diseases, Lancet Infect. Dis. 9 (2009) 45–56.

[3] A. Sanchez, S.G. Trappier, B. Mahy, C.J. Peters, S.T. Nichol, The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing, Proc. Natl. Acad. Sci. Unit. States Am. 93 (1996) 3602–3607.

[4] A. Sanchez, Z.-Y. Yang, L. Xu, G.J. Nabel, T. Crews, C.J. Peters, Biochemical analysis of the secreted and virion glycoproteins of Ebola virus, J. Virol. 72 (1998) 6442–6447.

[5] V.E. Volchkov, H. Feldmann, V.A. Volchkova, H.-D. Klenk, Processing of the Ebola virus glycoprotein by the proprotein convertase furin, Proc. Natl. Acad. Sci. Unit. States Am. 95 (1998) 5762–5767.

[6] S.A. Jeffers, D.A. Sanders, A. Sanchez, Covalent modifications of the Ebola virus glycoprotein, J. Virol. 76 (2002) 12463–12472.

[7] S. Baize, E.M. Leroy, M.-C. Georges-Courbot, M. Capron, J. Lansoud-Soukate, P. Debré, et al., Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients, Nat. Med. 5 (1999) 423–426.

[8] H. Feldmann, H.-D. Klenk, A. Sanchez, Molecular Biology and Evolution of Filoviruses. Unconventional Agents and Unclassified Viruses, Springer, 1993, pp. 81–100.

[9] R.J. Wool-Lewis, P. Bates, Characterization of Ebola virus entry by using pseudotyped viruses: identification of receptor-deficient cell lines, J. Virol. 72 (1998) 3155–3160.

[10] K.L. Warfield, K.A. Howell, H. Vu, J. Geisbert, G. Wong, S. Shulenin, et al., Role of antibodies in protection against Ebola virus in nonhuman primates immunized with three vaccine platforms, J. Infect. Dis. 218 (2018) S553–S564.

[11] J.A. Wilson, M. Hevey, R. Bakken, S. Guest, M. Bray, A.L. Schmaljohn, et al., Epitopes involved in antibody-mediated protection from Ebola virus, Science 287 (2000) 1664–1666.

[12] J. Duehr, T.J. Wohlbold, L. Oestereich, V. Chromikova, F. Amanat, M. Rajendran, et al., Novel cross-reactive monoclonal antibodies against Ebolavirus glycoproteins show protection in a murine challenge model, J. Virol. 91 (16) (2017) e00652-17JVI. 00652-17.

[13] S. Shahhosseini, D. Das, X. Qiu, H. Feldmann, S. Jones, M. Suresh, Production and characterization of monoclonal antibodies against different epitopes of Ebola virus antigens, J Virol Methods 143 (2007) 29.

[14] S. Shahhosseini, D. Das, X. Qiu, H. Feldmann, S.M. Jones, M.R. Suresh, Production and characterization of monoclonal antibodies against different epitopes of Ebola virus antigens, J Virol Methods 143 (2007) 29–37.

[15] J.E. Lee, M.L. Fusco, A.J. Hessell, W.B. Oswald, D.R. Burton, E.O. Saphire, Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor, Nature 454 (2008) 177–182.

[16] A.W. Purcell, J. McCluskey, J. Rossjohn, More than one reason to rethink the use of peptides in vaccine design, Nat. Rev. Drug Discov. 6 (2007) 404.

[17] R. Rappuoli, Reverse vaccinology, Curr. Opin. Microbiol. 3 (2000) 445–450.

[18] U.A. Ashfaq, B. Ahmed, De novo structural modeling and conserved epitopes prediction of zika virus envelop protein for vaccine development, Viral Immunol. 29 (7) (2016) 436–443.

[19] M. Khan, M. Hossain, S. Rakib-Uz-Zaman, M. Morshed, Epitope-based peptide vaccine design and target site depiction against Ebola viruses: an immunoinformatics study, Scand. J. Immunol. 82 (2015) 25–34.

