Advances in Vaccines & Vaccination Research
AVVR, 6(1): 146-154
www.scitcentral.com

ISSN: 2688-6545

Original Research Article: Open Access

Design an Expression Vector with a Polytope Sequence against Microfilarial Antigen WbBhp1

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Received October 16, 2022; Revised October 20, 2022; Accepted October 23, 2022

ABSTRACT

The risk of filarial infection due to distinct mosquitoes keeps posing health issues unless and until the dwellings of the human community along with the livestock are insulated from insects, which would be a hypothetical proposition. However, to prevent the filarial risk among the potential subjects, it would be constrained to design a vaccine against the microfilariae worm circulating in the bloodstream for a reasonable period. Therefore, in the present attempt, the authors explored online bioinformatics tools to design and construct an expression vector and in silico analysis of the attributes of the proposed polytope construct designed using 16.3 kD protein, Wb-Bhp-1.

Keywords: Microfilariae, Wb-Bhp-1, Polytope, Vaccine design, MDA

INTRODUCTION

A few insect vectors viz., *Aedes aegypti*, *Culex quinquefasciatus* and *Mansonia africana* carry microfilariae as an intermediate host [1,2]. They transmit 3rd instar microfilariae in the community predominantly in Asia, Africa and South America where the incidence of lymphatic filariasis is found more represented [3]. The primary host is the infected man wherein the adult filarial worms produce eggs. There are three prominent species of filarial worms. They are *Wucheraria bancrofti, Brugia malayi* and *Brugia timori* [4]. The adults of these nematodes find residence in the lymph vessels, obstruct lymphatic circulation and cause lymphoedema in the terminal parts of limbs causing a disease called elephantiasis.

The updates of lymphatic filariasis and hydrocoel from India are reported by NCVBDC (National Centre for Vector Borne Disease Control) and it is shown that in the year 2021, the reported cases were 525,440 and 144,615 respectively [5]. Among the 73 filarial endemic countries, Cromwell [6] developed a global dataset by georeferenced surveyed locations between the years 2000-2018 and reported that out of the estimated 199 million infected individuals with filariasis in the year 2000, there is a significant reduction in the number of individuals suffering from filarial infection to an estimated number of 51 million by 2018. This clearly indicates that the Global Program initiated by WHO to clear lymphatic filariasis with MDA (Mass Drug Administration) is in the process of achieving the set goal [7].

The microfilariae are being characterized by an antigen used for diagnosis namely 16.3 kD protein (Wb-Bhp-1) whose length is 147 amino acids. Greene [8] characterized this protein, named as per the filarial nomenclature and deposited in NCBI accession version UXF48056.1 with UniProt primary accession J9DKXO. These authors also reported that microfilariae infected subject's elicited immune responses by producing IgG4 antibodies against Wb-Bhp-1. Hence, it is conceived in the present study to develop a vaccine construct by designing a polytope vaccine candidate against Wb-Bhp-1 belonging to the microfilariae of *Wucheraria bancrofti* using online bioinformatics tools so as to be made available as a prophylactic regimen among the risk groups.

METHODS

The in-silico tools are employed in the present study to trace epitopes within Wb-Bhp-1 protein (https://www.ncbi.nlm.nih.gov/protein/2307719357) using

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Citation: Vijiha, Venkateswarulu TC, Krupanidhi S & Peele KA. (2023) Design an Expression Vector with a Polytope Sequence against Microfilarial Antigen WbBhp1. Adv Vaccines Vaccin Res, 6(1): 146-154.

IEDB NetMHCpan-4.1 Class II DR window [9]. The first five derived epitopes each with 15 amino acids length are classified as low adjusted percentile rank and are supposed to have high binding with MHC II **(Table 1)** [10,11]. Thus, these epitopes would elicit T-cell mediated immunity. The derived five epitopes are used in the present study as polytope by joining each of the epitopes with the two linker amino acids CS, which possibly acts as a substrate in the proteolytic digestion in the proteasome of a dendritic cell [12].

