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# Determining Clinical Thresholds for Donor HLA Eplet Compatibility to Predict Best Outcomes Following Lung Transplantation

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**Background.** Currently, the assessment of immunological risk in lung transplantation (LTx) does not completely consider HLA compatibility at the molecular level. We have previously demonstrated the association of HLA eplets in predicting chronic lung allograft dysfunction following LTx; however, the associations between HLA eplet mismatch (epMM) loads and overall survival are unknown. **Methods.** In this retrospective, single-center study, 277 LTx donor-recipient pairs were high resolution HLA typed and analyzed for HLA epMMs using HLAMatchmaker (version 3.1). LTx pairs were also assessed for the presence of the previously described risk epitope mismatches DQ2-DQA1\*05 and DQ7-DQA1\*05. **Results.** HLA class I epMMs were not associated with deleterious outcomes; however, lower HLA class II ( $\leq 19$ ), DQA1 ( $\leq 2$ ), and combined HLA class I and II ( $\leq 29$ ) epMM demonstrated an association with increased time to chronic lung allograft dysfunction and improved overall survival. The presence of a risk epitope mismatch was not associated with worse clinical outcomes. **Conclusions.** HLA epMM can risk-stratify LTx recipients and potentially guide donor-recipient matching and immunosuppression strategies.

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## INTRODUCTION

Lung transplantation (LTx) is an established treatment for patients with end-stage lung diseases, including those with chronic obstructive pulmonary disease, interstitial lung disease, and cystic fibrosis. However, outcomes following LTx have remained poor, with 5-y survival rates around 50% and

median post-LTx survival of just 6 y.<sup>1</sup> Although advancements have been made with identifying preformed HLA antibodies within the recipient before LTx, graft failure in the form of chronic lung allograft dysfunction (CLAD) remains the primary cause of mortality beyond 1-y.<sup>1</sup> Current pre-LTx immunological strategies involve the avoidance of preexisting donor-specific HLA antibodies (DSAs) that are of a strength likely to be detrimental to LTx outcomes.<sup>2,3</sup> However, this fails to account for HLA structural incompatibilities between donor and recipients that may lead to the development of post-LTx de novo donor-specific antibodies (dnDSAs), as well as other alloimmune responses that, together, are damaging to the lung allograft.<sup>4-6</sup>

HLA antibodies do not recognize the entire HLA molecule but rather short amino acid residues within them, defined as HLA eplets.<sup>2,7</sup> HLAMatchmaker is a computational program based on modeling of HLA crystal structures that compares sequences of HLA alleles and defines eplet mismatches (epMMs) between the recipient and donor.<sup>8-11</sup> Structural similarity between a recipient and donor, as defined by a low epMM score, is associated with better outcomes in kidney,<sup>12,13</sup> heart,<sup>14,15</sup> and LTx.<sup>6,16</sup> Previous LTx studies, albeit not at molecular level, have demonstrated that HLA compatibility decreases the risk of CLAD<sup>17-19</sup> and dnDSA development<sup>5,6,20</sup>; however, studies showing improved survival are limited.<sup>21</sup>

Additionally, so-called “high-risk” epitopes have been associated with dnDSA development.<sup>20</sup> McCaughan et al<sup>20</sup> identified that having the 45GE3 and 45EV/55PP eplets found

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on HLA-DQ2 and DQ7 with HLA-DQA1\*05 mismatches increased risk of dnDSA development 4.2-fold. However, this study only used dnDSA development as final outcome and was censored at 3-y posttransplantation.

In this retrospective study, we aimed to define the epMM thresholds, which best predicted long-term survival, and determine whether previously reported high-risk epitopes were associated with poorer outcomes following LTx. We hypothesized that the avoidance of high epMM between donor and recipients would be associated with decreased CLAD and improved survival following LTx and as such represents an important assessment of LTx immunological risk that should be considered at the time of transplant.

## MATERIALS AND METHODS

### Lung Transplant Cohort

The Alfred Hospital Ethics Committee (478/19) and Australian Red Cross Blood Service (02022019) approved this study.

All patients undergoing a primary bilateral lung transplant at the Alfred Hospital, Melbourne, between June 2008 and December 2015 and that had local clinical follow-up were considered for the study. In total, 336 transplants were considered, and after exclusions of pediatric (22), multiorgan transplant (4), and transplants across pretransplant HLA DSA (33), 277 transplants met criteria and constituted the final cohort (Figure 1).

The majority of patients received a standard triple immunosuppressant regimen consisting of tacrolimus, azathioprine or mycophenolate, and prednisolone, as has been previously described,<sup>22</sup> with induction therapy with an interleukin-2

antagonist reserved for patients with limited renal reserve. Patients at risk of cytomegalovirus (CMV) reactivation (either donor- or recipient-positive CMV serostatus) received prophylaxis with 2 wk of intravenous ganciclovir followed by oral valganciclovir for a further 5 mo.

All cohort participants were followed in the Alfred's lung transplant clinic with 3 mo reviews (including lung function testing) until death or the censor date of December 31, 2018.

