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In-silico Epitope Design against Chickenpox Virus (*Varicella zoster* Virus)

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Abstract: *Varicella zoster (VZ) is an infectious disease caused by a viral agent, varicella zoster virus (VZV), belonging to the sub-family Alphaherpesvirinae; VZV has a tropism for neurons leading to neurologic disease in humans. The purpose of this study was to generate a multi-epitope vaccine by combining five VZV surface proteins that will stimulate the immune response in humans. Immunoinformatics tools were used to predict B cell and T cell epitopes for the vaccine; population coverage of each T cell epitope's were analysed with results showing high population coverage scores. The vaccine contains a total of 409 amino acids; the vaccine was confirmed to be antigenic, and had low levels of allergenicity. The physical and chemical properties of vaccine were determined including hydrophilicity, stability, presence of aliphatic side chain amino acids, and thermal stability. The vaccine displayed a low level of homology (11%) to the human proteome. Secondary and tertiary vaccine structures were predicted, improved and verified through use of PSIPRED and the Ramachandran plot and analyzed for structural errors using the Procheck software program. Predicted vaccine docking yields significant docking scores. The improved DNA was cloned in pVAX1 vector using snap gene tool.*

Keywords: *Varicella zoster, Alphaherpesvirinae, Immunoinformatics tools, population coverage, PSIPRED, Ramachandran plot, Procheck software, docking.*

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Introduction:

Varicella zoster Virus (VZV) also known as human herpesvirus 3 (HHV-3) is a significant virus responsible for causing chickenpox (varicella) and shingles (herpes zoster). Since the virus transmits readily and can stay latent within the body for periods it remains a key public health issue (Daulagala & Noordeen, 2018).

VZV is a double stranded DNA virus with an envelope that influences various metabolic routes within the host cell such as the synthesis of biomolecules like nucleotides, lipids and amino acids (Zandi et al., 2022).

In spite of the successful vaccination programs, Varicella zoster virus remains a cause to public health challenge. Partial vaccine coverage, waning immunity over time, and viral reactivation causes "shingles" are major contributing factors, especially among older adults and individuals with weakened immune systems (Cohen et al., 1999).

Varicella can be treated by using antiviral drugs (Zheng et al.,2023), but their use is generally limited to those individuals, who are at high risk of severe disease, such as patients with weakened immune systems, neonates, or those with chronic medical conditions, due to the relatively low clinical benefit in otherwise healthy individual and the high cost of therapy. The most efficacious treatment for VZV infection involves the administration of anti-VZV antibodies. One of the most significant complications following herpes zoster is post- herpetic neuralgia, a condition characterized by severe neuropathic pain that can last for months after the resolution of the rash, and is often resistant to standard therapies (Kennedy and Gershon, 2018). Reactivation of VZV may also result in complications including meningoencephalitis, myelitis, Ramsay Hunt syndrome and zoster sine herpete (ZSH) which manifests symptoms in the absence of a rash (Testi et al., 2021).

Vaccinax, the live attenuated varicella vaccine, is administered to infants and young children aged 12 months and above to prevent infection from varicella. This vaccine consists of a genetically diverse population of attenuated VZV strains containing multiple polymorphic variants and remains the only licenced vaccine developed against a human herpesvirus. The attenuation results from mutations acquired during repeated passage in tissue culture, which weaken innate antiviral responses without compromising the viral's ability to establish infection in TR cells or dorsal root ganglia. During natural VZV infection, virus-host interactions are finely regulated to avoid excessive viral replication, a mechanism that benefits the virus by promoting its ongoing transmission and long-term persistence within the human population (Zerboni et al.,2014).

Progress in *In silico* (computer-aided) techniques has enhanced vaccine by reducing the time, cost, and experimental risks along with conventional laboratory based studies. These techniques are now widely used as alternatives to traditional culture-based vaccine development methods (Patronov and Doytchinova, 2013; Tomar et al., 2010). As a result, immunoinformatics based vaccine design offers a promising strategy for reducing the worldwide risk of varicella. Prediction of epitope is a key initial step in this process, which involves evaluating how well antigenic peptides bind to major histocompatibility complex (MHC) molecules. The increasing use of these immunoinformatic tools have also supported the development of more advanced and effective vaccination strategies (Yu et al.,2024; Suleman et al.,2023; Moin et al., 2022; Araf et al.,2022).

Polyvalent vaccines developed using these advanced strategies can help overcome critical challenges such as shingles reactivation in older age. In addition, these methods not only accelerate vaccine discovery but also deepen our understanding of virus-host immune interactions, enabling the design of more effective, personalized, and long lasting vaccine strategies **(**Oliver et al.,2021).

Materials and Methods:

1. **Data retrival:** Multiple sequences of VZV (Dumas strain) proteins were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/) in FASTA format.
2. **Subcellular localization and transmembrane topology:** Phobius tool (https://phobius.sbc.su.se/) is used to find signal peptides and membrane spanning regions in proteins. It was used to study where the viral proteins are located in the cell (Kall *et al.,*2007).

   CELLO v2.5 (https://cello.life.nctu.edu.tw/) was used to predict the location of these proteins inside the cell (Yu *et al.,*2006).

