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HLAMatchmaker-based strategy to identify acceptable HLA class I mismatches for highly sensitized kidney transplant candidates

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Abstract HLAMatchmaker determines HLA compatibility at the level of polymorphic amino acid triplets in antibody-accessible sequence positions. Recent studies have shown that among HLA-DR-matched kidney transplants, the HLA-A,B antigen mismatches which are compatible at the triplet level have almost identical graft survival rates as the zero-HLA-A,B antigen mismatches. This finding provides the basis of a new strategy to identify HLA-mismatched organs that have similar success rates as the zero-HLA-antigen mismatches. This report describes how in conjunction with the Acceptable Mismatch program in Eurotransplant, HLAMatchmaker can expand the pool of potential donors for highly sensitized patients, for whom it is very difficult to find a compatible transplant. Sera from 35 highly sensitized kidney transplant candidates with an average PRA of 96% were screened by lymphocytotoxicity with HLA-typed panels that included cells that were selectively mismatched for one or two HLA antigens for each patient. Acceptable and unacceptable HLA-A,B antigen mismatches were determined from the serum reactivity with the cell panel. HLAMatchmaker analysis was applied to identify additional HLA class I antigens that were matched at the triplet level. For each patient, we calculated the probability of finding a donor (PFD) in the different match categories from HLA gene frequencies in the kidney donor population. The median PFD for a zero-antigen mismatch was 0.025%. Matching at the triplet level increased the median PFD to 0.037% ($P=0.008$). The median PFD was 0.058% for a 0-1-triplet mismatch and 0.226% for a 0-2-triplet mismatch. Serum screening identified acceptable antigen mismatches for 28 of 35 highly sensitized patients, and the median PFD increased to 0.307% for a zero/acceptable antigen mismatch. The application of HLAMatchmaker permitted for 33 patients (or 92%) the identification of additional antigens that were acceptable at the triplet level, and the median PFD for a zero/acceptable triplet mismatch went up to 0.425%. Inclusion of one-triplet mismatches increased the median PFD to 1.112%. Validation studies have shown that patient sera reacted with none of the zero-triplet-mismatched antigens, 8-13% of the one-triplet mismatches, and 12-19% of the two-triplet mismatches. Although most antigens with one or two mismatched triplets appear acceptable to highly sensitized patients, a serum analysis must ascertain that the patient's antibodies do not recognize such mismatched triplets. HLAMatchmaker offers a useful strategy of identifying more

donors with acceptable HLA mismatches and could alleviate the problem of accumulation of highly sensitized patients on the transplant waiting list.

Keywords HLAMatchmaker · HLA antigen · Triplet · Kidney transplantation · Compatible organ donor

Introduction

The accumulation of highly sensitized patients on the kidney transplant waiting list presents a growing problem for many transplant programs. Not only is it difficult to find a suitable crossmatch-negative donor, but transplants in such patients are also generally less successful. Several strategies have been used to enhance transplantation of highly sensitized patients. One approach is to reduce serum antibody reactivity by exchange plasmapheresis and/or intravenous gammaglobulin treatment [1, 2, 3]. Many centers have participated in multi-laboratory strategies, such as the ROP trays in the United States [4], the SOS scheme in the United Kingdom [5], and the HIT program in Europe [6], to crossmatch sera from many sensitized patients so that the chances of finding compatible donors can increase.

Another approach is the Acceptable Mismatch program used in Eurotransplant [7, 8]. For each highly sensitized patient, serum screening includes the selection of specific panel cells that have only one HLA-A or HLA-B mismatch with the patient. Negative reactions identify HLA antigens that do not react with the patient's antibodies and are therefore acceptable mismatches. In this way, acceptable mismatches can even be found in patients whose sera have 100% PRA. This screening strategy requires access to a very large inventory of HLA-typed panel cells and can be less successful for patients with uncommon HLA antigens in their phenotypes. These problems can be overcome by adding the HLAMatchmaker algorithm to the Acceptable Mismatch program.

HLAMatchmaker is a computer algorithm that assesses HLA compatibility at the structural level by determining which and how many polymorphic amino acid triplets in antibody-accessible positions are shared between the donor and recipient [9, 10, 11]. It can identify HLA antigens that are mismatched by conventional criteria but share all their triplets with the patient and, therefore, such antigens should be considered fully compatible. This program has been particularly useful in the identification of compatible donors for highly sensitized patients.

Recent studies on two large multi-center databases of zero-HLA-DR-mismatched kidney transplants have shown that HLA matching at the triplet level benefits transplant outcome [12]. Grafts with 0-2-triplet mismatches had almost identical graft survival rates as the zero-HLA-A,B antigen mismatches defined by conventional criteria. The beneficial effect of triplet matching was seen for both non-sensitized and sensitized patients. The practical aspect of this finding is that matching at the triplet level will increase the availability of compatible donors, and this would especially benefit highly sensitized transplant candidates.

This report describes the application of HLAMatchmaker to the Acceptable Mismatch program for highly sensitized patients. This algorithm determines triplet mismatch acceptability from the negative reactions of patient sera with the HLA-typed panel and subsequently identifies additional antigens that are matched at the triplet level. This strategy increases the probability of finding suitably matched donors for highly sensitized patients.

