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# HLA class I amino acid sequence-based matching after interlocus subtraction and long-term outcome after deceased donor kidney transplantation

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ABSTRACT

We have shown previously that human leukocyte antigen (HLA) immunogenicity, defined by the physiochemical properties of mismatched amino acids, predicts humoral alloimmunity, and now report the effect on long-term graft survival after kidney transplantation. The influence of HLA-A and -B mismatch, number of amino acid mismatches (after interlocus subtraction) and their physiochemical (electrostatic and hydrophobic) disparity on the outcome of fully HLA matched and single HLA-A or -B mismatched deceased donor kidney transplants undertaken in the United Kingdom (1990–2005) were analyzed ($n$ = 5,247). Grafts with a single HLA-A or -B mismatch had significantly lower survival than fully matched transplants (81.9% vs 84.2% at 5 years, $p = 0.004$). However, single HLA-A or -B mismatched grafts with no or one amino acid mismatch had better survival than grafts with two or more amino acid mismatches (89.3% vs 81.8% at 5 years, HR 1.5, $p = 0.03$). The number of mismatched amino acids was an independent predictor of transplant survival after adjusting for the underlying HLA matching effect ($p = 0.02$). Physiochemical disparity scores correlated closely with amino acid mismatches and provided no additional predictive value. The immunogenicity of HLA class I alloantigens defined at the level of amino acid sequence correlates more closely with outcome after renal transplantation than conventional serologic HLA matching.



## 1. Introduction

Deceased donor kidney transplants that are well matched for human leukocyte antigen (HLA) are associated with superior outcomes, and several kidney allocation schemes incorporate HLA matching within their algorithm for sharing kidneys [1–6]. In the United Kingdom (UK), all deceased donor kidneys donated after brain death are allocated according to an evidence-based points system designed to provide equity of access while ensuring good HLA matching, particularly in young and highly sensitized recipients [7–10]. Consequently, approximately 60% of recipients receive an HLA-DR matched graft with no more than a single HLA-A or -B mismatch, based on broad serologic equivalent HLA specificities [8]. In the UK, no additional beneficial effect of HLA matching at a higher level of resolution has been observed, whereas both Eurotransplant and the Collaborative Transplant Study report that HLA matching using serologically defined split HLA specificities is associated with improved transplant outcomes [11–13].

Conventional approaches to HLA matching as currently used for kidney allocation schemes regard all HLA mismatches within a given locus as having equal weighting and do not consider the immunogenicity of a mismatch according to recipient HLA type. Early attempts to identify "permissible" HLA mismatches of low immunogenicity for a given recipient HLA type were based on analysis of outcome data for a large number of mismatched combinations [14,15]. This pragmatic approach showed initial promise, but further studies proved disappointing and it was abandoned [11,16].

More recently, detailed knowledge of the crystal structure and amino acid (aa) sequence of HLA molecules have provided a more rational basis on which to determine HLA immunogenicity. Based on this knowledge Duquesnoy et al. have developed a computer-based algorithm (HLAMatchmaker) that identifies immunogenic

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epitopes represented as accessible aa triplets or eplets on the surface of HLA [17–19]. A novel aspect of HLAMatchmaker is that HLA compatibility is assessed after intra- and inter-locus aa subtraction, on the assumption that triplet based epitopes expressed on donor alloantigens that are also present at the same position on recipient HLA are not immunogenic.

HLAMatchmaker is particularly effective for identifying compatible donors for highly sensitized patients and for predicting the likelihood of a humoral response against mismatched HLA alloantigens [20–22]. Retrospective analysis of UNOS and Eurotransplant databases has also suggested that HLAMatchmaker is able to identify HLA-A and -B mismatched grafts of low immunogenicity (0–2 triplet mismatches) in zero -DR mismatched kidney transplants where graft survival is equivalent to zero HLA-A and -B mismatched grafts [23]. However, the validity of this conclusion has been questioned, and it has been suggested instead that the apparent benefit of triplet matching is dependent on underlying serologic matching [24].

We recently reported a novel approach to defining HLA immunogenicity based on the number and physiochemical properties of aa mismatches (incorporating intralocus and interlocus subtraction) [25]. The occurrence and magnitude of the humoral response in highly sensitized patients correlated independently with the number of mismatched aa and the total electrostatic and hydrophobicity mismatch scores of mismatched HLA class I alloantigens. In the present study, we have examined data from the UK Transplant Registry to evaluate further approaches to HLA matching to improve graft survival after deceased donor kidney transplantation. The contribution to graft survival of serologically defined broad and split HLA specificities was determined, along with the number and physiochemical properties of mismatched aa.

