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 HLAMatchmaker 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.

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

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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*).

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A recent report describes a new matching strategy that considers the structural basis of HLA polymorphisms (*13*). HLAMatchmaker is a computer algorithm that assesses HLA compatibility by determining what and how many polymorphic amino acid triplets in antibody-accessible positions are shared between donor and recipient (*13–15*). It can identify HLA antigens that are mismatched by conventional criteria but share all their triplets with the patient and should therefore be considered fully compatible. A recent study examining patients in two separate registries found that patients with zero HLA-DR and zero to two HLA-A,B amino acid triplet mismatches had graft survivals essentially equal to zero HLA-A,B,DR antigen mismatches (*16*). These observations were made with both sensitized and nonsensitized patients and also with white and nonwhite recipients. This finding is important because it provides a strategy for identifying HLA-mismatched organs that potentially have outcomes similar to the zero-antigen mismatches.

This report describes a donor allocation strategy that is based on the beneficial effect of triplet matching on transplant outcome. The application of HLAMatchmaker increases the probability of finding compatible donors for kidney transplant candidates. These studies deal primarily with highly sensitized patients, for whom it is difficult to find conventionally matched organs (*17*).

METHODS

Patients and Laboratory Methods

This analysis was conducted with 54 highly sensitized kidney transplant candidates (including 12 African American patients) at the University of Pittsburgh Medical Center (UPMC). Their average panel reactive antibody (PRA) value was 93% (range, 87%–98%). This study included also a randomly selected group of 50 nonsensitized patients (including nine African Americans) with PRA values less than 3% PRA and who had been on the waiting list for at least 24 months.

HLA types were determined by standard lymphocytotoxicity or DNA-based methods, or both. Serum screenings were performed monthly by standard antihuman globulin-augmented (AHG) lymphocytotoxicity methods with HLA-typed panel cells from 50 to 60 donors. For each patient, the analysis determined from the pooled screening data on six or more consecutive serum samples what panel cells gave consistently negative reactions. The mismatched HLA antigens on such panel cells were considered acceptable.

Application of HLAMatchmaker to Identifying Donors with HLA Antigens Matched at the Amino Acid Triplet Level

HLAMatchmaker is a computer algorithm that assesses histocompatibility at the structural level as determined by polymorphic amino acid triplet sequences in alloantibody-accessible positions of HLA molecules (*14*). This algorithm incorporates the concepts that HLA antigens consist of strings of triplet-defined epitopes that have the potential of inducing humoral immune responses, and patients cannot produce specific antibodies to triplets on mismatched HLA antigens if such triplets are present in the same sequence location of any of the patient's own HLA molecules. Recent studies have validated these concepts (*15*, *18*).

As an example, consider patient 26, who typed as HLA-A24,-; B38,B35; Cw4,-. Table 1 shows the repertoire of self-triplets represented by the HLA antigens in the patient's phenotype. In the triplet notation system, amino acid residues are marked with the standard letter code: an uppercase letter corresponds to the residue in the numbered position of the protein sequence, whereas lowercase letters describe the nearest neighboring residues (*13*, *14*). Many triplets are marked with one or two residues because their neighboring residues are the same on all HLA-A,B,C chains and they are therefore not shown.

Five HLA antigens, A23, B39, B53, B59, and B71, are zero-triplet mismatches because all their triplets can be found in one or more of the patient's HLA antigens. For instance, the 9S triplet of A23 is present in the patient's A24 and Cw4 and the 9Y triplet of B39, B53, B59, and B71 is present in the patient's B35 and B38. Five HLA antigens are one-triplet mismatches: B51, B64, and B78 are mismatched for 171H, and B70 and B72 are mismatched for 66qIs (Table 1). Seven antigens are two-triplet mismatches and, often enough, different triplets are involved. B52 is mismatched for 66qIs and 171H; B55, B56, and B67 for 66qIy and 70aQa; B65 for 12aV and 171H; and B75 and B77 for 45Ma and 66qIs.

The triplet-matching algorithm provides a structural assessment of CREG matching. For instance, the B5 CREG (or B5C) consists of B18, B35, B51, B52, and B53 (*19*). Patient 26 types as B35 and all the other antigens in this group are considered CREG matches. These antigens have different levels of triplet compatibility: B53 has zero, B51 has one, B52 has two, and B18 has three mismatched triplets. Some of these CREG matches would be unacceptable if the patient had specific antibodies against one or more of such mismatched triplets.

Calculation of Probability of Finding a Donor with a Zero Mismatch

The probability of finding a donor (PFD) with a zero-HLA-A,B antigen mismatch can be calculated using the following formula: $PFD=(G_f$ patient's 1st HLA-A ag$+G_f$ patient's 2nd HLA-A ag$)^{2\times}(G_f$ patient's 1st HLA-B ag$+G_f$ patient's 2nd HLA-B ag$)^2$, where G_f represents the gene frequency of an HLA antigen in the donor population. The PFD calculations for the UPMC patients are based on gene frequencies in 98,800 white donors registered in the United Network for Organ Sharing (UNOS) database during 1987 to 2000. A computer program developed for this purpose can be downloaded from the HLAMatchmaker Web site at http//tpis.upmc.edu.

