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An immunoinformatic approach to universal therapeutic vaccine design against BK virus

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ABSTRACT

In kidney transplant recipients (KTRs) long-term immunosuppression leads to BK virus (BKV) reactivation, with an increased incidence of BKV-associated pathologies and allograft rejection. The current approaches to limit BKV infection include a reduction in immunosuppression and use of anti-BKV drugs, which are clinically sub-optimal and lead to undesirable therapeutic outcomes. Here, we adopted an immune-based approach to augment the endogenous BKV specific T-cells. Using *reverse vaccinology* based *in silico* analyses, we designed a peptide-based multi-epitope vaccine for BKV (MVBKV). A major advantage of our approach is that the selected epitopes show an affinity towards all the 12 superfamilies of HLA class I alleles and 27 reference alleles of HLA class II. This suggests MVBKV's universal nature and its potential effectiveness in a wide-population base. To improve MVBKV's immunogenic properties, a synthetic Toll-like Receptor (TLR) 4 peptide ligand (RS09) was added to the final vaccine construct. The sequences of the individual epitopes were molecularly linked to form a 3D-stable synthetic protein. Overall, our immunoinformatic-based approach led to the design of a new MVBKV vaccine, which remains to be validated experimentally.

1. Introduction

BK virus (BKV) is a small, non-enveloped double-stranded DNA virus. Most people get BKV infection during childhood, which persists in adults as a latent infection without any major symptoms [1–4]. However, immunosuppression in kidney transplant recipients (KTRs) leads to BKV reactivation and emergence of life-threatening conditions. An untreated BKV infection can lead to severe complications such as BKV-associated nephropathy (BKVAN), BKV-associated hemorrhagic cystitis (BKVHC), and ureteric stenosis [5–8]. While several factors contribute to an increased risk of BKV reactivation, it's prevalence varies between 11 and 43% in kidney transplant patients [9]. The single most important risk factor contributing to an opportunist BKV reactivation is the overall degree of immunosuppression after renal transplant, which causes the development of BKVAN leading to allograft dysfunction and rejection [10–12]. Therefore, decreasing immunosuppression (thereby boosting the body's immune system) to limit BKV infection is the mainstay therapy in KTRs. While this approach shows effectiveness in reducing the viral load, it also leads to the development of a non-specific immune response increasing the likelihood of allograft rejection. Currently, there are no satisfactory anti-viral drugs against BK viremia. Anti-BKV drugs to treat BK viremia (used either alone or in combination with immunosuppressive drugs) show variable clinical effectiveness in viral clearance and high incidence of side-effects [13]. In addition, excessive use of anti-microbial drugs may lead to the emergence of new drug-resistant BKV species posing a threat to global health [13]. To circumvent these limitations of traditional therapeutic approaches, we have developed a peptide-vaccine based immunotherapeutic approach. The multi-epitope vaccine for BKV (MVBKV) is expected to induce a BKV-specific cellular immune response, which offers advantages of low-toxicity and minimal effects on long-term allograft survival.

Cell-mediated immunity plays an important role in controlling BK infection [14]. In healthy individuals, an effective immune response against BK viremia is dependent on the induction of a stable antiviral memory T-cell response by the activation of both $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T-cell subtypes [15–17]. The most commonly detected BK specific T-cells epitopes are against the large T-antigen (LTA) and VP1 capsid protein [9,18–21]. $CD4^+$ T-cells predominate immune response to BKV and can control its reactivation through the generation of IFN-γ even in the absence of $CD8^+$ T-cells [15–17,22,23]. However, in most KTRs this natural

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immune response against BKV is not elicited due to an immunosuppressive state which reduces the endogenous T-cell number and IFN-γ production [24]. Supporting this, in patients where an immune response is mounted (despite a low T-cell count), there exists a strong inverse correlation between the strength of T-cell response and BK viral load [14,25–27]. These studies provide evidence suggesting the effectiveness of immune system modulation in clearing BK viremia in KTRs [16,26,28,29]. Notably, the majority immune response to BK viremia is conferred by cell-mediated immunity. Despite the presence of anti-BKV antibodies in BKV seropositive patients, an antibody-mediated response is insufficient to provide protection against BKV reactivation and its associated diseases [28,30].

