Immunoinformatics guided design of a universal epitope-based vaccine against Kaposi Sarcoma

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Abstract

The Kaposi sarcoma-associated herpesvirus (KSHV) is a virus that is identified as a direct carcinogen, causes Kaposi sarcoma, primary effusion lymphoma and multicentric Castleman disease. Despite this, there is no permanent treatment. This work intended to develop a multi-epitope vaccine aiming KSHV's key glycoproteins involved in viral entry. After applying rigorous immunoinformatics study and numerous immunological filters, the multi epitope vaccine was created, comprising of potent CTL, HTL and BCL epitope. A series of computational evaluations established the general dependability of the putative vaccine, and a molecular dynamics simulation established the vaccine's overall stability. Docking experiments revealed that the vaccination can make stable interactions with Toll-Like Receptors. Codon optimization and insertion into the cloning vector revealed that the vaccine could be expressed proficiently in the *E. coli* expression system. Finally, an immune simulation was done to assess the vaccine's potency to trigger an immune response.

1. Introduction

The Kaposi sarcoma (KS) is the soft tissue tumor that previously gained widespread attention as an AIDS-defining disease [1], this human malignancy is also linked with other medical conditions such as multicentric Castleman's disease (MCD), inflammatory cytokine syndrome (KICS), primary effusion lymphoma (PEL), and immunosuppression regarding an organ transplant [2]. The virus Kaposi sarcoma-associated herpesvirus (KSHV) is certainly the causative agent of Kaposi sarcoma malignancy, it is formerly known as Human Herpesvirus-8 (HHV-8). HHV-8 is also recognized as a human carcinogen, according to Global cancer statistics 2018, it is responsible for about 40,000 cancer cases and 20,000 deaths worldwide each year [3], and especially the AIDS patients have a 1,00,000-fold increased risk of contracting KS than the general population [4]. As the risk of KS malignancy cases linked with HIV and lymphoproliferative disorders are not only significantly common, but these malignancies can also occur in the absence of these coinfections in any immune-compromised, malnourished, or solid organ transplant recipient infected with HHV-8 [5].

KSHV is a gammaherpesvirus with a double-stranded DNA virus. This is a lymphtropic virus that encounters lytic replication in epithelial cells comprising skin, blood vessels, and organs. These viruses can remain dormant in their host cells for an indefinite period of time. HHV-8/KSHV has seven recognized subtypes (A, B, C, D, E, F, and Z) [6]. The HHV-8 genome is around 170 kb in size and encodes about 81 ORFs [7]. HHV-8 encompasses multiple viral proteins that are crucial for infecting, establishing latency, and cancer development, including the major latency antigen latency-associated Nuclear Antigen-1 (LANA), Kaposins are latent proteins that play a critical role in tumor formation, viral IL-6 (vIL-6), Rta protein (ORF50), viral cyclin (v-cyclin), viral IL-6 (vIL-6) and viral Fas ligand inhibitory protein (v-FLIP) [8]. Human herpesvirus-8 is typically transmitted sexually, and infection occurs rarely before puberty [9]. In Uganda, seropositivity to HHV-8 was closely linked with the frequency of transfusions, with a calculated risk of infection of 2.6 percent per transfused blood component [10].

The treatment for standard Kaposi sarcoma is retroviral therapy. However, not all cases of KS cancer are identical, neither will all patients react to the same treatment. So, to decrease the development of Kaposi sarcoma in all HHV-8 positive individuals a targeted antiviral therapy is needed. Study found that antiviral treatment with the ganciclovir and foscarnet family of medications that are powerful inhibitors of herpesvirus DNA polymerase, was efficacious in reducing KS lesions in HIV affected and non-affected patients but not in all cases [11]. Martín-Carbonero *et al*; (2004); demonstrated that intravenous pegylated liposomal doxorubicin is more effective than highly active antiretroviral treatment (HAART) alone in treating AIDS-KS patients [12]. However, other studies have found that pegylated liposomal therapies such as Doxil and Paclitaxel are effective against KS but also have a high rate of toxicity [13]. Additionally, intravenous liposomal tretinoin treatment in AIDS-KS patients exhibited a number of side effects and a partial response to Kaposi sarcoma lesions [14]. In terms of Transplant patients with KS respond to immune reconstitution, consequently decreasing the immunosuppressive dosage enhances graft rejection. In this event rapamycin predominantly inhibits tumor growth, resulting in stable condition rather than tumor regression [15, 16].

Vaccinations are the most appropriate method to prevent viral infections, and multi-epitope vaccines offer a broader immune response spectrum than single-epitope vaccines. HHV-8 vaccination has not yet been documented. Immune responses are one of the most critical aspects in fighting tumor progression in Kaposi sarcoma patients. Traditional vaccine design approaches include the addition of excess antigenic load and raises the risk of poor immunogenicity also expense [17]. The use of peptide-based vaccines, which have highly specific immune responses, can overcome these limitations. Progress in computational biology aid in designing an active vaccine synthesis [18].