   In addition, the TOPCONS web server (https://topcons.net/) was used to identify the transmembrane helices present in the proteins (Tsirigos *et al.,* 2015).
3. **Testing of Antigenicity and Allergenicity:** Antigenicity of the retrieved protein sequences was predicted using the VaxiJen v2.0 server to select antigenic proteins (Doytchinova et al.,2007).

   Similarly, allergenicity of the retrieved protein sequences was predicted using the AllerTOP server to select non-allergen proteins (Dimitrov et al.,2014).
4. **Analysis of Physiochemical properties:** Physicochemical properties of the viral protein were evaluated using the Expasy ProtParam tool to identify molecular weight, length of amino acid sequence, instability index, and Grand Average Hydropathocity (GRAVY) (Gasteiger et al.,2005).
5. **Homology check:** Homology check of the selected proteins was performed against the

human proteome (*Homo sapiens,* Taxid: 9606) using BLASTp to avoid autoimmunity.

Protein showing less or no similarity to human proteome were selected for vaccine design.

6. **Selection of proteins:** Three proteins which include tegument protein UL46 homolog (Q4JQW3), envelope glycoprotein B ORF 31 (Q6YP71) and Major capsid protein (CAH19069.1) were selected for epitope prediction based on different parameters such as antigenicity, allergenicity, physicochemical properties, homology check, subcellular localization and transmembrane topology.
7. **B-cell epitope prediction:** IEDB tool (BepiPred Linear Epitope Prediction 2.0) was used to predict the linear B-cell epitopes.
8. **T-cell epitope prediction:** MHC class I and class II epitope prediction were performed using the predicted B-cell epitopes sequences.

   MHC Class 1 epitope prediction

   MHC class I epitopes were predicted using "MHC class I epitope prediction tool" available on the IEDB server. Further, the "full HLA reference set" was selected to identify the alleles encoding MHC class I receptors (Reynisson *et al.,*2020).

   MHC Class 2 epitope prediction

   The MHC class II epitope prediction tool available on the IEDB server was used for MHC Class II epitope prediction. The "full HLA reference set" was selected to identify alleles encoding MHC class II receptors (Tsirigos *et al.,*2015).
9. **Antigenicity, Allergenicity, Solubility and Toxicity Analysis:** Predicted B-cell and T-cell epitopes were further evaluated for Antigenicity (VaxiJen), Allergenicity (AllerTOP), Solubility (Innovagen peptide calculator) and Toxicity (ToxinPred) (Rathore et al.,2024).
10. **Population coverage analysis:** Population coverage analysis of the antigenic, non-allergen, non-toxic, and water-soluble epitopes of T-cell was evaluated using the IEDB Population Coverage Tool. It was done to ensure that the selected epitopes could provide broad protection across multiple populations (Bui et al., 2006).
11. **Vaccine assemblage and construction:** T cell epitopes that showed highest population coverage scores were used for multi-epitope vaccine construction.

    Multi- epitope vaccine was constructed by linking the predicted B- cell and T- cell epitopes by using different linkers. Adjuvant was added at N- terminal end of the epitope. Similarly, 6x His tag was added at the C-terminal end. The order of linking is given in Table 1.

Table 1: Sequence of vaccine assemblage

1	Adjuvant	Cholera toxin B
2	Linker	EAAAK
3	MHC I epitopes (Each epitope separated by AAY linker)	1) SVSDQPYNR 2) DTHLKLQFR 3) DKNPKYNSV 4) DLLEPYYEDY 5) GQTKNPQVSI
4	MHC II epitopes (Each epitope separated by GPGPG linker)	1) AEVPVTSEKVNKKSK 2) ARSFSSDDRTRKSYD 3) AGVEARSIFPFGLRD 4) ALLTSRLTGLALRNR 5) DTVGQDVNAVEASSK 6)SSQNTTSTPHTDTVGQDVNAVEASSKAPLIQGS TADDADM
5	B cell epitopes (Each epitope separated by KK linker)	1) YTTGAEVPVTSEKVNKKS 2)YYSKWRNRDRPEYRRNLRFRRFFSSIHPNAAAGSGFNG 3) RTCPDYHLGK
8	6x His tag	HHHHHH

12. **Secondary structure prediction:** The PSIPRED server was used to predict the secondary structure of the vaccine construct to determine the alpha-helices, beta-sheets, and coils (Buchan *et al.,*2019).
13. **Tertiary structure prediction and refinement:** AlphaFold tool (https:// alphafold. com/) was used to predict the 3D structure of the vaccine construct. The

predicted 3D structure was visualized using Discovery Studio Visualizer (Shin et al.,2014).

The tertiary structure of the vaccine construct was further refined using the GalaxyRefine server (https://galaxy.seoklab.org/index.html) to improve structural accuracy (Wiederstein and Sippl, 2007).

The PDB file of the predicted protein structure was uploaded on Galaxy Refine, and Model 1 was selected for further analysis.

14. **Ramachandran plot:** Ramachandran plot of the refined vaccine model was generated using the PROCHECK server for structure evaluation. The refined protein PDB file was uploaded, and the distribution of phi (φ) and psi (ψ) torsion angles of amino acid residues were analyzed.
15. **Molecular docking and dynamic stability:** The structure of human Toll-like receptor (TLR; PDB ID: 2Z7O) was retrieved in PDB format from the PDB database and used as the receptor. The refined vaccine model was used as the ligand and molecular docking was performed using the HDOCK server to evaluate ligand–receptor interaction.