For patient 26 with the HLA-A24,-; B35,B38; Cw4,- phenotype, the PFD of a zero-HLA-A,B antigen mismatch is 0.007%, or 1 of 14,000 donors. The calculation of the PFD of a triplet match considers the addition of gene frequencies of other HLA antigens that are compatible at the triplet level. These PFD values were calculated with the following formula: $PFD=(G_f$ patient's 1st HLA-A ag$+G_f$ patient's 2nd HLA-A ag+Sum of G_f of other triplet-matched HLA-A antigens$)^{2\times}(G_f$ patient's 1st HLA-B ag$+G_f$ patient's 2nd HLA-B ag+Sum of G_f of other triplet-matched HLA-B antigens$)^2$.

Five antigens are zero-triplet mismatches for patient 26, namely, A23, B39, B53, B59, and B71 (Table 1). Their inclusion increases the PFD to 0.017%, or 1 of 6,000 donors. B51, B64, B70, B72, and B78 are one-triplet mismatches, and the PFD of a zero- to one-triplet mismatch is 0.038%, or 1 of 2,700 donors. With B52, B55, B56, B65, B67, B75, and B77 as two-triplet mismatches, the PFD of a zero- to two-triplet mismatch is 0.058%, or 1 of 1,700 donors. This example illustrates how triplet matching increases the availability of matched donors.

Inclusion of Serum Screening Results in Determining Probability of Finding Compatible Donors

Our serum screening analysis has focused on the identification of panel cells that give consistently negative reactions with the patient's sera. The unshared antigens on such panel cells can be considered as acceptable mismatches, and this will increase the chances for a suitably matched donor (*20*, *21*).

In the example cited above, the patient with the HLA-A24,-; B38,B35 phenotype had by AHG screening a 94% PRA with a 50-cell panel. Two panel cells with the types HLA-A23,A24;B27,B70 and HLA-A24,-;B60,- showed consistently negative reactions. Not unex-

Table 1. The self-triplet repertoire of patient 26 with the HLA-A24,–B35,B38,Cw4,–phenotype and the triplet strings of HLA antigens with zero, one, and two mismatched triplets[a]

	Position																																					
	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
Patient HLA antigens																																							
HLA-A24	S	sV	R	gR	A	Me	G	Ee	gKv	aHs	D	En	rla	aLr	A	S	G	K	R	I	tKr	W	aAh	aHv	Q	A	T	Dg	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B35	Y	aM	R	gR	A	Te	G	Rn	qlf	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	L	Ew	Y	Et	Q	dP	K	Pv	A	G	A	V	Ee
HLA-B38	Y	sV	R	gR	A	Ee	G	Rn	qlc	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	T	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-Cw4	S	sV	W	gR	A	Ge	G	Re	qKy	rQa	aD	Vn	rKl	IRg	D	P	G	N	R	I	tQr	W	aAr	aRe	R	A	T	Ew	Y	Et	Q	eH	K	Pv	A	G	A	V	Ee
Zero-triplet mismatches																																							
HLA-A23	S	sV	R	gR	A	Me	G	Ee	gKv	aHs	D	En	rla	aLr	A	S	G	K	R	I	tQr	W	aAr	aRv	L	A	T	Dg	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B39	Y	sV	R	gR	A	Ee	G	Rn	qlc	tNt	D	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	T	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B53	Y	aM	R	gR	A	Te	G	Rn	qlf	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	L	Ew	Y	Et	Q	dP	K	Pv	A	G	A	V	Ee
HLA-B59	Y	aM	R	gR	A	Ee	G	Rn	qlf	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B71	Y	aM	R	gR	A	Ee	G	Rn	qlc	tNt	Y	Es	rNl	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
One-triplet mismatches																																							
HLA-B51	Y	aM	R	gR	A	Te	G	Rn	qlf	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	H	Et	Q	dP	K	Pv	A	G	A	V	Ee
HLA-B64	Y	sV	R	gR	A	Ee	G	Rn	qlc	tNt	D	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B70	Y	aM	R	gR	A	Ee	G	Rn	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B72	Y	aM	R	gR	A	Ee	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B78	Y	aM	R	gR	A	Te	G	Rn	qlf	tNt	D	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	H	Et	Q	dP	K	Pv	A	G	A	V	Ee
Two-triplet mismatches																																							
HLA-B52	Y	aM	R	gR	A	Te	G	Re	qls	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	H	Et	Q	dP	K	Pv	A	G	A	V	Ee
HLA-B55	Y	aM	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNl	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B56	Y	aM	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B65	Y	aV	R	gR	A	Ee	G	Rn	qlc	tNt	D	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B67	Y	sV	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	T	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B75	Y	aM	R	gR	A	Ma	G	Rn	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B77	Y	aM	R	gR	A	Ma	G	Rn	qls	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee

[a] Mismatched triplets are shown by boxes.

pectedly, these negative panel cells shared some HLA antigens with the patient. The unshared HLA antigens, in this case A23, B27, B60, and B70, did not react with the patient's antibodies and were classified as acceptable antigen mismatches. The number of mismatched triplets was zero for A23, one for B70, five for B27, and nine for B60. Altogether, there were 12 mismatched triplets on these negative antigens; they are shown in the boxes of Table 2. None of them appeared to be specifically recognized by this patient's antibodies. These triplets were considered acceptable mismatches, and this information was incorporated by HLAMatchmaker to identify additional antigens with zero or acceptable triplet mismatches. Six such antigens were identified: B47, B48, B49, B50, B61, and B72. Their triplet strings are shown in Table 2.

After including A23, B27, B60, and B70 as acceptable antigen mismatches, the PFD of a zero or acceptable antigen mismatch for patient 26 increased to 0.069%, or 1 of 1,450 donors. By adding B47, B48, B49, B50, B61, and B72 as zero or acceptable triplet mismatches, the PFD of a zero or acceptable triplet mismatch increased to 0.115%, or 1 of 870 donors. This example illustrates how, after serum analysis, the application of HLAMatchmaker can increase compatible donor availability for highly sensitized patients.

RESULTS

Probabilities of Finding Donors for Highly Sensitized and Nonsensitized Patients

We have determined the PFD for 54 patients with PRA values greater than 85% and for 50 patients with PRA values less than 3%; the median values and ranges for the different match categories are shown in Table 3. The zero-HLA-A,B antigen mismatches had the lowest PFD values. The inclusion of antigens with zero-triplet mismatches increased the PFD only modestly. The PFD values were considerably higher if the one-triplet and two-triplet mismatches were included.

It is interesting to note that in all matching categories the PFD values were three to five times higher in nonsensitized than in highly sensitized patients. Three explanations can be offered as to why it seems more difficult to find well-matched donors for sensitized patients.

First, these patients have uncommon HLA phenotypes. We have determined how many times the patient's phenotype in each group had the following low-frequency antigens (G_f<2%) in UNOS white donors: HLA-A26, A29, A31, A33, A34, A36, A43, A66, A69, A74, A80 and HLA-B37, B38, B39, B41, B42, B46, B47, B48, B50, B52, B53, B54, B55, B56, B57, B59, B61, B63, B64, B67, B70, B71, B72, B73, B75, B76, B77, B78, B81. There were 59 low-frequency antigens in 54 highly sensitized patients, almost two times higher than the 31 low-frequency antigens in 50 nonsensitized patients (P=0.0009 by chi-square statistical analysis).

Second, the chances of finding a matched donor are smaller if the patient is homozygous for HLA-A or HLA-B loci, or both. Indeed, in sensitized patients, there were 23 cases in which HLA-A or HLA-B had a blank (i.e., only one defined antigen), but there were only 13 such cases in nonsensitized patients; however, this difference was statistically insignificant.

The third HLA-related factor deals with the repertoire of self-triplets in the patient. Patients with smaller repertoires can be expected to be more likely sensitized because they are exposed to a larger number of nonself-triplets. Highly sensitized patients had smaller self-triplet repertoires than nonsensitized patients (mean, 48±7 vs. 52±5 triplets; P=0.0007

TABLE 2. Triplet strings of HLA antigens with negative reactions with patient 26 sera and additional HLA antigens that were zero or acceptable triplet mismatches[a]

	Position																																						
	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
Patient HLA antigens																																							
HLA-A24	S	sV	R	gR	A	Me	G	Ee	gKv	aHs	D	En	rla	aLr	A	S	G	K	R	I	tKr	W	aAh	aHv	Q	A	T	Dg	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B35	Y	aM	R	gR	A	Te	G	Rn	qlt	tNt	Y	Es	rNl	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	L	Ew	Y	Et	Q	dP	K	Pv	A	G	A	V	Ee
HLA-B38	Y	sV	R	gR	A	Ee	G	Rn	qlc	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	T	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-Cw4	S	sV	W	gR	A	Ge	G	Re	qKy	rQa	a	Vn	rKl	IRg	D	P	G	N	R	I	tQr	W	aAr	aRe	R	A	T	Ew	Y	Et	Q	eH	K	Pv	A	G	A	V	Ee
Antigens that gave negative reactions with patient sera																																							
HLA-A23	S	sV	R	gR	A	Me	G	Ee	gKv	aHs	D	En	rla	aLr	A	S	G	K	R	I	tQr	W	aAr	aRv	L	A	T	Dg	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B27	H	sV	R	gR	A	Ee	G	Re	qlc	aKa	D	Ed	rTl	ILr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	E	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B60	H	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	Es	rNl	IRg	A	P	G	N	R	I	sQr	L	aAr	aRv	L	A	E	Ew	Y	Dk	E	dP	K	Pi	A	G	A	V	Ee
HLA-B70	Y	aM	R	gR	A	Ee	G	Rn	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
Additional antigens with acceptable triplet mismatches																																							
HLA-B47	Y	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	Ed	rTL	ILr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	E	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B48	Y	sV	R	gR	A	Ee	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	R	I	sQr	L	aAr	aRv	L	A	E	Ew	Y	Dk	E	dP	K	Pi	A	G	A	V	Ee
HLA-B49	H	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B50	H	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B61	H	sV	R	gR	T	Ke	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	R	I	tQr	W	aAr	aRv	L	A	E	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee
HLA-B72	Y	aM	R	gR	A	Ee	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee

[a] Acceptable triplet mismatches are shown in boxes.

