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ORIGINAL ARTICLE

Immune epitopes identification and designing of a multi-epitope vaccine against bovine leukemia virus: a molecular dynamics and immune simulation approaches

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Abstract

Background Bovine leukemia virus (BLV) is an oncogenic delta-retrovirus causing bovine leucosis. Studies on BLV have shown the association with human breast cancer. However, the exact molecular mechanism is neither known nor their appropriate preventative measure to halt the disease initiation and progression. In this study, we designed a multi-epitope vaccine against BLV using a computational analyses.

Methods Following a rigorous assessment, the vaccine was constructed using the T-cell epitopes from each BLV-derived protein with suitable adjuvant and linkers. Both physicochemistry and immunogenic potency as well as the safeness of the vaccine candidate were assessed. Population coverage was done to evaluate the vaccine probable efficiency in eliciting the immune response worldwide. After homology modeling, the three-dimensional structure was refined and validated to determine the quality of the designed vaccine. The vaccine protein was then subjected to molecular docking with Toll-like receptor 3 (TLR3) to evaluate the binding efficiency followed by dynamic simulation for stable interaction.

Results Our vaccine construct has the potential immune response and good physicochemical properties. The vaccine is antigenic and immunogenic, and has no allergenic or toxic effect on the human body. This novel vaccine contains a significant interactions and binding affinity with the TLR3 receptor.

Conclusions The proposed vaccine candidate would be structurally stable and capable of generating an effective immune response to combat BLV infections. However, experimental evaluations are essential to validate the exact safety and immunogenic profiling of this vaccine.

Keywords Bovine leukemia virus · T-cell epitopes · Vaccine design · Immune simulation · Molecular dynamics simulation

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Abbreviations

BLV	Bovine leukemia virus
CTL	Cytotoxic T lymphocyte

E. coli	*Escherichia coli*
GC	Guanine and cysteine
GRAVY	Grand average of hydropathicity
HTL	Helper T-lymphocyte
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-4	Interleukin-4
IL-10	Interleukin-10
II	Instability Index
MD	Molecular dynamics
MHC	Major histocompatibility complex
MW	Molecular weight
NCBI	National center for biotechnology information
TCRs	T-cell receptors
TLR3	Toll-like receptor 3
SOPMA	Self-optimized prediction method with alignment

Introduction

Bovine leukemia virus (BLV) is the etiologic agent of enzootic bovine leucosis, is closely related to human T-cell leukemia virus type 1 and 2 (HTLV-1 and HTLV-2), and causes the most common neoplastic disease of cattle [1, 2]. The pestilence depredation of BLV infection is extremely prevalent, appears in several regions of the world, and induces major commercial losses in dairy production and export business [3]. Erskine and Bartlett et al. had reported that BLV infections are associated with herd-level in high-performing dairy herds and cow longevity [4]. In 1995, because of BLV infection in USA, the amount of losses in the dairy industry was reported to $525 million annually [3]. Previous study had been reported that the economic loss per case of lymph sarcoma was estimated to be $412 [5], but there are no appropriate preventative measures to halt the disease initiation and progression. Also, BLV was highly associated with breast cancer tissues by white blood cells [6]. Recent studies indicate that a handful of evidence of BLV infection in breast tissue and approximately 18% of viral infection causes cancer patients to die worldwide [7]. In the absence of oncogenic property, BLV can integrate itself into the host cell genome which further disrupts the host gene but not the suppression of viral gene expression [8]. BLV infection is probably a human infection that transmits from dairy animals to humans as a Zoonotic infection. Despite having no proper investigation or public data about the chance of human to human transmission, the countries with the highest ingestion of dairy foodstuffs have the highest incidence of breast cancer which has been determined for decades; most probably transfer through blood and breast milk [9].

The structural gag-pol and env genes of BLV are responsible for the synthesis of this virus [10, 11], in which the gag gene elicits the precursor Pr45 Gag, forms three mature proteins [10, 12]. The matrix protein binds viral genomic RNA and interrelates with the lipid bilayer of the viral membrane [13]. BLV capsid protein is found in the serum of infected animals with high antibody titers and considered as the major target for the host immune response [14], and the nucleocapsid protein affix to the packaged genomic RNA [15]. The env gene encodes envelope glycoproteins which cleaved into a mature surface glycoprotein chain and transmembrane protein chain that are responsible for the fusion of virus membrane with the host cell membrane, and originate host–virus interaction [11].

Cytotoxic T lymphocyte epitopes [16] and CD4+ T-cell epitopes were found to diverge in cattle, mice, and sheep from the previous findings [17–19] by epitopes mapping using cattle with experimentally infected BLV and a library of overlapping peptides of BLV. The vaccine provides acquired immunity against contagious diseases like different types of viruses causing cancers [20]. Vaccines are designed to form an immune response against a harmful fatal foreign microbe and make a pre-preparation to invade those particles, to inhibit its toxicity or arise the assassination activity against the microbes. The vaccine was made an inhibitory memory response against furthermore similar microbe's effect on the body [21]. According to the previous investigation, a vaccine can prevent a future outbreak of those virus-associated natural microbes like viruses [22]. In previous vaccine, construction against metastatic breast cancer provides a direction to develop a peptide-based vaccine against BLV to prevent its infection of cattle's and human breast cells [23]. Previously, several attempts have been made to successfully formulate/design the multi-epitope vaccines against cancer and cancer-causing viruses [24–27].

In this study, we aim to construct an efficient vaccine candidate using the highly advanced computational framework with the simulation of immune interaction. Our predicted multi-epitope vaccine against BLV showed a strong immunogenic effect against BLV infections. Thus, the new strategy of vaccine development is a prime need that will contribute to finding a solution to BLV.

Materials and methods

The sequential steps followed to design and construct the multi-epitope vaccine are illustrated in Fig. 1.