Table 1. Prediction of epitope peptides from Wb-Bhp-1 protein using IEDB online software tool. Low adjusted rank = good binders.

Allele	Start	End	Length	Method used	Peptide	Adjusted Percentile Rank
HLA-DRB3*02:02	85	99	15	NetMHCIIpan	HLWLTINEEAVIEAK	3.7
HLA-DRB3*01:01	104	118	15	Consensus (comb.lib./smm/nn)	NGNYTMEAVGNFTEM	3.7
HLA-DRB3*01:01	103	117	15	Consensus (comb.lib./smm/nn)	ENGNYTMEAVGNFTE	3.8
HLA-DRB3*01:01	102	116	15	Consensus (comb.lib./smm/nn)	FENGNYTMEAVGNFT	3.8
HLA-DRB3*02:02	84	98	15	NetMHCIIpan	HHLWLTINEEAVIEA	4.2

Polytope construct sequence with linkers

HLWLTINEEAVIEAKCSNGNYTMEAVGNFTEMCSNG NYTMEAVGNFTECSFENGNYTMEAVGNFTCSHHLW LTINEEAVIEA

Determination of the attributes of the constructed polytope

The propensity of the constructed polytope to be an antigen is determined by VaxiJenV2 online tool. The potential to be an allergen is evaluated by the online tool namely AllerTop V2. Similarly, the potential of the polytope to be toxic is assessed by ToxinPred online tool. Phyre2 and PredictProtein.org online tools are employed to construct its homology model **(Figure 1)** and secondary structure **(Figure 2)** respectively**.** The instability index, aliphatic index, GRAVY (Grand Average of Hydropathicity) and half-life of the polytope construct is evaluated using ProtParam tool Expasy **(Table 2).** PDBsum web server (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html) is used to generate a Ramachandran plot **(Figure 3)** for the constructed polytope to observe the position of amino acids in the Phi and Psi axis [13]. The PDBsum is a visual database that provides an overview of the residues of each 3D structure deposited in the Protein Data Bank format.

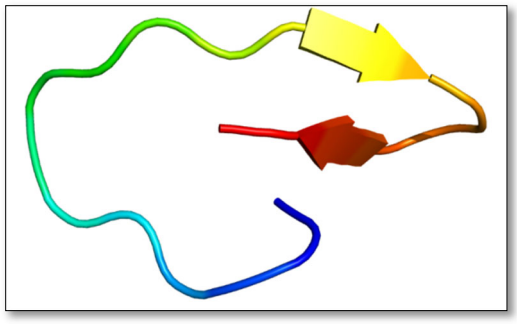

Figure 1. 3D predicted homology model of the polytope construct obtained using Phyre2 online server predominantly showing beta sheets and coil.

