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Multi Epitope Peptide Vaccine Prediction against Sudan Ebola Virus Using Immuno-Informatics Approaches

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Abstract

Sudan Ebola virus is single stranded negative sense RNA genome belonging to Filovirus Filoviridae family that causes hemorrhagic fever. There is no treatment or vaccine for it, thus the aim of this study is to design a peptide vaccine using immuoinformatics approaches to analyse the glycoprotein of the all strain of SUDV, to determine the conserved region which is further studied to predict all possible epitopes that can be used as a peptide vaccine. A total of 21 Sudan Ebola virus glycoprotein retrieved from NCBI database were aligned to determine the conservancy and to predict the epitopes using IEDB analysis resource. Three epitopes predicted as a peptide vaccine for B cell **(PPPPDGVR, ETFLQSPP, LQSPPIRE)**. For T cell four epitopes showed high affinity to MHC class I (**FLYDRLAST, IIIAIIALL, MHNQNALVC** and **RTYTILNRK**) and high coverage against Sudan and the whole world population. Also in MHC class II, Four epitopes that interact with most frequent MHC class II alleles (**FAEGVIAFL, FLRATTELR, FLYDRLAST** and **FVWVIILFQ**) with high coverage against Sudan and the whole world population. We recommend *in vivo* and *in vitro* study to prove the effectiveness of these predicted epitopes as a peptide vaccine.

Keywords: Sudan ebola virus (SUDV); Epitope; Peptide vaccine; Immune epitope database (IEDB)

Introduction

Ebola virus is belonging to Filoviruses Filoviridae family which is zoonotic pathogen that causes hemorrhagic fever for both human and nonhuman primate with high rate of death that exceeded 80% [1-8]. The first appearance of Ebola virus in Sudan, Yambuku, Nzara and Democratic Republic of Congo was in 1976 than it spread into a village near the Ebola River [2,4].

The first outbreak of Ebola virus was in Sudan, specifically in Nzara town in southern Sudan; as it started from a cotton factory and spread rapidly as a result of transmission from person to person of 15 generations leading to 284 infected individuals with 151 deaths. The second one was in Zaire (Democratic Republic of Congo) with fatality rate of 88%.

Ebola virus generally composed of single stranded negative sense RNA genome encoding a nucleoprotein (NP), viral proteins, a glycoprotein (GP) and the viral RNA-dependent RNA polymerase (L) [6].

The main Ebola virus glycoprotein (GP) is the only viral protein responsible for the attachment and immune response in the host cells which is found on the surface of the virus thus it's the main target for designing a vaccine, GP post-translationally yield GP1 and GP2 subunits [9-16].

Many studies shows that the GP plays an important role in Ebola virus infection by targeting the virus to the cells and allowing it to introduce its content into monocytes or macrophages which may lead to release of inflammatory cytokines [17]. Ebola virus stimulate immune system and inflammatory response at the same time leading to release of tumor necrosis factor (TNF) and interferon-γ (IFNγ) which in turn can disrupt some body tissues [18,19].

The first successful vaccine for Ebola virus developed in guinea pig using plasmid DNA, GP and sGP enhance cytotoxic and humoral responses but the efficacy of this DNA vaccine has been less effective in humans [17].

Our aim is to design a vaccine for Ebola virus using peptide of its glycoprotein as an immunogen to stimulate protective immune response. Survivors show high level of IgM and IgG response to antigen, a Russian investigator developed hyper immune horse serum and it was effective in baboons and guinea pigs but not in Cynomolgus monkeys. In addition, horse antibodies are not preferred for humans as some subclass of its IgG is immunogenic to humans [18].

Vaccine production that depends on biochemical experiments can be expensive, time consuming and not always work, although this vaccine formulation of attenuated or inactivated form of microorganism contains a few hundred of unnecessary proteins for the induction of immunity, that may cause allergenic or reactogenic responses [20,21].

Therefore, *in silico* prediction of epitopes of appropriate protein residues would help in production of peptide vaccine with powerful immunogenic and minimal allergenic effect [22,23]. This is the first study conducted to design a peptide vaccine against Sudan Ebola virus using an immunoinformatics approaches.

Materials and Methods

Protein sequence retrieval

A total of 21 Sudan Ebola virus strains' glycoprotein was retrieved from NCBI (http://www.ncbi.nlm.nih.gov/protein/?term=sudan+ebola+virus+glycoprotein) database in June 2016. These 21 strains sequences

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retrieved are from different parts of the world (include 11 collected from Uganda and 4 from Sudan). Retrieved glycoprotein strains and their accession numbers and area of collection are listed in (Table 1).

Phylogenetic and alignment

The retrieved sequences were conducted in Phylogenetic and alignment study to determine the common ancestor of each strain and the conservancy using different tools from (http://www.phylogeny.fr) [24]. The phylogenetic tree and alignment were presented in Figures 1 and 2.

Determination of conserved regions

The retrieved sequences were aligned to obtain conserved regions using multiple sequence alignment (MSA). Sequences aligned with the aid of ClustalW as implemented in the BioEdit program, version 7.0.9.0 [25] for finding the conserved regions among Ebola spike glycoprotein variants. Later on, the candidate epitopes were analyzed by different prediction tools from Immune Epitope Database IEDB analysis resource (http://www.iedb.org/) [25,26].

B-cell epitope prediction

B cell epitope is the portion of an immunogen, which interacts with B lymphocytes. As a result, the B-lymphocyte is differentiated into antibody-secreting plasma cell and memory cell. B cell epitope is characterized by being accessible and antigenic [27]. Thus, the classical propensity scale methods and hidden Markov model programmed softwares from IEDB analysis resource were used for the following aspects:

Prediction of linear B-cell epitopes: BepiPred from immune epitope database (http://toolsiedb.ofg/bcell/) [28] was used as linear B-cell epitopes prediction from the conserved region with a default threshold value of 0.35.

Prediction of surface accessibility: By using Emini surface accessibility prediction tool of the immune epitope database (IEDB) (http://tools.immuneepitope.org/tools/bcell/iedb) [29]. The surface accessible epitopes were predicted from the conserved region holding the default threshold value 1.000.

Prediction of epitopes antigenicity sites: (http://tools.immuneepitope.org/bcell/) [30] the kolaskar and tongaonker antigenicity method was used to determine the antigenic sites with a default threshold value of 1.016.

MHC class I binding predictions

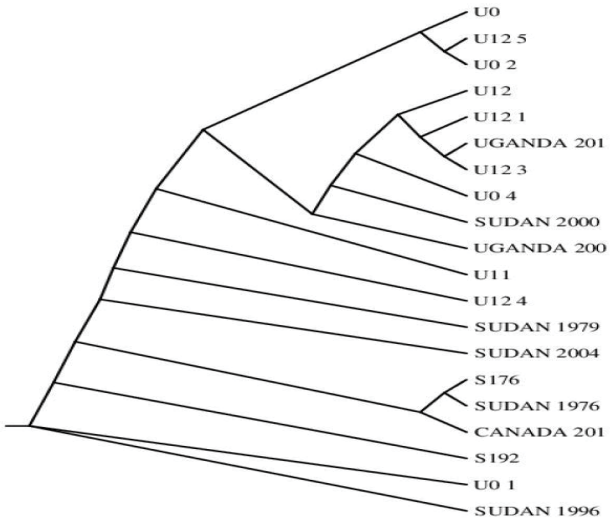
Analysis of peptide binding to MHC class I molecules was assessed by the IEDB MHC I prediction tool at http://tools.iedb.org/mhci/ n,MHC-I peptide complex presentation to T lymphocytes undergo several steps. The attachment of cleaved peptides to MHC molecules step was predicted. Prediction methods can be achieved by Artificial Neural Network (ANN), Stabilized Matrix Method (SMM) or Scoring Matrices derived from Combinatorial Peptide Libraries, ANN method was used [31-35]. Prior to prediction, all epitope lengths were set as 9mers, all conserved epitopes that bind to alleles at score equal or less than 100 half-maximal inhibitory concentration (IC50) is selected for further analysis [36].

MHC class II binding predictions

Analysis of peptide binding to MHC class II molecules was assessed by the IEDB MHC II prediction tool at http://tools.immuneepitope.org/mhcii/ [37,38]. For MHC-II binding predication, human allele

Accession Number	Date of collection	Country
ACR33190	1976	Sudan
ABY75325	2004	Sudan
Q66798	1996	Sudan
AAB37096	1996	Sudan
AAC54882	1996	Sudan
ALT19781	2000	Sudan
AFP28231	2011	Uganda
AAR11463	2000	Uganda
***YP_138523**	2000	Uganda
AAP88031	2000	Uganda
ALL26375	2015	Canada
AGB56678	1979	Sudan
AKB09538	2000	Uganda
AAU43887	2000	Uganda
ALH21228	1976	Sudan
AGL73446	2012	Uganda
AGL73439	2012	Uganda
AGL73432	2012	Uganda
AGL73425	2012	Uganda
AGL50928	2012	Uganda
Q7T9D9	2012	Uganda

Table 1: Virus strains retrieved and their accession numbers and area of collection. *Ref sequence



U0=UGANDA 2000
U0 1=UGANDA 2000 (1)
U0 2=UGANDA 2000 (2)
U0 4=UGANDA 2000 (4)
U12=UGANDA 2012
U12 1=UGANDA 2012 (1)
U12 3=UGANDA 2012 (3)
U12 4=UGANDA 2012 (4)
U12 5=UGANDA 2012 (5)
U11=UGANDA 2011
S176=SUDAN 1976
S192=SUDAN 1992

Figure 1: Cladogram shows the relationship between the different strains of SUDV.

references set were used. MHC class II groove has the ability to bind to peptides with different lengths. This variability in binding makes prediction as difficult as less accurate [39]. There are five prediction methods for IEDB MHC II prediction tool; SMM_align, NN- align, Compinatorial Libraries, Sturniolo's method and NetMHCIIpan in addition to the consensus method. SMM-align is a matrix-based method with extensions incorporating flanking residues outside of binding grooves, NN-align uses the artificial neural networks that allows for simultaneous identification of the MHC class II binding core epitopes and binding affinity, Compinatorial Libraries apply positional scanning combinatorial libraries approach which utilizes a pool of random peptide libraries to systematically measure the contribution to MHC binding from each amino acid at each of the nine positions at the binding peptide, Sturniolo's method and NetMHCIIpan predict peptide binding to HLA-DR molecule which make them less useful. The consensus approach combine the outcome of the three SMM-align, NN-align, Compinatorial Libraries methods which firstly run a random scan of Swiss-Prot proteins and achieve scores for 2,000,000 random peptides, thereafter, act as reference to rank new predictions. The consensus method uses the median rank of the three approaches as the final prediction score [40]. NN-algin method was used to predict MHC class II epitopes. All conserved epitopes that bind to many alleles at score equal or less than 1000 half-maximal inhibitory concentration (IC_{50}) is selected for further analysis.