    Dynamic stability was performed using tool ***elNemo.***
16. **Codon optimization:** The nucleotide sequence of the vaccine construct was optimized by using the J CAT server with *E. coli* K12 strain as the target organism.
17. **In silico Cloning:** The codon-optimized DNA sequence of the vaccine construct was cloned into the pVAX1 vector using the SnapGene tool (snapgene-viewer). Restriction sites, PsiI and KpnI in the vector and the target DNA sequence were digested and further the optimized vaccine gene was inserted into the pVax 1vector (Grote et al.,2005).

Results and Discussion:

1. The targeted protein for vaccine design: Based on the subcellular localization, antigenicity, allergenicity and the physiochemical characteristics of the 58 proteins of VZV, only the tegument protein UL46 homolog (Q4JQW3), envelope glycoprotein B ORF 31 (Q6YP71) and Major capsid protein (CAH19069.1) were used for epitopes prediction. The characteristics of these proteins demonstrated in Table 2.

Table 2. Distinct properties of the three selected proteins

Viral protein	Uniprot Entry	Molecular weight (Dalton)	Instability index#	Aliphatic Index	pI	No of amino acids	Extinction Coefficient	GRAVY †	Vaxijen Subcellular $	Vaxijen Subcellular localization
Tegument protein UL46 homolog	Q4JQW3	74272.94	39.9	80.51	7.95	661	99865	−0.395	0.4603	OMP
Envelope glycoprotein B	Q6YP71	105347.36	39.73	78.31	8.81	931	115060	−0.351	0.5 06	OMP
Capsid scaffolding protein	CAH19069.1	32766.54	38.55	57.15	6.44	302	22140	0.625	0.4833	OMP

2. Prediction of B cell Epitope: Based on the predictions, the UL46 homolog tegument protein had 17 linear conserved B-cell epitopes, the envelope glycoprotein B ORF had 26, and the major capsid protein had 13. The epitopes that were analyzed to be antigenic, non-allergenic and non-toxic were used in the vaccine as B-cell epitopes (Table 3).

3. MHC II and MHC I Epitope Prediction: Multiple epitopes interacting with MHC I molecule and MHC II were predicted. The epitopes which demonstrated antigenicity, nonallergic, nontoxic and highest population coverage scores were selected for the vaccine structure (Table 4)

Table 3. Epitope sequence

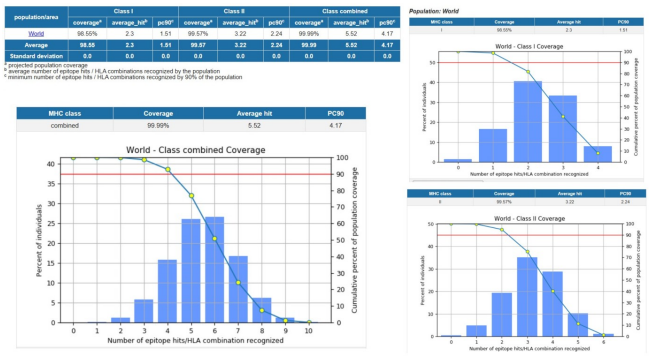
SELECTED PEPTIDES	MHC I	MHCII	B CELL
UL46	1) SVSDQPYNR 2) DTHLKLQFR	1)AEVPVTSEKVNKKSK 2)ARSFSSDDRTRKSYD	1)YTTGAEVPVTSEKVNKKS
ORF 31	1)DKNPKYNSV 2)DLLEPYYEDY	1)AGVEARSIFPFGLRD 2)ALLTSRLTGLALRNR	1)YYSKWRNRDRPEYRRNLRFRRFFSSIHPNAAAGSGFNG 2) RTCPDYHLGK
MAJOR CAPSID	1)GQTKNPQVSI	1)DTVGQDVNAVEASSK	1)SSQNTTSTPHTDTVGQDVNAVEASSKAPLIQGSTADDADM

Table 4: Results of Antigenicity, allergenicity, toxicity and solubility of epitopes

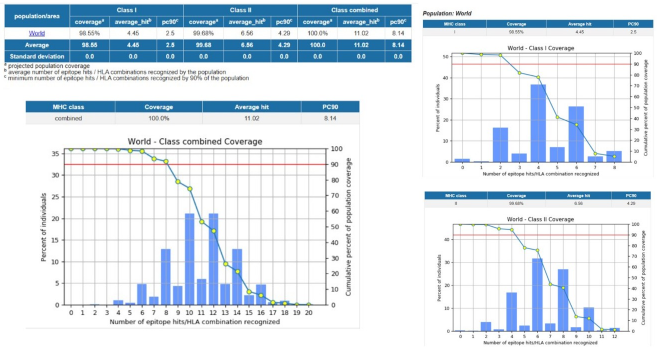
PEPTIDES	ANTIGENICITY	ALLERGENICITY	TOXICITY	SOLUBILITY
SVSDQPYNR	0.7825	Non allergen	Non toxic	Good water soluble
DTHLKLQFR	2.1704	Non allergen	Non toxic	Good water soluble
DKNPKYNSV	1.1112	Non allergen	Non toxic	Good water soluble
DLLEPYYEDY	0.4372	Non allergen	Non toxic	Good water soluble
GQTKNPQVSI	0.7225	Non allergen	Non toxic	Good water soluble
AEVPVTSEKVNKKSK	0.8836	Non allergen	Non toxic	Good water soluble
ARSFSSDDRTRKSYD	0.4798	Non allergen	Non toxic	Good water soluble
AGVEARSIFPFGLRD	1.5989	Non allergen	Non toxic	Good water soluble
ALLTSRLTGLALRNR	1.5466	Non allergen	Non toxic	Good water soluble
DTVGQDVNAVEASSK	0.5506	Non allergen	Non toxic	Good water soluble
YTTGAEVPVTSEKVNKKS	0.6894	Non allergen	Non toxic	Good water soluble
YYSKWRNRDRPEYRRNLRF	0.4099	Non allergen	Non toxic	Good water soluble
RRFFSSIHPNAAAGSGFNG				
RTCPDYHLGK	0.4514	Non allergen	Non toxic	Good water soluble
SSQNTTSTPHTDTVGQDVNAV	0.4131	Non allergen	Non toxic	Good water soluble
EASSKAPLIQGSTADDADM				

