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A Comparison of HLA Molecular Mismatch Methods to Determine HLA Immunogenicity

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Abbreviations: de novo donor specific antibody; dnDSA, eplet mismatch; EpMM, amino acid mismatch; AAMM, electrostatic mismatch; ESM, calculated panel reactive antibody; cPRA

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Abstract

Background

Antibody-mediated rejection is a major cause of premature graft loss in kidney transplantation. Multiple scoring systems are available to assess the HLA mismatch between donors and recipients at the molecular level, however, their correlation with the development of *de novo* donor-specific antibody (*dn*DSA) has not been compared in recipients on active immunosuppression.

Methods

HLA-DR$\beta_{1/3/4/5}$/$DQ_{\alpha1\beta1}$ molecular mismatch was determined using eplet analysis, amino acid mismatch, and electrostatic mismatch for 596 renal transplant recipients and correlated with HLA-DR/DQ *dn*DSA development. The molecular mismatch scores were evaluated in multivariate models of posttransplant *dn*DSA free survival.

Results

Eplet mismatch correlated with amino acid mismatch and electrostatic mismatch (R^2=0.85-0.96). HLA-DR *dn*DSA free survival correlated with HLA-DR eplet mismatch (HR 2.50 per 10 eplets mismatched, p<0.0001), amino acid mismatch (HR 1.49 per 10 amino acids mismatched, p<0.0001), and electrostatic mismatch (HR 1.23 per 10 units mismatched, p<0.0001). HLA-DQ dnDSA free survival correlated with HLA-DQ eplet mismatch (HR 1.98 per 10 eplets mismatched, p<0.0001), amino acid mismatch (HR 1.24 per 10 amino acids mismatched, p<0.0001), and electrostatic mismatch (HR 1.14 per 10 units mismatched, p<0.0001). All 3 methods were significant multivariate correlates of *dn*DSA development after adjustment for recipient age, baseline immunosuppression, and nonadherence.

Conclusion

HLA molecular mismatch represents a precise method of alloimmune risk assessment for renal transplant patients. This report highlights that the use of one method over the other is likely to be driven by familiarity and ease of use as highly correlated results are produced by each method.

Introduction

Antibody-mediated rejection (ABMR) is a major cause of allograft dysfunction and allograft loss in kidney transplantation.(1-3) Improvements in HLA histocompatibility assessment and HLA antibody screening methods have made it possible to avoid transplanting across known donor-specific antibodies (DSA), however, twenty to thirty percent of recipients develop *de novo* DSA (*dn*DSA) after 5-10 year of follow-up.(4) Therapy for late antibody mediated rejection is limited, therefore strategies to minimize *dn*DSA development through more precise HLA mismatch evaluation and through appropriate immunosuppression management are paramount.(5,6)

Advances in genetics and protein modelling have made it possible to compare donor-recipient HLA mismatch at the molecular level. Traditional HLA mismatch quantification is constrained by a limited range of possible values (0, 1, or 2 per locus) at the whole antigen level. However, assessment of HLA mismatch at the molecular level enables quantification of the degree of mismatch and in turn immunogenicity between donor-recipient HLA improving the precision of immunological risk assessment with *dn*DSA development as the immune response readout. One such approach, based on enumerating all mismatched amino acid sequence polymorphisms on donor HLA and scoring them according to their physicochemical properties, has been shown to be independently associated with *dn*DSA development after graft failure.(7) An extension of this work, to assess the impact of donor sequence polymorphisms on the HLA tertiary structure, suggested that surface exposed antibody epitopes have unique electrostatic potential profiles that help explain HLA cross-reactive antigen groups.(8) A different approach, namely HLA Matchmaker, identifies small patches of surface exposed mismatched amino acids named "eplets" on each HLA molecule, which are hypothesized to drive DSA specificity.(9) The

quantity of mismatched eplets between donor and recipient alleles has been shown to correlate with *dn*DSA development, rejection, chronic glomerulopathy, and graft loss.(6,10,11,12) Whether one of these methods is a superior correlate for *dn*DSA development in the setting of active immunosuppression has not been determined.

