Clin Transplant 2006: 20: 226–233 DOI: 10.1111/j.1399-0012.2005.00473.x

Clinical Transplantation

Predictive value of human leucocyte antigen epitope matching using HLAMatchmaker for graft outcomes in a predominantly African-American renal transplant cohort

Haririan A, Fagoaga O, Daneshvar H, Morawski K, Sillix DH, El-Amm JM, West MS, Garnick J, Migdal SD, Gruber SA, Nehlsen-Cannarella S. Predictive value of human leucocyte antigen epitope matching using HLAMatchmaker for graft outcomes in a predominantly African-American renal transplant cohort.
Clin Transplant 2006: 20: 226–233. © Blackwell Munksgaard, 2006

Abstract: The HLAMatchmaker program is based on the donor/recipient comparison of the polymorphic triplet amino-acid sequences of the antibody-accessible regions on the human leucocyte antigen (HLA) molecule. The previous reports on its predictive value for renal allograft outcomes are conflicting. We conducted a retrospective study in a predominantly African-American (AA) cohort (N = 101, 94% AA). HLA typing was performed by molecular methods and triplet matching using HLAMatchmaker. Study end points included graft survival and incidence of acute rejection. The relationship between the number of triplet mismatches (TMM) and the degree of HLA antigen MM was evaluated using Pearson's correlation coefficient. Logistic regression models were used to examine the association between triplet matching and the study end points. Kaplan–Meier and Cox proportional hazard models were used for graft survival analysis. The strongest relationship between the number of TMM and HLA antigen MM was observed for HLA-DQ (r = 0.88). The association between triplet matching at HLA-A, -B, -DR and -DRw HLA loci and the study end points was not statistically significant. However, after grouping, the unadjusted estimates of graft survival for those with more than 10 Class I TMM were significantly worse than the others (p = 0.03). Adjusting for the effect of donor source, recipient characteristics and the immunosuppressive regimen did not change this association (hazard ratio = 0.2, confidence interval = 0.04–1.1). We conclude that triplet matching using HLAMatchmaker can provide useful prognostic information in kidney transplantation and that more than 10 donor/recipient Class I HLA TMM is predictive of worse graft outcome.

Abdolreza Haririan[a], Omar Fagoaga[b], Hamidreza Daneshvar[a], Katherina Morawski[c], Dale H. Sillix[a], Jose M. El-Amm[a], Miguel S. West[c], James Garnick[d], Stephen D. Migdal[a], Scott A. Gruber[c] and Sandra Nehlsen-Cannarella[b]

[a]Division of Nephrology, Department of Medicine, [b]HLA Laboratory, Department of Pathology, [c]Section of Transplant Surgery, Department of Surgery, Wayne State University School of Medicine, Detroit, MI, USA, [d]Pharmacy Department, Harper University Hospital, Detroit, MI, USA

Key words: African-American – kidney transplantation – HLAMatchmaker – HLA antigen

Corresponding author: Abdolreza Haririan, MD, MPH, 22 South Greene Street, N3W143, Baltimore, MD 21201, USA.
Tel.: + 410 328 5720; fax: + 410 328 5685;
e-mail: ahariria@medicine.umaryland.edu

Accepted for publication 21 October 2005

In early 1970s, the dogma of human leucocyte antigen (HLA) matching between the donor and recipient of an allograft was established. This concept has since played an important role in the history and development of renal transplantation. Distributing deceased-donor (DD) organs on the basis of better HLA antigen (Ag) matching is still a key component of the allocation system. Morris et al. (1) studied the outcome of 6363 kidney transplants performed from 1986 to 1993 and showed the strong beneficial effect of HLA Ag matching. In the data collected from the Collaborative Transplant Study, Opelz (2) also found that HLA Ag matching had a highly significant impact on graft survival, even with a cold

ischaemic time of less than 12 h. However, over the past three decades, several revisions have been made in organ allocation schemes to balance the benefits of HLA Ag matching with equity among different racial backgrounds (3–7).

The HLAMatchmaker algorithm has provided an opportunity to evaluate the role of HLA matching at the amino-acid sequence level (8, 9). The program was originally developed to increase the probability of finding acceptable donors for highly sensitized candidates on the waiting list without extensive testing (10, 11). Subsequently, Duquesnoy et al. (12) showed that it may also be used to predict the risk of graft loss based on the number of Class I amino-acid triplet mismatches (TMM). However, the predictive role of TMM for graft survival has been disputed by Laux et al. (13). Moreover, these studies have focused on the role of Class I TMM in a non-African-American (AA) population. In this study, we analysed the distribution of Class I and II HLA TMM in a predominantly AA cohort of renal transplant recipients, and examined the value of TMM at different HLA loci as a predictor of graft survival and acute rejection (AR).