Population coverage calculation

All potential MHC I and MHC II binders of Sudan Ebola virus glycoprotein were assessed for population coverage against the whole world population and Sudan population with the selected MHC-I and MHC-II interacted alleles by the IEDB population coverage calculation tool at http://tools.iedb.org/tools/population/iedb_input [41].

Results

Phylogenetic

The phylogenetic tree revealed that the strains of SUDV that collected from Uganda in 2012, 2012 (1), 2012 (3) and 2011 could be the same one, while the one that collected from Canada 2015 could be the same strain of Sudan 1976.

Alignment

Prediction of B-cell epitope: The reference glycoprotein (GP) was subjected to Bepipred linear epitope, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity methods in IEDB, that predict the probability of specific regions in the protein to bind to B cell receptor, being in the surface and immunogenic, respectively.

In Bepipred Linear Epitope Prediction method; the average binders score of Glycoprotein to B cell was 0.267, with a maximum of 3.228 and a minimum of -3.132, thirty six epitopes were predicted eliciting B lymphocyte from the conserved regions and all values equal or greater than the default threshold 0.35. In Emini surface accessibility prediction; the average surface accessibility areas of the protein was scored as 1.000, with a maximum of 8.153 and a minimum of 0.030, twenty five epitopes were potentially in the surface by passing the default threshold 1.0.

In Kolaskar and Tongaonkar antigenicity; the average of the antigenicity was 1.016, with a maximum of 1.293 and minimum of 0.848, eight epitopes gave score above the default threshold 1.016. However, there are three epitopes successfully overlapped the three tools (PPPPDGVR, ETFLQSPP, LQSPPIRE). The result is illustrated in Table 2 below and Figures 3-5, and their positions in the structural level are shown in Figure 6.

Prediction of cytotoxic T-lymphocyte epitopes and interaction with MHC class I:

The reference glycoprotein strain was analyzed using IEDB MHC-1 binding prediction tool to predict T cell epitope suggested interacting with different types of MHC Class I alleles, based on Artificial Neural Network (ANN) with half-maximal inhibitory concentration (IC_{50}) ≤ 100; 65 peptides were predicted to interact with different MHC-1 alleles. The peptide **RTYTILNRK** from 580 to 588 had higher affinity to interact with 5 alleles (HLA-A*03:01, HLA-A*30:01, HLA-A*11:01, HLA-A*31:01, HLA-A*68:01), followed by **RLASTVIYR** from 164 to 172, and **YTENTSSYY** from 205 to 213 that had affinity to interact with 4 alleles for each. The epitopes and their corresponding MHC-1 alleles are shown in Table 3. Their positions in structural level are shown in Figure 7.

Prediction of T helper cell epitopes and interaction with MHC class II

The reference glycoprotein (GP) strain was analyzed using IEDB MHC-II binding prediction tool based on NN-align with half-maximal inhibitory concentration (IC_{50}) ≤ 1000; there were 116 predicted epitopes found to interact with MHC-II alleles. The peptide (core) FLRATTELR had high affinity to interact with twenty two alleles; HLA-DPB1*04:01, HLA-DPB1*02:01, HLA-DPB1*05:01, HLA-DPB1*04:02, HLA-DPA1*01, HLA-DPA1*02:01, HLA-DPA1*03:01, HLA-DQA1*05:01, HLA-DQB1*02:01, HLA-DQB1*03:01,HLA-DRB1*01:01,HLA-DRB1*03:01,HLA-DRB1*04:05,HLA-DRB1*07:01, HLA-DRB1*08:02, HLA-DRB1*04:01, HLA-DRB1*04:01, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*11:01, HLA-DRB4*01:01, HLA-DRB5*01:01. The results of top four epitopes are listed in Table 4 below and their positions are shown in Figure 8.

Analysis of the population coverage

Epitopes of glycoprotein (GP) that are suggested interacting with MHC-I and II alleles (especially high affinity binding epitopes and that can bind to different set of alleles) were selected for population coverage analysis. The results of population coverage of all epitopes in Sudan and world are listed in Table 5.

In MHC class I, Four epitopes that interact with most frequent MHC class I alleles (**FLYDRLAST, IIIAIIALL, MHNQNALVC** and **RTYTILNRK**) gave high percentage against Sudan and the whole world population by IEDB population coverage tool. The maximum population coverage percentage of these epitopes in World was 46.73% for **FLYDRLAST** and in Sudan was 67.96% for **MHNQNALVC**.

Also in MHC class II, Four epitopes that interact with most frequent MHC class II alleles (**FAEGVIAFL, FLRATTELR, FLYDRLAST** and **FVWVIILFQ**) gave high percentage against Sudan and the whole world population by IEDB population coverage tool. The maximum population coverage percentage of these epitopes in World was 99.72% for **FVWVIILFQ** and in Sudan was 97.36% for **FLRATTELR**. The result of population coverage of proposed epitopes in Sudan and whole word are listed in Table 6.

Discussion

Various studies support the assumption that a strong, specific and adaptive immune response is needed to survive from Ebola virus infection, as well as balanced response with respect to both humoral

Epitope	Start	End	Length	Surface accessibility[a]	Antigenicity score[b]
1*GSGVSTDIPSATKRWGFRSGVPP	72	94	23	0.291	1.003
1*VSTDIPSATKR	75	85	11	1.091	1.016
VSYEAGEWAE	97	106	10	0.614	1
2*KKPDGSECLPPPPDGVRG	114	131	18	1.369	1.018
2*PPPPDGVR	123	130	8	1.669	1.031
KAQGTGPCPGD	140	150	11	0.574	0.995
3*ETFLQSPPIREA	191	202	12	1.009	1.016
3*ETFLQSPP	191	198	8	1.204	1.032
3*LQSPPIRE	194	201	8	1.323	1.035
NYTENTSSYY	204	213	10	4.602	0.973
FGAQ	225	228	4	0.531	1.011
RPHT	246	249	4	2.108	0.988
KNL	295	297	3	1.218	0.985
QLR	300	302	3	1.285	1.046
NETEDDDA	314	321	8	3.981	0.881
SSR	323	325	3	1.616	0.966
GRISDRATR	329	337	9	1.581	0.944
DLVPK	341	345	5	0.857	1.099
PGM	348	350	3	0.696	0.921
PEGETTLPSQNSTEGRRV	356	373	18	4.124	0.964
VNTQETITE	375	383	9	1.214	0.973
SSSQI	406	410	5	0.792	1.041
SSSPT	412	416	5	1.456	1.002
SPE	420	422	3	1.648	0.979
TEE	438	440	3	1.988	0.87
TTPP	442	445	4	1.765	0.987
SPG	448	450	3	0.942	0.983
TTEAPTLTTPENITT	452	466	15	1.741	0.962
QESTSNGL	474	481	8	1.24	0.962
SRRQ	499	502	4	3.156	0.943
ATGKCNP	507	513	7	0.609	1.004
AQEQHNA	520	526	7	1.835	0.984
FGPGAEGIY	535	543	9	0.232	1.001
CIE	609	611	3	0.299	1.138
HDWTKN	613	618	6	2.292	0.913
NPLPNQDNDDNWWT	633	646	14	4.322	0.914

Table 2: B-cell epitopes prediction.

2* peptide from 114 to 131 gives higher score if it is shorten (123 to 130) in all tools

3* peptide from 191 to 202 gives higher score if it is shorten (191 to 198) or (194 to 201) in all tools

a: default threshold value 1.000

b: default threshold value 1.016

Position of peptides is according to position of amino acids in the glycoprotein (GP)

and cell mediated immunity [6,19,42,43]. Several vaccine attempts are in clinical trials now or preparing to; plasmid cocktail coding GP gene of EPOV, SUDV or both as well as NP gene of EPOV were used for vaccination of SUDV, although they were immunogenic at high doses and failed to induce robust cellular immunity. As well as recombinant viruses with different types of vectors that have been shown to confer protection against SUDV in nonhuman primate, or virus like particles (VLP) that provide additional advantage as safety administered by immunosuppressed individuals. In general, these studies are hopeful, but improvement is needed to achieve better outcomes [44-53].

To our knowledge, there is no peptide prediction has been conducted specifically for this virus so far. Peptide vaccination is a key role of combining a good desirable immune response and a minimal immunological side effect. There are many peptide vaccines under development, such as vaccine for human immunodeficiency virus (HIV), hepatitis C virus (HCV), malaria, foot and mouth disease, swine fever, influenza, anthrax, human papilloma virus (HPV), therapeutic anti-cancer vaccines, pancreatic cancer, melanoma, non-small cell lung cancer, advanced hepatocellular carcinoma, cutaneous T-cell lymphoma and B-Cell chronic lymphocytic leukaemia [54-67].

