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Immunoinformatics-Peptide Driven Vaccine and *In silico* Modeling for Duvenhage Rabies Virus Glycoprotein G

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Abstract

Background: Duvenahge rabies virus belongs to *Lyssavirus* genus family Rhabdoviridae causing fatal infection with no effective treatment available and no licensed DNA or peptide vaccine up to date. The aim of present study was to predict peptide vaccine for Duvenhage rabies virus.

Methods and materials: The sequences of Duvenahge virus was optioned from NCBI, and then it was subjected to many B cell and T cell tests from IEDB to realize the most promising peptides that could act as driven-peptide vaccine. Population coverage analysis was performed for selected peptides using IEDB and finally homology modeling and molecular docking studies were done to visualize the interaction with MHC1 molecules.

Result and conclusion: Among the tested peptides for T cell-test, this study projected an interesting epitope of T cell (YFLIGVSAV) that exhibit all-consuming of binding affinity as strong indicator to MHC-One and MHC-two alleles together, besides the binding to eighteen alleles through the population coverage 99.36% in the world. These results were further supported by molecular docking studies that show excellent interaction with MHC homo spins molecule with the lowest binding energy among tested peptides.

Only three B cell epitopes (AHYK, YTIPDKL and SLHNPYPDSH) were found to overlap all performed B cell tests by being linear, on the surface of glycoprotein G and being antigenic.

Keywords: Immunoinformatic; Duvenhage rabies virus; Glycoprotein G; *Lyssavirus*; Peptide vaccine; Epitope

Introduction

Lyssa viruses are representing a serious public health problem, especially in developing countries by causing lethal encephalitis in animals and humans. There is very few information on the way that lyssa viruses in general and Duvenhage virus caused disease [1]. This virus family has shape appear as a bullet, virion was envelop with a sense negatively RNA genome single strand which encodes for five proteins of viral: nucleoprotein, protein for matrix, phosphoprotein, glycoprotein and RNA- dependent RNA polymerase [2,3]. The incubation time is different, and the death is commonly occurred within six and eleven days after paralytic sign's forms, which limit treatment options [4]. Details of the lyssa virus's cycles like Duvenhage, Lagos bat, and Mokola viruses are unspecific [5,6]. The *Lyssavirus* genus of the family Rhabdoviridae consists of eleven additional virus species have been recognized within the genus *Lyssavirus*, which replicate in vertebrates, and mainly carried by bats except Mokola virus, and are restricted in special areas around the world [7]. African lyssa viruses include Mokola virus (MOKV), Lagos bat virus (LBV), and DUVV. European bat lyssa viruses 1 and 2 (EBLV 1 and 2 respectively), Irkut (IRKV), Aravan (ARAV), Khujant (KHUV) and West Caucasian bat virus (WCBV) cause cases in Europe and Asia. Australian bat *Lyssavirus* (ABLV) is restricted to Australia [8,9]. RABV (genotype 1) only bats isolated in South and North America, but rabies associated viruses have been bats isolated from another place. In Africa-countries, DUVV and LBV are bats associated with it, but Mokola virus is related with rodents and shrews [10]. Many susceptible vertebrates, sometimes have been found to be infected by rarely identified lyssaviruses, a human with Duvenhage virus [11,12]. The discovered of Duvenhage virus in South Africa in 1970 when the rabies was caused fatal like disease for a bitten person by a bat [13]. After that, they suggest that the virus isolated was a Miniopterusschreibersite for the reason that wide genus distribution in exposure area. The bat species previously identified as M. schreibersite in Africa is now known as Miniopterusnatalensis and then in 1986 the virus was isolated from an insectivorous bat, Nycteristhebaica in Zimbabwe. After 36 years later, DUVV was identified in human: in South Africa 2006 and subsequently in Kenya in 2007 [9]. Although most of the rabies infections are thought to be zoonotic, clinical cases have also been caused by Duvenhage virus, EBLV 1, EBLV 2, Australian bat *Lyssavirus*, Mokola virus and Irkut virus. Humans are likely to be susceptible to other rabies-related lyssa viruses [14]. Very few laboratories in African countries can diagnose species of rabies infection [9]. Some studies done to control measures and monitoring the spreading of lyssa viruses found that mongoose-related rabies in South Africa are different from classic rabies of dog [10]. Up to date no effective treatment is available for rabies infection. According to phylogeny, serological cross-reactivity and pathogenicity, lyssa viruses have been categorized to three phylogenic groups. Phylogroup I which consists of RABV, DUVV, EBLV1, EBLV2, ABLV, Aravan, Khujand and Irkut. Phylogroup II consists of MOKV and LBV, and Phylogroup III is WCBV [4]. All previous studies suggested that commercial vaccines

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protected against only the Phylogroup I lyssa viruses. Similarity in glycoprotein antigenic sites leads to cross protection between some *Lyssavirus* species by neutralizing antibodies [9]. Even with the presence of vaccines in effective form, rabies infection stays to kill as a minimum 55,000 of people every year, and many of the animal fatal infections. The highly mortality rate is reported in India with more than 16,000 deaths in just 2010 year 15. So, studying of new vaccines is important to produce an effective and low cost one instead of egg-based or cell-culture derived vaccines [15,16]. Vaccinating animals limited human cases, which transferring through dogs, bats and raccoons, in many countries [17,18]. Initial vaccines virus of rabies were formed in living tissue then substituted by vaccines produced in embryonated eggs and tissue culture. Vaccines of viral vector or DNA have been used successfully with greatly protection volume against the infection of rabies [19,20]. However, no one of these vaccines is lead to product a licensed for personal used until now 4. Furthermore, the immunization with P-G gene of rabies virus did not protect against viruses from genotypes 3 and [21-23].

Post-exposure prophylaxis consists of immediate cleansing of the wound after a minor animal injury [24,25], then taking of human rabies immunoglobulin and human rabies vaccine. If the people vaccinated before, fewer vaccine doses and no rabies immunoglobulin are given. If the patient is immunocompetent or immuno-suppressed or unvaccinated, Post-exposure prophylaxis is highly effective if it is begun soon after exposure. When rabies symptoms develop there is no ideal treatment is recommended, some treatments with aggressive therapy have a very high risk of failure, like vaccines, antiviral agents, antibodies to rabies virus, ketamine and the induction of a therapeutic coma are ineffective [14]. The replication cycle is depended on the highly Conservancy of the genome [26,27]. And due to the significance of the envelop matrix-protein M and glycoprotein G in virus infection and virus release [28]. G protein has the major role in the pathogenicity of genotype one lyssa viruses [29,30]. The outer membrane of glycoprotein G is only protein which is related in virus entry besides give a protective immune response [31]. Vaccines are designed for treatment of post exposures to the infection [32]. In animal studies, there is no combination treatment was done in animal models. Some limited access to post exposure prophylaxis may be expected. Put in Duvenhage Virus from a Bat in Kenya, the Treatment failure with Coma-Induction with Ketamine and Antiviral Drugs [33]. So, *in silico* modeling of epitopes protein remains would help in manufacture of peptide vaccine, which is highly immunogenic and with low allergenic effects [34,35]. Our goal is to project a vaccine for Duvenhage virus using peptide of its envelop glycoprotein G as an immunogenic part to encourage protective immunity.