The purpose of this analysis was to compare the eplet mismatch, amino acid mismatch, and electrostatic mismatch computational methodologies in a large cohort of well characterized renal transplant recipients for their correlation with the development of *dn*DSA posttransplant. Unique to this consecutive patient cohort is the strict exclusion of preexisting HLA DSA, the availability of high resolution donor and recipient HLA typing, immunosuppression adherence, serial sera obtained posttransplantation to characterize the timing of *dn*DSA onset, and long-term graft outcomes.

Methods:

Study Population

Approval was obtained from the IRB (H2011: 211) and was in adherence with the declaration of Helsinki. 654 adult and pediatric consecutive renal transplants between January 1999 and January 2015 were considered for inclusion. Patients with primary nonfunction (n=16), or pretransplant DSA (n=42) were excluded, leaving 596 recipients (adult n=541, pediatric n=55) for analysis. Recipients who moved (n=21) or died with a functioning graft (n=82) were censored at last follow-up. Standard maintenance immunosuppression consisted of a calcineurin inhibitor (tacrolimus (86%) or cyclosporin

(14%)), mycophenolate mofetil, and prednisone. Induction therapy with thymoglobulin (16%) or basiliximab (19%) was used in 35% of patients.

HLA Typing and Molecular Mismatch Identification

High-resolution (4-digit) Class II HLA typing (HLA-$DR_{\beta 1/3/4/5}$ and HLA-$DQ_{\alpha 1/\beta 1}$) was performed using sequence-specific oligonucleotide probes or sequence-specific primer technology (LABType HD SSO, Micro SSP, One Lambda). HLAMatchmaker software (HLA DRDQDP Matching version 2.0) was used to define Class II eplet mismatches (EpMM) between donors and recipients. The amino acid mismatch score (AAMS) and electrostatic mismatch score (EMS) for mismatched donor-recipient HLA combinations were determined using the Cambridge HLA Immunogenicity algorithm, as described previously.(7) For a given patient, when more than a single HLA mismatch was present within a locus, individual scores for each HLA mismatch were added to represent an overall immunogenicity score.

Antibody Monitoring

Posttransplant serum samples were collected and stored at 0,1, 2, 3, 6, 12, 18, and 24 months, then yearly, or at the time of biopsy for graft dysfunction, as routine clinical practice in our program since 1990. Since 2007 posttransplant surveillance for *dn*DSA was instituted for all renal transplant patients. DSA screening was performed using FlowPRA™ beads representing HLA-A, -B, -Cw, -DR, -DQ, and -DP antigens (One Lambda, Canoga Park, CA). If the screening assay was positive, determination of HLA antibody specificities was performed using FlowPRA™ single antigen class I and II beads (One Lambda, Canoga Park, CA) and analyzed according to the manufacture's

recommendations. HLA antibody specificities were validated using LABScreen™ single antigen beads (One Lambda, Canoga Park, CA) using a threshold mean fluorescence intensity ≥ 500 (mean fluorescence intensity ≥1000 initially or on a subsequent sample in 98% of cases).

Pretransplant all patients had remote and immediate pretransplant sera screened by FlowPRA and if positive evaluated by FlowPRA single antigen beads. Even if the FlowPRA screen was negative, patient sera were still evaluated by FlowPRA single antigen beads if there was elevated risk of sensitization (eg pregnancy, history of transfusion). To rule out a DSA pretransplant the mismatched donor antigens had to be represented on the single antigen beads. If donor specific antibodies were absent pretransplant, as determined by solid phase assays and a negative flow cross-match, and became detectable posttransplant they were classified as *dn*DSA. Patients with *dn*DSA had banked posttransplant serum tested to determine the approximate timing of *dn*DSA onset by FlowPRA single antigen beads. All patients continue to be prospectively tested for *dn*DSA according to the serum collection schedule outlined above to detect new *dn*DSA or to assess the persistence of existing *dn*DSA.