## 2. Subjects and methods

### 2.1. Study design

The influence of "broad" and "split" serologically defined HLA-A and -B mismatches, number of aa mismatches, and their physiochemical (electrostatic and hydrophobic) disparity on kidney transplant outcome were analyzed for adult recipients receiving kidneys from brain-dead deceased donors in the UK between 1990 and 2005. Because of the close relationships between the different approaches used to assess the level of HLA mismatch, we chose to study only zero HLA-A, -B, -DR (0.0.0) mismatched transplants and those with a single HLA-A or -B mismatch (1.0.0/0.1.0). This study design allowed a direct comparison of the different approaches to defining HLA mismatch, independent of their confounding interrelationships when multiple HLA mismatches are present.

### 2.2. Study population

The study population comprised 5,247 deceased donor kidney transplants, of which 2,051 (39%) had zero broad HLA-A, -B, -DR mismatches (0-BMM) and 3,196 (61%) had a single broad HLA-A or -B mismatch (1-BMM). Their median age was 44 years (range 1–81 years) and 4,020 (77%) were first grafts, and 1,227 (23%) were repeat transplantations. Of the recipients, 1524 (29%) were sensitized, defined as >15% panel-reactive antibodies determined by the referring center. Data on ethnicity were available for 3364 transplant recipients, of whom the large majority (3152, 94%) were Caucasian; during the study period, 96% of deceased organ donors reported to UK Transplant were Caucasian. Recipients who died or whose grafts failed within 1 day of transplantation have been excluded from the study group ($n = 149$, of whom 47 were 0-BMM and 102 were 1-BMM) on the basis that clinical complications were not attributable to the level of HLA mismatch. Donor and recipient HLA typing data at the level of split specificity were available for 4359 transplants, of which 1,700 (39%) had zero split mismatches (0-SMM), 2,256 (52%) had one split mismatch (1-SMM), and 403 (9%) had two or more split mismatches (>1-SMM). The number of mismatched aa (aaMM), electrostatic mismatch score (EMS) and hydrophobic mismatch score (HMS) was calculated for the 2,256 transplants with 1 split mismatch using inter-locus subtraction as described below. The influence of aa mismatch, EMS and HMS on transplant outcome was compared with 0-SMM transplants, which by definition, had mismatch scores of zero.

### 2.3. Determination of mismatched HLA alloantigen amino acid sequence polymorphisms

During the study period, standard UK practice was to undertake donor and recipient HLA-A, -B and -C, -DR, and -DQ typing by low-resolution polymerase chain reaction using sequence-specific primers (PCR-SSP) or sequence-specific oligonucleotide probes. To calculate the number of aaMM, each HLA class I specificity (defined at the level of split specificities) was assigned to the most common corresponding four-digit HLA allele as previously described [25]. A computer program was developed to perform interlocus aa sequence comparisons between the donor and recipient HLA-A and -B type and to identify the position and nature of all disparate aa. HLA-C typing was not routinely performed by all transplant centers and was not considered during interlocus subtraction. Analysis to determine the number of aaMM and physiochemical scores was performed for the entire $\alpha 1$ and $\alpha 2$ domains of HLA class I molecules.

### 2.4. Assignment of hydrophobicity and electrostatic scores for mismatched HLA-A and -B specificities

For each HLA-A and -B interlocus aa disparity, mismatched aa were each assigned a hydrophobicity value using the Hopp–Woods scale [33], and hydrophobicity mismatch value was determined as the difference in aa hydrophobicity values. Similarly, the difference between the isoelectric points (pI) of each mismatched aa was used to determine electrostatic mismatch values. In cases in which the donor and recipient HLA class I type carried several aa polymorphisms at the same position, the lowest aa hydrophobicity mismatch and lowest electrostatic mismatch value were used in the analysis of immunogenicity. For each mismatched HLA class I specificity, the aa hydrophobicity mismatched values and pI mismatch values were each summed to give a total HMS and EMS, respectively.

