IMMUNOLOGY

Donor HLA-DQ genetic and functional divergence affect the control of BK polyoma virus infection after kidney transplantation

Mathieu F. Chevalier[1]*†, Vincent Allain[1,2]†, Julien Gras[3]‡, Julien Racle[4]‡, Juliette Villemonteix[2]‡, Gillian Divard[5], Linda Feghoul[6], Constance Delaugerre[6], Jean-Michel Molina[3], Jean-Luc Taupin[2], Marie-Noelle Peraldi[5], David Gfeller[4], Cyrille Feray[7], Sophie Caillat-Zucman[1,2]*

BK polyomavirus (BKPyV) infection remains a major concern after kidney transplantation, increasing the risk of graft loss in the absence of specific antiviral agent now available. Here, we investigated the impact of HLA diversity on the control of posttransplant BKPyV replication. High HLA evolutionary divergence (HED) at the DQ locus in the donor was an independent predictor of BKPyV-free outcome. More generally, we highlighted the protective effect of highly divergent pairs of HLA-DQ heterodimers corresponding to heterozygous HLA-DQα01/non-DQα01 combinations. We then defined a functional divergence metrics assessed by the similarity of peptide-binding motifs between pairs of HLA-DQ molecules. Greater functional divergence correlated with the size of the BKPyV-derived DQ-bound immunopeptidome and a lower risk of BKPyV reactivation, thus providing a molecular basis for the observed genetic differences. Together, these data provide evidence for a direct link between donor HLA-DQ genetic and functional divergence, diversity of the DQ-bound immunopeptidome, and control of viral infection, likely reflecting stronger antiviral T cell responses.

INTRODUCTION

BK polyomavirus (BKPyV) is a ubiquitous virus of the *Polyomaviridae* family that persistently infects almost the entire human population from early childhood. After a usually asymptomatic primary infection, BKV establishes lifelong latency in uroepithelial and renal tubular epithelial cells. In healthy individuals, intermittent BKV reactivation with asymptomatic viruria may occur. In kidney transplant recipients, however, impairment of immune responses favors uncontrolled BKPyV replication and viremia, causing tubular epithelium damage that can progress to BKV-associated nephropathy (BKVAN), a frequent cause of graft loss (*1*, *2*). In the absence of specific antiviral agent now available, reducing immunosuppression with the aim of restoring effective antiviral immunity remains the cornerstone of management of BKPyV infection. However, it must be weighed against the risk of rejection and development of de novo donor-specific anti-human leukocyte antigen (HLA) antibodies (DSA). Therefore, early identification of patients at high risk of BKV replication is crucial to best tailor immunosuppression.

HLA class I and class II molecules play a critical role in antiviral immunity, by presenting virus-derived antigenic peptides to CD8 and CD4 T lymphocytes, respectively, which then trigger specific antiviral immune responses. Because of their high polymorphism, HLA molecules exhibit substantial interindividual variability in the spectrum of peptides they can present to T cells, which may directly affect the magnitude of the immune response. According to the heterozygote advantage, first demonstrated in viral infections, heterozygous HLA alleles present a broader array of antigenic peptides, thus promoting a more diverse and effective T cell response (*3*–*5*). HLA diversity can be refined using the HLA evolutionary divergence (HED) metrics, which quantifies the sequence divergence between the peptide binding domains of the two alleles of a given locus. The more different the peptide binding grooves of the two HLA alleles, the larger the immunopeptidome they can present to T cells, possibly increasing the likelihood of an efficient immune response. HED was positively correlated with the number of pathogen-derived peptides predicted to bind to the two corresponding HLA alleles (*6*). Furthermore, allele divergence at the HLA-B locus was negatively correlated with HIV viral load across chronically infected individuals (*5*). We and others demonstrated the relevance of HED as a predictor of outcome in various pathophysiological contexts such as response to anticancer treatment (*7*, *8*), vaccination (*9*, *10*), and solid organ (*11*) or hematopoietic stem cell transplantation (HSCT) (*12*–*14*).

Here, we hypothesized that HLA diversity might affect the risk of BKPyV replication after kidney transplantation. Our results indicate that HLA divergence at the DQ locus of the donor and, more precisely, heterozygosity for the two highly divergent DQα01 and non-DQα01 groups of HLA-DQαβ heterodimers are relevant proxies for the diversity of the BKPyV-derived immunopeptidome predicted to bind to donor DQ molecules and thereby predict the risk of BKPyV infection after kidney transplantation.

RESULTS

Cohort characteristics

Among patients who underwent kidney transplantation between January 2012 and March 2020, 531 patients were eligible for inclusion

[1]INSERM UMR 1342, Institut de Recherche Saint-Louis, Université Paris Cité, Paris, France. [2]Laboratory of Immunology, Saint-Louis Hospital, APHP, Paris, France. [3]Infectious Diseases Department, Saint-Louis Hospital, APHP, Paris, France. [4]Department of Oncology UNIL CHUV, Ludwig Institute for Cancer Research, University of Lausanne, Lausanne, Switzerland. [5]Nephrology-Transplantation Department, Saint-Louis Hospital, APHP, Paris, France. [6]Laboratory of Virology, Saint-Louis Hospital, APHP, Paris, France. [7]Centre Hépatobiliaire, Paul Brousse Hospital, Inserm U1193, Université Paris Sud, Villejuif, France.
*Corresponding author. Email: sophie.caillat-zucman@aphp.fr (S.C.-Z.); mathieu.chevalier@inserm.fr (M.F.C.)
†These authors contributed equally to this work.
‡These authors contributed equally to this work.

based on accurate screening for BKPyV DNAemia during the first 2 years after transplantation. Baseline characteristics of the patients are shown in Table 1. Median age at time of transplantation was 49 years. Pretransplant DSA were present in 29 patients (5.5%). Twelve patients (2.3%) did not receive antithymocyte globulin (ATG) induction because of HIV infection or past long-term immunosuppression.

During the 2-year follow-up, BKPyV DNAemia was diagnosed in 95 patients (17.9%, hereafter referred to as BKPyV-positive) at a median time of 137 days after transplantation. Of the 95 patients who experience BKPyV DNAemia, 23 patients (24.2% of BKPyV-positive, i.e., 4% of the entire cohort) developed BKVAN.

Overall, the risk of BKPyV DNAemia was significantly higher in older patients and in patients with a high number of HLA mismatches (Table 1 and Fig. 1, A and B). No other factor related to the recipient and/or the transplant was significantly associated with BKPyV DNAemia. In particular, the frequencies of de novo DSA and T cell– or antibody-mediated rejection occurring before the first BKPyV DNAemia (i.e., before preemptive reduction of immunosuppression) were similar in BKPyV-positive and BKPyV-negative patients.

HLA diversity and control of posttransplant BKPyV replication

HLA characteristics of the kidney transplant recipients did not evidence major differences between those who experienced BKPyV DNAemia or not. BKPyV DNAemia was associated positively with the presence of *A*23:01* [4.2% in BKPyV-negative and 8.4% in

Table 1. Cohort characteristics. M, male; F, female; NA, not applicable. Bold values denote statistical significance ($P < 0.05$ from the log-rank test).

Variables*		BKPyV^{+} ($n = 95$)	BKPyV^{neg} ($n = 436$)	Total ($n = 531$)	P value (log-rank)
Recipient age		54.1 [44.0, 64.3]	48 [36.6, 59.2]	48.9 [37.3, 59.8]	**0.0032**
Sex	M	62 (65.3)	277 (63.5)	339 (63.8)	0.5206
	F	33 (34.7)	159 (36.5)	192 (36.2)	
HIV	No	90 (94.7)	423 (97.0)	513 (96.6)	
	Yes	5 (5.3)	13 (3.0)	18 (3.4)	0.267
Donor age		52 [42.0, 65.5]	50 [38, 60]	51 [40, 61]	0.0529
Donor type	Living-related	13 (13.7)	84 (19.3)	97 (18.3)	0.2010
	Deceased	82 (86.3)	352 (80.7)	434 (81.7)	
ATG induction	No	3 (3.2)	9 (2.1)	12 (2.3)	0.5167
	Yes	92 (96.8)	427 (97.9)	519 (97.7)	
Rejection (before BKPyV)	No	86 (90.5)	366 (83.9)	452 (85.1)	0.1178
	Yes	9 (9.5)	70 (16.1)	79 (14.9)	
De novo DSA (before BKPyV)	No	71 (74.7)	347 (79.6)	418 (78.7)	0.2403
	Yes	24 (25.3)	89 (20.4)	113 (21.3)	
BKVAN	No	72 (75.8)	NA	508 (95.7)	NA
	Yes	23 (24.2)	NA	23 (4.3)	
Number of HLA mismatches	≤6	45 (47.9)	267 (61.2)	312 (58.9)	**0.0173**
	>6	49 (52.1)	169 (38.8)	218 (41.1)	
	Missing	1	0	1	
Antigen mismatch, HLA-A	0	8 (8.4)	58 (13.3)	66 (12.4)	0.3901
	1	41 (43.2)	205 (47)	246 (46.3)	
	2	46 (48.4)	173 (39.7)	219 (41.2)	
Antigen mismatch, HLA-B	0	6 (6.3)	39 (8.9)	45 (8.5)	0.1977
	1	35 (36.8)	160 (36.7)	195 (36.7)	
	2	54 (56.8)	237 (54.4)	291 (54.8)	
Antigen mismatch, HLA-C	0	9 (9.5)	59 (13.5)	68 (12.8)	0.2512
	1	47 (49.5)	179 (41.1)	226 (42.6)	
	2	39 (41.1)	198 (45.4)	237 (44.6)	
Antigen mismatch, HLA-DR	0	20 (21.1)	132 (30.3)	152 (28.6)	0.1756
	1	53 (55.8)	227 (52.1)	280 (52.7)	
	2	21 (22.1)	77 (17.7)	98 (18.5)	
	Missing	1	0	1	
Antigen mismatch, HLA-DQ	0	38 (40)	162 (37.2)	200 (37.7)	0.2772
	1	41 (43.2)	211 (48.4)	252 (47.5)	
	2	16 (16.8)	63 (14.4)	79 (14.9)	

*Continuous variables are expressed as median [interquartile range] and categorical variables as number (%) of individuals.