Statistics

Comparisons between baseline variables and clinical outcomes were done using Student's t-test for parametric continuous variables and Wilcoxon-rank test for nonparametric data. Chi-squared 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 ANOVA 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 correlates of *dnDSA* free survival. Akaike information criterion (AIC) was calculated with Cox models to allow model comparisons within specific cohorts. The ability of the models to correctly classify subjects for their actual outcomes (*dnDSA* development) was examined using time-dependent receiver operator characteristic curves and area under the curve (AUC) statistics. Variables for multivariate regression were selected on the basis of bivariate screening, with p values ≤0.2 used to identify candidates for inclusion in the final model. The proportional hazard assumption was not violated (assessed by both Schoenfeld residuals and Harrell's rho). Co-linearity was assessed and all variance inflation factors were less than 3. Statistical software used was R version 3.0.1 and JMP (version 12.2).

Results:

This consecutive cohort (n=596) represented a low immunological risk group (96% first transplant, <10% with cPRA >80%) by conventional criteria. Median follow-up was 87 months (range 18-210). HLA-DR or DQ *dn*DSA developed in 66 recipients (11%) at a median of 55 months (range of 6-170) posttransplant. At the time of *dn*DSA development 15/66 (23%) had HLA-DR *dn*DSA alone, 37/66 (56%) had HLA-DQ *dn*DSA alone, and 14/66 (21%) had both HLA-DR and DQ *dn*DSA. Significant correlates with Class II *dn*DSA were younger recipient and donor ages, Class II HLA-DR and DQ eplet mismatch, Class II HLA-DR and DQ amino acid mismatch, Class II HLA-DR and DQ electrostatic mismatch, greater cold ischemic time, CNI regimen (cyclosporine vs. tacrolimus), immunosuppression nonadherence, CNI coefficient of variation, and TCMR in the first year (Table 1).

Correlates of *dn*DSA Free Survival

The median number of HLA-DR$\beta_{1/3/4/5}$ EpMM, AAMM, and EMS were 10 (range 0-41), 15 (range 0-82), and 22 (range 0-147). HLA-DR$\beta_{1/3/4/5}$ EpMM (HR 2.50 per 10 mismatches, 95%CI 1.71-3.64, p<0.0001), AAMM (HR 1.49, 95%CI 1.25-1.76, p<0.0001), and EMS (HR 1.23, 95%CI 1.11-1.35, p<0.0001) were each significant univariate correlates of HLA-DR *dn*DSA free survival posttransplant (Table S1, SDC, http://links.lww.com/TP/B531).

The median number of HLA-DQ$_{\alpha 1\beta 1}$ EpMM, AAMM, and EMS was 13 (range 0-42), 18 (range 0-97), 24 (range 0-164). HLA-DQ $_{\alpha 1\beta 1}$ EpMM (HR 1.98 per 10 mismatches, 95%CI 1.53-2.58, p<0.0001), AAMM (HR 1.24, 95%CI 1.12-1.37, p<0.0001), and EMS (HR 1.14, 95%CI 1.07-1.21, p<0.0001) were each significant correlates of HLA-DQ *dn*DSA free survival posttransplant (Table S1, SDC, http://links.lww.com/TP/B531). Receiver operating characteristic curve analysis showed that all molecular mismatch methods had similar area under the curve scores (0.71 to 0.74) (Figure S1, SDC, http://links.lww.com/TP/B531).

There were strong correlations between intra-locus molecular mismatch scores (Figure 1). HLA-DR$\beta_{1/3/4/5}$ EpMM correlated with HLA-DRβ_1 AAMM (R_2=0.96), and EMS (R_2=0.85). HLA-DQ$\alpha_1\beta_1$ EpMM correlated with HLA- DQ$\alpha_1\beta_1$ AAMM (R_2=0.95), and EMS (R_2=0.90).

Multivariate Models

In multivariate analyses, each of the molecular mismatch scores were independent correlates of *dn*DSA development after adjustment for younger recipient age, cyclosporine vs. tacrolimus, and nonadherence, (Table 2). Akaike information criterion (a measure of the relative quality of multivariate statistical models) were similar among the molecular mismatch scores examined.