### 2.5. Statistical analysis

Graft loss from potentially HLA related causes was the outcome of interest in this study. Patients who died with a functioning graft during the study were censored for graft survival on the day of death ($n = 700$). Grafts that failed between days 2 and 7 after transplantation from non–HLA-matching–related causes were also censored on the day of failure ($n = 54$; 2 hyperacute rejection, 31 arterial or venous thrombosis, 20 other vascular or ureteric operative complications, and 1 nonviable kidney). Analysis focused on the influence of the five explanatory variables (BMM, SMM, aaMM, EMS, and HMS) on the time to graft failure. Exploratory analysis showed that many of these variables were correlated with each other, so it was not appropriate to include them in large multivariate models. For example, HMS and EMS were highly correlated ($p = 0.91$) and thus should not be entered into the same model. Initially we investigated the relationship between graft survival and explanatory variable categories by plotting Kaplan–Meier curves and comparing curves for different levels using the log-rank test. For this analysis, HMS and EMS were grouped in deciles.

**Table 1**
Relationship between explanatory variables and kidney allograft survival

| Variable | Category | Frequency | 1-Year survival (%) | 5-Year survival (%) |
|---|---|---|---|---|
| Broad HLA mismatches[a] | 000 | 2051 | 93.6 | 84.2 |
| overall ***p = 0.013*** | 100 or 010 | 3196 | 92.7 | 81.9 |
| Broad HLA mismatches[b] | 000 | 1893 | 93.3 | 84.2 |
| overall ***p = 0.004*** | 100 or 010 | 2466 | 92.4 | 81.9 |
| Split HLA mismatches[b] | 0 | 1700 | 93.4 | 84.2 |
| overall ***p = 0.004*** | 1 | 2256 | 92.7 | 82.2 |
| | 2+ | 403 | 90.9 | 81.5 |
| Amino acid mismatches[c] | 0–1 | 130 | 97.7 | 89.3 |
| overall ***p = 0.036*** | 2+ | 2126 | 92.4 | 81.8 |
| Hydrophobicity decile[c] | 1 | 228 | 96.4 | 86.8 |
| overall $p = 0.197$ | 2–10 | 2028 | 92.2 | 81.7 |
| Electrostatic charge decile[c] | 1 | 228 | 94.7 | 85.5 |
| overall $p = 0.487$ | 2–10 | 2028 | 92.4 | 81.8 |
| Alloantibody sensitization[b] | 0–15 | 3055 | 93.4 | 83.8 |
| overall ***p = 0.028*** | 16–50 | 468 | 92.1 | 83.7 |
| | >50 | 836 | 91.0 | 79.0 |
| Transplant number[b] | First | 3337 | 93.1 | 83.6 |
| overall ***p = 0.005*** | retransplant | 1022 | 91.7 | 80.6 |

*P*-values of significant variables are shown in bold.
Significance levels based on log-rank test.
[a]Based on entire study cohort.
[b]Based on cohort subset for which HLA typing at the spit specificity level was available.
[c]Based on the cohort subset with a single split HLA mismatch (1-SMM).

Informal assessment of proportional hazards assumptions was based on plots of log(-log(survival)). Cox regression was used to further model relationships between the probability of graft failure and the explanatory variables. Initially each explanatory variable was modeled separately. Thereafter a series of multivariable models were investigated to assess the additional value in incorporating HMS or EMS into models, including aaMM as well as the significance of the explanatory variables after recipient pretransplantation HLA sensitization and number of transplants were included into the models. Statistical significance was assessed using likelihood ratio tests for nested Cox regression models, which compares the fit of the model, including a variable with the fit of the model that excludes it. All analyses were completed in the statistical software package Splus version 8.1 for Windows (Insightful Corp., Seattle, WA).

## 3. Results

Overall transplant outcome for the entire study cohort was very good, with a 5-year graft survival rate of approximately 80% as might be expected for renal transplants with no mismatch at HLA-DR and no more than a single HLA-A or -B mismatch. As shown in Tables 1 and 2 and as illustrated in Figure 1A, transplants with zero broad HLA mismatches (0-BMM) showed a small but significant improvement in graft survival compared with those with a single HLA-A or -B mismatch (1-BMM). HLA matching based instead on split HLA specificities provided little

