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Prediction and Conservancy Analysis of Multiepitope Based Peptide Vaccine Against Merkel Cell Polyomavirus: An Immunoinformatics Approach

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Abstract

Merkel cell Polyomavirus is non-enveloped, dsDNA virus belonging to Polyomaviridae family linked to an uncommon aggressive skin malignancy. The poor prognosis and limited understanding of disease pathogenesis warrants innovative treatment. In this current study we aim to predict TB cell immunogenic epitopes from the VP1 protein of all merkel cell polyomavirus strain which will aid in effective epitope based vaccine design using immuoinformatics approaches. We retrieved 423 full-length VP1 protein sequences of merkel cell polyomavirus species from the NCBI database. These sequences were analyzed to determine the conserved region and were used to predict the epitopes using the IEDB immunoinformatics algorithms. For B cell three epitope were predicted as peptide vaccine (QEKTVY, KTVYPK, and QEKTVYP). For T cell the predicted Class-I peptides (SLFSNLMPK, LQMWEAISV and LLVKGGVEV) were found to cover the maximum number of MHC I alleles. The highest scoring Class II MHC binding peptides were (IELYLNPRM, ISSLINVHY and INSLFSNLM). Further experiments will need to be undertaken to confirm the potential of these predicted epitopes in a future efficacious vaccine development.

Keyword: Merkel cell polyomavirus (MCPYV); Epitope; Peptide vaccine; Immune epitope database IEDB

Introduction

Merkel cell polyomavirus is a recently discovered small non enveloped circular double-stranded DNA virus etiologically linked to an uncommon but highly lethal form of skin malignancy, Merkel cell carcinoma (MCC). MCPYV belongs to the Orthopolyomavirus genus of the Polyomaviridae family, which include mammalian polyomaviruses such as murine PyV (MPyV), simian virus (SV40) and the human polyomaviruses JC (JCPyV) and BK (BKPyV) [1-11]. The genome is 5.4 kb which constitute two regions; the early region which encodes the large tumor (T) and small T antigens, and the late region which comprise the structural viral proteins VP1, VP2 and VP3, which form the viral capsid. [2-5], However, VP1 makes up more than 70% of the total protein content of virus particles and is also called the major structural protein which is responsible for immunogenic response inside the host body [4,10]. Antibodies against vp1 protein is likely to be expressed in 90% of MCC tumors [11], thus it represent an ideal therapeutic candidate for designing immunoprophylactic vaccine [10,12,13].

Merkel cell carcinoma is an aggressive lethal neuroectodermal malignancy arising from mechanoreceptor Merkel cells [3,6,11,14,15]. MCC was first described by Cyril Toker in 1972, who noted a colored painless solid nodule within five different areas of two older men, who later died as a result of this tumor, and three older women, yet the pathogenesis and etiology of MCC remains poorly understood [3,6]. MCC is rare, but its incidence has tripled over the past two decades in the United States to 1500 cases per year and 2,500 new cases diagnosed in the E.U [11,14,16]. Epidemiological studies revealed that older, lighter-skinned, and immunosuppressed individuals, such as those infected with HIV and/or diagnosed with AIDS are more susceptible to infection [1,14,17-19]. In 2008, a novel merkel cell polyomavirus was discovered and found to be integrated and associated with 80% of MCC tumors [1,6,20,21], thus it has been confirmed to be the etiological agent behind six other viruses now known to be either directly or indirectly causes human cancer [7,11,22].

Developing an advanced vaccine for MCC, that specifically targets the immunogenic proteins, is of vital significance to overcome the devastating disease [23]. In the previous study a DNA vaccine which encoding large and small T virus antigen was developed and has shown that it is possible to induce both CD4+ and CD8+ T lymphocyte response [24]. However an epitope based peptide vaccine could be another possible candidate. The aim of this present study is to predict a promiscuous epitopes that bind to B cell as well as both classes of MHC molecules with a maximum number of HLA molecules in a given set of population in MCPYV protein using an immunoinformatics approach which is a prerequisite in the development of an epitope based vaccine design.

Epitopes based subunit vaccines offer a much stronger and measured immune response as well as avoid the possible fatal consequences of employing entire viral proteins and peptides [23,25,26]. The poor prognosis of MCC patients as well as the limited understanding of disease pathogenesis warrants innovative treatments to control MCC [24]. This is the first study concerning

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Merkel cell polyomavirus vp1 protein vaccine design using immunoinformatics tools.

Materials and Methods

Protein sequence retrieval

A set of available 97 virulent strains of Merkel Cell Polyomavirus (MCPYV) from different geographic regions were retrieved from the NCBI database. (https://www.ncbi.nlm.nih.gov/protein/?term=Merkel+cell+polyomavirus+VP1) .

These sequences were retrieved in October 2016 and selected for immunobioinformtic analysis. These sequences were isolated from different geographical areas (USA, Japan, China, Germany, France and Lithuania) from 1995-2011. The retrieved VP1 97 strains with length of 423 a.a and their accession number and collection area are listed in appendix (1).

Phylogenetic and alignment

The retrieved sequences were subjected to Phylogenetic and alignment study in order to determine the origin of each strain and the conservancy using different tools from **(http://www.phylogeny.fr)** [27]. The phylogenetic tree and alignment were presented in Figures 2 and 3.

Bepipred epitope	Start	End	Length	Emini surface threshold (1.00)	Antigenicity threshold (1.031)
MAPKRKASSTCKT	1	13	13	1.294	0.995
KRQC	15	18	4	1.217	1.057
GCCPN	23	27	5	0.179	1.108
GEDSI	48	52	5	0.679	0.951
VNSPDLPT	65	72	8	0.802	1.04
DLQPKGSSPDQPIKENLP	82	99	18	3.112	1.003
GAGIPVS	153	159	7	0.151	1.06
EPL	171	173	3	0.969	1.055
TTNGGPIT	189	196	8	0.542	0.933
MTPKNQGLDPQAKAKLDKDGNYP	205	227	23	4.957	0.972
PSKNENSRYYGSIQTGSQTP	235	254	20	5.346	0.973
GVGPLC	272	277	6	0.094	1.143
KVSGQPMEG	338	346	9	0.71	0.981
DNQ	348	350	3	2.04	0.886
LPG	363	365	3	0.554	1.063
EGSE	358	361	4	1.331	0.897
GQEKTVYPK *	377	385	9	2.448	1.013
QEKTVYP*	378	384	7	2.208	1.045
QEKTVY*	378	383	6	1.912	1.042
EKTVYP	379	384	6	1.707	1.05
EKTVYPK	379	385	7	2.549	1.033
KTVYPK*	380	385	6	1.971	1.063
SVAPA	387	391	5	0.397	1.117

Table 1: List of B-cell epitopes predicted by different scales fromVP1 protein in Merkel cell Polyomavirus;*Peptide from 377 to 385 gives higher score in Kolaskar and Tongaonkar antigenicity if it is shorten to 7 amino acids (378 to 384) or to 6 amino acids (378 to 383) & (380 to 385).

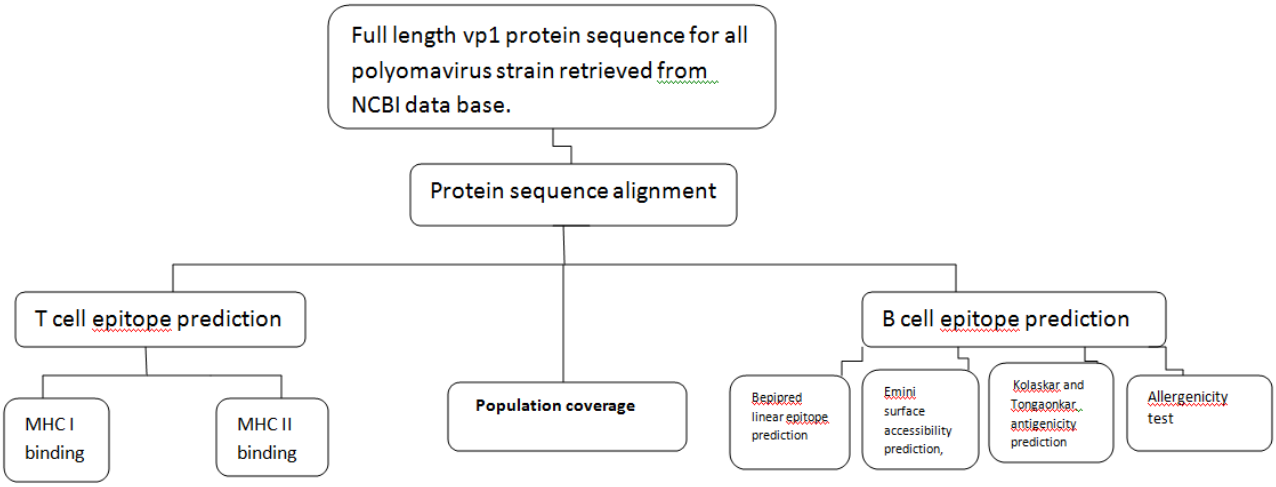

Figure 1: Flowchart representing the immunoinformatics prediction of potential B and T lymphocyte epitopes for the development of peptide vaccine targeting MCPYV.