Note: Threshold value is 0.4 for antigenicity

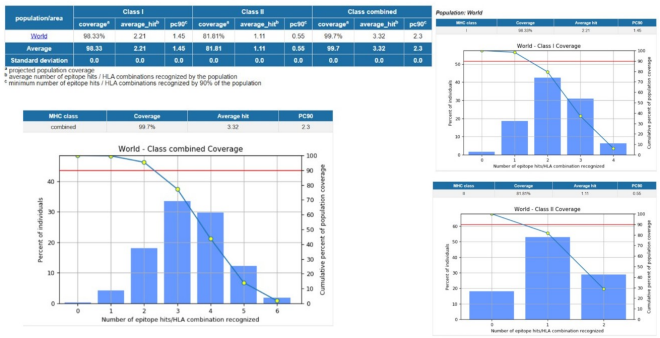
4. Population coverage analysis:



A



B



C

Fig. 1. Population coverage analysis: A- UL 46 tegument protein B- ORF31 enveloped glycoprotein B, C- major capsid scaffold protein

The highest population coverage was showed in ORF 31envelpoed glycoprotein B cell epitopes when interacted with combined MHC 1and MHC 2 alleles throughout the world (Fig. 1).

5. Final Vaccine Construct

10 20 30 40 50 60 70

MIKLKFGVFFTVLLSSAYAHGTPQNITDLCAEYHNTQ
IYTLNDKIFSYTESLAGKREMAIITFKNGAIFQ

VEVPGSQHIDSQKKAIERMKDTLRIAYLTEAKVEKLC
VWNNKTPHAIAAISMANEAAAKGQTKNPQVSIA

AYSVSDQPYNRAAYDTHLKLQFRAAYDKNPKYNSV
AAYDLLEPYYEDYAAYDTVGQDVNAVEASSKGPGP

GAEVPVTSEKVNKKSKGPGPGARSFSSDDRTRKSY
DGPGPGAGVEARSIFPFGLRDGPGPGALLTSRLTG

LALRNRGPGPGSSQNTTSTPHTDTVGQDVNAVEAS
SKAPLIQGSTADDADMKKYTTGAEVPVTSEKVNKK

SKKYYSKWRNRDRPEYRRNLRFRRFFSSIHPNAAA
GSGFNGKKRTCPDYHLGKHHHHHH

Total number of **ALIVMW** in sequence: 110

Fig. 2. Vaccine construct sequence

The Antigenicity of vaccine construct was recorded as 0.5765. The vaccine construct was found non allergen, nontoxic and good water soluble. Physiochemical properties of vaccine construct using protparam expassy tool was reported. 409 amino acids were found with molecular weight: 44869.28 (Fig 2).

6. Secondary structure prediction

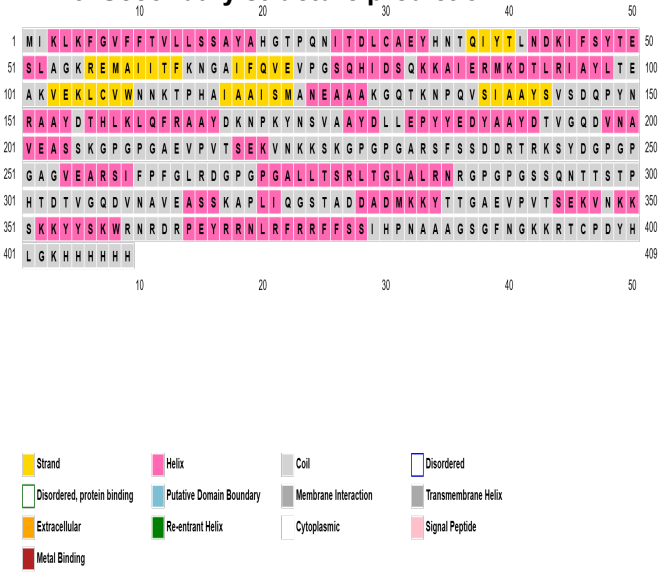


Fig. 3 Secondary structure of vaccine construct

The illustration displayed the predicted secondary structure and functional annotations applied to vaccine construct. The predicted secondary structure of vaccine majorly consists of alpha helices, coils and strand of B cell. The sequence includes several contiguous regions abundant in epitopes, particularly in areas that form helices, suggesting strong immunogenic potential (Fig. 3).

