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Evidence for the alloimmune basis and prognostic significance of Borderline T cell–mediated rejection

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Prognostic biomarkers of T cell–mediated rejection (TCMR) have not been adequately studied in the modern era. We evaluated 803 renal transplant recipients and correlated HLA-DR/DQ molecular mismatch alloimmune risk categories (low, intermediate, high) with the severity, frequency, and persistence of TCMR. Allograft survival was reduced in recipients with Banff Borderline (hazard ratio [HR] 2.4, $P = .003$) and Banff ≥ IA TCMR (HR 4.3, $P < .0001$) including a subset who never developed de novo donor-specific antibodies ($P = .002$). HLA-DR/DQ molecular mismatch alloimmune risk categories were multivariate correlates of Banff Borderline and Banff ≥ IA TCMR and correlated with the severity and frequency of rejection episodes. Recipient age, HLA-DR/DQ molecular mismatch category, and cyclosporin vs tacrolimus immunosuppression were independent correlates of Banff Borderline and Banff ≥ IA TCMR. In the subset treated with tacrolimus (720/803) recipient age, HLA-DR/DQ molecular mismatch category, and tacrolimus coefficient of variation were independent correlates of TCMR. The correlation of HLA-DR/DQ molecular mismatch category with TCMR, including Borderline, provides evidence for their alloimmune basis. HLA-DR/DQ molecular mismatch may represent a precise prognostic biomarker that can be applied to tailor immunosuppression or design clinical trials based on individual patient risk.

KEYWORDS

clinical research / practice, graft survival, histocompatibility, immunosuppression / immune modulation, kidney transplantation / nephrology, major histocompatibility complex (MHC), T cell–mediated rejection (TCMR), risk assessment / risk stratification

1 | INTRODUCTION

The US Food and Drug Administration (FDA) defines precision medicine as "an innovative approach to tailoring disease prevention and treatment that takes into account differences in people's genes, environments, and lifestyles."[1] Unfortunately, in transplantation two large registry studies observed that only 6% of prescription practice variation could be explained by patient-related risk factors whereas the

Abbreviations: ABMR, antibody-mediated rejection; dnDSA, de novo donor-specific antibody; TCMR, T cell–mediated rejection.

majority (30%-46%) of the variation was attributed to the transplant center effect.[2,3] The greatest obstacle to the adoption of precision medicine has been the lack of reliable prognostic or predictive biomarkers available at the time of transplantation to permit individualized treatment and monitoring strategies.[4-6] In the absence of early biomarkers clinicians have instead proposed risk prediction tools that include features present in injured grafts (eg, decreased glomerular filtration rate, proteinuria, histologic inflammation, and injury), which are the outcomes that ideally would be prevented by a robust early, prognostic/predictive biomarker.[7]

Immune-mediated allograft injury is the most common cause of death-censored allograft failure posttransplant.[8-12] In this context, and in the absence of pretransplant donor-specific memory, an essential requirement for a prognostic/predictive biomarker is an accurate assessment of the risk for a primary alloimmune response posttransplant. The outcome used to develop such a biomarker must accurately indicate a primary alloimmune response has occurred. For this purpose, de novo donor-specific antibody (dnDSA) surveillance has many favorable characteristics including the availability of highly sensitive noninvasive assays that correlate with antibody-mediated rejection (ABMR) and allograft loss. Using dnDSA free survival as an outcome, we previously developed an alloimmune prognostic/predictive biomarker through quantification of donor-recipient molecular mismatches for each HLA-DR and HLA-DQ molecule.[10] This approach increased the precision of risk assessment for dnDSA development from an area under the curve of 0.54 with traditional HLA antigen mismatch to an area under the curve of 0.84 with HLA molecular mismatch quantification. Moreover, we demonstrated that alloimmune risk categories, defined by a single molecule HLA-DR/DQ molecular mismatch score, clearly stratified risk for dnDSA, ABMR, and allograft survival.

Precise risk factors for T cell–mediated rejection (TCMR) in the modern era are poorly defined resulting in one-size-fits-all prevention/treatment strategies for most patients and high recurrence rates reported in serial biopsy studies.[11-15] As the underlying driver of alloimmunity is the dissimilarity between donor and recipient at the molecular level, we hypothesized that the alloimmune risk categories developed using dnDSA free survival would also apply to TCMR. Indeed, we reported that the HLA-DR/DQ molecular mismatch score correlated with Banff ≥ IA TCMR in the first year posttransplant.[10] In the present paper, we sought to determine whether the HLA-DR/DQ molecular mismatch risk categories correlated with the severity, frequency, and persistence of TCMR and with graft survival independent of dnDSA.[9-12]