Patients and methods

This is a retrospective cohort study of the effect of HLA triplet matching using the HLAMatchmaker algorithm on graft outcomes among adult renal allograft recipients who were transplanted at our centre between December 2001 and January 2004. Patients who lost their grafts owing to technical problems such as graft thrombosis or death in the perioperative period were excluded from this analysis. All patients received induction therapy with either thymoglobulin (ATG) or basiliximab (BSX) based on patient characteristics that included age, panel reactive antibody (PRA), transplant number and comorbidities such as cardiovascular disease or chronic hepatitis C. ATG was administered at a dose of 1.5 mg/kg/d for four to seven d starting intraoperatively, with dose adjusted for leucopenia and/or thrombocytopenia. BSX 20 mg was given on days 0 and 4. All patients were started on mycophenolate mofetil 2 g/d postoperatively, with subsequent dose adjustment for gastrointestinal side effects or leucopenia. Patients received intravenous methylprednisolone 250 mg intraoperatively, followed by doses of 200, 150 and 100 mg on subsequent days. Before July 2003, all patients were maintained on steroids, with tapering to oral prednisone 30 mg by one wk post-transplant, 10 mg/d at three months and 5 mg/d at 6–12 months. After July 2003, steroid was discontinued after the initial four intravenous doses for the vast majority of patients.

The third immunosuppressive agent was started within 24–48 h after surgery. During the first half of the study period, patients with immediate graft function received tacrolimus (TCL) and those with delayed graft function (DGF), defined as the need for haemodialysis during the first week post-transplant, received sirolimus (SRL). During the second half, all patients received TCL. The target trough levels for both drugs were 10–15 ng/mL during the first three months and 8–12 ng/mL thereafter. All patients received prophylactic trimethoprim/sulphamethoxazole and valganciclovir, as previously described (14). Patients were closely followed in the outpatient setting according to the recommended guidelines. An unexplained rise in serum creatinine was followed by ultrasound examination of the renal transplant, with biopsy as indicated. All episodes of AR were confirmed by biopsy and graded according to the Banff 97 schema. Antibody-mediated rejection (AMR) was diagnosed by positive staining of peritubular capillaries for C4d.

HLA typing was performed by low-resolution molecular methods for all donors and intermediate-resolution methods for recipients. Determination of the HLA match level (number of mismatched Ag) was accomplished in a traditional manner by comparing the HLA Ag of Class I (HLA-A, -B) and Class II (DR) of the recipient with that of the donor for each transplant episode. We also included matching at additional Class II loci, HLA-DRw 52, 53, 51 (HLA-DRB 3, 4, 5, respectively) and HLA-DQ. Then, using Duquesnoy's HLAMatchmaker computer-based algorithm (8), the HLA triplet matching was performed. In this scheme, matching is based on the most frequently occurring four-digit alleles in the general population.

The primary end points of the study were graft survival and the incidence of AR. Graft loss was defined as return to dialysis or death with a functioning graft. Patient characteristics examined included donor source, recipient age, gender, race, duration of end-stage renal disease (ESRD), transplant number, current (at the time of transplant) and peak PRA, the induction and initial maintenance agent, use of maintenance steroids and DGF. The number of HLA-A, -B, -DR and -DRw Ag MM was determined. Class I Ag MM was defined as the sum of HLA-A and -B MM. Total HLA MM was obtained by adding Class I and HLA-DR MM. The number of HLA-A, -B, -DR and -DRw TMM was determined for all patients, and Class I TMM was calculated as in HLA MM. Class II TMM was defined in this analysis as the sum of HLA-DR and -DRw MM, and the total TMM as the sum of Class I and II TMM. Repeat triplets within each class were counted. Data on HLA-DQ Ag and triplet MM

were collected for the patients who were typed accordingly.