**Table 2**
Single-variable Cox PH regression analysis of kidney allograft survival

| Variable | | Hazard ratio | 95% CI | Significance level |
|---|---|---|---|---|
| Broad HLA mismatches[a] | 000 | Reference | | **0.012** |
| | 100 or 010 | 1.16 | 1.03, 1.30 | |
| Broad HLA mismatches[b] | 000 | Reference | | **0.004** |
| | 100 or 010 | 1.20 | 1.06, 1.36 | |
| Split HLA mismatches[b] | 0 | Reference | | **0.004** |
| | 1 | 1.20 | 1.05, 1.37 | |
| | 2+ | 1.35 | 1.10, 1.66 | |
| Amino acid mismatches[c] | 0–1 | Reference | | **0.026** |
| | 2+ | 1.54 | 1.02, 2.31 | |
| Hydrophobicity decile[c] | 1 | Reference | | 0.187 |
| | 2–10 | 1.20 | 0.91, 1.59 | |
| Hydrophobicity[c] | Per decile | 1.00 | 0.99, 1.01 | 0.483 |
| Electrostatic charge decile[c] | 1 | Reference | | 0.482 |
| | 2–10 | 1.10 | 0.84, 1.44 | |
| Electrostatic charge[c] | Per decile | 1.00 | 0.99, 1.01 | 0.629 |
| Sensitization[b] | 0–15 | Reference | | **0.033** |
| | 16–50 | 1.06 | 0.87, 1.28 | |
| | >50 | 1.23 | 1.06, 1.43 | |
| Sensitization[b] | Per % | 1.00 | 1.00, 1.00 | **0.008** |
| Transplant number[b] | First | Reference | | **0.005** |
| | Re-transplant | 1.22 | 1.06, 1.39 | |
| Transplant number[b] | Per transplant | 1.15 | 1.05, 1.26 | **0.004** |

*P*-values of significant variables are shown in bold.
Significance levels refer to likelihood ratio test.
[a]Based on entire study cohort.
[b]Based on cohort subset for which HLA typing at the spit specificity level was available.
[c]Based on cohort subset with a single split HLA mismatch (1-SMM).

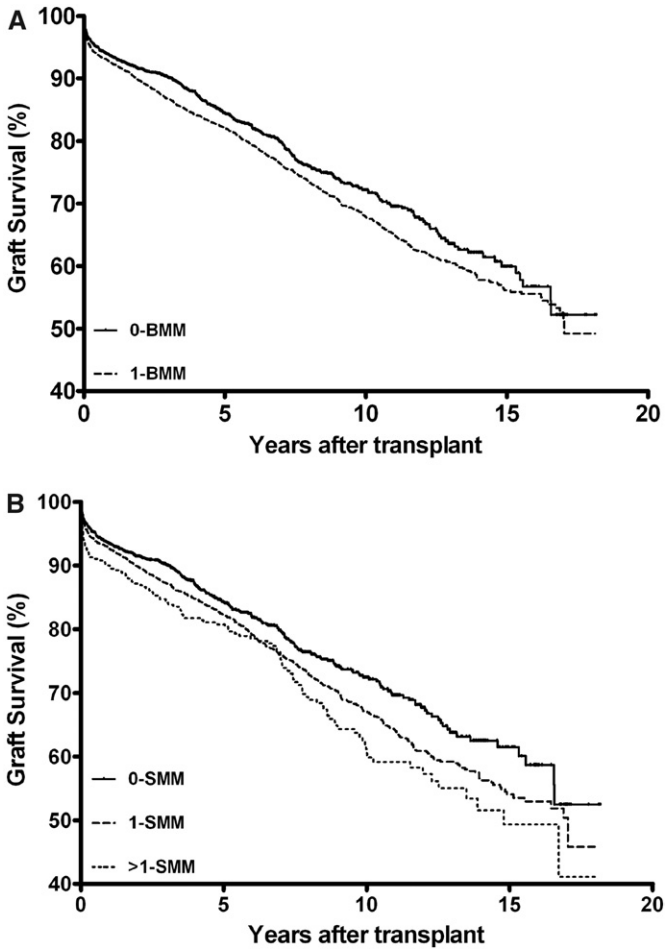


**Fig. 1.** Relationship between HLA matching for broad (A) and split (B) specificities and deceased donor kidney transplant survival. (A) Comparison of graft survival in 2051 0-BMM transplants and 3,196 1-BMM transplants ($p = 0.01$). (B) Comparison of graft survival in 1700 0-SMM transplants, 2,256 1-SMM transplants and 403 >1-SMM transplants ($p = 0.004$).

additional discrimination in terms of graft survival (Fig. 1B). Compared with renal transplants with a zero split mismatch (0-SMM), those with one split mismatch showed a reduction in graft survival; but there was no difference in graft survival between transplants with one and two or more split mismatches (Fig. 1B).