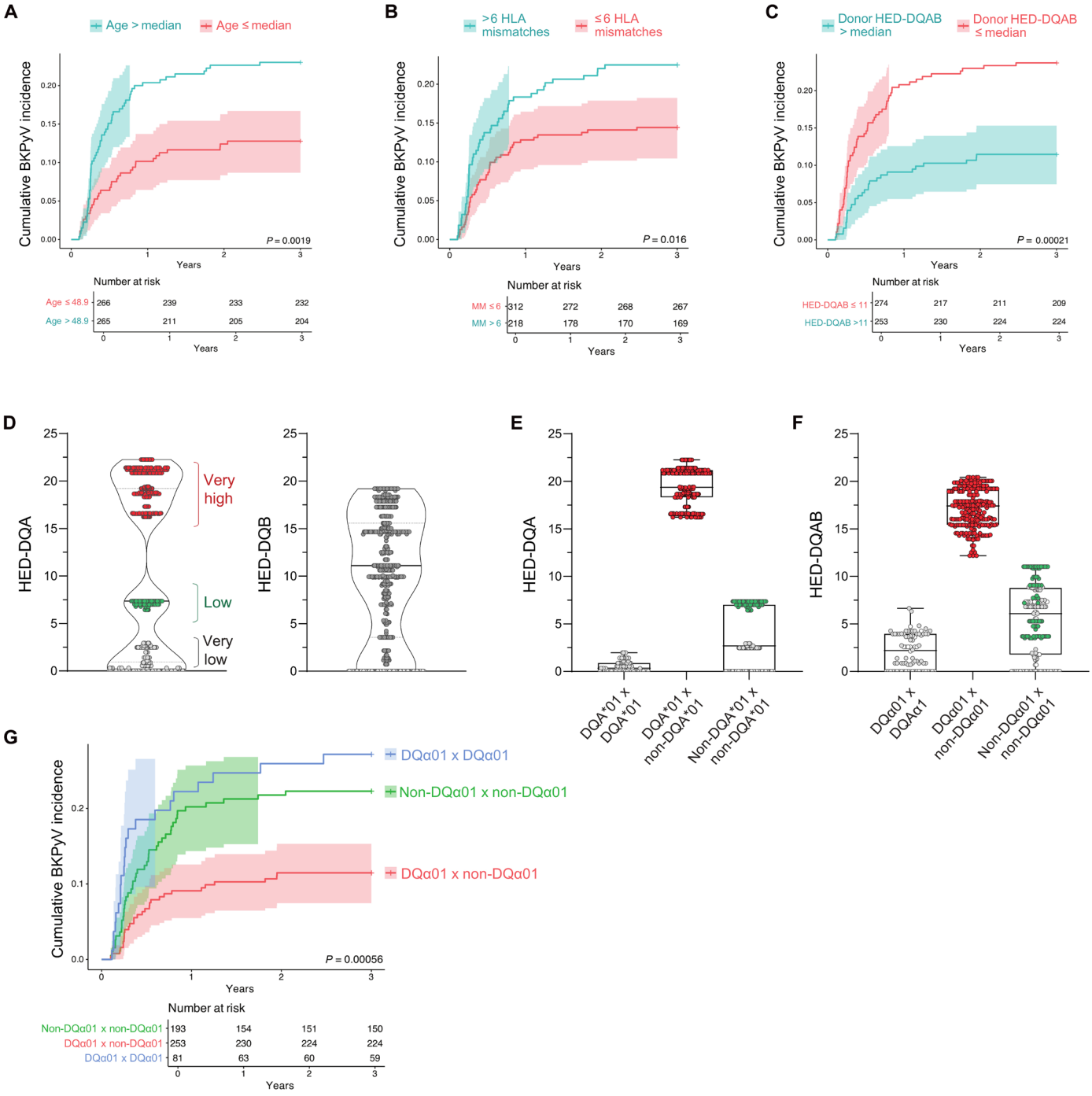

Fig. 1. Donor HLA-DQ diversity is associated with control of posttransplant BKPyV infection. (**A** to **C**) BKPyV cumulative incidence postkidney transplantation in patients (A) above or below median age, (B) with number of HLA mismatch above or below 6, and (C) with donor HED-DQAB above or below median. (**D**) Distribution of donor HED-DQA (left) and HED-DQB (right) values. (**E** and **F**) Donor HED-DQA (F) and HED-DQAB (G) in individuals with two alleles of the DQA1*01 supertype, an allele of the DQA1*01 supertype together with a non-DQA1*01 allele, or with two non-DQA1*01 alleles (i.e., of the DQA1*02, 03, 04, 05, or 06 supertype). (**G**) BKPyV cumulative incidence postkidney transplantation in patients with donors showing DQα01/DQα01, DQα01/non-DQα01, or non-DQα01/non-DQα01 pairs. *P* values from log-rank tests [(A) to (C) and (H)] are indicated on each graph. MM, mismatch.

BKPyV-positive, odds ratio (OR) = 2.18, $P = 0.015$] and negatively with *B*44:03* (4.8% versus 1.05%, OR = 0.20, $P = 0.029$) and *C*07* (37.1% versus 21%, OR = 0.55, $P = 0.019$), but these differences were not significant after Bonferroni correction for multiple testing. Furthermore, there was no impact of heterozygosity or HLA divergence (HED) at any HLA locus of the recipient on the risk of BKPyV DNAemia. Of note, median HED values were similar to those previously reported in other clinical settings (*10*, *12*, *14*–*16*).

We next analyzed HLA characteristics of the donors. No particular HLA allele was associated with BKPyV DNAemia. However, the risk of BKPyV DNAemia was lower in the case of donor heterozygosity at the DQA1 locus or DQB1 locus. Furthermore, donor HLA-DQ allotypes of BKPyV-negative recipients exhibited higher sequence divergence (median HED-DQAB, 13.5 in BKPyV-negative versus 7.0 in BKPyV-positive), reflecting differences at both the DQA1 locus (median HED-DQA, 16.2 versus 6.9) and DQB1 locus (median HED-DQB, 11.1 versus 9.5) (Table 2). Thus, cumulative incidence of BKPyV DNAemia was 11.4% in recipients of donors with high HED-DQAB (i.e., over the median) compared to 23.8% for those with low HED (Fig. 1C and fig. S1, A and B). This effect was not explained solely by differences in homozygosity between groups, since it remained significant when only considering donors heterozygous at the DQA1 and DQB1 loci (median HED-DQAB, 15.4 in BKPyV-negative versus 11.0 in BKPyV-positive) (fig. S1C). Of note, the impact of HLA-DQ allele divergence was even more pronounced in patients who developed BKVAN than in those who did not (HED-DQAB median 4.3 in BKPyV-positive with BKVAN compared to 7.33 in those without BKVAN and 13.5 in BKPyV-negative, $P = 0.0007$).

Together, these results suggest that HLA diversity at the donor DQA1 and DQB1 loci, assessed by high HED scores, contributes to the control of BKPyV replication after kidney transplantation.

Bimodal HED-DQA distribution defining two groups of DQ molecules

Examining the distribution of HED at all loci in the entire cohort showed that HED-DQA1 values were oddly distributed in three distinct clusters of very high, low, and very low divergence, in contrast to an almost continuous distribution of HED values at DQB1 and all other loci (Fig. 1D and fig. S1D). We therefore analyzed which pairs of heterozygous DQA1 alleles were present in these clusters and observed that highly divergent pairs (HED > 16) always consisted of an allele of the DQA1*01 supertype together with a non-DQA1*01 allele (i.e., allele of the DQA1*02, 03, 04, 05, or 06 supertype). Conversely, slightly divergent pairs consisted of either two alleles of the

Table 2. HLA divergence in recipients and donors of the study cohort. Bold values denote statistical significance ($P < 0.05$ from the log-rank test).

Variables*		BKPyV^{+} ($n = 95$)	BKPyV^{neg} ($n = 436$)	Total ($n = 531$)	*P* value (log-rank)
Recipient HLA characteristics:					
HED-A		7.9 [4.7, 10.4]	7.2 [4.2, 10.2]	7.3 [4.2, 10.2]	0.5881
	Missing	1	1	2	
HED-B		7.9 [5.7, 9.1]	7.7 [6.0, 9.6]	7.8 [6.0, 9.5]	0.6221
	Missing	1	1	2	
HED-C		5.2 [3.2, 7.2]	5.2 [3.3, 6.6]	5.2 [3.3, 6.7]	0.7950
	Missing	1	0	1	
HED-DRB1		9.9 [5.9, 13.9]	10.4 [6.5, 14.0]	10.3 [6.2, 14.0]	0.3481
	Missing	1	0	1	
HED-DQA1		7 [0.0, 19.1]	7.4 [0.6, 19.3]	7.4 [0.3, 19.2]	0.1044
	Missing	0	2	2	
HED-DQB1		9.9 [1.3, 14.7]	11.1 [3.6, 15.0]	11 [3.6, 15.0]	0.1621
Donor HLA characteristics:					
HED-A		7.4 [4.2, 10.3]	7.4 [4.8, 10.4]	7.4 [4.8, 10.4]	0.799
HED-B		8.1 [6.3, 9.6]	8 [6.1, 9.6]	8 [6.2, 9.6]	0.7350
HED-C		5 [3.4, 7.0]	5.3 [3.4, 7.2]	5.3 [3.4, 7.2]	0.8770
HED-DRB1		10.6 [4.5, 14.0]	10.9 [7.8, 14.3]	10.9 [7.4, 14.3]	0.0668
HED-DQA1		6.9 [0.0, 16.6]	16.2 [2.5, 20.9]	7.4 [1.1, 19.2]	**0.0001**
	Missing	1	3	4	
HED-DQB1		9.5 [0.6, 14.7]	11.1 [5.2, 16.3]	11.1 [3.6, 15.6]	**0.0350**
HED-DQAB		7.0 [2.0, 15.4]	13.5 [4.0, 17.5]	11.0 [3.7, 17.0]	**0.0003**
HLA-DQ groups	**α01/α01**	22 (23.4)	59 (13.6)	81 (15.4)	
	non-α01/non-α01	43 (45.7)	150 (34.6)	193 (36.6)	
	α01/non-α01	29 (30.9)	224 (51.7)	253 (48.0)	**0.0003**
	Missing	1	3	4	
Motif divergence (HLA-DQ)		0.87 [0.48–0.99]	0.92 [0.70–1.04]	0.90 [0.66–1.01]	**0.034**
	Missing	1	3	4	

*All variables are expressed as median [interquartile range].