2 | METHODS

The study cohort consisted of 868 consecutive renal transplant recipients transplanted between January 1999 and October 2018. Recipients were excluded for primary nonfunction (n = 24, 2.7%) or pretransplant donor-specific antibody (n = 41, 4.5%) leaving 803 patients for analysis. Standard immunosuppression consisted of a calcineurin inhibitor (CNI, tacrolimus [90%] or cyclosporin [10%]), mycophenolate mofetil, and prednisone. Induction therapy with thymoglobulin (22%) or basiliximab (24%) was used in 46% of patients. Details on serologic monitoring posttransplant have been reported previously and can be found in the supplemental methods.[16,17]

2.1 | HLA typing and epitope mismatch identification

Class II HLA typing (HLA-DRβ1/3/4/5 and HLA-DQα1/β1) was performed using sequence-specific oligonucleotide probes or sequence-specific primer technology (LABType® HD SSO, Micro SSP™, One Lambda, Canoga Park, CA). HLAMatchmaker software (HLA DRDQDP Matching version 2.2) was used to determine the eplet mismatch for each HLA-DR or HLA-DQ molecule individually and the single molecule eplet mismatch was used to categorize individuals into three alloimmune risk groups (low, intermediate, or high alloimmune risk) using previously described thresholds.[10]

2.2 | Rejection treatment

Recipients with dnDSA and/or acute rejection were treated by optimizing tacrolimus trough levels (8 ± 2 ng/mL) and mycophenolate dose (2 g/d as tolerated). A steroid bolus with taper was given when clinical or subclinical acute TCMR and/or ABMR was present on a biopsy. Occasionally, in cases with severe clinical TCMR, thymoglobulin was administered. For clinical ABMR, high dose intravenous immunoglobulin (2 g/kg) was given.

2.3 | Clinical and pathologic monitoring

Study recipients were followed at a single center in the adult or pediatric transplant clinic. Six-month protocol biopsies were performed on all consenting recipients. Renal biopsy was offered to all recipients with newly detected dnDSA since January 2008 as standard of care. Clinically indicated allograft biopsies were performed if proteinuria was ≥ 0.5 g/day or the serum creatinine rose ≥ 25% from baseline without a known cause. Banff Borderline rejection was diagnosed when interstitial inflammation score was ≥ 1 in the presence of tubulitis consistent with the Banff 1997 definition prior to the Banff 2005 update.[18-20] Patients who were identified as only having at most a Banff score of $i = 0$, $t \geq 1$ were considered as a distinct group from those with a diagnosis of Banff Borderline rejection. Histology was evaluated using Banff criteria by a single experienced renal transplant pathologist (IWG).

2.4 | Statistics

Comparisons between baseline predictors and clinical outcomes were done using Student's *t* test for parametric continuous variables

and Wilcoxon-rank test for nonparametric data. Chi-square or Fisher's exact tests were used to test categorical variables. Comparisons across multiple groups were done using Kruskal–Wallis test for nonparametric data and analysis of variance for parametric variables. Survival analysis was done by the Kaplan–Meier method using the log-rank test for significance. Cox proportional hazards model was used to evaluate predictors of TCMR free survival and dnDSA free survival. Variables for multivariate regression were selected based on bivariate screening, with *P* values ≤ .2 used to identify candidates for inclusion in the final model. Statistical software used was JMP Pro (version 15.0).

3 | RESULTS

This consecutive cohort (n = 803) had a mean follow-up of 86 months (median 83, range 6-239 months) and a median 10-year all-cause graft survival of 71% (death-censored graft survival 87%). A total of 2039 kidney allograft biopsies were performed in 605/803 (75%) of the cohort including 93% of those with death-censored graft loss. Banff Borderline or greater TCMR was present in 280/803 (34.8%) recipients whereas Banff ≥ IA TCMR occurred in 149/803 (18.6%). In 131/803 (16.3%) recipients the most severe TCMR phenotype was Banff Borderline (no subsequent or previous Banff ≥ IA TCMR biopsy). Compared to those with no TCMR those with Banff Borderline or Banff ≥ IA TCMR were younger, had longer cold ischemic time, and were more likely to have received cyclosporin and an interleukin (IL)-2 receptor antagonist (Table 1). Delayed graft function was more common in those with Banff ≥ IA TCMR. There was no difference in mean tacrolimus level in the first year between groups, however, tacrolimus coefficient of variation was increased in the TCMR groups (*P* = .0002). Although there was no significant difference in traditional HLA-A/B/DR mismatch, the HLA-DR/DQ molecular mismatch alloimmune risk category significantly correlated with the likelihood of TCMR (*P* < .0001).