7. Tertiary structure prediction

The predicted tertiary structure (Fig 4) showed that the designed vaccine construct has a well-defined organization of the various elements that make up the secondary structure, such as helices and sheets and loops. Each part is arranged in a stable, predictable fashion. The helices made up the majority of the protein's structural core, which was responsible for providing the structure with rigidity and overall stability. The sheets were arranged to create compact, orderly internal structures within the overall protein architecture. The loops are arranged such that they extend out from the core, providing an underlying degree of flexibility and improved access to the epitopes, which are critical for the vaccine design. The three-dimensional structure of the designed vaccine construct resembles that of a globular protein, with no signs of prominent steric clashes or unusual chain distortions, implying that the vaccine construct has a high probability of retaining stability and maintaining proper folding under physiological conditions and therefore will be applicable to downstream immunoinformatics and docking analysis.

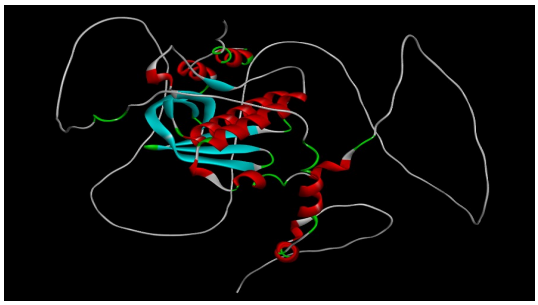


Fig. 4. Tertiary structure of vaccine construct

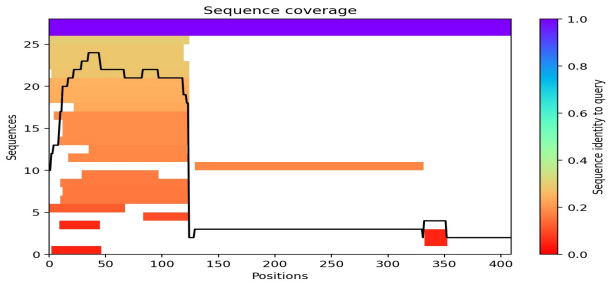


Fig. 5. Sequence coverage graph of vaccine construct

Sequence coverage graph of the vaccine construct showed that the N- terminal region (1–120) is well represented and conserved, indicating reliable

predictions while blank space signifies fewer homologs and reduced confidence (Fig 5).

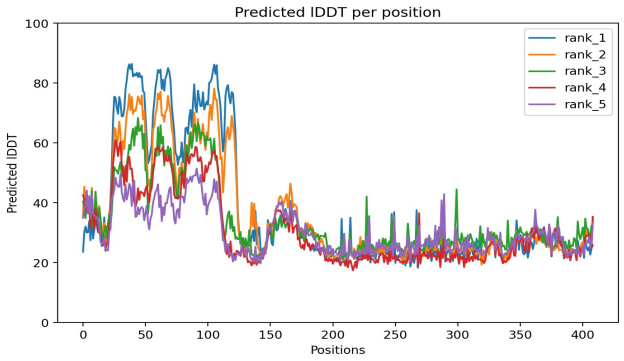


Fig. 6. Predicted IDDT of vaccine construct

This plot showed AlphaFold's predicted local accuracy (pLDDT/IDDT) for every position in protein across five ranked models. The first ~120 residues have high confidence (60–85), meaning a reliable and stable structure. After ~130, confidence drops to 20–40, indicating flexible, disordered, or poorly modeled regions. All five models showed the same pattern, confirming the reliability of the high-confidence N-terminal region. Overall, construct has a well-defined structured region followed by mostly flexible segments (Fig 6).

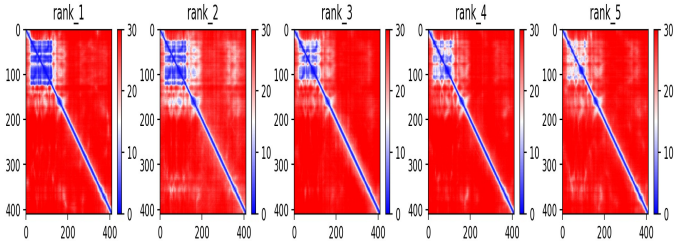


Fig. 7. Pae (Predicted aligned error) of vaccine construct

These PAE (Predicted Aligned Error) heatmaps showed how confidently AlphaFold predicts the relative positions of residues. The upper left region in the first ~120 residues indicated very low error and a well-defined, stable structure while rest of the region, after ~130 show high error, meaning these regions are flexible, disordered, or not reliably modeled. All five ranked models showed the same pattern, confirming a strong, stable N-terminal domain followed by loosely structured segments. The tertiary structure was refined with the help of Galaxy Refine and model score was recorded as 97% (Fig 7).

8. Ramachandran plot analysis of the model

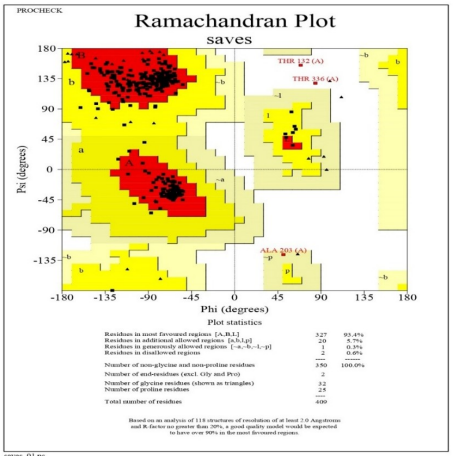


Fig.8. Ramachandran plot analysis

PROCHECK Ramachandran plot showed that 93.4% of the residues in the VZV vaccine construct land in the most favored regions—better than the usual 90% cutoff for high-quality models. That points to a stable, reliable structure. Another 5.7% of residues sit in allowed regions, and just 0.3% fall into generously allowed regions, so there's barely any structural deviation. Only two residues—just 0.6%—show up in disallowed regions, which is fine and doesn't mess with the model's stability (Fig 8).