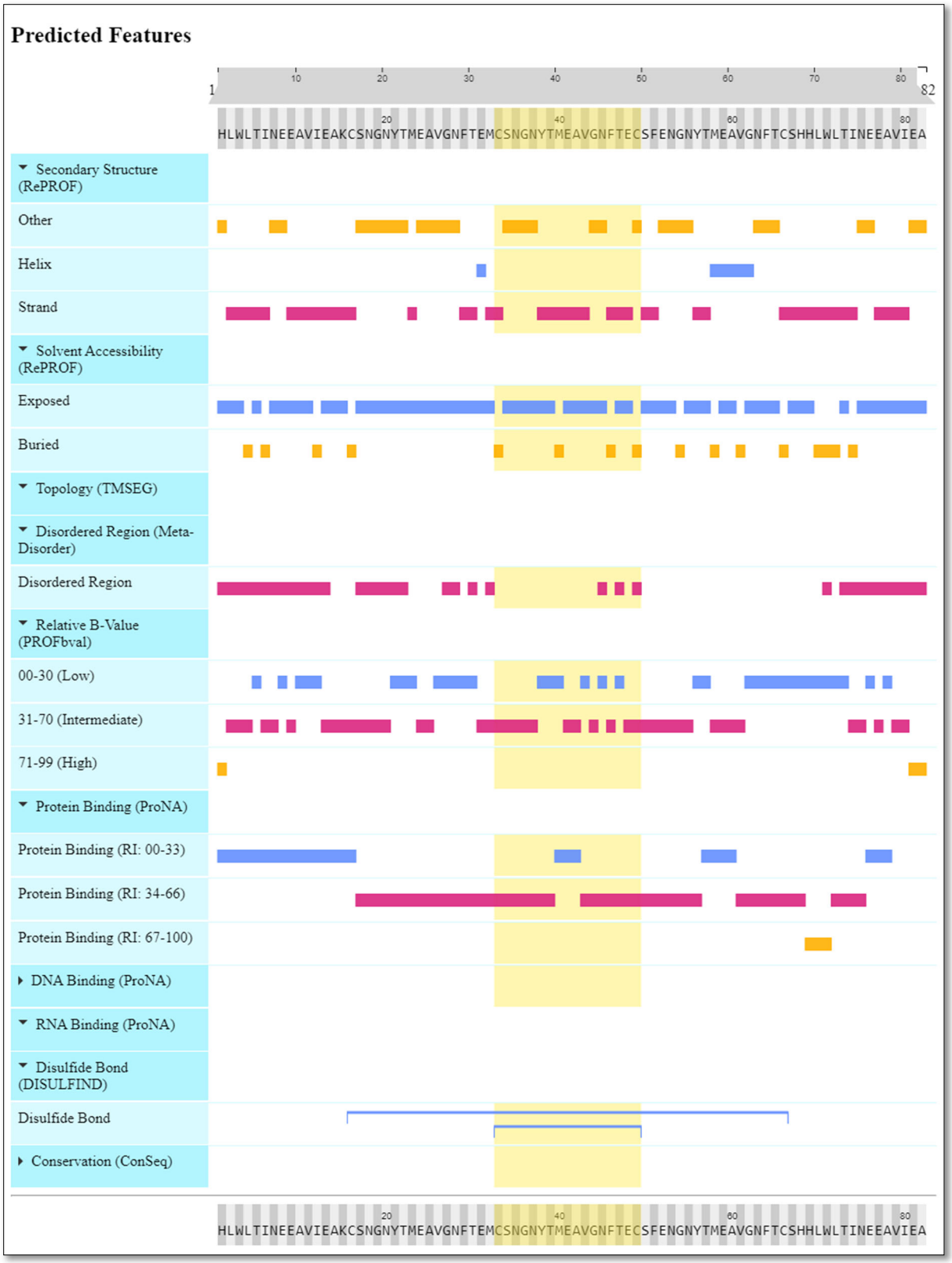

Figure 2A. Secondary structure predicted features of the constructed polytope of filarial antigen derived through https://predictprotein.org/visual_results?req_id=1nDPK2U2x$ZqI4zSHLSuB4fc40iDGYu/

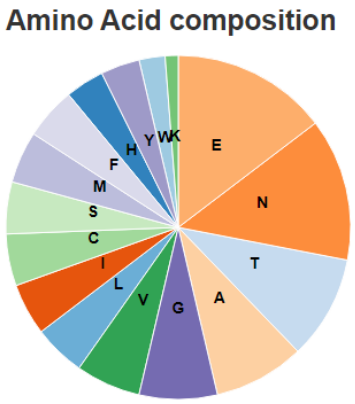

Figure 2B. The amino acid composition of the polytope construct out of a total of 82. https://predictprotein.org/home

Table 2. The physical and chemical parameters of the polytope construct. The values are obtained through https://web.expasy.org/cgi-bin/protparam/protparam.