An ideal strategy for the prevention and treatment of viral infections or tumor, a multi-epitope vaccine capable of activating both humoral and adaptive immune responses [19]. Numerous *in silico* and immunoinformatics techniques for the prediction of antigenic epitopes have been developed, with potential translational applications, these vaccinomics approaches are used to construct a multi-epitope vaccine against HHV-8 [20]. To strengthen the immune response against HHV-8, the multi-epitope vaccine should trigger Cytotoxic T lymphocytes (CTL), Helper T lymphocytes (HTL), induce cytokine production and create long lasting memory.

2. Materials And Methods

2.1 Selection of Human Herpesvirus-8 strain and protein sequence retrieval

As a repository for biomedical and genetic data, the server National Center for Biotechnology Information (NCBI) [21] makes significant contributions to research and health. This was used to identify and analyze Human Herpesvirus-8 strains in addition to related data such as genus, family, transmission, host, illness, genome, and proteome. UniProtKB [22] contains a lot of biological information about proteins. The database had five types of the envelope glycoprotein sequence of the Human herpesvirus 8 type P strain that had already been reviewed. A flow chart that shows how immunoinformatics techniques can be used to make a multi-epitope-based chimera vaccine has been shown below (Figure 1).

2.2 Screening of antigenic proteins

The antigenicity of a protein was determined using the VaxiJen v2.0 tool [23]. A thorough investigation has been conducted on the antigenic and physicochemical characteristics of the sequences that were discovered. Proteins that were antigenic and had the appropriate characteristics were carefully chosen for further investigation.

2.3 T cell epitopes identification

It was necessary to utilize the NetCTL 1.2 server [24] in order to identify cytotoxic T lymphocyte (CTL) epitopes for the proteins of Human Herpesvirus-8 that were chosen. There is a sensitivity and specificity range of 54-89% and 94-99% respectively, for the epitope predicted by the server. CTL epitopes were identified in this work using twelve MHC class I supertypes, which were utilized in the search for CTL epitopes. They received scores of 0.05 for TAP transport efficiency, 0.15 for C terminal cleavage, and 0.75 for the epitope identification threshold, accordingly. It was only recently that such an approach was devised for class I immunogenicity using 9-mer peptides [25]. The epitope identification tool TepiTool [26] was used to discover 15-mer helper T lymphocyte (HTL) epitopes. The IEDB suggested 27 HLA class II reference alleles for MHC class II alleles [27] in order to identify MHC class II alleles. It was used to predict HTL epitopes based on the IC50 value, which was set to less than or equal to 250 nM by default, which was used to predict HTL epitopes.

2.4 Linear B cell epitope identification

B cell lymphocyte (BCL) epitopes are critical components of the immune system that trigger an adaptive immunological response in the body. They have the potential to be employed as vaccine building blocks because they stimulate humoral immunity via the secretion of antigen-neutralizing Immunoglobulins. Linear and conformational receptors are the two kinds of surface receptors for B-cell epitopes that could be found on B cells' surfaces. The BCPred server was used to predict linear B-cell epitopes with a specificity of 75% for 20-mer linear B-cell epitopes. A total of 10 different epitope sequences for glycoproteins were predicted [28].

2.5 Selection of anticipated epitopes for further study

2.5.1 Prediction of B and T cell epitope's toxicity, antigenicity, allergenicity, transmembrane and immunogenicity topology

For the purpose of selecting the most appropriate B and T-cell epitopes, the toxicity, allergenicity and antigenicity of previously predicted BCL, CTL and HTL epitopes were examined. Unlike previous versions of the software, VaxiJen v2.0 categorizes antigens purely on the basis of protein physicochemical features rather than on sequence alignment. As the target organism and a default threshold value were both utilized, we were able to predict the epitopes that would be most effective. For the purpose of estimating the allergenicity of our putative epitopes, we utilized the AllerTOP v2.0 server [29]. It was only employed for the non-allergen epitopes that needed to be assessed for toxicity, so ToxinPred was used. It is possible to determine epitopes' cytotoxic potential by using the ToxinPred database [30], which also contains information on other essential physicochemical features. The transmembrane helices in epitopes were highlighted using the TMHMM v2.0 program. This approach was used to determine whether a certain epitope was located inside or outside the helices [31]. In the end, the immunogenicity of the epitopes was analyzed by the use of the IEDB server [32].

2.5.2 Prediction of HTL epitopes induced by IFN-, IL-4, and IL-10

Immune cytokines (interferon- γ [IFN- γ], interleukin-4 [IL-4], and interleukin-10 [IL-10]), which stimulate macrophages, dendritic cells, and natural killer cells in response to viruses, bacteria, and parasites. Those are vital cytokines in the regulation of both innate and adaptive immune responses. Not all HTL epitopes have the ability to induce cytokine production, however. It follows that including HTL epitopes into the vaccine is a key step in increasing the potency of the vaccine. As a consequence, the IFNepitope server [33] was utilized to discover HTL epitopes that triggered IFN production. In accordance with the Motif and SVM approaches, the server predicts epitopes that induce IFN

production.IL4pred server [34] and IL10pred server [35] are two web-based prediction servers that are used to forecast cytokines IL-4 and IL-10, respectively, in humans. Both programs used the SVM-based default technique.