DQA1*01 supertype (HED < 2) or two non-DQA1*01 alleles (HED 2.5 to 7.5) (Fig. 1E).

HLA-DQ molecules are heterodimers of α and β chain proteins encoded by the DQA1 and DQB1 genes, which can be formed as both cis and trans variants depending on whether the α and β chains are encoded on the same (cis) or opposite (trans) chromosomes. However, while all cis-encoded dimers are stably expressed at the cell surface, some trans-dimers are known to be unstable and thus not contributing to the DQ immunopeptidome (*17*–*19*). The DQα and DQβ molecules that can heterodimerize effectively fall into two mutually exclusive groups hereinafter designated as the DQα01 group (any DQA1*01 allele which can combine with any DQB1*05 or DQB1*06 allele) and the non-DQα01 group (any non-DQA1*01 allele which can combine with any DQB1*02, 03, or 04 allele) (*18*, *20*). When analyzing the HED-DQAB (i.e., mean of HED-DQA1 and HED-DQB1), highly divergent pairs of heterodimers (i.e., HED over the median) always consisted of heterozygous DQa01/non-DQa01 combinations similar to what was found with HED-DQA alone (Fig. 1F).

We therefore analyzed the distribution of the DQα01/DQα01, DQα01/non-DQα01, and non-DQα01/non-DQα01 pairs among donors and found that it significantly differed between donors of BKPyV-negative and BKPyV-positive recipients (fig. S1E), which here also remained significant when removing donors homozygous at the DQA1 and DQB1 loci (fig. S1F). Having a heterozygous DQα01/non-DQα01 donor strongly protected against the risk of posttransplant BKPyV DNAemia compared to a DQα01/DQα01 or non-DQα01/non-DQα01 donor (Fig. 1G). Together, these results indicate that combination of highly divergent DQα01 and non-DQα01 molecules in a donor is associated with a better control of BKPyV reactivation in the kidney transplant recipient.

HLA-DQ motif divergence as a molecular basis for BKPyV control

Our recent work accurately determined the binding specificity of any HLA class II allele (*21*), described by a motif that defines the 9-mer binding cores of peptides that can be presented by a given allele. We therefore determined whether the binding specificities of HLA-DQ alleles could recapitulate the differences observed between the DQα01 and non-DQα01 groups. Principal components analysis on all HLA-DQ heterodimers present in our dataset revealed that alleles from these two DQα groups clustered separately (Fig. 2A).

Considering this, we defined a motif divergence metrics, which measures on a continuous scale how divergent binding specificities of the donor HLA-DQ molecules are, to quantify the potential diversity of the peptides that can be presented by HLA-DQ molecules of each donor (Fig. 2B). We found that this motif divergence metrics was strongly correlated with HED (fig. S2A) and helped discriminating the patients with or without BKPyV reactivation (Fig. 2C and fig. S2B). In particular, greater motif divergence at the HLA-DQ was more protective against BKPyV reactivation, serving as a molecular basis for the differences observed previously based on HED and DQα groups.

Relationship between HED-DQAB, the BKPyV-derived immunopeptidome, and posttransplant BKPyV DNAemia

The BKPyV genome comprises three functional regions: a noncoding control region, an early region encoding the regulatory proteins small T antigen (sTag) and large T antigen (LTag), and a late region encoding the nonstructural agnoprotein and the capsid proteins VP1, VP2, and VP3. BKPyV-specific immune responses can target all proteins except the agnoprotein (*22*, *23*), but data on immunodominant epitopes are scarce and mostly limited to common class I alleles (*24*) or to in silico–predicted putative immunodominant epitopes (*25*).

To assess whether the sequence divergence between the peptide binding regions of two DQ molecules was related to the breadth of the corresponding BKPyV immunopeptidome, we evaluated the number of unique viral-derived peptides predicted to bind to DQ molecules present in the study cohort (fig. S2C). To this aim, we used the recently updated NetMHCIIpan-4.2 prediction algorithm, which includes large dataset highly enriched in DQ peptide ligands (*19*). A total of 68 cis-encoded and stable trans-encoded DQ molecules were considered for their ability to bind all unique 15-mer peptides derived from LTag, sTag, VP1, and VP2/3 proteins. Of note, VP3 is a truncated form of VP2 so VP3-derived peptides were not considered in the total number of BKV peptide binders to avoid counting them twice.

We first determined the number of predicted DQ-bound peptides across individuals of the entire cohort. Together, a median number of 152 (range, 62 to 235) unique BKPyV-derived DQ binders per individual was identified. The VP2 protein provided the highest number of DQ-bound peptides, followed by LTag (Fig. 2D). When normalized to the protein length, VP1 and sTag appeared poorly immunogenic by HLA-DQ presentation, while VP2-derived peptides and, more particularly, those not overlapping with the VP3 protein were the most likely to be presented by HLA-DQ molecules (Fig. 2E).

We next analyzed the relationship between the number of unique DQ-bound BKPyV peptides in a given donor and the corresponding HED-DQAB and motif divergence scores. The total number of predicted DQ binders was positively correlated with the sequence divergence between the corresponding HLA-DQ molecules, with VP2-derived peptides showing the strongest correlation (Fig. 2, F and G).

Analyzing the proportion of DQ binders shared by heterozygous HLA-DQ pairs showed a lower overlap between DQα01 and non-DQα01 molecules than between other combinations (fig. S2D). Thus, a high proportion of DQα01 binders was predicted to bind exclusively to DQα01 molecules (i.e., not shared with other DQ supertypes), while non-DQα01 binders were less specific to their respective DQA supertype (fig. S2E). Accordingly, the predicted BKPyV immunopeptidome of patients with DQα01/non-DQα01 pairs was broader than that of patients with DQα01/DQα01 or non-DQα01/non-DQα01 pairs (Fig. 2H). VP2-derived peptides contributed mostly to these differences (fig. S2F). These results confirm the high divergence of DQα01 and non-DQα01 supertypes with pairwise complementarity of associated peptide binding profiles.

Last, we tested whether the ability of a donor HLA-DQ pair to bind more BKPyV peptides was associated with control of BKPyV replication in the recipient. The number of predicted VP2-derived DQ binders was negatively associated with BKPyV DNAemia ($P = 0.035$) (Fig. 2I and table S1). Together, these results indicate that a highly divergent HLA-DQα01/non-DQα01 supertype pair in the donor (i) can bind a broader BKPyV immunopeptidome and (ii) is associated with a lower risk of posttransplant BKPyV DNAemia in the recipient, which likely reflects a stronger antiviral immune response.