No. of amino acids	M.W	pI	Instability index	Aliphatic index	Grand average of hydropathicity (GRAVY)	Estimated half-life
83	9277.20	4.12	26.12 (stable)	63.49	-0.227	3.5 h (mammalian reticulocytes, in vitro)

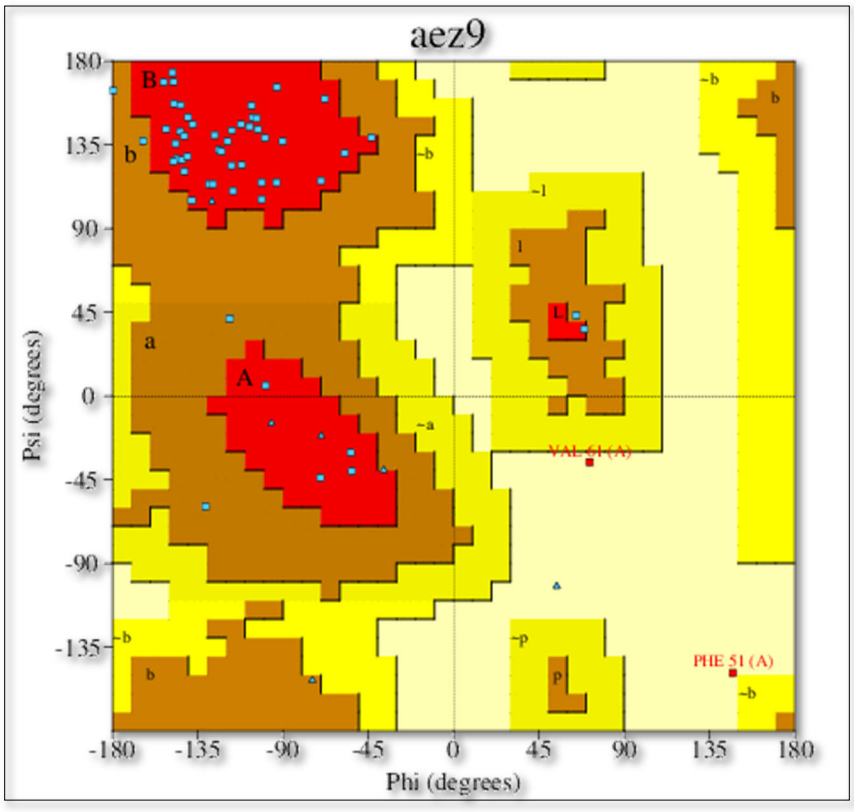

Figure 3. Ramachandran plot derived from PDBsum web server for the structure of polytope construct showing the distribution of residues clustered at beta-sheet region.

PROCHECK statistics (Table 3)

Table 3. Ramachandran Plot statistics.

	No. of residues	%age
Most favored regions [A,B,L]	48	87.3%*
Additional allowed regions [a,b,l,p]	5	9.1%
Generously allowed regions [~a,~b,~l,~p]	0	0.0%
Disallowed regions [XX]	2	3.6%
Non-glycine and non-proline residues	55	100.0%
End-residues (excl. Gly and Pro)	3	
Glycine residues	6	
Proline residues	0	
Total number of residues	64	

In-silico cloning of constructed polytope

The amino acid sequence of the constructed Wb-Bhp-1 polytope is translated into corresponding DNA sequence using Expasy online server (https://web.expasy.org/translate/). The obtained 249 bp DNA sequence is in-silicol igated in between HindII and HpaI restriction sites at 3' and 5' directions in Geneious Prime online tool in MCS of pET28(a) plasmid which is selected as the expression vector with T7 promoter and kanamycin resistance **(Figure 4)**. The constructed expression vector is to be transferred into the E.coli TOP10 cloning vehicle for *in vitro* generation of multiple copies of the polytope as a vaccine candidate.