TABLE 3. Effect of matching at the triplet level on the median values and ranges of PFD for 50 nonsensitized and 54 highly sensitized kidney transplant candidates on a local waiting list

Sensitization status	Zero-antigen mismatches	Zero-triplet mismatches	Zero- to one-triplet mismatch	Zero- to two-triplet mismatches
<3% PRA (n=50) (%)	0.046 (0.0001–0.572)	0.059 (0.001–1.284)	0.152 (0.001–0.224)	0.510 (0.004–7.179)
>85% PRA (n=54) (%)	0.009 (0.0001–0.650)	0.011 (0.0001–0.752)	0.042 (0.0001–2.238)	0.114 (0.0001–5.499)
Statistical significance by Mann-Whitney *U* test	$p=0.007$	$p=0.0017$	$p=0.0007$	$p=0.0004$

by Student two-tailed *t* test). Even if patients with HLA blanks in their phenotypes were excluded from analysis, there was still a significant difference: 51±5 versus 54±4 triplets ($P=0.009$).

A previous report describes considerable differences between the relative immunogenicity of polymorphic triplets (*15*). Triplet immunogenicity was determined as the ratio of the frequency of positive correlations (i.e., the presence of specific antibody) and the frequency of negative correlations (i.e., the absence of specific antibody) with the reactivity patterns of 127 high-PRA sera. This study yielded informative data about 77 triplets: 32 had high immunogenicity ratios (>1.0) and 45 had low immunogenicity ratios (<0.1) (*15*).

Highly sensitized patients had smaller numbers of high-immunogenicity triplets in their self-triplet repertoire than nonsensitized patients (mean, 9.3±4.4 vs. 11.4±4.2; $P=0.013$). The difference between the two patient groups became more pronounced if the analysis was performed for patients whose HLA-A,B phenotypes had no blanks: 8.7±4.6 versus 11.6±4.2 ($P=0.005$). These patient groups showed only minor differences in the numbers of low immunogenicity triplets (mean, 22.3±4.8 vs. 24.0±3.5; $P=0.04$) and for the HLA-A,B phenotypes without blanks (24.5±3.6 vs. 24.8±3.1; $P=0.65$). These findings indicate that the smaller repertoires of self-triplets in highly sensitized patients reflect primarily lower numbers of immunogenic triplets. Altogether, a more common occurrence of low-frequency antigens in the HLA phenotype; a greater homozygosity for HLA-A or HLA-B antigens, or both; and a lower proportion of immunogenic triplets in the self-triplet repertoires seem more prevalent in highly sensitized patients.

Triplet Matching for Highly Sensitized Patients

This analysis has focused on highly sensitized patients because it is much more difficult to find HLA-matched donors for them. In this group of 54 highly sensitized patients, there was considerable variability of PFD values for the zero-HLA-A,B antigen mismatches (Fig. 1). The median PFD was 0.011% and the range was 0.0001% to 0.65%. For 27 patients, the PFD was below 0.01%, or less than 1 of 10,000 donors; and only 9 had PFD above 0.1%, or more than 1 of 1,000 donors. Figure 1 shows also each patient's HLA-A,B phenotype, racial background, and PRA value. It can be readily noted that low PFD values pertain to phenotypes with many uncommon antigens. Although there was a trend toward lower PFD values in African Americans, there was no relationship between PFD and PRA. These results clearly indicate that it is unlikely that a zero-HLA-A,B antigen mismatch will become available for most of these highly sensitized patients.

HLAMatchmaker permits the inclusion of HLA antigens with compatible triplets, and Figure 2 illustrates how matching at the triplet level will increase the PFD for highly sensitized patients. The PFD values are shown on a log10 scale, and the differently shaded vertical bars show the cumulative effects of the zero-triplet, one-triplet, and two-triplet mismatches on the PFD for each patient.