[20] R. Dash, R. Das, M. Junaid, M.F.C. Akash, A. Islam, S.Z. Hosen, In silico-based vaccine design against Ebola virus glycoprotein, Comput. Biol. Chem. Adv. Appl.: AABC 10 (2017) 11.

[21] M.R. Dikhit, S. Kumar, B.R. Sahoo, R. Mansuri, A. Amit, M.Y. Ansari, et al., Computational elucidation of potential antigenic CTL epitopes in Ebola virus, Infect. Genet. Evol. 36 (2015) 369–375.

[22] R. Hossain, T. Yasmin, M.I. Hosen, A. Nabi, In silico identification of potential epitopes present in human adenovirus proteins for vaccine design and of putative drugs for treatment against viral infection, J. Immunol. Methods 455 (2018) 55–70.

[23] 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.

[24] M. Ali, R.K. Pandey, N. Khatoon, A. Narula, A. Mishra, V.K. Prajapati, Exploring dengue genome to construct a multi-epitope based subunit vaccine by utilizing immunoinformatics approach to battle against dengue infection, Sci. Rep. 7 (2017) 9232.

[25] U.A. Ashfaq, B. Ahmed, De novo structural modeling and conserved epitopes prediction of zika virus envelop protein for vaccine development, Viral Immunol. 29 (2016) 436–443.

[26] R. Sharmin, A.B.M.M.K. Islam, A highly conserved WDYPKCDRA epitope in the RNA directed RNA polymerase of human coronaviruses can be used as epitope-based universal vaccine design, BMC Bioinf. 15 (2014) 161.

[27] M. Lapelosa, E. Gallicchio, G.F. Arnold, E. Arnold, R.M. Levy, In silico vaccine design based on molecular simulations of rhinovirus chimeras presenting HIV-1 gp41 epitopes, J. Mol. Biol. 385 (2009) 675–691.

[28] M.R. Dikhit, S. Kumar, Vijaymahantesh, B.R. Sahoo, R. Mansuri, A. Amit, et al., Computational elucidation of potential antigenic CTL epitopes in Ebola virus,

Infect. Genet. Evol.: J. Mol. Epidemiol. Evol. Genet. Infect. Dis. 36 (2015) 369–375.

[29] S.M. Jones, H. Feldmann, U. Ströher, J.B. Geisbert, L. Fernando, A. Grolla, et al., Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses, Nat. Med. 11 (2005) 786–790.

[30] J.V. Kringelum, C. Lundegaard, O. Lund, M. Nielsen, Reliable B cell epitope predictions: impacts of method development and improved benchmarking, PLoS Comput. Biol. 8 (2012) e1002829.

[31] A. Marzi, R. Yoshida, H. Miyamoto, M. Ishijima, Y. Suzuki, M. Higuchi, et al., Protective efficacy of neutralizing monoclonal antibodies in a nonhuman primate model of Ebola hemorrhagic fever, PLoS One 7 (2012) e36192.

[32] S. Chakraborty, R. Chakravorty, M. Ahmed, A. Rahman, T. Waise, F. Hassan, et al., A computational approach for identification of epitopes in dengue virus envelope protein: a step towards designing a universal dengue vaccine targeting endemic regions, Silico Biol. 10 (2010) 235–246.

[33] A. Krogh, B. Larsson, G. Von Heijne, E.L. Sonnhammer, Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes, J. Mol. Biol. 305 (2001) 567–580.

[34] P.B. Lyonnais, Sopma Secondary Structure Prediction Method, (2005).

[35] D.W. Buchan, F. Minneci, T.C. Nugent, K. Bryson, D.T. Jones, Scalable web services for the PSIPRED protein analysis workbench, Nucleic Acids Res. 41 (2013) W349–W357.

[36] L.A. Kelley, S. Mezulis, C.M. Yates, M.N. Wass, M.J. Sternberg, The Phyre2 web portal for protein modeling, prediction and analysis, Nat. Protoc. 10 (2015) 845–858.