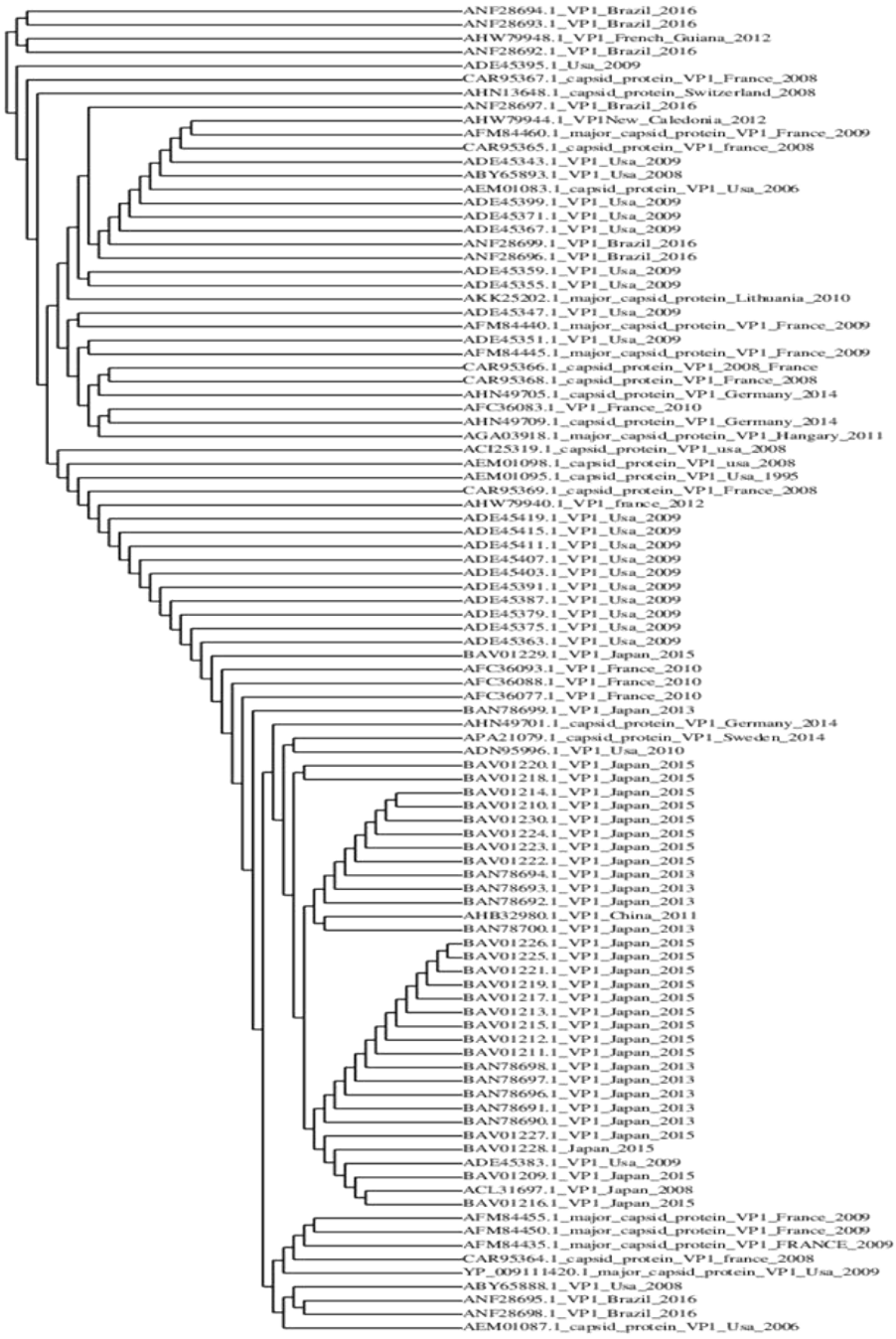

Figure 2: Cladogram shows the relationship between different MCPYV strains. The phylogenetic tree showed that YP-009111420 strain collected from USA (2009) could be related to the recent strains collected from Germany 2014, Brazil 2016.

Conserved regions determination

BioEdit sequence alignment editor (v7.0.9) were used to align the retrieved sequences to obtain conserved regions with the aid of ClustalW (Hall, 1999) by comparing the whole length amino acid of 97 VP1 strains against MCPYV reference sequence under gene bank accession number YP_009111420.1. 100% of identical and similar amino acid sequences were selected as a conserved region [28].

Prediction of B-cell epitopes

As the Immunogenic B cell epitopes interacts with B-lymphocytes, the B-lymphocyte is differentiated into antibody-secreting plasma cell and memory cell. B cell epitope is characterized by being accessible and antigenic [29]. B cell epitopes were predicted using tools from immune epitope data base analysis resource (IEDB-AR) (http://tools.iedb.org/bcell/) by Bepipred linear epitope prediction analysis [30,31]. The reference sequence was subjected to Bepipred linear epitope

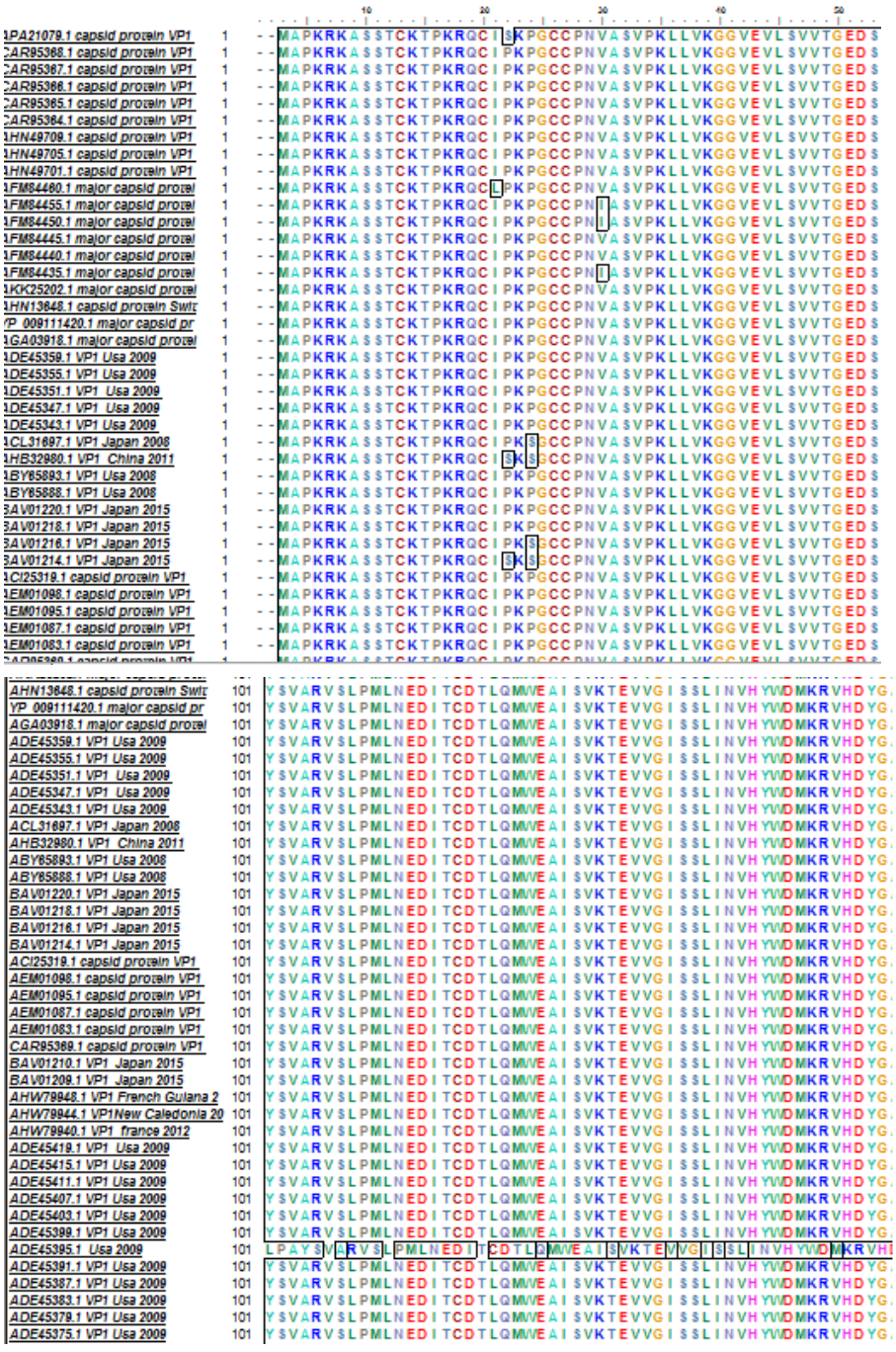

Figure 3: Representative multiple sequence alignment of whole length amino acid of 97 VP1 strains against the reference protein using (Bioedit tool v7.0.9); The highlighted sequence represents the most mutated region; Dot represents the conservancy between sequences.

prediction tool to predict the binding probability of specific regions in the protein to B cell receptor with a default threshold value of (0.393). The predicted epitopes were subjected to Bioedit tool and only 100% conserved epitopes were selected. Then IEDB tools were used to predict surface accessible epitopes by Emini surface accessibility prediction [32] and antigenicity by Kolaskar and Tongaonkar antigenicity method [33] with thresholds of 1.000 and 1.031 respectively.