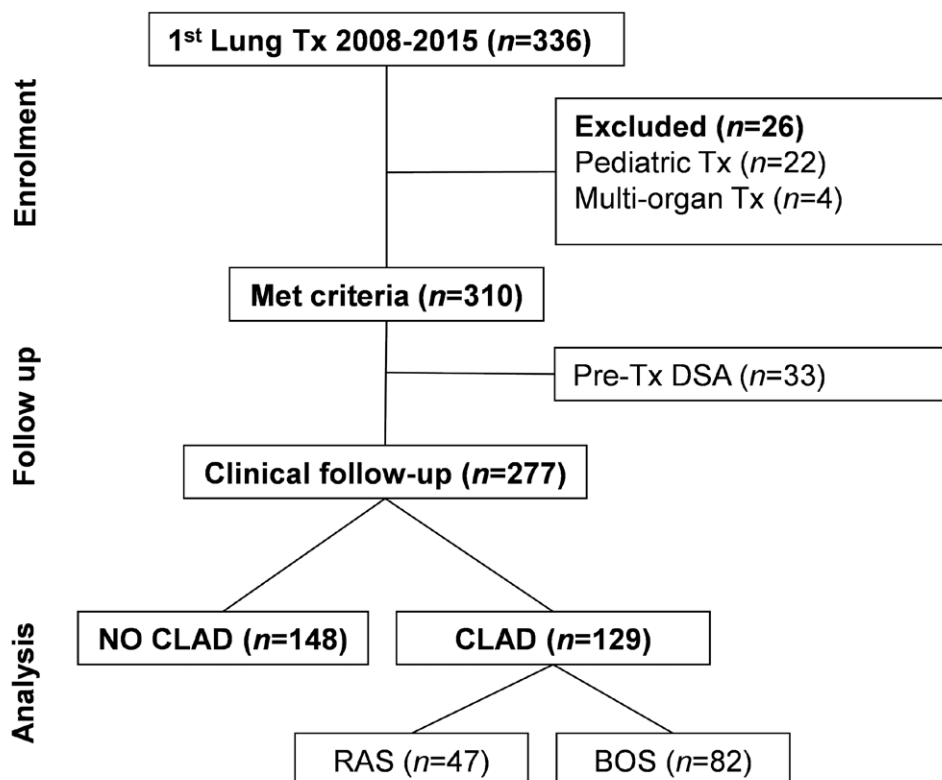
### HLA Typing

HLA typing for recipient and donors was retrospectively performed by Next Generation Sequencing (MIA FORA flex11, on Illumina Miseq). All HLA alleles were reported to 2-fields (HLA-A; B; C; DRB1; DRB345: DQB1; DQA1; DPB1; and DPA1). All testing was performed at the Victorian Transplantation and Immunogenetics Service (Melbourne, VIC, Australia).

### HLA Antibody Testing

Pretransplant, wait-listed patients were routinely screened pretransplant and every 6 mo with Luminex Mixed Screen (One Lambda Inc, Canoga Park) and by Single Antigen beads yearly. Sera were treated with hypotonic dialysis, absorption, or a combination of both in the event of suspected prozone effect. No specific desensitization was performed on pretransplant positive patients other than the utilization of intravenous immunoglobulin as a monthly infusion for patients with significant sensitization as assessed via a panel react antibody status of >95%. Mean fluorescence intensity  $\geq 2000$  was used for assignment of HLA DSA positivity.

A complement-dependent cytotoxicity T- and B-cell cross-match was prospectively performed for all transplants, and



**FIGURE 1.** Lung transplant cohort used for analysis. Summary of the final clinical cohort. BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; DSA, donor-specific antibody; RAS, restrictive allograft syndrome; Tx, transplantation.

any positive results were confirmed following treatment with dithiothreitol. The decision to proceed with LTx required a negative complement-dependent cytotoxicity T-cell cross-match result.

### Epitope Analysis

The HLA-Matchmaker 1000 pair program (ABC and DRDQDP eplet, version 3.1) was used to assess eplet compatibility (<http://www.epitopes.net/>). This HLA-Matchmaker version determines structurally based HLA compatibility for multiple transplant pairs simultaneously and also gives DQA1 and DPA1 epMMs independent to DQB1 and DPB1. Recipient and donor HLA typing was entered, and epMM load of each pairing was assigned. For our analysis, the total eplets were used; this includes both antibody verified and non-verified eplets. Where an HLA typed allele was not present in the program, the nearest HLA allele within exons 2 to 4 (class I) or exons 2 to 3 (class II) was assigned (eg, A\*01:37 to A\*01:01).

Risk epitope mismatches (REMs) were determined when the donor possessed either a 45GE3, present in DQ2, or 45EV/55PP, present in DQ7, eplet with a DQA1\*05 allele.<sup>20</sup> The presence or absence of REM with or without DQA1\*05 was used for further analysis of REMs association on outcomes.

### Clinical Outcomes

The primary clinical outcomes for the study were CLAD and survival. CLAD was defined as a sustained irreversible loss of forced expiratory volume in 1 s from baseline of  $\geq 20\%$ . Individuals meeting criteria for CLAD were further classified into 2 groups according to the pattern of loss based on the ratio of forced expiratory volume in 1 s to forced vital capacity (FER).<sup>23</sup> The obstructive phenotype defined by an FER  $< 70\%$  was bronchiolitis obliterans syndrome (BOS) or the nonobstructive by an FER  $> 70\%$  was restrictive allograft syndrome (RAS).<sup>23</sup>

### Statistical Analysis

Multivariate Cox proportional hazard models were used to determine associations between HLA mismatches and outcomes in survival analysis (time to CLAD and death) after adjustments for known risk factors (age, gender, disease type, and CMV status). Comparisons between low epMM and high epMM groups, as defined by tertiles, were used to assess the associations of epMM on outcomes. Overall statistical significance was determined with a statistical significance at  $\alpha = 0.05$  level. Kaplan-Meier survival curves were used to demonstrate time to CLAD and survival time. All statistical analysis were performed using SPSS (version 21).