Our strategy involves the use of peptide-based MVBKV vaccine to induce a strong BK-specific T-cell response. Using *reverse vaccinology* based computational tools, we have screened the entire BKV proteome and identified the most-antigenic BKV epitopes (in multiple proteins) for HLA class I and HLA class II molecules. By molecular-linking of the immunodominant antigenic peptide sequences, we designed a synthetic MVBKV protein. To further induce a robust immune response against BKV, we added a peptide adjuvant (RS09) in MVBKV's design. Here, we present the computational analyses leading to the design of therapeutic MVBKV subunit vaccine which can be used to control BK viremia.

2. Methods

2.1. Generation of BKV proteome database

BKV is a double-stranded DNA (dsDNA) polyomavirus with a ~5 kb genome [32]. There are genomic sequences of 317 BKV strains in the NCBI nucleotide database. BKV reference genome (NC_001538.1) was used to obtain reference proteins' sequence data for the present vaccine design. To collect all the sequences of BKV proteins, protein database of NCBI was used to create a separate local database. BKV genome has six proteins: agnoprotein, VP1, VP2, VP3, small T-antigen (STA) and LTA. The most antigenic BKV epitopes were identified by screening these six BKV proteins against reference HLA class I and HLA class II alleles.

2.2. The HLA class I binding prediction

To predict 9mer HLA class I epitopes in BKV proteins, publicly available web-based tool NetCTLv1.2, was used [33]. The selection criteria for identification of most antigenic HLA class I BKV epitopes was based on a high-overall combinatorial score of peptides and a high-intrinsic potential. The HLA class I alleles are classified into 12 superfamilies (A1, A2, A3, A24, A26, B7, B8, B27, B39, B44, B58, and B62). Potential BKV protein epitopes were identified by curating each of the six BKV proteins against each of the 12 HLA superfamily ($12 \times 6 = 72$ curations, as shown in the supplementary file). The threshold values used were, proteasomal C-terminal cleavage = 0.15; TAP transporter-associated efficiency = 0.0558; HLA-I binding-affinity >0.75. For each of the predicted epitopes, a combined score was obtained. Thereafter, *in silico* prediction of HLA class I epitope immunogenicity was done on the Immune Epitope Database and Analysis Resource (IEDB) server. Epitopes with positive immunogenicity values were selected for further analysis.

2.3. The HLA class II binding prediction

15mer HLA class II epitopes in BKV proteins were predicted using the IEDB server with default parameters. HLA class II alleles have been grouped into 27 reference alleles covering the entire population. The epitopes for HLA class II were computed based on consensus prediction approach, which uses a combination of stabilization matrix alignment and average relative binding matrix methods. The IEDB compares the predicted binding affinity with randomly selected peptides and assigns a percentile rank, where lower percentile rank corresponds to a higher binding affinity. Therefore, for each of the HLA class II reference allele, we selected the epitopes with lowest percentile ranks and IC50 > 500 nm.

2.4. Conservancy analyses

To identify conserved epitopes in each of the 317 BKV strains, conservancy analysis was performed for each of the HLA class I and HLA class II epitopes on the IEDB server. Presence of conserved epitopes is believed to be a superior approach providing maximum therapeutic utility and broader protection across multiple pathogenic strains, compared to the epitopes derived from highly-variable genomic regions. Our selected epitopes were 100% conserved across all 317 BKV strains. In case the epitopes did not show a 100% conservancy the next most conserved epitope was selected. Therefore, to represent 100% conservancy for all selected epitopes, for some of the HLA alleles more than one epitope was selected. Further, HLA distribution analysis was done to select T-cell epitopes which cover each of the 12 HLA class I superfamily and 27 reference alleles for HLA class II. Tables 1 and 2 show the selected epitopes representing a 100% conservancy for each of the HLA type. Further, highly conserved epitopes can sometimes show sequence homology across species. Any such similarity with human proteins will render a non-functional response. Therefore, to rule out any potential similarity between selected epitopes and human endogenous protein sequences, BLASTp on the selected peptides against human proteome was done to remove any peptide that show homology to human protein sequences. Using this criterion, only the epitope which showed <80% homology (for HLA class I) and <90% (for HLA class II) were selected.