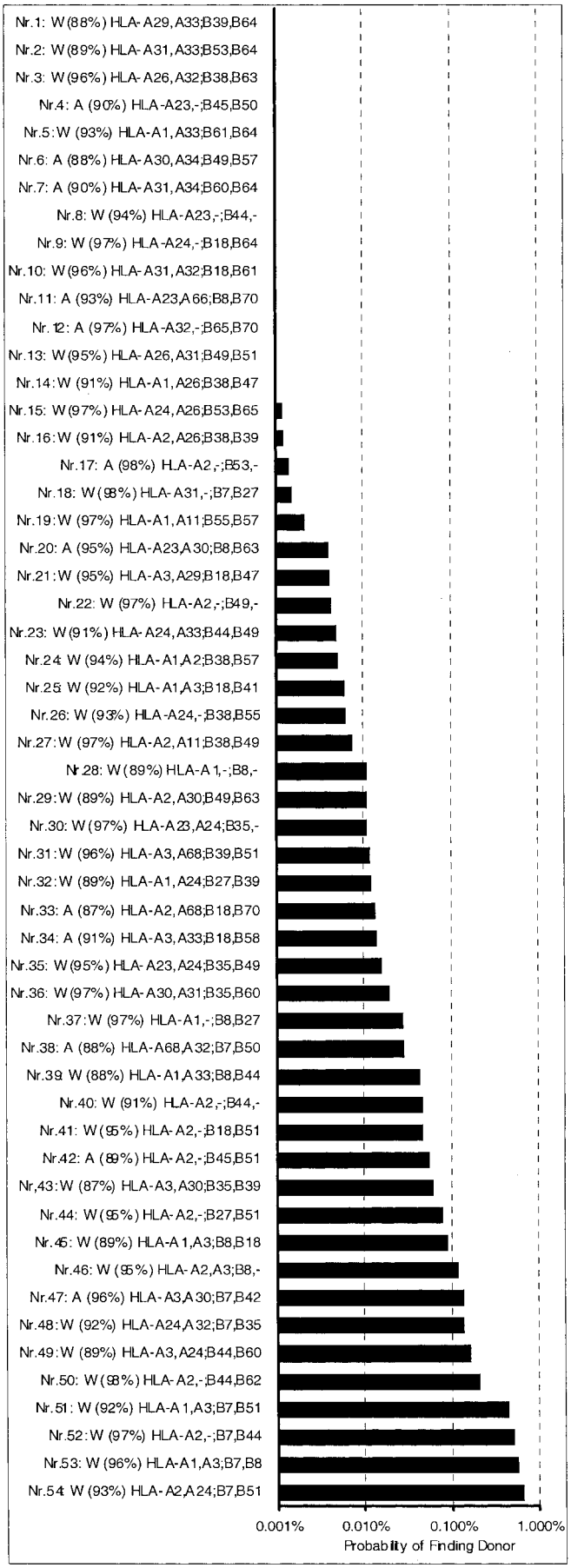
For one half of the patients, there were mismatched HLA antigens with zero-triplet mismatches; their inclusion increased the PFD for these patients by a median of 1.8-fold (range, 1.1–83.8). As stated above, the PFD of a zero-HLA-A,B antigen mismatch was below 0.01% (or <1 of 10,000 donors) for 27 patients. After including zero-triplet mismatches, there were 24 patients for whom the PFD remained below 0.01%. These findings indicate that the adding of a zero-triplet mismatch had a rather modest effect on matched donor availability.

HLA antigens with one-triplet mismatches were identified for 45 patients (83%). The PFD increased by a median of 3.8-fold (range, 1.1-fold–170.7-fold) after the one-triplet mismatches had been added to the zero-triplet mismatches. For 18 patients, the PFD of a zero- to one-triplet mismatch was still below 0.01%.

HLA antigens with two mismatched triplets were identified for 50 patients (93%). The PFD of a zero- to two-triplet mismatch was 13.7-fold higher than that of a zero- to one-triplet mismatch (range, 1.2-fold–502.8-fold). For only eight patients, the PFD of a zero- to two-triplet mismatch remained below 0.01%.

This analysis addressed the question of how many patients in each match category would have a greater than 0.1% PFD (i.e., >1 in 1,000 donors would be compatible with the patient). This was the case for 9 of 54 patients (16%) in the zero-antigen mismatch category, 13 (24%) of the zero-triplet mismatches, 21 (38%) of the zero- to one-triplet mismatches, and 28 (51%) of the zero- to two-triplet mismatches.

These findings suggest that triplet matching will markedly increase donor availability. This applies especially to the zero- to one-triplet mismatches and even more to the zero- to two-triplet mismatches. A potential limitation is that a sensitized patient may have antibodies to a mismatched triplet; this problem can be addressed by performing a detailed serum analysis for HLA-specific alloantibodies.



Probability of Finding Donors for Sensitized Patients after Serum Screening Analysis

For 37 of 54 highly sensitized patients, we identified acceptable antigen mismatches because their monthly sera demonstrated consistently negative reactions with panel cells expressing such HLA-A,B antigens. Twenty-nine patients were white and eight patients were African Americans. As expected, the median PFD went from 0.011% for the zero-antigen mismatches to 0.141% for the zero or acceptable antigen mismatches, or a 12.3-fold increase (Fig. 3). There were 21 patients (58%) for whom the PFD for a zero or acceptable antigen mismatch was greater than 0.1%, or more than 1 of 1,000 donors.