In this study, we aimed to determine the 100% conserved regions which are then investigated to predict the highly potential immunogenic epitopes for both B and T cells - the prime molecules of cell mediated and humoral immunity as vaccine candidates for the highly lethal SUDV infection using Spike glycoprotein(GP) as a target. SUDV GP is the key of cell attachment, entry and infectivity of the

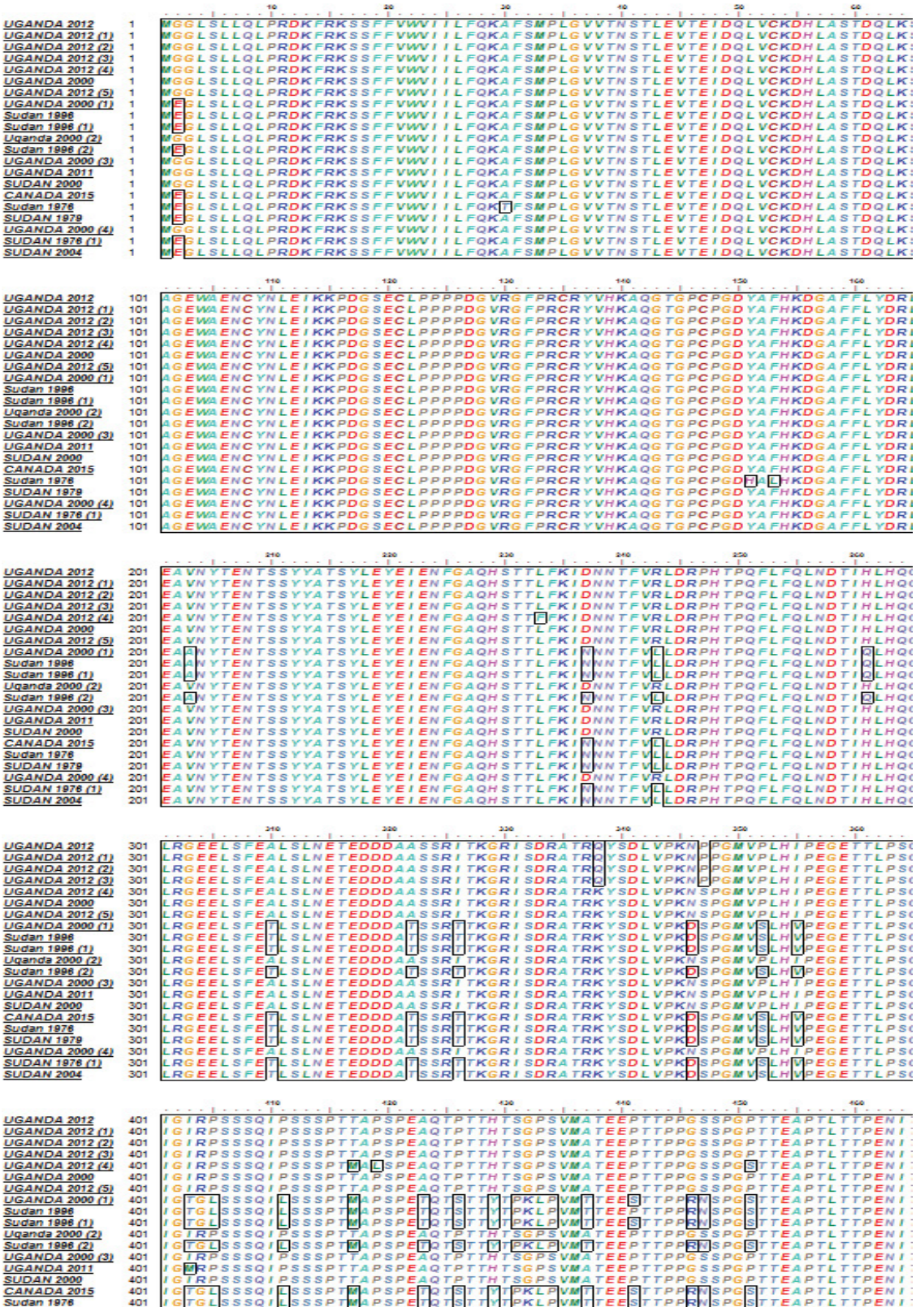


Figure 2: Multiple sequence alignment (the most mutated region) dots show the conservancy between sequences.

*The alignment is done using BioEdit tool

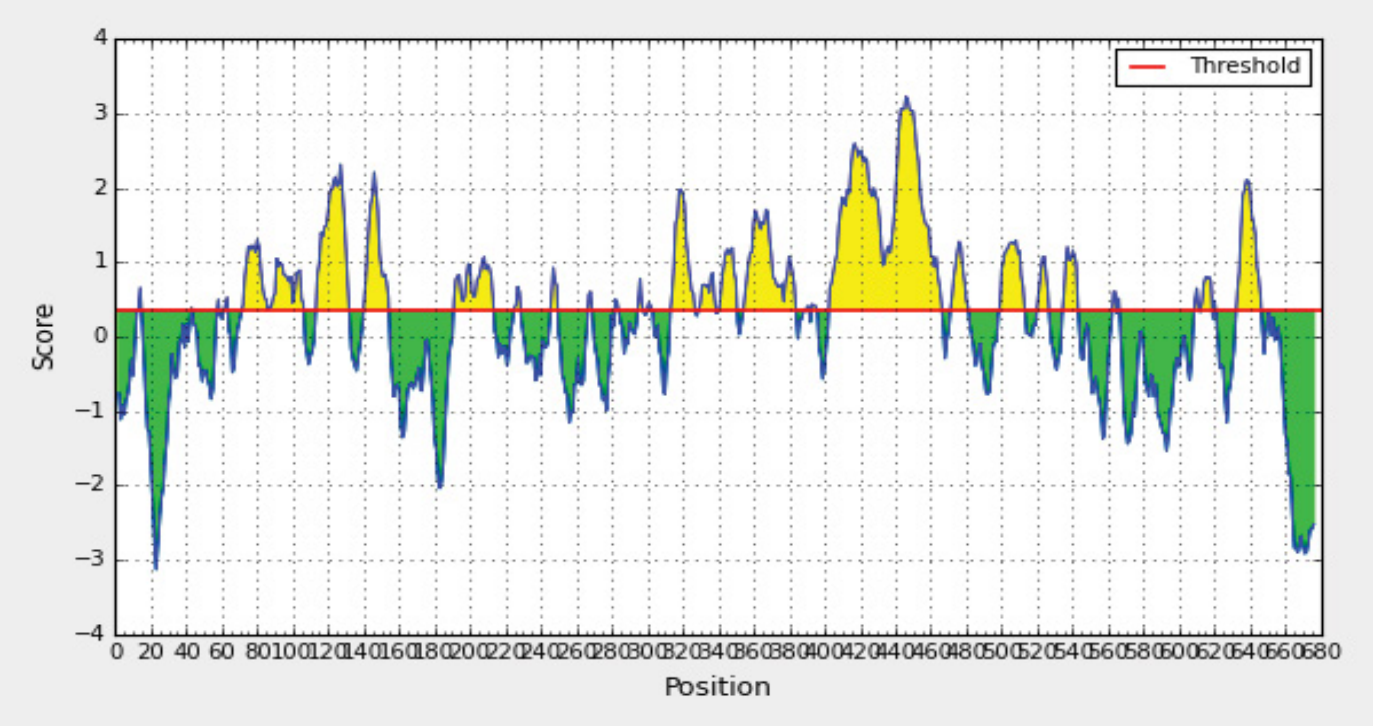


Figure 3: Bepipred linear epitope prediction.

Yellow areas above threshold (red line) are proposed to be a part of B cell epitope. While green areas are not

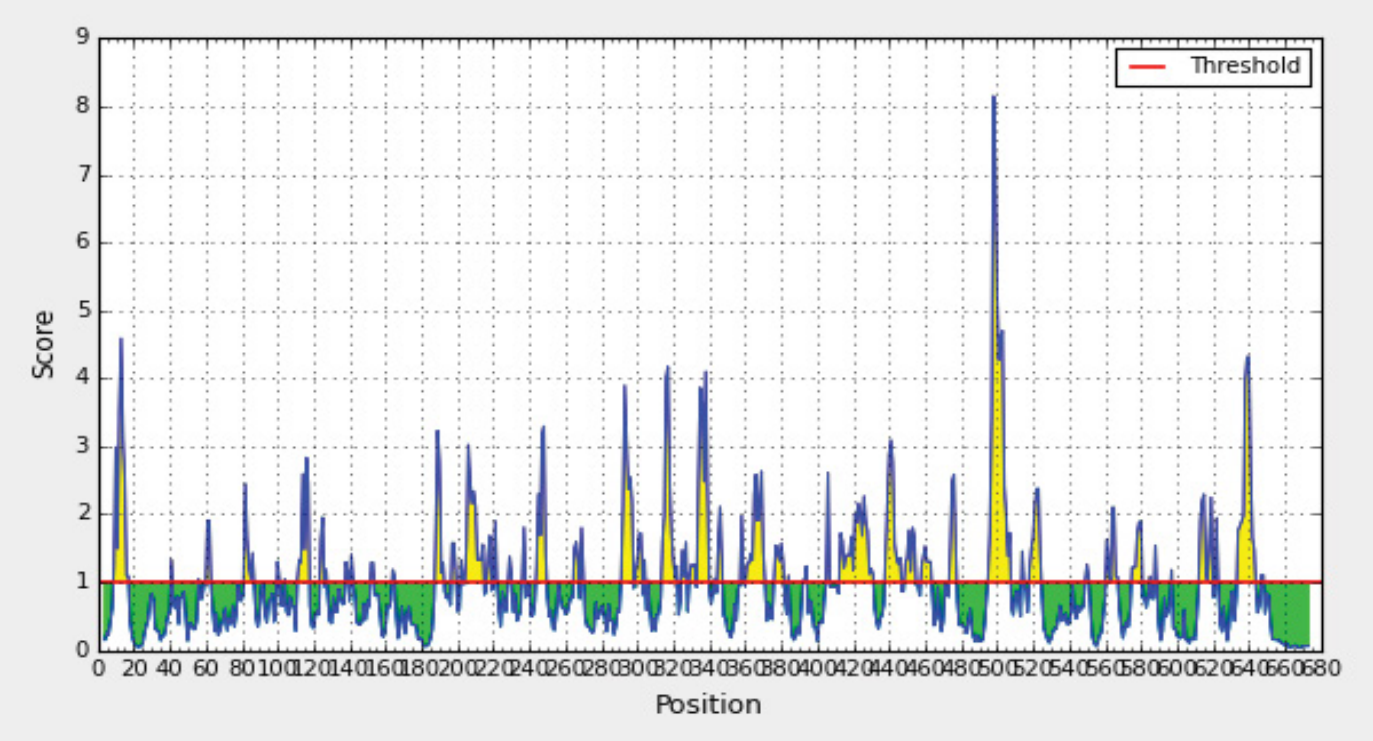


Figure 4: Emini surface accessibility prediction.

