oral tolerance to proteins. Proc Natl Acad Sci USA 2001; 98: 3310.

25. Gonnella PA, Chen Y, Inobe J, et al. In situ immune response in gut-associated lymphoid tissue (GALT) following oral antigen in TCR-transgenic mice. J Immunol 1998; 160: 4708.

26. Matsuura Y, Matsuoka T, Fuse Y. Ultrastructural and immunohistochemical studies on the ontogenic development of bronchus-associated lymphoid tissue (BALT) in the rat: Special reference to follicular dendritic cells. Eur Respir J 1992; 5: 824.

27. Behrens D, Lange K, Fried A, et al. Donor-derived soluble MHC antigens plus low-dose cyclosporine induce transplantation unresponsiveness independent of the thymus by down-regulating T cell-mediated alloresponses in a rat transplantation model. Transplantation 2001; 72: 1974.

28. Zavazava N, Kronke M. Soluble HLA class I molecules induce apoptosis in alloreactive cytotoxic T lymphocytes. Nat Med 1996; 2: 1005.

29. Niimi M, Roelen DL, Witzke O, et al. The importance of H2 haplotype sharing in the induction of specific unresponsiveness by pretransplant blood transfusions. Transplantation 2000; 69: 411.

30. Madsen JC, Superina RA, Wood KJ, et al. Immunological unresponsiveness induced by recipient cells transfected with donor MHC genes. Nature 1988; 332: 161.

31. Miller A, Lider O, Weiner HL. Antigen-driven bystander suppression after oral administration of antigens. J Exp Med 1991; 174: 791.

32. Weiner HL. Oral tolerance: Immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. Microbes Infect 2001; 3: 947.

33. Niimi M, Jones ND, Morris PJ, et al. Evidence that non-deletional mechanisms are responsible for inducing and maintaining unresponsiveness after intrathymic injection of non-professional antigen presenting cells. J Heart Lung Transplant 2000; 19: 576.

34. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. Annu Rev Immunol 1996; 14: 233.

35. Pearson TC, Alexander DZ, Winn KJ, et al. Transplantation tolerance induced by CTLA4-Ig. Transplantation 1994; 57: 1701.

36. Walunas TL, Lenschow DJ, Bakker CY, et al. CTLA-4 can function as a negative regulator of T cell activation. Immunity 1994; 1: 405.

37. Perez VL, Van Parijs L, Biuckians A, et al. Induction of peripheral T cell tolerance in vivo requires CTLA-4 engagement. Immunity 1997; 6: 411.

38. Zheng XX, Markees TG, Hancock WW, et al. CTLA4 signals are required to optimally induce allograft tolerance with combined donor-specific transfusion and anti-CD154 monoclonal antibody treatment. J Immunol 1999; 162: 4983.

39. Larsen CP, Elwood ET, Alexander DZ, et al. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature 1996; 381: 434.

40. Elwood ET, Larsen CP, Cho HR, et al. Prolonged acceptance of concordant and discordant xenografts with combined CD40 and CD28 pathway blockade. Transplantation 1998; 65: 1422.

41. Bumgardner GL, Li J, Heininger M, et al. Costimulation pathways in host immune responses to allogeneic hepatocytes. Transplantation 1998; 66: 1841.

42. Kishimoto K, Dong VM, Issazadeh S, et al. The role of CD154-CD40 versus CD28-B7 costimulatory pathways in regulating allogeneic Th1 and Th2 responses in vivo. J Clin Invest 2000; 106: 63.

43. Samoilova EB, Horton JL, Zhang H, et al. CTLA-4 is required for the induction of high dose oral tolerance. Int Immunol 1998; 10: 491.

# HLAMatchmaker: A MOLECULARLY BASED ALGORITHM FOR HISTOCOMPATIBILITY DETERMINATION. III. EFFECT OF MATCHING AT THE HLA-A,B AMINO ACID TRIPLET LEVEL ON KIDNEY TRANSPLANT SURVIVAL[1]

RENE J. DUQUESNOY,[2,6] STEVE TAKEMOTO,[3] PETER DE LANGE,[4] ILIAS I. N. DOXIADIS,[4]
GEZIENA M. TH. SCHREUDER,[4] GUIDO G. PERSIJN,[5] AND FRANS H. J. CLAAS[4]