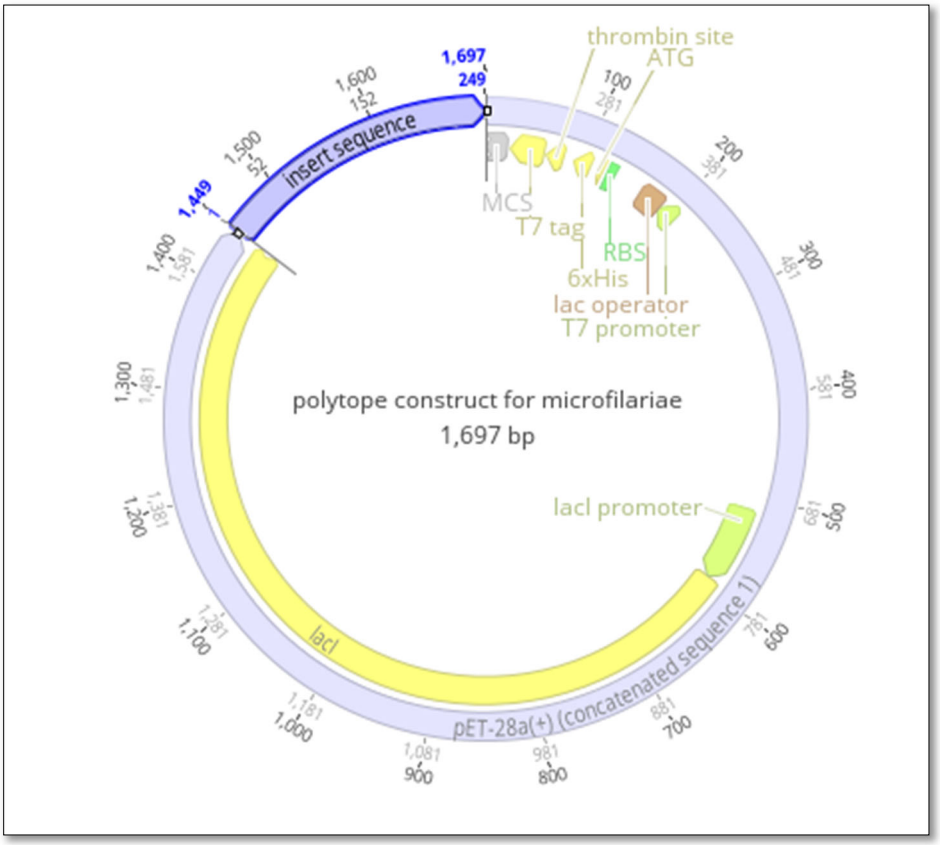

Figure 4. DNA of polytope construct is in-silico ligated at MCS of pET28(a) plasmid between HindII and HpaI restriction sites using Geneious Prime software tool (insert fragment highlighted in blue).

Molecular Docking

ClusPro 2.0 (https://cluspro.bu.edu/login.php?redir=/queue.php) web server is employed to perform docking of polytope construct against the Fab region of a typical IgM antibody **(Figures 5 & 6).** ClusPro 2.0 generates comparable results based on a large number of ligand/protein conformations; the scores of the top conformations interacting with the receptor grid are provided in ranked order **(Table 4)** [14,15].

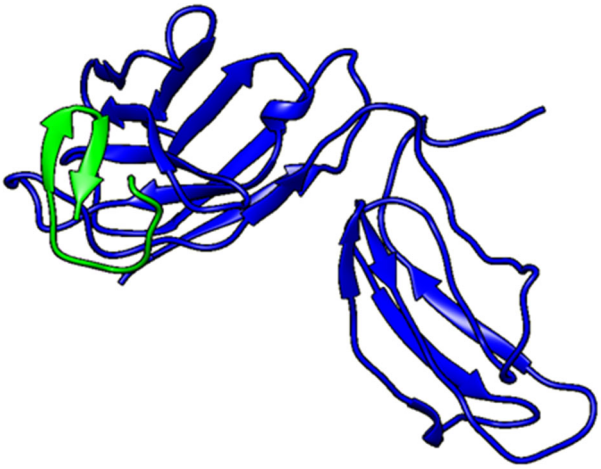

Figure 5. Top 1 Chimera generated image of Binding pose of polytope construct (green) with target- Fab region of IgM (blue) yields a score of 207.8 **(Table 4)**.