Transplants with a single split HLA-A or -B mismatch (1-SMM) were assessed to determine the potential impact of the number of mismatched amino acids (after interlocus subtraction) on graft survival. Of the 2,256 transplants with a single split mismatch, 130 (5.8%) had zero or one aaMM. Transplants with zero or one aaMM had a graft survival equivalent to those with 0-SMM, whereas those with two or more aaMM showed a significantly lower graft survival (Fig. 2, Table 1). When the physiochemical properties of the mismatched aa were considered, neither EMS nor HMS had any influence on graft survival.

A single-variable Cox proportional hazards (PH) regression analysis was undertaken to identify factors associated with graft survival (Table 2). In addition to the HLA-matching variables already described, pretransplantation allosensitization and second or subsequent renal transplants were associated with inferior graft survival. All of the explanatory variables examined in this analysis were closely related. For example, HLA matching for broad and split serologic specificities was associated with the number of mismatched aa and, in turn, with both HMS and EMS. In addition, retransplantation patients were more likely to be sensitized and to receive transplants with a zero HLA mismatch. We were particularly interested in determining whether HLA matching at the level of split specificities provided additional benefit over that of matching for broad serologic specificities, and whether the number of mismatched aa contributed independently to graft survival. Multivariate Cox proportional hazard (PH) regression analyses were performed for the study cohort subset for whom information at the HLA split specificity level was available ($n = 4359$; Table 3) and for the study subset with a single split HLA specificity mismatch (1-SMM), to examine the effect of matching at the aa sequence level ($n = 2256$; Table 4). After adjusting for matching at the level of broad HLA specificities, Cox regression analysis showed no additional benefit from matching at the level of split HLA specificities (Table 3, Model 3 vs Model 1, likelihood-ratio test, $p = 0.256$). In contrast, the number of mismatched aa provided the best indicator of transplant outcome. After adjusting for HLA matching at the level of broad HLA specificities, there was a significant increase in graft failure for transplants with two or more aaMM (Table 4, Model 3 vs Model 1, $p = 0.015$). Figure 3 illustrates the effect of aaMM in patients with 0-BMM. However, BMM and aaMM were closely related variables, such that the effect of broad matching was not significant indicating that aaMM was a stronger marker of graft outcome than broad HLA mismatches (Table 4, Model 3 vs Model 2, $p = 0.325$). In addition to HLA matching (at the broad or split specificity level), pretransplantation HLA sensitization and retransplantation were significant predictors of graft outcome. Importantly, the number of mismatched aa was strongly associated with graft outcome even after adjustment for other variables (Table 4).

## 4. Discussion

Kidney transplants that are closely matched for HLA-A, -B, and -DR have better long-term graft survival than those that are poorly matched for HLA, but the level of match required to produce worthwhile clinical benefit is controversial. In particular, there is uncertainty about whether resolving HLA mismatches at the level provided by conventional broad or split serologic specificities is optimal and whether further resolution of HLA mismatch at the molecular level provides additional clinical value. Our approach was to define HLA class I mismatch at the level of broad and split serologic specificities and at the level of aa disparity, based on published sequence data and assumptions regarding the most likely allele assigned for each HLA class I specificity within the ethnically homogeneous recipient and donor populations (>90% Caucasian).

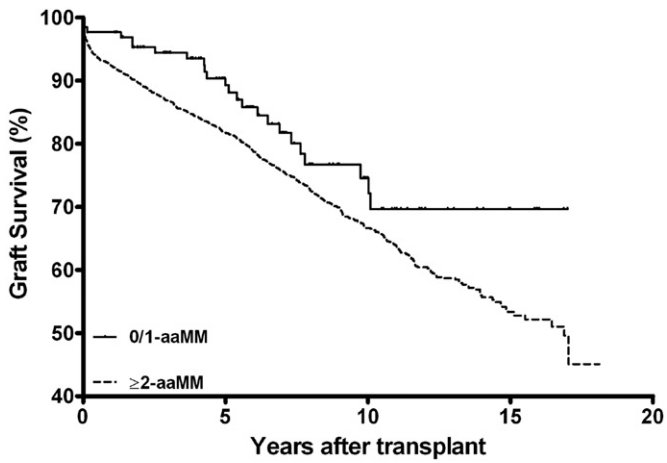


**Fig. 2.** Relationship between HLA matching at the level of amino acid mismatch (after inter-locus subtraction) and kidney transplant survival. Comparison of graft survival in 130 0/1-aaMM transplants and 2,126 ≥2-aaMM transplants ($p = 0.04$).