9. Docking analysis

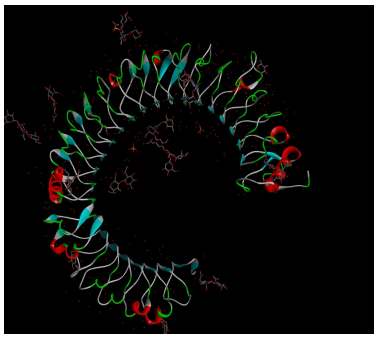


Fig.9. Receptor 2A0Z (Toll like receptor III)

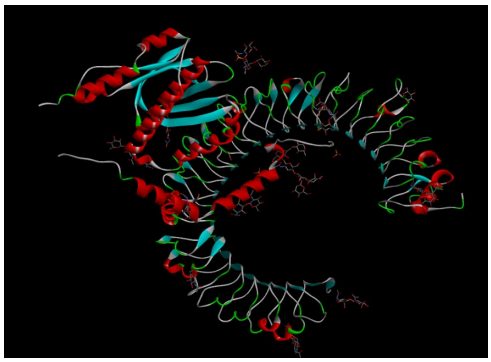


Fig.10. Model 1 of HDOCK Docking analysis

Toll-like receptor 3 (Fig 9) is used in construct for the VZV vaccine because this receptor specifically recognizes double-stranded RNA, which is a significant intermediate in the process of viral replication. This specificity leads to a robust antiviral innate and adaptive immune response, which makes the vaccine highly effective against the VZV virus.

This docked complex of receptor and ligands (Fig 10) was analyzed in Discovery Studio, which showed the final bound state between the receptor and ligand. The little clusters were noticed around the protein is the ligand or interacting residues, set in position based on how the model predicts they will bind.

Table 5. Summary of the top 10 models

Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-334.91	-324.31	-321.67	-311.57	-306.89	-303.03	-302.95	-298.21	-294.82	-294.49
Confidence Score	0.9758	0.9703	0.9687	0.9620	0.9584	0.9552	0.9552	0.9509	0.9477	0.9473
Ligand rmsd (Å)	81.55	97.25	91.69	99.46	90.43	83.55	92.98	62.15	96.05	53.36

The docking analysis turned up ten top-ranked models, with docking scores spread between -334.91 and -294.49 (Table 5). So a range of binding strengths between the ligand and the target were seen. The best model—Rank 1—scored -334.91, and its confidence score hit 0.9758. That is a strong signal that the predicted pose is reliable. Ligand RMSD values jumped around quite a bit, from 53.36 Å up to 99.46 Å, showing there's a fair amount of conformational change across these models. Still, all the top picks landed confidence scores over 0.94, which says a lot about how solid these docking predictions are. Out of the bunch, Rank 1 clearly stands out as the best candidate for further analysis.

10. Dynamic stability check of the docked protein

The dynamic stability of docked protein was checked with the help of *el Nemo*, where it showed the flexing and movement of different parts of the protein.

11. Codon optimization

A codon-optimized nucleotide sequence from a given protein sequence was generated with the help of J Cat Optimization tool (Fig 11) by considering the preferred codon usage for that organism. The JCat software produced a codon-optimized nucleotide sequence by optimizing key parameters, including GC content and Codon Adaptation Index (CAI), which increased the mRNA stability and translational efficiency and allowed for the removal of undesirable sequence motifs (i.e. ribosome binding sites) minimizing risk for possible cloning or expression issues.