Data are reported as counts or mean ± SD, as appropriate. The relationship between the distribution of the number of TMM and the degree of HLA Ag MM was determined by Pearson's correlation coefficient for four different loci. To evaluate the association between different degrees of HLA Ag and triplet matching and the study end points, logistic regression models were used. Stepwise multiple logistic regression models with p value less than 0.15 required for inclusion were employed to examine the independent predictive role of these variables after adjusting for age, sex, years of ESRD, current PRA, donor source, retransplant status, early graft function, induction and maintenance agents, maintenance steroids and the degree of MM at other HLA loci. The unadjusted effect of triplet matching on actuarial graft survival was estimated by the Kaplan–Meier method. For each HLA class, patients were divided into two groups: those with 10 or less (Group I) and those with greater than 10 mismatched triplets (Group II). This number was chosen as the threshold, after repeating the analysis for different numbers of TMM. Comparisons between the groups were made using the log-rank test. The Cox proportional hazard model was used to evaluate this effect after controlling for the effect of the confounding factors as above. Intercooled Stata 8.2 software package (Stata Corporation, College Station, TX, USA) was used for statistical analysis.

Results

Of the 106 patients who underwent kidney transplantation during the study period, 101 were eligible for this analysis. Patient characteristics are summarized in Table 1. Of note, 94% were AA, and mean duration of ESRD was 5.4 yr. Twenty percent had repeat transplants, 72% of the grafts were from DD and 38% of the donors were AA. The mean current and peak PRA levels were 9 and 26%, respectively. Seven patients had current PRA greater than 50%, and two more than 80% at the time of transplantation. Thirty-nine percent of cases experienced DGF: 51% received BSX for induction and 49% received ATG. Maintenance regimen included TCL in 75% and SRL in 25% of patients, and 21% underwent early steroid withdrawal.

The results of the donor/recipient HLA Ag and triplet matching, as well as Class I and II triplet repeats, are summarized in Table 2. The number of TMM for each degree of HLA Ag MM at four different loci and the correlation coefficient (r) between the two are shown in Fig. 1.

Table 1. Patient characteristics

	Overall	Group I	Group II
N	101	33	68
Age (yr)	47.5 ± 12.4	47.3 ± 11.5	47.6 ± 12.9
Gender			
Male	58 (57%)	15 (45%)	43 (63%)
Female	43 (43%)	18 (55%)	25 (37%)
Recipient race (AA)	95 (94%)	30 (91%)	65 (96%)
Donor race (AA)	38 (38%)	16 (48%)	22 (32%)
Years of ESRD	5.4 ± 4.0	4.9 ± 4.2	5.7 ± 3.9
Mean PRA			
Current/peak	9%/26%	9%/30%	9%/24%
Current PRA (%)			
>50	7 (7%)	3 (9%)	4 (6%)
>80	2 (2%)	0	2 (3%)
Retransplant	20 (20%)	7 (21%)	13 (19%)
Donor source			
DD	73 (72%)	19 (58%)	54 (79%)
LD	28 (28%)	14 (42%)	14 (21%)
DGF	39 (39%)	9 (27%)	30 (44%)
Induction			
ATG	52 (51%)	23 (70%)	29 (43%)
BSX	49 (49%)	10 (30%)	39 (57%)
Maintenance			
TCL	76 (75%)	26 (79%)	50 (74%)
SRL	25 (25%)	7 (21%)	18 (26%)
Maintenance steroid	79 (78%)	24 (73%)	55 (81%)

AA, African-American; ESRD, end-stage renal disease; PRA, panel reactive antibody; D, deceased donor; LD, living donor; DGF, delayed graft function; ATG, thymoglobulin; BSX, basiliximab; TCL, tacrolimus; SRL, sirolimus.

Patients were followed for 18.0 ± 8.2 months. During this period, there were five deaths, and 15 patients experienced graft loss. The incidence of AR was 24%, with five cases of AMR.

The role of HLA-A, -B and -DR Ag MM as predictors of graft loss was evaluated separately (Table 3). In univariate analysis, HLA-B, Class I, HLA-DR and total HLA Ag MM were found to be significantly associated with the risk of graft loss. However, after adjusting for the potential confounding variables, only HLA-B Ag MM was an independent predictor of graft loss. For each one

Table 2. The number of HLA antigen and triplet MM for different loci

HLA Ag MM		HLA TMM	
HLA-A MM	1.4 ± 0.6	HLA-A TMM	7.9 ± 5.6
HLA-B MM	1.5 ± 0.6	HLA-B TMM	7.0 ± 4.3
HLA-DR MM	1.3 ± 0.7	HLA-DR TMM	4.1 ± 3.1
Class I MM	2.9 ± 1.0	HLA-DRw TMM	3.9 ± 4.0
Class II MM	1.3 ± 0.7	Class I TMM	12.6 ± 6.5
Total MM	3.8 ± 1.6	Class II TMM	8.0 ± 6.1
		Total TMM	20.6 ± 9.6
		Class I TMM repeats	2.3 ± 2.3
		Class II TMM repeats	2.3 ± 2.7

HLA, human leucocyte antigen; MM, mismatch; TMM, triplet mismatch.