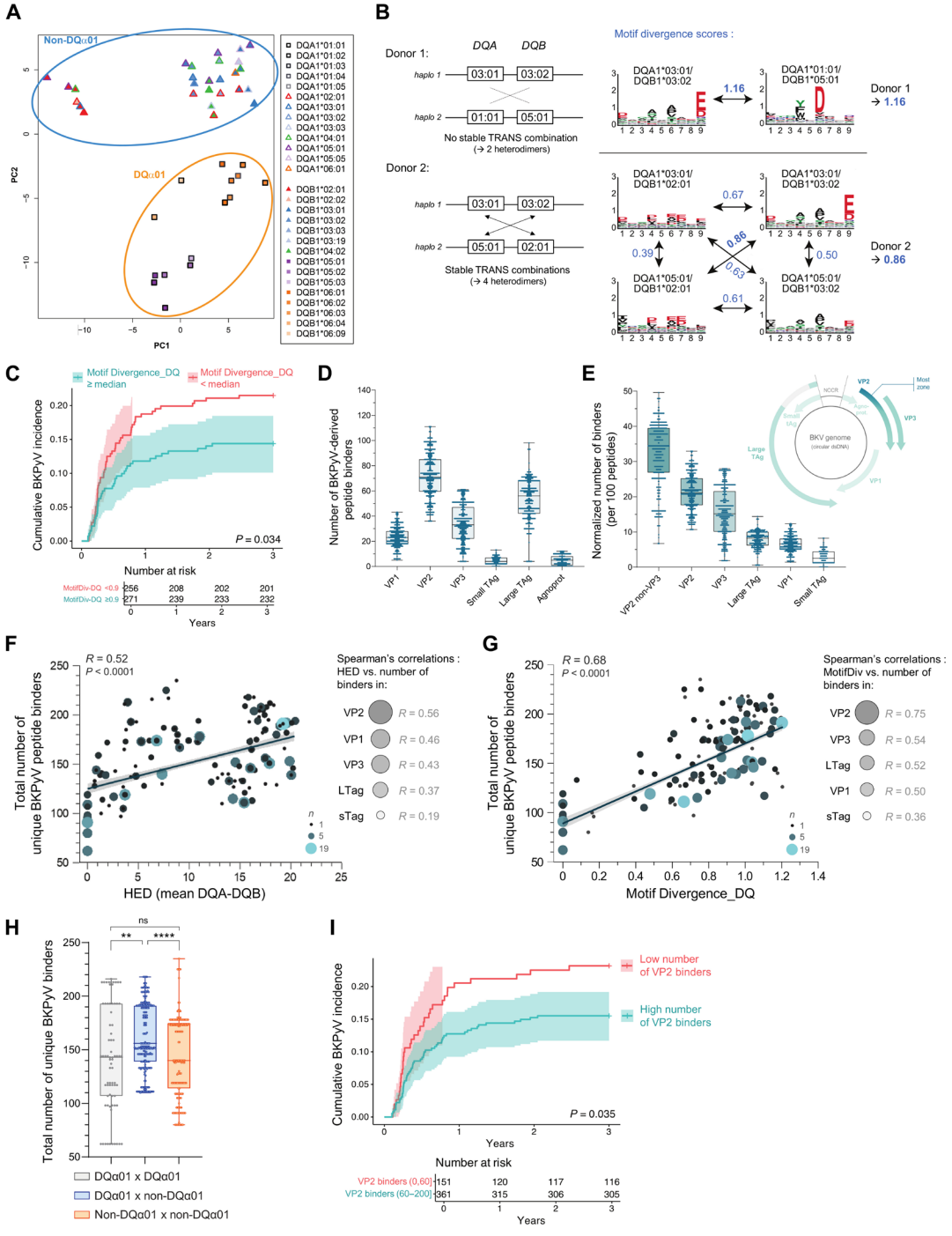

Fig. 2. Association of HLA-DQ genetic and motif divergence with the BKPyV-derived immunopeptidome. (**A**) Principal components (PC) analysis on the binding specificities from HLA-DQ molecules (outer colors: HLA-DQα chain, inner colors: HLA-DQβ chain). Squares: DQα01 group; triangles: non-DQα01 groups. (**B**) Example of motif divergence computed for the HLA-DQ molecules from two donors. Motif divergence is first computed between each pair of stable HLA-DQ heterodimers from the donor (for donor 1, this corresponds to a single comparison; donor 2 has four stable HLA-DQ heterodimers, resulting in six pairwise comparisons). Values from each pairwise comparison are indicated (arrows). The final motif divergence value assigned to the donor corresponds to the maximum value and is indicated in bold. (**C**) BKPyV cumulative incidence postkidney transplantation in patients stratified based on donor's HLA-DQ motif divergence. (**D** to **I**) Number of predicted DQ-bound peptides (15-mers overlapping the BKPyV proteome) determined for each donor of the entire cohort. (D) Number of unique peptides from the indicated BKPyV proteins predicted to bind the HLA-DQ molecules of each donor. (E) Number of unique predicted binders normalized to the length of the corresponding protein. (F and G) Correlation between the total number of predicted BKPyV-derived DQ-bound peptides and the HED-DQAB (F) or motif divergence (G) scores for each donor. Spearman's rank correlation coefficients (*R*) are indicated. (H) Total number of predicted BKPyV-derived peptide binders in donors from indicated groups. (I) BKPyV cumulative incidence according to the number of VP2 peptides predicted to bind donors' HLA-DQ molecules (using the optimal cutoff threshold, i.e., 60 peptides). *P* values from log-rank [(C) and (I)] or Mann-Whitney (H) tests are indicated on each graph. ns, not significant; $^{**}P < 0.01$ and $^{****}P < 0.0001$.

BKV variants

BKPyV is classified into four genotypes (I to IV) based on sequence variation of the VP1 gene. BKPyV-I subtype is largely predominant worldwide (80%) followed by genotype IV and rarely genotype II (*26*). Because almost all individuals are latently infected at adulthood, posttransplant BKPyV infection may be donor derived or as a result of reactivation of recipient latent infection. This raises the possibility that BKPyV-derived peptides presented to T cells may differ depending on the origin and subtype of the virus.

We, therefore, compared the repertoire of predicted DQ-bound peptides from the two most frequent BKPyV genotypes. As shown in fig. S3 (A and B), the number of unique DQ binders derived from BKPyV genotype I and genotype IV in a given donor was notably correlated. Consequently, as for BKPyV-I, the number of BKPyV-IV peptides predicted to bind to DQα01/non-DQα01 pairs was higher than for DQα01/DQα01 or non-DQα01/non-DQα01 pairs (fig. S3C). These data indicate that the source or subtype of BKPyV is unlikely to have a substantial impact on the breadth of the DQ-bound immunopeptidome and, therefore, on the control of posttransplant BKPyV replication.

Time-varying causal inference

In multivariable Cox proportional hazard analysis, the HLA-DQα01/non-DQα01 pair (i.e., high HED-DQAB) in the donor strongly protected from posttransplant BKPyV-DNAemia (adjusted hazard ratio, 0.49; 95% confidence interval, 0.30 to 0.80; $P = 0.005$), independently of recipient age and HLA mismatches (Fig. 3A). Similarly, HLA-DQ functional divergence assessed using the motif divergence score was also an independent predictor of posttransplant BKPyV infection (fig. S4). To ensure the baseline comparability between groups, we then performed a target trial emulation (table S2) (*27*, *28*), which allows to estimate the causal effect of an "intervention" while excluding the risk of confounding bias frequently encountered in observational studies. This approach confirmed a causal effect of donor HLA-DQ divergence on the cumulative incidence of BKPyV DNAemia (Fig. 3B). Together, these results indicate that a high diversity of donor HLA-DQ molecules, best exemplified by DQα01/non-DQα01 heterozygosity, is a strong predictor of BKPyV-free outcome in kidney transplant recipients.

DISCUSSION

BKPyV infection remains a concern after kidney transplantation due to the risk of progression to BKVAN-related graft loss. Apart from the nature and intensity of immunosuppression, BKPyV infection has been associated with factors related to the host (age, gender, and comorbidities) and/or the transplant (donor type, HLA mismatch, and acute cellular or humoral rejection). Moreover, studies in cohorts of varying sizes have identified protective or predisposing HLA alleles at different loci (including HLA-DQ), but most often the results have not been replicated across studies [reviewed in (*29*)].

Here, we studied the impact of HLA diversity on BKPyV infection in a large retrospective kidney transplant recipient cohort with systematic screening of plasma BKPyV replication, in which almost 20% of patients experienced BKPyV DNAemia, a proportion consistent with data in contemporary cohorts (*30*–*32*). We show that donor HLA-DQ diversity and, more particularly, heterozygosity for the highly divergent DQα01/non-DQα01 DQ groups are relevant proxies to predict the chance of a BKPyV-free transplant outcome.

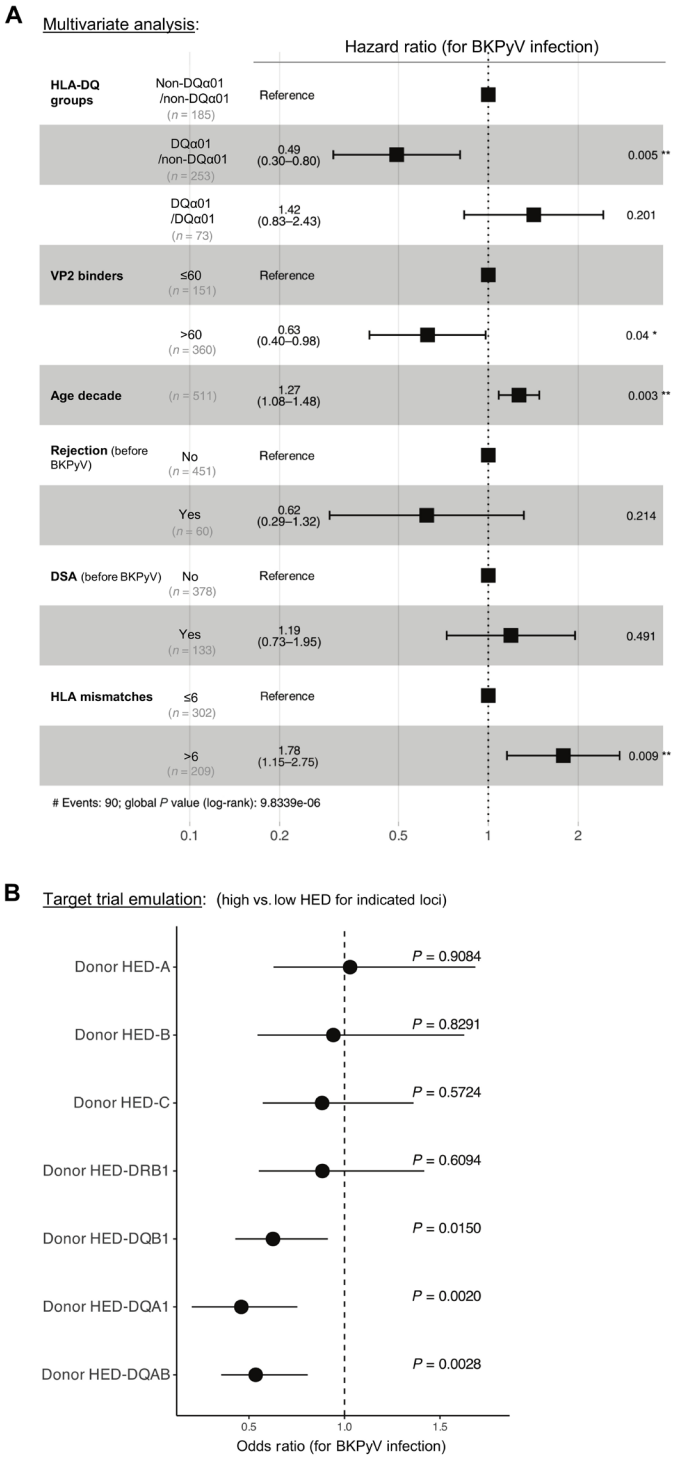

Fig. 3. Time-varying causal inference: Effect of HLA divergence on the risk of posttransplant BKPyV reactivation. (**A**) Multivariable analyses using Cox proportional hazard including HLA-DQ divergence (assessed by DQα combinations) and all variables statistically significant in the univariable analysis. Adjusted hazard ratios (95% confidence interval) are indicated for each variable with corresponding *P* values. (**B**) Target trial emulation (as described in table S2) including all covariates of the Cox model was performed using the g-method, to estimate the causal effect of donor HEDs on the BKPyV reactivation. Donor HEDs for indicated loci were compared by segregating individuals into two groups based on the median for each HED. $N = 511$ patients with no missing data in all variables were included in the analysis.

The divergent allele advantage assumes that more divergent allele pairs at a given HLA locus experience less overlap in the repertoire of bound antigenic peptides and therefore can present a broader range of potential antigens for T cell recognition, as first documented in HIV-infected individuals (*5*). A functional divergence metrics quantifying predicted peptide binding overlap between class I allele pairs further showed that greater functional divergence was associated with slower HIV disease progression (*33*). Here, HED and in silico prediction of the DQ-bound immunopeptidome allowed segregation of HLA-DQ allotypes into two discrete groups, DQα01 and non-DQα01, which are highly divergent with respect to the amino acid sequence of the α chain and physicochemical properties (*20*). We show that the proportion of peptide binding overlap between the DQα01 and non-DQα01 groups is lower than between other heterozygous combinations. Furthermore, when comparing the general peptide binding specificities between HLA-DQ heterodimers, we were able to identify and measure the divergence between the motifs (9-mer binding core) of peptides that can be presented by each HLA-DQ heterodimers. This allowed us to show that alleles of the DQα01 and non-DQα01 groups are functionally divergent and that donor HLA-DQ functional divergence was higher in patients without posttransplant BKPyV infection. These data provide a molecular basis for the differences observed when using the HED metrics and for the finding that DQα01/non-DQα01 pairs were predicted to present a broader BKPyV immunopeptidome to T cells. Of note, a similar approach using hierarchical clustering of experimentally determined peptide-binding motifs as a proxy for immunopeptidome divergence of HLA class I mismatches recently demonstrated its interest in predicting outcomes of HSCT (*34*).

