



# HLAMATCHMAKER: A Molecularly Based Donor Selection Algorithm for Highly Alloimmunized Patients

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**T**RANSPLANT candidates are generally considered highly alloimmunized if their serum panel-reactive antibody (PRA) activity exceeds 85%. Such patients can be transplanted successfully provided that the donor has no mismatched HLA antigens that react with the patient's alloantibodies. Serum screening against HLA-typed panels must be done to determine the alloantibody specificity spectrum so that unacceptable HLA antigen mismatches can be identified. This approach, however, does not work if the patient's serum has a very high PR A. Often, many highly sensitized patients remain on the waiting list with little prospect of a transplant because insufficient information is available about HLA mismatch acceptability and the probability of finding a zero antigen mismatch is very low.

This report shows an alternative strategy for identifying potential donors for highly sensitized patients. HLAMATCHMAKER is a computer-based algorithm that addresses amino acid sequence polymorphisms as critical components of immunogenic epitopes that can elicit alloantibodies. Such amino acids reside in sequence positions accessible to alloantibodies, namely the  $\alpha$  helices and  $\beta$  loops of the protein chain structure. The residues in the strands of the  $\beta$ -pleated sheets of the peptide-binding groove are excluded from this matching algorithm because they cannot make direct contact with alloantibodies. Many investigators have observed the presence of multiple amino acid-encoded antigenic determinants on the exposed parts of HLA class I molecules. This HLA matching algorithm is based on comparisons of linear sequences of amino acid triplets as motifs for potentially immunogenic epitopes.

## DESCRIPTION OF HLAMATCHMAKER ALGORITHM Amino Acid Triplet Polymorphisms in Antibody-Accessible Sites of HLA Class I Molecules

The assignment of antibody-accessible positions is based on the detailed descriptions of the crystalline structure of various HLA class I molecules (HLA-A2, -A68, and -B27).<sup>1-4</sup> The definition of the repertoire of triplets is based on published amino acid sequences of serologically defined HLA antigens (see the HLA Sequence Database of Steven Marsh, PhD, at [www.anthonynolan.com/hig/index.html](http://www.anthonynolan.com/hig/index.html)).

A total of 172 polymorphic triplets have been identified in the antibody-accessible positions of serologically defined HLA-A, -B, and -C molecules (Table 1). This list was the

result of a comparative analysis of the amino acid sequences of molecular equivalents of serologically defined HLA antigens. Triplet polymorphisms occur at 30 locations on HLA-A chains, 24 locations on HLA-B chains, and 19 locations on HLA-C chains. Each triplet is designated by its amino acid composition around a given position in the amino acid sequence. Amino acid residues are marked with the letter code; an uppercase letter corresponds to the residue in the numbered position, whereas a lowercase letter describes the nearest-neighbor residue. For instance, the triplet a65rNm represents an asparagine residue (N) in position 65 with arginine (r) in position 64 and methionine (m) in position 66 of the HLA-A chain. Many triplets are marked with one or two residues because their neighboring residues are monomorphic on all HLA class I chains and they are not shown. For instance, b12aM represents an alanine residue in position 11 and a methionine residue in position 12 on HLA-B chains. Triplet b41T has a threonine in position 41 and the two neighboring monomorphic residues are not shown.

## Determination of HLA Compatibility at the Amino Acid Triplet Level

The algorithm considers HLA class I polymorphisms of serologically defined antigens encoded by the HLA-A, -B, and -C loci to be very similar in structure and many positions in the amino acid sequence have identical residues. No comparisons are made with HLA-DR and HLA-DQ antigens because their amino acid sequences are very different from class I sequences.