Binding predictions for MHC class I

For prediction of peptides bind to MHC class I; the reference sequence was submitted in MHC-I Binding prediction tool http://tools.iedb.org/mhci/n in IEDB. In MHC-I peptide complex presentation to T lymphocytes several steps are involved. The cellular attachment of cleaved peptides to MHC molecules step was predicted. Prediction methods include Artificial Neural Network (ANN), Stabilized Matrix Method (SMM), or Scoring Matrices derived from Combinatorial Peptide Libraries (Comblib_Sidney2008), ANN method was used [34-38]. Epitopes lengths were set as 9 mers prior to prediction. The conserved epitopes which bind to alleles at score equal or less than 100 half-maximal inhibitory concentrations (IC50) were selected for further analysis [34].

Binding predictions for MHC class II

Peptide binding Analysis of MHC class II molecules was assessed by the IEDB MHCII prediction tool at http://tools.immuneepitope.org/mhcii/ [39,40]. Certain HLA-DR, HLA-DP, HLA-DQ alleles were analyzed. MHC class II groove has the ability to bind to peptides with different lengths. This binding variability makes the prediction difficult and less accurate [41]. MHC II binding prediction can be achieved using five different IEDB tools; SMM_align, NN- align, Compinatorial Libraries, Sturniolo's method and NetMHCIIpan in addition to the consensus method. NN-align method was used to predict MHC class II epitopes [35]. All conserved epitopes that bind to many alleles at score equal or less than 1000 half-maximal inhibitory concentration (IC50) is selected for further analysis.

Population coverage calculation

All MHC I and MHC II potential binders from Merkel Cell Polyomavirus VP1 capsid protein were assessed for population coverage analysis against the whole world population with the selected MHC I and MHC II interacted alleles using IEDB population coverage calculation tool at http://tools.iedb.org/tools/population/iedb_input [42]. Population coverage calculation is based on total HLA hits score that is obtained from IEDB, these data derived from the relative frequency of an allele at a particular locus in a population.

Assessment of epitope allergenicity

For allergenicity prediction AllerTOP v. 2.0 **(http://www.pharmfac.net/allertop)** was used [43]. So the predicted B cell epitopes and epitopes bind to MHC I & II are subjected to AllerTOP giving result either "probable allergen" or "probable non-allergen".

Homology modeling

Merkel Cell Polyomavirus VP1capsid protein 3D structure was obtained by RaptorX, (http/www.raptor.uch icago.edu) which uses advanced homology detection techniques to build protein 3D structures. UCSF Chimera (version 1.8) was used to visualize the 3D structure, Chimera currently available at the chimera web site (http://www.cgl.ucsf.edu/cimera). Further verification of the surface accessibility and hydrophilicity of predicted B lymphocyte epitopes was achieved, visualization of all predicted T cell epitopes in the structural level were also assessed [44,45].

Result

Prediction of B-cell epitope

VP1 capsid protein was subjected to Bepipred linear epitope prediction that predicts linear epitope, Kolaskar and Tongaonkar antigenicity and Emini surface accessibility prediction methods in IEDB, Figures 4,5,6.

In Bepipred Linear Epitope Prediction method; the average binders score of the protein to B cell was 0.393, with a maximum of 2.546 and a minimum of -1.464, all values equal or greater than the default threshold 0.393 were predicted to be a potential B cell binders.

In Emini surface accessibility prediction; the average surface accessibility areas of the protein was scored as 1.000, with a maximum of 5.749 and a minimum of 0.060, all values equal or greater than the default threshold 1.000 were potentially in the surface. The Kolasar and Tongaonkar antigenicity prediction; the average of the antigenicity was 1.031, with a maximum of 1.235 and minimum of 0.877; all values greater than 1.031 are potential antigenic determinants. The result of all conserved predicted B cell epitopes are shown in Table 1 and Figures 3-6.

MHC class 1 binding prediction

The VP1capsid protein was subjected to IEDB MHC-1 binding prediction tool. 29 peptides were predicted to interact with different MHC class 1 alleles using artificial neural network (ANN) method. The peptide SLFSNLMPK from 330 to 338 had higher affinity to interact with 4 alleles (HLA-A*03:01, HLA-A*11:01, HLA-A*30:01 & HLA-A*68:01). The predicted epitopes with their corresponding MHC1 alleles are listed in the Table 2, Figures 7 and 8.

MHC class II binding prediction

As in MHC I, the protein subjected to MHC- II binding prediction tool using NN-align method.156 predicted epitopes were found to interact with MHC II different alleles. The peptides that have higher affinity are listed below in the Table 3 and their positions in structural level are shown in Figure(9).

Population coverage analysis

Epitopes that are predicted to interact with MHC-I and II alleles were selected for population coverage analysis. The results of population coverage of all epitopes that bind to MHC I & II in the world are listed in

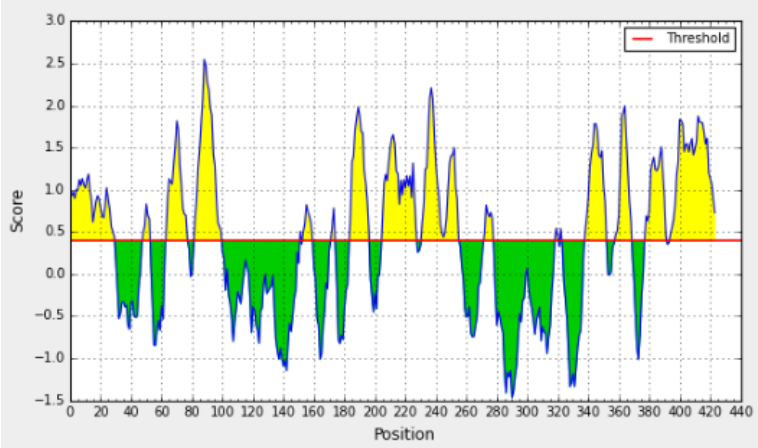

Figure 4: Bepipred Linear Epitope Prediction; Yellow areas above the red line(threshold) are proposed to be a part of B cell epitopes. While green areas are not.

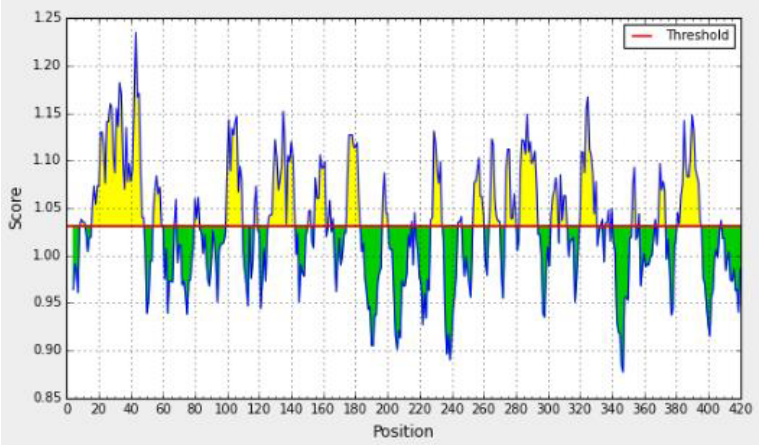

Figure 5: Kolaskar and Tongaonkar antigenicity prediction; Yellow areas above the red line (threshold) are proposed to be a part of B cell epitopes. While green areas are not.

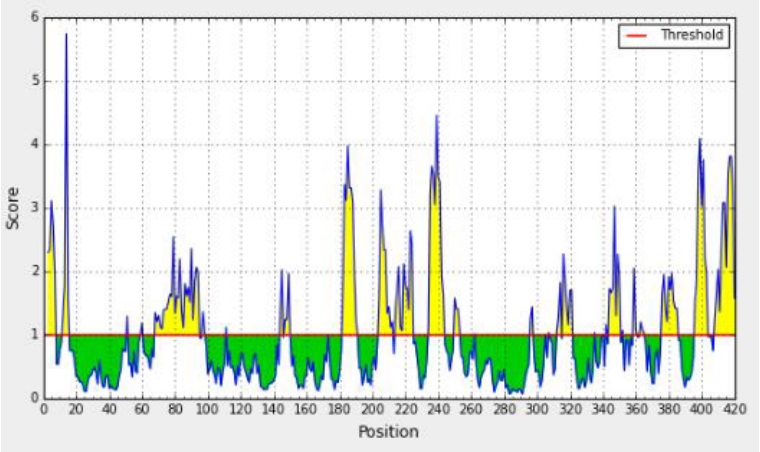

Figure 6: Emini surface accessibility prediction. Yellow areas above the red line (threshold) are proposed to be a part of B cell epitopes. While green areas are not.