Negative reactions with HLA antigens mean that these antigens have acceptable triplets that are not specifically recognized patient antibodies. HLAMatchmaker applies this information to identify additional antigens that are zero or acceptable triplet mismatches. Inclusion of such antigens increased the PFD to a median of 0.347%, or by 3.3-fold (Fig. 3). In this match category, there were 35 of 37 patients (95%) for whom the PFD exceeded 0.1%. These data illustrate how matching at the triplet level will increase the number of suitable matches if acceptable antigens have been found by serum screening analysis.

HLAMatchmaker can also identify donor HLA antigens that are mismatched for one or two triplets, whereas the other triplets are shared or acceptable mismatches. The inclusion of such antigens will further increase the PFD (data not shown), but one must ascertain that the patient's antibodies do not react with such mismatched triplets.

DISCUSSION

This report describes how the HLAMatchmaker algorithm can be applied to increase the allocation of compatible organs to highly sensitized patients. For each patient on a local waiting list, we have determined a PFD as a quantitative assessment of kidney allocation from compatible donors. Although many multicenter transplant programs have policies of mandatory sharing of cadaver kidneys with zero-HLA-A,B,DR antigen mismatches, our experience, summarized in Figure 1, shows that many highly sensitized patients are unlikely to receive transplants with such matches. Our data are limited to HLA-A and HLA-B antigens, and the requirement of a zero-HLA-DR mismatch would decrease the chances of a matched transplant even more. Thus, alternative matching strategies must be explored that would increase the number of HLA-compatible donors for highly sensitized patients. HLAMatchmaker offers an opportunity because, among the HLA-DR–matched kidney transplants, HLA-A,B matching at the amino acid triplet level appears to ensure the same high graft survival rates as the zero-antigen mismatches (*16*).

FIGURE 1. PFD with a conventional zero-HLA-A,B antigen mismatch for 54 highly sensitized patients. Each patient is identified by a unique number, racial background (A, African American; W, white), PRA values determined by AHG screening, and HLA-A,B phenotype. Patients are sorted from the lowest to the highest PFD values expressed as percentages on a log10 scale. No bars are seen for patients 1 through 14, because their PFD values were below 0.001%, or fewer than 1 of 100,000 donors.

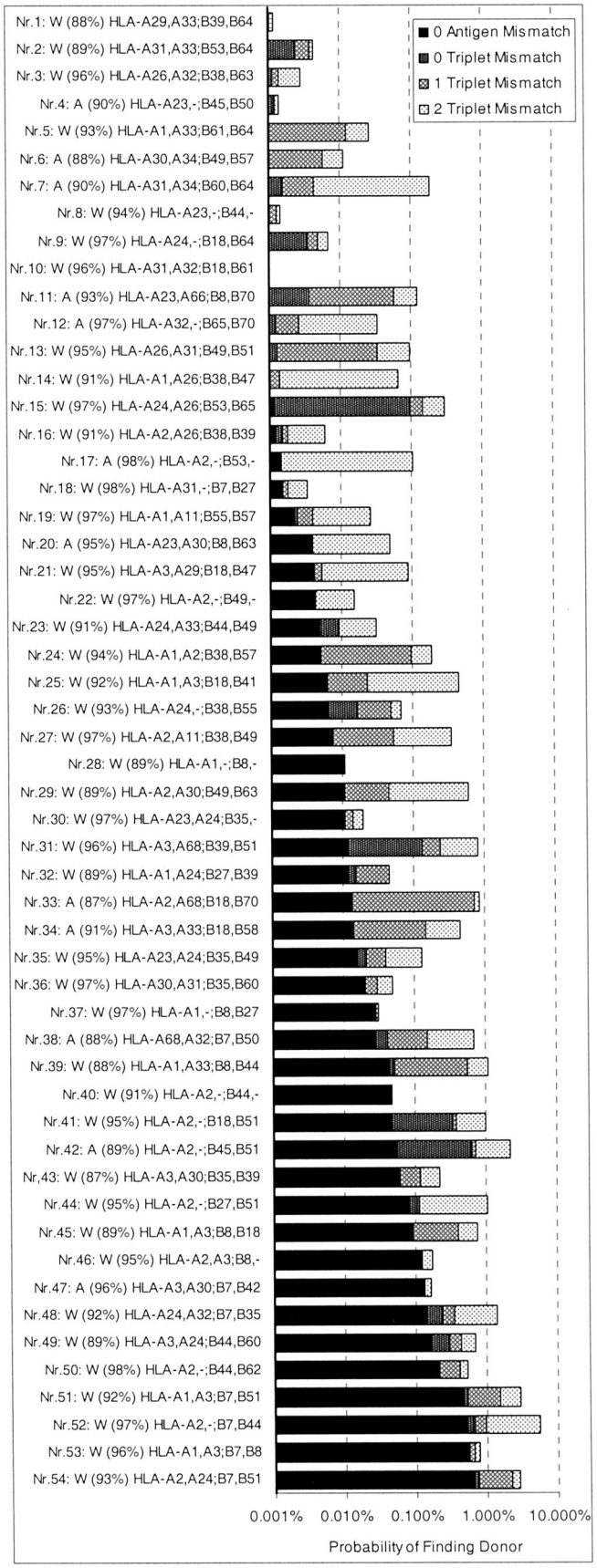


FIGURE 2. Effect of triplet matching on the PFD for 54 highly sensitized patients. (*Black bars*) PFD values for the zero-HLA-A,B antigen mismatches; (*stacked bars*) cumulative effects of matching at the various triplet levels, namely, from zero, zero to one, and zero to two triplets.