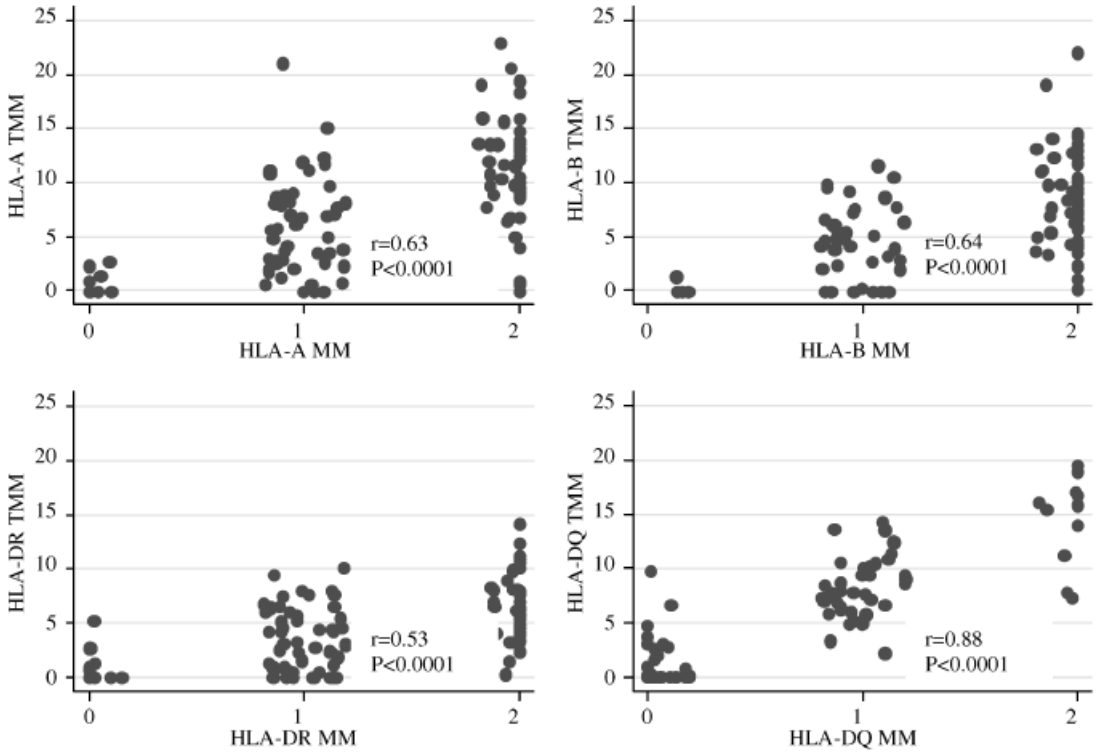

Fig. 1. Distribution of triplet mismatches (TMM) for each degree of human leucocyte antigen (HLA) antigen MM.

unit increase in the number of HLA-B MM, the risk of losing the graft increased by 4.7-fold (p = 0.035). Table 4 summarizes the results of examining the unadjusted association between the number of TMM and graft loss. Interestingly, the number of TMM in each of the categories did not have any significant association with the risk of graft loss. The number of repeated mismatched triplets also did not have any significant predictive value. Similar analyses were performed after excluding graft losses because of death, and identical results were obtained (data not shown).

Unadjusted graft survival was estimated according to the number of Class I and II TMM. Patients with Class I TMM greater than 10 (N = 68) had worse graft survival compared with those with 10 or less (N = 33) (p = 0.027) (Fig. 2). After adjusting for the confounders, this association was still significant (hazard ratio of graft loss = 0.21 for the latter vs. the former group, 95% confidence interval (CI) = 0.04–1.1, p = 0.07). Repeating the analysis for only AA patients reproduced similar results (data not shown). There was no significant association between Class II TMM and graft survival.

Table 3. OR of graft loss per HLA Ag MM using logistic regression models (N = 101)

Variable	OR	p value	95% CI	Adjusted OR[a]	p value	95% CI
HLA-A MM	2.1	0.11	0.86–5.1	–	0.83	–
HLA-B MM	3.2	0.046	1.0–9.8	4.7	0.035	1.1–20.1
Class I MM	1.9	0.036	1.0–3.4	2.1	0.071	0.94–4.5
HLA-DR MM	2.9	0.023	1.2–7.1	–	0.50	0.80–6.7
Total HLA MM	1.6	0.02	1.1–2.3	1.4	0.13	0.90–2.2

[a]Adjusted for donor source, recipient age, end-stage renal disease years, gender, retransplant status, induction and maintenance agent, use of maintenance steroid, current panel reactive antibody, delayed graft function, and MM at other HLA loci.