HLAMATCHMAKER applies two principles: (1) each HLA antigen represents a distinct array of polymorphic triplets as potential immunogenic epitopes; and (2) sensitized patients

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**Table 1. Repertoire of Polymorphic Triplets on HLA Class I Molecules**

Position	HLA-A	HLA-B	HLA-C
9	F S T Y	D H Y	D F S Y
12	sV	sV aM aV	sV aV
14	R	R	R W
17	gR gS	gR	gR sR
41	A	A T	A
45	Me kMe	Ee Te Ge Ke Ma GeV	Ge
56	G R E	G	G
62	Rn Qe Ee Lq Ge	Rn Re Ge	Re
66	rKv rNv gKv rNm	qKy qlc qIs qly qlf rNm	qKy qNy
70	aQs aHs	aQa tNt aKa aSa rQa	rQa
74	D N H iD	D Y	D aD
76	An Vd En Es	Es En Ed Vs Vg	Vs Vn
80	gTl rla	rla rNl rTl rTa	rNl rKi
82	aLr lRg	aLr lRg lLr	lRg
90	D A	A	A D
105	P S	P	P
107	G W	G	G
127	N K	N	N
131	R	R S	R
138	T	T	K T
142	I T	I	I
144	tKr tKh tQr	tQr tQl sQr	tQr
147	W	W L	W L
149	aVh aAh aAr tAh	aAr	aAr
151	vHa aHv aHe aRv aRw aRr aHa	aRv aRe	aRt aRe aRa
156	L W R Q	W L D R	L R W
158	A V	A T	A
163	R T dT E	L E T	T E L
166	Dg Ew	Ew Es Dg	Ew
171	Y H	Y H	Y
177	Et	Dt Et Dk	Kt Et
180	Q	Q E	Q
184	dP dA	dP	eP eH
186	K R	K	K
193	Av Pi	Pi Pv	Pv Pl
199	A	A V	A
207	G S	G	G
246	A S Va	A	A
248	V	V	V M
253	Ee Ke Qe	Ee	Ee Eq

Triplet selection was based on the sequences of serologically defined HLA antigens corresponding to: A\*0101 A\*0201 A\*0301 A\*1101 A\*2301 A\*2402 A\*2501 A\*2601 A\*2902 A\*3001 A\*3101 A\*3201 A\*3301 A\*3402 A\*3601 A\*4301 A\*6601 A\*6801 A\*6901 A\*7401 A\*8001 B\*0702 B\*0801 B\*1301 B\*1801 B\*2705 B\*3501 B\*3701 B\*3801 B\*3901 B\*4101 B\*4201 B\*4402 B\*4501 B\*4601 B\*4701 B\*4801 B\*4901 B\*5001 B\*5101 B\*5201 B\*5301 B\*5401 B\*5501 B\*5601 B\*5701 B\*5801 B\*5901 B\*4001 B\*4002 B\*1501 B\*1516 B\*1401 B\*1402 B\*6701 B\*1509 B\*1510 B\*1503 B\*7301 B\*1502 B\*1511 B\*1513 B\*7801 B\*8101 B\*8201 Cw\*0102 Cw\*0202 Cw\*0302 Cw\*0401 Cw\*0501 Cw\*0602 Cw\*0701 Cw\*0801.

cannot produce alloantibodies against triplet-encoded determinants expressed by their own HLA molecules. The algorithm assesses donor HLA compatibility through intralocus and interlocus comparisons, and determines which triplets on mismatched HLA molecules are different or shared with the HLA-A, -B, -C antigens of the patient.

As an example, consider the high PRA patient X, who types as HLA-A2, A31; B42, B53; Cw2, Cw7. There are two potential donors with three HLA-A, -B antigen mismatches: donor 1, HLA-A2,A30;B51,B60;Cw4,Cw7 and donor 2, HLA-A2,A32,B8,B55,Cw3,Cw6. Which donor would be preferred? Several donor HLA antigens have serologic

cross-reactivity with patient HLA antigens and, depending on what CREG matching criteria are used, one might prefer one of the donors or not.

The HLAMATCHMAKER algorithm uses a molecular approach to define HLA compatibility. This computer program converts each HLA antigen into a string of potentially immunogenic triplets and then determines what triplets on mismatched donor HLA antigens are shared or not shared with the patient's HLA antigens. Table 2 illustrates the results of two mismatched HLA antigens. The HLA-B60 of donor 1 shows six unshared triplets; they are in underlined bold font in sequence positions 9, 41, 45, 66, 144, and 177.