Discussion

Current assessment of donor-recipient histocompatibility and of the risk of humoral alloresponses after kidney transplantation is based on simple enumeration of HLA antigenic differences at individual class I and II loci without consideration of the relative immunogenicity of donor HLA mismatches according to the recipient HLA type. In the present study, we examined 3 different approaches for assessment of HLA class II immunogenicity ranging from simply enumerating the number of amino acid mismatches between donor and recipient HLA (AAMM), to counting the number of polymorphic surface accessible amino acid residues at discontinuous positions of donor HLA that cluster together to form a potential epitope (EpMM), to assessing the physicochemical disparity between the side chains of mismatched amino acids of donor and recipient HLA (EMS). Our study is the first to compare the capacity of these approaches to assess the risk of *dn*DSA development in a cohort of renal transplant patients on active immunosuppressive therapy and where the timing of *dn*DSA development posttransplant was monitored prospectively. The principal finding was that assessment of donor HLA immunogenicity based on AAMM, EpMM or EMS is superior to that of conventional HLA mismatch grade for assessing the risk of *dn*DSA development after kidney transplantation. We did not demonstrate an advantage in using one approach over another and, in this patient cohort, each method provided equivalent assessment of immunological risk associated with donor HLA class II mismatches.

Development of *dn*DSA after kidney transplantation is associated with rejection, accelerated eGFR decline, and graft loss.(1,13) Currently no therapies have been proven effective to eliminate *dn*DSA after its development nor prevent progression of allograft dysfunction,

therefore, prevention of *dn*DSA is of paramount importance.(5) Nonadherence with immunosuppression, younger recipient age, cyclosporine based immunosuppression regimens, early T-cell mediated rejection and HLA mismatch have been established as independent correlates of *dn*DSA development.(6,10,14,15,16) Molecular assessment of HLA immunogenicity has gained the interest of the transplant community due to its ability to outperform traditional whole molecule mismatch as a correlate of *dn*DSA development, transplant glomerulopathy, and graft survival.(6,10,11) Humoral responses after kidney transplantation are frequently directed against donor HLA class II alloantigens and our study suggests that assessment of HLA-DR and -DQ immunogenicity based on mismatched eplets produces similar results compared to simply enumerating the number of amino acid polymorphisms between donor and recipient HLA molecules. This is not surprising given that AMS and EpMS both reflect differences in donor-recipient amino acid sequence and a strong correlation between the 2 scoring systems has been demonstrated in this and other studies.(7) EMS integrates information on the number of mismatched amino acids and the differences in electrostatic charge of their side chains and it is, therefore, correlated to the AAMM score. Previous studies suggested that consideration of the electrostatic charge of amino acid polymorphisms on donor HLA-A and -B alloantigens might provide useful information regarding their immunogenic potential,(7,17) but this and other studies do not support an advantage in using EMS, over EpMM and AAMM, for assessing the risk of DSA responses against HLA class II mismatches.(7,18) Larger studies and assessment of HLA electrostatic properties at the tertiary level are warranted to further explore the relationship between donor-recipient HLA physicochemical differences and humoral alloresponses after transplantation.

HLAMatchmaker defines eplets by considering each polymorphic amino acid at or near the surface of the molecule and then asks the question what other polymorphic amino acids are nearby (3Å radius).(9) This small patch of polymorphic amino acids is known as an eplet and its specific name is derived from the amino acids involved (ie 52PQ). By comparison, the amino acid mismatch software developed by Kosmoliaptsis et al aligns the amino acid sequence of donor and recipient HLA alleles and counts the number of mismatched amino acids irrespective of their position in three-dimensional space (as no advantage was previously demonstrated by exclusion of surface inaccessible polymorphisms).(17) The physicochemical approach compares the isoelectric points of mismatched amino acids between donor and recipient alleles and the differences are summed to represent an overall electrostatic mismatch score. The strong correlations between eplet mismatch, amino acid mismatch, and electrostatic mismatch (Figure 1) are expected given that each scoring system examines a similar, but not identical, list of polymorphic amino acids (nonsurface exposed residues are excluded in HLAMatchmaker).