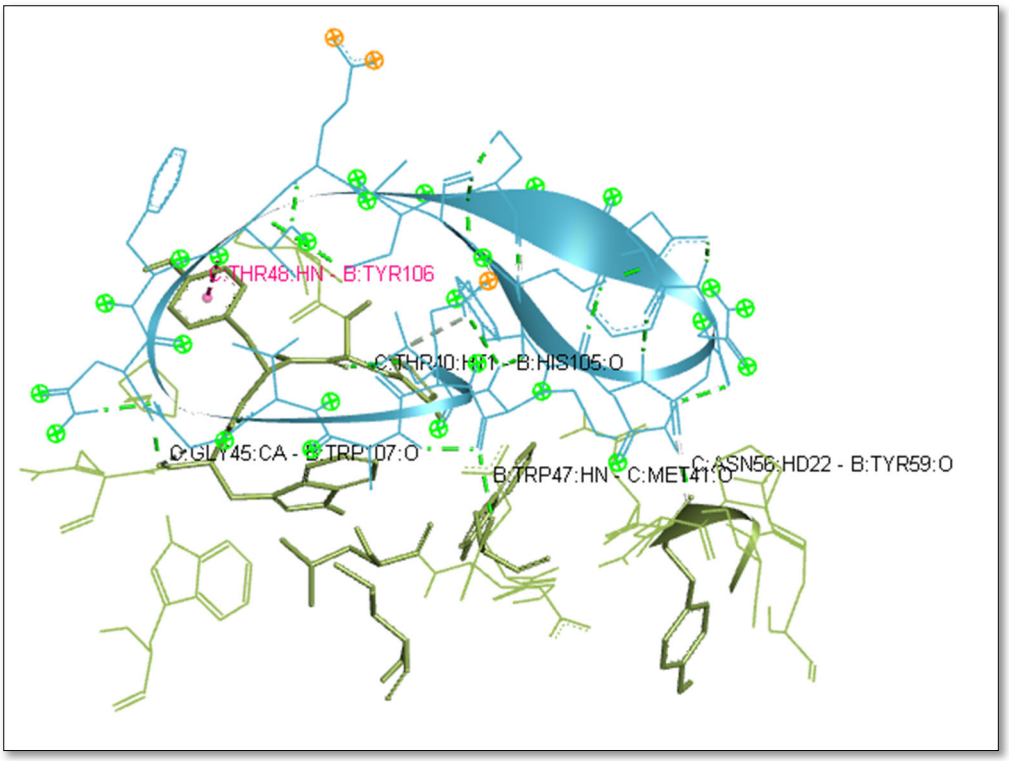

Figure 6. The docking of polytope construct and a typical immunoglobulin (IgM) is done using ClusPro2.0 online server. The image is retrieved from Discovery Studio. The generated pose represents a few active residue interactions between polytope (blue) and antibody (green) consisting of pi-donor hydrogen bonds (pink) and conventional hydrogen bonds (black). The remaining hydrogen bond interactions are given in **Table 4**.

Table 4. Top 10 docking scores of polytope construct with FAb region of IgM.