2.5.3 Population coverage and conservancy analysis of the epitopes

Due of the vast differences in immune profiles amongst populations, the multi-epitope vaccine should be efficient in combating the virus. As a result, the population coverage of the specified CTL epitopes was determined using the Population Coverage tool on the IEDB website [36].

Also, The conservancy analysis tool of IEDB was also used to identify the most conserved CTL epitopes across strains [37]. The conservancy was determined by focusing on the antigenic protein's identity. Epitopes with 100% conservation were chosen for vaccine development.

2.6 Construction of multi-epitope vaccine candidate

The epitopes that matched all of the parameters were utilized to build the vaccine design that was ultimately selected. Beginning with a Methionine at the Carboxy-terminal end of the recommended vaccine, which is then linked to a β defensin-2 adjuvant [38] (a TLR4 agonist) by an EAAAK linker. When used in conjunction with a vaccine, adjuvants may boost its immunogenicity. A short peptide (RKKRRQRRR) derived from the HIV-TAT protein [39] was inserted immediately after the adjuvant, which may improve cell penetration and facilitate in MHC class I processing. GGGS linkers were distributed throughout the chimera sequence in order to provide 3D structural stability. Conjugation of CTL epitopes is accomplished by the inclusion of an AAY linker in the contract [20]. In order to aid in the cellular processing of the vaccine, the unique linkers AAY and HEYGAEALERAG, which function as cleavage sites for the proteasomal and lysosomal systems, respectively, were inserted [40-42]. Another adjuvant RS-09 [43] was added after the last epitope to further boost immunity even more.

2.7 Analysis of immunogenic and physicochemical characteristics

A series of immunogenicity tests were performed using the VaxiJen 2.0, AllerTop 2.0, and ToxinPred servers in order to validate the immunogenic properties (antigenicity, allergenicity, and toxicity) of the newly proposed vaccine. Expasy's ProtParam tool was used to display the vaccine's functional characteristics based on the vaccine's molecular weight, aliphatic index, pI, hydropathicity, instability index, GRAVY values, and predicted half-life in *E. coli*. It is possible that insufficient protein solubility may prevent therapeutic protein expression in *E. coli*, and it is most probable that this will be the most common reason for insufficient protein production, which would significantly increase the expense of experimental research. To analyze the solubility of the designed vaccine, the researchers employed SoluProt 1.0, a freely available web server [44].

2.8 Prediction of the secondary structure

For the secondary structure prediction of the proposed vaccine, PSIPRED 4.0 was utilized [45]. With the vaccine sequence as a guide, it predicts the formation of the helix, sheet, and coil structures in the vaccine candidate sequence.

2.9 Prediction of the tertiary structure and refinement of the vaccine

A 3D structure of the intended vaccine was created with the aid of the Robetta web server [46], which was necessary due to a lack of predesigned structures. RoseTTAFold made use of deep learning to correctly predict the three-dimensional structure of every input sequence that was given to it. Robetta created five models on the basis of amino acid sequence that was provided. The model with the lowest estimated error was selected for examination, and it was further refined using the GalaxyRefine option on the GalaxyWEB server [47] to get the lowest possible error estimate. GalaxyRefine runs MD simulations on the structure in order to continually perturb it and subsequently relax it, as seen in the following figure: It was decided which model will be used from five updated models based on the lowest MolProbity Clash score. The original and updated structures were structurally validated using the SAVES v6.0 webserver's ERRAT [48] and PROCHECK [49] options. PROCHECK contains a Ramachandran plot, which allows for the straightforward presentation of residues in the most favorable, considerably acceptable, widely permitted, and disallowed regions in order to evaluate the validity of the vaccine model. Further, with the aid of the ProSA webserver, it was possible to evaluate the likelihood of structural and modeling mistakes in the refined mode [50].

2.10 Docking with the TLR4 receptor

The proposed vaccine's affinity for TLR4 was evaluated via the use of molecular docking. The three-dimensional structure of TLR4 (PDB ID: 4G8A) Downloaded from the RCSB PDB site [51] and prepared for docking using the Discovery studio software. In order to dock with the vaccine, only the "A" Chain of TLR4 was preserved. Docking was achieved via the use of the online docking server ClusPro 2.0 [52]. ClusPro constructs docking complexes in three steps: rigid body docking using billions of conformational data, grouping the 1000 lowest energy structures by RMSD, and refining the selected structures using energy reduction. A second docking server, Hawkdock [53] was used for docking and MMGBSA analysis to further increase the confidence in the studies. The complex with the highest ranking was selected, and the subsequent protein-protein interaction of the docked complex was analyzed using another web-based resource, PDBsum [54].