From a general point of view, our results highlight the hitherto undescribed contribution of the DQA1 locus to the diversity of the immunopeptidome. Whereas sequence variation in the peptide binding domain of HLA-DR and HLA-DP molecules is primarily defined by their β chains, both DQα and DQβ chains are polymorphic and contribute to the binding specificity of DQ molecules. For obscure reasons, the role of DQA1 has been largely underestimated so far. HLA-DQA1 genotyping is usually not performed in clinical studies, and predicting HLA-DQ ligands without defining precisely the nature of the DQαβ molecules (i.e., cis and stable trans heterodimers) may appear challenging. Here, we used the recently updated NetMHCIIpan-4.2 prediction algorithm, which includes a large dataset highly enriched in peptides eluted from DQ molecules (i.e., endogenous peptides that have been physiologically processed and presented on the cell surface) and therefore strongly improves the predictive performance compared to previous versions of this algorithm (*19*). Moreover, we exclusively considered stable DQαβ heterodimers for peptide binding prediction, thus limiting inclusion of peptides which cannot be presented in vivo. Notably, we recently showed that high HLA-DQ divergence is also critical for vaccine responsiveness in liver transplant patients (*10*), further supporting the contribution of HLA-DQ restricted presentation in antigen-specific T cell responses.

BKPyV-specific T cell responses have been thoroughly investigated and seem essential in controlling posttransplant BKPyV replication. Several studies reported higher BKPyV-specific T cell immunity in kidney transplant patients with resolved BKPyV viremia/viruria compared to patients with ongoing/persisting infection (*35*–*40*), with some reports highlighting the importance of CD4 T cell responses (*41*–*47*). An increase in BKPyV-specific CD4 (but not CD8) T cell responses was found to be concomitant with viral clearance (*41*). Not only quantitative but also qualitative features of BKPyV-specific CD4 T cells may be of importance for rapid clearance of BKPyV reactivation, namely, polyfunctionality (i.e., production of ≥2 effector cytokines) (*43*, *44*), as well as higher clonal diversity and lower exhaustion (*45*). Furthermore, in addition to their indirect role in providing help to cytotoxic CD8 T cells, BKPyV-specific CD4 T cells may also play a direct role in viral clearance through cytotoxic capacity (*41*, *46*, *47*). Together, these data are consistent with our results on the contribution of HLA class II diversity to the control of BKPyV infection, further arguing for a major role of CD4 T cells.

Noteworthy is our observation that the VP2 protein may predominantly contribute to the DQ-bound peptide repertoire. While BKPyV-specific cytotoxic CD8 T cells are mostly described as targeting VP1 and LTag, data on the antigen specificity of CD4 T cell responses are scarce and rarely include analysis of the five proteins separately. Higher frequency and magnitude of CD4 T cell responses directed to VP3 (which is a truncated form of VP2) than to other proteins were observed in patients with previous severe infection (*44*).

Because of the persistence of BKPyV in the renourinary tract, the kidney donor is thought to be a major source of viral transmission in recipients with posttransplant infection. However, the latent recipient strain remains largely predominant in the bladder, the native kidneys, and ureters from where it may reactivate, lead to BKPyV DNAemia, and further colonize the allograft (*48*, *49*). We show that the source and the subtype of BKPyV are unlikely to influence the profile of the immunopeptidome, as the majority of DQ-bound peptides were shared by the most divergent BKPyV genotypes, suggesting that cross-reactivity for T cell response may occur. This is consistent with the observation that BKPyV-positive transplant recipients have a strong increase in neutralizing antibody (NAb) titers directed not only against the replicating strain but also cross-reactive against the other genotypes (*49*, *50*).

The reason why HLA-DQ diversity plays a more predominant role than that of HLA-DR remains elusive. Whether this is related to differences in the level of constitutive or induced HLA-DQ expression on different cell types of the graft, to the total number of cis- or trans-encoded HLA-DQ molecules, or to different intracellular pathways following interactions with CD4 T cells, remains to be investigated [as reviewed in (*51*)]. Of note, de novo HLA-DQ antibodies are most frequently observed after solid organ transplantation and are associated with worse graft outcomes than all other HLA antibodies (*51*, *52*). The risk of BKPyV infection was associated with HLA-DQ divergence in the donor but not in the recipient. Donor antigen-presenting cells (APCs) persist in the graft for several weeks after transplantation and are progressively replaced by host APCs. Moreover, renal microvascular endothelial cells from normal kidney cortex express HLA-DQ molecules (*53*), with further enhanced expression in inflammatory environments such as in the allograft (*54*). HLA-DQ may also be expressed on renal tubular cells in pathological conditions (*55*). As recently demonstrated (*56*), kidney transplants contain tissue-resident memory T cells of both donor and recipient origin, which express T cell receptors with specificities against common viruses including BKPyV and may contribute to local antiviral protection. Whether HLA-DQ–restricted T cells of the donor or the recipient are involved in the local control of BKPyV infection is an intriguing issue that may depend on whether BKPyV is transmitted through the graft or a latent

virus in the recipient. Besides, a more diverse immunopeptidome resulting from a higher donor HLA-DQ divergence could also favor the recruitment of preexisting, cross-reactive recipient memory T cells recognizing the same peptides presented by donor or recipient HLA-DQ molecules. An additional level of complexity arises from the frequent cross-reactivity between virus-specific and HLA-alloreactive T cells (*57*–*59*). Shortly after transplantation, HLA-peptide complexes expressed on donor cells within the graft can directly activate recipient alloreactive and/or virus-specific T cells. In addition, recipient APCs can be cross-dressed with intact donor HLA-peptide complexes, amplifying alloimmune response (*60*, *61*). One may also hypothesize that recipient APCs cross-dressed with donor DQ molecules could present BKPyV peptides to recipient T cells in lymphoid organs. Although we found no significant association between BKPyV DNAemia and allograft rejection, the potential cross-reactivity of HLA-DQ–restricted alloreactive and BKPyV-specific T cell responses should be further investigated.

This study has some limitations worth noting: (i) It was conducted retrospectively and is subject to inherent sources of bias, although this was reduced by the use of a target trial emulation approach; (ii) while the overall sample size was relatively large, results may have to be validated in a second cohort; (iii) BKPyV-derived peptide data are based on in silico prediction and may require further experimental validation using immunopeptidome characterization of BKPyV-infected cells; (iv) whether HLA-DP diversity may play a role, as shown in the HSCT setting (*9*, *62*) could not be investigated in this study (not typed in the context of solid organ transplant). Last, we did not directly demonstrate that higher HLA-DQ divergence is associated with stronger BKPyV-specific CD4 T cell responses and/or higher NAb titers. Thus, future studies to investigate relationships between these parameters are needed.

In conclusion, our study provides strong evidence for the functional relevance of HLA-DQ diversity in the control of viral infection. The lack of highly divergent HLA-DQ supertype pairs, i.e., DQα01/non-DQα01 heterozygosity, in the donor could serve as a reliable predictor of posttransplant BKPyV infection, thereby allowing early stratification of patients into categories at low risk or high risk. Such information is very easily accessible since the DQα01 and non-DQα01 supertypes can be ascribed even from low-resolution typing (i.e., at the antigen level) usually performed in the organ transplantation setting. This information could provide a good opportunity for personalized monitoring of BKPyV DNAemia and initiation of prophylactic or prompt therapeutic intervention when warranted. More generally, our results could guide the design of improved adoptive cellular strategies (e.g., BKPyV-CTL therapy) most likely to elicit antipolyomavirus protective immunity in other clinical settings such as BKPyV-associated hemorrhagic cystitis infection after HSCT (*63*) or infection with the closely related JC polyomavirus (*64*).

METHODS

Patients

We retrospectively studied all consecutive adult kidney transplant recipients who underwent transplantation at St-Louis Hospital between January 2012 and March 2020 and had a systematic screening for BKPyV DNAemia for at least 2 years posttransplant. According to our local institutional protocols, induction therapy was based on ATG, except for patients with HIV infection or past long-term immunosuppression who received basiliximab. Maintenance immunosuppression consisted of a calcineurin inhibitor (tacrolimus or ciclosporin A), mycophenolate, and tapered corticosteroids. Routine biopsies were systematically performed at 3, 12, and 24 months after transplantation and additionally in case of de novo acute kidney injury or substantial proteinuria, suspicion of acute rejection, or BKVAN. DSA were assessed before transplantation, at 3 months posttransplant and then at least yearly or in case of suspicion of acute rejection. Clinical data were collected prospectively during routine clinical follow-up in electronic medical records.

Systematic detection and quantification of BKPyV DNA were performed every month for the first 6 months posttransplant and then every 3 months until the end of year 2. The same plasma BKPyV polymerase chain reaction (PCR) assay (BK Virus R-GENE PCR Kit, bioMérieux, France) was used throughout the study period. The lower limit of detection of the assay was 500 copies/ml. Sustained BKPyV replication was defined as a BKPyV DNAemia > 3 weeks and/or >4 $\log_{10}$ copies/ml. BKVAN was defined by biopsy-proven diagnosis according to the to the Banff Working Group criteria (*65*).

Once eligible patients were identified, data including demographics, transplantation characteristics, pre- and posttransplant DSA, T cell–mediated or antibody-mediated allograft rejection, and outcomes were extracted from the electronic medical record. Patients provided written consent for the use of their samples for research purpose at the time of registration on the transplant waiting list. The study was conducted in accordance with the Declaration of Helsinki and approved by the review board of Saint-Louis Hospital, Assistance Publique Hôpitaux de Paris.