Yellow areas above threshold (red line) are proposed to be a part of B cell epitope. While green areas are not

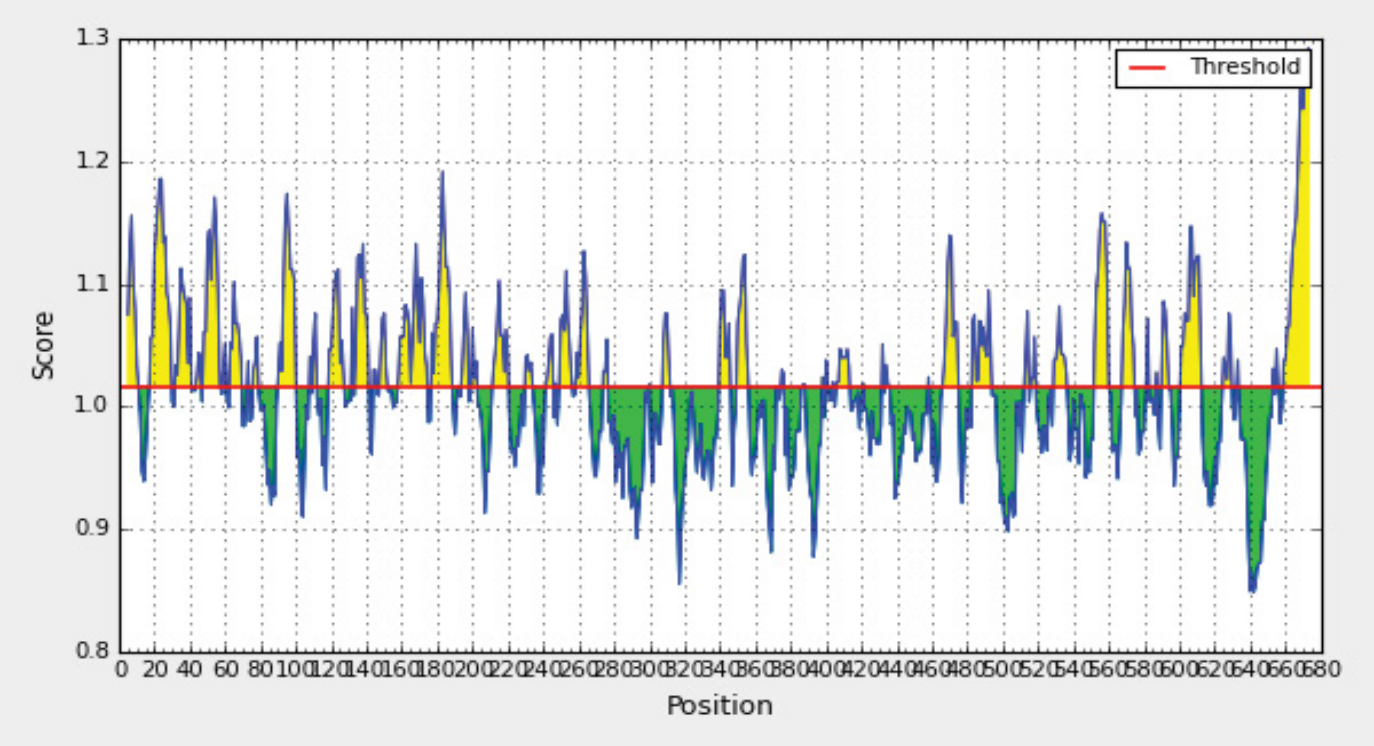


Figure 5: Kolaskar and Tongaonkar antigenicity prediction.

Yellow areas above threshold (red line) are proposed to be a part of B cell epitope. While green areas are not

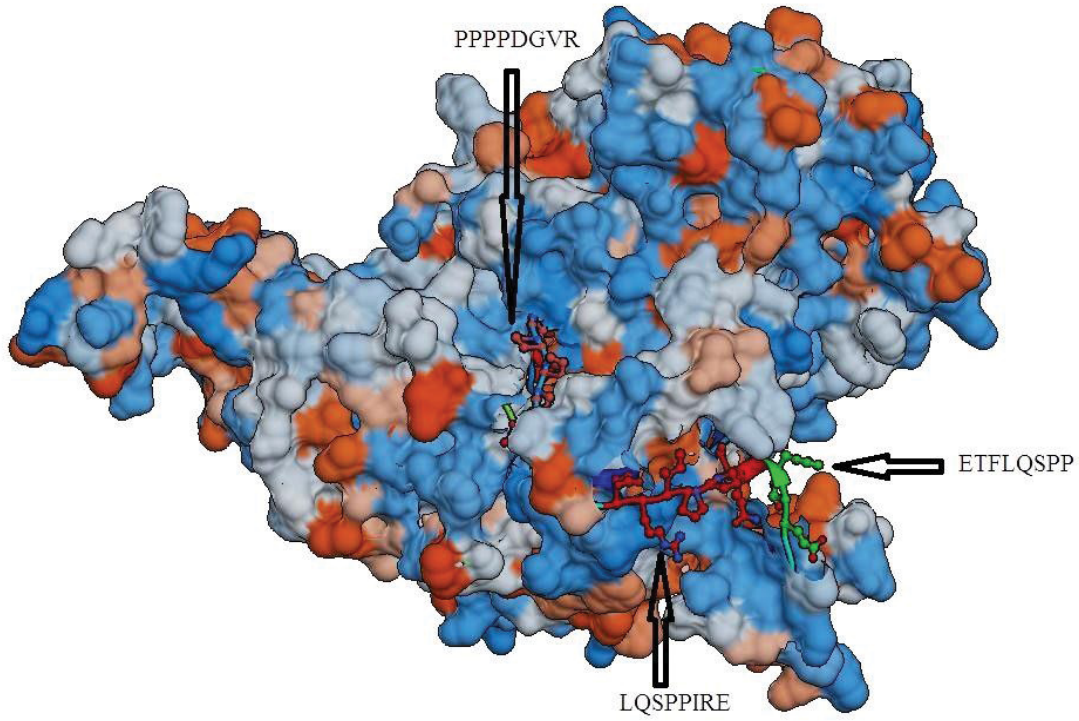


Figure 6: B-cell epitopes proposed.

Position of proposed conserved B cell epitopes in structural level of glycoprotein of Sudan Ebola virus

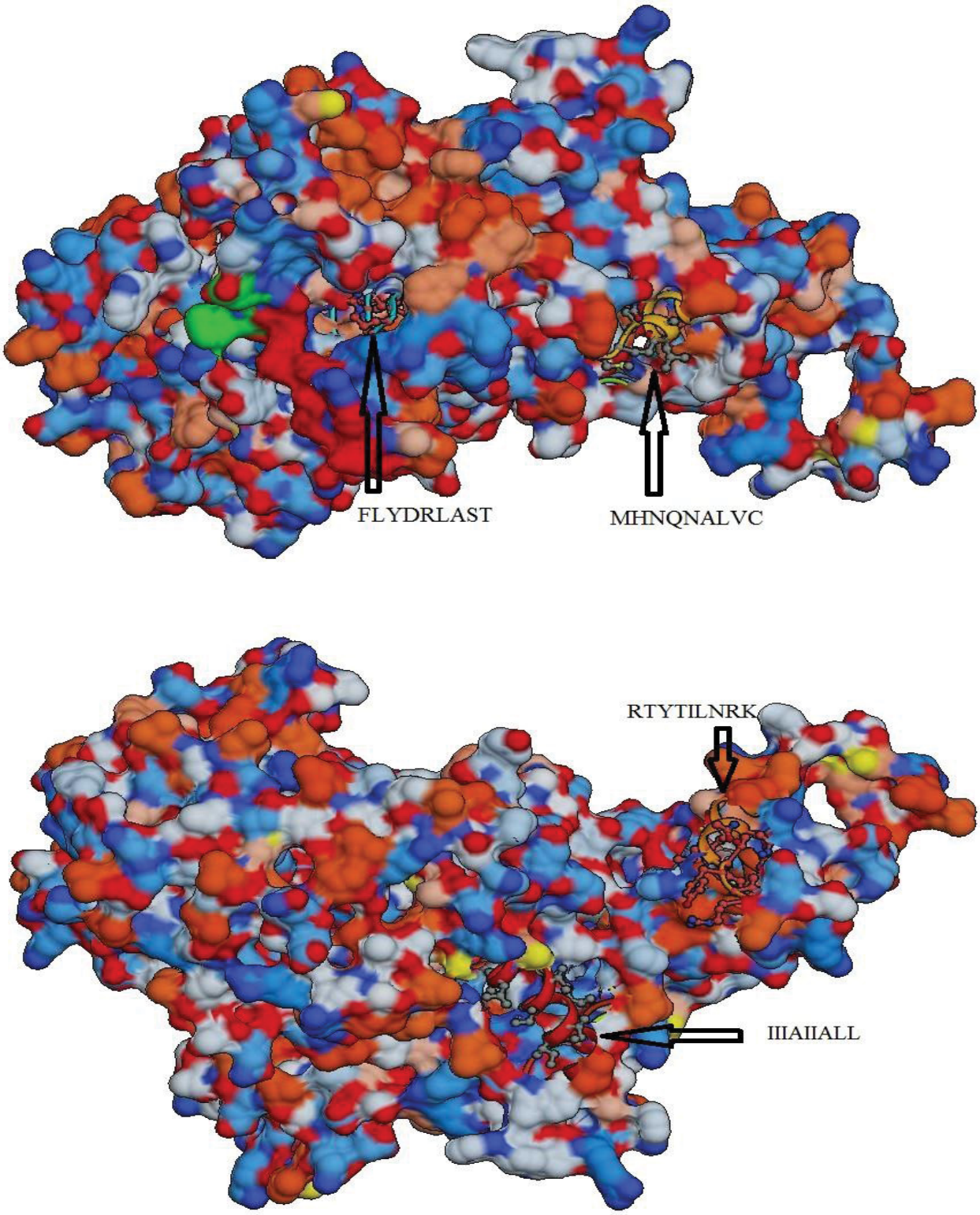


Figure 7: T cell epitopes proposed that interact with MHC I.