**Table 2. Identification of Unshared Triplets on Mismatched HLA Antigens HLA-B60 and HLA-B8 for an HLA-A2, -A31, -B42, -B53, -Cw2, -Cw7 Recipient**

Patient	HLA: Allele	Position																			
		9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	138
A2	A*0201	F	sV	R	gR	A	Me	G	Ge	rKv	aHs	H	Vd	gTI	IRg	A	A	W	K	R	T
A31	A*3101	T	sV	R	gR	A	Me	R	Qe	rNv	aHs	iD	Vd	gTI	IRg	A	S	G	N	R	T
B42	B*4201	Y	sV	R	gR	A	Ee	G	Rn	qlf	aQa	D	Es	rNI	IRg	A	P	G	N	R	M
B53	B*5301	Y	aM	R	gR	A	Te	G	Rn	qlf	tNt	Y	En	rla	aLr	A	P	G	N	S	M
Cw2	Cw*0202	Y	aV	R	gR	A	Ge	G	Re	qKy	rQa	D	Vn	rKI	IRg	A	P	G	N	R	T
Cw7	Cw*0701	D	aV	R	gR	A	Ge	G	Re	qKy	rQa	aD	Vs	rNI	IRg	D	P	G	N	R	T
Donor HLA																					
B60	B*4001	<u>H</u>	aM	R	gR	<u>I</u>	<u>Ke</u>	G	Re	<u>qls</u>	tNt	Y	Es	rNI	IRg	A	P	G	N	R	M
B8	B*0801	D	aM	R	gR	A	Ee	G	Rn	qlf	tNt	D	Es	rNI	IRg	A	P	G	N	R	M

  

Patient	HLA: Allele	Position																			
		142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
A2	A*0201	T	tKh	W	aAh	aHv	L	A	T	Ew	Y	Et	Q	A	K	Av	A	S	A	V	Q
A31	A*3101	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	P	K	Av	A	S	s	V	Q
B42	B*4201	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	P	K	Pi	A	G	A	V	E
B53	B*5301	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	P	K	Pv	A	G	A	V	E
Cw2	Cw*0202	I	tQr	W	aAr	aRe	W	A	E	Ew	Y	Et	Q	Eh	K	Pv	A	G	A	V	E
Cw7	Cw*0701	I	tQr	L	aAr	aRa	L	A	T	Ew	Y	Et	Q	Ep	K	PI	A	G	A	V	eQ
Donor HLA																					
B60	B*4001	I	<u>sQr</u>	L	aAr	aRv	L	A	E	Ew	Y	<u>Dk</u>	E	P	K	Pi	A	G	A	V	E
B8	B*0801	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	P	K	Pi	A	G	A	V	E

Mismatched triplets are underlined, bold.

HLA-B60 is an undesirable mismatch because this high PRA patient's antibodies are likely to react with one or more of these mismatched triplets. On the other hand, the HLA-B8 antigen of donor 2 shares all of its triplets with the patient's HLA antigens; there are no mismatched triplets in the antibody-accessible sequence positions. HLA-B8 is therefore a compatible mismatch because the patient cannot make alloantibodies against self-triplets.

For patient X, the numbers of mismatched triplets of the all mismatched HLA antigens of these donors were as follows: donor 1, A30 (4), B51 (1), B60 (6), Cw4 (3) (total of 13 different triplets); and donor 2, A32 (0), B8 (0), B55 (0), Cw3 (0), Cw6 (0) (total of 0 triplets). Thus, for this high PRA patient, donor 2 is much more compatible at the molecular level than donor 1.

A total of seven HLA antigens with zero mismatched triplets can be identified for the HLA-A2,A31; B42,B53; Cw2,Cw7 type of patient X. They are A32, A74, B8, B35,

B55, B56, and B59. Any donor with an HLA type originating from this group and the patient's own HLA antigens is fully compatible at the molecular level of antibody-accessible sequence positions.