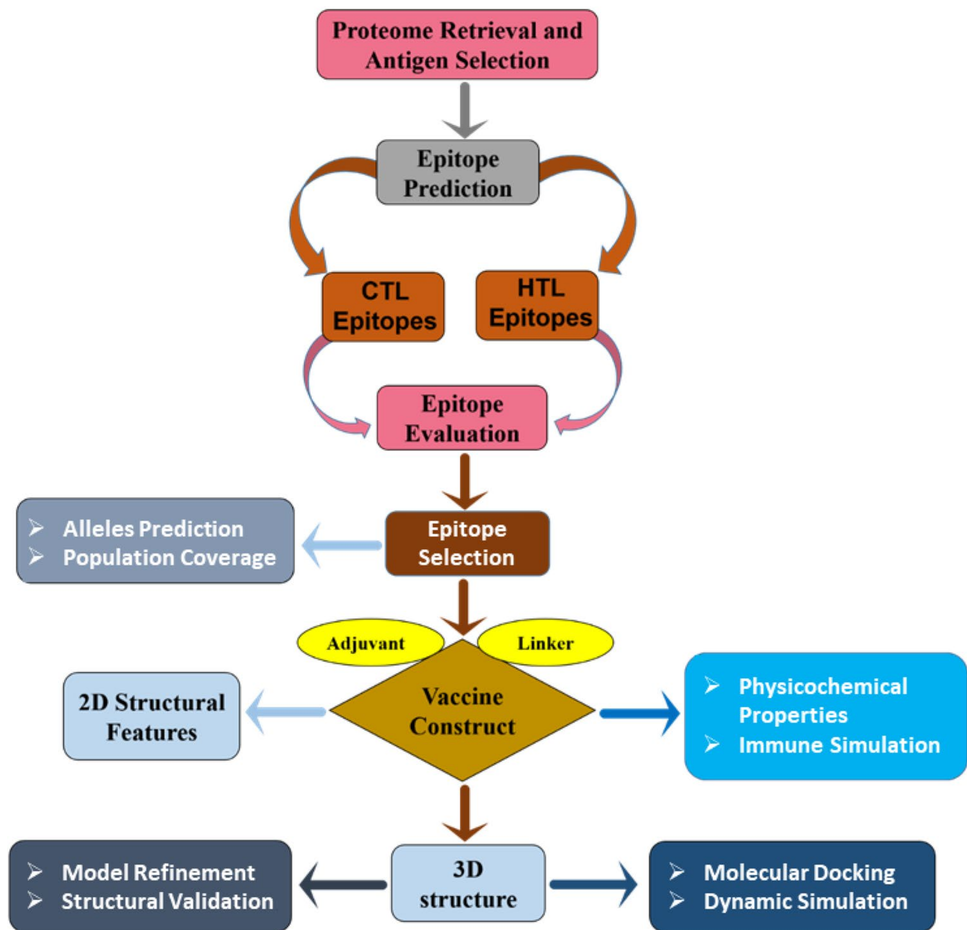

Fig. 1 Graphical representation of study workflow

Protein sequences retrieval

Protein sequences of BLV (Proteome ID: UP000202838) were extracted from the UniProt database (http://www.uniprot.org) [28]. A total of six protein names were available—Gag polyprotein, Gp60 SU, p18, p34, Pr66, and RT-IN. A total of 2,252 sequences contained in the dataset and selected protein sequences were principally chosen by antigenicity. The prediction of antigenicity is using the VaxiJen v-2.0 and ANTIGENpro server at threshold 0.5. All protein data are downloaded in FASTA format.

Antigenic properties of target proteins

The protein sequences of the total proteins were submitted in the VaxiJen v-2.0 server (http://www.ddg-pharmfac.net/Vaxijen/VaxiJen/VaxiJen.html) at threshold 0.5 for the prediction of antigenicity [29]. We also predicted antigenicity using ANTIGENpro (http://www.scratch.proteomics.ics.uci.edu/) server [30]. The prediction of antigenicity is an important step to isolate when attempting to isolate the most antigenic protein sequences.

CTL and HTL epitopes prediction

Most cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) are expressed in T-cell receptors that can recognize a specific antigen [31]. CTL and HTL epitopes were predicted by submitting FASTA sequences of the target proteins to the NetCTL-1.2 (http://www.cbs.dtu.dk/services/NetCTL/) [32] at 1.0 threshold (combine score) and MHC-II binding tool (http://tools.iedb.org/mhcii/) [33] of IEDB server where percentile score was set to 5.

CTL and HTL epitopes evaluation

The reasonableness of CTL epitopes exists in their immunogenic potential. Consequently, it is considered as the center of the vaccine ability [34]. All predicted CTL epitopes were assessed for their immunogenicity, antigenicity, allergenicity, and toxicity utilizing MHC-I immunogenicity (http://tools.iedb.org/immunogenicity/) [35], VaxiJen v-2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [29], AllergenFPv-1.0 (http://ddg-pharmfac.net/AllergenFP/) [36], and ToxinPred servers (http://crdd.osdd.net/raghava/toxinpred/) [37], respectively. Epitopes having antigenic,

allergenic, and/or toxic properties were eliminated, since they can compromise the aim and development of the vaccine.

The cytotoxic T-cell epitopes can stimulate the HTL by releasing different types of cytokines such as interferon-gamma (IFN-γ), interleukin-4 (IL-4), and interleukin-10 (IL-10) [38]. For the prediction of IFN-γ in the target protein, we used the IFNepitope server (http://crdd.osdd.net/raghava/ifnepitope/) [39]. The support vector machine (SVM) and a motif hybrid method were used to perform the prediction. Next, IL-4 and IL-10 inducers were identified with the IL4pred (http://crdd.osdd.net/raghava/il4pred/) and IL10pred servers (http://crdd.osdd.net/raghava/il10pred/predict.php), respectively. The IL4pred and IL10pred operations were carried out based on hybrid methods with other parameters kept as default [40, 41]. Finally, we analyzed their antigenicity using the VaxiJen v-2.0 server [29].

Population coverage

CTL and HTL alleles of selected epitopes were analyzed for exploring the population coverage of the constructed vaccine. Epitopes of CTL and HTL were submitted to the IEDB database tools for MHC-I-binding [42] and MHC-II-binding [43] alleles, respectively. With the HLA alleles, the prospective coverage of the population was appraised using the IEDB population coverage tool [44].