Materials and Methods

Sequence of protein recovery

A total of 26 Africa strains of rabies Duvenhage virus strains' glycoprotein was retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/protein) in November 2016. These 12 strains sequences were recovered from many parts in the world (consist of 11 were isolated from South Africa, Zimbabwe and one from Holland-Netherlands). Glycoprotein strains were Retrieved and area of isolated or collection and their accession numbers are listed in Table 1.

Determinant of conserve regions

The aligned of retrieved sequences was done to obtain conserved regions using multiple-sequence alignment (MSA). Sequences aligned were supported by Clustal-W as Applier in the BioEdit-software, version 7.0.9.1 (Hall, 1999) to find the conserved regions in all 12 retrieved in Rabies spike glycoprotein G sequences Figure 1. At the same time; then

Accession Number	Date of collection	Country
YP_007641405.1]	1971	South Africa
ABZ81220.1	1981	South Africa
AMR44683.1	1986	South Africa
ABZ81215.1	1971	South Africa
AMM70635.1	1971	South Africa
AFK93191.1	2007	Netherlands
ACF32425.1	1970	South Africa
ACF32424.1	1970	South Africa
ACF37213.1	2006	Pilanesberg,South Africa
Q91C28.1	N.A*	Zimbabwe, South Africa
AAK97862.1	1981	South Africa
AAK97861.1	1970	South Africa
Ref sequence. N.A: not available.		

Table 1: Virus Strains retrieved and their Accession numbers and area of collection.

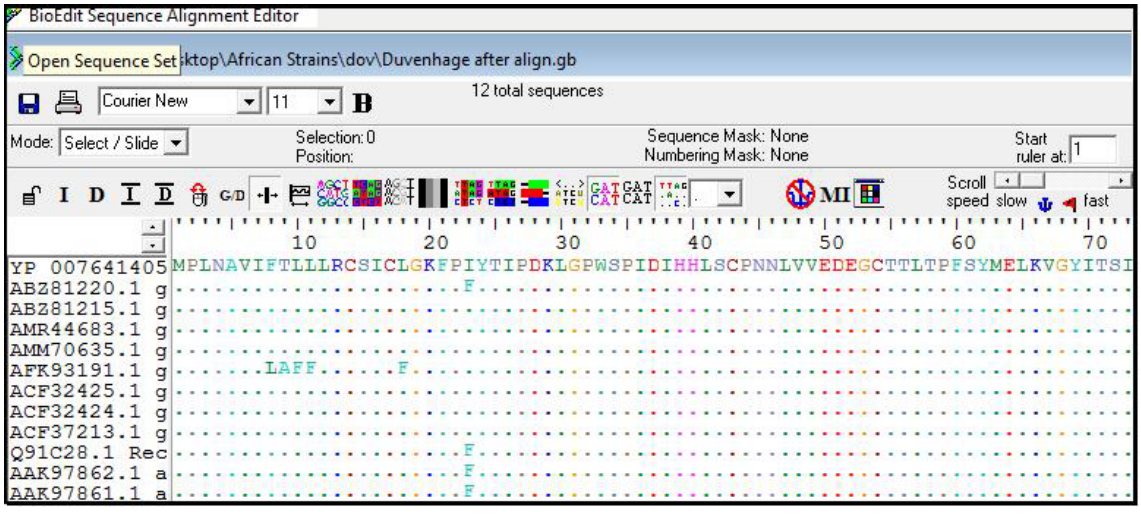

Figure 1: Allignment using Bioedit software for showing of conservancy.

analyzed these candidates epitopes by various prediction tools from Immune Epitope Database 'IEDB' examination resource (http://www.iedb.org/) [36,37].

Prediction of B-cell epitopes

The B-cell epitope is the part of an immunogenic, which determine with B- lymphocytes. B-cell epitope was categorized by hydrophilic reachable and in a turn of beta region. Thus, the classical susceptibility scale methods and hidden programmed for Markov model software's from IEDB as resource of analysis were utilized for the sequential tests [37,38] (Figures 2-3).

Linear Prediction of B-cell epitopes

BepiPred-test from immune epitope database (http://tools.iedb.org/bcell/result/) [39] it has been done as linear prediction of B-cell epitopes when selected from conserved region with a default threshold value of 0.148.

Surface accessibility prediction

via prediction of Emini surface accessibility tool of IEDB (http://tools.iedb.org/bcell/result/) [40]. Epitopes of the surface accessible were predicted from the region they were conserved and the default threshold holding value was 1.0.

Antigenicity prediction of epitopes sites

The antigenicity method of kolaskar and tongaonker was proposed to determine the sites of antigenic epitopes with a default threshold value of 1.042, (http://tools.iedb.org/bcell/result/) [41].

Binding predictions of MHC class I

The peptide binding Analysis to Major Histocompatibility complex class I molecules was evaluated by the IEDB MHC-I estimated tool at (http://tools.iedb.org/mhci/), MHC-I peptide molecules presentation to T lymphocytes submit to a number of steps. The linking of fragment peptides to MHC molecules step was proposed its reached by Artificial neural network (ANN) method [42,43]. Previous to prediction, all lengths of epitope was set as 9 mers, all internationally conserved epitopes that binding to alleles at score less than or equal to 500 half-maximal inhibitory concentration (IC50) was obtained for additional analysis [43].

Binding predictions MHC class II

Binding analysis of peptide to Major Histocompatibility complex class II molecules was evaluated by the IEDB MHC-II estimated tool at (http://tools.iedb.org/mhcii/) [44,45]. In this step, references of human allele were used. In MHC class II groove has the capability to binding

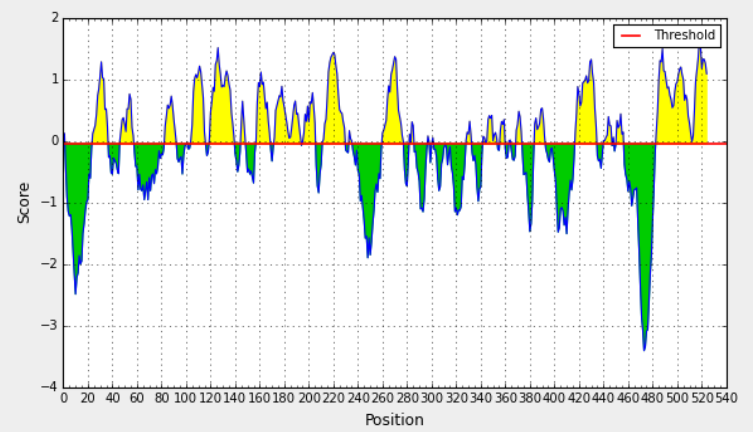

Figure 2: Bepipred Linear Epitope Prediction, Yellow areas above threshold (red line) are proposed to be a part of B cell epitopes and the green areas are not.