Patients and methods

Patients

This analysis was conducted on 35 highly sensitized kidney transplant candidates evaluated by the Eurotransplant Reference Laboratory. Their average PRA was 96% (range: 85-100%). HLA typing was done by lymphocytotoxicity and/or molecularly based methods. Serum samples were screened by direct lymphocytotoxicity with a panel of 50-60 HLA-typed cells. These sera were also tested with selected HLA-typed panel cells mismatched for one HLA-A or HLA-B antigen with the patient. Serum reactivity with such panel cells will permit the identification of acceptable and unacceptable antigen mismatches. This serum screening strategy is a component of the Eurotransplant Acceptable Mismatch program for highly sensitized kidney transplant candidates [8, 13].

Principle of the HLAMatchmaker algorithm

HLAMatchmaker considers each HLA antigen as a string of amino acid triplet-defined epitopes that have the potential of inducing humoral immune responses. Allosensitized patients cannot produce 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 [9, 10, 11]. This algorithm assesses compatibility between donor and recipient by determining which triplets on donor HLA antigens are present or absent on any of the patient's own HLA antigens. It can identify HLA antigens that share all their triplets with the HLA phenotype of the patient and, therefore, must be considered fully compatible at the epitope level. Other HLA antigens are considered acceptable if the patient's antibodies do not recognize any mismatched triplet of such antigens.

Calculation of the probability of finding a matched donor

The inclusion of HLA antigens with triplet matches will increase the chances of finding donors with the appropriate HLA phenotypes.

Table 1 The self-triplet repertoire of patient 25 with the HLA-A1,All; B7,B52; Cw7, - phenotype and the triplet let strings of four HLA antigens that are zero-triplet let mismatches

		Position:	9	11	16	41	45	56	62	66	70	74	76	80	82	90	105	107	127
Self-Antigen	A1	A*0101	9F	11Sv	16Gr	41A	45kMe	56G	62Qe	66rNm	70aHs	74D	76An	80gTL	82lRg	90D	105P	107G	127N
Self-Antigen	A11	A*1101	9Y	11Sv	16Gr	41A	45rMe	56G	62Qe	66rNv	70aQs	74D	76Vd	80gTL	82lRg	90D	105P	107G	127N
Self-Antigen	B7	B*0702	9Y	11Sv	16Gr	41A	45rEe	56G	62Rn	66qIy	70aQa	74D	76Es	80rNl	82lRg	90A	105P	107G	127N
Self-Antigen	B52	B*5201	9Y	11Am	16Gr	41A	45rTe	56G	62Re	66qIs	70tNt	74Y	76En	80rIa	82aLr	90A	105P	107G	127N
Self-Antigen	Cw7	C*0701	9D	11Av	16Gr	41A	45rGe	56G	62Re	66qNy	70rQa	74AD	76Vs	80rNl	82lRg	90D	105P	107G	127N
0 Triplet Mismatch	A36	A*3601	9F	11Sv	16Gr	41A	45kMe	56G	62Qe	66rNm	70aHs	74D	76An	80gTL	82lRg	90D	105P	107G	127N
0 Triplet Mismatch	B55	B*5501	9Y	11Am	16Gr	41A	45rEe	56G	62Rn	66qIy	70aQa	74D	76Es	80rNl	82lRg	90A	105P	107G	127N
0 Triplet Mismatch	B70	B*1509	9Y	11Am	16Gr	41A	45rEe	56G	62Re	66qIs	70tNt	74Y	76Es	80rNl	82lRg	90A	105P	107G	127N
0 Triplet Mismatch	B72	B*1503	9Y	11Am	16Gr	41A	45rEe	56G	62Re	66qIs	70tNt	74Y	76Es	80rNl	82lRg	90A	105P	107G	127N
		Position:	131	142	144	147	149	151	156	158	163	166	171	177	180	183	186	193	199
Self-Antigen	A1	A*0101	131R	142I	144tKr	147W	149aVh	151vHa	156R	158V	163R	166Dg	171Y	177Et	180Q	183P	186K	193Pi	199A
Self-Antigen	A11	A*1101	131R	142I	144tKr	147W	149aAh	151aHa	156Q	158A	163R	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
Self-Antigen	B7	B*0702	131R	142tI	144tQr	147W	149aAr	151aRe	156R	158A	163E	166Ew	171Y	177Dk	180E	183P	186K	193Pi	199A
Self-Antigen	B52	B*5201	131S	142tI	144tQr	147W	149aAr	151aRe	156L	158A	163L	166Ew	171H	177Et	180Q	183P	186K	193Pv	199A
Self-Antigen	Cw7	C*0701	131R	142tI	144tQr	147L	149aAr	151aRa	156L	158A	163T	166Ew	171Y	177Et	180Q	183Ep	186K	193Pl	199A
0 Triplet Mismatch	A36	A*3601	131R	142I	144tKr	147W	149aVh	151vHa	156R	158V	163T	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
0 Triplet Mismatch	B55	B*5501	131S	142tI	144tQr	147W	149aAr	151aRe	156L	158A	163T	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
0 Triplet Mismatch	B70	B*1509	131S	142tI	144tQr	147W	149aAr	151aRe	156L	158A	163L	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
0 Triplet Mismatch	B72	B*1503	131S	142tI	144tQr	147W	149aAr	151aRe	156L	158A	163L	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A

The probability of finding a donor (PFD) with such matches can be 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 × (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 whereby G_f represents the gene frequency of an HLA antigen in the donor population. The PFD calculations were made with gene frequencies in 28,500 Eurotransplant donors during the 1987–1999 period. A PFD calculation software program can be downloaded free of charge from the HLAMatchmaker website (http://tpis.upmc.edu).