## RESULTS

### Lung Transplant Cohort

Complete recipient and donor demographics are shown in Table 1. Of the 277 LTx included in the study, 55% of recipients and 55% of donors were male with mean age for recipients being 52 y ( $\pm 14$  y) compared with 45 y ( $\pm 16$  y) for donors. The most common indication for LTx was chronic obstructive pulmonary disease (48%). Ninety percent of recipients had a calculated panel reactivity antibody of  $< 50\%$ , whereas 6 (2%) had  $> 95\%$  calculated panel reactivity antibody and classed as highly sensitized. The majority of patients

**TABLE 1.**  
Recipient/donor demographics

N = 277	n (%) / mean (SD)
Recipient	
Age, y	52 ( $\pm 14$ )
Male	151 (55)
Diagnoses	
COPD	133 (48)
ILD	50 (18)
CF	55 (20)
PH	17 (6)
Other	22 (8)
Sensitization (cPRA)	
$< 50\%$	248 (90)
51%–79%	17 (6)
80%–94%	5 (2)
$> 95\%$	6 (2)
Donor	
Age, y	45 ( $\pm 16$ )
Male	153 (55)
Pathway	
DCD	80 (29)
DBD	197 (71)
Clinical outcome	
Time of follow-up, y	5.0 ( $\pm 2.4$ )
CLAD	129 (47)
RAS	47 (36)
BOS	82 (64)
Died during follow-up	97 (35)

Pretransplant criteria used for compatibility assessment and posttransplant clinical follow-up. BOS, bronchiolitis obliterans syndrome; CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; COPD, chronic obstructive pulmonary disease; cPRA, calculated panel reactivity antibody; DBD, donation after brain death; DCD, donation after circulatory death; ILD, interstitial lung disease; PH, pulmonary hypertension; RAS, restrictive allograft syndrome.

(n = 197) followed a donation after brain death pathway. The mean follow-up time of recipients to censor of cohort was 5.0 y ( $\pm 2.4$  y). One hundred twenty-nine recipients developed CLAD posttransplant, 47 defined as RAS and 82 with BOS, whereas 97 patients died during follow-up.

### HLA Mismatch and LTx Outcome

Overall, this cohort included several alleles considered rare or less common within local population. Of the total 238 unique HLA alleles (2-field used for epMM calculations), 14 were represented only once, and the total epMM between the rarer and the more common alleles range from 0 to 5 (seen with DRB1\*13:21) epMM; furthermore, no recipient-donor pairing would have changed the epMM groupings used in this study if the common allele was used.

Although LTx patients with high HLA antigen mismatches (agMMs) (HLA-A, B, C, DRB1345, DQA1, DQB1, DPA1, DPB1) were more likely to have a higher epMM score, determining structural compatibility at the antigen rather than the molecular level has a reduced discriminating ability in assessing donor-recipient compatibility (Figure 2A). A patient who might traditionally be deemed compatible based on a low HLA agMM score can have widely differing epMM scores and as such be assessed as structurally incompatible based on a high epMM score. For example, at the molecular level, a patient with a high eplet score of 30 epMM may have as few as 6 or as high as 12 agMMs when looking at all HLA loci