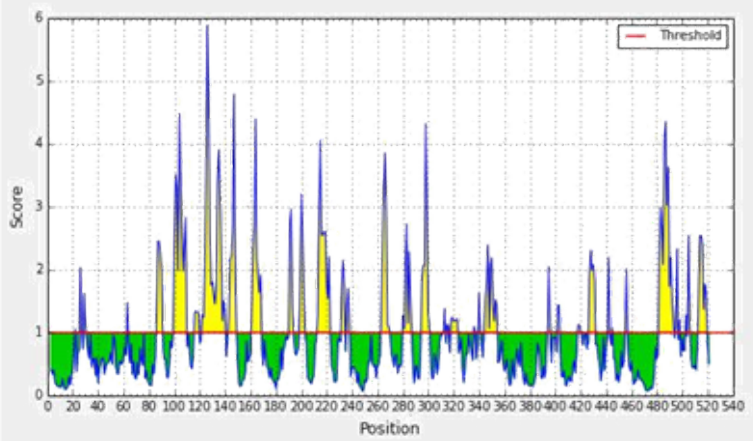

Figure 3: Emini surface accessibility prediction, Yellow areas above threshold (red line) is proposed to be a part of B cell epitopes and the green areas are not.

to peptides with various lengths. This binding variability makes difficult prediction as fewer accurate [46]. IEDB take a five methods of prediction for MHC II as tool of prediction, we used the artificial neural networks method 'NN- align' that allows for simultaneously identifying binding core epitopes of the MHC class II and affinity to binding. Method of the consensus uses the medium ranking of the three methodologies as the final score of prediction [47]. All epitopes were preserved as conserves that binding to various alleles at equal score or less than 1000 half-maximal inhibitory concentration (IC50) it was selected for additional analysis (Figures 4-6).

Population coverage calculation

All probable binders of MHC I and MHC II from rabies virus of Africa duvenhage strain Glycoprotein was evaluated the coverage of population compared to the whole world and Africa population with a selection of MHC-I and MHC-II related alleles by calculation

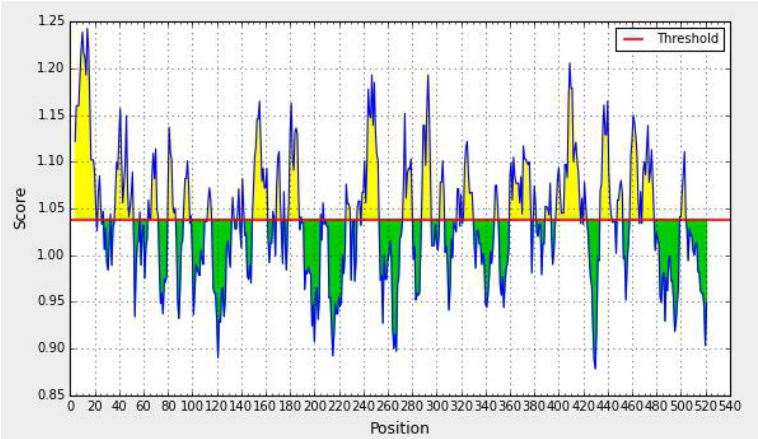

Figure 4: Kolaskar and Tongaonkar antigenicity prediction, Yellow areas above threshold (red line) are proposed to be a part of B cell epitope while green areas are not.

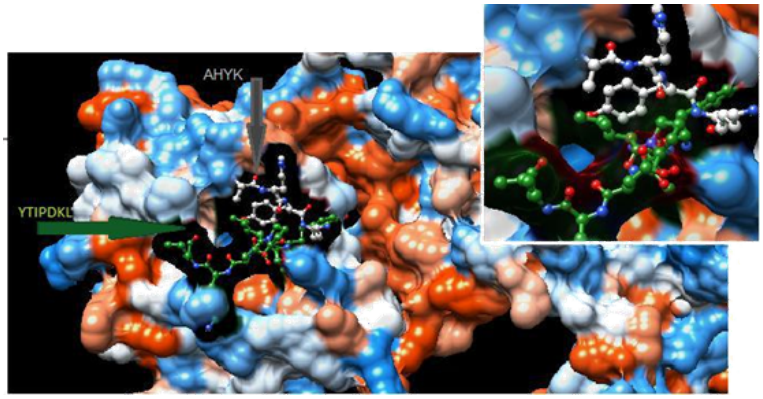

Figure 5: Structural position of the most promising conserved B cell epitopes of glycoprotein G of Duvenhage rabies virus.

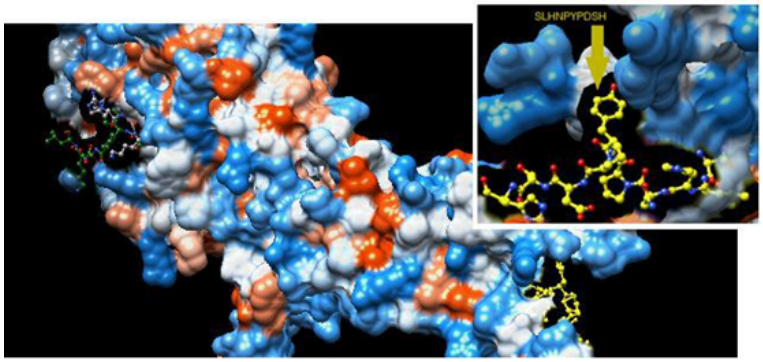

Figure 6: Third Structure position of the most promising conserved B cell epitopes of glycoprotein G of Duvenhage rabies virus.

of the IEDB population coverage tool at http://tools.iedb.org/tools/population/iedb_input [48].

Homology modeling

The modeling has been done by Raptor X structure prediction server. Which is submitted the reference sequence of duvenhage strain on 2016-12-21. The files result is received at 2016-12-22, which containing the predicted 3D model and image are attached noreply@raptorx.uchicago.edu.

Then we used visualized tool of UCSF Chimera (version 1.8) to get a 3D-structures of protein, this tool was presently available within the Chimera package and accessible from the chimera web site (http://www.cgl.ucsf.edu/cimera) [49].

Docking

MHCI alleles Epitopes was predicted to get bind with fewer average of percentile-rank were designated as the ligands, PEP- FOLD as online tool of peptide modeling it has been used to given epitopes structure [50]. The MHCI allele's 3D structure was achieved from PatchDock algorithm stimulated by object recognition and segmentation techniques of image used in Computer Visualization. It tries to match two parts by selection one part and search for the matching one as complementary [51]. The FireDock server statements used for problem of refinement in protein-protein docking resolutions; The method at the same time targets the flexibility problem and scoring of solutions was produced by docking algorithms as fast rigid-body [52-54].

Results

Prediction of B-cell epitope

The Duvenhage rabies glycoprotein (GP) reference sequence was examined by Bepipred linear epitope prediction, Kolaskar and Tongaonkar antigenicity and Emini surface accessibility methods in IEDB, to confine the epitope linearity, which is surface rise and the immunogenicity respectively supported test (Figures 7-9).

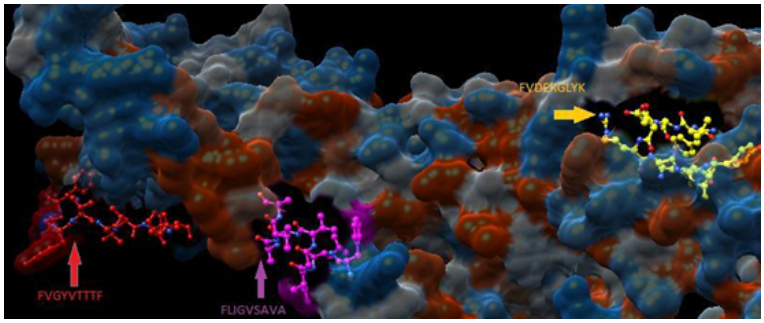

Figure 7: Structural Position of the three most promising conserved epitopes at glycoprotein G of Duvenhage rabies virus that interact with MHC I alleles.