2.5. MVBKV design

Cluster analysis was done to identify the overlapping regions in the epitopes with the selection of overlapping peptides for final vaccine construct. The selected HLA class I and HLA class II epitopes were linked together by using molecular linkers to form the MVBKV vaccine construct. Use of linkers help in stable folding of the polypeptide and facilitate the immunological processing and presentation of the epitopes. Once the vaccine epitopes were identified, based on the previously published studies [34,35], epitopes for each of the selected HLA class I (9mer), HLA class II (15mer) or overlapping peptides were linked using EAAAK sequences. To further improve MVBKV's immunogenicity, Toll-like receptor (TLR) 4 ligand APPHALS [34] was added on MVBKV's N-terminal and linked by EAAAK sequences (Fig. 1A). The final MVBKV subunit vaccine (including TLR4 ligand) consists of 479 amino acids (Fig. 1A).

2.6. Biophysiochemical characterization and molecular docking

Biophysiochemical properties were determined using ProtParam and PSERD server to assess MVBKV's isoelectric point, half-life, and instability index. Solubility and toxicity analyses of final MVBKV construct, showed it to be non-allergen, as predicted by the hybrid approach on Algpred. Further, analyses of MVBKV sequence, secondary structure (using RAMPAGE) and tertiary structure (using RaptorX) were done to assess the quality of the predicted 3D-model. After an optimal MVBKV 3D-structure was obtained, molecular docking studies were done on the ClusPro server to predict the functional binding. MVBKV's adjuvant RS09 binding with its receptor TLR4 (Fig. 1D), as well as binding of the

Table 1
Predicted 9mer HLA class I epitopes in BKV proteins.

Epitope sequence	HLAI allele	Length	Conservancy	Immunogenicity	Protein
DPRHWGPSL	B7	9	100.00% (325/325)	0.19093	VP2
ELQRRTERF	B8	9	100.00% (325/325)	0.21616	VP2
LQRRTERFF	B62	9	100.00% (325/325)	0.31301	VP2
QRRTERFFR	B27	9	100.00% (322/322)	0.36928	VP2
TERFFRDSL	B44	9	100.00% (322/322)	0.21938	VP2
TPHRHRVSA	B7	9	100.00% (319/319)	0.08098	LTA
VPKRRYWLF	B8	9	100.00% (319/319)	0.20946	LTA
FLEETTWTI	A1	9	100.00% (325/325)	0.41721	VP2
QIDFRPKIY	A1	9	100.00% (319/319)	0.06072	LTA
FLTPHRHRV	A2	9	100.00% (319/319)	0.1392	LTA
IIFFLTPHR	A3	9	100.00% (319/319)	0.19108	LTA
KTLQARFVR	A3	9	100.00% (319/319)	0.08958	LTA
VPKRRYWLF	A24	9	100.00% (319/319)	0.20946	LTA
NIIFFLTPH	A26	9	100.00% (319/319)	0.29084	LTA
PMERLTFEL	B39	9	100.00% (319/319)	0.26762	LTA
YGTEEWESW	B58	9	100.00% (319/319)	0.4072	LTA

mer = amino acid.

Table 2
Predicted 15mer HLA class II epitopes in BKV proteins.