Patients with isolated mild tubulitis (Banff i0,t1 in the absence of glomerulitis, vasculitis, or peritubular capillaritis) were not classified as Banff Borderline; however, they were assessed independently. There were 50/803 recipients where isolated Banff i0,t1 was the most severe TCMR phenotype. There were no significant differences in any of the peritransplant recipient characteristics listed in Table 1 for recipients with the isolated Banff i0t1 phenotype compared to the No TCMR group (data not shown). There was no difference in dnDSA free survival (*P* = .89) or allograft survival (*P* = .34) in the isolated Banff i0t1 phenotype compared to the No TCMR group. Therefore, for the purposes of all subsequent analysis these patients were grouped with the No TCMR group. Recurrent disease contributed to allograft loss in 15% of recipients; however, there was no significant difference in the rate of recurrent disease across the No TCMR, Borderline TCMR, and Banff ≥ IA TCMR groups (data not shown).

TABLE 1 Recipient demographics

	Most severe T cell–mediated rejection phenotype			
	None (n = 523)	Banff borderline (n = 131)	Banff ≥ IA (n = 149)	*P* value
First transplant	95%	97%	92%	.1724
Recipient age (y)	47.1 ± 14.8	39.3 ± 18.6	40.9 ± 17.9	<.0001
Donor age (y)	41.6 ± 15.0	40.5 ± 14.8	40.2 ± 14.9	.4855
Living donor	50%	46%	42%	.1958
Ethnicity (Caucasian vs other)	66%	63%	65%	.7943
Cold ischemic time (h)	6.1 ± 4.9	7.2 ± 5.6	7.7 ± 5.9	.0206
Delayed graft function	12%	12%	23%	.0024
Induction therapy				.0019
None	58%	51%	42%	
IL-2 receptor antagonist (basiliximab)	18%	30%	39%	
Anti-thymocyte globulin (Thymoglobulin)	24%	19%	19%	
Tacrolimus vs cyclosporin	95%	87%	72%	<.0001
Cyclosporin mean 0-12 mo	339 ± 48	376 ± 41	372 ± 25	.0187
Tacrolimus mean 0-12 mo (n = 720)	9.9 ± 1.2	9.9 ± 1.2	9.9 ± 1.3	.6941
Tacrolimus or cyclosporin CV 0-12 mo	34.1 ± 11.1	37.0 ± 12.0	37.8 ± 12.3	.0002
HLA-A/B/DR/DQ antigen mismatch	4.8 ± 2.4	4.9 ± 2.0	5.3 ± 2.0	.0856
HLA-DR/DQ molecular mismatch risk category				<.0001
Low	29%	14%	16%	
Intermediate	35%	43%	34%	
High	36%	43%	50%	

Abbreviations: CV, coefficient of variation; IL, interleukin; TCMR, T cell–mediated rejection.

3.1 | Banff borderline and Banff ≥ IA TCMR correlate with allograft survival

Death-censored allograft survival was significantly reduced in recipients whose most severe TCMR phenotype was Banff Borderline (hazard ratio [HR] 2.42, 95% confidence interval [CI] 1.35-4.34, $P = .003$) or Banff ≥ IA TCMR (HR 4.28, 95% CI 2.62-7.03, $P < .0001$, Figure 1A) compared to recipients without TCMR. Recipients with Banff ≥ IA TCMR had lower allograft survival compared to those with Banff Borderline TCMR (HR 1.77 95% CI 1.04-3.03, $P = .03$, Figure 1). There was no significant difference in 10-year death-censored allograft survival between recipients whose most severe TCMR was Banff IA (5.6% of cohort), Banff IB (6.9%), Banff 2A (5.5%), or Banff IIB (0.5%) TCMR (data not shown, $P = .22$). The correlation between TCMR and allograft survival remained significant when patients who developed dnDSA (n = 95) were excluded (n = 708, $P = .002$, Figure 1B).

3.2 | HLA-DR/DQ alloimmune risk categories correlate with dnDSA development and TCMR

HLA-DR/DQ single molecule eplet mismatch scores were used to categorize patients into low, intermediate, or high alloimmune risk categories using previously published thresholds.[10] HLA-DR/DQ dnDSA free survival correlated with alloimmune risk categories; Intermediate risk vs low risk (HR 10.18, 95% CI 2.40-43.21, $P = .002$); and high risk vs low risk (HR 20.80, 95% CI 5.05-85.74, $P < .0001$, Figure S1) in this expanded cohort. Alloimmune risk category also correlated with Banff Borderline ($P = .01$), Banff ≥ IA ($P = .0005$), and Banff ≥ IB ($P = .004$, Figure 2) TCMR free survival.

3.3 | CNI levels

Calcineurin inhibitor (Tacrolimus n = 45 881, Cyclosporin n = 7031) trough levels were available in 97% of the study cohort. The mean number of trough levels analyzed per recipient was 139 for cyclosporin (median 135) and 100 for tacrolimus (median 93). As shown in Table S1, the mean calcineurin trough levels were the same or increased in the intermediate and high alloimmune risk groups compared to the low-risk group. Mean CNI levels in the 30 or 90 days prior to biopsy were not statistically different for No TCMR, Banff Borderline TCMR, or Banff ≥ IA TCMR groups (Table S2). However, CNI coefficient of variation was increased in recipients with Banff Borderline or Banff ≥ IA TCMR (Table 1) compared to the No TCMR group.