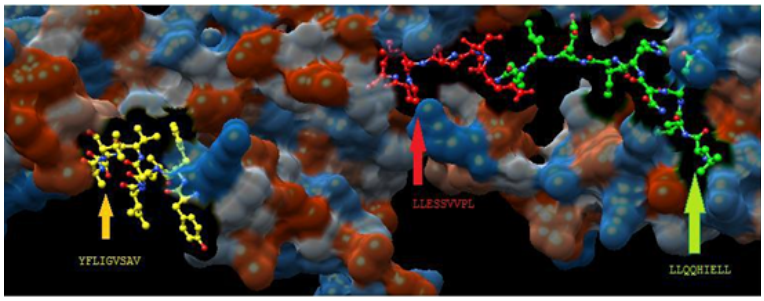

Figure 8: Molecular position of the most promising three epitopes of Duvenhage rabies virus that binds MHC II alleles.

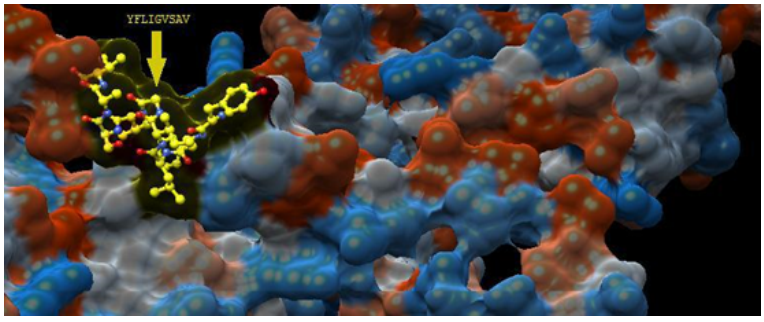

Figure 9: Molecular position of the most promising epitope for population coverage that binds both MHC1 and MHC11 alleles.

Cytotoxic T-lymphocyte epitopes Prediction and interaction with MHC-1

The duvenhage rabies virus reference sequence of glycoprotein G was examined using IEDB MHC-1 tool of binding prediction to predict T cell epitopes to given interact proposed with types of different MHC I alleles built on ANN- align by (IC50) ≤ 500, the list of epitopes were a conserved and their MHC-1 alleles corresponding are shown in (Tables 2-4).

T helper cell epitopes Prediction and interaction with MHC II alleles

The Duvenhage rabies virus reference sequence of glycoprotein G was examined using IEDB MHC II tool of binding prediction based on NN-align by half-maximal inhibitory concentration (IC50) ≤ 1000; In supplementary- Table I were shown the list of all epitopes and their correspondent binding MHC II alleles while the list most promising epitopes that had Required to binding affinity with MHC II alleles along with their positions in the glycoprotein G Duvenhage rabies virus were presented in (Table 5).

Population Coverage Analysis

The test of Population coverage was done to observe the all epitopes world coverage binds to MHC1 alleles and MHC11 alleles also to joined

Peptide	Start	End	Length	Emini Score	Antigenicity Score
YTIPDKLGPWSPI	24	36	13	0.614	1.03
YTIPDKL	24	30	7	1.156	1.047
LVVEDEGCTTL	47	57	11	0.131	1.085
VVTEAETYT	83	91	9	0.813	1.047
FRPSVNSCRDA	106	116	11	0.631	0.998
NWKIAGDPRYEESLHNPYPDSHWL	118	141	24	4.164	0.996
SLHNPYPDSH	130	139	10	2.229	1.024
VADMDAYDKK	158	167	10	1.768	0.996
ISPGS	181	185	5	0.553	1.023
PFCPTNHEYT	186	195	10	1.109	1.034
WMPESSNPGIS	197	207	11	0.733	0.958
MGKKATK	215	221	7	1.955	0.923
EWCSPDQL	269	276	8	0.637	1.045
WVSLPQVNN	259	267	9	0.458	1.061
PGFGK	328	332	5	0.753	0.967
AHYK	346	349	4	1.599	1.065
EIIP	356	359	4	0.488	1.055
GCLKAGGRCYPH	362	373	12	0.148	1.074
LGPGG	384	388	5	0.355	0.987
HPLADPSTVFKNDDEAE	416	432	17	2.27	1.001
HLPDTNQKISG	438	448	11	1.372	0.996
KSKP	488	491	4	3.076	0.984
PVELT	493	497	5	0.679	1.091

Table 2: List of conserved peptides with their surface accessibility score and antigenicity score.

Epitope	Start	End	Length	Surface accessibility score	Antigenicity score
AHYK	346	349	4	1.599	1.065
SLHNPYPDSH	130	139	10	2.229	1.042
YTIPDKL	24	30	7	1.156	1.047

Table 3: List of conserved peptide that passes Bepipred, Emini surface and Koalaskar tests with their length and scores.

Peptide	Start	End	Alleles	IC 50	Percentile rank
HHLSCPNNL	39	47	HLA-	15.19	0.3
			B*39:01		
			HLA-	220.64	0.1
			B*38:01		
HLSCPNNLV	40	48	HLA-	314.16	0.7
			A*02:01		
			HLA-	390.85	1.5
			A*02:06		
			HLA-	67.93	0.7
			A*68:02		
LSCPNNLVV	41	49	HLA-	132.13	0.3
			C*12:03		
			HLA-	83.41	0.1
			C*15:02		
VEDEGCTTL	49	57	HLA-	7.43	0.1
			B*40:01		
			HLA-	51.45	0.4
			C*05:01		
DEGCTTLTP	51	59	HLA-	485.41	0.4
			B*18:01		
CTTLTPFSY	54	62	HLA-	54.44	0.2
			A*01:01		
			HLA-	43.56	0.2
			A*29:02		
			HLA-	115.27	0.4
			A*30:02		
			HLA-	419.12	0.9
			B*35:01		
			HLA-	106.58	0.3
			B*58:01		
TTLTPFSYM	55	63	HLA-	127.07	1
			A*02:06		
			HLA-	109.17	0.7
			A*68:02		
			HLA-	240.51	0.8
			C*14:02		
			HLA-	408.8	0.5
			C*15:02		
LTPFSYMEL	57	65	HLA-	117.08	0.8
			A*68:02		
			HLA-	84.58	0.3
			C*14:02		
TPFSYMELK	58	66	HLA-	484.2	0.4
			A*11:01		
			HLA-	70.16	0.6
			A*68:01		
SYMELKVGY	61	69	HLA-	175.38	0.4
			A*29:02		
			HLA-	185.99	0.8
			C*14:02		
YMELKVGYI	62	70	HLA-	283.51	0.7
			A*02:01		
			HLA-	246.31	0.4
			C*12:03		
MELKVGYIT	63	71	HLA-	47.56	0.1
			B*18:01		
			HLA-	65.75	0.2
			B*40:02		
KVGYITSIK	66	74	HLA-	49.25	0.2
			A*03:01		
			HLA-	52.57	0.4