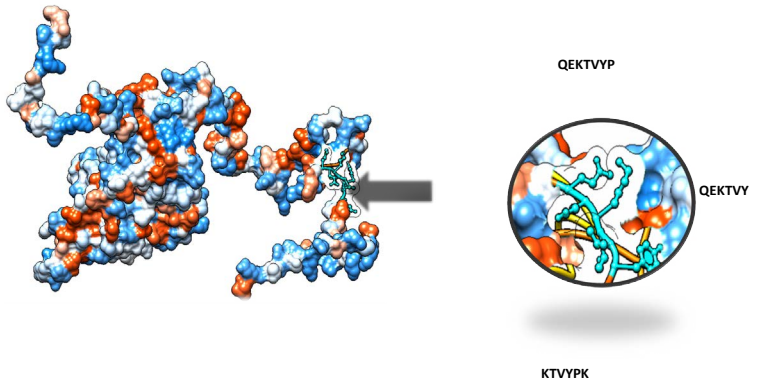

Figure 7: Position and interactive visualization of proposed conserved B cell epitopes in structural level of vp1 major capsid protein of MCPYV; Modeling was done using **(chimera 1.8**) software**.**

(Tables 4 and 5) respectively. The proposed epitopes with their coverage results are shown in Table 6.

Allergenicity test

The proposed B cell epitopes & those bind with different set of MHC I and II alleles were subjected to AllerTOP 2.0 software to avoid production of IgE antibodies as possible. The results are listed in Table 7.

Peptide	Start	End	Length	Allele	ANN_ic50*	percentile rank
APKRKASST	2	10	9	HLA-B*07:02	97.43	0.3
ASVPKLLVK	29	37	9	HLA-A*11:01	21.32	0.3
AYSVARVSL	100	108	9	HLA-C*14:02	54.49	0.2
DSITQIELY	50	58	9	HLA-A*26:01	49.81	0.1
DTLQMWEAI	118	126	9	HLA-A*32:01	48.77	0.3
EAISVKTEV	124	132	9	HLA-A*68:02	5.32	0.2
EVVGISSLI	131	139	9	HLA-A*26:01	31.33	0.1
				HLA-A*68:02	2.95	0.2
FSNTLTTVL	259	267	9	HLA-B*39:01	55.09	0.3
				HLA-C*15:02	77.15	0.1
GVNYHMFAI	160	168	9	HLA-A*02:06	59.59	0.7
				HLA-A*32:01	88.82	0.3
ISSLINVHY	135	143	9	HLA-B*58:01	54.83	0.3
ITCDTLQMW	115	123	9	HLA-B*57:01	73.45	0.3
				HLA-B*58:01	18.43	0.3
KENLPAYSV	95	103	9	HLA-B*40:02	11.87	0.1
				HLA-B*44:02	77.75	0.1
KRKASSTCK	4	12	9	HLA-A*30:01	27.32	0.4
LLVKGGVEV*	34	42	9	HLA-A*02:01	83.35	0.5
				HLA-A*02:06	31.73	0.6
LPRYFNVTL	305	313	9	HLA-B*07:02	5.97	0.1
				HLA-B*35:01	66.6	0.4
LQMWEAISV*	120	128	9	HLA-A*02:01	21.99	0.4
				HLA-A*02:06	4.27	0.1
				HLA-B*39:01	74.99	0.3
MPKVSGQPM	336	344	9	HLA-B*07:02	6.8	0.1
				HLA-B*08:01	59.3	0.2
				HLA-B*35:01	18.27	0.2
NEDITCDTL	112	120	9	HLA-B*40:01	25.59	0.2
NPYPVVNLI	320	328	9	HLA-B*51:01	68.27	0.1
				HLA-B*53:01	78.09	0.3
NVHYWDMKR	140	148	9	HLA-A*31:01	93.09	0.5
				HLA-A*68:01	11.03	0.1
QMWEAISVK	121	129	9	HLA-A*03:01	72.45	0.2
RVHDYGAGI	148	156	9	HLA-A*30:01	28.75	0.4
RYFNVTLRK	307	315	9	HLA-A*11:01	39.91	0.4
				HLA-A*30:01	11.58	0.2
				HLA-A*31:01	52.51	0.4
RYYGSIQTG	242	250	9	HLA-C*14:02	77.25	0.3
SKNENSRYY	236	244	9	HLA-C*06:02	90.48	0.1
SLFSNLMPK*	330	338	9	HLA-A*03:01	8.36	0.1
				HLA-A*11:01	5.05	0.2
				HLA-A*30:01	51.63	0.5
				HLA-A*68:01	43.73	0.6
SSLINVHYW	136	144	9	HLA-B*57:01	9.42	0.1
				HLA-B*58:01	4.82	0.1
SVARVSLPM	102	110	9	HLA-A*68:02	52.81	0.7
				HLA-B*07:02	75.63	0.2
				HLA-B*15:01	53.52	0.2
				HLA-B*35:01	73.5	0.4
TEVVGISSL	130	138	9	HLA-B*40:01	10.2	0.1
				HLA-B*40:02	46.89	0.2

Table 2: list of epitopes that had binding affinity to MHC Class I alleles;*Proposed epitopes. ANN_ic50*the half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness for successful binding of peptide to MHC molecule by the Artificial Neural Network method.

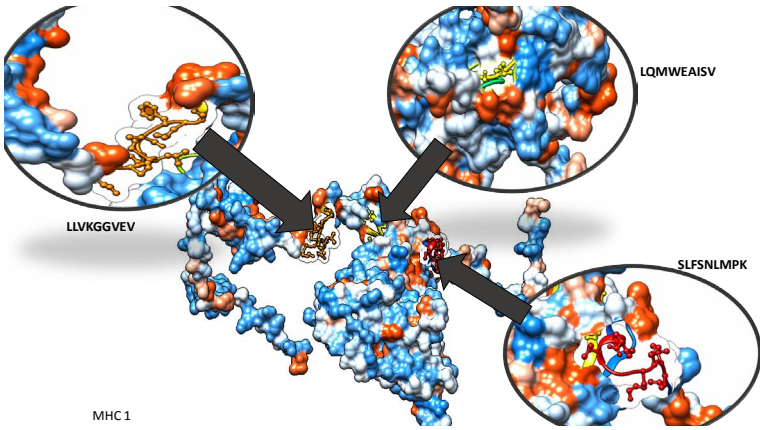

Figure 8: Position and interactive visualization of proposed conserved T cell epitopes that interact with MHC I in structural level of vp1 of MCPYV. Modeling was done using **(chimera 1.8**) software**.**

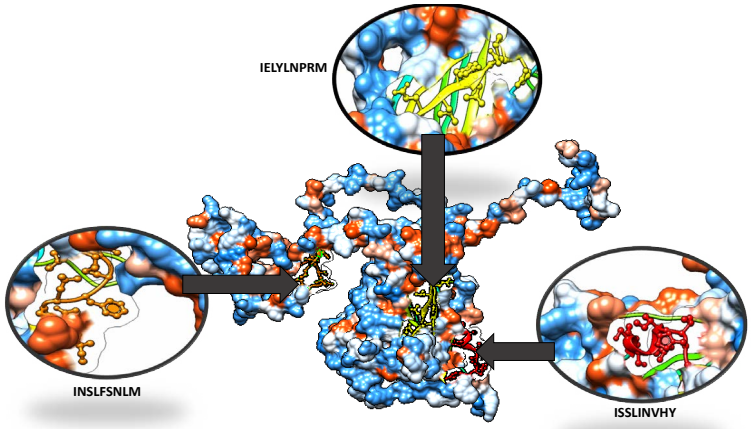

Figure 9: Position and visualization of proposed conserved T cell epitopes that interact with MHC 1I in structural level of vp1 protein of MCPYV. (Modeling was done using **(chimera 1.8**) software**.**

Epitope	Start	End	Allele	Peptide	IC50	Percentile Rank
IELYLNPRM	55	63	HLA-DPA1*02:01/DPB1*01:01	TQIELYLNPRMGVNS	512.2	31.11
				IELYLNPRMGVNSPD	722	36.84
			HLA-DQA1*01:01/DQB1*05:01	ITQIELYLNPRMGVN	188.6	4.11
				SITQIELYLNPRMGV	202	4.37
				TQIELYLNPRMGVNS	220.7	4.72
				QIELYLNPRMGVNSP	254.1	5.32
				DSITQIELYLNPRMG	284.2	5.83
				EDSITQIELYLNPRM	352.1	6.93
				IELYLNPRMGVNSPD	501.6	9.06
			HLA-DRB1*01:01	DSITQIELYLNPRMG	154	36.59
				EDSITQIELYLNPRM	276.9	46.71
			HLA-DRB1*04:01	SITQIELYLNPRMGV	67.6	5.42
				DSITQIELYLNPRMG	73.9	5.97
				EDSITQIELYLNPRM	75.6	6.11
				ITQIELYLNPRMGVN	76.8	6.22
				TQIELYLNPRMGVNS	160.6	12.51
				QIELYLNPRMGVNSP	230.4	16.76
				IELYLNPRMGVNSPD	361.6	23.28
			HLA-DRB1*04:05	SITQIELYLNPRMGV	52.7	5.15
				EDSITQIELYLNPRM	56.4	5.55
				ITQIELYLNPRMGVN	56.9	5.6