HLAMatchmaker-based strategy of identifying acceptable mismatches

The following example illustrates the application of HLAMatchmaker to the Acceptable Mismatch program in Eurotransplant. Patient 25 types as HLA-A1,A11; B7,B52; Cw7,- and Table 1 shows his repertoire of self-triplets. Four HLA antigens, A36, B55, B70, and B72, are zero-triplet mismatches because all their triplets can be found in one or more of the patient's HLA antigens*. Any combination of these antigens and the patient's own antigens would constitute a compatible HLA phenotype of a potential donor. The PFD with such zero-triplet match would be 0.110%, whereas the PFD of a zero-HLA-A,B-antigen mismatch would be 0.084%.

HLAMatchmaker identified for this patient eight HLA antigens with one mismatched triplet and 11 HLA antigens with two mismatched triplets. Table 2 shows which triplets are mismatched. For instance, B51 and B78 are both mismatched for 66qIf, and the two B14 splits B64 and B65 as well as B71 are mismatched for 66qIc. B35, B53, and B59 have the same pair of mismatched triplets: 66qIf + 151aRv, whereas B48 and B81 are mismatched for 144sQr and 151aRv. The data in Table 1 and Table 2 also illustrate triplet match differences between serologically defined antigens in the B15 group. These antigens cross-react with the patient's B52 antigen. B70 and B72 are zero-triplet mismatches, B71 is mismatched for 66qIc, and B75 and B77 have a 45rMa mismatch. B63 has 45rMa + 70aSa, and B62 and B76 have 45rMa + 156 W.

Previous studies have shown that HLA-A,B-mismatched kidney transplants with 0-2-triplet mismatches have similar graft survivals as the zero-HLA-A,B-antigen mismatches [14]. These findings indicate that one-triplet and two-triplet mismatches confer a high degree of HLA compatibility. For this patient, the PFD is 0.401% for a 0-1-triplet mismatch and 1.022% for a 0-2-triplet mismatch.

Table 2 HLA antigens that have one and two mismatched triplets for the HLA-A1,All; B7,B52; Cw7, - phenotype

Antigens with One-Triplet Mismatch	Mismatched Triplet	Antigens with Two-Triplet Mismatches	Mismatched Triplets
B51 (B*5101)	66qIf	B18 (B*1801)	9H, 151aRv
B56 (B*5601)	151aRv	B35 (B*3501)	66qIf, 151aRv
B64 (B*1401)	66qIc	B48 (B*4801)	144sQr, 151aRv
B65 (B*1402)	66qIc	B53 (B*5301)	66qIf, 151aRv
B71 (B*1510)	66qIc	B54 (B*5401)	45GeV, 151aRv
B75 (B*1502)	45rMa	B59 (B*5901)	66qIf, 151aRv
B77 (B*1513)	45rMa	B62 (B*1501)	45rMa, 156W
B78 (B*7801)	66qIf	B63 (B*1516)	45rMa, 70aSa
		B67 (B*6701)	151aRv, 158T
		B76 (B*1511)	45rMa, 156W
		B81 (B*8101)	144sQr, 151aRv

* 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. For instance, the triplet 45kMe represents a methionine residue (M) in position 45 with lysine (k) in position 44 and glutamic acid (e) in position 46 in the amino acid sequence. 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. As an example, 12aM represents an alanine residue in position 11 and a methionine residue in position 12. The triplet 9S has a serine in position 9, and the two neighboring monomorphic residues are not shown.

Table 3 Determination of acceptable triplet mismatches for HLA antigens A30 and B8 that gave negative reactions wirb the sera for patient 25 and the description of triplet strings of additional HLA antigens that are zero/acceptable mismatches