Designing of epitope-based vaccine construct

The epitope-based peptide vaccine was developed with the appropriate linkers and adjuvant [45, 46]. Toll like receptor (TLR3) agonist, namely β-defensin, was added at the N-terminal of the vaccine with EAAAK linker [47, 48]. We used TLR3 as a receptor for vaccine construction as it can recognize the virus [49]. The adjuvant is an immunological agent to response immune system and produces long-lasting immunity [45]. Previously, many studies demonstrated that GPGPG and AAY linkers [50, 51] were added between predicted HTL and CTL epitopic sequences, respectively, and produced junctional immunogenicity, thereby allowing the rational design construction of a potent poly-epitope vaccine [52]. Arai et al. reported that the EAAAK linker was incorporated between epitopes and adjuvant for improving bioactivity of fused protein and reaching a high level of expression [53].

Secondary structure features

The secondary structure features such as alpha-helix, extended sheet, beta turn, and random coil were predicted through the SOPMA tool (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) [54]. All SOPMA secondary structure analysis tool parameters were kept defaults (Number of conformational states as 4, helix, sheet, turn, and coil; threshold values 8 and Window width: 17).

Structure modeling, refinement, and validation

GalaxyTBM online tool (http://galaxy.seoklab.org/) [55] used to generate the 3D structure of the linear vaccine constructs, and the Galaxy Refine web server (http://galaxy.seoklab.org/) [56] was used to refine the model. The GalaxyRefine server enhances the local structural quality using CASP10 refinement techniques. This algorithm primarily reconstructs side chains and accomplishes side-chain repacking followed by molecular dynamics simulation to achieve overall structural relaxation. The obtained 3D structure was visualized with BIOVIA Discovery Studio 2020.

Validation of modeled structure was performed based on the Z-score as assessed by ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) [57]. The Z-score of an anticipated model defines the structural quality of a model based on the protein structure elucidated by NMR and X-ray crystallography. Finally, the overall structural quality was validated by a Ramachandran plot analysis, followed by the PROCHECK server (https://servicesn.mbi.ucla.edu/PROCHECK/) [58].

Pharmacological properties of the vaccine

The immunogenicity, antigenicity, and allergenicity of the final vaccine construct were analyzed by the IEDB Immunogenicity tool, VaxiJenv-2.0, and AllergenFP v-1 servers, respectively. Molecular weight, aliphatic index, instability index, isoelectric point, half-life, and GRAVY values were evaluated using the ExPASasy ProtParam tool [59]. Finally, vaccine construct was submitted to the SOLpro server (http://scratch.proteomics.ics.uci.edu/) for solubility assessment [60].

Vaccine–receptor docking and interaction

Molecular docking is the interaction of a ligand with its receptor and consequently forecasts the stable adduct [61]. In addition, it can also predict the binding affinity of two molecules based on certain scoring functions. For our docking simulation, we chose TLR3 as the receptor and the specific PDB file (PDB id: 2A0Z) was downloaded from RCSB-Protein Data Bank. The TLR3 receptor was paired with the vaccine model (which acts as the ligand) that we had already refined [62]. The processing and water remove from TLR3 using BIOVIA Discovery Studio 2020. The ClusPro v-2.0 server was used to measure the binding affinity between the multi-epitope vaccine and TLR3 receptor with the help

of molecular docking [63]. The docked structure and vaccine–receptor interaction was visualized by Discovery Studio and PDBSum server, respectively.

In-silico molecular dynamic simulation

The complex structure of the selected vaccine-receptor complex candidate was evaluated using 100 ns molecular dynamic simulations (MDS) to evaluate their binding stability to the desired protein to the active site cavity of the protein [64, 65]. The MDS of the receptor–ligand complex was performed using the 'Desmond v6.3 Program' in Schrödinger 2020-3 under Linux framework to evaluate the thermodynamic stability of the receptor–ligand complex [66, 67]. To solve the system, a predetermined TIP3P water model was used, with an orthorhombic periodic boundary box form with a box distance of 10 Å assigned to both sides to retain a specific volume. After constructing the solvated system containing protein in complex with the ligand, the system has been minimized and relaxed using the default protocol introduced within the Desmond module with OPLS_2005 force field parameters [65]. In protein preparation wizard: Initially, protein preprocesses by adding hydrogens, create disulfide bonds, fill in the missing side chains, and delete waters using Epik (pH 7.0 ± 2.0) and optimize by PROPKA pH: 7.0. In model system for simulation run, simulation time = 100 ns, trajectory intervals = 100 ps, total number of frame = 1000, Ensemble class = NPT, temperature = 300 K, and one atmospheric (1.01325 bar) pressure. Finally, the simulation was carried out for 100 ns, and RMSF, RMSD, and protein secondary structure elements from the trajectories were analyzed to reveal the stability of the vaccine complex.

Immune simulation analysis

For analyzing the immunological response, the sequence of the vaccine construct was submitted to the C-IMMSIM server and the immunological response results were downloaded [68]. The lowest suggested dose of vaccine was two times, dose-1 and dose-2 with an interval of 1 month [69]. The three consecutive injections were administered with time steps of 1, 84, and 168 where 1 time steps = 8 h.); and simulation steps were 300. Other immune simulation criteria were kept default.

Table 1 The selected protein name and antigenicity score of BLV

Accession ID	Proteins name	Antigenicity score		Remark
		VaxiJen	ANTIGEN-pro	
Q85490	Gag polyprotein	0.51	0.963546	Selected
Q77YG2	Gp60 SU	0.6561	0.687863	Selected
O92813	p18	0.4349	0.938062	Non-selected
O56232	p34	0.2963	0.778874	Non-selected
Q9WJP3	Pr66	0.4410	0.944496	Non-selected
O92812	RT-IN	0.4214	0.6666078	Non-selected

Results

Retrieving of target protein sequences and antigenicity prediction

The target sequence of BLV was retrieved in FASTA format from the UniProt database. The available six proteins were Gag polyprotein, Gp60 SU, p18, p34, Pr66, and RT-IN. The highest antigenicity score was found for Gag polyprotein and Gp60 by VaxiJen v2.0 and ANTIGENpro servers (Table 1).