**Table 3**
Multiple variable Cox PH regression analyses of kidney allograft survival in cohort subset for which HLA typing at the spit specificity level was available

| Model | Variables included | | Hazard ratio | 95% Confidence interval | Comparison and significance level |
|---|---|---|---|---|---|
| M1 | Broad HLA matching | 000 | Reference | | 0.004 |
| | | 010 or 100 | 1.20 | 1.06, 1.36 | |
| M2 | Split HLA matching | 0 | Reference | | 0.002 |
| | | 1+ | 1.22 | 1.07, 1.39 | |
| M3 | Broad and split HLA matching | 010 or 100 | 1.01 | 0.74, 1.38 | M3–M2 $p = 1.00$ |
| | | 1+ | 1.21 | 0.88, 1.67 | M3–M1 $p = 0.256$ |
| M4 | Sensitization | 0–15 | Reference | | 0.033 |
| | | 16–50 | 1.06 | 0.87, 1.28 | |
| | | >50 | 1.23 | 1.06, 1.43 | |
| M5 | Broad HLA matching and sensitization | 010 or 100 | 1.23 | 1.09, 1.40 | M5–M4 $p = 0.001$ |
| | | 16–50 | 1.08 | 0.89, 1.31 | M5–M1 $p = 0.009$ |
| | | >50 | 1.27 | 1.09, 1.48 | |
| M6 | Transplant number | First | Reference | | 0.005 |
| | | Re-transplant | 1.22 | 1.06, 1.39 | |
| M7 | Broad HLA matching and transplant number | 010 or 100 | 1.23 | 1.08, 1.39 | M7–M6 $p = 0.001$ |
| | | Re-transplant | 1.25 | 1.09, 1.43 | M7–M1 $p = 0.002$ |

The results of the present study show that for recipients of a kidney allograft matched for HLA-DR and bearing a single HLA-A or -B mismatch, split serologic specificities provide no additional predictive value for graft survival over that observed for broad serologic specificities. In contrast, defining HLA disparity at the level of aa mismatch after interlocus subtraction allowed further discrimination with respect to transplant outcome: grafts with only zero or one aa mismatch had superior graft survival compared with those with multiple aa mismatches. We have previously shown, in highly sensitized patients, that the physiochemical properties (hydrophobicity and electrostatic charge) of mismatched aa independently predict the humoral response to mismatched HLA class I alloantigens [25]. However, transplant outcome is a considerably more complex end point for immunogenicity than the presence of an alloantibody response alone; and, in the present study, the physiochemical properties of aaMM did not provide additional predictive value.

Duquesnoy and Claas et al., in a study that analyzed the outcome of kidney transplants reported to the UNOS and Eurotransplant registries, suggested that HLA matching at the level of aa triplets (using the HLAMatchmaker program) provided improved graft survival over that defined by conventional serologic analysis [23]. However, the validity of these findings was challenged by Opelz et al., who suggested that because the original analysis included recipients with multiple HLA mismatches, the apparent benefit from aa triplet matching was attributable instead to underlying differences in the number of mismatched HLA specificities [24]. In the present study, we confined our analysis to kidney transplants with a single HLA-A or -B mismatch to avoid the potential confounding effect of multiple HLA mismatches. We also defined HLA class I disparity at the level of single aa differences rather than aa triplets because our previous analysis of HLA class I immunogenicity in highly sensitized patients showed that the major benefit of the HLAMatchmaker program was attributable to the novel concept of interlocus subtraction rather than the use of triplet over single aa mismatches.

We and others have shown previously that the immunogenicity of a mismatched alloantigen in the context of recipient HLA type can be predicted from the number of aa mismatches [21,22,25,26] and their physiochemical properties [25]. These studies, and those of Duquesnoy et al., along with the results of the present study, all support the hypothesis that the degree of HLA disparity between a given donor and recipient pair is better expressed at the level of aa disparity (after interlocus subtraction) than by the current approach, which assigns a simple mismatch grade based on the number of mismatched donor antigens at each HLA loci regardless of potential differences in their immunogenicity according to recipient HLA type.