3.4 | Timing of dnDSA and TCMR

De novo DSA developed posttransplant in 95 recipients against Class I alone (n = 14), Class II alone (n = 62), or Class I and II (n = 19). The timing of dnDSA relative to TCMR was known for 82/95 (86%) cases whereas in 13 cases there was no biopsy done at the time of dnDSA onset (n = 9) or concomitant BK viremia complicated the histologic assessment (n = 4). At least one TCMR episode preceded or occurred concomitant with dnDSA development in 67/82 (82%) of recipients in which the timing was known. Only four recipients developed dnDSA without preceding or concurrent TCMR (including biopsy at the time of dnDSA development) and then developed their first TCMR at a later time point. De novo DSA free survival was reduced in recipients whose most severe TCMR was Banff Borderline (HR 3.03, 95% CI 1.70-5.34, $P = .0002$, Figure 3) and Banff ≥ IA TCMR (HR 6.40, 95% CI 3.97-10.33, $P < .0001$) compared to recipients with no TCMR (recipients who developed dnDSA before TCMR excluded, n = 4). De novo DSA development was significantly more common in recipients with Banff ≥ IA TCMR compared to recipients whose most severe TCMR was Banff Borderline (HR 2.12, 95% CI 1.27-3.55, $P = .004$). The timing of TCMR episodes relative to dnDSA development did not correlate with allograft survival ($P = .13$ data not shown).

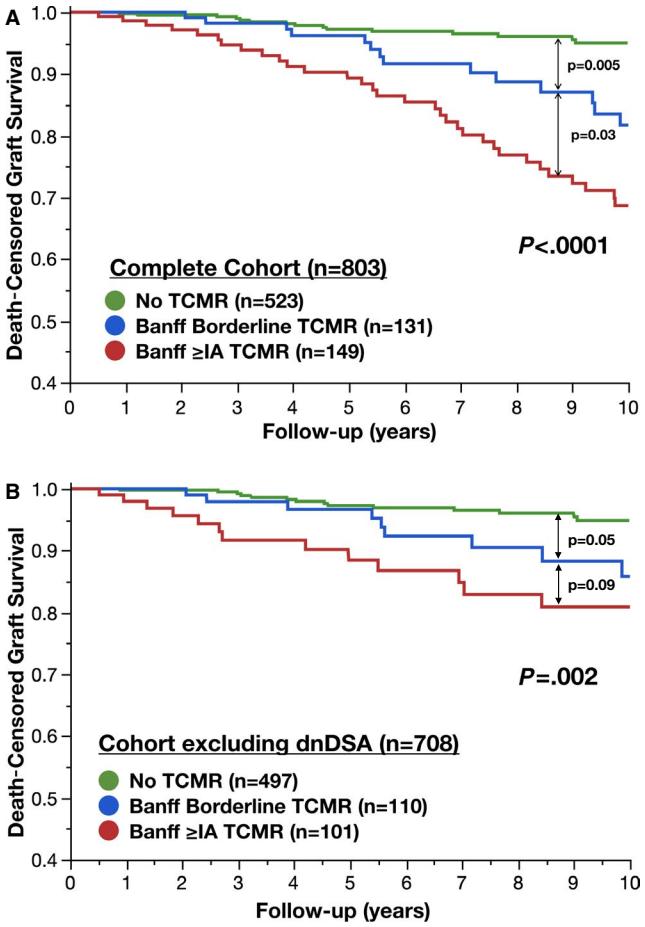

FIGURE 1 Allograft survival by most severe T cell–mediated rejection (TCMR) phenotype. Recipients with Banff Borderline and Banff ≥ IA T cell–mediated rejection had significantly reduced death-censored allograft survival (A). After exclusion of recipients who developed de novo donor-specific antibodies posttransplant, recipients (n = 708) with Banff Borderline and Banff ≥ IA T cell-mediated rejection had significantly reduced death-censored allograft survival (B)