>EMBOSS_001

ATGATCAAGCTGAAGTTCGGCGTGTTCTTCACCG
TGCTGCTGAGCAGCGCCTACGCCCAC

GGCACCCCCCAGAACATCACCGACCTGTGCGCC
GAGTACCACAACACCCAGATCTACACC

CTGAACGACAAGATCTTCAGCTACACCGAGAGCC
TGGCCGGCAAGAGGGAGATGGCCATC

ATCACCTTCAAGAACGGCGCCATCTTCCAGGTGG
AGGTGCCCGGCAGCCAGCACATCGAC

AGCCAGAAGAAGGCCATCGAGAGGATGAAGGAC
ACCCTGAGGATCGCCTACCTGACCGAG

GCCAAGGTGGAGAAGCTGTGCGTGTGGAACAAC
AAGACCCCCCACGCCATCGCCGCCATC

AGCATGGCCAACGAGGCCGCCGCCAAGGGCCAG
ACCAAGAACCCCCAGGTGAGCATCGCC

GCCTACAGCGTGAGCGACCAGCCCTACAACAGG
GCCGCCTACGACACCCACCTGAAGCTG

CAGTTCAGGGCCGCCTACGACAAGAACCCCAAGT
ACAACAGCGTGGCCGCCTACGACCTG

CTGGAGCCCTACTACGAGGACTACGCCGCCTACG
ACACCGTGGGCCAGGACGTGAACGCC

GTGGAGGCCAGCAGCAAGGGCCCCGGCCCCGG
CGCCGAGGTGCCCGTGACCAGCGAGAAG

GTGAACAAGAAGAGCAAGGGCCCCGGCCCCGGC
GCCAGGAGCTTCAGCAGCGACGACAGG

ACCAGGAAGAGCTACGACGGCCCCGGCCCCGGC
GCCGGCGTGGAGGCCAGGAGCATCTTC

CCCTTCGGCCTGAGGGACGGCCCCGGCCCCGGC
GCCCTGCTGACCAGCAGGCTGACCGGC

CTGGCCCTGAGGAACAGGGGCCCCGGCCCCGGC
AGCAGCCAGAACACCACCAGCACCCCC

CACACCGACACCGTGGGCCAGGACGTGAACGCC
GTGGAGGCCAGCAGCAAGGCCCCCCTG

ATCCAGGGCAGCACCGCCGACGACGCCGACATG
AAGAAGTACACCACCGGCGCCGAGGTG

CCCGTGACCAGCGAGAAGGTGAACAAGAAGAGC
AAGAAGTACTACAGCAAGTGGAGGAAC

AGGGACAGGCCCGAGTACAGGAGGAACCTGAGG
TTCAGGAGGTTCTTCAGCAGCATCCAC

CCCAACGCCGCCGCCGGCAGCGGCTTCAACGGC
AAGAAGAGGACCTGCCCCGACTACCAC

CTGGGCAAGCACCACCACCACCACCAC

Fig 11: Codon-optimized nucleotide sequence

12. CLONING THE IMPROVED DNA INTO pVAX1 VECTOR USING SNAP GENE TOOL

The sequence of the improved DNA insert (Fig 12) was prepared for insertion into the expression vector pVAX1 using compatible restriction enzyme sites as identified in SnapGene (Fig 13). Both the optimized coding sequence and pVAX1 contained KpnI and PstI restriction sites that allow for directional cloning. The final version of the insert-plasmid construct provided a detailed representation of the engineered pVAX1 insert plasmid to ensure the designed cloning strategy was structurally correct for subsequent expression experiments (Fig 14).

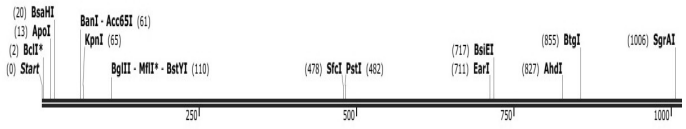


Fig.12. Improved DNA

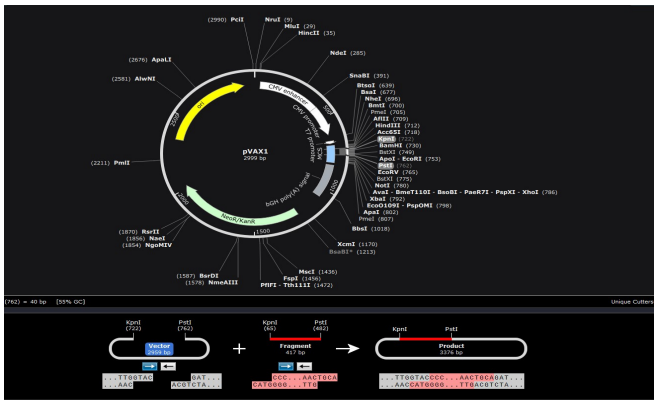


Fig 13: Cloning using Snap gene tool

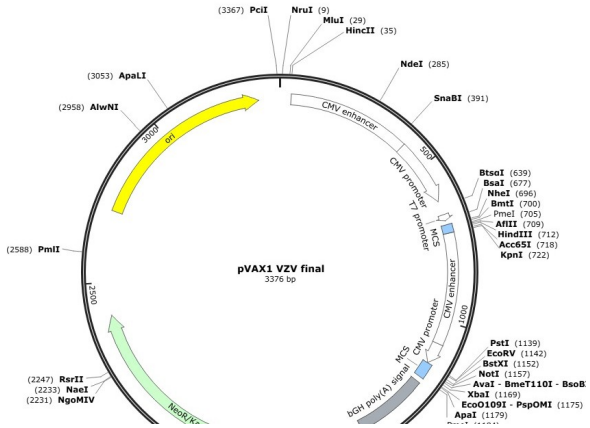


Fig. 14: Final cloned vaccine of VZV

Conclusion:

In this study, a multi-epitope vaccine against Varicella Zoster Virus (VZV) was successfully designed using immunoinformatics and structural bioinformatics techniques. Three highly conserved viral proteins (UL46, glycoprotein B, and major capsid protein) were selected for B-cell and T-cell epitope prediction. The selected B-cell and T-cell epitopes showed high antigenic properties, were non-allergenic, non-toxic, and provided broad population coverage.

The final vaccine construct demonstrated good physicochemical stability, high antigenic properties, non-allergen, non-toxic, proper secondary and tertiary structures, and high structural quality as confirmed by Ramachandran plot analysis. In molecular docking and dynamic simulations, strong and stable interaction with Toll-like receptor 3 (TLR-3) showed its potential to cause an effective immune response. Codon optimization along with in-silico cloning into the pVAX1 expression vector further confirmed its experimental potential. Overall, the developed vaccine demonstrates promise as a safe and efficient option against VZV and necessitates additional in-vitro and, in-vivo testing.