HLA genotyping

HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 typing at two-field resolution was available in all kidney transplant recipients and their donors. HLA-C and HLA-DQA1 alleles were imputed from the HaploSFHI online tool (www.sfhitools.fr/HaploSFHI) as reported (*66*). HLA-DPA1 and HLA-DPB1 typing was not considered because it is not performed in the organ transplant setting. The corresponding amino acid sequences of the peptide binding region (encoded by exons 2 and 3 for class I genes and exon 2 for class II genes) were extracted from the IMGT/HLA database (*67*). The sequence divergence (HED) between the two HLA alleles at each locus was computed for all allele pairs encountered in the recipients and their donors. HED was measured using the Grantham distance (*6*), a quantitative pairwise distance accounting for the physicochemical differences between two amino acids. For DQA1, the amino acid at position 56 was ignored in the HED calculation because of a deletion mutation in several alleles. HED-DQAB was defined as the mean of HED-DQA1 and HED-DQB1 in each patient.

HLA mismatch was calculated for each recipient as the number of antigenic differences with the donor at each locus and the total number of differences. The midasHLA R package was used to assess HLA alleles association with BKPyV outcome (logistic regression analysis with a dominant inheritance model) (*68*).

Analysis of HLA-DQ binding specificities

The binding specificities from all HLA-DQ heterodimers present in our data were retrieved from MixMHC2pred-2.0 (*21*). Specifically, the position weight matrices (PWMs) estimated by MixMHC2pred for human alleles were obtained (http://mixmhc2pred.gfellerlab.org/PWMdef), and these were transformed to position probability matrices (PPMs) by multiplying the PWM values from each amino acid

by their frequency in the human proteome. The PPM from an HLA-DQ heterodimer corresponds to a matrix (9 × 20) of the frequency of each amino acid at each of the nine positions of the binding site, describing the binding specificity of a given heterodimer. Principal components analysis was then performed on these matrices to compare the binding specificities between HLA-DQ heterodimers.

The motif divergence between HLA-DQ heterodimers from a donor was defined as the Euclidean distance between the PPMs describing these heterodimers. Specifically, if $PPM^{a}_{i,l}$ corresponds to the given probability of the amino acid i at position l of the peptide binding core describing the binding specificity of the HLA-DQ heterodimer a from the donor (these values are obtained as described in the previous paragraph, directly from MixMHC2pred-2.0 webserver), then the motif divergence between heterodimers a and b is obtained as

$$\mathrm{MotifDiv}^{a-b} = \sqrt{\sum_{l=1}^{9}\sum_{i=1}^{20}\left(\mathrm{PPM}^{a}_{i,l} - \mathrm{PPM}^{b}_{i,l}\right)^{2}}$$

For donors fully homozygote at HLA-DQ, a value of 0 was considered. For donors fully heterozygote at HLA-DQ, the maximum distance between all stable heterodimers was considered [i.e., between the up to four heterodimers formed between an HLA-DQA and an HLA-DQB of the donor, keeping only heterodimers known to be stable (*19*, *69*), thus corresponding to up to six such comparisons], as illustrated by the example shown in Fig. 2B.

In silico prediction of BKV peptides bound to HLA-DQ molecules

The referenced proteins of genotype I BKPyV (organism ID: 1891762) were retrieved from the UniProt proteome database (https://uniprot.org/proteomes/). The NetMHCIIpan-4.2 server (*19*) was used to predict HLA-DQ binding capacity of all possible 15-mer peptides derived from the five BKPyV antigenic proteins, LTag, sTag, VP1, and VP2/3. For each protein, the number of peptides with a percentile rank of eluted ligand prediction scores of <5% for strong and weak binders and <1% for strong binders only, was determined across DQ molecules found in more than two individuals (i.e., with a frequency of >0.5%) from the study cohort.

Statistical analysis

Data were summarized as median and interquartile range for quantitative variables (HED values show nonnormal distribution) and number and percentage for categorical variables. Comparison between groups was evaluated by unpaired *t* test or Mann-Whitney test for quantitative variables and Fisher's exact test for categorical variables. *P* values below 0.05 were considered as significant. Kaplan-Meier curves were constructed, and cumulative incidence of BKPyV DNAemia was compared according to all included variables using the log-rank test. Correlation between continuous variables was calculated using nonparametric Spearman test. Associations of HLA phenotype frequencies with BKPyV DNAemia were evaluated using a two-sided Fisher's exact test with the Bonferroni correction.

Multivariable analyses were performed using Cox proportional hazard including variables statistically significant ($P < 0.05$) in the univariable analysis. For variables necessarily occurring after the starting point (e.g., de novo DSA and rejection), we used a time-dependent Cox model with time-based merge datasets to avoid the risk of immortal time bias.

To estimate the causal effect of HED on the cumulative incidence of BKPyV DNAemia while excluding the risk of confounding bias inherent in observational studies, we designed a target emulated trial by (i) fitting a Cox regression, (ii) duplicating the dataset in two counterfactual datasets, (iii) applying the outcome model to predict each individual's outcome in the two counterfactual datasets, (iv) establishing the estimand using the g-method (table S2) (*70*). All analyses were performed with R 4.0 using "RISCA" packages.

Supplementary Materials

This PDF file includes:

Figs. S1 to S4
Tables S1 and S2

REFERENCES AND NOTES

1. N. Babel, H. D. Volk, P. Reinke, BK polyomavirus infection and nephropathy: The virus-immune system interplay. *Nat. Rev. Nephrol.* **7**, 399–406 (2011).
2. A. Cohen-Bucay, S. E. Ramirez-Andrade, C. E. Gordon, J. M. Francis, V. C. Chitalia, Advances in BK virus complications in organ transplantation and beyond. *Kidney Med.* **2**, 771–786 (2020).
3. M. Carrington, G. W. Nelson, M. P. Martin, T. Kissner, D. Vlahov, J. J. Goedert, R. Kaslow, S. Buchbinder, K. Hoots, S. J. O'Brien, *HLA* and HIV-1: Heterozygote advantage and *B*35-Cw*04* disadvantage. *Science* **283**, 1748–1752 (1999).
4. M. R. Thursz, D. Kwiatkowski, C. E. M. Allsopp, B. M. Greenwood, H. C. Thomas, A. V. S. Hill, Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. *N. Engl. J. Med.* **332**, 1065–1069 (1995).
5. J. Arora, F. Pierini, P. J. McLaren, M. Carrington, J. Fellay, T. L. Lenz, HLA heterozygote advantage against HIV-1 is driven by quantitative and qualitative differences in HLA allele-specific peptide presentation. *Mol. Biol. Evol.* **37**, 639–650 (2020).
6. F. Pierini, T. L. Lenz, Divergent allele advantage at human MHC genes: Signatures of past and ongoing selection. *Mol. Biol. Evol.* **35**, 2145–2158 (2018).
7. D. Chowell, C. Krishna, F. Pierini, V. Makarov, N. A. Rizvi, F. Kuo, L. G. T. Morris, N. Riaz, T. L. Lenz, T. A. Chan, Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy. *Nat. Med.* **25**, 1715–1720 (2019).
8. J. Delyon, L. Biard, M. Renaud, M. Resche-Rigon, J. Le Goff, S. Dalle, V. Heidelberger, L. Da Meda, L. Toullec, G. Carcelain, S. Mourah, S. Caillat-Zucman, V. Allain, M. Battistella, C. Lebbe, PD-1 blockade with pembrolizumab in classic or endemic Kaposi's sarcoma: A multicentre, single-arm, phase 2 study. *Lancet Oncol.* **23**, 491–500 (2022).
9. J. Villemonteix, V. Allain, E. Verstraete, D. Jorge-Cordeiro, G. Socie, A. Xhaard, C. Feray, S. Caillat-Zucman, HLA-DP diversity is associated with improved response to SARS-Cov-2 vaccine in hematopoietic stem cell transplant recipients. *iScience* **26**, 106763 (2023).
10. C. Feray, V. Allain, C. Desterke, B. Roche, A. Coilly, S. Caillat-Zucman, HLA-DQ diversity is associated with humoral response to vaccines in patients awaiting or after liver transplantation. *Gastroenterology* **166**, 915–917.e3 (2024).
11. C. Feray, J.-L. Taupin, M. Sebagh, V. Allain, Z. Demir, M.-A. Allard, C. Desterke, A. Coilly, F. Saliba, E. Vibert, D. Azoulay, C. Guettier, A. Chatton, D. Debray, S. Caillat-Zucman, D. Samuel, Donor HLA class 1 evolutionary divergence is a major predictor of liver allograft rejection: A retrospective cohort study. *Ann. Intern. Med.* **174**, 1385–1394 (2021).
12. S. Pagliuca, C. Gurnari, C. Hercus, S. Hergalant, S. Hong, A. Dhuyser, M. D'Aveni, A. Aarnink, M. T. Rubio, P. Feugier, F. Ferraro, H. E. Carraway, R. Sobecks, B. K. Hamilton, N. S. Majhail, V. Visconte, J. P. Maciejewski, Leukemia relapse via genetic immune escape after allogeneic hematopoietic cell transplantation. *Nat. Commun.* **14**, 3153 (2023).
13. M. Roerden, A. Nelde, J. S. Heitmann, R. Klein, H. G. Rammensee, W. A. Bethge, J. S. Walz, HLA evolutionary divergence as a prognostic marker for AML patients undergoing allogeneic stem cell transplantation. *Cancers* **12**, 1835 (2020).
14. P. Merli, P. Crivello, L. Strocchio, R. M. Pinto, M. Algeri, F. Del Bufalo, D. Pagliara, M. Becilli, R. Carta, S. Gaspari, F. Galaverna, F. Quagliarella, G. Boz, M. L. Catanoso, E. Boccieri, M. Troiano, K. Fleischhauer, M. Andreani, F. Locatelli, Human leukocyte antigen evolutionary divergence influences outcomes of paediatric patients and young adults affected by malignant disorders given allogeneic haematopoietic stem cell transplantation from unrelated donors. *Br. J. Haematol.* **200**, 622–632 (2023).
15. Q. L. Wang, T. M. Wang, C. M. Deng, W. L. Zhang, Y. Q. He, W. Q. Xue, Y. Liao, D. W. Yang, M. Q. Zheng, W. H. Jia, Association of HLA diversity with the risk of 25 cancers in the UK Biobank. *EBioMedicine* **92**, 104588 (2023).
16. S. Pagliuca, C. Gurnari, H. Awada, A. Kishtagari, S. Kongkiatkamon, L. Terkawi, M. Zawit, Y. Guan, T. LaFramboise, B. K. Jha, B. J. Patel, B. K. Hamilton, N. S. Majhail, S. Lundgren, S. Mustjoki, Y. Saunthararajah, V. Visconte, T. A. Chan, C. Y. Yang, T. L. Lenz, J. P. Maciejewski, The similarity of class II HLA genotypes defines patterns of autoreactivity in idiopathic bone marrow failure disorders. *Blood* **138**, 2781–2798 (2021).