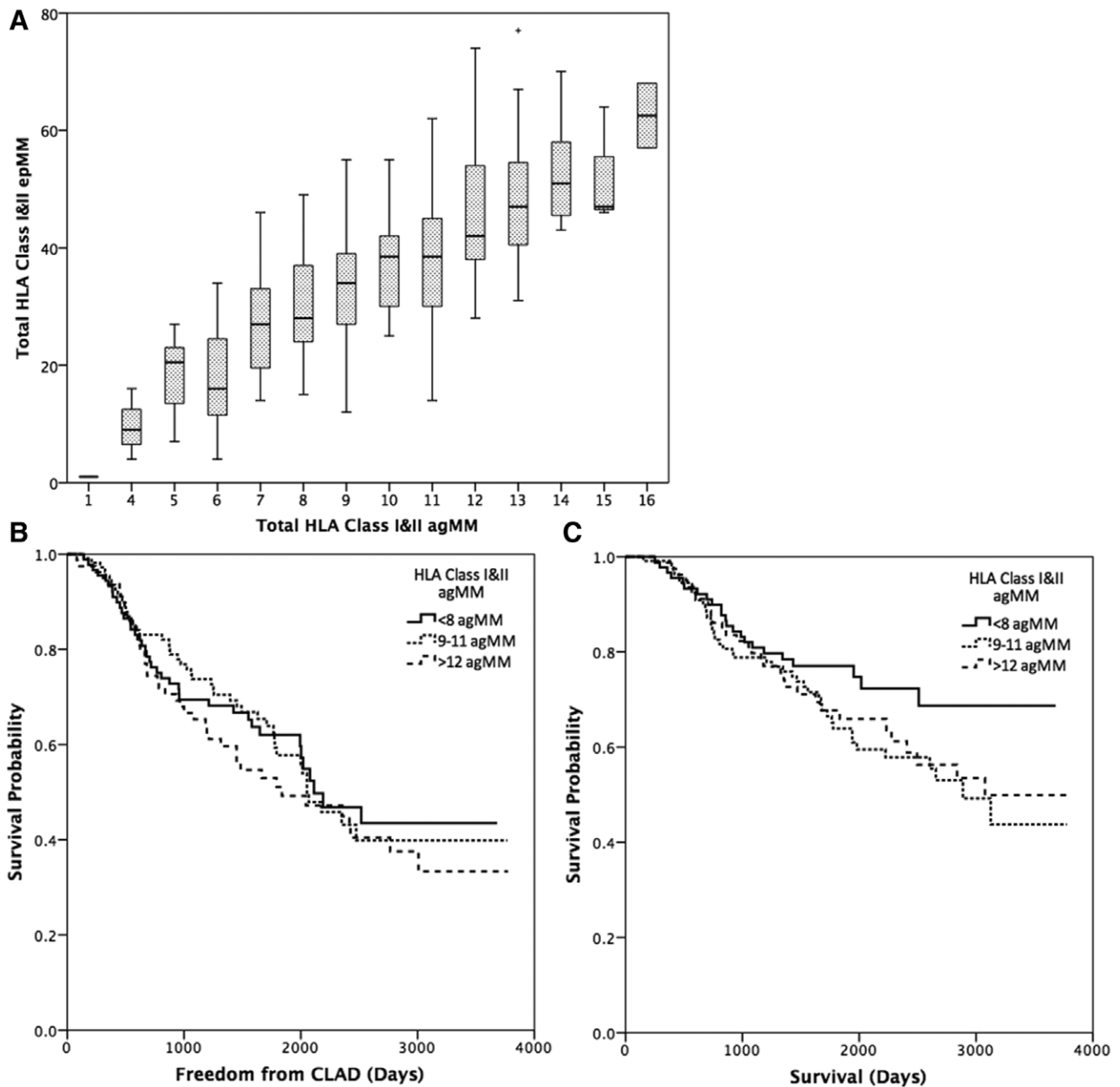
(A-DPA) (Figure 2A). HLA eplets provide a more linear and therefore more discriminating assessment of HLA compatibility between recipient and donor pairs. The number of HLA agMMs was not found to be significantly associated with either time to CLAD (Figure 2B) or overall patient survival (Figure 2C).

### HLA epMM and Association With LTx Outcomes

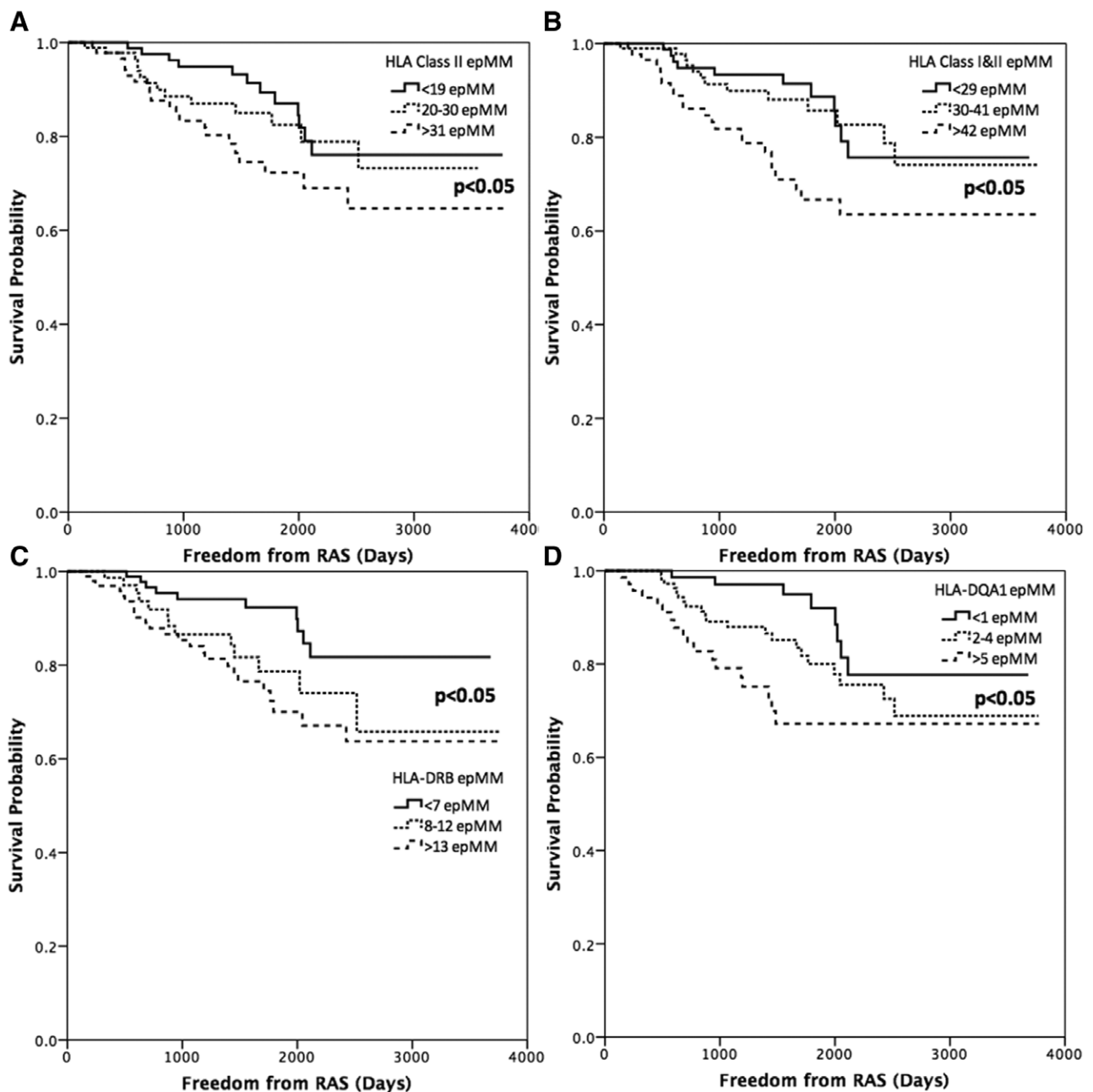
Lower HLA class II ( $\leq 19$ ) epMM and lower total HLA class I and II ( $\leq 29$ ) epMM were both significantly associated with increased freedom from RAS ( $P = 0.044$  and  $P = 0.021$ , respectively) (Figure 3A and B) in the multivariate