Position of proposed conserved T cell epitopes that interact with MHC I in structural level of glycoprotein of Sudan ebola virus

Epitope	Start	End	Allele	ANN-ic50*	Percentile Rank
AAGIAWIPY	526	534	HLA-B*35:01	37	1
AEGVIAFLI	177	185	HLA-B*40:01	39	0.7
			HLA-B*40:02	49	0.7
AENCYNLEI	105	113	HLA-B*40:01	27	0.5
			HLA-B*40:02	61	0.8
			HLA-B*44:02	18	0.2
ATSYLEYEI	214	222	HLA-A*68:02	68	1.7
			HLA-A*32:01	90	0.7
DAASSRITK	320	328	HLA-A*68:01	15	0.4
DGAFFLYDR	156	164	HLA-A*68:01	63	1.3
EPHDWTKNI	611	619	HLA-C*12:03	72	2.1
ETFLQSPPI	191	199	HLA-A*68:02	8	0.4
ETTQALQLF	564	572	HLA-A*26:01	11	0.2
EVTEIDQLV	44	52	HLA-A*68:02	4	0.2
FAEGVIAFL	176	184	HLA-A*68:02	96	2.1
			HLA-A*02:06	50	2.4
			HLA-C*12:03	12	0.5
FFVWVIILF	19	27	HLA-A*23:01	29	0.3
			HLA-A*29:02	73	0.8
FLFQLNDTI	252	260	HLA-A*02:01	25	1
			HLA-A*02:06	67	3
			HLA-C*12:03	15	0.6
FLRATTELR	572	580	HLA-A*68:01	98	1.7
FLYDRLAST	160	168	HLA-A*02:01	11	0.5
			HLA-A*02:06	7	0.6
			HLA-C*12:03	48	1.8
FSMPLGVVT	31	39	HLA-C*12:03	68	2.1
GLMHNQNAL	546	554	HLA-A*02:01	84	2.2
GTGPCPGDY	143	151	HLA-A*30:02	31	0.4
GVIAFLILA	179	187	HLA-A*02:06	30	1.8
GVRGFPRCR	128	136	HLA-A*30:01	85	1.7
HLASTDQLK	56	64	HLA-A*68:01	76	1.4
HTPQFLFQL	248	256	HLA-A*68:02	37	1.3
IALLCVCKL	666	674	HLA-C*12:03	58	1.9
IHDFIDNPL	627	635	HLA-B*39:01	71	0.9
IIALLCVCK	665	673	HLA-A*11:01	57	1.2
			HLA-A*68:01	66	1.3
IIIAIIALL	661	669	HLA-A*02:01	40	1.4
			HLA-A*68:02	38	1.3
			HLA-A*02:06	41	2.2
ILGSLGLRK	489	497	HLA-A*03:01	40	0.3
KAIDFLLRR	588	596	HLA-A*11:01	50	1.1
			HLA-A*31:01	33	0.9
KCNPNLHYW	510	518	HLA-B*58:01	27	0.5
			HLA-B*57:01	32	0.2
KFRKSSFFV	13	21	HLA-A*30:01	3	0.2
KINQIIHDF	622	630	HLA-A*32:01	32	0.4
KRWGFRSGV	84	92	HLA-B*27:05	23	0.2
KSSFFVWVI	16	24	HLA-A*32:01	10	0.2
			HLA-B*58:01	10	0.2
KSSFFVWVI	16	24	HLA-C*15:02	87	0.9
LAKPKETFL	186	194	HLA-C*12:03	79	2.3

LANETTQAL	561	569	HLA-B*35:01	11	0.4
			HLA-C*12:03	24	0.9
LMHNQNALV	547	555	HLA-A*02:01	46	1.5
LQLPRDKFR	7	15	HLA-A*31:01	46	1.1
MHNQNALVC	548	556	HLA-B*39:01	45	0.7
			HLA-C*06:02	68	0.4
			HLA-C*07:01	41	0.5
NADIGEWAF	282	290	HLA-B*35:01	22	0.7
NFAEGVIAF	175	183	HLA-B*35:01	31	0.8
NPNLHYWTA	512	520	HLA-B*08:01	87	0.6
NQNALVCGL	550	558	HLA-A*02:06	97	3.5
			HLA-B*39:01	37	0.6
QLRGEELSF	300	308	HLA-B*15:01	94	1.3
RLASTVIYR	164	172	HLA-A*03:01	49	0.4
			HLA-A*11:01	43	0.9
			HLA-A*31:01	6	0.2
			HLA-A*68:01	73	1.4
RPHTPQFLF	246	254	HLA-B*07:02	31	0.5
RRWGGTCRI	595	603	HLA-B*27:05	21	0.2
RTYTILNRK	580	588	HLA-A*03:01	22	0.2
			HLA-A*30:01	15	0.5
			HLA-A*11:01	15	0.2
			HLA-A*31:01	12	0.4
			HLA-A*68:01	48	1.1
SATKRWGFR	81	89	HLA-A*31:01	24	0.8
			HLA-A*68:01	67	1.3
SSFFVWVII	17	25	HLA-A*68:02	20	0.8
			HLA-A*32:01	74	0.7
SSYYATSYL	210	218	HLA-A*68:02	21	0.8
			HLA-C*15:02	30	0.3
STDIPSATK	76	84	HLA-A*11:01	33	0.8
TELRTYTIL	577	585	HLA-B*40:01	13	0.3
			HLA-B*40:02	73	0.8
TPENITTAV	460	468	HLA-B*07:02	75	0.9
TQALQLFLR	566	574	HLA-A*31:01	42	1.1
			HLA-A*68:01	85	1.6
TSSYYATSY	209	217	HLA-B*15:01	60	0.9
TTELRTYTI	576	584	HLA-A*32:01	65	0.6
TTPENITTA	459	467	HLA-A*68:02	64	1.7
			HLA-A*68:02	22	0.9
VIAFLILAK	180	188	HLA-A*03:01	33	0.3
			HLA-A*11:01	17	0.3
VVTNSTLEV	37	45	HLA-A*02:06	39	2.1
WTKNITDKI	615	623	HLA-A*68:02	65	1.7
YEIENFGAQ	220	228	HLA-B*18:01	33	0.3
YTENTSSYY	205	213	HLA-A*01:01	6	0.2
			HLA-A*29:02	56	0.8
			HLA-A*30:02	45	0.5
			HLA-C*12:03	84	2.4
YTILNRKAI	582	590	HLA-C*12:03	19	0.8
YYATSYLEY	212	220	HLA-A*29:02	3	0.2

Table 3: List of epitopes that had binding affinity with the MHC Class I alleles.

*ANN ic50 is the inhibitory concentration needed for successful binding of peptide to MHC molecule by the Artificial Neural Network method. The lower number of epitope is the better

Position of peptides is according to position of amino acids in the glycoprotein (GP)

Core Sequence	Start	End	Peptide Sequence	Allele	IC50	Rank
FAEGVIAFL	176	184	FAEGVIAFLILAKPK	HLA-DPA1*01:03/DPB1*02:01	448.4	20.28
				HLA-DQA1*01:01/DQB1*05:01	525.3	9.38
				HLA-DQA1*05:01/DQB1*03:01	88.1	13.65
				HLA-DRB1*04:05	569.2	31.35
				HLA-DRB1*07:01	458	32.04
				HLA-DRB1*04:01	528.3	29.85
				HLA-DRB1*09:01	658.2	30.42
			GVNFAEGVIAFLILA	HLA-DPA1*01/DPB1*04:01	644.9	17.17
				HLA-DPA1*01:03/DPB1*02:01	215.1	13.45
				HLA-DPA1*02:01/DPB1*05:01	659.3	13.01
				HLA-DQA1*01:01/DQB1*05:01	385.5	7.44
				HLA-DQA1*05:01/DQB1*02:01	502.5	11.22
				HLA-DRB1*04:05	503.4	29.39
				HLA-DRB1*07:01	129.3	16.67
				HLA-DRB1*08:02	903	20.71
				HLA-DRB1*04:01	269.9	18.87
				HLA-DRB1*09:01	133.6	9.09
				HLA-DRB5*01:01	733	38.49
			IYRGVNFAEGVIAFL	HLA-DPA1*01:03/DPB1*02:01	213	13.37
				HLA-DPA1*02:01/DPB1*01:01	384.3	26.62
				HLA-DQA1*01:01/DQB1*05:01	688.4	11.43
				HLA-DQA1*05:01/DQB1*02:01	299.3	6.73
				HLA-DRB1*09:01	121.3	8.31
				HLA-DRB1*15:01	586.7	31.5
			NFAEGVIAFLILAKP	HLA-DPA1*01:03/DPB1*02:01	349.8	17.73
				HLA-DPA1*02:01/DPB1*05:01	723.9	13.98
				HLA-DQA1*01:01/DQB1*05:01	449.9	8.36
				HLA-DQA1*05:01/DQB1*02:01	667.5	14.57
				HLA-DQA1*05:01/DQB1*03:01	60.6	10.43
				HLA-DRB1*04:05	680.8	34.4
				HLA-DRB1*07:01	346.6	28.14
				HLA-DRB1*04:01	379.8	24.08
				HLA-DRB1*09:01	370.7	20.8
			RGVNFAEGVIAFLIL	HLA-DPA1*01:03/DPB1*02:01	180.9	12.11
				HLA-DPA1*02:01/DPB1*05:01	596.5	12.01
				HLA-DQA1*01:01/DQB1*05:01	431.2	8.09
				HLA-DQA1*05:01/DQB1*02:01	360.6	8.15
				HLA-DRB1*04:05	479.5	28.62
				HLA-DRB1*07:01	80.3	12.25
				HLA-DRB1*08:02	586.3	14.2
				HLA-DRB1*04:01	281.1	19.46
				HLA-DRB1*09:01	131.9	8.99
				HLA-DRB5*01:01	749.8	38.82
			VNFAEGVIAFLILAK	HLA-DPA1*01:03/DPB1*02:01	227.8	13.91
				HLA-DPA1*02:01/DPB1*05:01 10.81	525.8	10.81
				HLA-DQA1*01:01/DQB1*05:01	404.3	7.71
				HLA-DQA1*05:01/DQB1*02:01	581.7	12.86
				HLA-DRB1*04:05	566.4	31.27
				HLA-DRB1*07:01	179	20.16
				HLA-DRB1*04:01	332.6	21.98
				HLA-DRB1*09:01	219.1	13.93
				HLA-DRB5*01:01	695.1	37.7