Using the Akaike information criterion to compare EpMM, AAMM and EMS in multivariate models of *dn*DSA development (Table 2) revealed a small advantage to the HLAMatchmaker model. However, all 3 molecular mismatch methods had similar discrimination measures (AUC) and have been shown to outperform traditional HLA antigen matching in this and in previous reports, and a clinically meaningful difference of using one method over another to correlate with *dn*DSA development in clinical practice is doubtful. 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 a larger independent cohort. We acknowledge that this analysis focused on Class II *dn*DSA

because, in our cohort, *dn*DSA against donor HLA Class I mismatches alone was infrequent (2% of cohort) and only 1 patient in the entire cohort suffered allograft failure after developing isolated Class I *dn*DSA. HLA-$DP_{\alpha 1 \beta 1}$ dnDSA development was tracked in this cohort (data not shown), however, was too infrequent for meaningful analysis. Development of mature humoral alloimmunity is dependent upon T cell help through linked recognition of HLA derived peptides presented in the context of recipient HLA class II molecules. Recent reports suggest that the presentation of allopeptides by HLA-DR correlate with *dn*DSA development.(19) Although early in development, this may be a promising area for future research. Forthcoming studies, should also explore the immunogenicity of individual donor HLA, as determined by molecular mismatch methods, and the risk of *de novo* HLA-specific antibody development as a time-dependent variable accounting for the effect of relevant confounders.

In conclusion, HLA molecular mismatch methods enable precise assessment of alloimmune risk associated with renal transplantation. Donor HLA amino acid mismatch, electrostatic mismatch and eplet mismatch were each significant multivariate correlates of *dn*DSA development. Relevant studies in larger independent cohorts are warranted but, at present, the use of one method over the other is likely to be driven by familiarity and ease of use as highly correlated results are produced by each method.

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Figure Legends:

Figure 1. There was a strong correlation between eplet mismatch and amino acid mismatch (top row) and electrostatic mismatch (bottom row) scores at the HLA-DR$\beta_{1/3/4/5,}$ HLA-DQ $_{\alpha 1\beta 1}$ loci.