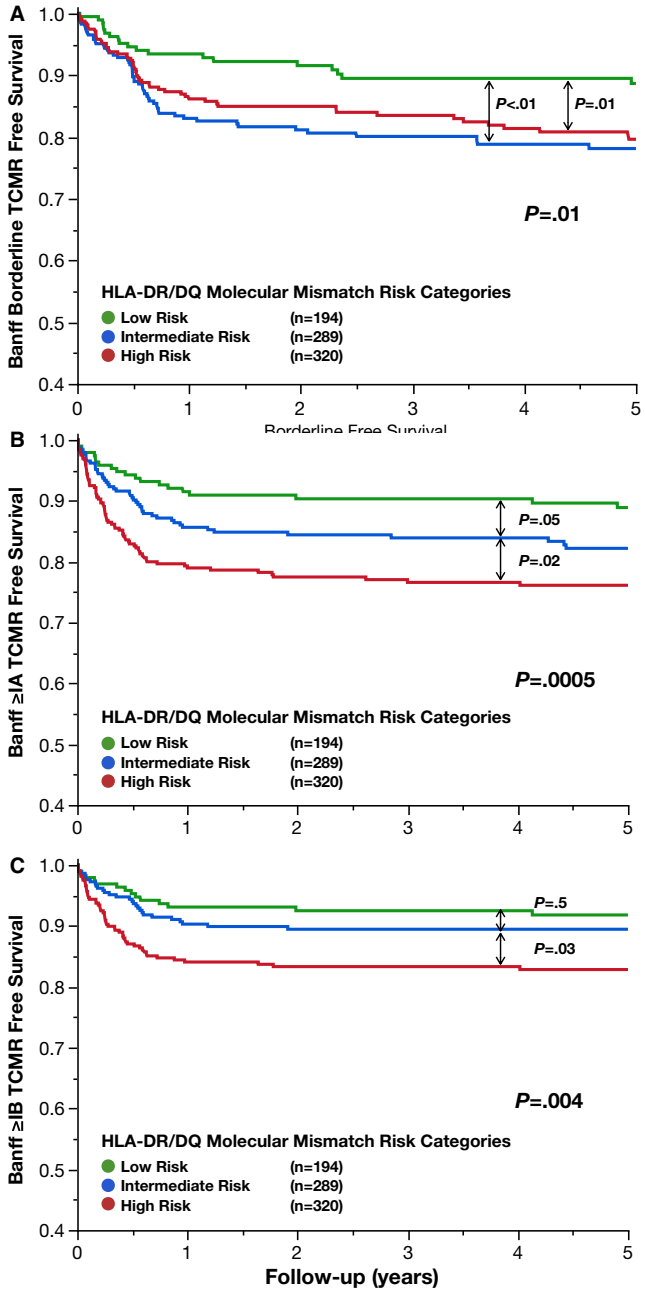

FIGURE 2 Banff Borderline, Banff ≥ IA, Banff ≥ IB T cell–mediated rejection (TCMR) free survival by alloimmune risk category. Low, intermediate, or high HLA-DR/DQ alloimmune risk categories correlated with Banff Borderline, Banff ≥ IA, and Banff ≥ IB T cell–mediated rejection free survival posttransplant

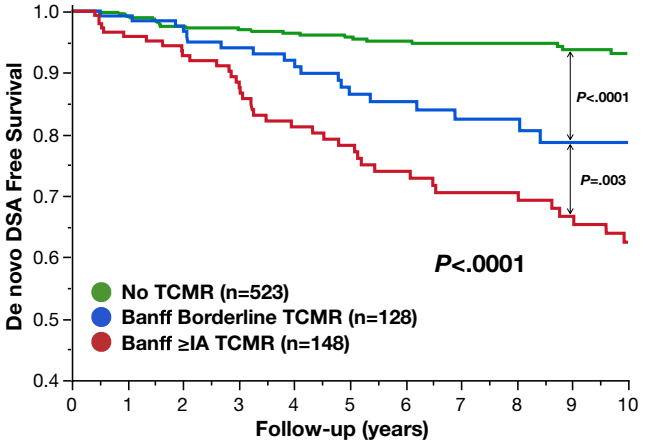

FIGURE 3 De novo DSA free survival by T cell–mediated rejection (TCMR) phenotype. De novo donor-specific antibody (dnDSA) development posttransplant correlated with T cell–mediated rejection. Recipients who developed dnDSA without preceding or concurrent TCMR (including biopsy at the time of dnDSA development) and then developed their first TCMR episode post-dnDSA onset were excluded from analysis (n = 4)

3.5 | Multivariate correlates of Banff borderline TCMR and Banff ≥ IA TCMR

Univariate correlates of Banff Borderline TCMR free survival were recipient age, induction therapy, tacrolimus vs cyclosporin therapy, and HLA-DR/DQ alloimmune risk category (Table 2). Multivariate independent correlates of Banff Borderline TCMR free survival were recipient age (HR 0.97, 95% CI 0.96-0.98, $P < .0001$), tacrolimus vs cyclosporin therapy (HR 0.37, 95% CI 0.22-0.63, $P < .0001$), and HLA-DR/DQ alloimmune risk category (intermediate vs low risk, HR 2.91, 95% CI 1.73-4.89, $P < .0001$ and high vs low risk, HR 2.82, 95% CI 1.66-4.77, $P = .0001$). If recipients who developed dnDSA were excluded from the analysis, all three risk factors were still independent multivariate correlates of Banff Borderline TCMR (n = 708, $P < .0001$, Table S4).