References:

Araf Y, Moin AT, Timofeev VI, Faruqui NA, Saiara SA, Ahmed N, *et al.*(2022). Immunoinformatic Design of a Multivalent Peptide Vaccine Against Mucor mycosis: Targeting FTR1 Protein of Major Causative Fungi. *Front Immunol.*13:863234.

Buchan DWA, Jones DT.(2019). The PSIPRED Protein Analysis Workbench: 20 years on. *Nucleic Acids Res*. 47(W1):W402–7.

Bui H-H, Sidney J, Dinh K, Southwood S, Newman MJ, Sette A. (2006). Predicting population coverage of T-cell epitope-based diagnostics and vaccines. *BMC Bioinformatics.*7:153.

Cohen, J. I., Brunell, P. A., Straus, S. E., and Krause, P. R. (1999). Recent advances in varicella-zoster virus infection. *Ann. Intern. Med.* 130, 922–932.

Daulagala S. L., and Noordeen F. (2018). Epidemiology and factors influencing varicella infections in tropical countries including Sri Lanka. *Virus*. 29: 277–284.

Dimitrov I, Bangov I, Flower DR, Doytchinova I. (2014).AllerTOP v.2--a server for in silico prediction of allergens. *J Mol Model.* 20(6):2278.

Doytchinova IA, Flower DR. (2007). VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics*.8:4.

Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, *et al*. (2005). Protein Identification and Analysis Tools on the ExPASy Server. In: John M. W (ed). *The Proteomics Protocols Handbook. Humana Press;* p. 571–607.

Grote A, Hiller K, Scheer M, Munch R, Nortemann B, Hempel DC, *et al*. (2005). JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. *Nucleic Acids Research*;33.

Käll L, Krogh A, Sonnhammer ELL. (2007). Advantages of combined transmembrane topology and signal peptide prediction--the Phobius web server. *Nucleic Acids Res.* 35.

Kennedy PGE, Gershon AA. (2018). Clinical Features of Varicella-Zoster Virus Infection. *Viruses.* 10(11):609.

Moin AT, Patil RB, Tabassum T, Araf Y, Ullah MA, Snigdha HJ, *et al.*(2022). Immunoinformatics Approach to Design Novel Subunit Vaccine against the Epstein-Barr *Virus. Microbiol Spectr.*10(5):e0115122.

Oliver S. L., XingY., ChenD.H., Roh S. H., PintilieG. D., Bushnell D. A., *et al*. (2021). The N-terminus of varicella-zoster virus glycoprotein B has a functional role in fusion. *PLoSPathog*. 17:e1008961.

Patronov A, Doytchinova I. (2013). T-cell epitope vaccine design by immunoinformatics. *Open Biol.* 3(1):120139.

Rathore AS, Choudhury S, Arora A, Tijare P, Raghava GPS. (2024). ToxinPred 3.0: An improved method for predicting the toxicity of peptides. *Comput Biol Med*. 179:108926.

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.

Shin WH, Lee GR, Heo L, Lee H, Seok C. (2014). Prediction of Protein Structure and Interaction by GALAXY Protein Modeling Programs. *Biodesign.* 2:1–11.

Suleman M, Khan SH, Rashid F, Khan A, Hussain Z, Zaman N, *et al*. (2023). Designing a multi-epitopes subunit vaccine against human herpes virus 6A based on molecular dynamics and immune stimulation. *Int J Biol Macromol*. 244:125068.

Testi I, Aggarwal K, Jaiswal N, Dahiya N, Thng ZX, Agarwal A, *et al*.(2021). Antiviral Therapy for Varicella Zoster Virus (VZV) and Herpes Simplex Virus (HSV)-Induced Anterior Uveitis: A Systematic Review and Meta-Analysis. *Front Med (Lausanne).*8:686427.

Tomar N, De RK. (2010).Immunoinformatics: an integrated scenario. *Immunology.* 131(2):153–68.

Tsirigos KD, Peters C, Shu N, Käll L, Elofsson A. (2015). The TOPCONS web server for consensus prediction of membrane protein topology and signal peptides. *Nucleic Acids Res.* 43(W1):W401–7.

Wiederstein M, Sippl MJ.(2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res.* 2007;35.

Yu C, Wu Q, Xin J, Yu Q, Ma Z, Xue M, *et al*. (2024) Designing a smallpox B-cell and T-cell multi-epitope subunit vaccine using a comprehensive immunoinformatics approach. *Microbiol Spectr*. 12(6):e0046524.

Zandi M., Shokri S., Mahmoudvand S., Hosseinzadeh Adli A., Mohammadi R., and Haddadi A. (2022). Interplay between cellular metabolism and DNA viruses*. J. Med. Virol*. 94, 5163–5173.

Zerboni L, Sen N, Oliver SL, Arvin AM. (2014). Molecular mechanisms of varicella zoster virus pathogenesis. Nat Rev Microbiol. 12(3):197–210.

Zheng Q, Wang D, Lin R, Chen Y, Huang H, Xu Z, *et al*. (2023). Mendelian randomization analysis suggests no associations of human herpes viruses with amyotrophic lateral sclerosis. *Front Neurosci*. 17:1299122.