Epitope sequence	HLAII allele	Length	Conservancy	Protein
RRTERFFRDSLARFL	HLA-DRB3*01:01	15	100.00% (325/325)	VP2
TERFFRDSLARFLEE	HLA-DPA1*01:03/DPB1*02:01	15	100.00% (325/325)	VP2
RRTERFFRDSLARFL	HLA-DRB1*07:01	15	100.00% (325/325)	VP2
RTERFFRDSLARFLE	HLA-DRB3*02:02	15	100.00% (325/325)	VP2
ERFFRDSLARFLEET	HLA-DRB5*01:01	15	100.00% (325/325)	VP2
RTERFFRDSLARFLE	HLA-DPA1*02:01/DPB1*14:01	15	100.00% (325/325)	VP2
TERFFRDSLARFLEE	HLA-DPA1*02:01/DPB1*01:01	15	100.00% (325/325)	VP2
QRRTERFFRDSLARF	HLA-DRB1*03:01	15	100.00% (322/322)	VP2
DSLARFLEETTWTIV	HLA-DRB1*04:01	15	100.00% (322/322)	VP2
TERFFRDSLARFLEE	HLA-DRB1*09:01	15	100.00% (322/322)	VP2
TERFFRDSLARFLEE	HLA-DQA1*04:01/DQB1*04:02	15	100.00% (322/322)	VP2
TERFFRDSLARFLEE	HLA-DPA1*02:01/DPB1*01:01	15	100.00% (322/322)	VP2
GGDEDKMKRMNTLYK	HLA-DRB1*08:02	15	100.00% (323/323)	VP2
GGDEDKMKRMNTLYK	HLA-DRB1*11:01	15	100.00% (323/323)	VP2
MLCQLRLRHLNRKFL	HLA-DRB1*15:01	15	100.00% (323/323)	VP2
DEDKMKRMNTLYKKM	HLA-DRB5*01:01	15	100.00% (323/323)	VP2
NSEFLLEKRILQSGM	HLA-DRB1*03:01	15	100.00% (319/319)	VP2
MERLTFELGVAIDQY	HLA-DQA1*03:01/DQB1*03:02	15	100.00% (319/319)	VP2
MERLTFELGVAIDQY	HLA-DQA1*04:01/DQB1*04:02	15	100.00% (319/319)	VP2
TVGLYQQSGMALELF	HLA-DRB1*01:01	15	99.69% (324/325)	VP2
VNTFVNNIQYLDPRH	HLA-DRB1*04:05	15	99.69% (324/325)	VP2
PQWMLPLLLGLYGTV	HLA-DRB1*12:01	15	100.00% (325/325)	VP2
TYAVIAGAPGAIAGF	HLA-DRB1*13:02	15	100.00% (325/325)	VP2
TYAVIAGAPGAIAGF	HLA-DQA1*05:01/DQB1*03:01	15	100.00% (325/325)	VP2
QWFGLDLTEETLQWW	HLA-DQA1*05:01/DQB1*02:01	15	98.76% (319/323)	STA
PCMLCQLRLRHLNRK	HLA-DRB4*01:01	15	100.00% (323/323)	STA
EDVFLLLGMYLEFQY	HLA-DQA1*05:01/DQB1*02:01	15	99.69% (318/319)	LTA
LLLGMYLEFQYNVEE	HLA-DQA1*01:01/DQB1*05:01	15	99.69% (318/319)	LTA
DVFLLLGMYLEFQYN	HLA-DQA1*01:01/DQB1*05:01	15	99.69% (318/319)	LTA
RILQSGMTLLLLLIW	HLA-DQA1*01:02/DQB1*06:02	15	100.00% (319/319)	LTA
KRILQSGMTLLLLLI	HLA-DPA1*01/DPB1*04:01	15	100.00% (319/319)	LTA
LQNSEFLLEKRILQS	HLA-DPA1*03:01/DPB1*04:02	15	100.00% (319/319)	LTA
CKGVNKEYLLYSALT	HLA-DPA1*02:01/DPB1*05:01	15	100.00% (319/319)	LTA
PQTYAVIAGAPGAIA	HLA-DRB1*09:01	15	100.00% (325/325)	LTA

mer = amino acid.

individual peptides (3D-peptide structure was predicted on PEPFOLD server) with respective HLA alleles show optimal binding characteristics. For data presented in this paper, we selected the structure with the least center and lowest energy weighted score.