Univariate correlates of Banff ≥ IA TCMR free survival were recipient age, cold ischemic time, delayed graft function, induction therapy, tacrolimus vs cyclosporin therapy, and HLA-DR/DQ alloimmune risk category (Table 3). Multivariate independent correlates of Banff ≥ IA TCMR free survival were recipient age (HR 0.98, 95% CI 0.97-0.99, $P = .0006$), delayed graft function (HR 2.11, 95% CI 1.43-3.12, $P = .0002$), tacrolimus vs cyclosporin therapy (HR 0.19, 95% CI 0.13-0.28, $P < .0001$), and HLA-DR/DQ alloimmune risk category (intermediate vs low risk, HR 2.40, 95% CI 1.46-3.96, $P = .0006$ and high vs low risk, HR 3.07, 95% CI 1.90-4.96, $P < .0001$). In a sensitivity analysis of recipients treated with tacrolimus maintenance immunosuppression (n = 720) significant multivariate predictors of Banff Borderline or greater TCMR were recipient age (HR 0.97, 95% CI 0.97-0.98, $P < .0001$), tacrolimus CV (HR 1.02, 95% CI 1.01-1.03, $P = .0006$), and HLA-DR/DQ alloimmune risk category (intermediate vs low risk, HR 1.60, 95% CI 1.09-2.35, $P = .02$ and high vs low risk, HR 1.78, 95% CI 1.23-2.59, $P = .002$, Table S5).

3.6 | Recurrent TCMR

More than one biopsy was performed in 660/803 (82%) of the cohort. Recipients with TCMR (Borderline or greater) all had more than one biopsy. In recipients whose most severe rejection was Banff Borderline TCMR the number of Borderline rejection episodes correlated with death-censored allograft survival (HR 1.31 per rejection, 95% CI 1.18-1.43, $P < .0001$). The mean number of Banff Borderline

TABLE 2 Correlates of Banff Borderline TCMR vs no TCMR

	Univariate		Multivariate	
	Hazard ratio	*P* value	Hazard ratio	*P* value
First transplant vs two or more	1.45 (0.54-3.95)	.4303		
Recipient age (y)	0.97 (0.96-0.98)	<.0001	0.97 (0.96-0.98)	<.0001
Donor age (y)	0.99 (0.98-1.00)	.5105		
Living donor	0.84 (0.60-1.19)	.3268		
Ethnicity (Caucasian vs other)	0.87 (0.61-1.24)	.4411		
Cold ischemic time (h)	1.03 (1.0-1.07)	.0348		
Delayed graft function	1.14 (0.67-1.95)	.6412		
Induction therapy vs none	1.53 (1.08-2.16)	.0162		
Tacrolimus vs cyclosporin	0.41 (0.25-0.68)	.0006	0.37 (0.22-0.63)	<.0001
Alloimmune risk category (low, intermediate, high)				
Intermediate vs low	2.32 (1.39-3.86)	.0013	2.91 (1.73-4.89)	<.0001
High vs low	2.15 (1.29-3.58)	.0034	2.82 (1.66-4.77)	.0001

TCMR, T cell–mediated rejection.

TABLE 3 Correlates of Banff ≥ IA TCMR vs no TCMR

	Univariate		Multivariate	
	Hazard ratio	*P* value	Hazard ratio	*P* value
First transplant vs two or more	0.61 (0.34-1.10)	.1216		
Recipient age (y)	0.98 (0.99-1.0)	.0001	0.98 (0.97-0.99)	.0006
Donor age (y)	0.99 (0.93-1.00)	.3192		
Living donor	0.73 (0.53-1.0w)	.0634		
Ethnicity (Caucasian vs other)	0.94 (0.67-1.32)	.7249		
Cold ischemic time (h)	1.05 (1.02-1.08)	.0015		
Delayed graft function	2.17 (1.48-3.17)	<.0001	2.11 (1.43-3.12)	.0002
Induction therapy vs none	2.03 (1.47-2.83)	<.0001		
Tacrolimus vs cyclosporin	5.37 (3.74-7.70)	<.0001	0.19 (0.13-0.28)	<.0001
Alloimmune risk category (low, intermediate, high)				
Intermediate vs low	1.85 (1.13-3.02)	.0146	2.40 (1.46-3.96)	.0006
High vs low	2.51 (1.57-4.01)	.0001	3.07 (1.90-4.96)	<.0001

TCMR, T cell–mediated rejection.

TCMR rejections significantly increased across the low, intermediate, and high HLA-DR/DQ alloimmune risk categories (0.29, 0.49, 0.59 rejection episodes per recipient respectively, $P = .005$, Table S3). The association between HLA-DR/DQ alloimmune risk categories and the number of Borderline rejection episodes remained significant after adjustment for recipient age and cyclosporin vs tacrolimus treatment ($P = .0002$, data not shown).