***Background.*** **HLAMatchmaker is a recently developed computer-based algorithm to determine donor-recipient HLA compatibility at the molecular level. Originally designed for highly alloimmunized patients, this algorithm is based on the concept that immunogenic epitopes are represented by amino acid triplets on exposed parts of protein sequences of HLA-A, -B, and -C chains accessible to alloantibodies. Donor HLA compatibility is determined by intralocus and interlocus comparisons of triplets in polymorphic sequence positions. For most patients, HLAMatchmaker can identify certain mismatched HLA antigens that are zero-triplet mismatches to the patient's HLA phenotype and should, therefore, be considered fully histocompatible. The present study was designed to determine how class I HLA matching at the triplet level affects kidney transplant outcome.**

***Methods.*** **We analyzed two multicenter databases of zero-HLA-DR–mismatched kidneys transplanted from 1987 to 1999. One database consisted of 31,879 primary allografts registered by U.S. transplant centers in the United Network for Organ Sharing database and the other consisted of 15,872 transplants in the Eurotransplant program.**

***Results.*** **HLA-A,B mismatched kidneys that were compatible at the triplet level exhibited almost iden-**

tical graft survival rates as the zero-HLA-A,B antigen mismatches defined by conventional criteria. This beneficial effect of triplet matching was seen for both nonsensitized and sensitized patients and also for white and nonwhite patients.

*Conclusions.* These findings suggest that the application of HLAMatchmaker will increase the number of successful transplants, at least in the HLA-DR match combinations.

In kidney transplantation, the number of mismatched HLA-A, -B, and -DR antigens generally determines the degree of donor HLA compatibility. Many studies have shown that zero-HLA antigen–mismatched transplants have the highest success rates, and these findings have led to the allocation policy of mandatory sharing of zero-mismatched cadaver kidneys. Although only 9% of all cadaver kidney transplants in the United Network for Organ Sharing (UNOS) are zero-antigen mismatches (1), an organ allocation based on HLA matching is disadvantageous for racial minority groups who have different HLA antigen frequencies than the donor pool (2–5).

Another method for assessing donor-recipient compatibility considers public epitopes that are shared by HLA antigens belonging to so-called cross-reactive antigen groups (CREGs) (6–8). Such epitopes are largely determined by alloantibodies, and they have been defined for class I antigens encoded by the HLA-A and HLA-B loci. CREG matching considers the broader groups of HLA-A,B antigens with shared public epitopes rather than the specific HLA antigens. It provides an alternative HLA-based organ allocation system that would benefit more transplant candidates and provide more access to better-matched organs for minorities (9).

Several studies have suggested CREG matching is associated with graft survival rates that are better or comparable to those allocated on HLA antigen matching (10–18), but others have not found a beneficial effect of CREG matching (19–23). The controversy about CREG matching is in part a result of the difficulty in defining the exact spectrum of public epitopes on all HLA antigens. The CREG matching system in UNOS uses 10 CREGs, but the actual number of public specificities seems to be considerably higher (6–8,18,24). Amino acid sequence information on HLA antigens has led to the identification of polymorphic residues that correspond to many public epitopes and private HLA antigens. Matching at the HLA residue level in retrospective analysis resulted in graft outcome similar to zero HLA-B, -DR–mismatched grafts (15,25), but a prospective analysis has not demonstrated a comparable benefit (26).

This report deals with an alternative approach to determine donor-recipient HLA compatibility at the structural level. HLAMatchmaker is a computer algorithm that considers each HLA antigen as a string of polymorphic amino acid triplets in antibody-accessible positions of HLA molecules (27–29). HLA compatibility is assessed at the structural level by determining the differences in triplet combinations between donor and recipient HLA molecules. HLAMatchmaker can identify HLA antigens that are mismatched by conventional criteria, but because they share all their triplets with the patient, they must be considered fully compatible at the epitope level. This program has been particularly useful in the identification of compatible donors for highly sensitized patients (30,31).

The present study was designed to determine how class I HLA matching defined by HLAMatchmaker affects kidney transplant outcome. The analysis was performed with the UNOS and the Eurotransplant databases of zero-HLA-DR–mismatched transplants. We have found that HLA-A,B mismatching at the triplet level is associated with almost identical graft survival rates as the zero HLA-A,B antigen mismatches defined by conventional criteria.

## MATERIALS AND METHODS

We analyzed two large cadaver kidney transplant databases. One consisted of 31,879 zero-DR–mismatched kidneys transplants in the United States from 1987 to 1999 and reported to the UNOS Scientific Registry. The second database comprised 15,872 zero-DR–mismatched kidney transplants in Austria, Belgium, Germany, Luxembourg, The Netherlands, and Slovenia from 1987 to 1999 and reported to the Eurotransplant Registry. Sensitized patients with a pretransplantation panel-reactive antibody (PRA) of more than 50% were considered separately from nonsensitized patients with a PRA less than 10%.