2.11 Molecular dynamics simulation

Molecular Dynamics simulation is a great computational tool for studying a protein's structural stability at the atomic level [55]. In this study, we used the GROMACS [56] software package (version 2021.04) to conduct an MD simulation of our constructed vaccine using the final pdb file. For this, first, the protein was solvated in a cubic box with SPC/E water molecule using the CHARMM36 force field [57]. The whole system was optimized by using proper periodic boundary conditions and neutralized by adding 3 chloride ions. The entire system was then equilibrated using the steepest descent approach with 5000 iterations for 1ns using canonical ensembles (NVT and NPT). Finally, the MD simulation was executed for 100ns by maintaining Berendsen thermostat [58] with no pressure coupling at a constant 300K temperature and 1atm pressure. The last step of MD simulation includes the analysis RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation) and Rg (Radius of gyration) of the protein from the simulation trajectory.

2.12 Immune simulation

To further validate the immunogenicity and immune reaction status of the proposed vaccine, in silico immune simulations were executed using the C-ImmSim server [59]. C-ImmSim is an agent-based method that predicts immune epitopes by using a position-specific scoring matrix (PSSM) and immune interactions by using machine learning strategies. This server also simulates the three vital functional elements of the mammalian system: bone marrow, lymph node, and thymus [59]. In this study, Random seed was set to 12345 while simulation volume and simulation step were set to 10 and 1050, respectively. Each of the three injections was given at a four-week interval, with the time steps of 1, 84, and 168. The remaining parameters were kept at their default settings [60].

2.13 Codon optimization for in-silico cloning of the constructed vaccine

The procedure of reverse translation and adaptation of multi-epitope vaccine codons was carried out using the Java Codon Adaptation Tool (JCat) [61] in order to maximize vaccine production in a suitable host organism. The host organism for the projected vaccine candidate's expression was determined by E. coli K12 strain in this study. In addition, the server response contains a codon adaptation index (CAI), the cDNA sequence, and the ratio of GC content. Finally, using the SnapGene software, the EcoRI restriction enzyme was introduced to the 5' end of the vaccine nucleotide sequences as an initial cleavage site, and BamHI was added to the 3' end of the vaccine nucleotide sequences, and the resulting cloning vector pET-28 (+) was used to incorporate the vaccine nucleotide sequences.

3. Results

3.1 Protein sequence retrieval and estimate of antigenicity

The five Envelope glycoproteins (gB, gM, gN, gH, gL) of the Human Herpesvirus 8 type P (isolate GK18, strain HHV-8) were extracted from UniProtKB and used to predict B and T-cell epitopes in order to create multi-epitope vaccine sequences. The structural proteins were predicted to be highly antigenic by VaxiJen server, with antigenicity values of 0.4483, 0.5387, 0.5099, 0.5391, and 0.6528 respectively, all exceeding the default threshold value of 0.4.

3.2 Cytotoxic and Helper T-cell epitopes prediction

3.2.1 Potential cytotoxic T lymphocyte epitopes

Each cytotoxic T lymphocyte (CTL) epitope was 9 amino acids in length, and a total of 1055 distinct CTL epitopes were listed utilizing the Glycoprotein sequences through the NetCTL v1.2 platform. Among these CTL epitopes, 19 were found highly antigenic, non-allergic, non-toxic, show outside topology, and immunogenic. Based on 100% conservation and binding affinity of epitopes with MHC-I alleles, a total of ten CTL epitopes (Table 1) were selected among those potential candidates.

Table 1
Final epitopes of CTL for multi-epitope based vaccine formation

Protein	Epitope	Position	NetCTL Score	Antigenicity	Immunogenicity	Allergenicity	Toxicity	Topology	Conservancy
gB	IVIAIILII	741	0.8967	0.5861	0.40585	NA	NT	Outside	100%
gB	LATLGTVIL	7	0.8898	0.4536	0.18358	NA	NT	Outside	100%
gH	MPTFTTVGY	356	0.9664	1.1929	0.25938	NA	NT	Outside	100%
gH	VPLPHVTYI	569	1.0572	0.6850	0.0862	NA	NT	Outside	100%
gL	FLVAVVRRRL	83	1.1278	0.5869	0.20575	NA	NT	Outside	100%
gL	FAVHSIHFA	41	0.8187	1.2734	0.10583	NA	NT	Outside	100%
gM	KLLFVAVIM	16	0.7659	0.5995	0.30383	NA	NT	Outside	100%
gM	LALTVVWYI	249	0.9807	0.9868	0.2993	NA	NT	Outside	100%
gN	ATFFYLVL	90	1.1841	0.6524	0.17444	NA	NT	Outside	100%
gN	TASTVALAL	2	1.176	0.7568	0.07589	NA	NT	Outside	100%

3.2.2 Prediction of helper T lymphocyte epitopes

A total of 1489 epitopes were identified in the Glycoproteins, but only 7 distinct (15-mers) epitopes for helper T lymphocyte (HTL) were extracted from the Glycoproteins sequences (Table 2). In addition to other criteria, they were chosen for their low IC50 value (250 nM), antigenicity, non-allergenicity, non-toxicity, outer topology, IFN- γ positivity, and ability to induce HTL epitopes in response to interleukin-4 [IL-4] and interleukin-10 [IL-10] cytokines.