			A*11:01		
			HLA-	35.29	0.4
			A*30:01		
			HLA-	420.59	0.6
			A*31:01		
TSIKVSGFT	71	79	HLA-	146.56	1.1
			A*68:02		
KVSGFTCTG	74	82	HLA-	219.97	0.5
			A*32:01		
			HLA-	244	0.8
			A*30:01		
TEAETYTNF	85	93	HLA-	32.55	0.1
			B*18:01		
			HLA-	77.9	0.2
			B*40:01		
TEAETYTNF	85	93	HLA-	156.19	0.1
			B*44:02		
			HLA-	467	0.4
			B*40:02		
EAETYTNFV	86	94	HLA-	33.82	0.6
			A*68:02		
			HLA-	325.47	0.4
			C*12:03		
AETYTNFVG	87	95	HLA-	470.09	0.4
			B*40:02		
ETYTNFVGY	88	96	HLA-	65.11	0.1
			A*25:01		
			HLA-	405.23	0.3
			A*01:01		
			HLA-	7.41	0.1
			A*26:01		
			HLA-	47.88	0.6
			A*68:01		
			HLA-	94.31	0.2
			A*29:02		
			HLA-	144.33	1.1
			A*68:02		
			HLA-	289.84	0.8
			A*30:02		
TYTNFVGYV	89	97	HLA-	110.78	0.7
			A*68:02		
			HLA-	96.3	0.3
			C*14:02		
YTNFVGYVT	90	98	HLA-	159.65	1.1
			A*68:02		
			HLA-	477.54	0.5
			C*15:02		
FVGYVTTTF	93	101	HLA-	337.71	0.4
			A*23:01		
			HLA-	115.41	0.3
			A*32:01		
			HLA-	18.33	0.2
			B*35:01		
			HLA-	99.41	0.2
			B*15:01		
			HLA-	206.88	0.7
			B*53:01		
			HLA-	247.75	1
			C*03:03		
			HLA-	494.71	0.5
			B*58:01		
			HLA-	87.09	0.2
			C*12:03		

			HLA-	251.83	0.7
			C*05:01		
VGYVTTTFR	94	102	HLA-	28.73	0.4
			A*31:01		
			HLA-	265.97	1.4
			A*68:01		
GYVTTTFRR	95	103	HLA-	45.64	0.4
			A*31:01		
YVTTTFRRR	96	104	HLA-	53.03	0.6
			A*68:01		
			HLA-	107.18	0.5
			A*31:01		
TTFRRRHFR	99	107	HLA-	15.23	0.3
			A*11:01		
			HLA-	150.56	0.3
			A*03:01		
			HLA-	2.97	0.1
			A*31:01		
			HLA-	4.34	0.1
			A*68:01		
			HLA-	137.46	0.8
			A*30:01		
FRRRHFRPS	101	109	HLA-	71.14	0.2
			B*08:01		
RRRHFRPSV	102	110	HLA-	28.06	0.4
			A*30:01		
			HLA-	147.32	0.5
			B*27:05		
HFRPSVNSC	105	113	HLA-	113.41	0.7
			A*30:01		
SVNSCRDAY	109	117	HLA-	60.55	0.2
			A*30:02		
			HLA-	335.2	0.5
			A*29:02		
			HLA-	47.04	0.3
			B*35:01		
			HLA-	307.58	0.8
			B*15:01		
NSCRDAYNW	111	119	HLA-	41.25	0.3
			B*58:01		
			HLA-	251.39	0.3
			B*57:01		
SCRDAYNWK	112	120	HLA-	39.7	0.4
			A*30:01		
CRDAYNWKI	113	121	HLA-	243.88	0.5
			B*27:05		
WKIAGDPRY	119	127	HLA-	165.13	0.4
			A*29:02		
			HLA-	316.37	0.8
			B*35:01		
YEESLHNPY	127	135	HLA-	397.61	0.5
			A*29:02		
			HLA-	4.96	0.1
			B*18:01		
			HLA-	188.53	0.8
			B*35:01		
			HLA-	433.38	0.3
			B*15:02		
			HLA-	348.49	0.5
			C*12:03		
NPYPDSHWL	133	141	HLA-	277.75	0.8
			B*35:01		
			HLA-	288.16	0.5

			B*07:02		
			HLA-	248.57	0.7
			B*39:01		
			HLA-	98.73	0.4
			B*53:01		
			HLA-	213.91	1
			C*03:03		
YPDSHWLRT	135	143	HLA-	358.65	0.8
			B*35:01		
DSHWLRTVK	137	145	HLA-	127.98	0.9
			A*68:01		
WLRTVKTTK	140	148	HLA-	222.81	0.4
			A*03:01		
KTTKESLLI	145	153	HLA-	225.79	0.8
			A*30:01		
			HLA-	44.67	0.3
			B*58:01		
			HLA-	39.44	0.1
			C*15:02		
TTKESLLII	146	154	HLA-	230.23	0.8
			A*30:01		
KESLLIISP	148	156	HLA-	91.49	0.2
			B*40:02		
SLLIISPSV	150	158	HLA-	6.74	0.1
			A*02:01		
			HLA-	28.53	0.6
			A*02:06		
PSVADMDAY	156	164	HLA-	480.82	0.9
			A*30:02		
MDAYDKKLY	161	169	HLA-	263.3	0.5
			A*29:02		
PFCPTNHEY	186	194	HLA-	79.07	0.2
			A*29:02		
MPESSNPGI	198	206	HLA-	430.37	0.9
			B*35:01		
			HLA-	47.23	0.2
			B*53:01		
NPGISCDIF	203	211	HLA-	407.55	0.9
			B*35:01		
GISCDIFTR	205	213	HLA-	348.3	0.4
			A*11:01		
			HLA-	116.13	0.7
			A*68:01		
			HLA-	132.48	0.5
			A*31:01		
DIFTRSMGK	209	217	HLA-	156.93	0.3
			A*03:01		
			HLA-	234.21	0.4
			A*11:01		
			HLA-	22.88	0.5
			A*68:01		
RSMGKKATK	213	221	HLA-	45.49	0.4
			A*11:01		
			HLA-	62.14	0.2
			A*03:01		
			HLA-	16.18	0.4
			A*31:01		
			HLA-	59.31	0.5
			A*30:01		
QLCGFVDER	224	232	HLA-	154.3	1.2
			A*68:01		
GFVDERGLY	227	235	HLA-	11.55	0.2
			A*29:02		
			HLA-	459.99	0.9
			A*30:02		
FVDERGLYK	228	236	HLA-	62.05	0.4
			A*11:01		
			HLA-	291.55	0.5
			A*03:01		
			HLA-	480.7	0.3
			A*01:01		
			HLA-	301.3	1.6
			A*68:01		
			HLA-	68.6	0.4
			C*05:01		
DERGLYKSL	230	238	HLA-	22.93	0.1
			B*18:01		
RLCGISGLR	245	253	HLA-	251.77	0.4
			A*03:01		
			HLA-	38.77	0.4
			A*31:01		
RLMDGSWVS	253	261	HLA-	52.59	0.5
			A*02:01		
			HLA-	79.18	0.7
LMDGSWVSL			A*02:06		
			HLA-	260.76	0.5
			A*32:01		
LMDGSWVSL	254	262	HLA-	20.81	0.6
			A*02:06		
			HLA-	34.65	0.5
			A*02:01		
			HLA-	61.65	0.3
			A*32:01		
			HLA-	83.67	0.3
			B*39:01		
GSWVSLPQV	257	265	HLA-	152.46	1.2
			A*02:06		
FTKSISFRR	311	319	HLA-	4.78	0.1
			A*68:01		
			HLA-	12.31	0.3
			A*31:01		
KSISFRRLS	313	321	HLA-	307.01	0.8
			A*30:01		
RKLVPGFGK	324	332	HLA-	186.86	0.5
			B*27:05		
KLVPGFGKA	325	333	HLA-	47.95	0.7
			A*02:06		
LVPGFGKAY	326	334	HLA-	460.89	0.7
			A*29:02		
			HLA-	215.75	0.2
			B*15:02		
			HLA-	245.2	0.8
			B*35:01		
			HLA-	364.34	0.8
			B*15:01		
			HLA-	264.81	0.8
			C*14:02		
			HLA-	368.88	0.5
			C*12:03		
TLMEAEAHY	340	348	HLA-	107.76	0.2
			A*29:02		
			HLA-	28.25	0.1
			B*15:01		
			HLA-	349.06	0.3
			B*15:02		
	341	349	HLA-	145.36	0.4