In recipients who had Banff ≥ IA TCMR, the number of Banff ≥ IA TCMR episodes also correlated with death-censored allograft survival (HR 1.90 per rejection episode, 95% CI 1.61-2.20, $P < .0001$). The mean number of Banff ≥ IA TCMR rejection episodes significantly correlated with low, intermediate, and high HLA-DR/DQ alloimmune risk categories (0.20, 0.27, 0.38 rejection episodes per recipient respectively, $P = .004$, Table S3). The association between HLA-DR/DQ alloimmune risk categories and number of Banff ≥ IA rejection episodes remained significant after adjustment for recipient age, cyclosporin vs tacrolimus, and delayed graft function ($P = .01$, data not shown). When evaluating the number of Banff ≥ Borderline TCMR episodes that occurred prior to dnDSA development there was a significant correlation

between the number of rejection episodes and dnDSA development (HR 1.28, 95% CI 1.17-1.38, $P < .0001$). In the cohort treated with tacrolimus (n = 720) the number of Banff Borderline or greater TCMR rejection episodes also continued to be associated with HLA-DR/DQ alloimmune risk category after adjusting for recipient age and tacrolimus CV.

4 | DISCUSSION

The key finding in this study is that in the absence of donor-specific memory (ie, no preformed DSA by solid phase single antigen bead assay) alloimmune risk assessment for TCMR can be more precisely understood through quantification of HLA-DR/DQ mismatches at the molecular level. The independent correlation between HLA molecular mismatch and Banff Borderline TCMR suggests that Borderline rejection is part of a spectrum of alloimmune-mediated inflammation and not simply a response to injury. The increased incidence of TCMR and recurrent TCMR across HLA-DR/DQ alloimmune risk categories indicate possible utility as a prognostic biomarker for precision medicine as well as for stratification or enrichment in clinical trial design.

Whereas some have argued that TCMR has limited relevance as a correlate of allograft loss,[9,21] others have shown a correlation between early clinical or subclinical TCMR and functional decline, interstitial fibrosis and tubular atrophy, and allograft loss.[12-13,22-25] Furthermore, multiple studies have reported that early TCMR correlates with later development of dnDSA, ABMR, and chronic glomerulopathy.[26-32] We found a significant correlation between those with Banff Borderline TCMR or Banff ≥ IA TCMR and death-censored allograft survival (Figure 1). Importantly, although TCMR preceded or was concomitant with dnDSA development in some cases, the correlation between TCMR and graft loss persisted when recipients who developed dnDSA were excluded from the analysis (Figure 1B).

In contrast to the original Banff criteria for Borderline rejection, there are little data available to support the prognostic importance of isolated mild tubulitis (*i0t1*) biopsies added in the Banff 2005 report. Mehta et al reported that $i + t > 0$ biopsies (excluding Banff ≥ IA) had higher serum creatinine and Banff chronicity scores at 12 months compared to $i + t = 0$ biopsies; however, 21% of the *i0t1* patients progressed to more severe forms of TCMR after the initial biopsy.[13] We found that in recipients where isolated mild tubulitis *i0t1* was the most severe phenotype there was no association with dnDSA development or graft loss. Furthermore, traditional risk factors of alloimmunity did not correlate with the Banff i0t1 phenotype.

Although diagnostic criteria for Banff TCMR have been in place for more than 25 years, the pretransplant risk factors that correlate with TCMR in the current era remain poorly understood. Early reports from the 1990's identified HLA antigen mismatch, delayed graft function, and immunosuppression adequacy as correlates of TCMR.[33-38] Prospective randomized surveillance biopsy trials also documented that early detection and treatment of TCMR in the first three months decreased the incidence of late TCMR (12 months) and preserved GFR.[38] However, the lack of comprehensive HLA typing or sensitive solid phase assays to rule out donor-specific memory limit the applicability of these older reports. Elevated panel reactive antibody (PRA) and repeat transplantation were once thought to be risk factors for alloimmunity. However, recent work using state-of-the-art antibody assessment in combination with comprehensive HLA typing (ie, including HLA-C, HLA-DQ, and HLA-DP) has shown that when preformed DSA are ruled out, neither PRA nor repeat transplant are prognostic of allograft outcomes.[27,39-41] In the current study, we show that HLA-DR/DQ molecular mismatch category is a significant univariate and multivariate correlate of Banff Borderline TCMR and Banff ≥ IA TCMR. The correlation of HLA-DR/DQ molecular mismatch with Banff Borderline TCMR free survival and recurrent Banff Borderline TCMR provides evidence for the alloimmune basis of Banff Borderline rejection as opposed to a nonspecific injury response. Furthermore, the same predictors of dnDSA (younger age, HLA-DR/DQ molecular mismatch, inadequate immunosuppression)—an indisputable donor-specific response—are also independent predictors of both Banff Borderline and Banff ≥ IA TCMR. This makes sense in the context of the immune system as we understand it where CD4 T cell help is a requirement for B cell activation, the production of antibody secreting plasma cells, as well as CD8 T cell activation.[42,43]