				DSITQIELYLNPRMG	59	5.82
				TQIELYLNPRMGVNS	94.9	9.19
				QIELYLNPRMGVNSP	258.6	19.74
				IELYLNPRMGVNSPD	344.3	23.65
			HLA-DRB1*04:04	QIELYLNPRMGVNSP	507.2	34.95
			HLA-DRB1*07:01	SITQIELYLNPRMGV	845.5	41.52
			HLA-DRB1*09:01	ITQIELYLNPRMGVN	178.3	11.77
				TQIELYLNPRMGVNS	187.3	12.25
				SITQIELYLNPRMGV	232.8	14.6
				DSITQIELYLNPRMG	558	27.41
				EDSITQIELYLNPRM	674.9	30.92
			HLA-DRB1*13:02	DSITQIELYLNPRMG	290.8	12.48
				EDSITQIELYLNPRM	296.5	12.63
			HLA-DRB1*15:01	SITQIELYLNPRMGV	12.5	0.66
				ITQIELYLNPRMGVN	12.6	0.67
				TQIELYLNPRMGVNS	14.4	0.85
				EDSITQIELYLNPRM	15.4	0.97
				DSITQIELYLNPRMG	15.6	1
				QIELYLNPRMGVNSP	18.8	1.37
				IELYLNPRMGVNSPD	74.9	7.66
			HLA-DRB4*01:01	ITQIELYLNPRMGVN	304.7	19.69
				SITQIELYLNPRMGV	328.5	20.75
				DSITQIELYLNPRMG	347.6	21.58
				TQIELYLNPRMGVNS	373	22.64
				QIELYLNPRMGVNSP	757.7	35.01
				IELYLNPRMGVNSPD	847.4	37.22
			HLA-DRB5*01:01	ITQIELYLNPRMGVN	444.5	31.48
				SITQIELYLNPRMGV	548.9	34.33
				DSITQIELYLNPRMG	803	39.84
ISSLINVHY	135	143	HLA-DRB1*01:01	VVGISSLINVHYWDM	36.6	17.63
				TEVVGISSLINVHYW	50.5	21.37
				EVVGISSLINVHYWD	61.2	23.74
				VGISSLINVHYWDMK	86.3	28.17
				GISSLINVHYWDMKR	165.3	37.72
			HLA-DRB1*04:01	VVGISSLINVHYWDM	91.8	7.45
				EVVGISSLINVHYWD	102	8.27
				TEVVGISSLINVHYW	104.1	8.44
				KTEVVGISSLINVHY	108.9	8.81
				VGISSLINVHYWDMK	144.7	11.42
				GISSLINVHYWDMKR	223.4	16.36
				ISSLINVHYWDMKRV	448.1	26.85
			HLA-DRB1*04:05	TEVVGISSLINVHYW	161	14.13
				EVVGISSLINVHYWD	178.7	15.28
				VVGISSLINVHYWDM	179.3	15.32
				KTEVVGISSLINVHY	190.4	16.01
				VGISSLINVHYWDMK	226.5	18.05
				GISSLINVHYWDMKR	354.6	24.09
				ISSLINVHYWDMKRV	717.1	35.32
			HLA-DRB1*04:04	GISSLINVHYWDMKR	109.6	12.87
				ISSLINVHYWDMKRV	298.3	26.2
			HLA-DRB1*07:01	KTEVVGISSLINVHY	55.7	9.44
				TEVVGISSLINVHYW	75.8	11.77
				EVVGISSLINVHYWD	124.7	16.31
			HLA-DRB1*08:02	VVGISSLINVHYWDM	549.4	13.36
			HLA-DRB1*09:01	VVGISSLINVHYWDM	647	30.09
				EVVGISSLINVHYWD	682.2	31.13
				KTEVVGISSLINVHY	699.1	31.57

				TEVVGISSLINVHYW	753.9	33.05
				VGISSLINVHYWDMK	774.1	33.53
			HLA-DRB1*11:01	VVGISSLINVHYWDM	77.7	11.7
				VGISSLINVHYWDMK	123	15.55
				EVVGISSLINVHYWD	139.6	16.72
				TEVVGISSLINVHYW	206.7	20.64
				GISSLINVHYWDMKR	208	20.71
				ISSLINVHYWDMKRV	282	24.04
				KTEVVGISSLINVHY	328.4	25.82
			HLA-DRB1*15:01	GISSLINVHYWDMKR	154.7	14.1
				KTEVVGISSLINVHY	178	15.63
				TEVVGISSLINVHYW	182.7	15.93
				EVVGISSLINVHYWD	188.3	16.28
				VGISSLINVHYWDMK	214.9	17.78
				VVGISSLINVHYWDM	222	18.16
			HLA-DRB4*01:01	TEVVGISSLINVHYW	198.6	14.23
				KTEVVGISSLINVHY	203.3	14.49
				EVVGISSLINVHYWD	352.5	21.78
			HLA-DRB5*01:01	VVGISSLINVHYWDM	342.9	28.19
				GISSLINVHYWDMKR	370	29.12
				VGISSLINVHYWDMK	389.5	29.77
				EVVGISSLINVHYWD	477.6	32.44
				TEVVGISSLINVHYW	496.9	32.98
INSLFSNLM	328	336	HLA-DPA1*02:01/DPB1*05:01	LINSLFSNLMPKVSG	986.1	17.6
			HLA-DQA1*05:01/DQB1*02:01	PVVNLINSLFSNLMP	605.8	13.35
				VNLINSLFSNLMPKV	684.9	14.9
				VVNLINSLFSNLMPK	687	14.94
				YPVVNLINSLFSNLM	803.7	17.1
			HLA-DQA1*05:01/DQB1*02:01	NLINSLFSNLMPKVS	985.7	20.22
			HLA-DRB1*01:01	VNLINSLFSNLMPKV	10.1	5.27
				NLINSLFSNLMPKVS	10.8	5.8
				YPVVNLINSLFSNLM	11.1	6.03
				VVNLINSLFSNLMPK	12.2	6.81
				LINSLFSNLMPKVSG	12.5	7.01
				PVVNLINSLFSNLMP	15.1	8.66
			HLA-DRB1*04:01	VNLINSLFSNLMPKV	12.6	0.37
				VVNLINSLFSNLMPK	15.1	0.56
				NLINSLFSNLMPKVS	15.7	0.6
				PVVNLINSLFSNLMP	20.8	1.04
				LINSLFSNLMPKVSG	21.2	1.08
				YPVVNLINSLFSNLM	26.8	1.6
				INSLFSNLMPKVSGQ	37.8	2.64
			HLA-DRB1*04:05	YPVVNLINSLFSNLM	13	0.53
				PVVNLINSLFSNLMP	17.1	0.95
				VVNLINSLFSNLMPK	19.1	1.18
				VNLINSLFSNLMPKV	19.7	1.25
				NLINSLFSNLMPKVS	27.3	2.16
				LINSLFSNLMPKVSG	45.3	4.33
				INSLFSNLMPKVSGQ	72	7.09
			HLA-DRB1*07:01	YPVVNLINSLFSNLM	11.4	1.98
				PVVNLINSLFSNLMP	16	3.01
				VVNLINSLFSNLMPK	23.4	4.52
				VNLINSLFSNLMPKV	26.9	5.16
				NLINSLFSNLMPKVS	41.7	7.47
				LINSLFSNLMPKVSG	62.6	10.25
				INSLFSNLMPKVSGQ	102.1	14.38
			HLA-DRB1*09:01	NLINSLFSNLMPKVS	68.6	4.65

				LINSLFSNLMPKVSG	78.9	5.43
				VNLINSLFSNLMPKV	79.8	5.5
				VVNLINSLFSNLMPK	104.3	7.19
				PVVNLINSLFSNLMP	148	9.98
				YPVVNLINSLFSNLM	159.9	10.66
			HLA-DRB1*11:01	VNLINSLFSNLMPKV	139.4	16.7
				VVNLINSLFSNLMPK	256.6	22.99
			HLA-DRB1*15:01	PVVNLINSLFSNLMP	164.5	14.76
				VVNLINSLFSNLMPK	177.2	15.59
			HLA-DRB4*01:01	YPVVNLINSLFSNLM	72.5	5.63
				PVVNLINSLFSNLMP	73.2	5.69
				VVNLINSLFSNLMPK	83.5	6.51
				VNLINSLFSNLMPKV	92.3	7.19
				NLINSLFSNLMPKVS	149.5	11.22
				LINSLFSNLMPKVSG	179.5	13.11
				INSLFSNLMPKVSGQ	203.6	14.51
			HLA-DRB5*01:01	VNLINSLFSNLMPKV	12.4	3.03
				VVNLINSLFSNLMPK	16.5	4.08
				PVVNLINSLFSNLMP	21.8	5.28
				NLINSLFSNLMPKVS	21.8	5.28
				LINSLFSNLMPKVSG	36	7.92
				INSLFSNLMPKVSGQ	63.6	11.69

Table 3: List of the proposed epitopes that had binding affinity to MHC Class II alleles.

Epitope	Coverage class I	Total HLA hits
APKRKASST	12.78%	1
ASVPKLLVK	15.53%	1
AYSVARVSL	3.04%	1
DSITQIELY	5.82%	1
DTLQMWEAI	4.61%	1
EAISVKTEV	2.50%	1
EVVGISSLI	8.25%	2
FSNTLTTVL	7.04%	2
GVNYHMFAI	6.51%	2
ISSLINVHY	3.42%	1
ITCDTLQMW	7.26%	2
KENLPAYSV	10.93%	2
KRKASSTCK	3.89%	1
LLVKGGVEV	40.60%	2
LPRYFNVTL	20.62%	2
LQMWEAISV	42.23%	3
MPKVSGQPM	29.99%	3
NEDITCDTL	7.81%	1
NPYPVVNLI	9.87%	2
NVHYWDMKR	11.03%	2
QMWEAISVK	16.81%	1
RVHDYGAGI	3.89%	1
RYFNVTLRK	23.91%	3
RYYGSIQTG	3.04%	1
SKNENSRYY	15.52%	1
SLFSNLMPK	38.86%	4
SSLINVHYW	7.26%	2
SVARVSLPM	29.93%	4
TEVVGISSL	11.13%	2
Epitope set	**94.16%**	

Table 4: Population coverage of all epitopes in MHC class I.