Figure 1. Eplet Mismatch Correlates with Electrostatic and Amino Acid Mismatch

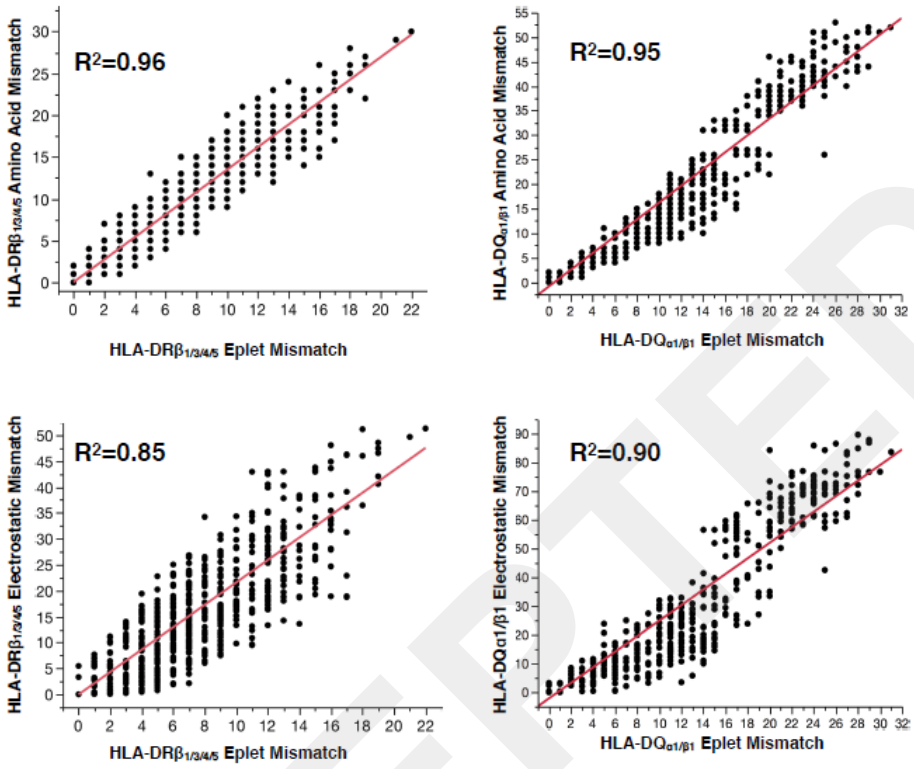

Table 1. Recipient Characteristics

	HLA-DR or DQ *dn*DSA (n=66)	No HLA-DR or DQ *dn*DSA (n=530)	p value
First Transplant	97%	96%	0.9196
Recipient Age at Transplant (years)	33.6±17.6	44.6±15.6	<0.0001
Donor Age (years)	36.6±14.9	40.7±14.7	0.0438
Living Donor	41%	50%	0.1712
Recipient Ethnicity (Caucasian vs. other)	76%	65%	0.0718
Cold Ischemic Time (hours)	8.7±5.7	6.8±5.4	0.0035
Delayed Graft Function	14%	12%	0.6544
Non-Adherence	41%	11%	<0.0001
Cyclosporin vs. Tacrolimus regime	39%	11%	<0.0001
Calcineurin inhibitor coefficient of variation	39.6±13.5	33.7±13.3	0.0083
HLA-DRB1 Mismatch	1.4±0.5	1.2±0.7	0.1381
HLA-DRB1/3/4/5 Mismatch	2.4±0.9	2.1±1.3	0.1838
HLA-DQB1 Mismatch	1.2±0.5	1.1±0.7	0.2492
HLA-DQA1/B1 Mismatch	2.3±0.9	2.2±1.4	0.5443
HLA-DRB1/3/4/5 Eplet Mismatch	14.1±7.3	11.0±9.2	0.0014
HLA-DQA1/B1 Eplet Mismatch	17.5±8.1	13.0±10.4	0.0002
HLA-DRB1/3/4/5 Amino Acid Mismatch	23.2±14.9	18.4±17.5	0.0026

HLA-DQA1/B1 Amino Acid Mismatch	35.1±25.6	29.5±29.7	0.0136
HLA-DRB1/3/4/5 Electrostatic Mismatch	30.7±17.8	23.2±22.7	0.0002
HLA-DQA1/B1 Electrostatic Mismatch	47.7±29.6	35.2±37.4	<0.0001
Episodes of TCMR ≥ Borderline in 0-12 months	1.4±1.4	0.6±1.1	<0.0001
Episodes of TCMR ≥ 1A in 0-12 months	0.6±0.8	0.2±0.5	<0.0001

Table 2. Multivariate Correlates of *dn*DSA Development

A. Eplet Mismatch	**DR *dn*DSA**		**DQ *dn*DSA**		**DR or DQ d*n*DSA**	
	n=596, 29 events		**n=596, 51 events**		**n=596, 66 events**	
	HR	**p value**	**HR**	**p value**	**HR**	**p value**
Recipient Age at Transplant (years)	0.97 (0.95-0.99)	0.0192	0.97 (0.95-0.98)	0.0018	0.97 (0.96-0.99)	0.0006
Nonadherence	3.07 (1.40-6.52)	0.0058	3.11 (1.71-5.58)	0.0002	3.09 (1.83-5.15)	<0.0001
Cyclosporin vs. Tacrolimus	2.14 (0.93-4.70)	0.0722	1.97 (1.06-3.52)	0.0251	2.28 (1.35-3.78)	0.0023
HLA-DRB1/3/4/5 Eplet Mismatch (per 10 mismatches)	2.79 (1.84-4.27)	<0.0001				
HLA-DQA1/B1 Eplet Mismatch (per 10 mismatches)			2.00 (1.52-2.67)	<0.0001		
HLA-DRB1/3/4/5 + HLA-DQA1/B1 Eplet Mismatch (per 10 mismatches)					1.37 (1.18-1.58)	<0.0001
Akaike Information Criterion (AIC) for the Model		**300.7**		**539.6**		**703.6**