models after adjustments for known risk factors. Of the HLA class II loci when independently analyzed, lower DRB1 ( $\leq 7$ ) and DQA1 ( $\leq 2$ ) epMM contributed the most toward the total class II association with freedom from RAS. DRB1 ( $P = 0.010$ ) and DQA1 ( $P = 0.006$ ) were both significantly associated with increased freedom of RAS after adjustments (Figure 3C and D).

Both lower HLA class II ( $\leq 19$ ) and total class I and II ( $\leq 29$ ) epMM were significantly associated with improved overall survival ( $P = 0.002$  and  $P = 0.004$ , respectively) (Figure 4A and B). Again, lower DRB1 ( $\leq 7$ ) and DQA1 ( $\leq 2$ ) epMM were significantly associated with overall survival when analyzed independently ( $P = 0.010$  and  $P = 0.048$ ) (Figure 4C and D).



**FIGURE 2.** HLA agMMs' association with HLA epMM load and outcomes (CLAD/survival). A, Association between total agMMs (A, B, C, DRB1345, DQA1, DQB1, DPA1, DPB1) and total HLA epMM load (class I and II). B, Kaplan-Meier analysis of freedom from CLAD and HLA agMM classified in tertiles. No significance was attained when comparing low agMM ( $\leq 8$ ) and high agMM ( $\leq 12$ ) with freedom from CLAD. C, Kaplan-Meier analysis of overall patient survival and HLA agMM classified in tertiles. There was no significance between low agMM ( $\leq 8$ ) and high agMM ( $\leq 12$ ) groups and overall survival after adjustments for known risk factors ( $P = 0.20$ ). For this analysis, all HLA loci were used to define agMM; therefore, the potential minimum is 0 agMM and maximum 18 agMM. agMM, antigen mismatch; CLAD, chronic lung allograft dysfunction; epMM, eplet mismatch.



**FIGURE 3.** Association of epMM and RAS. A, Kaplan-Meier analysis of HLA class II epMM to freedom from RAS ( $P < 0.05$ ). B, Kaplan-Meier analysis of HLA class I and II epMM to freedom from RAS ( $P < 0.05$ ). C, Kaplan-Meier analysis of HLA DRB1345 epMM to freedom from RAS ( $P < 0.05$ ). D, Kaplan-Meier analysis of HLA DQA1 epMM to freedom from RAS ( $P < 0.05$ ). epMM, eplet mismatch; RAS, restrictive allograft syndrome.

However, lower DQB1 ( $\leq 7$ ) epMM was also significantly associated with improved overall survival after adjustments for risk factors ( $P = 0.041$ ) (Figure 4E). Overall, no HLA epMM groups were associated with freedom from BOS (Table 2). When analyzed independently, neither HLA class I, HLA DPB1, nor DPA1 was associated with either freedom from CLAD or overall survival in any statistical models.