HLA, human leucocyte antigen; Ag, antigen; MM, mismatch; OR, odds ratio; CI, confidence interval.

Table 4. OR of graft loss per TMM using a univariate logistic regression model (N = 101)

Variable	OR	p value	95% CI
HLA-A TMM	1.03	0.51	0.94–1.12
HLA-B TMM	1.07	0.28	0.95–1.20
Class I TMM	1.04	0.28	0.97–1.1
Class I TMM repeats	0.91	0.44	0.74–1.18
HLA-DR TMM	1.02	0.82	0.87–1.20
HLA-DRw TMM	1.08	0.23	0.95–1.21
Class II TMM	1.04	0.37	0.96–1.13
Class II TMM repeats	0.96	0.68	0.79–1.16
Total HLA TMM	1.05	0.11	0.99–1.11

TMM, triplet mismatch; OR, odds ratio; CI, confidence interval; HLA, human leucocyte antigen.

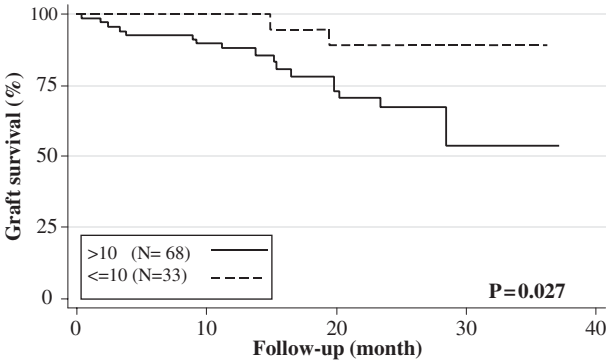

Fig. 2. Unadjusted graft survival for Class I triplet matching using Kaplan–Meier method.

Data on HLA-DQ Ag and triplet matching were available for 76 patients. Similar analyses were conducted to test the association of these variables with the risk of graft loss. HLA-DQ, Class II and total TMM were found to be independent predictors of graft loss in this subgroup [odds ratio (OR) = 1.19, 1.13 and 1.11, p = 0.029, 0.045 and 0.034, respectively]. We also showed that the degree of HLA-DQ Ag MM was independently associated with the risk of poor graft outcome (OR of graft loss = 5.8 for increments of one, p = 0.012, 95% CI = 1.47–23.1).

The role of different degrees of HLA Ag and triplet matching as a risk factor for AR was evaluated after adjusting for other potential risk factors. Neither the number of Ag MM nor the number of TMM was found to be an independent predictor of AR for each of the categories as defined above (data not shown).

Discussion

The presence of anti-HLA antibodies, because of previous blood transfusion, organ transplantation or pregnancy, is generally considered a contraindication to transplantation from a donor carrying those HLA Ag because of the risk of hyperacute AMR (15). Monthly monitoring of the waitlisted recipient's sera for presence of HLA-specific antibodies is a key factor in evaluating the acceptability of an HLA-mismatched available organ. With higher degrees of sensitization, expressed by PRA, the probability of finding a compatible organ declines. Therefore, patients with high PRA, especially those with PRA > 80%, generally have to wait a long time to receive an optimally matched allograft.

To increase the probability of identifying HLA Ag-compatible organs for highly allosensitized patients without the need for extensive serum screening, Duquesnoy (8) and Duquesnoy and Marrari (9) developed the HLAMatchmaker computer algorithm, designed to compare intra- and interlocus polymorphic amino-acid triplet sequences in the alloantibody-accessible positions of HLA molecules. This algorithm is based on two principles: (1) each HLA Ag represents a distinct string of polymorphic triplets as potential immunogens that can induce specific alloantibodies and (2) patients do not produce antibodies against triplets on mismatched HLA Ag if such triplets are present on their own HLA molecules. By comparing patients' triplet repertoire with that of potential donors who are not compatible at the HLA Ag level, acceptable HLA Ag MM can be identified. Lobashevsky et al. (16) analysed serum samples from 39 highly sensitized (PRA $\geq$ 85%) patients with ESRD. The results suggested that flow cytometry crossmatch compatibility of a particular highly immunized donor/recipient combination depends on the degree of immunogenic TMM (TMMI), particularly the highly TMMI. By considering the HLA types of panel cells that give negative reactions with the patient's serum, and contain mismatched triplets that are apparently not recognized by the patient's antibodies, the probability of finding an acceptable HLA Ag-mismatched donor will increase without further serum screening with additional panel cells. Duquesnoy et al. (10) applied the HLAMatchmaker algorithm to 54 highly sensitized kidney transplant candidates with PRA 87–98% at the University of Pittsburgh Medical Center. They showed that this program could be used to calculate the probability of finding a suitable donor for many highly sensitized patients. The median probability for finding a zero-HLA-A,B Ag-mismatched donor was 0.011%. With the application of the algorithm, allowing for up to two TMM, the probability increased by 12.3-fold. Moreover, after adding the data on acceptable Ag MM identified from negative reactions in PRA testing, there was a further increase by 3.3-fold. Similar results were reproduced in a subsequent study conducted on 35 highly sensitized kidney transplant candidates evaluated by the Eurotransplant Reference Laboratory (11).