Table 2
Final epitopes of HTL for multi-epitope based vaccine formation

Protein	Position	Epitope	IC50	Antigenicity	Allergenicity	Toxicity	IFN- γ	IL-4	IL-10
gM	149-163	KHVLMAAFVYCIHFC	110.28	0.4872	NA	NT	Positive	Inducer	Inducer
gL	37-51	LPLFFAVHSIHFAADP	9.22	0.9577	NA	NT	Positive	Inducer	Inducer
gH	503-517	FLGLRYDLHPAKLRA	31.09	1.0503	NA	NT	Positive	Inducer	Inducer
gH	495-509	IYRAFSPCFLGLRYD	39.44	1.1001	NA	NT	Positive	Inducer	Inducer
gH	302-316	AQEAPDIFLVLFQM	136.4	0.4019	NA	NT	Positive	Inducer	Inducer
gB	14-28	ILLVCFCAGAAHSRG	41.74	0.73	NA	NT	Positive	Inducer	Inducer
gB	205-219	AEPGWFPGIYRVRTT	234.94	0.7156	NA	NT	Positive	Inducer	Inducer

3.3 Prediction of line B-lymphocyte epitopes

According to preliminary findings, there were a total of 31 epitopes for B-cell lymphocytes (BCL), with just one of them meeting the requirements, which included being antigenic, non-allergenic, and nontoxic. However, just one B-cell epitope (Table 3) was utilized in the vaccine composition, and it was also identified in the vaccine composition's outer topology as well.

Table 3
Final epitopes of BCL for multi-epitope based vaccine formation

Protein	Position	Epitope	Score	Antigenicity	Allergenicity	Toxicity	Topology
gN	43-62	SARSPGPTSVTTPGFYDVA	1	0.8762	NA	NT	Outside

3.4 Population coverage and conservancy analysis of epitopes

A universal vaccination with a wide range of coverage needs epitopes that are conserved across several strains. It was decided to utilize the IEDB Epitope Conservancy Analysis tool to evaluate epitopes across different strains of Human herpesvirus 8 type P with a sequence identity threshold of 100%. To construct a vaccine, only CTL epitopes with 100% conservation were considered for inclusion. It was necessary to use the IEDB MHC-I Binding Prediction tool to find the alleles that would be most likely to bind with each predicted CTL epitope in order to assess the population encompassed by those epitopes.

The IEDB MHC-I Binding Prediction tool was used to find the alleles that may optimally associate with each anticipated CTL epitope. They had the best coverage for Europe (99.45%) and the lowest for Central Africa (85.24%). Other large areas got over 90% coverage (Figure 2).

3.5 Construction of multi-epitope vaccine sequence

The developed vaccine includes 18 of the most effective epitopes from three classes; 10 CTL, 7 HTL, and 1 B-cell selected from a pool of predicted epitopes. The CTL, HTL, and B-cell epitopes were connected together using the EAAAK, GGGS, and AAY linkers. With the goal of boosting immunogenicity, the vaccine sequence was supplemented with the human β defensin-2 adjuvant, the PADRE sequence, the TAT peptide, and the one-of-a-kind linker "HEYGAELERAG," among other components. Ultimate length of the vaccine was found to be 402aa (Figure 3).

3.6 Physiochemical properties, antigenicity, allergenicity, toxicity, solubility prediction of vaccine candidate

According to the results of the ProtParam study (Table 4), the estimated molecular weight of the vaccine candidate was 42990.32 Da (43 kDa) and the isoelectric point was 9.73 Da (43 kDa). There are a total of 18 negative amino acid residues (Asp + Glu) and 43 positive amino acid residues (Arg + Lys) in this polypeptide. A number of additional criteria were taken into account, including the aliphatic index and the grand average of hydropathicity (GRAVY) value, both of which were determined to be 89.28 and 0.267, respectively, and were found to be significant. Moreover, along with the physicochemical features of the constructs, immunological efficiency also has been evaluated. There was an antigenicity score of 0.5359 (antigenic) for the suggested sequences, as well as non-allergenicity and a solubility score of 0.768 for the proposed vaccine sequences.