		Position:	9	11	16	41	45	56	62	66	70	74	76	80	82	90	105	107	127
Self-Antigen	A1	A*0101	9F	11Sv	16Gr	41A	45kMe	56G	62Qe	66rNm	70aHs	74D	76An	80gTL	82lRg	90D	105P	107G	127N
Self-Antigen	A11	A*1101	9Y	11Sv	16Gr	41A	45rMe	56G	62Qe	66rNv	70aQs	74D	76Vd	80gTL	82lRg	90D	105P	107G	127N
Self-Antigen	B7	B*0702	9Y	11Sv	16Gr	41A	45rEe	56G	62Rn	66qIy	70aQa	74D	76Es	80rNl	82lRg	90A	105P	107G	127N
Self-Antigen	B52	B*5201	9Y	11Am	16Gr	41A	45rTe	56G	62Re	66qIs	70tNt	74Y	76En	80rIa	82aLr	90A	105P	107G	127N
Self-Antigen	Cw7	C*0701	9D	11Av	16Gr	41A	45rGe	56G	62Re	66qNy	70rQa	74AD	76Vs	80rNl	82lRg	90D	105P	107G	127N
Negative Antigen	A30	A*3001	**9S**	11Sv	**16S**	41A	45rMe	**56R**	62Qe	66rNv	70aQs	74D	76Vd	80gTL	82lRg	90A	**105S**	107G	127N
Negative Antigen	B8	B*0801	9D	11Am	16Gr	41A	45rEe	56G	62Rn	**66qIf**	70tNt	74D	76Es	80rNl	82lRg	90A	105P	107G	127N
Zero/Acc Triplet Mismatch	B35	B*3501	9Y	11Am	16Gr	41A	45rTe	56G	62Rn	**66qIf**	70tNt	74Y	76Es	80rNl	82lRg	90A	105P	107G	127N
Zero/Acc Triplet Mismatch	B42	B*4201	9Y	11Sv	16Gr	41A	45rEe	56G	62Rn	66qIy	70aQa	74D	76Es	80rNl	82lRg	90A	105P	107G	127N
Zero/Acc Triplet Mismatch	B51	B*5101	9Y	11Am	16Gr	41A	45rTe	56G	62Rn	**66qIf**	70tNt	74Y	76En	80rIa	82aLr	90A	105P	107G	127N
Zero/Acc Triplet Mismatch	B53	B*5301	9Y	11Am	16Gr	41A	45rTe	56G	62Rn	**66qIf**	70tNt	74Y	76En	80rIa	82aLr	90A	105P	107G	127N
Zero/Acc Triplet Mismatch	B56	B*5601	9Y	11Am	16Gr	41A	45rEe	56G	62Rn	66qIy	70aQa	74D	76Es	80rNl	82lRg	90A	105P	107G	127N
Zero/Acc Triplet Mismatch	B59	B*5901	9Y	11Am	16Gr	41A	45rEe	56G	62Rn	**66qIf**	70tNt	74Y	76En	80rIa	82aLr	90A	105P	107G	127N
Zero/Acc Triplet Mismatch	B78	B*7801	9Y	11Am	16Gr	41A	45rTe	56G	62Rn	**66qIf**	70tNt	74D	76Es	80rNl	82lRg	90A	105P	107G	127N

		Position:	131	142	144	147	149	151	156	158	163	166	171	177	180	183	186	193	199
Self-Antigen	A1	A*0101	131R	142I	144tKr	147W	149aVh	151vHa	156R	158V	163R	166Dg	171Y	177Et	180Q	183P	186K	193Pi	199A
Self-Antigen	A11	A*1101	131R	142I	144tKr	147W	149aAh	151aHa	156Q	158A	163R	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
Self-Antigen	B7	B*0702	131R	142tI	144tQr	147W	149aAr	151aRe	156R	158A	163E	166Ew	171Y	177Dk	180E	183P	186K	193Pi	199A
Self-Antigen	B52	B*5201	131S	142tI	144tQr	147W	149aAr	151aRe	156L	158A	163L	166Ew	171H	177Et	180Q	183P	186K	193Pv	199A
Self-Antigen	Cw7	C*0701	131R	142tI	144tQr	147L	149aAr	151aRa	156L	158A	163T	166Ew	171Y	177Et	180Q	183Ep	186K	193Pi	199A
Negative Antigen	A30	A*3001	131R	142I	144tQr	147W	149aAr	**151aRw**	156L	158A	163T	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
Negative Antigen	B8	B*0801	131R	142tI	144tQr	147W	149aAr	**151aRv**	**156D**	158A	163T	166Ew	171Y	**177Dt**	180E	183P	186K	193Pi	199A
Zero/Acc Triplet Mismatch	B35	B*3501	131S	142tI	144tQr	147W	149aAr	**151aRv**	156L	158A	163L	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
Zero/Acc Triplet Mismatch	B42	B*4201	131R	142tI	144tQr	147W	149aAr	**151aRv**	**156D**	158A	163T	166Ew	171Y	**177Dt**	180E	183P	186K	193Pi	199A
Zero/Acc Triplet Mismatch	B51	B*5101	131S	142tI	144tQr	147W	149aAr	151aRe	156L	158A	163L	166Ew	171H	177Et	180Q	183P	186K	193Pv	199A
Zero/Acc Triplet Mismatch	B53	B*5301	131S	142tI	144tQr	147W	149aAr	**151aRv**	156L	158A	163L	166Ew	171Y	177Et	180Q	183P	186K	193Pv	199A
Zero/Acc Triplet Mismatch	B56	B*5601	131S	142tI	144tQr	147W	149aAr	**151aRv**	156L	158A	163L	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
Zero/Acc Triplet Mismatch	B59	B*5901	131S	142tI	144tQr	147W	149aAr	**151aRv**	156L	158A	163T	166Ew	171Y	177Et	180Q	183P	186K	193Pi	199A
Zero/Acc Triplet Mismatch	B78	B*7801	131S	142tI	144tQr	147W	149aAr	151aRe	156L	158A	163L	166Ew	171H	177Et	180Q	183P	186K	193Pv	199A