LMEAEAHYK			A*11:01		
			HLA-	400.58	0.5
			A*03:01		
EIIPSKGCL	356	364	HLA-	187.52	1.2
			A*68:02		
			HLA-	255.04	1
			C*03:03		
IIPSKGCLK	357	365	HLA-	235.98	0.4
			A*03:01		
			HLA-	359.4	0.4
			A*11:01		
CLKAGGRCY	363	371	HLA-	30.31	0.1
			B*15:02		
			HLA-	111.18	0.3
			B*15:01		
RCYPHHNGI	369	377	HLA-	325.09	0.5
			A*32:01		
			HLA-	340.81	1
			C*03:03		
			HLA-	139.45	0.3
			C*12:03		
			HLA-	212.84	0.4
			C*15:02		
CYPHHNGIF	370	378	HLA-	80.76	0.5
			A*24:02		
			HLA-	429.94	0.4
			A*23:01		
			HLA-	36.57	0.2
			C*14:02		
			HLA-	440.64	0.3
			C*07:02		
YPHHNGIFF	371	379	HLA-	6.45	0.1
			B*35:01		
			HLA-	51.13	0.2
			B*07:02		
			HLA-	20.72	0.1
			B*53:01		
			HLA-	166.81	0.9
			C*03:03		
			HLA-	59.23	0.2
			C*12:03		
			HLA-	144.26	0.7
			C*14:02		
	392	400	HLA-	171.15	0.5
IPEMQSALL			B*07:02		
QSALLQQHI	396	404	HLA-	191.67	0.5
			B*58:01		
ALLQQHIEL	398	406	HLA-	68.57	0.5
			A*02:01		
			HLA-	193.81	1.3
			A*02:06		
LLQQHIELL	399	407	HLA-	199.84	0.7
			A*02:01		
			HLA-	352.77	1.5
			A*02:06		
IELLESSVV	404	412	HLA-	86.85	0.2
			B*40:02		
LLESSVVPL	406	414	HLA-	87.01	0.5
			A*02:01		
			HLA-	271.05	1.4
			A*02:06		
HPLADPSTV	416	424	HLA-	150.55	0.8
			B*35:01		
			HLA-	310.77	0.5
			B*07:02		
			HLA-	128.22	0.6
			B*39:01		
			HLA-	282.85	0.7
			B*53:01		
PLADPSTVF	417	425	HLA-	143.69	0.1
			B*15:02		
			HLA-	325.79	0.8
			B*15:01		
			HLA-	244.72	1
			C*03:03		
LADPSTVFK	418	426	HLA-	37.37	0.4
			A*11:01		
			HLA-	87.2	0.6
			A*68:01		
			HLA-	24.07	0.4
			C*05:01		
TVFKNDDEA	423	431	HLA-	174.93	1.2
			A*68:02		
DTNQKISGI	441	449	HLA-	397.11	1.3
			A*68:02		
KISGIDLGL	445	453	HLA-	135.24	1.1
			A*02:06		
			HLA-	329.2	0.8
			A*02:01		
			HLA-	255.09	0.5
			A*32:01		
LPEWKRYFL	453	461	HLA-	42.96	0.2
			B*07:02		
			HLA-	177.79	0.5
			B*08:01		
PEWKRYFLI	454	462	HLA-	106.51	0.2
			B*40:02		
KRYFLIGVS	457	465	HLA-	329.77	0.5
			B*27:05		
RYFLIGVSA	458	466	HLA-	118.86	0.7
			A*30:01		
YFLIGVSAV	459	467	HLA-	456.79	1.6
			A*02:06		
			HLA-	14.97	0.2
			C*14:02		
			HLA-	162.44	0.4
			C*12:03		
FLIGVSAVA	460	468	HLA-	20.1	0.4
			A*02:01		
			HLA-	27.56	0.6
			A*02:06		
			HLA-	301.65	1.3
			A*68:02		
			HLA-	367.04	0.8
			B*15:01		
LTRKVSVIS	496	504	HLA-	141.8	0.8
			A*30:01		
KGNGPVPSW	505	513	HLA-	9.76	0.1
			B*58:01		
			HLA-	274.24	0.3
			B*57:01		
GPVPSWESY	508	516	HLA-	81.03	0.4
			B*35:01		

Table 4: List of conserved epitopes and their corresponding MHC-1 alleles along with their position in glycoprotein, IC50 and percentile rank.