17. W. W. Kwok, P. Thurtle, G. T. Nepom, A genetically controlled pairing anomaly between HLA-DQ alpha and HLA-DQ beta chains. *J. Immunol.* **143**, 3598–3601 (1989).
18. W. W. Kwok, S. Kovats, P. Thurtle, G. T. Nepom, HLA-DQ allelic polymorphisms constrain patterns of class II heterodimer formation. *J. Immunol.* **150**, 2263–2272 (1993).
19. J. B. Nilsson, S. Kaabinejadian, H. Yari, B. Peters, C. Barra, L. Gragert, W. Hildebrand, M. Nielsen, Machine learning reveals limited contribution of trans-only encoded variants to the HLA-DQ immunopeptidome. *Commun. Biol.* **6**, 442 (2023).
20. C. Maguire, P. Crivello, K. Fleischhauer, D. Isaacson, A. Casillas, C. S. M. Kramer, H. C. Copley, S. Heidt, V. Kosmoliaptsis, M. Meneghini, M. Gmeiner, J. Schold, Y. Louzoun, A. R. Tambur, Qualitative, rather than quantitative, differences between HLA-DQ alleles affect HLA-DQ immunogenicity in organ transplantation. *HLA* **103**, e15455 (2024).
21. J. Racle, P. Guillaume, J. Schmidt, J. Michaux, A. Larabi, K. Lau, M. A. S. Perez, G. Croce, R. Genolet, G. Coukos, V. Zoete, F. Pojer, M. Bassani-Sternberg, A. Harari, D. Gfeller, Machine learning predictions of MHC-II specificities reveal alternative binding mode of class II epitopes. *Immunity* **56**, 1359–1375.e13 (2023).
22. D. Leuenberger, P. A. Andresen, R. Gosert, S. Binggeli, E. H. Strom, S. Bodaghi, C. H. Rinaldo, H. H. Hirsch, Human polyomavirus type 1 (BK virus) agnoprotein is abundantly expressed but immunologically ignored. *Clin. Vaccine Immunol.* **14**, 959–968 (2007).
23. T. Schachtner, K. Muller, M. Stein, C. Diezemann, A. Sefrin, N. Babel, P. Reinke, BK virus-specific immunity kinetics: A predictor of recovery from polyomavirus BK-associated nephropathy. *Am. J. Transplant.* **11**, 2443–2452 (2011).
24. M. Cioni, C. Leboeuf, P. Comoli, F. Ginevri, H. H. Hirsch, Characterization of immunodominant BK polyomavirus 9mer epitope T cell responses. *Am. J. Transplant.* **16**, 1193–1206 (2016).
25. A. G. Lara-de-Leon, R. Mora-Buch, E. Canto, C. Pena-Gomez, F. Rudilla, Identification of candidate immunodominant epitopes and their HLA-binding prediction on BK polyomavirus proteins in healthy donors. *HLA* **104**, e15722 (2024).
26. Y. Yogo, C. Sugimoto, S. Zhong, Y. Homma, Evolution of the BK polyomavirus: Epidemiological, anthropological and clinical implications. *Rev. Med. Virol.* **19**, 185–199 (2009).
27. M. A. Hernan, Methods of public health research - strengthening causal inference from observational data. *N. Engl. J. Med.* **385**, 1345–1348 (2021).
28. E. L. Fu, Target trial emulation to improve causal inference from observational data: What, Why, and How? *J. Am. Soc. Nephrol.* **34**, 1305–1314 (2023).
29. M. Burek Kamenaric, V. Ivkovic, I. Kovacevic Vojtusek, R. Zunec, The role of HLA and KIR immunogenetics in BK virus infection after kidney transplantation. *Viruses* **12**, 1417 (2020).
30. R. Rogers, K. Bodziak, F. Cheema, M. Chobanian, Z. Ebcioglu, S. Gabardi, S. Ghai, H. Gilligan, R. Gohh, N. Goyal, G. Kavalam, B. Mehri, N. Rodig, A. Rossi, J. Singh, E. Wood, C. S. Tan, M. Pavlakis, Implementing a regional standardized BK polyomavirus screening protocol across eleven transplant centres. *Transpl. Int.* **34**, 2680–2685 (2021).
31. E. Cleenders, P. Koshy, E. Van Loon, K. Lagrou, K. Beuselinck, G. Andrei, M. Crespo, K. De Vusser, D. Kuypers, E. Lerut, K. Mertens, O. Mineeva-Sangwo, P. Randhawa, A. Senev, R. Snoeck, B. Sprangers, C. Tinel, A. Van Craenenbroeck, J. van den Brand, M. Van Ranst, G. Verbeke, M. Coemans, M. Naesens, An observational cohort study of histological screening for BK polyomavirus nephropathy following viral replication in plasma. *Kidney Int.* **104**, 1018–1034 (2023).
32. M. E. Seifert, R. B. Mannon, A. Nellore, J. Young, A. C. Wiseman, D. J. Cohen, V. R. Peddi, D. C. Brennan, C. J. Morgan, K. Peri, I. Aban, R. J. Whitley, J. W. Gnann Jr., A multicenter prospective study to define the natural history of BK viral infections in kidney transplantation. *Transpl. Infect Dis.* **26**, e14237 (2024).
33. M. Viard, C. O'Huigin, Y. Yuki, A. A. Bashirova, D. R. Collins, J. M. Urbach, S. Wolinsky, S. Buchbinder, G. D. Kirk, J. J. Goedert, N. L. Michael, D. W. Haas, S. G. Deeks, B. D. Walker, X. Yu, M. Carrington, Impact of HLA class I functional divergence on HIV control. *Science* **383**, 319–325 (2024).
34. P. Crivello, E. Arrieta-Bolanos, M. He, T. Wang, S. Fingerson, S. M. Gadalla, S. Paczesny, S. G. E. Marsh, S. J. Lee, S. R. Spellman, Y. T. Bolon, K. Fleischhauer, Impact of the HLA immunopeptidome on survival of leukemia patients after unrelated donor transplantation. *J. Clin. Oncol.* **41**, 2416–2427 (2023).
35. A. Chakera, S. Bennett, S. Lawrence, O. Morteau, P. D. Mason, C. A. O'Callaghan, R. J. Cornall, Antigen-specific T cell responses to BK polyomavirus antigens identify functional anti-viral immunity and may help to guide immunosuppression following renal transplantation. *Clin. Exp. Immunol.* **165**, 401–409 (2011).
36. S. E. Prosser, R. J. Orentas, L. Jurgens, E. P. Cohen, S. Hariharan, Recovery of BK virus large T-antigen-specific cellular immune response correlates with resolution of bk virus nephritis. *Transplantation* **85**, 185–192 (2008).
37. C. Leboeuf, S. Wilk, R. Achermann, I. Binet, D. Golshayan, K. Hadaya, C. Hirzel, M. Hoffmann, U. Huynh-Do, M. T. Koller, O. Manuel, N. J. Mueller, T. F. Mueller, S. Schaub, C. van Delden, F. H. Weissbach, H. H. Hirsch, Swiss Transplant Cohort Study, BK polyomavirus-specific 9mer CD8 T cell responses correlate with clearance of BK viremia in kidney transplant recipients: First report from the swiss transplant cohort study. *Am. J. Transplant.* **17**, 2591–2600 (2017).
38. S. Binggeli, A. Egli, S. Schaub, I. Binet, M. Mayr, J. Steiger, H. H. Hirsch, Polyomavirus BK-specific cellular immune response to VP1 and large T-antigen in kidney transplant recipients. *Am. J. Transplant.* **7**, 1131–1139 (2007).
39. E. Mutlu, S. Koksoy, D. Mutlu, V. T. Yilmaz, H. Kocak, A. Dinckan, G. Suleymanlar, M. Gultekin, Quantitative analysis of BKV-specific CD4+ T cells before and after kidney transplantation. *Transpl. Immunol.* **33**, 20–26 (2015).
40. T. Schachtner, M. Stein, A. Sefrin, N. Babel, P. Reinke, Inflammatory activation and recovering BKV-specific immunity correlate with self-limited BKV replication after renal transplantation. *Transpl. Int.* **27**, 290–301 (2014).
41. B. J. D. Weist, P. Wehler, L. El Ahmad, M. Schmueck-Henneresse, J. M. Millward, M. Nienen, A. U. Neumann, P. Reinke, N. Babel, A revised strategy for monitoring BKV-specific cellular immunity in kidney transplant patients. *Kidney Int.* **88**, 1293–1303 (2015).
42. T. Schmidt, C. Adam, H. H. Hirsch, M. W. W. Janssen, M. Wolf, J. Dirks, P. Kardas, T. Ahlenstiel-Grunow, L. Pape, T. Rohrer, D. Fliser, M. Sester, U. Sester, BK polyomavirus-specific cellular immune responses are age-dependent and strongly correlate with phases of virus replication. *Am. J. Transplant.* **14**, 1334–1345 (2014).
43. H. Trydzenskaya, A. Sattler, K. Muller, T. Schachtner, C. Dang-Heine, P. Friedrich, P. Nickel, J. Hoerstrup, R. Schindler, A. Thiel, M. F. Melzig, P. Reinke, N. Babel, Novel approach for improved assessment of phenotypic and functional characteristics of BKV-specific T-cell immunity. *Transplantation* **92**, 1269–1277 (2011).
44. K. Mueller, T. Schachtner, A. Sattler, S. Meier, P. Friedrich, H. Trydzenskaya, C. Hinrichs, R. Trappe, A. Thiel, P. Reinke, N. Babel, BK-VP3 as a new target of cellular immunity in BK virus infection. *Transplantation* **91**, 100–107 (2011).
45. U. Stervbo, M. Nienen, B. J. D. Weist, L. Kuchenbecker, J. Hecht, P. Wehler, T. H. Westhoff, P. Reinke, N. Babel, BKV clearance time correlates with exhaustion state and T-cell receptor repertoire shape of BKV-specific T-cells in renal transplant patients. *Front. Immunol.* **10**, 767 (2019).
46. W. Zhou, M. Sharma, J. Martinez, T. Srivastava, D. J. Diamond, W. Knowles, S. F. Lacey, Functional characterization of BK virus-specific CD4+ T cells with cytotoxic potential in seropositive adults. *Viral Immunol.* **20**, 379–388 (2007).
47. B. J. D. Weist, M. Schmueck, H. Fuehrer, A. Sattler, P. Reinke, N. Babel, The role of $CD4^+$ T cells in BKV-specific T cell immunity. *Med. Microbiol. Immunol.* **203**, 395–408 (2014).
48. O. Mineeva-Sangwo, J. Marti-Carreras, E. Cleenders, D. Kuypers, P. Maes, G. Andrei, M. Naesens, R. Snoeck, Polyomavirus BK genome comparison shows high genetic diversity in kidney transplant recipients three months after transplantation. *Viruses* **14**, 1533 (2022).
49. J. Gras, M. L. Nere, M. N. Peraldi, L. Bonnet-Madin, M. Salmona, J. L. Taupin, F. Desgrandchamps, J. Verine, E. Brochot, A. Amara, J. M. Molina, C. Delaugerre, BK virus genotypes and humoral response in kidney transplant recipients with BKV associated nephropathy. *Transpl. Infect. Dis.* **25**, e14012 (2023).
50. M. Solis, A. Velay, R. Porcher, P. Domingo-Calap, E. Soulier, M. Joly, M. Meddeb, W. Kack-Kack, B. Moulin, S. Bahram, F. Stoll-Keller, H. Barth, S. Caillard, S. Fafi-Kremer, Neutralizing antibody-mediated response and risk of BK virus-associated nephropathy. *J. Am. Soc. Nephrol.* **29**, 326–334 (2018).
51. M. Meneghini, A. R. Tambur, HLA-DQ antibodies in alloimmunity, what makes them different? *Curr. Opin. Organ Transplant.* **28**, 333–339 (2023).
52. D. Isaacson, J. D. Schold, M. W. Gmeiner, H. C. Copley, V. Kosmoliaptsis, A. R. Tambur, HLA-DQ mismatches lead to more unacceptable antigens, greater sensitization, and increased disparities in repeat transplant candidates. *J. Am. Soc. Nephrol.* **33**, 2293–2305 (2022).
53. K. A. Muczynski, D. M. Ekle, D. M. Coder, S. K. Anderson, Normal human kidney HLA-DR-expressing renal microvascular endothelial cells: Characterization, isolation, and regulation of MHC class II expression. *J. Am. Soc. Nephrol.* **14**, 1336–1348 (2003).
54. A. R. Cross, J. Lion, P. Loiseau, D. Charron, J. L. Taupin, D. Glotz, N. Mooney, Donor specific antibodies are not only directed against HLA-DR: Minding your Ps and Qs. *Hum. Immunol.* **77**, 1092–1100 (2016).
55. C. A. Muller, J. Markovic-Lipkovski, T. Risler, A. Bohle, G. A. Muller, Expression of HLA-DQ, -DR, and -DP antigens in normal kidney and glomerulonephritis. *Kidney Int.* **35**, 116–124 (1989).
56. D. M. Hullegie-Peelen, H. Tejeda Mora, D. A. Hesselink, E. M. J. Bindels, T. P. P. van den Bosch, M. C. Clahsen-van Groningen, M. Dieterich, S. Heidt, R. C. Minnee, G. M. G. M. Verjans, M. J. Hoogduijn, C. C. Baan, Virus-specific T_{RM} cells of both donor and recipient origin reside in human kidney transplants. *JCI Insight* **8**, e172681 (2023).
57. A. L. Amir, L. J. A. D'Orsogna, D. L. Roelen, M. M. van Loenen, R. S. Hagedoorn, R. de Boer, M. A. W. G. van der Hoorn, M. G. D. Kester, I. I. N. Doxiadis, J. H. F. Falkenburg, F. H. J. Claas, M. H. M. Heemskerk, Allo-HLA reactivity of virus-specific memory T cells is common. *Blood* **115**, 3146–3157 (2010).
58. L. D'Orsogna, H. van den Heuvel, C. van Kooten, S. Heidt, F. H. J. Claas, Infectious pathogens may trigger specific allo-HLA reactivity via multiple mechanisms. *Immunogenetics* **69**, 631–641 (2017).
59. G. E. Karahan, F. H. J. Claas, S. Heidt, Heterologous immunity of virus-specific T cells leading to alloreactivity: Possible implications for solid organ transplantation. *Viruses* **13**, 2359 (2021).
60. A. D. Hughes, D. Zhao, H. Dai, K. I. Abou-Daya, R. Tieu, R. Rammal, A. L. Williams, D. P. Landsittel, W. D. Shlomchik, A. E. Morelli, M. H. Oberbarnscheidt, F. G. Lakkis,