Serum screening of patient 25 showed negative reactions with A30 and B8 cells. Inclusion of these antigens as acceptable mismatches increases the PFD from 0.084 to 0.316%. A30 has five mismatched triplets, 9S, 16S, 56R, 105S, and 151aRw, and B8 has four mismatched triplets, 66qIf, 151aRv, 156D, and 177Dt (Table 3). These triplets did apparently not react with patient's antibodies, and they are therefore considered as acceptable mismatches. HLAMatchmaker identifies seven additional HLA antigens, B35, B42, B51, B53, B56, B59, and B78, whose triplets are shared with the patient or are acceptable mismatches (Table 3). Inclusion of these antigens as acceptable mismatches increases the PFD about fourfold: from 0.316 to 1.237%. This example illustrates how HLAMatchmaker increases the pool of acceptable mismatches for highly sensitized patients.

After entering A30 and B8 as negative antigens, HLAMatchmaker identified 10 HLA-A,B antigens with one mismatched triplet and seven HLA-A,B antigens with two mismatched triplets, whereas the remaining triplets of these antigens are either shared with the patient or are acceptable mismatches. Table 4 shows the similarities and differences between mismatched triplets of these antigens. The two-triplet mismatches include three HLA-C antigens. For patient 25, the PFD of a 0-1-triplet mismatch is 1.357% and the PFD of a 0-2-triplet mismatch is 6.034%. Although these one-triplet- and two-triplet-mismatched antigens appear quite compatible at the structural level, one must ascertain that the patient does not have specific antibodies to the mismatched triplets.

Table 4 HLA antigens that have one and two mismatched triplet lets for patient 25 whereas the other triplet lets are shared or acceptable mismatches

Antigens with One-Triplet Mismatch	Mismatched Triplet	Antigens with Two-Triplet Mismatches	Mismatched Triplets
B18 (B*1801)	9H	A3 (A*0301)	151aHe, 163dT
B48 (B*4801)	144sQr	B38 (B*3801)	66qIc, 158T
B54 (B*5401)	45GeV	B39 (B*3906)	66qIc, 158T
B64 (B*1401)	66qIc	B58 (B*5801)	62Ge, 70aSa
B65 (B*1402)	66qIc	B62 (B*1501)	45rMa, 156W
B67 (B*6701)	158T	B63 (B*1516)	45rMa, 70aSa
B71 (B*1510)	66qIc	B76 (B*1511)	45rMa, 156W
B75 (B*1502)	45rMa	Cw1 (C*0102)	66qKy, 183Eh
B77 (B*1513)	45rMa	Cw12 (C*1203)	66qKy, 183Eh
B81 (B*8101)	144sQr	Cw14 (C*1402)	66qKy, 183Eh

Results

Probabilities of finding donors for sensitized patients before serum screening analysis

Figure 1 summarizes the PFD values for the different match categories. These values are shown on a log10 scale, and the differently shaded vertical bars show the cumulative effect of the zero-triplet, one-triplet, and two-triplet mismatches on the PFD of each patient. The range of PFD values for zero-HLA-A,B-antigen mismatches was more than 1000-fold, and for 11 patients the PFD was below 0.01% or less than 1 in 10,000 donors. Their HLA phenotypes seemed to have a high proportion of low-frequency antigens.

The median PFD for a zero-antigen mismatch was 0.025%. Matching at the triplet level increased the median PFD to 0.037% ($P=0.008$). The median PFD was 0.058% for a 0-1-triplet mismatch and 0.226% for a