HLAMATCHMAKER permits fine tuning of the matching protocol at the level of HLA antigen cross-reactivity (CREG matching). Many HLA antigens with zero-triplet mismatches are serologically cross-reactive with the patient's HLA antigens, but other cross-reactive HLA antigens have one or several triplet mismatches. Table 3 illustrates how cross-reacting HLA antigens may have different numbers of mismatched triplets, depending on HLA type of the patient. This example summarizes the findings for the HLA-A2,A31; B42,B53; Cw2,Cw7 phenotype of patient X and another phenotype HLA-A2,A32; B35; B55; Cw3,Cw6 of patient Y (both patients have HLA-A and -B antigens in the same CREGs). Table 3 shows that patient Y has fewer zero-triplet mismatches than patient X and other

**Table 3. Cross-reacting HLA Antigen Mismatches Have Different Numbers of Unshared Triplets for Patients X and Y**

HLA Type of Patient X	Number of Nonshared Triplets for Cross-Reacting Antigens	HLA Type of Patient Y	Number of Nonshared Triplets for Cross-Reacting Antigens
A2	A68 (2) A69 (1)	A2	A68 (2) A69 (1)
A31	A29 (3) A30 (4) A32 (0) A33 (2) A74 (0)	A32	A29 (4) A30 (5) A31 (3) A33 (4) A74 (0)
B42	B7 (2) B8 (0) B27 (6) B54 (1) B55 (0) B56 (0) B60 (6) B61 (4)	B55	B7 (4) B8 (3) B27 (7) B42 (3) B54 (1) B56 (0) B60 (9) B61 (5)
B53	B35 (0) B49 (4) B50 (4) B51 (1) B52 (2) B57 (3) B58 (2) B70 (1)	B35	B49 (5) B50 (4) B51 (2) B52 (3) B53 (1) B57 (4) B58 (3) B70 (1)

**Table 4. Identification of Unshared Triplets on Mismatched HLA Antigens of Panel Cells That React Negatively With High PRA Serum of Patient Z**

	Position																			
	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	138
<b>Patient HLA</b>																				
A2	Y	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	IRg	D	P	G	N	R	T
A11	Y	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	IRg	D	P	G	N	R	T
B7	Y	sV	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNI	IRg	A	P	G	N	S	M
B38	Y	sV	R	gR	A	Ee	G	Rn	qlc	tNt	Y	En	rla	aLr	A	P	G	N	S	M
Cw2	Y	aV	R	sR	A	Ge	G	Re	qKy	rQa	D	Vn	rKI	IRg	A	P	G	N	R	T
Cw7	D	aV	R	gR	A	Ge	G	Re	qNy	rQa	AD	Vs	rNI	IRg	D	P	G	N	R	T
<b>Neg reactions</b>																				
A1	<b>E</b>	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	IRg	A	<b>S</b>	G	N	R	T
A33	<b>T</b>	sV	R	gR	A	Me	G	Rn	rNv	<b>aHs</b>	<b>iD</b>	Vd	gTL	IRg	A	<b>S</b>	G	N	R	T
B8	Y	<b>aM</b>	R	gR	A	<b>Te</b>	G	Rn	<b>qlf</b>	tNt	D	Es	rNI	IRg	A	P	G	N	S	M
Cw8	Y	aV	R	gR	A	Ge	G	Re	qKy	rQa	D	Vs	rNI	IRg	A	P	G	N	R	T

  

	Position																			
	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
<b>Patient HLA</b>																				
A2	I	tKr	W	aAh	aHa	Q	A	R	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
A11	I	tKr	W	aAh	aHa	Q	A	R	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
B7	I	tQr	W	aAr	aRv	L	T	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
B38	I	tQr	W	aAr	aRv	L	T	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
Cw2	I	tQr	W	aAr	aRe	W	A	E	Ew	Y	Et	Q	eH	K	Pv	A	G	A	V	E
Cw7	I	tQr	L	aAr	aRa	L	A	T	Ew	Y	Et	Q	eP	K	PI	A	G	A	V	E
<b>Neg reactions</b>																				
A1	I	tKr	W	aAh	<b>aHe</b>	L	A	<b>dT</b>	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
A33	I	tQr	W	aAr	aRv	L	A	T	Ew	<b>H</b>	Et	Q	dP	<b>R</b>	<b>Av</b>	A	<b>S</b>	<b>S</b>	V	<b>Q</b>
B8	I	tQr	W	aAr	aRe	L	A	L	Ew	<b>H</b>	Et	Q	dP	K	Pv	A	G	A	V	E
Cw8	I	tQr	W	aAr	<b>aRt</b>	L	A	T	Ew	Y	Kt	Q	eH	K	Pv	A	G	A	V	E

Unshared triplets underlined, bold.

cross-reacting antigens often have greater numbers of mismatched triplets. By conventional compatibility criteria there is a three-A, -B-antigen mismatch between the HLA types X and Y in each direction. An analysis with the HLAMATCHMAKER algorithm shows that Y is a zero-triplet mismatch for X and, therefore, would be fully compatible at the level of structurally defined antibody-accessible epitopes. Conversely, the mismatched HLA antigens of X have a total of 15 unshared triplets for Y, and this level of incompatibility would likely be unacceptable for a high PRA patient. This example illustrates the concept that marked differences may occur in the molecularly defined HLA compatibility of various CREG matches.