Identification of CTL and HTL epitopes

In this study, the NetCTL-1.2 server was employed for the prediction of the CTL epitopes, whereas the HTL epitopes was predicted using the Net MHC-II pan 3.2 server. After evaluation, only 5 unique CTL epitopes were found to be as antigenic, immunogenic, non-toxic, and non-allergenic, which were considered for vaccine construction (Table S1). Similarly, we found only 4 unique HTL epitopes to be IFN-γ, IL-4, IL-10, and antigenic, which were considered for construct design (Table S2). Finally, we identified only 9 unique epitopes for vaccine design (Table 2).

Population coverage

Successful HLA allele calculations are essential when developing a vaccine that will be effective worldwide. The selected CTL and HTL epitopes along with their binding alleles were used for population coverage estimation. The total combined (CTL and HTL) epitopes have a good significant percentage of coverage across the World (98.04%), Europe (98.70%), South Asia (95.95%), India (94.58%), Russia (98.55%), and North America (98.61%). This result indicated that our designed vaccine can be used worldwide (Fig. 2 and Table S3).

Table 2 Final selected CTL and HTL epitopes of BLV

CTL epitopes				
Epitopes	Antigenicity	Immunogenicity	Toxicity	Allergenicity
SYVEFVNRL	1.1064	0.29179	No	No
QACAHWAPK	1.1052	0.28842	No	No
GIFTLTWEI	0.6583	0.34824	No	No
GLTGINVAV	1.245	0.22791	No	No
NRRGLDWLY	1.7751	0.24144	No	No
HTL epitopes				
Epitopes	IFN-γ	IL-4	IL-10	Antigenicity
CAHWAPKVKQPAILV	POSITIVE	Yes	Yes	0.9935
DLKNYIHWFHKTQKK	POSITIVE	Yes	Yes	0.7997
PSHWKRDCPTLKSKN	POSITIVE	Yes	Yes	1.2702
DWLNLRQSAQRLNPR	POSITIVE	Yes	Yes	1.0392

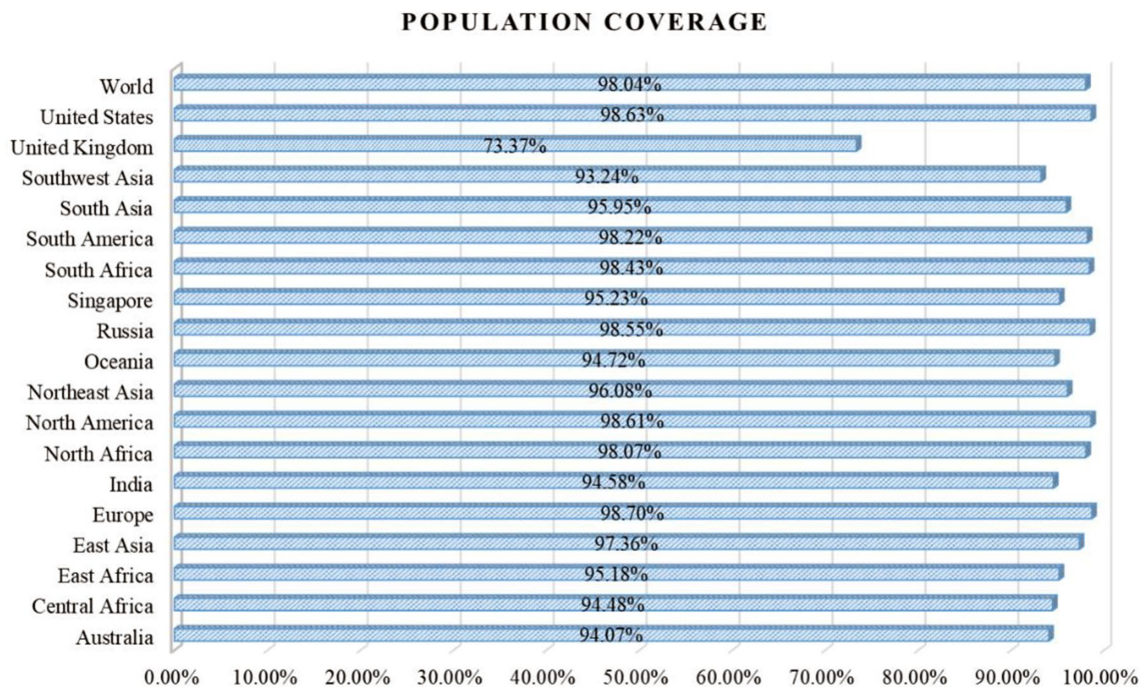

Fig. 2 Worldwide population coverage map by T-cell epitopes based on their combined CTL- and HTL epitoptes-binding alleles

Vaccine formulating, 2D and 3D structure modeling, and refinement

For multi-epitope vaccine designing, we have considered five CTL epitopes (by immunogenicity = 0.2, combined score = 1, and antigenicity = 0.5) from every type of proteins that are immunogenic, non-allergenic, and non-toxic, as shown in Table 2. On the other hand, at HTL epitope selection, we screened cytokine-inducing properties, overlapped epitopes, and found three types of cytokine and the highest antigenicity (Table 2). Therefore, 5 CTL and 4 HTL epitopes are merged by AAY and GPGPG linkers, respectively. Furthermore, TLR3 agonist β-defensin was added as an adjuvant using EAAAK linkers. The arrangement of different epitopes along with their joining linkers is shown in Fig. 3A. The final vaccine construct comprises 187 amino acid residues.