Serologic HLA-A and HLA-B typing information of recipients and donors was retrieved from the databases, and the HLA antigens were converted into strings of polymorphic triplets applying a previously described computer algorithm (29). There are 113 polymorphic triplets distributed over 30 positions in HLA-A molecular sequence and 27 positions in the HLA-B molecular sequence. HLAMatchmaker assesses compatibility between donor and recipient by determining what triplets on donor HLA antigens are present or absent on any of the patient's own HLA antigens. A triplet in a given sequence position of a donor HLA antigen is considered a match if the recipient has this triplet in that position of any of its own HLA class I antigens. HLA compatibility is assessed by determining the total number of mismatched triplets on the HLA antigens of the donor. Certain HLA antigens are mismatched by conventional criteria but turn out to be fully compatible at the triplet level (29). The Microsoft Excel version of the HLAMatchmaker program can be downloaded from the http://tpis.upmc.edu website.

This triplet-matching program was adapted to the UNOS and Eurotransplant databases, and standard Kaplan-Meier methods were applied to determine actuarial graft survival rates with death considered graft loss in the various match groups. Statistical significance was determined by log-rank comparisons of survival rates using two-sided $P$ values.

## RESULTS

This analysis was performed with two databases of zero-HLA-DR–mismatched transplants. The distribution of the HLA-A,B antigen mismatches was different between the UNOS and Eurotransplant databases; this might be related to differences in organ allocation policies in these programs. UNOS had a higher percentage of zero-HLA-A,B mismatches than Eurotransplant: 31.1% versus 21.6% (Table 1); this might be a result of the larger waiting list of patients in UNOS (32). On the other hand, the higher percentages of one- and two-HLA-A,B antigen mismatches in Eurotransplant reflects a greater emphasis on minimizing the number of mismatched HLA antigens in the mismatches (24). Overall, the average number of HLA-A,B mismatches was slightly higher for the UNOS transplants than the Eurotransplant cases (1.80 versus 1.65). The 5-year graft survival rates for zero-HLA-DR–mismatched transplants were 71.4% in recipients in the UNOS database and 76.8% in patients in the Eurotransplant database.

TABLE 1. Proportions of zero-HLA-DR–mismatched kidney transplants with different numbers of HLA-A,B antigen mismatches in the UNOS and Eurotransplant databases

| Number of mismatched HLA-A,B antigens[a] | UNOS | Eurotransplant |
|---|---|---|
| 0 | 31.1% | 21.6% |
| 1 | 9.1% | 22.3% |
| 2 | 21.8% | 30.6% |
| 3 | 24.9% | 20.2% |
| 4 | 13.1% | 5.3% |

[a] Average number of mismatched HLA-A,B antigens: UNOS, 1.80; Eurotransplant, 1.65.

Figure 1 shows the graft survival rates for zero-HLA-DR–mismatched recipients, stratified by the number of HLA-A,B antigen mismatches. As expected, the zero mismatches had the highest success rates for both UNOS and Eurotransplant patients and graft survival decreased in a stepwise manner with increasing numbers of HLA-A,B antigen mismatches.
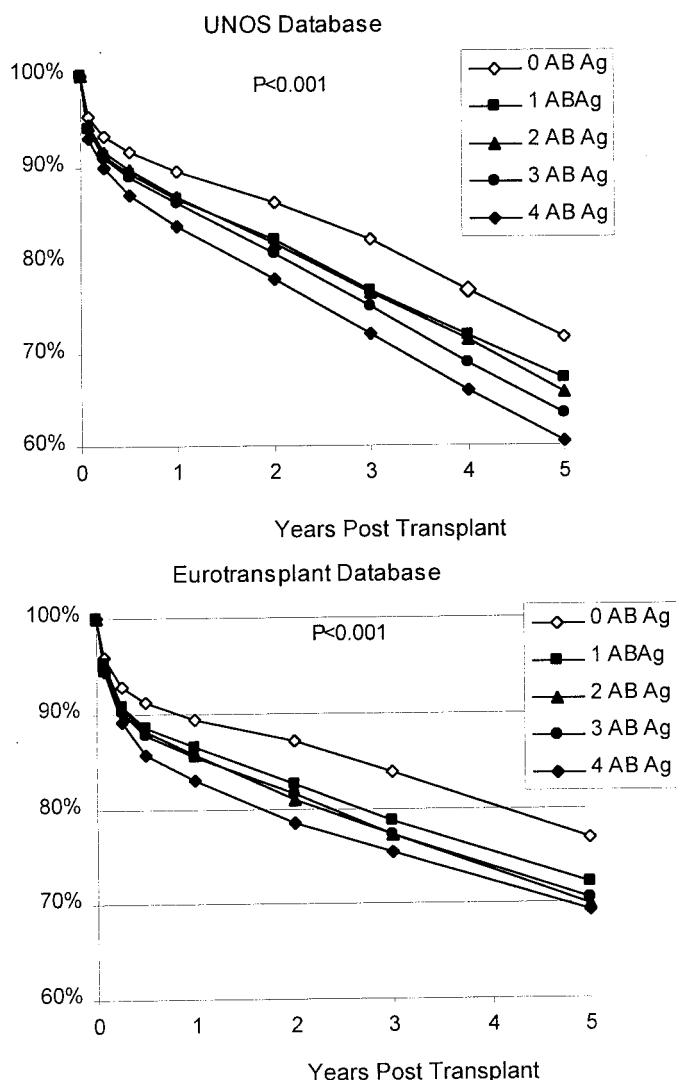