Increasingly TCMR recurrence or persistence has been reported in serial biopsy studies; however, definite risk factors have not been well elucidated.[12-15] In this study we found that the number of Banff Borderline and Banff ≥ IA TCMR rejection episodes per recipient was independently correlated with the HLA-DR/DQ alloimmune risk categories after adjustment for other covariates (recipient age, cyclosporin vs tacrolimus, delayed graft function). The standard approach to most TCMR treatment is a short course of increased steroids. However, given that age and HLA-DR/DQ alloimmune risk are fixed variables, their association with risk may help explain the persistence of rejection in some recipients and suggests close follow-up (with or without repeat biopsy) may be required after a TCMR episode in these individuals.

Tacrolimus and mycophenolate, with or without steroids, is standard of care immunosuppression based on evidence of reduced clinical rejection and improved graft survival.[44-47] In a large registry cohort Nankivell et al also found both medications were associated with reduced subclinical TCMR.[14] In the current study we confirmed that tacrolimus use correlated with the prevention of Banff Borderline TCMR (HR 0.33, $P < .0001$) and Banff ≥ IA TCMR (HR 0.18, $P < .0001$) compared to cyclosporin after adjustment for recipient age, delayed graft function, and alloimmune risk category.

Although tacrolimus trough level variation is not synonymous with inadequate tacrolimus exposure, it has been correlated with rejection, dnDSA development, and graft loss.[48-50] In our study mean CNI trough levels were no different in recipients with and without TCMR. With an average of more than one hundred trough

levels per recipient it is not surprising that fluctuations in medication levels that may have clinical significance will be unaccounted for by average trough levels and emphasize the need for alternative metrics. Tacrolimus coefficient of variation may be associated with nonadherence, drug interactions, fluid shifts, or physician-directed dosing alterations.[48] In the current study tacrolimus coefficient of variation was a significant independent correlate of any Banff Borderline or greater TCMR (P = .018) after adjusting for other covariates confirming previous work and extending it to Banff Borderline TCMR.[48,50-53]

Younger recipient age has been correlated with the risk of TCMR, dnDSA, and ABMR in the past independent of donor age and nonadherence.[16-17,54,55] Age-dependent metabolic and immunosuppressive effects of tacrolimus have been demonstrated in murine and human $CD4^+$ T cells.[56,57] In addition, recipient age and cytochrome P3A5*1 genotype were the two multivariate correlates with the largest effect on tacrolimus trough levels in a human study showing that a 50% CNI dose reduction was required in older recipients to reach the same trough levels as younger recipients.[58] Unfortunately, in the context of precision medicine little is known regarding specific age thresholds that may be important or how to use recipient age to select trough level targets.

5 | LIMITATIONS

Due to the relatively small sample size and the associated risk of type II error, risk quantification should be interpreted with caution and should be validated in an independent cohort. Histology was available in 75% of recipient's posttransplant; however, 93% of the death-censored graft loss occurred in the cohort with at least one biopsy. Although HLA-DP typing was available for only 576/803 of the cohort, there was no significant correlation between HLA-DP MM and TCMR free survival (P = .32) after adjusting for alloimmune risk category. Mean tacrolimus and cyclosporin level may not capture periods of nonadherence associated with TCMR. Methods of risk stratification will need to be tested prospectively and in independent cohorts including those with varying ethnicities to confirm their general applicability.

6 | CONCLUSION

The underlying driver of all alloimmune injury, and the need for immunosuppression to mitigate that injury, is donor-recipient dissimilarity at the molecular level. HLA-DR/DQ single molecule molecular mismatch evaluation is a precise method to quantify this difference that correlates with dnDSA development, ABMR, TCMR, and graft loss. Favorable characteristics of this potential prognostic biomarker include that it is cost effective, sensitive, widely available (in silico test using existing FDA-approved HLA typing methods), reproducible and available at the time of transplant. In this study we report that a recipient's HLA molecular mismatch risk category independently correlates with Banff Borderline TCMR, Banff ≥ IA TCMR, and recurrent TCMR. In the absence of pretransplant DSA, TCMR is often the earliest indication of alloimmune reactivity; thus, developing a prognostic biomarker of TCMR could serve as a drug development tool for risk stratification or enrichment in phase II/III clinical trials.[59] Beyond clinical trials, once fully validated, the HLA-DR/DQ single molecule molecular mismatch score has the potential to transition immune monitoring and therapy in kidney transplantation from an empiric to a precision medicine framework that could be rapidly implemented by the transplant community.

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DISCLOSURE

The authors of this manuscript have conflicts to disclose as described by the *American Journal of Transplantation*. DNR is a consultant with Astellas Pharma. PWN is a consultant with Astellas Pharma and Vitaeris Inc.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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