Epitope	Coverage class II	Total HLA hits	Epitope	Coverage class II	Total HLA hits
KRKASSTCK	0.00%	1	GAGIPVSGV	0.00%	2
VPKLLVKGG	10.54%	2	AGIPVSGVN	0.00%	1
PKLLVKGGV	0.00%	1	IPVSGVNYH	18.41%	2
KLLVKGGVE	18.23%	1	PVSGVNYHM	17.82%	2
LLVKGGVEV	28.79%	3	VSGVNYHMF	18.23%	3
LVKGGVEVL	34.26%	7	SGVNYHMFA	27.90%	3
VKGGVEVLS	6.40%	3	VNYHMFAIG	0.00%	1
KGGVEVLSV	0.00%	1	NYHMFAIGG	4.77%	2
GGVEVLSVV	0.00%	1	YHMFAIGGE	24.10%	4
GVEVLSVVT	18.23%	1	HMFAIGGEP	9.32%	5
VEVLSVVTG	18.15%	4	MFAIGGEPL	0.00%	1
EVLSVVTGE	0.00%	2	FAIGGEPLD	56.92%	10
VLSVVTGED	3.02%	1	AIGGEPLDL	0.00%	1
LSVVTGEDS	18.23%	3	IGGEPLDLQ	0.00%	3
VVTGEDSIT	0.00%	1	GEPLDLQGL	0.00%	2
VTGEDSITQ	27.97%	2	PLDLQGLVL	0.00%	2
GEDSITQIE	0.00%	1	QGLVLDYQT	11.53%	1
EDSITQIEL	0.00%	2	TTNGGPITI	0.00%	1
DSITQIELY	0.00%	4	TNGGPITIE	0.00%	2
SITQIELYL	28.79%	5	LGRKMTPKN	4.77%	1
ITQIELYLN	20.57%	4	GRKMTPKNQ	21.43%	2
TQIELYLNP	4.77%	1	NQGLDPQAK	11.53%	1
IELYLNPRM*	65.84%	12	LDPQAKAKL	18.41%	1
WYTYTYDLQ	22.06%	6	NSRYYGSIQ	18.41%	1
YTYTYDLQP	18.47%	5	SRYYGSIQT	4.77%	1
YTYDLQPKG	26.80%	5	RYYGSIQTG	0.00%	1
TYDLQPKGS	11.53%	1	YYGSIQTGS	29.38%	6
YDLQPKGSS	10.54%	1	YGSIQTGSQ	22.06%	3
PKGSSPDQP	0.00%	1	VLQFSNTLT	59.12%	11
KGSSPDQPI	18.23%	1	LQFSNTLTT	42.33%	10
IKENLPAYS	57.34%	10	QFSNTLTTV	0.00%	1
KENLPAYSV	11.53%	1	FSNTLTTVL	50.51%	11
NLPAYSVAR	18.23%	2	SNTLTTVLL	11.53%	1
LPAYSVARV	30.29%	6	NTLTTVLLD	0.00%	2
PAYSVARVS	0.00%	1	TLTTVLLDE	0.00%	5
AYSVARVSL	27.73%	5	LTTVLLDEN	7.71%	4
YSVARVSLP	28.85%	5	TVLLDENGV	11.53%	1
SVARVSLPM	34.78%	2	VLLDENGVG	27.97%	3
VARVSLPML	19.66%	8	LDENGVGPL	6.69%	1
ARVSLPMLN	10.54%	4	ENGVGPLCK	0.00%	2
RVSLPMLNE	3.02%	1	LCKGDGLFI	61.09%	6
VSLPMLNED	4.77%	2	CKGDGLFIS	0.00%	1
SLPMLNEDI	14.37%	4	GDGLFISCA	0.00%	2
LPMLNEDIT	4.77%	2	IVGFLFKTS	17.84%	6
LNEDITCDT	17.84%	2	VGFLFKTSG	36.31%	6
EDITCDTLQ	0.00%	2	GFLFKTSGK	11.21%	2
DITCDTLQM	0.00%	3	ALHGLPRYF	15.05%	4
ITCDTLQMW	27.97%	8	LHGLPRYFN	24.10%	3
TCDTLQMWE	0.00%	1	HGLPRYFNV	11.53%	1
CDTLQMWEA	11.53%	2	LPRYFNVTL	43.71%	8
DTLQMWEAI	0.00%	2	PRYFNVTLR	4.77%	2
TLQMWEAIS	7.04%	3	RYFNVTLRK	0.00%	2
LQMWEAISV	52.19%	8	WVKNPYPVV	49.39%	7
MWEAISVKT	43.78%	4	KNPYPVVNL	20.95%	2
WEAISVKTE	16.52%	7	PYPVVNLIN	3.02%	1
AISVKTEVV	33.10%	5	YPVVNLINS	7.04%	3

SVKTEVVGI	34.26%	3	PVVNLINSL	11.30%	2
VKTEVVGIS	2.33%	2	VVNLINSLF	41.67%	8
TEVVGISSL	0.00%	3	VNLINSLFS	38.62%	7
EVVGISSLI	44.03%	4	NLINSLFSN	0.00%	4
VVGISSLIN	45.82%	10	LINSLFSNL	35.36%	9
VGISSLINV	11.30%	4	INSLFSNLM*	65.37%	11
GISSLINVH	0.00%	1	NSLFSNLMP	4.77%	2
ISSLINVHY*	69.46%	11	SLFSNLMPK	0.00%	1
SSLINVHYW	0.00%	1	LFSNLMPKV	41.13%	10
SLINVHYWD	0.00%	2	FSNLMPKVS	25.65%	4
LINVHYWDM	34.26%	8	NLMPKVSGQ	0.00%	1
INVHYWDMK	4.77%	4	LMPKVSGQP	0.00%	1
NVHYWDMKR	18.41%	2	MPKVSGQPM	13.72%	3
VHYWDMKRV	28.79%	5	KVSGQPMEG	0.00%	1
HYWDMKRVH	10.54%	1	EEVRIYEGS	0.00%	1
YWDMKRVHD	4.77%	2	PDIVRFLDK	0.00%	1
WDMKRVHDY	11.53%	1	IVRFLDKFG	20.57%	6
RVHDYGAGI	29.99%	4	VRFLDKFGQ	10.54%	3
VHDYGAGIP	0.00%	1	RFLDKFGQE	0.00%	2
HDYGAGIPV	44.03%	4	FLDKFGQEK	11.53%	2
DYGAGIPVS	0.00%	4	LDKFGQEKT	18.41%	1
YGAGIPVSG	27.70%	4	FGQEKTVYP	26.27%	3
Epitope set	**81.94%**				

Table 5: Population coverage of all epitopes in MHC class II.

Epitope	Coverage Class I	Total HLA hits	Epitope	Coverage Class II	Total HLA hits
LLVKGGVEV	40.60%	**2**	IELYLNPRM	65.84%	**12**
LQMWEAISV	42.23%	**3**	ISSLINVHY	69.46%	**11**
SLFSNLMPK	38.86%	**4**	INSLFSNLM	65.37%	**11**
Epitope set	**70.30%**		**Epitope set**	**73.11%**	

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

B cell epitopes	Result	MHC class I epitopes	Result	MHC class II epitopes	Result
QEKTVYP	probable allergen	**LLVKGGVEV**	Probable allergen	**IELYLNPRM**	Probable Non allergen
KTVYPK	probable allergen	**LQMWEAISV**	Probable allergen	**ISSLINVHY**	Probable allergen
QEKTVY	probable non-allergen	**SLFSNLMPK**	Probable Non-allergen	**INSLFSNLM**	Probable allergen

Table 7: Result of Allergenicity Test of predicted B cell and MHC class I & II epitopes.

Discussion

In the current study we have successfully predicted a promiscuous epitopes for designing subunit based vaccine. The immune system appears to be playing a critical role in MCC biology with increasing evidence of virus-specific cellular and humoral immune responses that influence the prognosis of MCC patients. Newer strategies are currently being used to treat cancer, among these peptide vaccines which serve as a promising anticancer candidates as they target tumor cell and induce specific T cell response to tumor cell [1,13,46-48].

To best of our knowledge there is no effective approved vaccine against this virus, however previously a DNA vaccine encoding large or small T antigen as well as VP1 virus like particles have been developed. These type of vaccine have been shown to possess a protective specific CD4+\CD8+ T cell response in vaccinated mice, despite that subunit vaccine production which target a specific immunogenic protein would be helpful in generating adequate immune response inside the host body. Furthermore, a murine model expressing tumor cell line from B 16 mouse melanoma was created by Gomez et al. would be useful in clinical setting to address the efficacy of our predicted vaccine [17,24,49-51].