Core Sequence	Start	End	Peptide Sequence	Allele	IC50	Rank
YTIPDKLGP	24	32	FPIYTIPDKLGPWSP	HLA-DRB1*11:01	157	17.83
			PIYTIPDKLGPWSPI	HLA-DRB1*11:01	248.5	22.63
LGPWSPIDI	30	38	DKLGPWSPIDIHHLS	HLA-DRB1*04:04	744.2	41.77
			PDKLGPWSPIDIHHL	HLA-DRB1*04:04	806.5	43.27
			PDKLGPWSPIDIHHL	HLA-DRB1*09:01	441.1	23.44
			DKLGPWSPIDIHHLS	HLA-DRB1*09:01	564	27.6
			IPDKLGPWSPIDIHH	HLA-DRB1*09:01	709.1	31.86
			KLGPWSPIDIHHLSC	HLA-DRB1*09:01	715.1	32.03
			TIPDKLGPWSPIDIH	HLA-DRB1*09:01	976.6	38.45
			LGPWSPIDIHHLSCP	HLA-DRB1*09:01	976.6	38.45
			KLGPWSPIDIHHLSC	HLA-DRB4*01:01	571.2	29.76
			LGPWSPIDIHHLSCP	HLA-DRB4*01:01	620.7	31.26
			DKLGPWSPIDIHHLS	HLA-DRB4*01:01	639.6	31.81
			PDKLGPWSPIDIHHL	HLA-DRB4*01:01	671.7	32.72
WSPIDIHHL	33	41	KLGPWSPIDIHHLSC	HLA-DRB1*01:01	95.9	29.61
			LGPWSPIDIHHLSCP	HLA-DRB1*01:01	120.9	32.9
			DKLGPWSPIDIHHLS	HLA-DRB1*01:01	136.9	34.77
			PDKLGPWSPIDIHHL	HLA-DRB1*01:01	290.9	47.58
			GPWSPIDIHHLSCPN	HLA-DRB1*01:01	484.9	56.24
			PWSPIDIHHLSCPNN	HLA-DRB1*01:01	939.9	66.37
			PDKLGPWSPIDIHHL	HLA-DRB1*07:01	454.1	31.91
			DKLGPWSPIDIHHLS	HLA-DRB1*07:01	642.4	37.06
			KLGPWSPIDIHHLSC	HLA-DRB1*07:01	871	41.97
			WSPIDIHHLSCPNNL	HLA-DRB1*11:01	157	17.83
			PWSPIDIHHLSCPNN	HLA-DRB1*11:01	248.5	22.63
DIHHLSCPN	37	45	GPWSPIDIHHLSCPN	HLA-DRB1*04:04	693.7	40.56
IHHLSCPNN	38	46	PIDIHHLSCPNNLVV	HLA-DRB1*04:04	36.5	3.77
			IDIHHLSCPNNLVVE	HLA-DRB1*04:04	38.9	4.15
			SPIDIHHLSCPNNLV	HLA-DRB1*04:04	40.9	4.43
			WSPIDIHHLSCPNNL	HLA-DRB1*04:04	48.9	5.54
			DIHHLSCPNNLVVED	HLA-DRB1*04:04	53.7	6.18
			PWSPIDIHHLSCPNN	HLA-DRB1*04:04	61.5	7.28
			IHHLSCPNNLVVEDE	HLA-DRB1*04:04	70	8.41
HHLSCPNNL	39	47	PIDIHHLSCPNNLVV	HLA-DRB1*07:01	203	21.56
			IDIHHLSCPNNLVVE	HLA-DRB1*07:01	294	26
			SPIDIHHLSCPNNLV	HLA-DRB1*07:01	384.9	29.57
			DIHHLSCPNNLVVED	HLA-DRB1*07:01	462.1	32.16
			WSPIDIHHLSCPNNL	HLA-DRB1*07:01	527.4	34.06
			IDIHHLSCPNNLVVE	HLA-DRB1*09:01	767.7	33.37
			IHHLSCPNNLVVEDE	HLA-DRB1*07:01	769.7	39.99
			PIDIHHLSCPNNLVV	HLA-DRB1*09:01	844.1	35.36
			IDIHHLSCPNNLVVE	HLA-DRB1*15:01	219.8	18.05
			PIDIHHLSCPNNLVV	HLA-DRB1*15:01	225.5	18.35
			SPIDIHHLSCPNNLV	HLA-DRB1*15:01	296.8	21.79
			DIHHLSCPNNLVVED	HLA-DRB1*15:01	331.3	23.24
			WSPIDIHHLSCPNNL	HLA-DRB1*15:01	383.1	25.25
			PIDIHHLSCPNNLVV	HLA-DRB1*13:02	388.6	14.95
			IHHLSCPNNLVVEDE	HLA-DRB1*15:01	520.5	29.66
			HHLSCPNNLVVEDEG	HLA-DRB1*15:01	912	38.57
HLSCPNNLV	40	48	IDIHHLSCPNNLVVE	HLA-DRB1*01:01	180.3	39.14
			DIHHLSCPNNLVVED	HLA-DRB1*01:01	210	41.74
			IHHLSCPNNLVVEDE	HLA-DRB1*01:01	223.3	42.82
			PIDIHHLSCPNNLVV	HLA-DRB1*01:01	232.5	43.54
			SPIDIHHLSCPNNLV	HLA-DRB1*01:01	568.2	58.76
			HHLSCPNNLVVEDEG	HLA-DRB1*01:01	659.2	61.06
LSCPNNLVV	41	49	IDIHHLSCPNNLVVE	HLA-DRB1*13:02	307.3	12.93
			DIHHLSCPNNLVVED	HLA-DRB1*13:02	325.8	13.42
			IHHLSCPNNLVVEDE	HLA-DRB1*13:02	350.8	14.04
			HHLSCPNNLVVEDEG	HLA-DRB1*13:02	519.6	17.8

Table 5: the most epitopes list that had Binding affinity with MHC II alleles along with their positions in the glycoprotein G Duvenhage rabies virus, sequence of peptide binding alleles, IC50 and rank.

MHC 1 and MHC11 as well as a selection of the best promise epitopes for each test.

Population coverage for MHC1

All epitopes in population coverage results binding to MHC1 alleles of Duvenhage rabies virus was shown in supplementary-Table (II) and the results of population coverage for the most candidates promising three peptides was shown on (Table 6).

MHC-1 peptides		MHC-11 Peptides	
Epitopes	The Coverage	Epitopes	The Coverage
FLIGVSAVA	47.38%	LLESSVVPL	99.12%
FVDERGLYK	53.45%	LLQQHIELL	99.20%
FVGYVTTTF	47.28%	YFLIGVSAV	99.36%
Epitope set	56.71%		99.68%

Table 6: Most promising 3 epitopes in population coverage binds to MHC1 alleles & MHC11 alleles of Duvenhage rabies virus along with their population coverage in the world. The epitope coverage set is 99.99% for class1 and 11 together.

Population coverage for MHC11

All peptides in population coverage results binding to MHC11 alleles with total HLA hits and their coverage were shown in supplementary-Table (II) and the results of candidates three epitopes promising were presented on (Table 6).

Population coverage for both MHC1 and MHC11 alleles

The performed of this test to candidate most one promising epitope (YFLIGVSAV) and it was establishing to bind 12 alleles with the world coverage of 99.36% (Figures 10-12).

Docking

Many servers used as the complementary tool to give final resolute by achieved two input options for ligand and receptor of (2 PDB) files with each docking solution, The output is a list of ranked in all the input

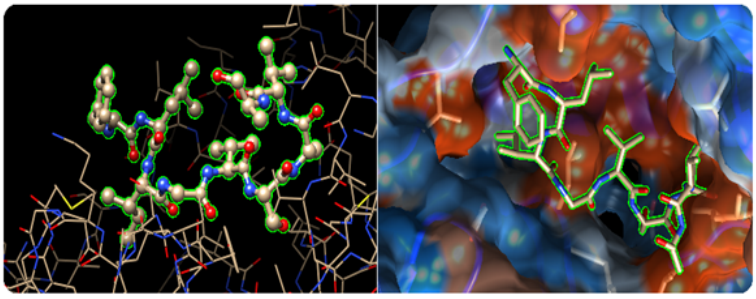

Figure 10: Interaction of FLIGVSAVA epitope with MHC homo sapiens. Left: Binding interaction of ligand shown as stick and balls structure while the receptor shown as lines. Right: stick contacts with receptor which shown as surface.

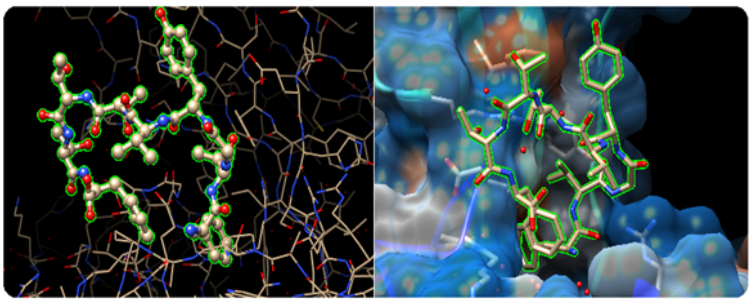

Figure 11: Interaction of FVGYVTTTF epitope with MHC homo sapiens. Left: Binding interaction of ligand shown as stick and balls structure while the receptor shown as lines. Right: stick contacts with receptor which shown as surface.