Table 4
Physicochemical features and antigenicity, allergenicity, toxicity of the vaccine

Properties	Assessment
Number of amino acids	402
Molecular Weight	42 kDa
Theoretical pI	9.73
Atomic composition	C ₁₉₈₅ H ₃₀₄₈ N ₅₄₀ O ₅₀₀ S ₁₆
Estimated half-life (in <i>E. coli</i>)	>10 hours
Aliphatic index	89.28
Instability index	36.20 (Stable)
Grand average of hydropathicity (GRAVY)	0.267
Antigenicity	0.5359
Allergenicity	Non-allergen
Toxicity	Non-toxic
Solubility	0.768 (Soluble)

3.7 Prediction of secondary structure

The α -helix, β -strand, and random coil characteristics of the final vaccine candidate were analyzed using the PSISPREP server. On the server, it was determined that the vaccine had 46.52% of the residues that were in the α -helix conformation, 13.93% that were in the β -strand conformation, and 39.55% that were in the coil structure conformation. Figure 4 shows the structural properties in the alpha-helix, beta-strand, and random coils of the protein.

3.8 Tertiary structure, refinement, and validation analysis of the final candidate vaccine

Robetta generated five 3D structural models for the supplied protein sequence. The first predicted model (Figure 5) was chosen for further investigation since it had the lowest estimated error rate. The GalaxyRefine was also employed to improve the previous 3D model obtained

from Robetta. After the refining process, the fraction of residues within the ideal region has increased, resulting in five better models being produced. From there, the third model that featured the lowest MolProbity and Clash scores were retrieved as the chosen vaccine model because it was relatively better than all other models. To validate the refined 3D structure, the Procheck tool in SAVES.v6 server was used. Detailed analysis of the Ramachandran plot of the final modeled vaccine illustrated that the protein has residues of 94.8% in the most favorable areas, 3.7% in the additional allowed areas, 0.9% in generously allowed areas, and 0.6% in disallowed areas. Each value is far better than the Ramachandran plot evaluation of the initial model, which has 84.5% in favorable areas, and 14.1% in allowed areas.

3.9 Molecular docking of refined protein with immune receptor TLR-4

To analyze the binding interactions between the constructed vaccine and TLR4, docking simulation was done through the online ClusPro docking server. Cluspro produced 29 clusters for the docked complex. Cluster 0 had the lowest energy score (-1424.5) and was selected for further evaluation. PDBsum analysis (Figure 6) showed that the vaccine-TLR4 complex formed 18 hydrogen bonds, 6 salt-bridges, and 193 non-bond interactions. MMGBSA Analysis from HawkDock showed the free binding energy to be -67.02 (kcal/mol) which is again an indication of stable complex formation.

3.10 Molecular dynamics analysis of final vaccine candidate

The simulation trajectory was used to calculate the RMSD, RMSF values of the vaccine. RMSD is used to examine whether or not a protein is stable by computing the Deviations from its beginning position to its final conformation. The RMSD analysis depicted in the Figure shows initial fluctuations of the protein till 20ns of the simulation, after that the protein reached an equilibration. The overall RMSD value (Figure 7) of the vaccine stayed within the 0.8-1.2nm region without any extreme fluctuations throughout the simulation. This indicates the protein to be structurally stable during the simulation. RMSF analysis from the simulation can help to explain the average fluctuation of amino acids over time. The RMSF values (Figure 8) again remained consistent throughout the simulation, with no unexpected fluctuations. The RMSF values stayed between 0.2nm to 0.5nm throughout the simulation indicating the absence of any unwanted extreme flexible regions.

3.11 In silico immune response simulation

Results from C-ImmSim showed that the immune response in the second and third reactions was relatively high compared to the first dose of the vaccine as expected. Increased numbers of Natural Killer cells and Macrophages were observed throughout the simulated time period (Figure 9A). Antigen concentrations were reported to be high during the primary immune stimulation responses.

There was a comparable increase in T-cytotoxic cell and Th cell reactions with the formation of memory (Figure 9B). Based on Figure 9B, there was a considerable upsurge in immunoglobulin activities (IgM + IgG, IgM, IgG1+IgG2) with rapid antigen clearance. A significant increase in the population of B-cells, including memory B-cells after vaccination, suggests the possibility of isotype switching and the formation of long-term memory and long-lasting immune response. IFN- γ , TGF- β , IL-10, and IL-12 levels were also substantially higher in secondary and tertiary responses than in primary ones, indicating a strong immune response.

3.12 Codon optimization and silico cloning process of the final candidate

According to the results of the study, the customized nucleotide sequence of the vaccine construct has a CAI score of 0.972 (codon adaptation index) and is highly expressive in the E. coli strain K12. The range of 0.8-1.0 is considered to be a desirable value for the codon adaptation index. Furthermore, the concentration of GC (Guanine-Cytosine) was determined to be 56.05%, which is within the acceptable range of 30–70% for effective translation, according to the findings. The predicted DNA sequences for the vaccine were cloned into pET-28a(+) cloning vector plasmids using the SnapGene program, with EcoRI serving as the beginning site restriction enzyme and BamHI serving as the end site restriction enzyme. The cloned plasmid, which contained the vaccine DNA sequences that had been created, was discovered to be 6575 bp in length (Figure 10). It is indicated in red, along with restriction sites. 6x histidine residues and thrombin are found in this plasmid, which will aid in the purification of the vaccine and its subsequent downstream processing.