Peptide Vaccination produces profound and long lasting modifications in the adaptive immune system comprising T and B cells. Peptide vaccines are intrinsically safer than alternative vaccine formulations. Moreover, they will allow focusing solely on relevant epitopes, avoiding those that lead to non-protective responses [48]. Currently, there is an increasing interest in developing vaccines based on synthetic peptides. Peptide vaccines under various phases of trial and development, the vast majority of them related to cancers. [52-64].

In the present study we choose our predicted epitopes to be effective peptide antigens for both B and T cells. We selected 100% conserved sequence identity to VP1 major capsid protein. Several studies revealed its ability to induce potential immune response in MCC positive tumors [11,13].

In our case we choose our predicted B cell epitopes to be potential and strong immunogenic peptide antigens for B cell, the length of the predicted epitopes ranged from 3 to 23 amino acids. According to Linear B cell epitope prediction tool available from IEDB these epitopes were found to be above the threshold scores in Bepipred linear epitope prediction, Emini surface accessibility, Kolaskar and Tongaonkar antigenicity, were analyzed based on methods of the IEDB. Epitopes

illustrated in Table 1, are the only conserved regions among all retrieved strains of MCPYV vp1 protein that have been reported in NCBI database until 20th October 2016 and have high probability of activating humoral immune response. However, epitope **QEKTVYP*** from 378 to 384 was found to have the highest score, followed by **KTVYPK** from 380 to 385 and **QEKTVY** from 378 to 383 as summarized in Table 1. These findings indicated that these epitopes are surface accessible and antigenic.

Studies have shown T-cells to be important mediators of MCPyV-specific immune Surveillance thus, T cell epitope prediction was performed based on the probability of MHC-peptide ligand formation and presentation to different T cell populations [65,66]. In the development of universal vaccine, capable of inducing adequate immune response against all circulating strains, alleles binding affinity and accurate characterization of population coverage are highly recommended. A total of 29 conserved peptides in MHC class 1 were selected to bind to multiple HLA alleles as shown in Table 2, among these **LLVKGGVEV, LQMWEAISV and SLFSNLMPK** have high binding affinity as well as high percentage coverage (HLA-A*02:01, HLA-A*02:06), (HLA-A*02:01, HLA-A*02:06 and HLA-B*39:01), (HLA-A*03:01, HLA-A*11:01, HLA-A*30:01 and HLA-A*68:01) respectively. While the highest scoring MHC class II were **(IELYLNPRM), (ISSLINVHY) and (INSLFSNLM)** as shown in Table 3. Moreover, epitope **LLVKGGVEV** and **LQMWEAISV** has successfully predicted to interact with **HLA-A*02:01** the most prevalent major histocompatibility complex (MHC) class I allele family in humans, presenting at high frequencies in all ethnic populations. Interestingly, MHC I epitope 330 **SLFSNLM** 336 has succeded to elicit MHC II response as seen in Table 3. This epitope was found to successfully bind to several **HLA-D, P**, and **Q** alleles indicating that further attention need to be targeted to this region. Furthermore 225**NYPIEVWCPDPSK**237 and 245**GSIQTGSQTPTVL**257 were suggested before by Iyer et al. [67]. The later 245 **(GSIQTGSQ)** 252 has successfully interacted with HLA-DRB1*01:01 which provides instructions for making a protein that plays a critical role in the immune system.

Allergic reactions are triggered when allergens cross-link preformed **IgE** bound to the high-affinity receptor **FcεRI** on mast cells. So mast cells act as alert the immune system to local infection [68]. Responses to allergens in humans are very heterogeneous and involve recognition of a large number of epitopes [69]. Thus; we subjected predicted B and T cells to allergenicity test, among the 9 predicted epitopes it was concluded that two of them (**SLFSNLMPK** and **IELYLNPRM**) have the potential to be real epitopes, in MHC1 and MHCII respectively as their probable non allergic effect. On the other hand epitopes that are predicted to activate B cell **KTVYPK** and **QEKTVYP** were found to have low potential to be a real epitope, as their probable allergic effect which needs further experimental investigation.

Conclusion

The increasing incidence of human viral infections warrants the design of innovative treatment. With the recent advances in the field of bioinformatics, newer strategies are being devised to control and fight infectious diseases [70].

Merkel cell carcinoma is an aggressive devastating disease that warrants the need of developing effective protective vaccine. Several epitopes were proposed in this study especially **SLFSNLMPK** that successfully bind with high affinity to both MHC classes. In addition to **(GSIQTGSQ)** that is suggested before by lyer et al. as adoptive immunotherapy [67]. Further *in vitro* and *in vivo* studies will need to be undertaken in order to confirm the effectiveness of these predicted epitopes as peptide vaccine.

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References

1. Moens U, Rasheed K, Abdulsalam I, Sveinbjørnsson B (2015) The role of Merkel cell polyomavirus and other human polyomaviruses in emerging hallmarks of cancer. Viruses 7:1871-901.
2. Li TC, Iwasaki K, Katano H, Kataoka M, Nagata N, et al. (2015) Characterization of self-assembled virus-like particles of Merkel cell polyomavirus. PloS one 10: e0115646.
3. Stakaitytė G, Wood JJ, Knight LM, Abdul-Sada H, Adzahar NS, et al. (2014) Merkel cell polyomavirus: Molecular insights into the most recently discovered human tumour virus. Cancers 6: 1267-1297.
4. Sadeghi M (2014) Molecular Biology and Clinical Occurrence of Emerging Human Polyomaviruses.
5. Krumbholz A, Bininda-Emonds OR, Wutzler P, Zell R (2009) Phylogenetics, evolution, and medical importance of polyomaviruses. Infect Genet Evol 9: 784-799.
6. Spurgeon ME, Lambert PF (2013) Merkel cell polyomavirus: a newly discovered human virus with oncogenic potential. Virology 435: 118-130.
7. Moschos S, Chang Y, Moore PS (2011) Merkel Cell Polyomavirus: The Seventh Human Cancer Virus. Annu Rev Pathol 7: 123-144.
8. Martel-Jantin C, Filippone C, Tortevoye P, Afonso PV, Betsem E, et al. (2014) Molecular epidemiology of merkel cell polyomavirus: evidence for geographically related variant genotypes. J Clin Microbiol 52: 1687-1690.
9. Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL, Buck CB (2010) Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. Cell Host Microbe 7: 509-515.
10. Pastrana DV, Tolstov YL, Becker JC, Moore PS, Chang Y, et al. (2009) Quantitation of human seroresponsiveness to Merkel cell polyomavirus. PLoS pathogens 5: e1000578.
11. Moshiri AS, Nghiem P (2014) Milestones in the staging, classification, and biology of Merkel cell carcinoma. J Natl Compr Canc Netw 12:1255-1262.
12. Touzé A, Gaitan J, Arnold F, Cazal R, Fleury MJ, et al. (2010) Generation of Merkel cell polyomavirus (MCV)-like particles and their application to detection of MCV antibodies. J Clin Microbiol 48: 1767-1770.
13. Bhatia S, Afanasiev O, Nghiem P (2011) Immunobiology of Merkel cell carcinoma: implications for immunotherapy of a polyomavirus-associated cancer. Curr Oncol Rep 13: 488-497.
14. Wang TS, Byrne PJ, Jacobs LK, Taube JM (2011) Merkel cell carcinoma: update and review. Semin Cutan Med Surg 30: 48-56.
15. Wendzicki JA, Moore PS, Chang Y (2015) Large T and small T antigens of Merkel cell polyomavirus. Curr Opin Virol 11: 38-43.
16. Beatty S, O'Neill L (2016) European Medicines Agency Validates the Marketing Authorization Application for Avelumab for the Treatment of Metastatic Merkel Cell Carcinoma.
17. Gomez B, He L, Tsai YC, Wu TC, Viscidi RP, Hung C-F (2013) Creation of a Merkel cell polyomavirus small T antigen-expressing murine tumor model and a DNA vaccine targeting small T antigen. Cell Biosci 3: 29.
18. Engels EA, Frisch M, Goedert JJ, Biggar RJ, Miller RW (2002) Merkel cell carcinoma and HIV infection. The Lancet 359: 497-498.
19. Minutilli E, Mulè A (2016) Merkel cell polyomavirus and cutaneous Merkel cell carcinoma. Future Science 2: 1-5 .
20. Feng H, Shuda M, Chang Y, Moore PS (2008) Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 319: 96-100.
21. Starrett GJ, Marcelus C, Cantalupo PG, Katz JP, Cheng J, et al. (2017) Merkel Cell Polyomavirus Exhibits Dominant Control of the Tumor Genome and Transcriptome in Virus-Associated Merkel Cell Carcinoma. mBio 8: e02079-16.
22. Coursaget P, Samimi M, Nicol JT, Gardair C, Touzé A (2013) Human Merkel

cell polyomavirus: virological background and clinical implications. APMIS 121: 755-769.