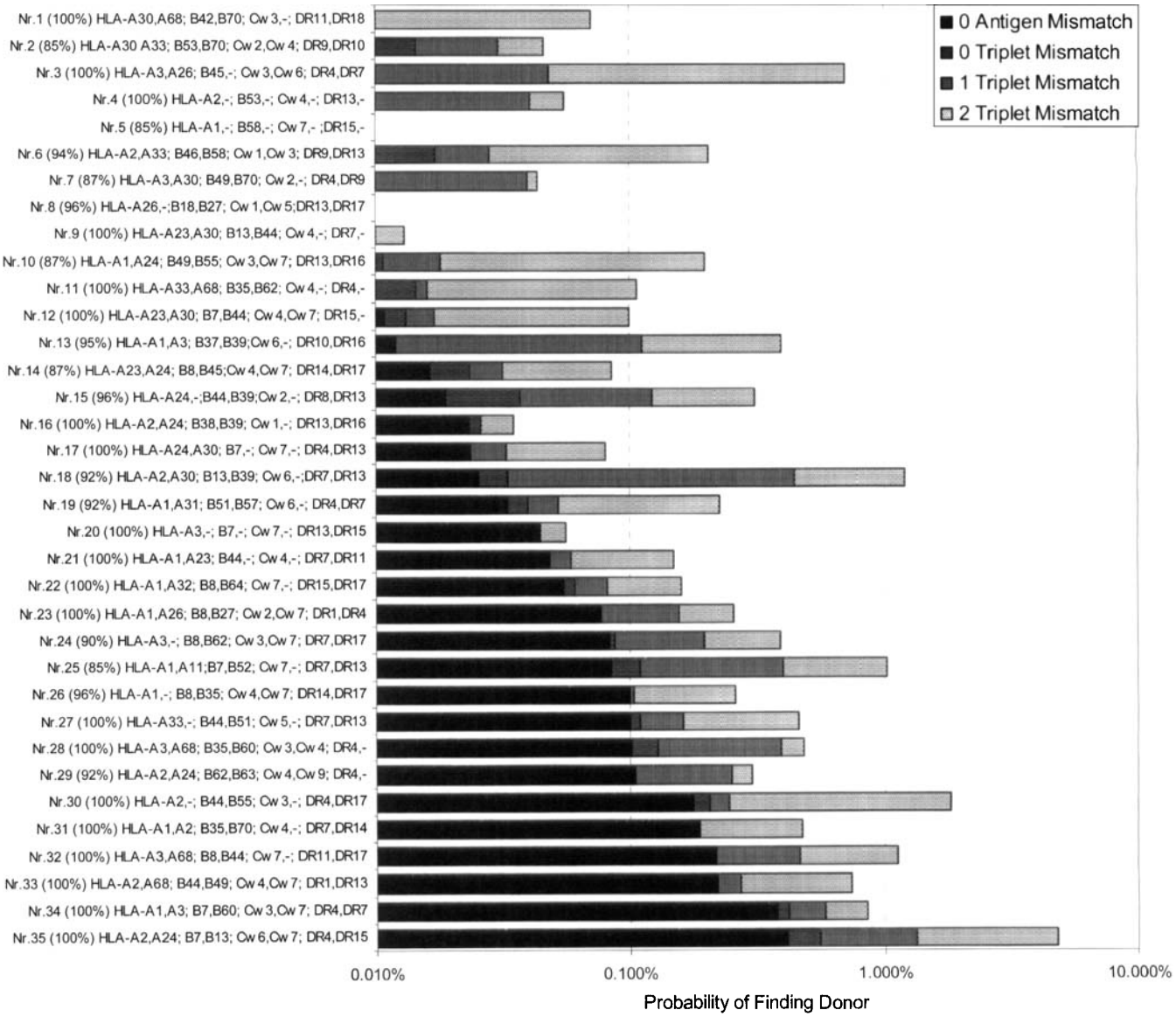

Fig. 1 Effect of triplet matching on the probability of finding a donor (PFD) for 35 highly sensitized patients. Each patient is identified by a unique number, the %PRA determined by CDC screening and HLA-A,B phenotype. Patients are sorted from the lowest to the highest PFD values (on a log10 scale) of finding a donor with a zero-antigen mismatch expressed as percentages. The *black bars* represent PFD values for the zero-HLA-A,B-antigen mismatches; for patients 1-11 these PFD values were $<0.01\%$ or less than 1 in 10,000 donors. The *stacked bars* represent the cumulative effects of matching at the various triplet levels, namely from 0, to 0-1, and to 0-2 triplets. For two patients, patients 5 and 8, the PFD in all match categories remained below 0.01%

0-2-triplet mismatch. We also determined how many patients in each match category would have a PFD of greater than 0.1%, i.e., more than 1 in 1000 donors would be compatible to the patient. This was the case for six patients (17%) in the zero-antigen mismatch category, for nine (26%) of zero-triplet mismatches, for 14 (40%) of 0-1-triplet mismatches, and for 29 (83%) of 0-2-triplet mismatches.

These findings suggest that triplet matching will markedly increase the availability of compatible donors. It should be noted that for six patients (or 17%) the HLAMatchmaker algorithm did not offer a significant advantage over conventional antigen matching criteria. A detailed serum analysis of these high-PRA patients may present the only opportunity to identify acceptable mismatches so that the chances of finding a suitably matched donor will be higher.

Probability of finding donors for sensitized patients after serum screening analysis

Serum screening identified acceptable antigen mismatches for 28 of 35 highly sensitized patients. Figure 2