HLAMATCHMAKER can perform this type of analysis for any HLA phenotype. It permits the identification of HLA antigens fully compatible at the antibody-accessible triplet level.

**Analysis of Serum Reactivity Patterns and Identification of Acceptable HLA Antigen Mismatches**

HLAMATCHMAKER can also incorporate information about the patient's serum reactivity with an HLA-typed panel. The information pertains to the negative reactions of panel cell preparations with high PRA sera. For example, patient Z has a 96% PRA with a 50-cell panel. He types as

HLA-A2,A11;B7,B38;Cw7, and HLAMATCHMAKER analysis shows that the only HLA antigens with zero-triplet mismatches are low-frequency antigens A69, B39 and B67. This means that it would be very difficult to identify a compatible donor for patient Z.

The PRA information may, however, permit the identification of additional HLA antigens with zero-triplet mismatches. This patient's serum has a 96% PRA with a 50-cell panel and negative reactions were seen with two cells with the following types: HLA-A1,A2;B7,B8;Cw7, and HLA-A2,A33;B7,B39; Cw7,Cw8. Because a high PRA is due to HLA-specific antibodies, one can expect that the negative cells will share HLA antigens with the patient. The other HLA antigens on the negative panel cells, in this case A1, A33, B8, and Cw8, carry unshared triplets, but none can apparently be recognized by the patient's antibodies. These 17 unshared triplets are shown in bold underlined font in Table 4. HLAMATCHMAKER applies this information to identify additional acceptable HLA antigen mismatches for patient Z. The following HLA antigens have unshared triplets that cannot be recognized by the patient's antibodies: A32, A36, A74, B42, B54, B55, B59, B64, and B65. These HLA antigens, together with the mismatched antigens on the negative panel cells (A1, A33, B8, and Cw8)

and the antigens with zero-triplet mismatches (A69, B39, and B67), comprise the group of acceptable HLA antigens for patient Z. Any donor mismatch for only acceptable HLA antigens can be expected to give a negative cross-match with patient Z serum. An ongoing analysis of cross-match data on high PRA patients has produced preliminary results that are consistent with the validity of this approach for identifying compatible donors.

#### Relative Immunogenicity of HLA Triplets

HLA mismatch acceptability may also be assessed with information about the immunogenicity of polymorphic triplets. During recent years, the concept has emerged that highly sensitized patients produce a limited repertoire of alloantibodies specific for the more immunogenic private and public HLA epitopes.<sup>5-7</sup> Although most highly sensitized patients have been exposed to many mismatched triplets, their antibody reactivity patterns reveal specificity to a limited number of immunogenic triplets, whereas other triplets do not induce an antibody response and therefore must be nonimmunogenic for the patient. A recent study on high PRA sera has demonstrated significant differences in the immunogenicity of triplets (manuscript submitted). HLA antigens that have only unshared triplets with low immunogenicity may also become acceptable mismatches for a high PRA patient.

#### CONCLUSIONS

HLAMATCHMAKER is an easy-to-use computer program for determining donor HLA antigen acceptability for highly

sensitized patients. This "in silico" compatibility test permits determination of the structural basis of an HLA antigen mismatch. It is based on intralocus and interlocus comparisons of amino acid triplets on antibody-accessible sites of HLA class I antigens. It permits the identification of HLA-A and -B antigens that are usually considered to be mismatched for a given HLA type, but turn out to be fully compatible at the triplet level.

This matching algorithm has been designed primarily for highly sensitized patients awaiting an organ transplant or requiring HLA-compatible platelet transfusion support. A program demo is posted on the website of the Transplantation Pathology Internet Service of the University of Pittsburgh Medical Center (<http://tpis.upmc.edu>).

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