FIGURE 1. Five-year graft survival rates of zero-HLA-DR–mismatched primary kidney transplants with zero to four HLA-A,B antigen mismatches in the UNOS and Eurotransplant databases.

*Analysis of the UNOS Data*

The primary goal of this study was to compare the graft survival rates of the zero-HLA-A,B antigen mismatches with those HLA-A,B antigen mismatches that are compatible at the triplet level. Of the 21,270 zero-HLA-DR–mismatched cases with HLA-A,B antigen mismatches reported to the UNOS database, there were 183 cases that had zero-triplet mismatches. Graft survival rates were nearly identical between the patients with zero-triplet mismatches and those with zero-HLA-A,B,DR antigen mismatches. Recipients with one-triplet (n=32) and two-triplet mismatches (n=36) had similar survival rates, so we combined the zero-, one-, and two-triplet mismatches in a triplet match group (n=251). Figure 2 shows that this group had almost identical survival rates as the zero-HLA-A,B antigen mismatches. Recipients with 3- to 10-triplet mismatches and those with greater than 10-triplet mismatches had significantly lower graft survival rates (*P*<0.001).

The beneficial effect of matching at the triplet level was seen in both white and nonwhite recipients (Table 2). Two-year graft survival rates are shown because the number (n=64) of nonwhite recipients (mainly African Americans) was too small for a longer follow-up interval. It should also be noted that the graft survival rates of the 3 to 10 and the greater than 10 triplet-mismatched groups were lower for nonwhite and white recipients.

The impact of triplet matching on sensitized and nonsensitized patients was also examined in the UNOS database analysis. Overall, sensitized patients with PRA greater than 50% (n=4,033) had poorer graft survival than unsensitized patients with PRA less than 10% (n=22,280). It is interesting to note that although the numbers are small, when stratified in this way, patients with zero- to two-triplet mismatches had graft survival rates similar to the zero-HLA-A,B antigen–mismatched transplants and strikingly higher than those with more than two-triplet mismatches (Table 3). These data suggest that the beneficial effect of triplet matching applies equally to sensitized and nonsensitized transplant recipients.
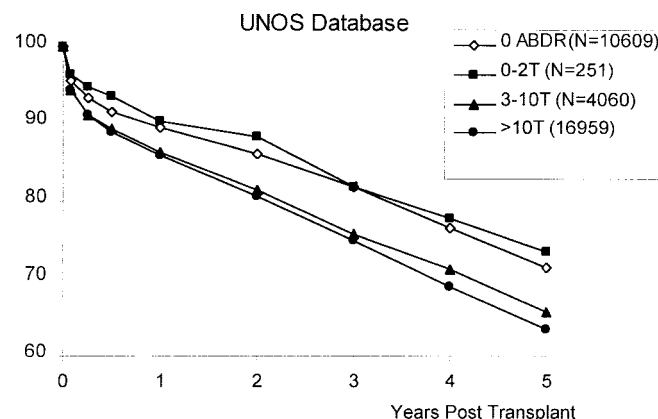


FIGURE 2. Effect of HLA-A,B triplet matching on 5-year graft survival rates of zero-HLA-DR–mismatched kidney transplants in the UNOS database.