One shortfall of this program is that the assignment of triplets to the HLA Ag of patients and panel cells lacks precision because it is based on amino-acid sequence information of what was estimated to be the most common molecularly defined allele of each serologically defined HLA Ag. In the US, with a large number of AA waitlisted patients who have different molecular variants of the serologically defined HLA types (17, 18), this may not be a correct assumption.

In this report, we examined the degree of donor/recipient HLA compatibility in a predominantly

AA cohort using the HLAMatchmaker algorithm. More than 60% of the donors were non-AA. This analysis showed that there were 13 Class I triplet amino-acid MM on average between donor and recipient, and eight TMM in Class II. This program is better developed for analysing Class I TMM, and the majority of previous studies have been performed in HLA-DR-matched donor/recipient combinations, studying the impact of HLA-A and -B TMM on graft outcomes. Although data for Class II triplet epitopes are not yet as comprehensive, we used the program in its current form to analyse the TMM profile for both HLA classes in our patients. To our knowledge, this is the first report of such an analysis, which not only includes Class II, but also applies this algorithm to a predominantly AA transplant population. We demonstrated that the number of HLA Ag MM is not a reliable predictor of the number of TMM, and there is a wide overlap among the numbers of TMM for each degree of Ag MM. The correlation between the two was strongest for the HLA-DQ locus.

We also studied the predictive value of HLA Ag and TMM for graft loss and AR. At the Ag level, HLA-B MM was the only independent predictor for graft loss in our cohort. We did not observe a significant association between the total number of HLA Ag MM and the risk of graft loss. When the effect of TMM was analysed with regard to graft loss, we did not find a significant independent predictive role for the absolute number of triplet matching for any of the HLA classes or loci. Comparison of the graft survival estimates between patients with more than 10 Class I TMM and those with equal to or less than 10 revealed a statistically significant difference between the two groups.

To examine the predictive role of TMM for graft survival, Duquesnoy et al. (12) analysed 31,879 zero-DR-mismatched kidney transplants reported to the UNOS Scientific Registry between 1987 and 1999, and 15 872 kidney transplants reported to the Eurotransplant Registry during the same period. For both cohorts, patient and graft survival decreased in a stepwise manner with increasing numbers of HLA-A,B Ag MM. Of the 21 270 zero-DR-mismatched cases with HLA-A,B Ag MM reported to the UNOS database, 251 patients with up to two TMM had survival rates virtually identical to those with zero-HLA-A,B Ag MM, regardless of race or degree of sensitization. Among 1905 patients with up to four TMM in the Eurotransplant series, graft survival was not statistically different from those with zero HLA-A,B Ag MM. Bohringer et al. (19) studied 545 patients with primary penetrating keratoplasty, who were not matched for HLA-DR. They found a relative risk of 1.8 for developing graft rejection per HLA-A,B TMM. The group with up to 13 TMM had a significantly longer rejection-free graft survival than the remaining more poorly matched keratoplasties ($p = 0.05$).

To further evaluate this association, Laux et al. (13) conducted a study on 16 997 Caucasian patients matched for HLA-DR and typed for HLA-A and -B Ag at the split resolution who received a DD kidney transplant between 1991 and 2001. The authors chose six TMM and five TMMI for differentiation of 'good' and 'poor' matches. They were unable to confirm a statistically significant correlation between triplet epitope matching and graft survival. In this analysis, kidney transplants with zero HLA-A and -B Ag MM had significantly better graft outcomes than those with zero TMMI. Among HLA-DR-matched patients, a significantly better graft outcome was observed for the group with <6 TMM than that with 7–12 TMM. However, the former group was much better matched at the serologic Ag level. Thus, they concluded that the effect described by Duquesnoy et al. (12) was mainly caused by the 'conventional' HLA Class I Ag MM effect rather than by a specific influence of MM at the amino-acid triplet level.