4. Discussion

Kaposi sarcoma cancer is a cancer that affects people who have a weakened immune system. It is a life-threatening illness that affects adults in particular. The standard retroviral and anticancer drugs, including as chemotherapy, are used to treat KS cancer all over the world, although they are not always effective in treating it. Aside from that, chemotherapy with pegylated liposomal doxorubicin or paclitaxel is the most often used treatment for advanced-stage KS cancer, despite the fact that it is infrequently effective [1]. Due to the fact that Kaposi sarcoma cancer is not similarly expressed in all instances, those various therapeutic approaches provide very limited protection against Kaposi sarcoma cancer in adults, who account for the majority of Kaposi sarcoma cancer cases globally. As a consequence, a new

candidate vaccine against HHV-8 is urgently required, one that can protect a large proportion of the population while also providing good results against the virus. The advancement of reverse vaccinology, as well as the availability of genomics and proteomics information, aid in the formulation of vaccines. Additionally, as compared to conventional vaccine design, effective adoption of bio-informatics techniques is advantageous [62]. Because it has the potential to be utilized for in-silico epitope prediction the identification of immunogenic antigens is a critical stage in the vaccine design process [63]. Immunogenic antigens are distinguished by their ability to adhere and stimulate immune cells, as well as their ability to function as immuno-dominant antigens [64]. We used computational methods to conduct an organized and comprehensive evaluation of the five surface proteins of virus glycoproteins (gB, gM, gN, gH, and gL) that make up HHV-8 vaccines. Our study team predicted in total of 18 (CTL, HTL and linear BCL) epitopes using bioinformatics approaches, which we think may have the capacity to induce both cellular and humoral response [63]. The construction of multi epitope vaccine relies on the prediction of Human Herpes Virus-8 (B-cell and T-cell) epitopes sequences, which are important in the development of biological and cellular functions as well as it predicted to be stimulating for innate and adaptive immunity in vitro. Vaccine design relies on BCL epitope prediction [65], as it distinguish the antigen–antibody interaction site[66]. According to the findings of this study, a 20-mer linier B-cell epitope was predicted using the BCPred server. It is important for T-cell epitopes to be found in order for adaptive immunity to initiate. The NetCTL and Tepitool servers predicted 9-mer CTL and 15-mer HTL epitope that triggered adaptive immunity functions appropriately with MHC molecules [67]. So, finding epitopes that can be cooperated by MHC is very important in predicting which T-cell epitopes are potent [26]. Additionally, identification of CD4+ and CD8+ T cells is crucial for designing vaccines based on multiple epitopes [68].

Finally, our predicted BCL, CTL and HTL epitopes from extracted glycoprotein sequences and linked together with AAY and GGGs linkers [69] to develop an multi-epitope based vaccine. To ability to develop immune response in vaccine sequences, GGGs, GP GPG, and AAY linkers were incorporated between chosen epitopes, allowing for the systematic construction of a candidate vaccine sequence [70]. Additionally, the EAAAK linker joined among the epitope's amino acid sequences and the adjuvants to optimize vaccine production and bioactivity. The MHC Class I, MHC Class II, IFN- γ , IL-4, IL-10, and line B cell epitopes were identified by assessing various physicochemical and structural parameters of the produced vaccine. Lower allergenicity and higher antigenicity in the protein vaccine has increased its effectiveness as a potential vaccine. Adjuvants are used to balance the lower immunogenicity in construct [71]. The multi - epitope vaccine design has a molecular weight of 43kDa and a calculated pI of 9.73, indicating that it is alkaline. The aliphatic index indicates that the protein contains aliphatic side chains, indicating probable hydrophobicity. The score has been assessed to be highly soluble following expression and to have a high immunogenicity. Primarily, the solubility of overexpressed recombinant proteins in the E. coli host is crucial for several biochemical and functional investigations [72].

Then secondary and tertiary structures for the vaccine candidate sequence were predicted. Secondary structure analysis revealed that the protein was composed mostly of 46.52% α -helices and 39.55% coils. The unfolded protein domains and α -helical coiled-coil peptides have been suggested to be antigenic in structure. Both structural categories have been demonstrated to fold into the recipient's natural forms, enabling them to be identified by antibodies produced in reaction to infection [73]. The tertiary structure of the vaccine candidate has significantly improved during refining and exhibited suitable properties as determined by the detailed Ramachandran plot data. The Ramachandran plot reveals that 94.8% of residues originate in preferred areas, while 3.7% additional allowed areas with less 0.9% residues generously allowed areas; this implies that the overall model's value is acceptable. Screening for immunoreactivity by serological studies is a critical aspect of validating a potential vaccine [71]. Additionally, to ascertain the vaccine's ability to bind to TLRs expressed on various immune cells, the docking of the candidate vaccine was done with TLR-4. The findings indicated that the developed vaccine binds well to TLR-4 and has a strong probability of inducing both innate and adaptive immune responses.