In the UNOS point system of cadaver kidney allocation, highly sensitized patients receive four extra points to improve their ranking status on the waiting list. A most revealing aspect of our analysis is the wide range of PFD values for the zero-HLA-A,B antigen mismatches. For about one half of the highly sensitized patients, the PFD was below 0.01%, or less than 1 of 10,000 donors. It seems evident that many highly sensitized patients have practically no chance of receiving transplants with a zero-HLA-A,B–mismatched kidney. Such patients can be found on almost any local waiting list, and awarding them four extra points seems to offer them no special benefit regarding organ allocation. The use of a PFD-based point system would permit a more equitable organ allocation within the group of highly sensitized patients.

The application of the triplet matching algorithm will increase the PFD for many highly sensitized patients, especially if HLA antigens with one or two mismatched triplets are included. It must of course be ascertained that the patient's antibodies do not react with such mismatched triplets, and this can be determined by serum screening analysis with informative HLA-typed panels and by sensitive crossmatches with donor cells.

This study has several limitations. The assignment of triplets to HLA antigens lacks 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 more accurate assignments of polymorphic triplets. The analysis did not consider triplet polymorphism of HLA-C antigens because serologic typing does not yield reliable HLA-C typing information. With currently available DNA typing methods for HLA-C and the application of the triplet-matching algorithm, it will become possible to fully assess the role of HLA-C antigens in humoral sensitization and in compatible donor searches for highly sensitized patients.

A principal goal of serum screening should be the identification of HLA antigens that are acceptable mismatches for sensitized patients. Such antigens can be identified on panel cells that give negative reactions by sensitive screening methods. These negative reactions must be consistent among the different serum samples from the patient. Acceptable HLA antigen mismatches may have mismatched triplets that are apparently not recognized by patient antibodies. HLA-Matchmaker can incorporate this information to identify additional HLA antigens with zero or acceptable triplet mismatches, and this will lead to a further increase of the PFD for most patients.

This analysis was performed on highly sensitized patients, and most of them had serum PRA values that were above 90%. In routine laboratory settings, screening of such high PRA sera yields little information about antibody specificity and, often enough, there are no panel cells that exhibit negative reactions consistently enough that acceptable antigen mismatches can be identified. This was the case for about one third of highly sensitized patients on our waiting list. The use of selected panel cells that are mismatched for only one HLA antigen has been most useful in the determination of accept-

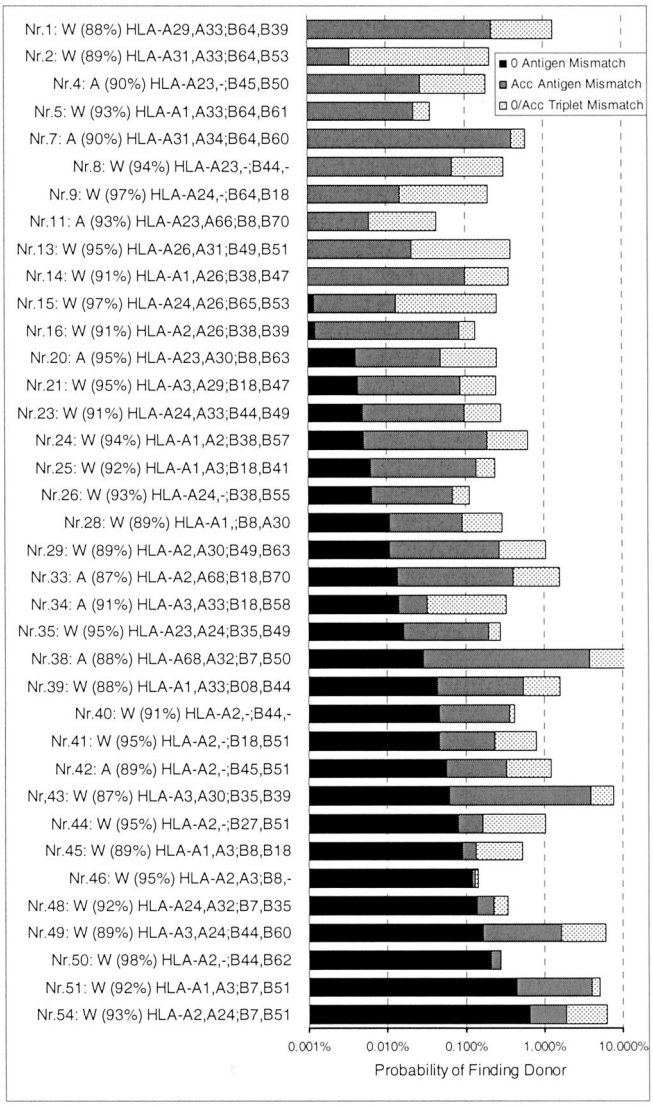


FIGURE 3. PFD values for 36 highly sensitized patients whose serum screens yielded information about acceptable HLA antigen mismatches. (*Black bars*) PFD values for the zero-HLA-A,B antigen mismatches; (*stacked bars*) cumulative effects of matching for acceptable antigen mismatches and zero or acceptable triplet mismatches.