Moreover, when we examined the TMM data in the group of patients whose HLA-DQ matching results were available, there was a significant association between HLA-DQ, Class II, and total number of TMM and the risk of graft loss. A similar association was also observed between the number of HLA-DQ Ag MM and graft loss. As the numbers of HLA-DQ Ag MM and TMM were highly correlated, the significance of the latter's association with graft outcome may merely reflect the effect of HLA-DQ Ag MM.

Tong et al. (20) studied the effect of HLA-DQB1 matching among 63 kidney donor/recipient pairs on graft survival. Survival rates, estimated by the Kaplan–Meier method, were significantly higher for pairs with two matches than for those with one or zero matches. They concluded that determining HLA-DQB1 molecular alleles is important for assessing graft survival. In another study, Freedman et al. (21) analysed the effect of matching for serologically defined HLA-DQ Ag on graft survival in 12 050 recipients of first kidney transplants. After adjusting for donor and recipient characteristics, cyclosporine A use, and HLA-A, -B and -DR matching, there was no significant reduction in survival for increasing levels of HLA-DQ MM.

Dankers et al. (22) studied sera from 144 renal allograft recipients who had undergone transplantation between 1973 and 2000, and returned to the Eurotransplant waiting list after graft loss. They found a strong correlation between the number of

donor/recipient TMM and the percentage of patients that produce donor-specific antibodies. Therefore, they concluded that implementation of triplet matching in a kidney allocation scheme might reduce the incidence of AMR. In our patient population, donor/recipient TMM was not found to be predictive of episodes of AR, irrespective of the type, or AMR. Considering that cellular mechanisms mediate rejection in the majority of AR cases, the important question that remains to be answered is whether triplet amino-acid polymorphisms as defined in the HLAMatchmaker algorithm are also the target of T-cell allorecognition. Bohringer et al. (19) noted that their findings in patients with keratoplasties suggest that epitopes eliciting cellular and antibody-mediated immune responses seem to be highly inter-related or even identical. Dankers et al. (23) analysed 108 kidney transplant donor/recipient combinations registered at the Eurodonor Foundation. All individuals were typed for HLA Class I and II on a high-resolution level by DNA-based typing. All pairs had a single HLA Class I MM and were matched for HLA-DRB1 and HLA-DQB1. Analysis of cytotoxic T-lymphocyte (CTL) precursor tests showed that the frequency against the zero-triplet-mismatched patients was not significantly lower than that against those with five or more TMM. The authors concluded that HLAMatchmaker is not a suitable tool for predicting alloreactive CTL response *in vitro*. This is probably because of the fact that this program considers only triplets on antibody-accessible positions, whereas CTLs also recognize other epitopes on the HLA molecule, including the bound peptides.

We acknowledge that our study group is relatively small and the analysis is not powered to show statistically significant association between the absolute number of TMM and graft outcomes as examined. However, it is notable that patients with more than 10 Class I HLA TMM had worse graft survival than the others. In contrast to prior large database analyses, we were able to control for the effect of other potential confounding factors, including patient characteristics and immunosuppressive agents. As we showed in this analysis, the estimates of graft survival, as stated in one of these reports (12), were significantly dependent on the number of Class I TMM. The other limitation of our study is the fact that triplet assignments in the HLAMatchmaker program are based on the most common molecularly defined amino-acid sequence of the serologically defined alleles. Consequently, the assigned triplets in different loci for AA patients using this program may not completely correspond to the actual epitopes expressed by the recipients.

In conclusion, our analysis showed that among our predominantly AA kidney transplant recipients, the majority of whom received organs from non-AA donors, there is a significant number of MM at the amino-acid triplet level. Moreover, the number of HLA Ag MM between donor and recipient is not highly correlated with the number of TMM at the main three loci. The correlation is strongest for the HLA-DQ locus. We could show a statistically significant independent association between categorized Class I TMM and graft survival. The observed association between graft loss and the number of HLA-DQ TMM could be explained by a similar association with HLA-DQ Ag MM. Further studies are needed to examine the independent predictive value of Class I as well as Class II TMM using the HLAMatchmaker algorithm in organ transplantation, especially in AA recipients.