3. Results

3.1. HLA class I and HLA class II epitope selection

The IEDB predicted 23 HLA class I epitopes for agnoprotein, 99 for VP1, 135 for VP2, 91 for VP3, 55 for STA and 233 for LTA (supplementary file). Of note, using our selection criteria agnoprotein did not show any reliable epitope for superfamily A1. Conservancy analyses for identification of conserved BKV epitopes followed by HLA distribution analyses of the conserved epitopes, identified 15 unique HLA class I epitopes. Table 1 lists 9mer epitope sequences for HLA class I alleles showing 6 epitopes in VP2 and 10 epitopes in LTA proteins, respectively. Notably, epitope VPKRRYWLF showed optimal binding characteristics with two HLA class I (A24 and B8) superfamilies. Further, each of the HLA class I superfamily is represented at least once in Table 1.

For HLA class II alleles, we selected 25 unique 15mer epitopes in VP2, STA and LTA proteins (Table 2). The total number of epitopes

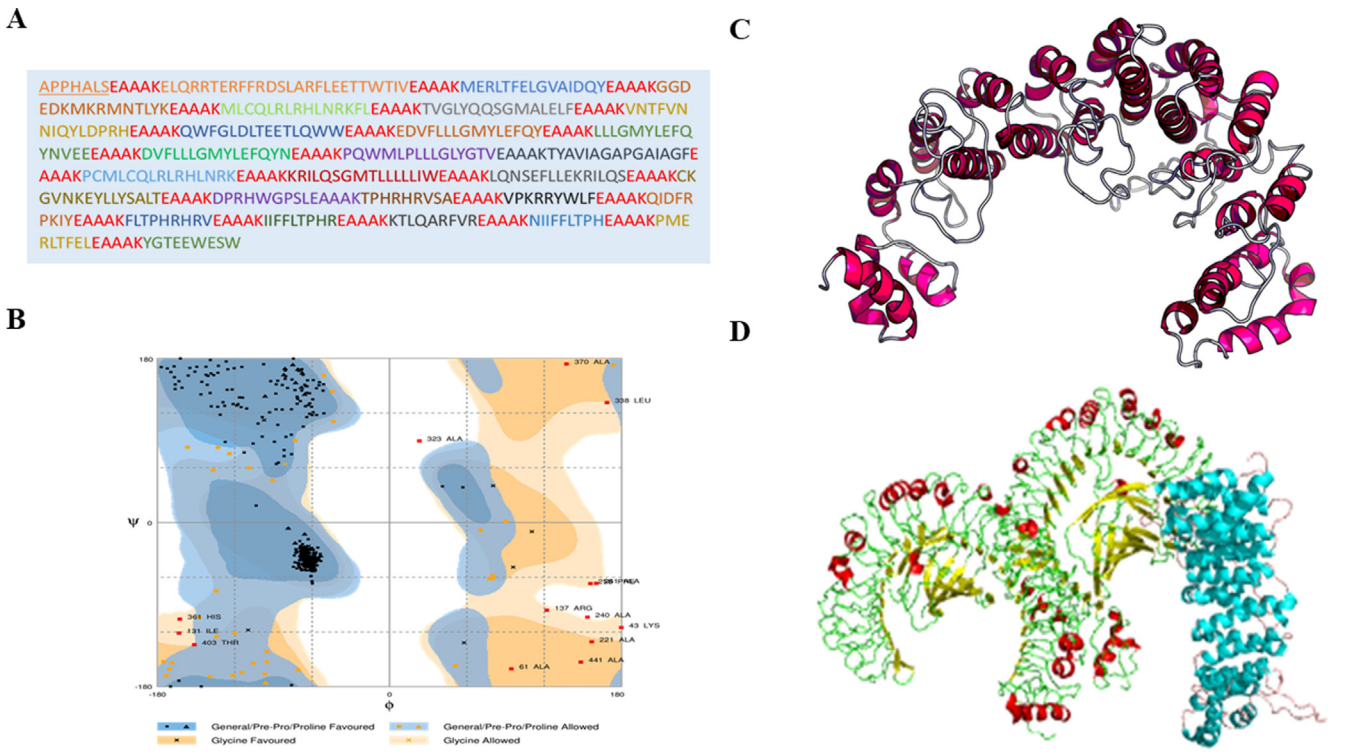


Fig. 1. (A) MVBKV vaccine sequence consisting of 479 amino acids, with first 7 amino acids for TLR4 binding. Molecular linker sequence (EAAAK) are shown in red. Epitopes for HLA class I are 9mer and HLA class II are 15mer long respectively. (B) Ramachandran plot analyses of modeled structure shows 89.7%, 7.3% and 2.9% in favored, allowed and outlier regions respectively. (C) MVBKV 3D-model after homology modeling. (D) Docked complex of TLR4 (PDB ID:4G8A) with subunit vaccine construct. TLR4 is shown in red whereas blue is MVBKV vaccine as a ligand in the docked complex (ClusPro server). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(34) in Table 2 represent binding of some of the epitopes with multiple HLA class II alleles. Importantly, each of the 27 reference alleles of HLA class II molecules are represented in our vaccine construct. Therefore, our selected epitopes demonstrate the binding potential with the maximum number of HLA alleles and highest population coverage rate for all the geographical areas.

Tables 1 and 2 also lists the immunogenicity score of the epitopes, positive score indicating a greater probability to induce an immune response. Next, after checking the solubility, toxicity, and allergenicity (supplementary file), the selected epitopes were subjected to further vaccine designing.

3.2. MVBKV 3D-structure prediction and structural studies

Galaxy server was used to predict the 3D-structure of the final vaccine construct using a template-based approach. The server provided several possible structures ranking with the significance values. The top rank model uses the alpha-chymotrypsin (2vchA) as a template with a p-value of 6.57e-04. The predicted model is a single domain with 61% b-helix, 2% beta sheet and 35% coils (Fig. 1B). Secondary structure studies using Ramachandran plot found 89.7% residues fall in the favored region, 7.3% in the allowed region and 2.9% in the outlier region of the R-plot, which suggest the stable nature of the designed construct (Fig. 1C). The final vaccine construct (MVBKV) is 479 amino acids long, water-soluble and has a half-life time 4.4 h *in vitro* as predicted in mammalian reticulocyte.