Overall, secondary structural feature prediction results indicate 44.39% are random coils, 21.39% are extended strand, 24.06% are α-helix, and 10.16% are β-turn (Fig. 3B). The vaccine construct was modeling was done through GalaxyWeb server, where the modeled vaccine structure of BLV

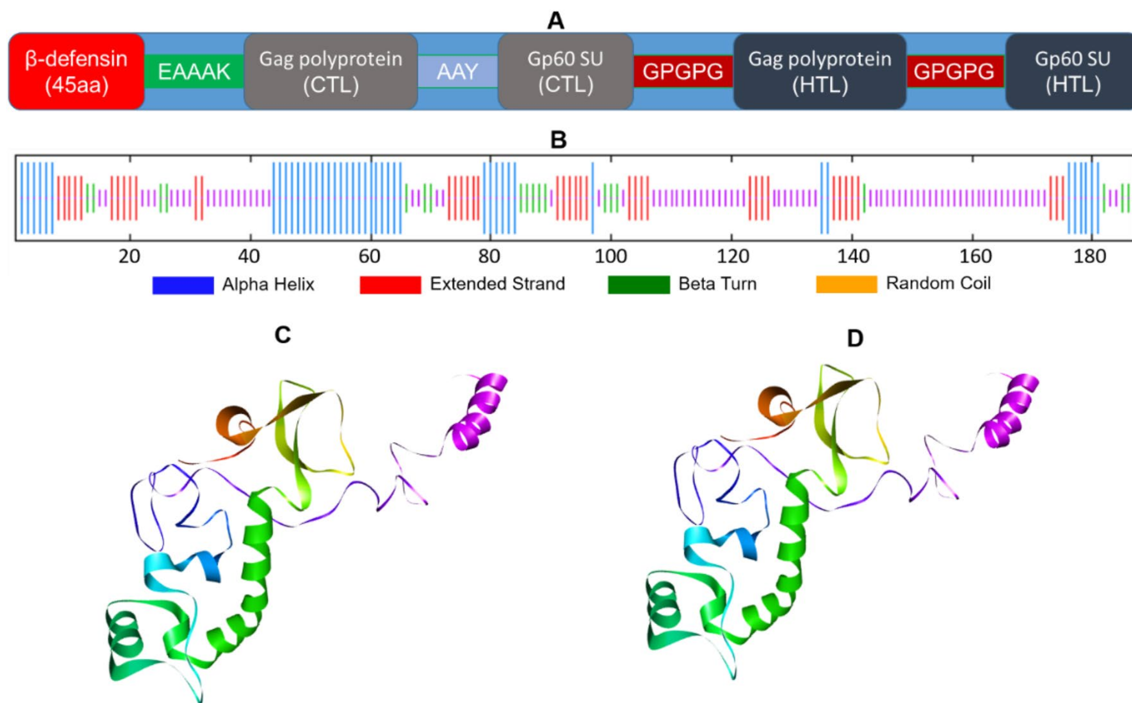

Fig. 3 **A** Vaccine construct of BLV. **B** The secondary structural properties of the vaccine. **C** Initial 3-D model of the final vaccine. **D** Refine the 3-D model of the final vaccine

using the template 1JVR and structure was visualized Discovery Studio 2020 (Fig. 3C). The 3D structure was refine performed by the Galaxy Refine server, which generated five models. Among all of the refined 3-D structures, model 2 was selected for better quality, and structure was visualized Discovery Studio 2020 **(**Fig. 3D and Table S4).

Validation of final vaccine model

Validation of the initial and refined models was examined using ProSA-Web and PROCHECK servers. According to the Ramachandran plot most favored (MFR), additional allowed (AAR), generously allowed (GAR), and disallowed regions (DS) score is 90.0%, 6.7%, 2.0%, and 0.7% in initial vaccine model. On the other hand, refine vaccine model Ramachandran plot score is 92.0%, 5.3%, 2.0%, and 0.7% (Fig. 4A and B). The Z-scores of the initial and refined model were − 4.44 and − 4.58, respectively, suggesting that the overall quality falls within the X-ray-derived structure region. (Fig. 4C and D).

Physicochemical, immunogenic, antigenic, allergenic, solubility, and properties of final vaccine construct

The physicochemical properties of a vaccine must be determined to assess efficiency and safety. The theoretical pI values were found to be 9.98, which suggested that the candidate is thermostable. The GRAVY score is − 0.53 which specifies the candidate is hydrophilic and can interact with the aqueous environment. The instability index was calculated as 33.40, which classified the protein as being stable. Antigenicity, immunogenicity, allergenicity, and solubility were determined to be antigenic, high immunogenic, non-allergenic, and good soluble (Table 3). These evaluations suggest that our vaccine construct might be an ideal vaccine for BLV.

Molecular docking of vaccine with receptor

The interaction between the TLR3 and refine final vaccine was evaluated using the ClusPro v-2.0 online server, and a total of 30 models were generated by ClusPro v-2.0 online server. Among the top-ten complexes generated when examining the interaction between the vaccine and TLR-4, model 0 was characterized as having the best lowest energy (− 1038.8) and interaction member was 50 (Fig. 5). The vaccine–receptor complex view and interaction were visualized by Discovery Studio 2020 and PBDSum.