Molecular dynamics simulations were utilized to further assess the stability and dynamics of the TLR-4 and vaccine docking complex, with the RMSD and RMSF plots reflecting the complex's steady state binding properties [74]. The immune simulation data showed an increase in memory B-lymphocytes and helper cell lines following repeated antigen exposure. A multi-epitope vaccination induced antigen-specific immune responses against KSHV. Other innate immune cells, such as macrophages and dendritic cells, also performed well in our study.

It is necessary at this point to express the recombinant protein in an appropriate host in order to proceed. The E. coli K12 strain expression methods are the favored choice for recombinant protein expression [75]. Both the GC content 56.05% and the CAI score 0.972 were advantageous for high-level protein expression and optimization in bacteria. The next step is to produce this peptide in a bacterial system and perform the various immunological assays necessary to corroborate the immunoinformatics study findings.

5. Conclusion

The causative agent, Human Herpes Virus-8 (HHV-8), causes cancer when a patient's immune system is unable to fight against the virus, particularly immune impaired HIV patients and organ transplant recipients. This illness deteriorates the patients' condition at a time when medication is ineffective. While people suffer from Kaposi sarcoma malignancy, there is now no pharmacological option accessible to treat a

broad spectrum of KS patients. Novel vaccine candidates that cannot be obtained only via preclinical, in vitro, or animal trials. We used computational methodologies to produce a multi-epitope vaccine against HHV-8 that is highly immunogenic and has the required features for use as a carrier vaccine. Numerous techniques were used rigorously to generate a multi epitope vaccination. The evaluation of population coverage and conservancy, molecular docking, molecular dynamics simulations, and immunity simulations demonstrates that the newly designed vaccine is well accepted and safe in all parameters. Now, our chimeric multi epitope vaccination must be analyzed in the laboratory to see if it is suitable for real-world use in the treatment of Kaposi sarcoma cancer.

Declarations

Author Contributions

Sumaiya: Methodology, Experiment Design, Data curation, Manuscript Writing, Molecular Dynamics Simulation

Mahmudul Hasan: Data curation, Results analysis

Md. Mashiur Rahaman: Experiment Design, Manuscript Writing, Molecular Dynamics Simulation

G. M. Nurnabi Azad Jewel: Project Supervision, Formal analysis Data curation

Hammadul Hoque: Conceptualization, Supervision, Formal analysis, and Reviewing

Conflict of Interest

The authors declare no conflict of interest.

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Figures

Figure 1

Process flow diagram

Figure 2

Global coverage of selected CTL epitopes

MEAAAKRKKRRQRRRGGGSGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKCCR
 RKKEAAAKAKFVAAWTLKAAAGGGSIVIAIILIIAAYLATLGTVILAAYMPTFTTVGYAAYVPLP
 HVTYIAAYFLVAVVRRLLAAYFAVHSIHFAAAYKLLFVAVIMAAAYLALT VVWYIAAYATFFYL VYL
 AAYTASTVALALHEYGAELERAGKHVLM AAFVYCIHF CGPGPGLPLFFAVHSIHFA DP GPGP
 GFLGLRYDLHPAKLRAGPGPGIYRAFSPCFLGLRYD GPGPGAQE AAPDIFLVLFQM GPGPGIL
 LVCFCAGAAHSRGGPGPGAEPGWFPGIYRVRTTKKSARPSPGPTSVTTPGFYDVAEAAAKAPP
 HALSHEYGAELERAGHHHHHH

Figure 3

An overview of the vaccine candidate sequence having 10 CTL, 7 HTL, and 1 BCL epitopes, with an EAAAK linker attached to the N-terminal Methionine. The AAY, GPGPG, and KK linkers were connected to the CTL, HTL, and BCL epitopes respectively.

Figure 4

Graphical illustration of the secondary structural characterization of the multi-epitope vaccine. The anticipated protein has 46.52% alpha helical formation, 13.93% beta-strand, and 39.55% coil structure.

Figure 5

(A) Refined Tertiary structure of the designated vaccine. Ramachandran plot of the initial modelled vaccine (B) and after refinement (C).

Figure 6

Analyses of the docked complex (A) 3D structure (B) Interface statistics synopsis (C) Bond interaction between TLR4 (chain-A) receptor residues and vaccine (chain-B) residues

Figure 7

RMSD plot from dynamics simulation showing the structural stability

Figure 8

The RMSF plot exhibiting no extreme fluctuations

Figure 9

Vaccine immune response simulations. (A) NK cells and macrophages were adequately activated. (B) C-ImmSim server showed an adaptive immune response comprising cytotoxic T cell, T helper, B cell, and antibody responses as well as cytokine generation following vaccination doses.

Figure 10

The vaccine nucleotide sequences were cloned into the pET28a (+) expression vector using EcoRI (192 bp) and BamHI (1398 bp) as start and end sites. Red region indicate gene product of the vaccine and black indicates the rest of the plasmid.