[37] A. Drozdetskiy, C. Cole, J. Procter, G.J. Barton, JPred4: a protein secondary structure prediction server, Nucleic Acids Res. 43 (Web Server issue) (2015) W389–W394 gkv332, 1.

[38] J.E.P. Larsen, O. Lund, M. Nielsen, Improved method for predicting linear B-cell epitopes, Immunome Res. 2 (2006) 1.

[39] Y. EL-Manzalawy, D. Dobbs, V. Honavar, Predicting linear B-cell epitopes using string kernels, J. Mol. Recognit. 21 (2008) 243–255.

[40] T.M. Fieser, J.A. Tainer, H.M. Geysen, R.A. Houghten, R.A. Lerner, Influence of protein flexibility and peptide conformation on reactivity of monoclonal anti-peptide antibodies with a protein alpha-helix, Proc. Natl. Acad. Sci. Unit. States Am. 84 (1987) 8568–8572.

[41] E.A. Emini, J.V. Hughes, D. Perlow, J. Boger, Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide, J. Virol. 55 (1985) 836–839.

[42] P. Koehl, M. Levitt, Structure-based conformational preferences of amino acids, Proc. Natl. Acad. Sci. Unit. States Am. 96 (1999) 12524–12529.

[43] H. Singh, G. Raghava, ProPred1: prediction of promiscuous MHC Class-I binding sites, Bioinformatics 19 (2003) 1009–1014.

[44] H. Singh, G. Raghava, ProPred: prediction of HLA-DR binding sites, Bioinformatics 17 (2001) 1236–1237.

[45] T. Maruyama, L.L. Rodriguez, P.B. Jahrling, A. Sanchez, A.S. Khan, S.T. Nichol, et al., Ebola virus can be effectively neutralized by antibody produced in natural human infection, J. Virol. 73 (1999) 6024–6030.

[46] E. Davidson, C. Bryan, R.H. Fong, T. Barnes, J.M. Pfaff, M. Mabila, et al., Mechanism of binding to Ebola virus glycoprotein by the ZMapp, ZMAb, and MB-003 cocktail antibodies, J. Virol. 89 (2015) 10982–10992.

[47] A.I. Flyak, X. Shen, C.D. Murin, H.L. Turner, J.A. David, M.L. Fusco, et al., Cross-Reactive and potent neutralizing antibody responses in human survivors of natural ebolavirus infection, Cell 164 (2016) 392–405.

[48] A.M. Henao-Restrepo, I.M. Longini, M. Egger, N.E. Dean, W.J. Edmunds, A. Camacho, et al., Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial, Lancet 386 (2015) 857–866.

[49] I.D. Milligan, M.M. Gibani, R. Sewell, E.A. Clutterbuck, D. Campbell, E. Plested, et al., Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia ankara-vectored Ebola vaccines: a randomized clinical trial, Jama 315 (2016) 1610–1623.

[50] S. Wu, A. Kroeker, G. Wong, S. He, L. Hou, J. Audet, et al., An adenovirus vaccine expressing Ebola virus variant makona glycoprotein is efficacious in Guinea pigs and nonhuman primates, J. Infect. Dis. 214 (2016) S326–S332.

[51] Q. Zhang, M. Gui, X. Niu, S. He, R. Wang, Y. Feng, et al., Potent neutralizing monoclonal antibodies against Ebola virus infection, Sci. Rep. 6 (2016) 25856.

[52] A. Patronov, I. Doytchinova, T-cell epitope vaccine design by immunoinformatics, Open Biol. 3 (2013) 120139.

[53] Y. Wang, W. Wu, N.N. Negre, K.P. White, C. Li, P.K. Shah, Determinants of antigenicity and specificity in immune response for protein sequences, BMC Bioinf. 12 (2011) 251.

[54] D.J. Barlow, M.S. Edwards, J.M. Thornton, Continuous and discontinuous protein antigenic determinants, Nature 322 (1986) 747–748.

[55] B. Shrestha, M.S. Diamond, Role of CD8+ T cells in control of West Nile virus infection, J. Virol. 78 (2004) 8312–8321.

[56] 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.