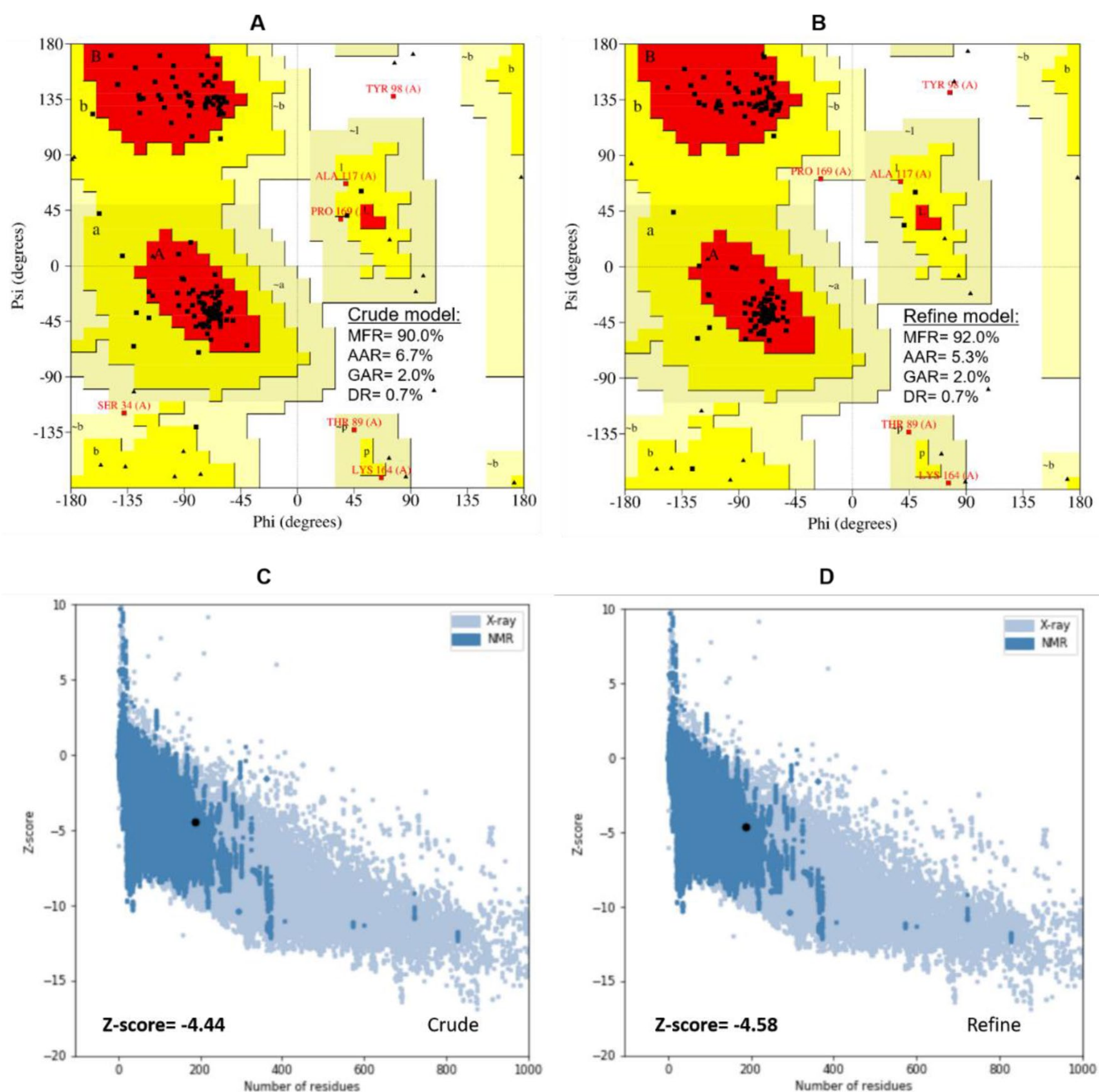

Fig. 4 **A**–**B** Analysis of Ramachandran plot PROCHECK server. The MFR, AAR, GAR, and DR was represented the most favored, additional allowed, generously allowed, and disallowed regions of vaccine. **C**–**D** 3-D structure validation with a Z-score by Pro-SA server

Molecular dynamics simulation of vaccine–TLR3 complex

The root-mean-square deviation (RMSD) of both the vaccine complex and vaccine was calculated. The average RMSD value for the vaccine complex were 6.188 Å, which demonstrates the structural stability during the interaction. From Fig. 6A, it can be observed that the vaccine complex has the initial increase of RMSD descriptors till 75 ns, and after that, it stopped its upper trend. A lower degree of fluctuation was observed for both, which may be responsible for structural integrity and/or to allow firm binding. Moreover, the protein flexibility across the amino acid residues was evaluated through the root-mean-square fluctuation (RMSF) score. The RMSF profile of the vaccine complex indicates maximum amino acid residues from complexes that an RMSF profile below 23.08 Å and greater change was observed for fewer residues. This result from Fig. 6B defines the vaccine complex stability and stiffness.

Table 3 Physicochemical properties, antigenic, immunogenic, allergenic, and soluble of the final vaccine protein

Evaluating parameters	Assessment	Remark
Number of amino acids	187	–
Molecular weight	20,716.03 Dalton	Good
Theoretical pI	9.98	Basic
Chemical formula	$C_{931}H_{1452}N_{278}O_{243}S_9$	–
Total number of atoms	2913	–
Instability index	33.40	Stable
Grand average of hydropathicity (GRAVY)	− 0.533	Hydrophilic
Antigenicity	0.7026	Antigenic
Allergenicity	Probable non-allergen	No
Immunogenicity	Positive	Immunogenic
Solubility	0.951102	Soluble

Immune simulation

The immunogenic profile of the multi-peptide vaccine is shown in Fig. 7. Immune simulation results showed that the secondary and tertiary response was generated considerably higher compared to the primary response (Fig. 7A). The antigenic concentration is decreased and the immunoglobulin activity is significantly increased by revealed secondary and tertiary responses and multiple B-cell iso-types were found. This result indicates the formation of possible iso-type (Fig. 7B) and similar higher response displayed by helper T and cytotoxic T-cell population during vaccination (Fig. 7C, D). Higher macrophage activity was formed by the natural killer (NK) and dendritic cells (Fig. 7E, F).

Discussion

The neoteric maleficent emergence of BLV in human breast cancer creates a life-threatening situation to the worldwide public health cancer cases with increased mortality and morbidity of human life, which influenced us to design this multi-peptide vaccine. Since there is no evidence of a peptide vaccine against BLV, our vaccine candidate can be considered a good model to develop vaccine formulation against oncogenic delta-retrovirus, which may offer a better outcome than the conventional treatment [70]. To stimulate a strong immunogenic effect against BLV and its perilous contagious infection, we constructed this novel vaccine candidate with a rigorous assessment