**TABLE 2.** Triplet matching and two-year graft survival rates of zero-DR–mismatched kidneys in white and nonwhite recipients in the UNOS database

| | White recipients | | Nonwhite recipients | |
|---|---|---|---|---|
| | n | Graft survival rate | n | Graft survival rate |
| 0 A,B Antigen mismatches | 9199 | 86.1% | 1410 | 84.6% |
| 0–2 Triplet mismatches | 187 | 88.6% | 64 | 87.6% |
| 3–10 Triplet mismatches | 3037 | 82.1% | 1023 | 78.7% |
| >10 Triplet mismatches | 11464 | 82.2% | 5495 | 76.9% |

**TABLE 3.** Triplet matching and two-year graft survival rates of zero-DR–mismatched kidneys in sensitized and non-sensitized recipients in the UNOS database

| | Nonsensitized patients | | Sensitized patients | |
|---|---|---|---|---|
| | n | Graft survival rate | n | Graft survival rate |
| 0 A,B Antigen mismatches | 6970 | 87.4% | 2055 | 81.2% |
| 0–2 Triplet mismatches | 173 | 89.2% | 44 | 83.4% |
| 3–10 Triplet mismatches | 2789 | 83.7% | 508 | 71.7% |
| >10 Triplet mismatches | 12348 | 82.1% | 1426 | 74.3% |

### Analysis of the Eurotransplant Data

The Eurotransplant database had a somewhat different distribution of HLA-A,B antigen mismatches than the UNOS database. Although the proportion of zero-antigen mismatches was smaller (i.e., 21.6% vs. 31.1%), Eurotransplant had higher percentages of one-antigen mismatches (22.3% vs. 9.1%) and two-antigen mismatches (30.6% vs. 21.8%) than UNOS (Table 1). Also, the numbers of cases with zero and few triplet mismatches were considerably higher and this permitted a more detailed analysis of the association of graft survival and the number of triplet mismatches. Table 4 compares the graft survival rates during a 5-year interval between the zero-HLA-A,B antigen mismatches (n=3426) and the HLA-A,B mismatches grouped according to the number of mismatched triplets. Overall, graft survival correlated significantly with the number of mismatched triplets (P<0.001). The zero-triplet mismatches (n=231) and the one-triplet mismatches (n=218) had almost the same graft survival rates as the zero-HLA-A,B antigen mismatches. For instance, the 5-year graft survival rate was 76.0% for the combined group of zero- and one-triplet mismatches (n=449) and 76.8% for the zero-HLA-A,B antigen mismatches. The two-triplet mismatch group had a lower graft survival rate than the zero- and one-triplet mismatches, but the difference

was statistically not significant. Interestingly, the graft survival rates of the three- and four-triplet mismatch groups were similar to those of the zero- and one-triplet mismatches. Mismatching for five and more triplets was associated with significantly lower graft survival rates (P<0.001).

Altogether, these results demonstrate that matching at the triplet level is associated with excellent kidney transplant survival and that mismatching for up to two triplets has no significant adverse effect. The numbers of cases with high serum PRA values were too small for a meaningful analysis of a triplet matching effect on graft survival in sensitized patients. Also, insufficient numbers of cases were available in the Eurotransplant database to compare white and nonwhite recipients.

### DISCUSSION

This analysis of two databases of zero-HLA-DR–mismatched kidney transplants provides evidence that HLA-A,B antigen mismatches with compatible amino acid triplets have similar graft survival rates to those with zero-HLA-A,B antigen mismatches. The practical implication of this finding is the possible use of the HLAMatchmaker algorithm to increase the number of well-matched transplants. Furthermore, triplet matching may benefit sensitized patients and nonwhite transplant candidates, for whom it is difficult to find donors with good matches.

In the UNOS database of zero-HLA-DR mismatches, the number of zero- to two-triplet mismatches represented only 251 (or about 1%) of the 21,270 HLA-A,B antigen–mismatched cases. In Eurotransplant, almost 7% of the 12,374 HLA-A,B antigen-mismatched cases had zero- to two-triplet mismatches. This finding indicates that the triplet matching algorithm will increase the availability of HLA-compatible donors. In an accompanying article, we address this issue and how the application of HLAMatchmaker will increase the probability of finding a suitable donor (33).