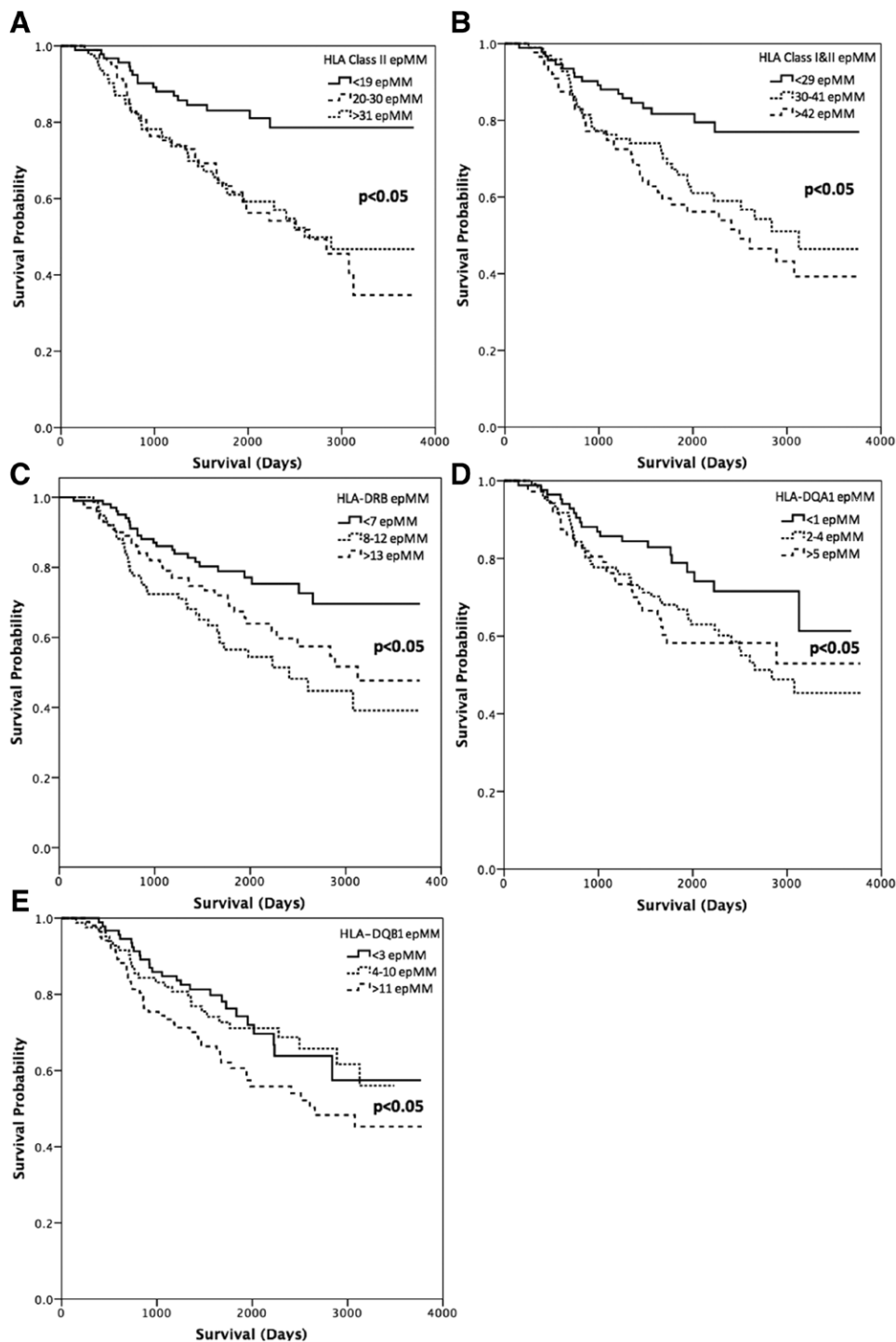
### REMs

To determine the association with the risk epitopes (45GE3 or 45EV/55PP), transplants that occurred with either the REM (DQ2 or DQ7) mismatches, with and without DQA1\*05 mismatch, were independently analyzed. Transplants with either

the DQ2 or DQ7 REM were present in 73 (26%) of donors (Table 3), whereas 16 (5%) of donors had the DQ2 or DQ7 mismatch without a DQA1\*05. Neither of the REM groups was associated with freedom from RAS or BOS (Figure 5A) or overall patient survival (Figure 5B).

### DISCUSSION

In this study, we have confirmed an association between HLA structural compatibility, as defined by a low epMM score, and freedom from CLAD but importantly have also demonstrated an association with improved overall patient survival. More significantly, we have defined an epMM



**FIGURE 4.** Association of epMM and overall survival. A, Kaplan-Meier analysis of HLA class II epMM to overall patient survival ( $P < 0.05$ ). B, Kaplan-Meier analysis of HLA class I & II epMM to overall patient survival ( $P < 0.05$ ). C, Kaplan-Meier analysis of HLA DRB1345 epMM to overall patient survival ( $P < 0.05$ ). D, Kaplan-Meier analysis of HLA DQA1 epMM to overall patient survival ( $P < 0.05$ ). E, Kaplan-Meier analysis of HLA DQB1 epMM to overall patient survival ( $P < 0.05$ ). epMM, eplet mismatch.

threshold (19 for HLA class II and 29 for class I and II) that is associated with improved outcomes, specifically overall patient survival. Although HLA epMM loads have previously been shown to predict post-LTx outcomes that include the development of dnDSA<sup>6,24</sup> and CLAD,<sup>16</sup> the association of HLA eplets on long-term patient survival has yet to be well characterized. Much of the work done to determine the utility of HLA eplet matching in transplantation has been performed

with renal transplants<sup>12,13,25</sup> with major concerns on how to define epitopes or whether to use epMM loads or only eplets with higher immunogenicity.<sup>26-28</sup> In contrast, in the setting of LTx, the clinical urgency and need for a lifesaving transplant often require consideration of a less than immunologically perfect donor organ. Notwithstanding this limitation, knowledge of HLA structural compatibility, as determined by a peri-transplant epMM algorithm could ultimately be used to direct