			YRGVNFAEGVIAFLI	HLA-DPA1*01/DPB1*04:01	942.1	21.11
				HLA-DPA1*01:03/DPB1*02:01	218.3	13.56
				HLA-DPA1*02:01/DPB1*05:01	823.7	15.43
				HLA-DQA1*01:01/DQB1*05:01	526.5	9.39
				HLA-DQA1*04:01/DQB1*04:02	835.5	13.31
				HLA-DQA1*05:01/DQB1*02:01	323.7	7.29
				HLA-DRB1*04:05	505.5	29.46
				HLA-DRB1*07:01	71.6	11.33
				HLA-DRB1*08:02	631.6	15.16
				HLA-DRB1*04:01	312.3	21.01
				HLA-DRB1*09:01	143.5	9.71
				HLA-DRB5*01:01	766.9	39.16
FLRATTELR	572	580	ALQLFLRATTELRTY	HLA-DPA1*01:03/DPB1*02:01	282.8	15.74
				HLA-DPA1*02:01/DPB1*05:01	203.4	4.49
				HLA-DPA1*03:01/DPB1*04:02	92.1	9.14
				HLA-DQA1*05:01/DQB1*02:01	635.1	13.94
				HLA-DQA1*05:01/DQB1*03:01	723.2	42.09
				HLA-DRB1*01:01	15.6	8.96
				HLA-DRB1*03:01	25.5	1.49
				HLA-DRB1*04:05	24.2	1.79
				HLA-DRB1*07:01	38.4	6.97
				HLA-DRB1*08:02	906.5	20.78
				HLA-DRB1*04:01	39.3	2.79
				HLA-DRB1*09:01	143.8	9.72
				HLA-DRB5*01:01	10.7	2.57
			FLRATTELRTYTILN	HLA-DPA1*01/DPB1*04:01	995.1	21.75
				HLA-DPA1*02:01/DPB1*05:01	203.4	4.49
				HLA-DRB1*01:01	58	23.06
				HLA-DRB1*03:01	234.3	8.87
				HLA-DRB1*04:05	73.3	7.21
				HLA-DRB1*04:01	116.1	9.36
				HLA-DRB1*11:01	359.3	26.89
				HLA-DRB5*01:01	43.7	9.11
			LFLRATTELRTYTIL	HLA-DPA1*01:03/DPB1*02:01	431	19.87
				HLA-DPA1*02:01/DPB1*05:01	156	3.38
				HLA-DRB1*01:01	30.1	15.51
				HLA-DRB1*03:01	77.9	4.12
				HLA-DRB1*04:05	50.6	4.92
				HLA-DRB1*04:01	75.4	6.09
				HLA-DRB1*11:01	208.9	20.75
				HLA-DRB4*01:01	419.1	24.48
				HLA-DRB5*01:01	24.8	5.9
			LQLFLRATTELRTYT	HLA-DPA1*01/DPB1*04:01	363.7	12.34
				HLA-DPA1*01:03/DPB1*02:01	300.9	16.29
				HLA-DPA1*02:01/DPB1*05:01	182.2	4
				HLA-DPA1*03:01/DPB1*04:02	61	6.76
				HLA-DQA1*05:01/DQB1*02:01	836.3	17.68
				HLA-DQA1*05:01/DQB1*03:01	844.1	44.84
				HLA-DRB1*01:01	12.3	6.87
				HLA-DPA1*01:03/DPB1*02:01	300.9	16.29
				HLA-DPA1*02:01/DPB1*05:01	182.2	4
				HLA-DPA1*03:01/DPB1*04:02	61	6.76
				HLA-DQA1*05:01/DQB1*02:01	836.3	17.68

				HLA-DQA1*05:01/DQB1*03:01	844.1	44.84
				HLA-DRB1*01:01	12.3	6.87
				HLA-DRB1*03:01	18.8	1.06
				HLA-DRB1*04:05	26.3	2.04
				HLA-DRB1*07:01	45.2	7.97
				HLA-DRB1*08:02	862	19.92
				HLA-DRB1*04:01	35.3	2.42
				HLA-DRB1*09:01	127	8.67
				HLA-DRB5*01:01	10	2.37
			QALQLFLRATTELRT	HLA-DPA1*01:03/DPB1*02:01	285.5	15.82
				HLA-DPA1*02:01/DPB1*05:01	352.9	7.66
				HLA-DQA1*05:01/DQB1*02:01	574.9	12.72
				HLA-DQA1*05:01/DQB1*03:01	741.4	42.52
				HLA-DRB1*01:01	23.5	12.91
				HLA-DRB1*03:01	45.1	2.64
				HLA-DRB1*04:05	24.9	1.88
				HLA-DRB1*07:01	36.5	6.69
				HLA-DRB1*04:01	49.2	3.74
				HLA-DRB1*09:01	207.8	13.36
				HLA-DRB5*01:01	13.4	3.3
			QLFLRATTELRTYTI	HLA-DPA1*01/DPB1*04:01	414.6	13.35
				HLA-DPA1*02:01/DPB1*05:01	150	3.24
				HLA-DRB1*01:01	15.8	9.07
				HLA-DRB1*03:01	35	2.09
				HLA-DRB1*04:05	33.1	2.88
				HLA-DRB1*08:02	896.3	20.58
				HLA-DRB1*04:01	47.1	3.53
				HLA-DRB1*11:01	113.9	14.85
				HLA-DRB5*01:01	14.9	3.69
			TQALQLFLRATTELR	HLA-DPA1*01/DPB1*04:01	714.7	18.16
				HLA-DQA1*05:01/DQB1*02:01	636.2	13.96
				HLA-DQA1*05:01/DQB1*03:01	786.5	43.58
				HLA-DRB1*01:01	35.7	17.36
				HLA-DRB1*03:01	73.6	3.94
				HLA-DRB1*04:05	25.1	1.9
				HLA-DRB1*07:01	40.4	7.26
				HLA-DRB1*04:01	60.1	4.75
				HLA-DRB1*09:01	320.7	18.66
				HLA-DRB5*01:01	16	3.96
FLYDRLAST	160	168	AFFLYDRLASTVIYR	HLA-DPA1*01:03/DPB1*02:01	4.2	0.3
				HLA-DPA1*02:01/DPB1*01:01	26.8	2.45
				HLA-DPA1*03:01/DPB1*04:02	6.8	0.39
				HLA-DQA1*05:01/DQB1*03:01	118.6	16.59
				HLA-DRB1*03:01	18.7	1.06
				HLA-DRB1*04:05	72.6	7.15
				HLA-DRB1*08:02	251	5.78
				HLA-DRB1*04:01	27.8	1.69
				HLA-DRB3*01:01	19.7	1.22
				HLA-DRB5*01:01	158.6	19.59
			DGAFFLYDRLASTVI	HLA-DPA1*01:03/DPB1*02:01	3.4	0.18
				HLA-DPA1*02:01/DPB1*01:01	22	1.84
				HLA-DQA1*05:01/DQB1*03:01	141.4	18.46
				HLA-DRB1*03:01	23	1.33
				HLA-DRB1*04:05	64.4	6.34
				HLA-DRB1*08:02	295.1	6.95

				HLA-DRB1*04:01	26.3	1.56
				HLA-DRB3*01:01	12.2	0.7
				HLA-DRB5*01:01	147.3	18.86
			FFLYDRLASTVIYRG	HLA-DPA1*03:01/DPB1*04:02	10.9	0.98
				HLA-DQA1*05:01/DQB1*03:01	136	18.04
				HLA-DRB1*03:01	27.8	1.62
				HLA-DRB1*04:05	109.2	10.38
				HLA-DRB1*08:02	195.3	4.25
				HLA-DRB1*04:01	43	3.13
				HLA-DRB3*01:01	39.4	2.32
				HLA-DRB5*01:01	226.2	23.35
			FLYDRLASTVIYRGV	HLA-DPA1*01/DPB1*04:01	520.8	15.25
				HLA-DPA1*01:03/DPB1*02:01	170.6	11.68
				HLA-DPA1*02:01/DPB1*01:01	144	13.97
				HLA-DPA1*03:01/DPB1*04:02	66.9	7.26
				HLA-DQA1*05:01/DQB1*03:01	163.7	20.14
				HLA-DRB1*03:01	55.8	3.13
				HLA-DRB1*04:05	138.6	12.6
				HLA-DRB1*08:02	204.4	4.5
				HLA-DRB1*04:01	64.4	5.13
				HLA-DRB1*11:01	341.1	26.26
				HLA-DRB3*01:01	73	3.65
				HLA-DRB5*01:01	370.8	29.14
			GAFFLYDRLASTVIY	HLA-DPA1*01:03/DPB1*02:01	3.3	0.16
				HLA-DPA1*02:01/DPB1*01:01	23.6	2.04
				HLA-DPA1*03:01/DPB1*04:02	6.9	0.4
				HLA-DQA1*05:01/DQB1*03:01	126	17.2
				HLA-DRB1*03:01	13.2	0.64
				HLA-DRB1*04:05	57.6	5.68
				HLA-DRB1*08:02	209.1	4.63
				HLA-DRB1*04:01	21.5	1.11
				HLA-DRB3*01:01	11.6	0.64
				HLA-DRB5*01:01	124.3	17.23
			HKDGAFFLYDRLAST	HLA-DPA1*01:03/DPB1*02:01	4.5	0.34
				HLA-DPA1*02:01/DPB1*01:01	27.6	2.55
				HLA-DQA1*05:01/DQB1*03:01	194.9	22.25
				HLA-DRB1*01:01	61.6	23.82
				HLA-DRB1*03:01	113.2	5.49
				HLA-DRB1*04:05	113.7	10.74
				HLA-DRB1*08:02	767.5	18.03
				HLA-DRB1*04:01	69.5	5.59
				HLA-DRB3*01:01	18.1	1.12
				HLA-DRB5*01:01	263.4	25.08
			KDGAFFLYDRLASTV	HLA-DPA1*01:03/DPB1*02:01	3.6	0.21
				HLA-DQA1*05:01/DQB1*03:01	171.3	20.68
				HLA-DRB1*01:01	22.8	12.6
				HLA-DRB1*03:01	47.7	2.75
				HLA-DRB1*04:05	92	8.95
				HLA-DRB1*07:01	907.8	42.68
				HLA-DRB1*08:02	483.3	11.75
				HLA-DRB1*04:01	54.2	4.21
				HLA-DRB3*01:01	14.5	0.86
				HLA-DRB5*01:01	216.7	22.85
FVWVIILFQ	20	28	FFVWVIILFQKAFSM	HLA-DPA1*01/DPB1*04:01	178.7	7.94
				HLA-DPA1*03:01/DPB1*04:02	42.1	4.95