Why do the zero-triplet and few-triplet mismatched transplants appear so successful? Designed originally for highly sensitized patients (27,28), the HLAMatchmaker algorithm considers only triplets that are in antibody-accessible positions of the HLA molecular structure. HLA-specific antibodies play a major role in graft rejection (34), and matching at the humoral immune level (i.e., for epitopes recognized by such antibodies) can be expected to improve graft survival. HLA compatibility must also consider cellular immune mechanisms of graft rejection, such as class I HLA-specific cytotoxic T lymphocytes and indirect allorecognition of processed donor class I HLA antigens presented by recipient CD4 T cells. Although HLA matching at the cellular immune level must use different structural criteria (they are currently not defined), this humoral immunity-based matching strategy

**TABLE 4.** Effect of HLA-A,B triplet mismatching on graft survival of zero-HLA-DR–mismatched kidneys in Eurotransplant

| Mismatch group | 0 AB Ag | 0 trp | 1 trp | 2 trp | 3 trp | 4 trp | 5–9 trp | 10–19 trp | 20–29 trp |
|---|---|---|---|---|---|---|---|---|---|
| Number of recipients | 3426 | 231 | 218 | 377 | 450 | 629 | 3448 | 6078 | 943 |
| % Graft survival after | | | | | | | | | |
| 1 yr | 89.3 | 89.2 | 86.7 | 85.7 | 89.1 | 88.4 | 85.1 | 85.4 | 84.3 |
| 2 yr | 87.1 | 85.1 | 84.3 | 82.1 | 86.7 | 84.1 | 81.5 | 80.6 | 80.0 |
| 3 yr | 83.7 | 82.3 | 80.7 | 78.3 | 82.4 | 81.1 | 77.4 | 76.7 | 76.1 |
| 5 yr | 76.8 | 75.6 | 76.4 | 72.9 | 75.4 | 75.9 | 69.9 | 69.8 | 68.5 |

Ag, antigen; trp, triplet.

permits the identification of triplet matches among the zero-HLA-DR mismatches that have the same high success rates as the conventional zero-HLA-A,B,DR mismatches.

We have also obtained evidence that kidney transplants mismatched for one or a few triplets may have equal graft survival rates as the zero-triplet mismatches. The Eurotransplant data seem to indicate excellent survival rates of kidney transplants with up to four mismatched triplets. These findings raise questions about the immunogenicity of triplets, and some of them may not play a determining role in matching. Indeed, a recent serologic analysis of sera from highly sensitized patients has identified several triplets with low immunogenicity in terms of specific antibody formation (*29*).

In this study, the assignment of triplets to HLA antigens does lack precision because HLA typing was performed largely by serologic methods that cannot test for molecular subtypes. DNA-based typing will permit the definition of HLA subtypes and accurate assignments of polymorphic triplets.

The triplet polymorphism of HLA-C was not considered in this analysis because no reliable HLA-C typing information was available in the two databases. Many HLA-C antigens are in linkage disequilibrium with certain HLA-B antigens (*35*). Therefore, a zero-HLA-B antigen mismatch is likely a zero-HLA-C antigen mismatch. On the other hand, a donor HLA-A,B phenotype with one or more mismatched antigens may more likely also have a HLA-C antigen incompatibility even if the mismatched HLA-A,B antigens are triplet matches. Fortunately, many incompatible HLA-C antigens are zero-triplet or a few-triplet mismatches (*28*). The exception is HLA-Cw7, for which the polymorphic triplet repertoire is different than for the other HLA-C antigens. With currently available DNA typing methods for HLA-C typing and the application of the triplet-matching algorithm, it might become possible to fully assess the role of HLA-C matching in transplantation.

Many triplets seem to be equivalent to public epitopes expressed by CREGs of HLA antigens. For most patients, HLAMatchmaker can identify mismatched HLA antigens that are fully compatible at the triplet level. Many of them cross-react with HLA antigens of the patient, and this is in accordance with the CREG matching concept in kidney transplantation. CREG matching emphasizes the sharing of public epitopes between donor and recipient HLA antigens. Certain HLA antigens, however, carry private epitopes with considerable immunogenic potential and mismatching for them might be detrimental. This may explain why the beneficial effect of CREG matching on kidney transplant outcome remains controversial (*10,16,17,20,21,23*). HLAMatchmaker permits a fine tuning of the CREG matching algorithm because it considers the structural organization of public and private epitopes.