**TABLE 2.****Associations between HLA epMM and outcomes**

ep	MM	CLAD					
		RAS		BOS		Survival	
		HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Class I	≤9	1.17 (0.81-1.68)	0.42	1.20 (0.86-1.48)	0.43	1.26 (0.94-1.58)	0.14
Class II	≤19	1.44 (1.01-2.05)	<b>&lt;0.05</b>	1.07 (0.82-1.41)	0.61	1.49 (1.16-2.21)	<b>&lt;0.05</b>
DRB1345	≤7	1.58 (1.12-2.24)	<b>&lt;0.05</b>	0.97 (0.75-1.27)	0.86	1.04 (1.01-1.08)	<b>&lt;0.05</b>
DQB1	≤4	1.13 (0.79-1.60)	0.51	1.15 (0.88-1.50)	0.31	1.30 (1.01-1.67)	<b>&lt;0.05</b>
DQA1	≤2	1.78 (1.18-2.69)	<b>&lt;0.05</b>	1.14 (0.80-1.54)	0.40	1.32 (1.00-1.73)	<b>&lt;0.05</b>
DPB1	0	0.81 (0.59-1.17)	0.26	0.82 (0.62-1.08)	0.15	0.91 (0.71-1.17)	0.48
DPA1	0	0.77 (0.34-1.75)	0.53	0.56 (0.28-1.36)	0.11	0.87 (0.50-1.53)	0.64
I & II	≤29	1.57 (1.07-2.31)	<b>&lt;0.05</b>	1.20 (0.90-1.60)	0.22	1.49 (1.14-1.95)	<b>&lt;0.05</b>

HRs reported after adjustment for known risk factors (age, gender, disease type, cytomegalovirus status, and sensitization) in a multivariate Cox proportional hazard model analysis. Tertiles were used to define epMM groups, with the threshold for low epMM group reported. Low epMM groups were compared with high epMM groups in each category. HR reported as the increased risk of outcome between groups. BOS and RAS were independently assessed for associations, and statistically significant associations were reported in bold.

BOS, bronchiolitis obliterans syndrome; CI, confidence interval; CLAD, chronic lung allograft dysfunction; epMM, eplet mismatch; HR, hazard ratio; RAS, restrictive allograft syndrome.

**TABLE 3.****Incidence of REM and incidence of outcomes divided by REM categories**

REM group	n (%)	CLAD					
		RAS		BOS		Survival	
		HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
No REM	189 (69)	Reference	–	Reference	–	Reference	–
DQ2/DQ7 only	15 (5)	<sup>a</sup>	<sup>a</sup>	1.31 (0.82, 2.07)	0.26	1.17 (0.77, 1.78)	0.67
REM+ DQA1*05	73 (26)	1.23 (0.65, 2.35)	0.52	1.26 (0.77, 2.05)	0.36	1.16 (0.74, 1.80)	0.51

No incidence of RAS was diagnosed for any subjects in the DQ2/DQ7 only group.

Associations between REM and time to CLAD (RAS/BOS) or death was calculated using multivariate Cox proportional hazard models after adjustments for known risk factors (age, gender, disease type, cytomegalovirus status, and sensitization). None of the groups had significant associations with any outcomes used in this study.

BOS, bronchiolitis obliterans syndrome; CI, confidence interval; CLAD, chronic lung allograft dysfunction; HR, hazard ratio; RAS, restrictive allograft syndrome; REM, risk epitope mismatch.

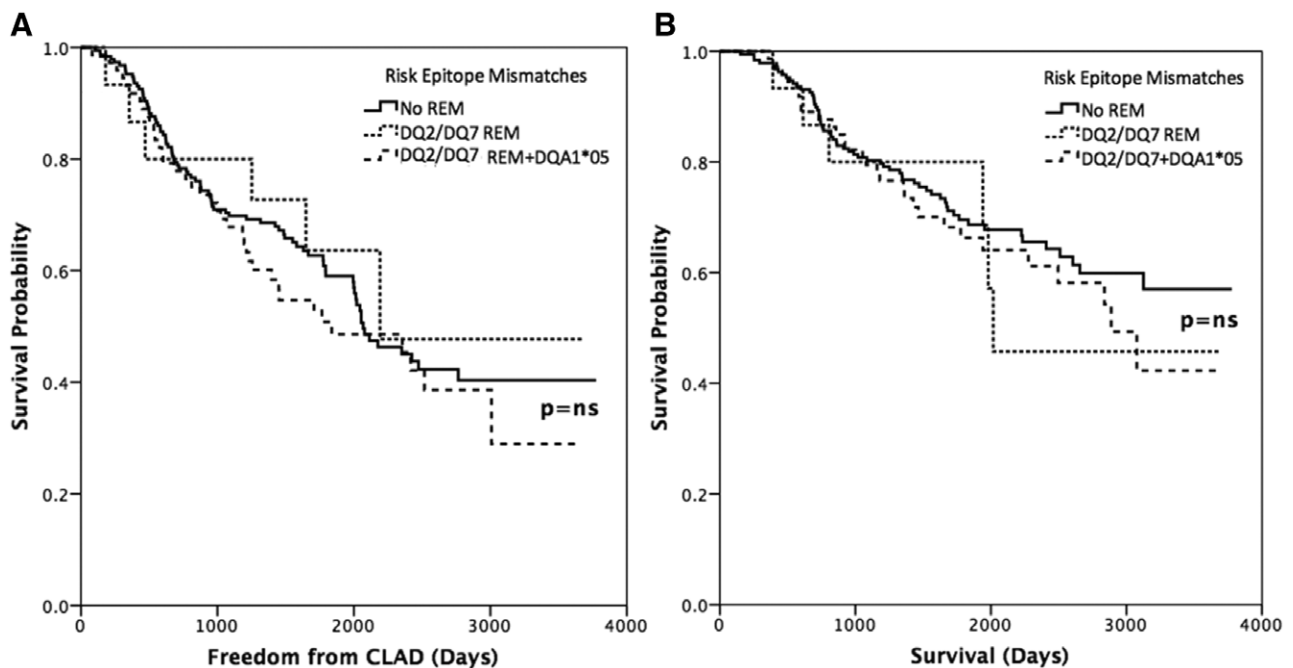
donors toward the pool of the lowest risk recipients. Of our cohort, approximately one third of patients was transplanted against an HLA structurally compatible donor as defined using a lower HLA class II epMM. A better understanding of immunological risk may also allow better tailoring of postimmunosuppression. Unique and rare HLA types in recipients and donors would impact the chance of having a compatible transplant pair. This is an obvious concern for waitlist recipients who may have a rarer HLA allele than that of the local donor population, where the concern would be finding a lower epMM donor to transplant while not extending wait times. However, the use of eplets to define compatibility may increase compatible donor pools, as the rarer allele can often be seen as low as a 0 epMM to the more common allele, as we have seen here. The use of lower epMM to determine LTx compatibility provides a decrease risk of rejection (RAS) and improved overall survival. Therefore, the added knowledge of the epMM load, even in the presence of a rarer HLA allele, would allow clinical teams could customize post-LTx monitoring and preemptive therapeutic strategies.<sup>29</sup>