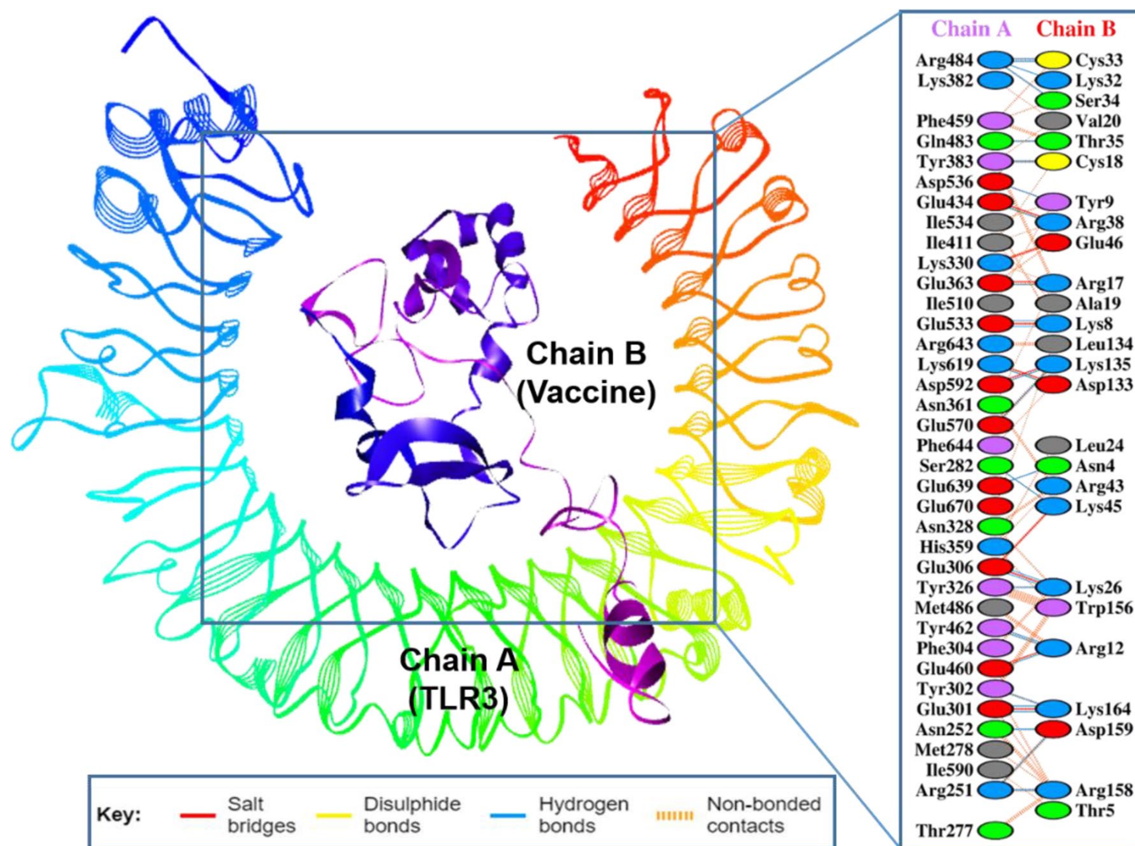

Fig. 5 The binding interaction between the designed vaccine candidate and TLR3 immune receptor

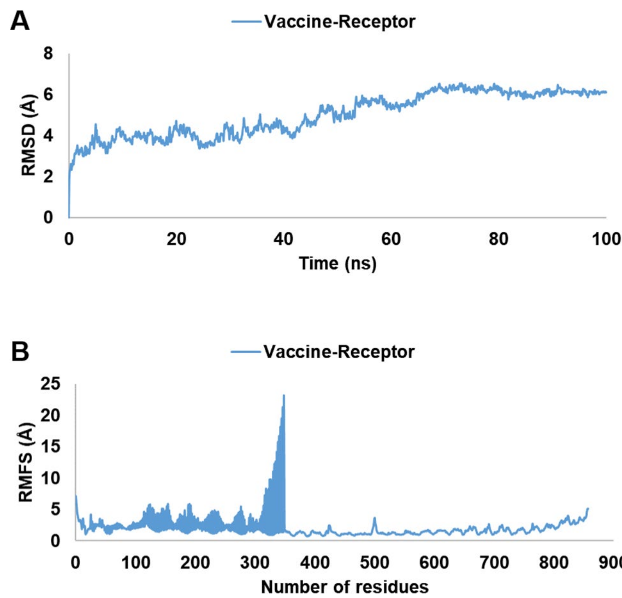

Fig. 6 Molecular dynamic simulation of the multi-epitope vaccine complex. **A** The RMSD plot of the backbone atoms of the complexes. **B** The RMSF plot of the multi-epitope docked vaccine candidate

of its stability, selectivity, and reduction of any undesired immune response ordained using computer immunology. Scientific consensus indicates the importance of a vaccine which lies in its safety and efficacy, so that it can deliver acquired immunity against viral invasion to exclude infectious diseases [20, 71]. Our designed vaccine that maybe reflects the reorganization capabilities of these virus particles demonstrated an extraordinary significance to protect human breast cancer and cattle diseases.

As well, our designed vaccine has several advantages compared to conventional and single-epitope vaccines owing to the following distinctive characteristics: (a) comprising multiple MHC epitopes (both class I and II), so that can be recognized by several T-cell receptors; (b) contains overlapping CTL, and HTL epitopes; (c) has of poly-epitopes from target virulent protein; (d) an immunostimulator (adjuvant) for providing the long-lasting immune response [72–76]. The multi-epitope vaccine design is a new field that has already gained importance, and vaccines developed using this method have not only demonstrated in vivo efficacy with protective immunity [77–79], but have also undergone phase-I clinical trials [79–82].

During antiviral immune responses, CTL and HTL cell responses will most likely be directed against both structural and non-structural proteins, since all viral proteins are available for processing and presentation on infected cells' HLA molecules [83, 84]. Therefore, we identified 5 CTL epitopes and 4 HTL epitopes as potential targets for vaccine development. In our approach, we find the best population