## REFERENCES

1. Takemoto S, Terasaki P, Gjertson D, et al. Twelve years' experience with national sharing of HLA-matched cadaveric kidneys for transplantation. N Engl J Med 2000; 343: 1078–1084.
2. Milford EL, Ratner L, Yunis EJ. Will transplant immunogenetics lead to better graft survival in blacks? Racial variability in the accuracy of tissue typing for organ donation: the fourth American workshop. Transplant Proc 1987; 19: 30–33.
3. Perez LM, Schulman B, Davis F, et al. Organ donation in three major American cities with large Latino and black populations. Transplantation 1988; 46: 553–559.
4. Gaston R, Ayres J, Dooley L, et al. Racial equity in renal transplantation: the disparate impact of HLA-based allocation. JAMA 1993; 270: 1352.
5. Zachary AA, Braun WE, Hayes JM, et al. Effect of HLA matching on organ distribution among whites and African-Americans. Transplantation 1994; 57: 1115–1119.
6. Rodey GE, Fuller TC. Public epitopes and the antigenic structure of the HLA molecules [review]. Crit Rev Immunol 1987; 7(3): 229–267.
7. Konoeda Y, Terasaki P, Wakisaka A, et al. Public determinants on HLA indicated by pregnancy antibodies. Transplantation 1986; 41: 253–259.
8. Duquesnoy RJ, White LT, Fierst JW, et al. Multiscreen serum analysis of highly sensitized renal dialysis patients for antibodies toward public and private class I HLA determinants: implications for computer-predicted acceptable and unacceptable donor mismatches in kidney transplantation. Transplantation 1990; 50(3): 427–437.
9. Takemoto S, Terasaki PI, Gjertson DW, et al. Equitable allocation of HLA-compatible kidneys for local pools and minorities [see comments]. N Engl J Med 1994; 331(12): 760–764.
10. Takemoto SK, Cecka JM, Terasaki PI. Benefits of HLA-CREG matching for sensitized recipients as illustrated in kidney regrafts. Transplant Proc 1997; 29(1–2): 1417.
11. Takemoto S, Terasaki PI. Refinement of permissible HLA mismatches. Clin Transpl 1994: 451–466.
12. Thompson JS, Bryne JE, Hempel HO, et al. Computer algorithm that predicts both acceptable and unacceptable private and public HLA class I antigens in highly sensitized patients. Transplant Proc 1993; 25(1 Pt 1): 251–254.
13. Sanfilippo F, Vaughn WK, Spees EK, et al. The effect of HLA-A, -B matching on cadaver renal allograft rejection comparing public and private specificities. Transplantation 1984; 38(5): 483–489.
14. Takemoto S, Terasaki PI, Cecka JM, et al. Survival of nationally shared, HLA-matched kidney transplants from cadaveric donors: The UNOS Scientific Renal Transplant Registry. N Engl J Med 1992; 327: 834–838.
15. Takemoto S, Gjertson DW, Terasaki PI. HLA matching: maximizing the number of compatible transplants. Clin Transpl 1993: 521–531.
16. McKenna RM, Lee KR, Gough JC, et al. Matching for private and public HLA epitopes reduces acute rejection and improves two-year renal allograft function. Transplantation 1998; 66: 38–42.
17. Thompson J, Thacker L, Takemoto S. CREG matching for first kidney transplants performed by Seopf Centers between October 1987 and September 1995: an analysis of outcome and prospective benefit. Transplant Proc 1997; 29(1–2): 1435–1438.
18. Thompson J, Thacker L, Takemoto S. The influence of conventional and cross-reactive group HLA matching on cardiac transplant outcome: an analysis from the United Network of Organ Sharing Scientific Registry. Transplantation 2000; 69: 2178–2186.
19. Scantlebury V, Gjertson D, Eliasziw M, et al. Influence of HLA and CREG matching in African-American primary cadaver kidney recipients: UNOS 1991–1995. Transplant Proc 1997; 29(8): 3733–3736.
20. Starzl TE, Eliasziw M, Gjertson D, et al. HLA and cross-reactive antigen group matching for cadaver kidney allocation. Transplantation 1997; 64(7): 983–991.
21. Wujciak T, Opelz G. Evaluation of the permissible mismatch concept. Transplant Int 1996; 9(suppl 1): S8–S10.
22. Fernandez-Fresnedo G, Pastor JM, Ruiz JC, et al. Differences in anti-CREG antibody formation between transplanted and non-transplanted renal patients. Transplantation 1999; 67: 1188–1193.
23. Stobbe I, Van der Meer-Prins EMW, De Lange P, et al. Cross-reactive group matching does not lead to a better allocation and survival of donor kidneys. Transplantation 2000; 70: 157–161.
24. McKenna RM, Takemoto SK. Improving HLA matching for kidney transplantation by use of CREGs. Lancet 2000; 355: 1842–1843.
25. Takemoto S, Gjertson DW, Terasaki PI. HLA matching: a comparison of conventional and molecular approaches. Clin Transpl 1992: 413–434.
26. Takemoto S, Tolleris C, Klohe E, et al. Two-year analysis of the UNOS CREG allocation variance [abstract]. Presented at the 27th Annual ASHI Meeting, 2001. Hum Immunol 2001; 62: 534.
27. Duquesnoy RJ. HLAMatchmaker: a molecularly based donor selection algorithm for highly alloimmunized patients. Transplant Proc 2001; 33: 493–497.
28. Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. Hum Immunol 2002; 63: 339–352.