References

1. Morris PJ, Johnson RJ, Fuggle SV, Belger MA, Briggs JD. HLA Task Force of the Kidney Advisory Group of the United Kingdom Transplant Support Service (UKTSSA). Analysis of factors that affect outcome of primary cadaveric renal transplantation in the UK. Lancet 1999: 354: 1147.
2. Opelz G. Very short ischemia is not the answer. Nephrol Dial Transplant 2002: 17: 715.
3. Welsh KI, Bunce M. HLA typing, matching and crossmatching in renal transplantation. In: Morris PJ, ed. Kidney Transplantation, Chapter 10. Philadelphia: W.B. Saunders, 2001: 135.
4. Hiesse C, Pessione F, Houssin D. The case to abandon human leukocyte antigen matching for kidney allocation: would it be wise to throw out the baby with the bathwater? Transplantation 2004: 77: 623.
5. Ting A, Edwards LB. Human leukocyte antigen in the allocation of kidneys from cadaveric donors in the United States. Transplantation 2004: 77: 610.
6. http://www.optn.org/PoliciesandBylaws/policies/pdfs/policy_7.pdf; accessed on May 16, 2005.
7. Su X, Zenios SA, Chakkera H, Milford EL, Chertow GM. Diminishing significance of HLA matching in kidney transplantation. Am J Transplant 2004: 4: 1501.
8. Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. Hum Immunol 2002: 63: 339.
9. Duquesnoy RJ, Marrari M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. II. Verification of the algorithm and determination of the relative immunogenicity of amino acid triplet-defined epitopes. Hum Immunol 2002: 63: 353.
10. Duquesnoy RJ, Howe J, Takemoto S. HLAmatchmaker: a molecularly based algorithm for histocompatibility determination. IV. An alternative strategy to increase the number of compatible donors for highly sensitized patients. Transplantation 2003: 75: 889.
11. Duquesnoy RJ, Witvliet M, Doxiadis II, de Fijter H, Claas FH. HLAMatchmaker-based strategy to identify acceptable HLA class I mismatches for highly sensitized kidney transplant candidates. Transplant Int 2004: 17: 22.

12. Duquesnoy RJ, Takemoto S, De Lange P et al. HLA-Matchmaker: a molecularly based algorithm for histocompatibility determination. III. Effect of matching at the HLA-A,B amino acid triplet level on kidney transplant survival. Transplantation 2003: 75: 884.
13. Laux G, Mytilineos J, Opelz G. Critical evaluation of the amino acid triplet-epitope matching concept in cadaver kidney transplantation. Transplantation 2004: 77: 902.
14. Haririan A, Morawski K, Sillix DH et al. Induction therapy with basiliximab versus thymoglobulin in African-American kidney transplant recipients. Transplantation 2005: 79: 716.
15. Sumitran-Holgersson S. HLA-specific alloantibodies and renal allograft outcome. Nephrol Dial Transplant 2001: 16: 897.
16. Lobashevsky AL, Senkbeil RW, Shoaf JL et al. The number of amino acid residues mismatches correlates with flow cytometry crossmatching results in high PRA renal patients. Hum Immunol 2002: 63: 364.
17. Cao K, Hollenbach J, Shi X, Shi W, Chopek M, Fernández-Viña MA. Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States reveals high levels of diversity in these loci and contrasting distribution patterns in these populations. Hum Immunol 2001: 62: 1009.
18. Zachary AA, Leffell MS, Johnson A, Rose SM, Bias WB. HLA antigens, alleles, and haplotypes among African-Americans. Transplant Proc 1997: 29: 3706.
19. Bohringer D, Reinhard T, Duquesnoy RJ et al. Beneficial effect of matching at the HLA-A and -B amino-acid triplet level on rejection-free clear graft survival in penetrating keratoplasty. Transplantation 2004: 77: 417.
20. Tong JY, Hsia S, Parris GL et al. Molecular compatibility and renal graft survival; the HLA-DQB1 genotyping. Transplantation 1993: 55: 390.
21. Freedman BI, Thacker LR, Heise ER, Adams PL. HLA-DQ matching in cadaveric renal transplantation. Clin Transplant 1997: 11: 480.
22. Dankers MK, Witvliet MD, Roelen DL et al. The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched human leukocyte antigens. Transplantation 2004: 77: 1236.
23. Dankers MK, Heemskerk MB, Duquesnoy RJ et al. HLAMatchmaker algorithm is not a suitable tool to predict the alloreactive cytotoxic T-lymphocyte response *in vitro*. Transplantation 2004: 78: 165.