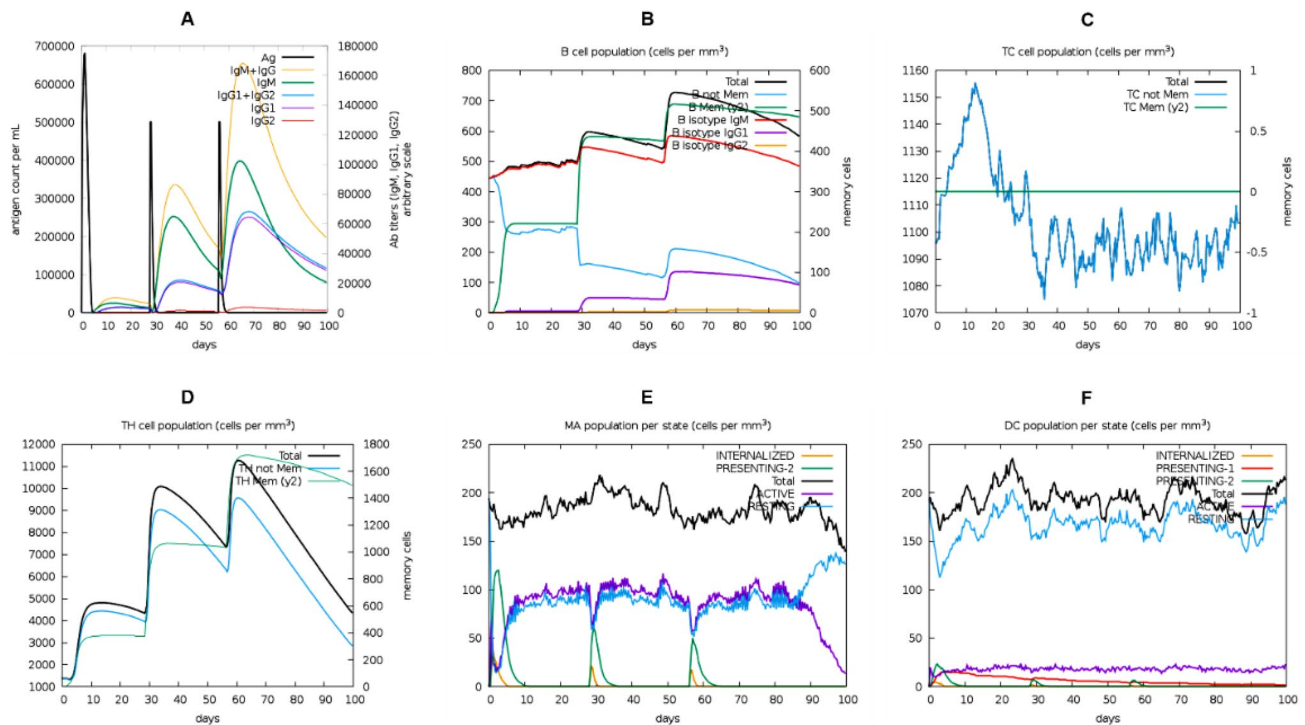

Fig. 7 Immunological response evaluation of vaccine in host using the C-IMMSIM online platform: **A** The virus, the immunoglobulins and the immune-complexes, **B** B-cell population, **C** TC cell population, **D** TH cell population, **E** Concentration of cytokines and interleukins, and **F** percentage (%) and amount (cells/mm^3) of Th1 mediated

coverage in the world (98.04%) with both MHC-1 and 2 alleles making it a good vaccine candidate for the population worldwide. The vaccine's effectiveness depends on the population in which the vaccination is used [85].

The top 5 antigenic CTL and 4 HTL epitopes were selected to design multi-epitopes vaccine with AAY and GPGPG linker, respectively. Linkers are used in vaccine construction for the incorporation between the epitopes and an adjuvant also uses to bust up the activities of vaccine construction [86, 87]. Finally, vaccine construction is accumulated 187 amino acid residues. Our vaccine construct was predicted using a solubility assessing tool to be soluble inside host *E. coli*. Solubility is a vital characteristic of any recombinant vaccine as a physiochemical property [88–91]. The designed vaccine's properties were found to be acidic, soluble, non-allergenic and highly antigenic. In multi-epitope vaccine design, allergenicity plays an important role [92–94]. We evaluated the allergenicity before vaccine design; hence, no available allergenic epitopes in the vaccine construct. Therefore, the designed vaccine will not be an allergic reaction to the human body [95]. In our newly finding novel peptide vaccine, its binding affinity is predicted by molecular docking and we get the appropriate interaction between the vaccine and TLR3 cell surface receptor [96]. The simulation trajectories with the TLR3 receptor and the vaccine were calculated to gain knowledge about the well binding strength and contact [96]. Since the vaccine consisted of CTL and HTL epitopes, it could induce the activation of the respective immune cells in the host, which may further lead to the activation of other immune cells through complex signaling [97]. In the case of the immune simulation study, it was confirmed that our designed vaccine candidate could generate an appropriate immune response in secondary exposure after the final injection. The molecular dynamics simulation study of the vaccine candidate was conducted to confirm their stable nature at atomistic conditions. The simulation data by combining RMSD and RMSF descriptors from trajectories correlate with the structural rigidity of the vaccine complexes. The RMSD and RMSF profile of the vaccine candidates was below 6.188 Å for most of the simulation time. These results define the vaccine complexes integrity and less mobility at the simulation conditions. Recently, many other studies are devoted to design multi-epitope vaccines against pathogens such as SARS-CoV-2 [51, 98], Kaposi sarcoma [99], onchocerciasis [46], *Leishmania* [50], and dengue [50]. We believed that BLV multi-epitope vaccine that we designed in this study could reduce the upstream processing efforts of the BLV vaccine development.

Conclusions

In this study, we computationally designed a multi-epitope vaccine against BLV as prophylaxis to human breast cancer. Our vaccine construct had a potential immune response and good physicochemical properties. Additionally, the vaccine is antigenic and immunogenic as well as has no allergenic and toxic effect on the human body. This novel vaccine contains a significant interaction and binding affinity with the TLR3 receptor. However, further investigations of both in vitro and in vivo are to ensure its true potential to fight BLV infection.

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Author contributions AS and MSR designed the project; AS performed the analysis of data; AS evaluated and interpreted the results; AS, NSM, ZN, and TMK prepared the draft manuscript; AS, NSM, ZN, and MSR critically reviewed and finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability Table S1: The predicted CTL epitopes utilizing NetCTL-1.2 server. Table S2: The predicted HTL epitopes utilizing IEDB MHC-II server. Table S3: The worldwide population coverage map by T-cell epitopes. Table S4: Predicted top five refined models with their different criteria of BLV epitope-based vaccine.

Declarations

Conflict of interest The authors declare that they have no competing of interests.

Ethical approval Not applicable.

Consent for publication Not applicable.

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