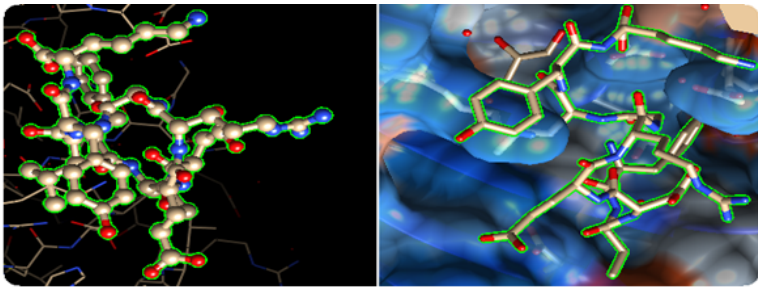

Figure 12: Interaction of FVDERGLYK epitope with MHC homo sapiens. Left: Binding interaction of ligand shown as stick and balls structure while the receptor shown as lines. Right: stick contacts with receptor which shown as surface.

Epitopes	Attractive VdWc	Repulsive VdWb	Global Energya
		25.74	
FLIGVSAVA	-24.34		-22.98
		7.51	
FVDERGLYK	-18.89		-34.28
		14.17	
FVGYVTTTF	-23.14	Repulsive VdWb	-20.44

a: energy needed for binding the lowest is better
b: replusive interaction of van der waals ranking
c: attractive interaction of van der Waals ranking

Table 7: Selected three best result with lowest binding of global-energy score necessary for all epitopes besides the attractive and Repulsive softened van der Waals interactions VdW.

resolutions. All epitopes were docked Epitomized a Molecular Structural Shape, which have good affinity to binding with lowered binding global energy, the method we are used at the same time targets to problem of flexibility and recording of solutions created by fast rigid body docking algorithms. This gave a set of up to 15 possible docking candidates of three promising peptides bind with MHC1 Homo sapiens. In Table 7 selected three best result with lowest binding of global-energy score necessary for all epitopes besides the attractive and Repulsive softened van der Waals interactions VdW. The developed complex structure is created for up to three candidate's low-energy for individually structure. Epitope FVGYVTTTF is showing the best one binding energy Figure 10.

Discussion

The importance of selecting Duvenhage strain 'genus *Lyssavirus*' in the present study was based on many contributed including high mortality rate and the used of traditional method to produce rabies vaccine without deeply testing studies on the immune system response can be selected a conserve peptide to give a universal covering of population and who have made a strong binding between MHC homo spins with these peptides. Some study that supports previous studies indicating all African *Lyssavirus* species that can be lethal when inoculated *via* the i. m. route and that the pathogenicity of a *Lyssavirus* species should not be concluded based on a single isolate [53].

In this study we used, the immunoinformatics tools to predicate the candidate conserved peptides to give a good binding and faster recognized by immune system, especially in B and T lymphocytes in adaptive immune response to produce specific antibodies against Duvenhage virus. All protein's sequences of glycoprotein G were selected from NCBI website were aligned to get a conserved region that representative Duvenhage *Lyssavirus* isolates is shown in Figure 1. Then used reference sequence of target strain "Duvenhage rabies virus glycoprotein.

G" to be subjected in Bepipred linear epitope prediction test, Kolaskar and Tongaonkar antigenicity test and Emini surface accessibility test using IEDB, to define binding affinity to B cell, presence in the surface and to exam the immunogenicity.

In Bepipred test, were presented of 23 conserved epitopes that have the affinity of binding to B cell while there are 17 epitopes surface predicted that according to Emini test for surface accessibility and 18 epitopes presence antigenic when detected by Kolaskar-Tongaonkar antigenicity test but only three epitopes established as overlap all performed B Cell tests (AHYK, YTIPDKL and SLHNPYPDSH). Furthermore, the reference Duvenhage rabies sequence of glycoprotein G was tested by IEDB-MHC 1 of bind predicted tool to obtain the predict result T cell epitope was proposed to interact with various types of MHCI alleles were subjected on Artificial neural network (ANN) through the half-maximal inhibitory concentration (IC50) ≤ 500; 23 conserved peptides were predicted interact with various MHC-1 proposed alleles. The peptide FVGYVTTTF from 93 into 101 had greater affinity to interact with 9 alleles (HLA-A*23:01, HLA-A*32:01, HLA-B*35:01, HLA-B*15:01, HLA-B*53:01, HLA-C*03:03, HLA-B*58:01, HLA-C*12:03, HLA-C*05:01), followed by ETYTNFVGY From 88 to 96 that binds seven alleles (HLA-A*25:01, HLA-A*01:01, HLA-A*26:01, HLA-A*68:01, HLA-A*29:02, HLA-A*68:02, HLA-A*30:02). These two peptides can be supposed about as a promising peptide vaccine for Duvenhage rabies virus that's assured after performed for the population coverage database.

The analyzed for reference glycoprotein G strain was performed *via* IEDB MHC II prediction of binding tool established with half-maximal inhibitory concentration (IC50) on NN-align ≤ 1000; around 39 conserved epitopes remained predicted found to interact with alleles of MHC-II, The peptides LLESSVVPL, LLQQHIELL and YFLIGVSAV required the affinity to bind the maximum number to these alleles of MHC11.

The Results in World population coverage for total binding epitopes to MHC1 alleles were 99.51% and the peptides have most promising were FVGYVTTTF, FVDERGLYK and FLIGVSAVA with population coverage in the world 56.71% and total HLA hits 9, 5 and 4 respectively.

World population coverage result for totally epitopes that take binding affinity to MHC11 alleles was 99.99% while population coverage in world of the best promising three epitopes LLESSVVPL, LLQQHIELL and YFLIGVSAV was 99.68% with HLA hits 16, 18 and 18 respectively. The peptide YFLIGVSAV shows excellent population coverage outcomes for MHC1 and MHC11 alleles together of (99.36%) with total HLA hits 18 different alleles.

Recommendations and Conclusions

In the current study, we achieve that to our information this was the first one study to suggest peptide vaccine through previous steps for Duvenhage rabies virus up to now.

Furthermore, the immuno-informatics methods representing a smart *in silico* tools, it's gives a highly supported outputting epitopes-driven vaccines for communicable pathogens could be extended to efficacy trials stage in humans, after demonstration a several effective result in animal models must be doing as the next step in our vision. In the summary, we are in the procedure to developing a vaccine of epitope-based through the present study to get a trial promising epitopes vaccine; in this article designates our *in silico* progress through many pathway to reach our goal. We trust that our rabies vaccine of immunoinformatics-driven peptides development approach could have been many good expected over other approaches: (1) rapidity to response, (2) safety and low adverse allergic significant, (3) broadimmunogenicity (delivery of conserve multiple epitopes, derived a specific protein, faster in recognition when predicated on B cell, MHC1, MHC11 tools) and (4) world coverage with good docking to MHC homo spins properties.

So among the different test for peptides to both B cell and T cell this study planned an interesting T-cell epitope (YFLIGVSAV) that has a strong bind affinity to MHC1 and MHC11 alleles together, and it can be binding with 18 distinctive alleles with population coverage of the world (99.36%), indeed this candidate epitope-based vaccines are adept to inducing more potent reactions than vaccines of the whole

protein. We also trusted that the methods defined here, which will lead to development of a multi-epitopes Duvenhage vaccine, could being a step in the right direction over the development of a kind of safer, good effective bio defense with this promising candidate epitope vaccines. We recommend to doing further studies to propose a peptide driven vaccine for the other strains of Rabies *Lyssavirus*, here will be a possible to find public conserved candidates epitopes for multiple strains. In addition, we recommend applying these peptides to evaluate the *in vitro* and *in vivo* methods.

Competing Interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

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