Cluster	Members	Representative	Weighted Score
0	133	Center	-200.8
		Lowest Energy	-207.8
1	95	Center	-180.3
		Lowest Energy	-200.1
2	79	Center	-177.4
		Lowest Energy	-206.8
3	77	Center	-177.2
		Lowest Energy	-213.4
4	66	Center	-176.9
		Lowest Energy	-209.7
5	65	Center	-182.0
		Lowest Energy	-219.0
6	57	Center	-180.9
		Lowest Energy	-199.7
7	54	Center	-184.1
		Lowest Energy	-196.9
8	53	Center	-184.5
		Lowest Energy	-207.4
9	35	Center	-181.7
		Lowest Energy	-196.2
10	30	Center	-181.7
		Lowest Energy	-195.3

RESULTS AND DISCUSSION

The amino acid sequence of the filarial antigen Wb-Bhp-1 developed by Greene et al [8] is used in the present study to derive the 15-mer epitopes in IEDB software tool which gave several numbers of combinatorial peptide sequences, of which, the Low adjusted rank peptides are reported to be good binders and such five are selected which are compatible to HLA class II DR B alleles and further constructed a polytope containing 82 amino acids with CS linkers (**Table 1**). The CS linkers are provided in the polytope to enable the same for proteolytic digestion in the proteasome of macrophage/dendritic cell by cysteine or serine proteases [2]. The physical and chemical attributes of the polytope construct reveal that the polytope is antigenic as authenticated by the VaxiJen V2 by yielding a value of 0.7456. Both AllerTop V2 and ToxinPred tools yielded the outcome as non-allergic and non-toxin respectively. Further, the polytope construct is found to be stable shown by an instability index value of 26.12, aliphatic index of 63.49 and an estimated half-life of 3.5 hours, sufficient period enough in the host to be identified by TLR2/4 [16] and to be encountered by a dendritic cell **(Table 2)**.

The Phyre2 (Protein Homology/analogy Recognition Engine) and PredictProtein.org tools have yielded respectively the homology model **(Figure 1)** and the secondary structure of the constructed polytope. Upon submission in Phyre2 server revealed the presence of 4.0% alpha helix, 74% beta strands and 10% disordered which suggest that the polytope is a stable folded potential antigen to be chosen for the vaccine preparation. Furthermore, the PredictProtein.org yielded important observations on the quality of the polytope construct namely that the secondary structure is predominantly beta strand, exposed residues are significantly more compared to buried residues and protein

binding residues are found to be predominant as shown in (**Figure 2)**. The Ramachandran plot also revealed the distribution of residues in the most favored region clustered at beta-sheet region **(Figure 3).** The molecular docking of the polytope construct with the typical Fab of IgM revealed the binding pose with a score of 207.8 **(Table 3)** in ClusPro 2.0 indicating that the active residues (blue) have shown interaction with antibody (green) represented by pi-donor hydrogen bonds (pink) and conventional hydrogen bonds (black) along with the hydrogen bond interactions as given in **Table 3** and **Figures 5 & 6**. The aforementioned attributes authenticate that the constructed polytope would be a vaccine candidate, which upon formulation will be used as a prophylactic regimen among the filarial endemic group.

To manufacture the vaccine candidate, the up-stream protocol requires a design of gene construct with a cloning vehicle. As shown in the **Figure 4** the translated DNA of the polytope construct is *in-silico* ligated in pET28(a) plasmid which upon transferring into *E.coli* TOP10 cloning vehicle in a suitable bacterial growth medium will enrich the quantity of polytope which upon down-streaming steps, formulating with TLR2/4 agonist, stabilizers and preservatives, vaccine for filarial worm could be formulated for pre-clinical trials. The vaccine against filarial worm is quintessential requirement as elephantiasis is a disease with a serious social stigma in addition to illness. Further, microfilariae elicit IgG4 immune response [8,17,18]. The memory B-cells built will be a repertoire to neutralize Wb-Bhp-1 antigen of a freshly infected microfilariae which measures 177-230 µm and 5-7 µm in length and width respectively [19] and whose pre-patent period in bloodstream lasts several months [20] which would elicit both complement mediated and ADCC (antibody dependent cell cytotoxicity) response to arrest the intensity of lymphoedema.