				HLA-DQA1*04:01/DQB1*04:02	676	10.77
			FRKSSFFVWVIILFQ	HLA-DPA1*03:01/DPB1*04:02	76.1	8.01
				HLA-DQA1*03:01/DQB1*03:02	471.5	8.14
				HLA-DQA1*04:01/DQB1*04:02	800.8	12.78
				HLA-DRB1*04:05	381.9	25.16
			FVWVIILFQKAFSMP	HLA-DPA1*01/DPB1*04:01	187.4	8.18
				HLA-DPA1*03:01/DPB1*04:02	63.6	6.97
				HLA-DQA1*01:01/DQB1*05:01	989.5	14.75
				HLA-DQA1*04:01/DQB1*04:02	998.4	15.78
			KSSFFVWVIILFQKA	HLA-DPA1*01/DPB1*04:01	112.7	5.77
				HLA-DPA1*03:01/DPB1*04:02	36	4.28
				HLA-DQA1*04:01/DQB1*04:02	405.3	6.16
				HLA-DRB1*04:05	484.5	28.79
			RKSSFFVWVIILFQK	HLA-DPA1*01:03/DPB1*02:01	26	3.02
				HLA-DPA1*02:01/DPB1*01:01	43.3	4.5
				HLA-DPA1*03:01/DPB1*04:02	46.9	5.45
				HLA-DQA1*03:01/DQB1*03:02	387.4	6.47
				HLA-DQA1*04:01/DQB1*04:02	410.2	6.25
				HLA-DRB1*04:05	453.4	27.74
			SFFVWVIILFQKAFS	HLA-DPA1*01/DPB1*04:01	140.1	6.72
				HLA-DPA1*03:01/DPB1*04:02	32.9	3.93
				HLA-DQA1*04:01/DQB1*04:02	534.7	8.4
				HLA-DQA1*05:01/DQB1*03:01	905	46.11
				HLA-DRB1*04:05	187.6	15.84
				HLA-DRB1*07:01	870.6	41.96
			SSFFVWVIILFQKAF	HLA-DPA1*01/DPB1*04:01	117	5.92
				HLA-DPA1*03:01/DPB1*04:02	30.6	3.65
				HLA-DQA1*03:01/DQB1*03:02	180.6	2.35
				HLA-DQA1*04:01/DQB1*04:02	413.8	6.31
				HLA-DQA1*05:01/DQB1*03:01	804.3	43.98
				HLA-DRB1*04:05	327.5	22.96
				HLA-DRB1*07:01	740.6	39.32

Table 4: List of top four epitopes that had binding affinity with the Class II alleles.

Position of peptides is according to position of amino acid in the Envelope glycoprotein

virus. Several recent studies conclude the ability of GP of SUDV alone to induce strong humoral and cellular immune response against Sudan Ebola Virus [6,68-70].

Conservancy in GP protein in SUDV was found promising for peptide vaccine design. However, as limitations to the current study; the few numbers of SUDV glycoprotein variants that was available to use is minimizing the significance of this conservancy.

To determine a potential and effective peptide antigen for B cell, epitopes should get above threshold scores in Bepipred linear epitope, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction methods in IEDB. Epitopes illustrated in Table 2, are the only conserved regions from all retrieved strains of SUDV Spike glycoprotein that are available in NCBI database until 1st June 2016 and have high probability of activating humoral immune response. Epitope 114 KKPDGSECLPPPPDGVRG 131 is overlapping the three predicted tools as well as its last 9mers PPPPDGVRG indicating that this region is probably promising.

Since the immune response of T cell is long lasting response comparing with B cell, where the antigen can easily escape the antibody memory response [71] and considering that $CD8^+$ T and $CD4^+$ T cell responses play a major role in antiviral immunity [72], designing a vaccine against T cell epitope is much more promising. Among 65 conserved T cell epitopes predicted to interact with MHC Class I as shown in Table 3, epitope MHNQNALVC has succeeded to interact with only three MHC I alleles under the selected threshold. However, this epitope is very promising as it interacted with HLA-C*06:02 and HLA-C*07:01 that are very frequent among Sudanese population [73-75]. As well as FLYDRLAST that had successfully predicted to bind with good affinity to HLA-A*02:01 - the world wide predominant MHC I allele which is capable of eliciting strong CTL responses. 246 RPHTPQFLF 254 is proposed by different in silico prediction studies, Interestingly this epitope in addition to TPENITTAV are the only epitopes that are successfully predicted to bind to HLA-B*07 - the allele concluded by Sanchez et al. [76] as inducing lifesaving robust cellular immune response among SUDV survivors

MHC I epitope FLYDRLAST is showing high potentials to induce MHC II response as seen in Table 4, as it was found to successfully bind to several HLA-D, P and Q alleles indicating that further attention need to be targeted to this region. All proposed MHC I and MHC II epitopes as illustrated were better chosen to serve the best population coverage percentage as well as the lowest number of peptides to be used as multi epitope vaccine against the highly lethal Sudan Ebola Virus.

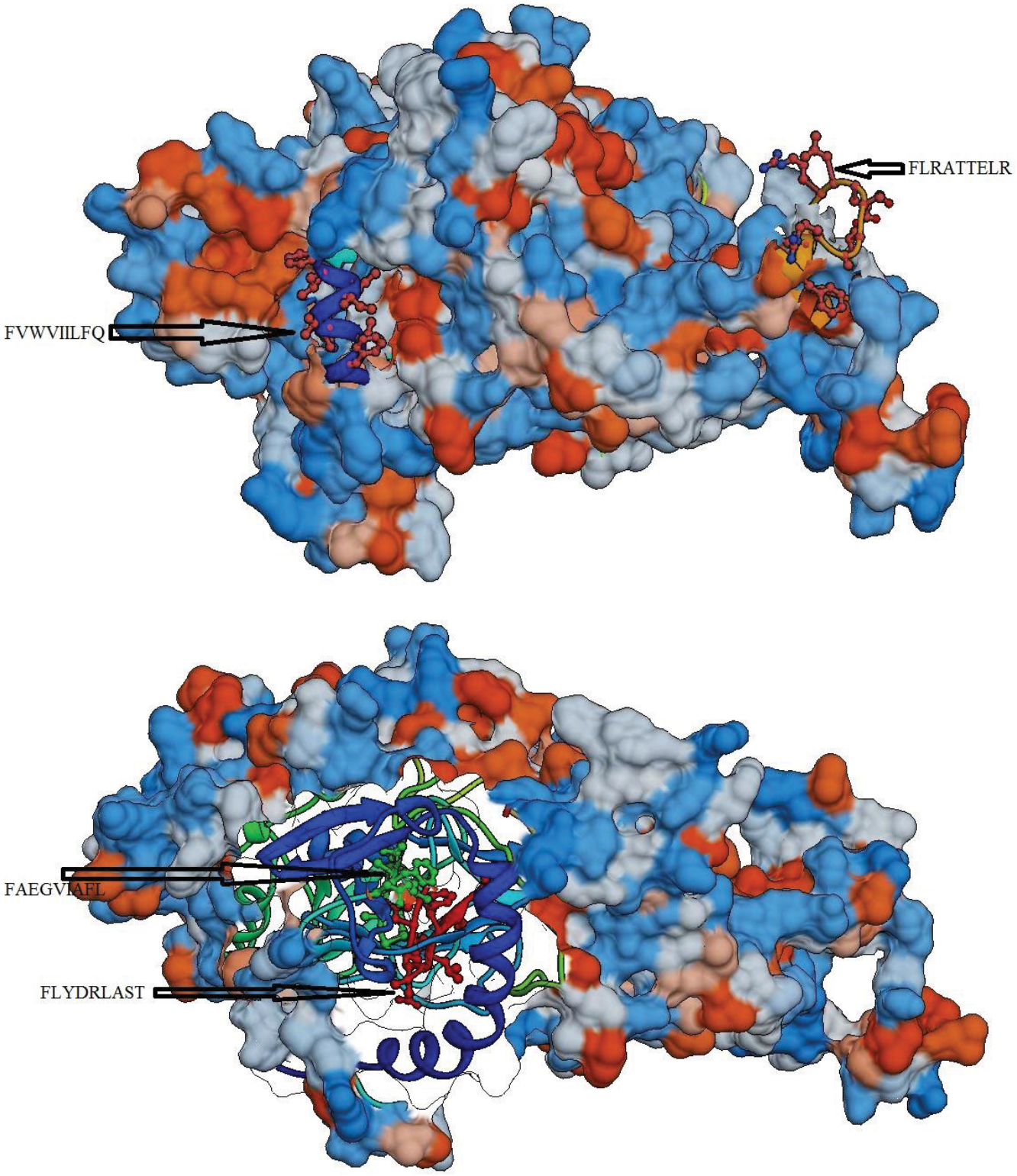


Figure 8: T cell epitopes proposed that interact with MHC II.