Our study group included more than 3000 single HLA-A or -B mismatched transplants, of which more than 2500 were also resolved at the level of split HLA specificities, thereby allowing a robust analysis of the relative benefits of matching at broad and split HLA specificities. Of the single HLA-mismatched transplants defined at the level of split HLA specificity, only 6% had zero or 1 aa

**Table 4**
Multiple variable Cox PH regression analyses of kidney allograft survival in cohort subset with a single split HLA mismatch (1-SMM)

| Model | Variables included | | Hazard ratio | 95% Confidence interval | Comparison and significance level[a] |
|---|---|---|---|---|---|
| M1 | Broad HLA matching | 000 | Reference | | 0.967 |
| | | 010 or 100 | 1.01 | 0.72, 1.40 | |
| M2 | Amino acid mismatches | 0–1 | Reference | | **0.026** |
| | | 2+ | 1.54 | 1.02, 2.31 | |
| M3 | Broad and amino acid HLA mismatches | 010 or 100 | 0.83 | 0.58, 1.19 | M3–M2 $p = 0.325$ |
| | | 2+ | 1.67 | 1.08, 2.60 | M3–M1 $p = 0.015$ |
| M4 | Sensitization | 0–15 | Reference | | 0.021 |
| | | 16–50 | 1.12 | 0.87, 1.45 | |
| | | >50 | 1.36 | 1.10, 1.67 | |
| M5 | Amino acid mismatches and sensitization | 2+ | 1.53 | 1.02, 2.31 | M5–M4 $p = 0.027$ |
| | | 16–50 | 1.14 | 0.88, 1.47 | M5–M2 $p = 0.022$ |
| | | >50 | 1.35 | 1.09, 1.67 | |
| M6 | Transplant number | First | Reference | | 0.004 |
| | | Re-transplant | 1.31 | 1.10, 1.58 | |
| M7 | Amino acid mismatches and transplant number | 2+ | 1.52 | 1.01, 2.29 | M7–M6 $p = 0.033$ |
| | | Re-transplant | 1.31 | 1.09, 1.57 | M7–M2 $p = 0.005$ |

*P*-values of significant variables are shown in bold.
[a]Significance levels refer to likelihood ratio test comparing models.

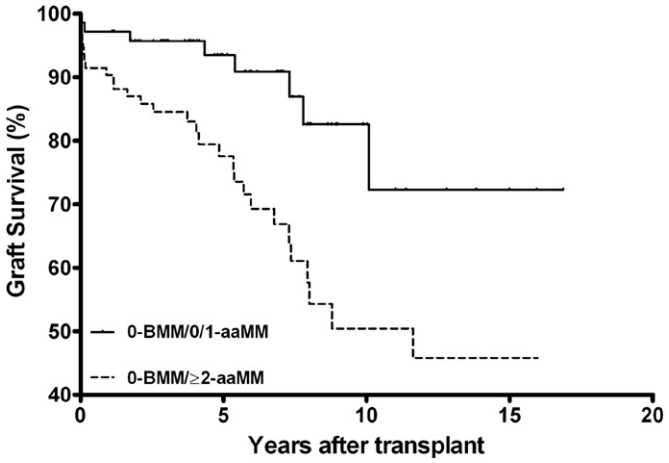


**Fig. 3.** Relationship between amino acid mismatches and kidney transplant survival in patients fully matched at the broad HLA specificity level. Comparison of graft survival in single split HLA specificity mismatched kidney transplants (1-SMM) that were matched at the broad HLA specificity level (0-BMM) according to the number of amino acid mismatches (72 0/1-aaMM/0-BMM transplants and 97 ≥2-aaMM/0-BMM transplants). Cox regression analysis, after adjusting for HLA matching at the level of broad HLA specificities, showed there was a significant increase in graft failure for transplants with two or more mismatched amino acids ($p = 0.015$).

mismatch (after interlocus subtraction), which limits the practical implications of our findings in terms of incorporating them into a kidney allocation scheme. However, our results, taken together with those of Duquesnoy et al., [23] provide important proof of concept that assessment of histocompatibility at the aa sequence level using interlocus subtraction is superior to conventional serologically based HLA matching. Further analyses are now required in large cohorts of renal transplant recipients to examine the effect of aa mismatching at HLA class II loci as well as transplants with multiple HLA class I and class II mismatches.

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