23. Ayub G, Waheed Y, Najmi MH (2016) Prediction and conservancy analysis of promiscuous T-cell binding epitopes of Ebola virus L protein: An in silico approach. Asian Pacific J Trop Dis 6: 169-173.
24. Zeng Q, Gomez BP, Viscidi RP, Peng S, He L, et al. (2012) Development of a DNA vaccine targeting Merkel cell polyomavirus. Vaccine 30: 1322-1329.
25. Allen T, Giridhar M (2014) A Review on Role of Peptide Vaccines in Cancer Therapy Treatment of Cancer Disease by Peptide Vaccine.
26. Terry FE, Moise L, Martin RF, Torres M, Pilotte N, et al. (2015) Time for T? Immunoinformatics addresses vaccine design for neglected tropical and emerging infectious diseases. Expert Rev Vaccines 14: 21-35.
27. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, et al. (2008) Phylogeny. fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36: W465-W9.
28. Hall TA (1999) editor BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series London: Information Retrieval Ltd., c1979-c2000.
29. Hasan MA, Hossain M, Alam J (2013) A computational assay to design an epitope-based Peptide vaccine against Saint Louis encephalitis virus. Bioinform Biol Insights 7: 347.
30. Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, et al. (2015) The immune epitope database (IEDB) 3.0. Nucleic Acids Res 43: D405-D12.
31. Larsen JEP, Lund O, Nielsen M (2006) Improved method for predicting linear B-cell epitopes. Immunome Res 2: 2.
32. Emini EA, Hughes JV, Perlow D, Boger J (1985) Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol 55: 836-839.
33. Kolaskar A, Tongaonkar PC (1990) A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett 276: 172-174.
34. Kim Y, Ponomarenko J, Zhu Z, Tamang D, Wang P, et al. (2012) Immune epitope database analysis resource. Nucleic Acids Res: gks438.
35. Nielsen M, Lundegaard C, Worning P, Lauemøller SL, Lamberth K, et al. (2003) Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. Protein Sci 12: 1007-1017.
36. Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, et al. (2008) NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8–11. Nucleic Acids Res 36: W509-W12.
37. Peters B, Sette A (2005) Generating quantitative models describing the sequence specificity of biological processes with the stabilized matrix method. BMC Bioinformatics 6: 132.
38. Sidney J, Assarsson E, Moore C, Ngo S, Pinilla C, et al. (2008) Quantitative peptide binding motifs for 19 human and mouse MHC class I molecules derived using positional scanning combinatorial peptide libraries. Immunome Res 4: 2.
39. Wang P, Sidney J, Dow C, Mothe B, Sette A, et al. (2008) A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. PLoS Comput Biol 4: e1000048.
40. Wang P, Sidney J, Kim Y, Sette A, Lund O, et al. (2010) Peptide binding predictions for HLA DR, DP and DQ molecules. BMC Bioinformatics 11: 568.
41. Srivastava PN, Jain R, Dubey SD, Bhatnagar S, Ahmad N (2016) Prediction of epitope-based peptides for vaccine development from coat proteins GP2 and VP24 of Ebola virus using immunoinformatics. International Journal of Peptide Research and Therapeutics. 22: 119-133.
42. Bui HH, Sidney J, Dinh K, Southwood S, Newman MJ, et al. (2006) Predicting population coverage of T-cell epitope-based diagnostics and vaccines. BMC bioinformatics 7: 153.
43. Dimitrov I, Naneva L, Doytchinova I, Bangov I (2013) AllergenFP: allergenicity prediction by descriptor fingerprints. Bioinformatics 2013: btt619.
44. Källberg M, Wang H, Wang S, Peng J, Wang Z, et al. (2012) Template-based protein structure modeling using the RaptorX web server. Nature protocols 7: 1511-1522.
45. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, et al. (2004) UCSF Chimera-a visualization system for exploratory research and analysis. J Comput Chem 25: 1605-1612.
46. Xiao YF, Jie MM, Li BS, Hu CJ, Xie R, et al. (2015) Peptide-Based Treatment: A Promising Cancer Therapy. J Immunol Res 2015: 761820.
47. Li W, Joshi MD, Singhania S, Ramsey KH, Murthy AK (2014) Peptide vaccine: progress and challenges. Vaccines 2: 515-536.
48. Reche P, Flower DR, Fridkis-Hareli M, Hoshino Y (2015) Peptide-based immunotherapeutics and vaccines 2015. J Immunol Res 2015.
49. Ramqvist T, Dalianis T (2010) Lessons from immune responses and vaccines against murine polyomavirus infection and polyomavirus-induced tumours potentially useful for studies on human polyomaviruses. Anticancer Res 30: 279-284.
50. Tegerstedt K, Franzén AV, Andreasson K, Joneberg J, Heidari S, et al. (2005) Murine polyomavirus virus-like particles (VLPs) as vectors for gene and immune therapy and vaccines against viral infections and cancer. Anticancer Res 25: 2601-2608.
51. Gomez BP, Wang C, Viscidi RP, Peng S, He L, et al. (2012) Strategy for eliciting antigen-specific CD8+ T cell-mediated immune response against a cryptic CTL epitope of merkel cell polyomavirus large T antigen. Cell Biosci 2: 36.
52. Liu Y, McNevin J, Zhao H, Tebit DM, Troyer RM, et al. (2007) Evolution of human immunodeficiency virus type 1 cytotoxic T-lymphocyte epitopes: fitness-balanced escape. J Virol 81: 12179-12188.
53. Kolesanova EF, Sanzhakov MA, Kharybin ON (2013) Development of the schedule for multiple parallel “difficult” Peptide synthesis on pins. International J Pept 2013: 1-9.
54. Epstein JE, Giersing B, Mullen G, Moorthy V, Richie TL (2007) Malaria vaccines: are we getting closer. Current opinion in molecular therapeutics 9: 12-24.
55. Volpina OM, Gelfanov VM, Yarov AV, Surovoy AY, Chepurkin AV, et al. (1993) New virus-specific T-helper epitopes of foot-and-mouth disease viral VP1 protein. FEBS letters 333: 175-178.
56. Tarradas J, Monsó M, Munoz M, Rosell R, Fraile L, et al. Partial protection against classical swine fever virus elicited by dendrimeric vaccine-candidate peptides in domestic pigs. Vaccine 29: 4422-4429.
57. Stanekova Z, Kiraly J, Stropkovska A, Mikušková T, Mucha V, et al. (2010) Heterosubtypic protective immunity against influenza A virus induced by fusion peptide of the hemagglutinin in comparison to ectodomain of M2 protein. Acta Virol 55: 61-67.
58. Oscherwitz J, Yu F, Cease KB (2010) A synthetic peptide vaccine directed against the 2β2–2β3 loop of domain 2 of protective antigen protects rabbits from inhalation anthrax. J Immunol 185: 3661-3668.
59. Solares AM, Baladron I, Ramos T, Valenzuela C, Borbon Z, et al. (2011) Safety and immunogenicity of a human papillomavirus peptide vaccine (CIGB-228) in women with high-grade cervical intraepithelial neoplasia: first-in-human, proof-of-concept trial. ISRN obstetrics and gynecology 2011: 1-9.
60. Bernhardt S, Gjertsen M, Trachsel S, Møller M, Eriksen J, et al. (2006) Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: a dose escalating phase I/II study. Br J Cancer 95: 1474-1482.
61. Brunsvig PF, Aamdal S, Gjertsen MK, Kvalheim G, Markowski-Grimsrud CJ, Sve I, et al. (2006) Telomerase peptide vaccination: a phase I/II study in patients with non-small cell lung cancer. Cancer Immunol Immunother 55: 1553-1564.
62. Kyte JA, Gaudernack G, Dueland S, Trachsel S, Julsrud L, et al. (2011) Telomerase peptide vaccination combined with temozolomide: a clinical trial in stage IV melanoma patients. Clin Cancer Res 17: 4568-4580.
63. Greten TF, Forner A, Korangy F, N'Kontchou G, Barget N, et al. (2010) A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. BMC cancer 10: 209.
64. Kyte JA, Trachsel S, Risberg B, thor Straten P, Lislerud K, et al. (2009) Unconventional cytokine profiles and development of T cell memory in long-term survivors after cancer vaccination. Cancer Immunol Immunother 58: 1609-1626.
65. Tenzer S, Peters B, Bulik S, Schoor O, Lemmel C, et al. (2005) Modeling the MHC class I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class I binding. Cell. Mol. Life Sci 62: 1025-1037.
66. Loureiro J, Ploegh HL (2006) Antigen presentation and the ubiquitin-proteasome system in host–pathogen interactions. Adv Immunol 92: 225-305.

67. Iyer JG, Afanasiev OK, McClurkan C, Paulson K, Nagase K, et al. (2011) Merkel cell polyomavirus-specific CD8+ and CD4+ T-cell responses identified in Merkel cell carcinomas and blood. Clin Cancer Res 17: 6671-6680.

68. Janeway CA, Travers P, Walport M, Shlomchik MJ (1997) Immunobiology: the immune system in health and disease: Current Biology Singapore.

69. Oseroff C, Sidney J, Vita R, Tripple V, McKinney DM, et al. (2012) T cell responses to known allergen proteins are differently polarized and account for a variable fraction of total response to allergen extracts. J Immunol 189: 1800-1811.

70. Soria-Guerra RE, Nieto-Gomez R, Govea-Alonso DO, Rosales-Mendoza S (2015) An overview of bioinformatics tools for epitope prediction: implications on vaccine development. J Biomed Inform 53: 405-414.