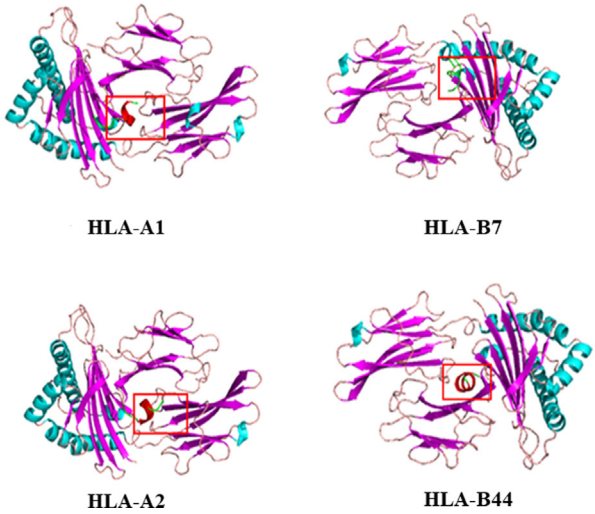
3.3. Molecular docking studies

Molecular docking studies on the ClusPro server to predict the binding of MVBKV's adjuvant (RS09) with TLR4 receptor (PDB ID: 4G8A), showed that the RS09 dock snugly in TLR4 binding sites. The center and lowest energy weighted scores are 916.5 and −918.8 respectively (Fig. 1D). Molecular docking of the individual epitopes with representative HLA class I alleles (HLA -A1, -A2, -B7, -B44), and representative HLA class II alleles (HLA – DRB1*1:01, -DRB3*02:02, -DRB5*01:01, -DRB1*03:01, HLA-DRB1*04:01) showed optimal binding characteristics (Figs. 2 and 3). Thus, the molecular docking studies showing stable interactions between the individual epitopes and their respective HLA alleles support our approach for MVBKV design. High-resolution structure of other HLA alleles for docking studies was not freely available for analyses.

4. Discussion

In the present study, we have applied a reverse vaccinology-based *in silico* approach to identify the most-antigenic epitopes in the BKV proteome. Though computational approaches have been utilized to identify vaccine targets for a number of microbial pathogens (including HIV, leishmaniosis, mycobacterium, hepatitis B and HPV [36–43], only limited studies have addressed the efficacy of these approaches in BK viremia. Given that individuals with an intact immune system can effectively control BKV reactivation, immunotherapeutic approaches have the potential to limit viral load in KTRs, without showing the adverse effects of the current therapeutic regimen [27,44,45]. Supporting this, a recent clinical phase II study at the National Heart Lung, and Blood Institute, NHLBI (NCT02108522) [46] demonstrated the effectiveness of adoptively transferred BKV-specific T-cells to prevent BK viremia in hematopoietic stem cell transplant (HSCT) patients. The use of overlapping peptides and adoptive T-cell expansion was also successful against multiple viruses (including BKV), frequently infect-

A

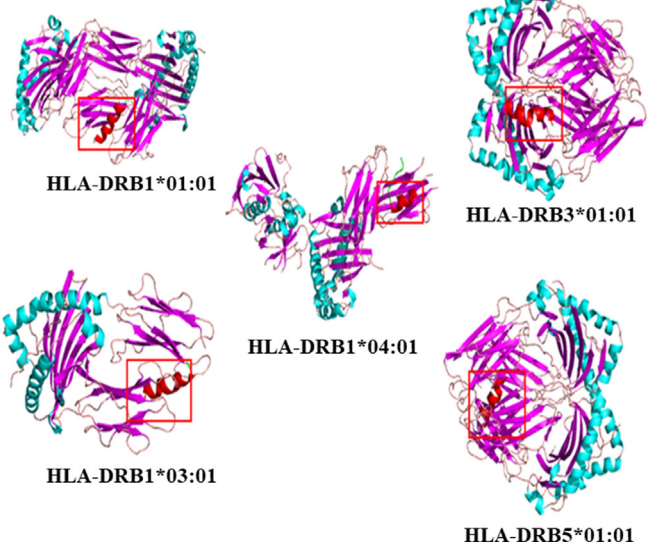


B

HLA I superfamily	Cluster	Members	Representative	Weighted Score
HLA A1	0	224	Center	-539.9
			Lowest Energy	-658.7
HLA A2	0	394	Center	-663.0
			Lowest Energy	-791.4
HLA B7	0	328	Center	-493.1
			Lowest Energy	-645.2
HLA B44	0	750	Center	-672.4
			Lowest Energy	-721.8

Fig. 2. (A) Docked complex of HLA class I allele with respective peptide. Peptide is shown in red color box in the docked complex obtained from ClusPro server. (B) Representative center and lowest energy weighted score of HLA-peptide docked complex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

A



B

HLA II Allele	Cluster	Members	Representative	Weighted Score
DRB5-0101	0	268	Center	-847.8
			Lowest Energy	-998.1
DRB1-0301	0	378	Center	-861.7
			Lowest Energy	-882.2
DRB1-0101	0	348	Center	-826.4
			Lowest Energy	-845.7
DRB3-0101	0	201	Center	-1096.1
			Lowest Energy	-1096.1
DRB1-0401	0	280	Center	-673.2
			Lowest Energy	-802.9