B. Amino Acid Mismatch	DR *dn*DSA n=596, 29 events		DQ *dn*DSA n=596, 51 events		DR or DQ d*n*DSA n=596, 66 events	
	HR	p value	HR	p value	HR	p value
Recipient Age at Transplant (years)	0.97 (0.95-0.99)	0.0209	0.97 (0.95-0.98)	0.0015	0.97 (0.96-0.99)	0.0006
Nonadherence	3.02 (1.38-6.46)	0.0067	3.37 (1.87-6.00)	<0.0001	3.22 (1.91-5.43)	<0.0001
Cyclosporin vs. Tacrolimus	2.44 (1.05-5.48)	0.0382	2.03 (1.11-3.61)	0.0219	2.35 (1.40-3.88)	0.0017
HLA-DRB1/3/4/5 Amino Acid Mismatch (per 10 mismatches)	1.57 (1.31-1.89)	<0.0001				
HLA-DQA1/B1 Amino Acid Mismatch (per 10 mismatches)			1.24 (1.12-1.39)	<0.0001		
HLA-DRB1/3/4/5 + HLA-DQA1/B1 Amino Acid Mismatch (per 10 mismatches)					1.12 (1.05-1.19)	0.0008
Akaike Information Criterion (AIC) for the Model	**302.9**		**549.4**		**710.2**	

C. Electrostatic Mismatch	DR *dn*DSA n=596, 29 events		DQ *dn*DSA n=596, 51 events		DR or DQ d*n*DSA n=596, 66 events	
	HR	**p value**	**HR**	**p value**	**HR**	**p value**
Recipient Age at Transplant (years)	0.97 (0.95-0.99)	0.0309	0.97 (0.95-0.99)	0.0015	0.97 (0.96-0.99)	0.0006
Nonadherence	2.94 (1.32-6.33)	0.0090	3.34 (1.86-5.95)	<0.0001	3.30 (1.95-5.49)	<0.0001
Cyclosporin vs. Tacrolimus	2.47 (1.06-5.52)	0.0367	2.02 (1.11-3.61)	0.0230	2.37 (1.41-3.92)	0.0014
HLA-DRB1/3/4/5 Electrostatic Mismatch (per 10 mismatches)	1.25 (1.13-1.38)	<0.0001				
HLA-DQA1/B1 Electrostatic Mismatch (per 10 mismatches)			1.15 (1.07-1.22)	<0.0001		
HLA-DRB1/3/4/5 + HLA-DQA1/B1 Electrostatic Mismatch (per 10 mismatches)					1.01 (1.00-1.01)	0.0016
Akaike Information Criterion (AIC) for the Model		**307.4**		**549.2**		**709.0**

Figure S1. Receiver operating characteristic curves for *dn*DSA development are shown for HLA-DR$\beta_{1/3/4/5}$ and HLA-DQ$_{\alpha1\beta1}$ with each of the three molecular mismatch methods.