Position of proposed conserved T cell epitopes that interact with MHC II in structural level of glycoprotein of Sudan Ebola virus

Epitope	Coverage World class I	Coverage Sudan Class I	Total HLA hits	Epitope (core sequence)	Coverage World Class II	Coverage Sudan Class II	Total HLA hits
AAGIAWIPY	8.42%	6.67%	1	AAGIAWIPY	85.67%	50.60%	4
AEGVIAFLI	11.13%	2.35%	2	ADIGEWAFW	76.04%	46.62%	2
AENCYNLEI	18.29%	3.80%	3	AEGVIAFLI	97.78%	75.00%	9
ATSYLEYEI	7.05%	20.37%	2	AFFLYDRLA	56.18%	41.45%	5
DAASSRITK	5.83%	6.14%	1	AGIAWIPYF	83.57%	60.80%	4
DGAFFLYDR	5.83%	6.14%	1	AKPKETFLQ	43.67%	0.91%	2
EPHDWTKNI	10.31%	18.71%	1	ALVCGLRQL	27.48%	19.42%	2
ETFLQSPPI	2.50%	10.07%	1	ASTVIYRGV	42.10%	0.00%	3
ETTQALQLF	5.82%	3.24%	1	DDNWWTGWR	31.46%	26.56%	2
EVTEIDQLV	2.50%	10.07%	1	DFIDNPLPN	27.48%	19.42%	2
FAEGVIAFL	14.29%	26.90%	3	DKFRKSSFF	92.37%	55.19%	6
FFVWVIILF	9.21%	13.77%	2	ELRTYTILN	60.83%	26.88%	5
FLFQLNDTI	46.73%	39.93%	3	ENTSSYYAT	76.04%	46.62%2	2
FLRATTELR	5.83%	6.14%	1	EVTEIDQLV	55.49%	33.36%	4
*FLYDRLAST	46.73%	39.93%	3	EWAENCYNL	76.04%	46.62%	2
FSMPLGVVT	10.31%	18.71%	1	EWAFWENKK	35.07%	9.27%	2
GLMHNQNAL	39.08%	26.10%	1	*FAEGVIAFL	99.67%	97.24%	21
GTGPCPGDY	2.43%	5.19%	1	FFLYDRLAS	97.74%	87.28%	10
GVIAFLILA	1.95%	0.00%	1	FFVWVIILF	90.63%	68.64%	9
GVRGFPRCR	3.89%	11.73%	1	FIDNPLPNQ	27.48%	19.42%	2
HLASTDQLK	5.83%	6.14%	1	FLFQLNDTI	98.84%	88.42%	19
HTPQFLFQL	2.50%	10.07%	1	FLILAKPKE	96.20%	66.42%	13
IALLCVCKL	10.31%	18.71%	1	FLLRRWGGT	78.80%	46.62%	4
IHDFIDNPL	2.75%	5.86%	1	FLQSPPIRE	90.90%	80.22%	15
IIALLCVCK	20.88%	9.26%	2	*FLRATTELR	99.69%	97.36%	21
*IIIAIIALL	42.53%	34.71%	3	*FLYDRLAST	99.38%	95.87%	19
ILGSLGLRK	16.81%	8.81%	1	FRKSSFFVW	98.46%	88.10%	16
KAIDFLLRR	20.45%	8.69%	2	*FVWVIILFQ	99.72%	95.94%	18
KCNPNLHYW	7.26%	8.67%	2	FWENKKNLS	41.91%	23.70%	3
KFRKSSFFV	3.89%	11.73%	1	GAFFLYDRL	83.07%	65.03%	9
KINQIIHDF	4.61%	10.88%	1	GVIAFLILA	27.48%	19.42%	2
KRWGFRSGV	4.78%	1.26%	1	HKDGAFFLY	43.67%	0.91%	2
KSSFFVWVI	11.94%	20.99%	3	HNAAGIAWI	81.77%	43.45%	8
LAKPKETFL	10.31%	18.71%	1	HTPQFLFQL	80.85%	56.06%	8
LANETTQAL	17.86%	24.13%	2	IAIIALLCV	78.56%	47.62%	6
LMHNQNALV	39.08%	26.10%	1	IALLCVCKL	69.87%	38.55%	7
LQLPRDKFR	5.36%	5.56%	1	IAWIPYFGP	76.04%	46.62%	2
*MHNQNALVC	35.14%	67.96%	3	IENFGAQHS	74.98%	46.20%	6
NADIGEWAF	8.42%	6.67%	1	IGEWAFWEN	35.07%	9.27%	2
NFAEGVIAF	8.42%	6.67%	1	IGITGIIIA	74.96%	40.71%	6
NPNLHYWTA	10.55%	6.21%	1	IHDFIDNPL	92.12%	78.01%	14
NQNALVCGL	4.64%	5.86%	2	IIAIIALLC	27.48%	19.42%	2
QLRGEELSF	8.44%	1.04%	1	IIHDFIDNP	75.68%	69.37%	7
RLASTVIYR	40.03%	22.68%	4	IIIAIIALL	87.90%	86.32%	8
RPHTPQFLF	12.78%	3.60%	1	ILAKPKETF	91.22%	65.33%	6
RRWGGTCRI	4.78%	1.26%	1	ILGSLGLRK	95.36%	82.55%	16
*RTYTILNRK	43.03%	32.96%	5	ILNRKAIDF	57.32%	22.87%	7
SATKRWGFR	11.03%	11.52%	2	INADIGEWA	90.79%	83.03%	9
SSFFVWVII	7.05%	20.37%	2	INQIIHDFI	87.75%	76.88%	11
SSYYATSYL	6.81%	14.72%	2	ITGIIIAII	59.73%	37.57%	7
STDIPSATK	15.53%	3.22%	1	IYRGVNFAE	97.85%	79.39%	15
TELRTYTIL	11.13%	2.35%	2	IYTEGLMHN	84.50%	57.57%	8
TPENITTAV	12.78%	3.60%	1	KAIDFLLRR	97.93%	88.76%	12
TQALQLFLR	11.03%	11.52%	2	KDGAFFLYD	93.44%	56.81%	6
TSSYYATSY	8.44%	1.04%	1	KFRKSSFFV	82.94%	59.48%	8

TTELRTYTI	4.61%	10.88%	1	KINQIIHDF	35.07%	9.27%	2
TTPENITTA	2.50%	10.07%	1	KKNLSEQLR	35.07%	9.27%	3
TVTGILGSL	2.50%	10.07%	1	KPKETFLQS	35.07%	9.27%	2
VIAFLILAK	30.92%	11.89%	2	KSSFFVWVI	56.97%	45.17%	5
VVTNSTLEV	1.95%	0.00%	1	LAKPKETFL	46.90%	22.87%	3
WTKNITDKI	2.50%	10.07%	1	LANETTQAL	94.36%	66.27%	9
YEIENFGAQ	7.32%	3.89%	1	LASTDQLKS	35.12%	32.24%	3
YTENTSSYY	30.96%	34.82%	4	LASTVIYRG	55.64%	33.74%	6
YTILNRKAI	10.31%	18.71%	1	LEVTEIDQL	94.75%	75.18%	12
YYATSYLEY	3.89%	3.35%	1	LEYEIENFG	31.32%	33.96%	2
Epitope set	**98.19%**	**97.94%**		LFLRATTEL	96.07%	80.79%	9
				LHYWTAQEQ	52.68%	12.50%	6
				LITSTVTGI	72.55%	51.10%	9
				LKSVGLNLE	71.70%	31.28%	9
				LLQLPRDKF	62.71%	45.47%	5
				LNRKAIDFL	76.85%	42.90%	6
				LQLPRDKFR	43.67%	0.91%	3
				LRATTELRT	93.26%	72.21%	10
				LRGEELSFE	89.36%	55.64%	5
				LRTYTILNR	90.57%	61.95%	12
				LVCGLRQLA	52.81%	21.85%	4
				LYDRLASTV	35.07%	9.27%	2
				NFAEGVIAF	86.45%	55.04%	10
				NITTAVKTV	42.10%	0.00%	3
				NLHYWTAQE	47.36%	34.83%	5
				NQNALVCGL	34.55%	0.00%	2
				NRKAIDFLL	89.24%	76.26%	7
				NSTLEVTEI	47.25%	3.57%	4
				NWWTGWRQW	76.04%	46.62%	2
				QALQLFLRA	89.03%	53.33%	4
				QFLFQLNDT	54.34%	27.71%	4
				QIIHDFIDN	27.48%	19.42%	2
				QLANETTQA	34.55%	0.00%	2
				RKAIDFLLR	97.74%	87.28%	10
				RKSSFFVWV	63.19%	34.70%	4
				RLASTVIYR	90.02%	52.71%	8
				SFFVWVIIL	50.17%	0.91%	3
				SNGLITSTV	78.05%	43.64%	6
				SSFFVWVII	35.07%	9.27%	2
				STIGIRPSS	10.54%	15.91%	1
				SYEAGEWAE	80.26%	48.53%	4
				SYYATSYLE	98.97%	88.09%	15
				TELRTYTIL	89.03%	53.33%	4
				TLEVTEIDQ	92.24%	75.68%	7
				TQALQLFLR	79.70%	28.48%	9
				TSSYYATSY	39.14%	27.29%	3
				TTQALQLFL	87.65%	76.26%	6
				VCGLRQLAN	82.82%	47.95%	9
				VIAFLILAK	74.46%	50.35%	7
				VSYEAGEWA	92.47%	83.56%	5
				VTNSTLEVT	93.86%	68.35%	6
				VVTNSTLEV	96.12%	74.93%	10
				VWVIILFQK	84.96%	60.53%	6
				WAENCYNLE	93.44%	56.81%	6
				WRQWIPAGI	88.10%	74.46%	9
				WWTGWRQWI	31.46%	26.56%	2
				YATSYLEYE	98.95%	93.65%	15

				YDRLASTVI	71.90%	29.10%	8
				YLEYEIENF	99.31%	94.14%	16
				YRGVNFAEG	96.66%	85.29%	10
				YYATSYLEY	99.38%	93.52%	16
				Epitope set	**99.99%**	**99.22%**	

Table 5: Population coverage of all epitopes in both MHC class I and II in Sudan and the world.

*Proposed epitopes

Epitope	Coverage World Class I	Coverage Sudan Class I	Total HLA hits	Epitope (core sequence)	Coverage World Class II	Coverage Sudan Class II	Total HLA hits
FLYDRLAST	46.73%	39.93%	3	FAEGVIAFL	99.67%	97.24%	21
IIIAIIALL	42.53%	34.71%	3	FLRATTELR	99.69%	97.36%	21
MHNQNALVC	35.14%	67.96%	3	FLYDRLAST	99.38%	95.87%	19
RTYTILNRK	43.03%	32.96%	5	FVWVIILFQ	99.72%	95.94%	18
Epitope set	**85.08%**	**91.30%**		**Epitope set**	**99.97%**	**99.22%**	

Table 6: Population coverage of proposed epitopes in both MHC class I and II in Sudan and the world.

Conclusion

As the increase of incidence of viral infections by new lethal viruses and infection of human by viruses that earlier recognized as a zoonotic, the need of new available technology increases. Bioinformatics techniques cover this need and reduce the time and effort consumed in designing of new vaccines and therapies.

Sudan Ebola virus is life threatening infection which enforces the need of developing a protective vaccine. The fact that all Ebola species accompanied with high mortality rates increases the need of developing a vaccine against all filoviruses. Several epitopes proposed in this study especially FLYDRLAST which is suggested before by Srivastava et al. [77], to be a peptide vaccine against Ebola virus, could be a powerful multi epitope vaccine against SUDV after *in vivo* and *in vitro* verifications.

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