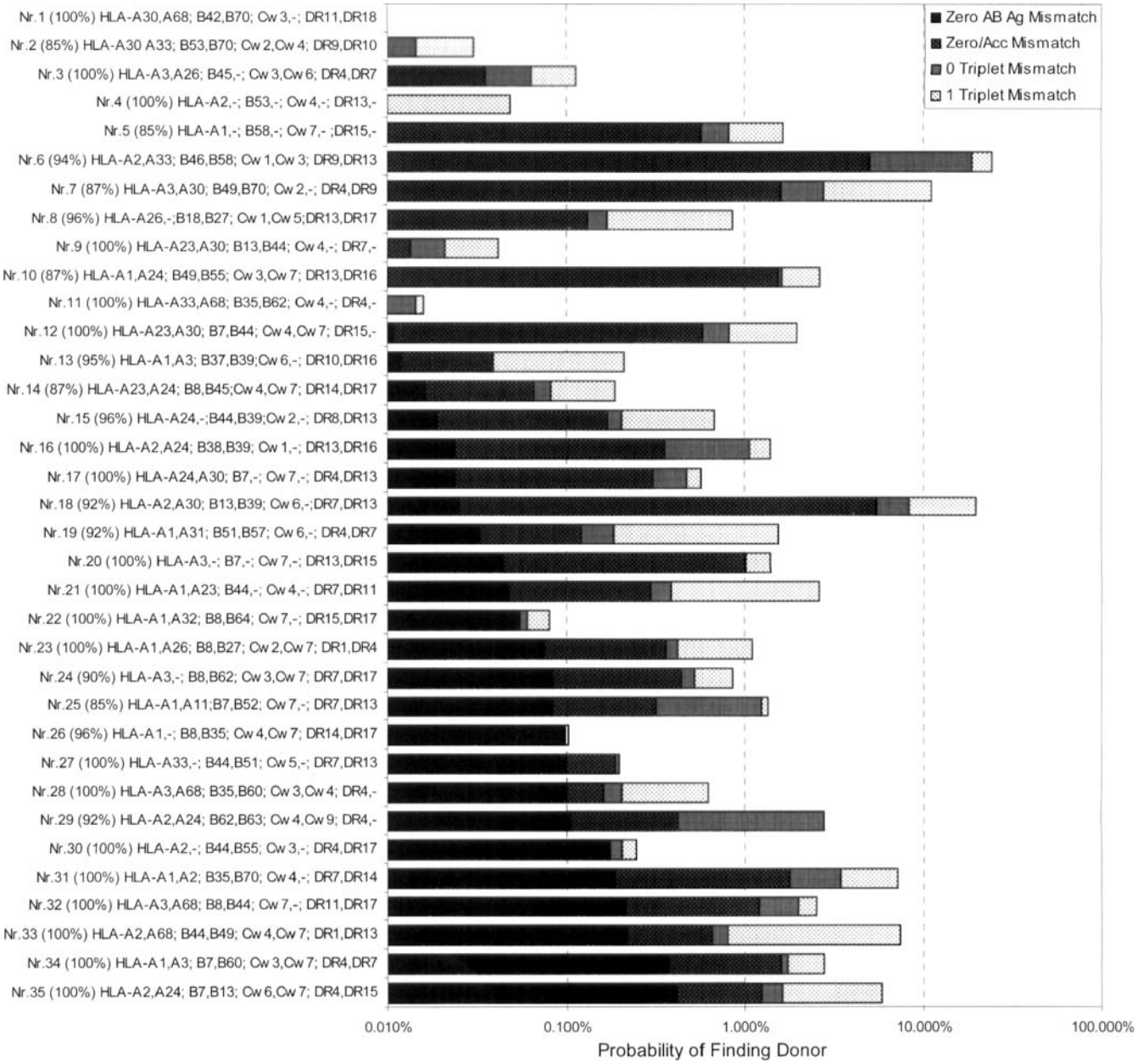

Fig. 2 Probability of finding a donor (PFD) values for 33 highly sensitized patients whose serum screens yielded information about acceptable HLA antigen mismatches. The *black bars* represent PFD values for the zero-HLA-A,B-antigen mismatches and the *stacked bars* the cumulative effects of matching for acceptable antigen mismatches and zero/acceptable triplet mismatches. For patient identification, see legend of Fig. 1

shows the changes in PFD values after acceptable antigens were included. The median PFD for the total group of 35 patients went from 0.025% for a zero-antigen mismatch to 0.307% for a zero/acceptable antigen mismatch. The application of HLAMatchmaker permitted for 33 patients (or 92%) the identification of additional antigens that were acceptable at the triplet level, and the median PFD for a zero/acceptable triplet mismatch went up to 0.425%. HLAMatchmaker can also identify donor HLA antigens that are mismatched for one triplet whereas the other triplets are shared or acceptable mismatches. Figure 2 shows that the inclusion of such one-triplet mismatches led to a considerable increase of the PFD for many patients, as the median PFD went to 1.112%.

The inclusion of antigens with two mismatched triplets further increased the median PFD to 2.592% (data not shown). An important consideration is that the patient's antibodies should not react with mismatched triplets.

Table 5 Validation of mismatched triplet acceptability

	Numbers of Unacceptable Antigens with		
	Zero-Triplet Mismatches	One-Triplet Mismatches	Two-Triplet Mismatches
No Serum Screening:	0/70 Zero/Acceptable Triplet Mismatches	14/164 Zero/Acceptable Triplet Mismatches + One Mismatched Triplet	29/236 Zero/Acceptable Triplet Mismatches + Two Mismatched Triplets
After Identification of Acceptable Antigen Mismatches:	0/108	30/237	43/235

Validation studies

The comprehensive serum screening with selected HLA-typed panel cells generated for each patient a list of unacceptable HLA antigens. This information was used to validate the triplet matching algorithm. In this group of high-PRA patients, a total of 508 HLA-A,B antigens had been identified by serological testing as unacceptable mismatches.