able antigen mismatches (*20*, *21*), but this approach requires access to a large inventory of HLA-typed panel cells. The use of HLAMatchmaker permits an effective strategy of selecting informative panel cells that are mismatched for only a few triplets because such cells are more likely to give negative reactions with the patient's serum (*22*). Other investigators have also suggested the usefulness of HLAMatchmaker in finding crossmatch-negative donors for high-PRA patients (*23*, *24*).

This analysis has shown that the PFD values for zero-antigen and the zero-, one-, or two-triplet mismatches were significantly lower for the highly sensitized patients than the nonsensitized patients. This means that it is more difficult to find HLA-compatible donors for highly sensitized patients. We have identified three HLA-related factors that seem to contribute to this problem. The HLA phenotypes of highly sensitized patients contain more low-frequency antigens and seem to exhibit a greater homozygosity for HLA-A or HLA-B antigens, or both. Also, their self-triplet repertoires have smaller proportions of immunogenic triplets. This means that during a sensitizing event such as a transplant, blood transfusion, or pregnancy, these patients might have been exposed to greater numbers of foreign HLA antigens, including those that carry more immunogenic triplets. This information about the patient's HLA phenotype might be useful in estimating the risk for HLA sensitization induced by random donor blood transfusions.

CONCLUSION

The application of HLAMatchmaker coupled with comprehensive serum screening will increase the probability of finding compatible donors. This strategy has the potential for alleviating the problem of accumulation of highly sensitized patients on the transplant waiting list. The Eurotransplant Reference Laboratory has recently adapted the HLAMatchmaker algorithm in the mandatory allocation program of organs with acceptable mismatches (*25*).

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. 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.
3. 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.
4. Takemoto S, Gjertson DW, Terasaki PI. HLA matching: Maximizing the number of compatible transplants. Clin Transpl 1993: 521–531.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. Wujciak T, Opelz G. Evaluation of the permissible mismatch concept. Transpl Int 1996; 9(suppl 1): S8–S10.
11. 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.
12. 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.
13. Duquesnoy RJ. HLAMatchmaker: A molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. Hum Immunol 2002; 63: 339–352.
14. Duquesnoy RJ. HLAMatchmaker: A molecularly based donor selection algorithm for highly alloimmunized patients. Transplant Proc 2001; 33: 493–497.
15. 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.
16. Duquesnoy RJ, Takemoto S, De Lange P, et al. 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. Transplantation 2003; 75(6): 884–889.
17. Wolfe R, Ashby V, Milford E, et al. Differences in access to cadaveric renal transplantation in the United States. Am J Kidney Dis 2000; 36: 1025–1033.
18. Duquesnoy RJ, Wituliet M, Doxiadix IIN, de Fijter H, Claas FHJ. HLA-Matchmaker-based strategy to identify acceptable HLA-Class I mismatches for highly sensitized kidney transplant candidates. Transpl Int 2003 (in press).
19. Rodey GE, Fuller TC. Public epitopes and the antigenic structure of the HLA molecules [review]. Crit Rev Immunol 1987; 7(3): 229–267.
20. Claas FH, van Leeuwen A, van Rood JJ. Hyperimmunized patients do not need to wait for an HLA identical donor [review]. Tissue Antigens 1989; 34(1): 23–29.
21. Claas FHJ, De Meester J, Witvliet MD, et al. Acceptable HLA mismatches for highly immunized patients. Rev Immunogenet 1999; 1: 351–358.
22. Duquesnoy R, Marrari M, Awadalla Y, et al. HLAMatchmaker based serum analysis for identifying acceptable and unacceptable HLA antigen mismatches for highly sensitized patients [Abstract]. Hum Immunol 2002; 63(Suppl): S85.
23. Lobashevsky AL, Senkbeil RW, Shoaf JL, et al. The number of amino acid residues mismatches correlates with flow cytometry crossmatching results in high PRA patients. Hum Immunol 2002; 63: 365–373.
24. Vorhaben R, Pervis K, Lavingia B, et al. Predictive values for a negative crossmatch of sensitized renal recipients: Triplet amino acid matching vs CREG matching [abstract]. Hum Immunol 2001; 62(suppl 1): S32.
25. Class FHJ. Predictive parameters for in vivo alloreactivity. Transpl Immunol 2002; 10: 137–142.