Cross-dressed dendritic cells sustain effector T cell responses in islet and kidney allografts. *J. Clin. Invest.* **130**, 287–294 (2020).
61. F. Zeng, A. E. Morelli, Extracellular vesicle-mediated MHC cross-dressing in immune homeostasis, transplantation, infectious diseases, and cancer. *Semin. Immunopathol.* **40**, 477–490 (2018).
62. T. Meurer, P. Crivello, M. Metzing, M. Kester, D. A. Megger, W. Chen, P. A. van Veelen, P. van Balen, A. M. Westendorf, G. Homa, S. E. Layer, A. T. Turki, M. Griffioen, P. A. Horn, B. Sitek, D. W. Beelen, J. H. F. Falkenburg, E. Arrieta-Bolanos, K. Fleischhauer, Permissive HLA-DPB1 mismatches in HCT depend on immunopeptidome divergence and editing by HLA-DM. *Blood* **137**, 923–928 (2021).
63. A. Olson, R. Lin, D. Marin, H. Rafei, M. H. Bdiwi, P. F. Thall, R. Basar, A. Abudayyeh, P. Banerjee, F. M. Aung, I. Kaur, G. Abueg, S. Rao, R. Chemaly, V. Mulanovich, G. Al-Atrash, A. M. Alousi, B. S. Andersson, P. Anderlini, Q. Bashir, K. M. Castro, M. Daher, I. M. Galvan, C. Hosing, J. S. Im, R. B. Jones, P. Kebriaei, I. Khouri, R. Mehta, J. Molldrem, Y. Nieto, B. Oran, U. Popat, M. Qazilbash, G. Rondon, N. Saini, B. Spencer, S. Srour, D. Washington, M. Barnett, R. E. Champlin, E. J. Shpall, K. Rezvani, Third-party BK virus-specific cytotoxic T lymphocyte therapy for hemorrhagic cystitis following allotransplantation. *J. Clin. Oncol.* **39**, 2710–2719 (2021).
64. A. Sempere, N. Castillo, F. Rudilla, S. Querol, E. Enrich, C. Prat-Vidal, M. Codinach, F. Cofan, V. Torregrossa, F. Dieckmann, M. Bodro, Successful BK virus-specific T cell therapy in a kidney transplant recipient with progressive multifocal leukoencephalopathy. *Am J Transplant* **24**, 1698–1702 (2024).
65. V. Nickeleit, H. K. Singh, P. Randhawa, C. B. Drachenberg, R. Bhatnagar, E. Bracamonte, A. Chang, W. J. Chon, D. Dadhania, V. G. Davis, H. Hopfer, M. J. Mihatsch, J. C. Papadimitriou, S. Schaub, M. B. Stokes, M. F. Tungekar, S. V. Seshan, Banff Working Group on Polyomavirus Nephropathy, The Banff Working Group Classification of Definitive Polyomavirus Nephropathy: Morphologic definitions and clinical correlations. *J. Am. Soc. Nephrol.* **29**, 680–693 (2018).
66. R. Lhotte, V. Letort, C. Usureau, D. Jorge-Cordeiro, PHRC Acorghla Consortium, J. Siemowski, L. Gabet, P.-H. Cournede, J.-L. Taupin, Improving HLA typing imputation accuracy and eplet identification with local next-generation sequencing training data. *HLA* **103**, e15222 (2024).
67. J. Robinson, J. A. Halliwell, J. D. Hayhurst, P. Flicek, P. Parham, S. G. E. Marsh, The IPD and IMGT/HLA database: Allele variant databases. *Nucleic Acids Res.* **43**, D423–D431 (2015).
68. M. Migdal, D. F. Ruan, W. F. Forrest, A. Horowitz, C. Hammer, MiDAS—Meaningful immunogenetic data at scale. *PLOS Comput. Biol.* **17**, e1009131 (2021).
69. E. W. Petersdorf, M. Bengtsson, M. Horowitz, C. McKallor, S. R. Spellman, E. Spierings, T. A. Gooley, P. Stevenson, International Histocompatibility Working Group in Hematopoietic Cell Transplantation, HLA-DQ heterodimers in hematopoietic cell transplantation. *Blood* **139**, 3009–3017 (2022).
70. A. Chatton, F. Le Borgne, C. Leyrat, F. Gillaizeau, C. Rousseau, L. Barbin, D. Laplaud, M. Leger, B. Giraudeau, Y. Foucher, G-computation, propensity score-based methods, and targeted maximum likelihood estimator for causal inference with different covariates sets: A comparative simulation study. *Sci. Rep.* **10**, 9219 (2020).

Acknowledgments: We thank J. Zuber (Necker Hospital, Paris, France) for critical review of the manuscript. **Funding:** We acknowledge that we received no funding in support of this research. **Author contributions:** Study design: M.F.C., V.A., C.F., and S.C.-Z. Methodology: M.F.C., J.R., D.G., C.F., and S.C.-Z. Resources: G.D., L.F., J.-M.M., S.C.-Z., C.D., M.-N.P., J.G., and J.-L.T. Data curation: L.F., M.-N.P., G.D., and C.F. Formal analysis: M.F.C., J.R., V.A., J.V., and C.F. Visualization: M.F.C., J.R., C.F., and S.C.-Z. Writing—original draft: M.F.C., V.A., and S.C.-Z. Writing—review and editing: All authors. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 24 September 2024
Accepted 29 January 2025
Published 5 March 2025
10.1126/sciadv.adt3499