Figure S1. Receiver Operating Characteristic Curves

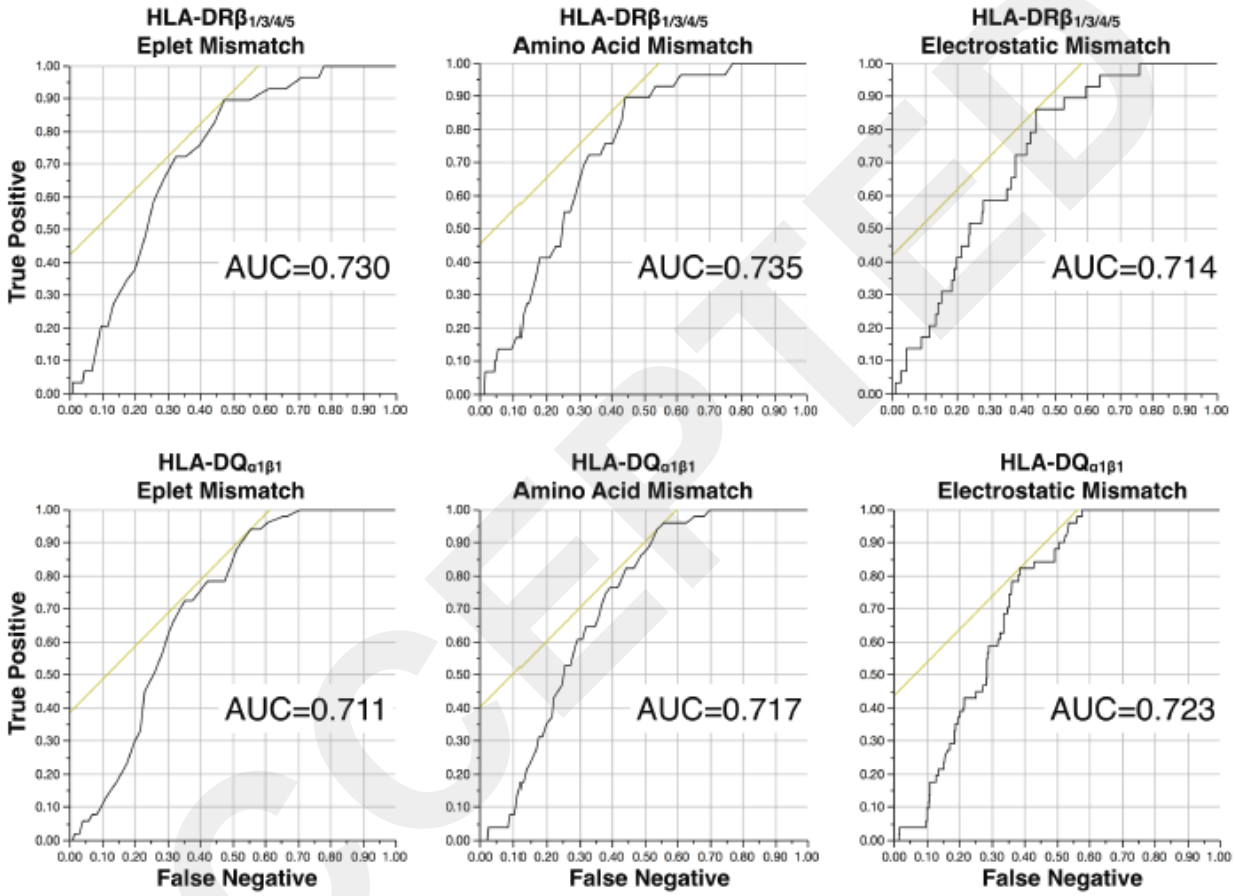

Table S1. Univariate Molecular Mismatch Scores Predict *dn*DSA Free Survival Posttransplant

A. HLA-DR$\beta_{1/3/4/5}$ *dn*DSA Free Survival

	Range	Hazard Ratio	p value
HLA-DR$\beta_{1/3/4/5}$ Eplet Mismatch (per 10 mismatches)	0-41	2.51 (1.71-3.64)	<0.0001
HLA-DR$\beta_{1/3/4/5}$ Amino Acid Mismatch (per 10 mismatches)	0-82	1.49 (1.25-1.76)	<0.0001
HLA-DR$\beta_{1/3/4/5}$ Electrostatic Mismatch (per 10 mismatches)	0-147	1.23 (1.11-1.35)	<0.0001

B. HLA-DQ$_{\alpha1\beta1}$ *dn*DSA Free Survival

	Range	Hazard Ratio	p value
HLA-DQ$_{\alpha1\beta1}$ Eplet Mismatch (per 10 mismatches)	0-42	1.98 (1.53-2.58)	<0.0001
HLA-DQ$_{\alpha1\beta1}$ Amino Acid Mismatch (per 10 mismatches)	0-97	1.24 (1.12-1.37)	<0.0001
HLA-DQ$_{\alpha1\beta1}$ Electrostatic Mismatch (per 10 mismatches)	1-164	1.14 (1.07-1.21)	<0.0001