Fig. 3. (A) Docked complex of HLA class II allele with respective peptide. Peptide has been shown in red color box in the docked complex obtained from ClusPro server. (B) Representative center and lowest energy weighted score of HLA-peptide docked complex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ing immunocompromised patients [47]. Another study showed overlapping peptide pool for 9mer $CD8^+$ epitopes [44] or 15mer $CD4^+$ epitopes [45] in early viral gene region (EVGR) to be effective in KTRs. Further, an adenoviral vector-based approach was also effective in T-cell immunotherapy for transplant patients [48].

Our study represents several advancements over previous immunotherapeutic approaches against BK viremia. This is the first study to design an epitope-based fusion-peptide BKV vaccine (MVBKV) by identifying the most-antigenic epitopes in the entire BKV proteome. Further, our analyses include an overlap among the peptide-binding specificities for each of the HLA class I and class II alleles, suggesting MVBKV's universal nature and potential binding in a wide-population base, irrespective of patients' HLA type. Our analyses also extended to include epitopes conserved among all currently known BKV strains, suggesting higher protection against BK viremia. To our knowledge, except for a single previously published epitope (FLTPHRHRV) [49], the list of HLA class I and class II epitopes presented in this paper are unique and have not been previously reported. A major limitation of the present study is, however, the lack of any experimental data validating the *in silico* predicted epitopes. A review of the previously published reports show that the success of computationally predicted epitopes is variable and dependent on a number of factors (for e.g., the complexity and size of the pathogen's genome and dataset, stringency of selection criteria in epitope filtering and method/s of experimental validation, HLA type frequency etc.), with an

average of 25–60% computationally identified epitopes that can be experimental validated [50–52]. Thus, the next step in the process of MVBKV's validation will involve actual testing of immunogenicity and therapeutic efficacy.

Since the development of the meningococcus vaccine in 2000, immunoinformatic approaches are becoming increasingly popular as the first step in the identification of relevant immunodominant epitopes for further experimental validation. In addition to allowing a faster and robust screening of the entire proteome, *in silico* approaches also provide useful predictive numerical scores on several vaccine traits such as peptide antigenicity, sub-cellular localization, conservancy, stable configuration etc., which are impossible to obtain using traditional approaches, but nonetheless essential in establishing vaccine profile and reducing downstream failure rate. Additionally, when compared to the whole proteins, epitope-based approaches generate a more potent and sustained T-cell response, by efficient processing of only relevant HLA epitopes. Further, use of molecular linkers to engineer a multivalent subunit vaccine eases any potential issues with the syntheses, delivery, and handling of peptide pool. The molecularly linked fusion-peptide vaccine is currently in clinical trials for cytomegalovirus [53]. A newer untested aspect of MVBKV design is the presence of a synthetic adjuvant (RS09), which binds and activate the TLR4 receptor on antigen presenting cells. RS09 mimics lipopolysaccharide (LPS), a natural TLR4 ligand, and its use is a safer and more effective advancement over traditional vaccination approaches (such as using Freund's adjuvant) [31]. Whether RS09 is tolerable in transplant patients remains to be tested, however, RS09 has been shown to be effective in animal studies following intranasal immunization against HIV-1 adenoviral vector [54].

In summary, MVBKV offers a simple, fast and effective way to manage BK viremia in KTRs irrespective of HLA type or BKV strain subtypes. Further immunogenicity and efficacy testing of MVBKV needs to be done to fully evaluate the clinical translational potential of the identified epitopes. Once validated, MVBKV is also expected to provide cross-protection against the reactivation of JC virus, another polyomavirus commonly seen in transplant patients with high sequence similarity to BKV [55].

Author contribution

VK conceived the idea. Both the authors performed the data acquisition, analyses and wrote the manuscript. Both authors approved the final article.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.04.096.

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