HLAMatchmaker defined a total of 70 HLA-A,B antigens as zero-triplet mismatches, but none of them had been listed as unacceptable (Table 5). After the serological identification of acceptable antigen mismatches from the serum screening data, HLAMatchmaker defined a total of 108 additional antigens with zero/acceptable triplet mismatches, but again, none of them had been identified as unacceptable antigens.

We also determined how many antigens with one or two mismatched triplets were unacceptable. Before serum screening, HLAMatchmaker defined a total of 164 HLA-A,B antigens with one mismatched triplet, and serum screening identified 14 of them (or 8%) as unacceptable antigens (Table 5). About 12% of the 236 two-triplet mismatches were identified by serum analysis as unacceptable antigens.

After serological identification of acceptable antigens, HLAMatchmaker determined which additional HLA antigens had one ($n=237$) or two ($n=235$) mismatched triplets whereas the other triplets on these antigens were shared with or acceptable to the patient. About 13% of these one-triplet mismatches and 19% of these two-triplet mismatches were identified by serum screening analysis as unacceptable. Although these findings suggest that most antigens with one or two mismatched triplets appear acceptable to highly sensitized patients, we must ascertain by serum analysis that the patient's antibodies do not react with such mismatched triplets.

Discussion

The principal goal of serum screening of highly sensitized patients is to identify HLA antigens that are acceptable mismatches so that suitable donors can be identified. This analysis was done on patients with extremely high PRA values, many of which were 100%. In a routine laboratory setting, screening of such high-PRA sera against a regular panel yields very little information about antigen mismatch acceptability. Often enough, there are no informative panel cells that permit the identification of acceptable antigen mismatches. The use of selected panel cells that are mismatched for only one HLA antigen has been most useful in the determination of acceptable antigen mismatches [8, 13]. The Acceptable Mismatch protocol for organ allocation has markedly increased the transplantation rate for highly sensitized patients, and kidney transplant survivals are very good [15].

Serum screening with selectively mismatched panel cells requires access to a large inventory of HLA-typed panel cells, but this approach is not feasible for most clinical laboratories. It is also difficult to find informative panel cells for patients who type for uncommon HLA antigens. The application of HLAMatchmaker will enhance the Acceptable Mismatch protocol in three ways:

First, we have shown that without serum screening, HLAMatchmaker can identify for many highly sensitized patients mismatched antigens with no or few triplet mismatches. Such antigens can be considered as acceptable mismatches because triplet-matched kidney transplants have the same survival rates as the zero-antigen mismatches [12]. This study shows that matching at the triplet level will increase the availability of matched transplants for most patients.

Second, after serum screening to identify acceptable antigen mismatches, HLAMatchmaker determines from the serum reactivity patterns which mismatched triplets are acceptable to the patient. The inclusion of additional antigens that are acceptable mismatches at the triplet level will further increase donor organ availability.

Third, HLAMatchmaker permits a more effective serum screening 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. A recently developed triplet-matching-based serum reactivity analysis program per-

mits the identification of acceptable and unacceptable antigens for highly sensitized patients without the need of a very large panel [14].

These findings are similar to the data for a group of highly sensitized patients in Pittsburgh whose sera had been screened by anti-human-globulin-augmented lymphocytotoxicity with a regular HLA-typed cell panel [16]. The PFD values for the different match categories were 3-5 times lower for highly sensitized patients than non-sensitized patients. These differences were also seen for the Eurotransplant patients (data not shown), and they appeared to reflect a prevalence of low-frequency antigens in the patient's HLA phenotype, a greater degree of homozygosity, and a lower proportion of immunogenic triplets in the self-triplet repertoires of highly sensitized patients [16]. Since it is much more difficult to find suitable donors for highly sensitized patients, it is imperative to conduct a detailed serum screening analysis. The cohort of patients described in this paper presented a particular challenge because many of them had 100% PRA values. With the combination of screening with selected panel cells and HLAMatchmaker we could identify acceptable mismatches and significantly increase the number of potential donors for most of these very highly sensitized patients.

The application of HLAMatchmaker will increase the transplantation rate for many highly sensitized patients, especially if we include HLA antigens with one or two mismatched triplets. We must, of course, ascertain 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. Recent studies by other investigators have verified HLA-Matchmaker in predicting crossmatch-negative donors for sensitized patients [17, 18]. The validation study in this report shows that a small but significant proportion of one-triplet and two-triplet mismatches are unacceptable because the patient's antibodies react with them. The remaining one-triplet and two-triplet mismatches are more likely to give negative reactions with the patient's serum.

A limitation of this study is that the assignment of triplets to HLA antigens lacks precision because HLA typing was done largely by serological methods that cannot test for molecular subtypes. Especially for HLA-C antigens, which are difficult to type for serologically, we had incomplete information about triplet polymorphisms. DNA-based typing will permit the definition of HLA subtypes and more accurate assignments of polymorphic triplets. This will also permit an assessment of the role of HLA-C antigens in humoral sensitization and in compatible donor searches for highly sensitized patients.

The Eurotransplant Reference Laboratory has recently begun to implement the combination of HLA-Matchmaker and the Acceptable Mismatch program to identify suitable donors for highly sensitized patients. Thus far, eight patients have received a transplant; seven kidneys are still functioning well, and one kidney was rejected after 18 months.

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