29. 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: 353–363.

30. Witvliet MD, Doxiadis IIN, Schreuder GMT, et al. Validation of the HLA-Matchmaker concept [EFI Meeting abstract]. Eur J Immunogenet 2002; 29: 128.

31. 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: 364–374.

32. Mickey M, Cook D, Terasaki P. Recipient pool sizes for prioritized HLA matching. Transplantation 1989; 47: 401–403.

33. Duquesnoy R, 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(6): 889–897.

34. McKenna RM, Takemoto S, Terasaki PI. Anti-HLA antibodies after solid organ transplantation. Transplantation 2000; 69: 319–326.

35. Prasad VK, Heller G, Kernan NA, et al. The probability of HLA-C matching between patient and unrelated donor at the molecular level: estimations based on the linkage disequilibrium between DNA typed HLA-B and HLA-C alleles. Transplantation 1999; 68: 1044–1050.

# HLAMATCHMAKER: A MOLECULARLY BASED ALGORITHM FOR HISTOCOMPATIBILITY DETERMINATION. IV. AN ALTERNATIVE STRATEGY TO INCREASE THE NUMBER OF COMPATIBLE DONORS FOR HIGHLY SENSITIZED PATIENTS[1]

RENE J. DUQUESNOY,[2,4] JUDY HOWE,[2] AND STEVE TAKEMOTO[3]

*Background.* HLAMatchmaker is a computer algorithm that determines human leukocyte antigen (HLA) compatibility at the level of polymorphic amino acid triplets in antibody-accessible sequence positions. Recent studies have shown that HLA-DR–matched kidney transplant recipients with zero to two triplet mismatches had almost identical graft survival rates as those with zero HLA-A,B,DR antigen mismatches. This report describes how HLAMatchmaker can be used to identify more compatible donors for highly sensitized patients.

*Methods.* The HLAMatchmaker program was used to calculate the probability of finding a donor (PFD) with zero, one, or two triplet mismatches for 54 highly sensitized patients waiting for a kidney transplant and having panel reactive antibody (PRA) values greater than 85% and 50 randomly selected nonsensitized patients with PRA values less than 3%.

*Results.* There was a wide variability for PFD values for the two patient cohorts. If only donors with zero HLA-A,B mismatches were deemed acceptable for recipients, the median PFD of a zero-antigen mismatch was 0.046% for nonsensitized patients and 0.009% for highly sensitized patients ($P=0.007$). Half of the highly sensitized patients had a PFD below 0.01%, or fewer than 1 in 10,000 donors would have zero antigen mismatches. Application of HLAMatchmaker identified additional HLA antigens with zero-triplet mismatches for 27 patients, resulting in a 1.8-fold increase in PFD. Considering additional antigens with one-triplet or two-triplet mismatches increased the PFD by an additional 3.8-fold and 13.7-fold, respectively. Acceptable antigen mismatches for 37 of the 54 highly sensitized patients were identified by consistently negative reactions in serum screens, and their addition resulted in a 12.7-fold increase of the PFD to a median of 0.141%. Applying these acceptable antigens to the HLAMatchmaker algorithm identified additional antigens with zero or acceptable triplet mismatches and their inclusion increased the PFD by 3.3-fold to 0.347%.

*Conclusions.* HLAMatchmaker offers a valuable strategy for identifying more suitably HLA-matched donors and has the potential for alleviating the problem of accumulation of highly sensitized patients on the transplant waiting list.

The beneficial effect of human leukocyte antigen (HLA) matching on kidney transplantation is well known. A zero-HLA-A,B,DR antigen mismatch is associated with the highest survival rates of cadaver kidney transplants and mandates the obligatory sharing of such matched kidneys. Nevertheless, small proportions of cadaver kidney transplants are matched at this level (*1*). During recent years, the concept of HLA matching within cross-reacting groups (CREG) of HLA-A and HLA-B antigens has been applied to increase the allocation of suitably matched kidneys (*2–7*). Many studies have shown, however, that CREG-matched kidneys have lower graft survivals than the zero-antigen mismatches (*8–12*).