As we have previously demonstrated, high HLA-DQAB epMM scores strongly predicted adverse clinical outcomes.<sup>16</sup> However, the newest version of HLA-Matchmaker defines DQ eplets as either DQB1 or DQA1, and with this, we have shown that it was lower DQA1 epMM, which predicted increased freedom from CLAD (RAS). The split of the DQB1 and DQA1 eplets has potentially highlighted the importance of considering

DQA1 in any HLA compatibility assessment. Interestingly, the REM included the use of DQA1 eplets; in our study, this was not shown to be a significant predictor of CLAD or overall survival. However, with these results that demonstrated that higher DQA1 epMMs increased the risk of CLAD and decreased overall survival, in addition to reports of DQA1 mismatching increasing risk of dnDSA development,<sup>20</sup> further investigation into the role of DQA1 in LTx is warranted.

Recognizing HLA eplets with high immunogenicity that may be mismatched between recipients and donors is of interest, as it may be associated with poorer posttransplant outcomes. Previous studies have highlighted potential high-risk epitopes.<sup>20,24</sup> However, in our study with extended patient follow-up, crossing these REM, in LTx did not increase the risk of CLAD or overall patient survival.

Although these thresholds were significantly associated with long-term survival in our cohort, we understand these results are limited to this release of HLA-Matchmaker and based on our own center's LTx experience. However, the significance of improved outcomes following LTx with lower epMM loads is valuable, as it demonstrates a long-term benefit of selecting patients with lower epMM loads. The use of eplet compatibility is still contentious in renal allocation,<sup>26,28</sup> yet for thoracic transplants, centers could incorporate an HLA epMM score in the decision process where multiple potential recipients are considered. Therefore, the inclusion of an epMM compatibility would steer clinicians away from selecting higher-risk recipients.



**FIGURE 5.** Association of REM and outcomes (CLAD/survival). A, Kaplan-Meier survival analysis of REM groups and freedom from CLAD. No significant association with any REM group and freedom from CLAD was reached. B, Kaplan-Meier survival analysis of REM groups and overall patient survival of time to outcome. No significant association with any REM group and overall survival was reached. CLAD, chronic lung allograft dysfunction; ns, not significant; REM, risk epitope mismatch.

Reduced costs and improved efficiency of full gene HLA typing of patients on waitlists have allowed the realization that HLA epMM could be used routinely in the pretransplant compatibility assessment of transplants. Although an accurate, rapid, high resolution HLA typing for deceased donors has not yet been fully realized, there are signs that it could be used in the future.<sup>30</sup> However, we feel that the use of 2-field HLA typing for donors would still allow an improved risk assessment for LTx, specifically in determining epMM load and identifying the lowest risk recipients. We also acknowledge that intermediate 2-field donor HLA typing may not be as accurate with ethnic diversities in some populations consisting of rarer HLA alleles; however, including a “provisional” epMM load at the time of transplant would not be the only factor to transplant but a possible aid in better understanding the immunological risks.

Although many studies have reported higher epMM loads increasing the risk of dnDSA development, our omission of dnDSA development as an outcome is a limitation. Earlier transplants in this cohort did not receive routine posttransplant antibody screening, and screening was for clinical indication of antibody-mediated rejection. Therefore, associations of epMM loads on this dnDSA would be biased. We accept this is a limitation of this extended cohort. However, we have previously reported on higher epMM loads increasing risk of dnDSA,<sup>6</sup> and we believe the association of epMM to overall survival and freedom from CLAD (RAS) are important determinants in patient selection. Therefore, epMM load would greatly improve the accuracy of immunological risk prediction in the pretransplant setting and warrant consideration.

In conclusion, we have identified HLA epMM thresholds that define lower immunological risk and are associated with the best long-term survival. Selection toward lower-risk patients could allow for lower immunosuppression treatments and tailored posttransplant monitoring.

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