ACKNOWLEDGEMENTS

The authors acknowledge VFSTR University for providing facilities through Centre of Excellence in the Department of Biotechnology.

REFERENCES

1. Roy N (2018) Elimination of lymphatic filariasis India Updates and way forward. Manipal J Med Sci 9: 1-3.
2. Nutman TB (2001) Blood-borne Filarial Infections: Wuchereriabancrofti, Brugiamalayi, Brugiatimori, Loa loa, Mansonellaperstans and Mansonellaozzardi. Principles and Practise of Clinical Parasitology pp: 433-455.
3. Horton J, Witt C, Ottesen EA, Lazdins JK, Addiss DG, et al. (2000) An analysis of the safety of the single dose, two drug regimens used in programmes to eliminate lymphatic filariasis. Parasitology 121(S1): S147-S160.
4. Babu S, Nutman TB (2014) Immunology of lymphatic filariasis. Parasite Immunol 36(8): 338-346.
5. NVBDCP newsletter. Volume 4, Issue 4, April 2022. Available online at: https://nvbdcp.gov.in/Doc/Newsletter-13-May.pdf
6. Cromwell EA, Schmidt CA, Kwong KT, Pigott DM, Mupfasoni D, et al. (2020) The global distribution of lymphatic filariasis, 2000-18: A geospatial analysis. Lancet Glob Health 8(9): e1186-e1194.
7. Ottesen EA, Hooper PJ, Bradley M, Biswas G (2008) The Global Programme To Eliminate Lymphatic Filariasis: Health Impact After 8 Years. 2(10): e317.
8. Greene SE, Fischer K, Choi YJ, Curtis KC, Budge PJ, et al. (2022) Characterization of a novel microfilarial antigen for diagnosis of *Wuchereria bancrofti* infections. PLoS Negl Trop Dis 16(5): e0010407.
9. Reynisson B, Alvarez B, Paul S, Peters B, Nielsen M (2020) NetMHCpan-4.1 and NetMHCIIpan-4.0: Improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. Nucleic Acids Res 48(W1): W449-W454.
10. Wang P, Sidney J, Dow C, Mothé 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(4): e1000048.
11. 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.
12. Sreerama K (2021) Conserved envelope protein of nCoV2 as the possible target to design polytope vaccine. Explor Immunol 1: 155-165.
13. Subramanian E (2001) Ramachandran GN. Nat Struct Mol Biol 8: 489-491.
14. Vajda S, Yueh C, Beglov D, Bohnuud T, Mottarella SE, et al. (2017) New additions to the ClusPro server motivated by CAPRI. Proteins 85(3): 435-444.
15. Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, et al. (2017) The ClusPro web server for protein-protein docking. Nature Protoc 12(2): 255-278.
16. Fouzder C, Mukhuty A, Das S, Chattopadhyay D (2019) TLR Signaling on Protozoan and Helminthic Parasite Infection. In (Ed.), Toll-like Receptors. Intech Open.
17. Prodjinotho UF, Hoerauf A, Adjobimey T (2019) IgG4 antibodies from patients with asymptomatic bancroftian filariasis inhibits the binding of IgG1 and IgG2 to C1q in a Fc-Fc-dependent mechanism. Parasitol Res 118(10): 2957-2968.

18. Adjobimey T, Hoerauf A (2010) Induction of immunoglobulin G4 in human filariasis: An indicator of immunoregulation. Ann Trop Med Parasitol 104(6): 455-464.

19. CDC (2018) Parasites - Lymphatic Filariasis. Biology - Life Cycle of *Wuchereria bancrofti*. Available online at: https://www.cdc.gov/parasites/lymphaticfilariasis/biology_w_bancrofti.html

20. https://www3.paho.org/hq/index.php?option=com_content&view=article&id=5855:2011-general-information-lymphatic-filariasis&Itemid=4195&lang=en