

HLAMatchmaker: A Molecularly Based Algorithm for Histocompatibility Determination.

I. Description of the Algorithm

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ABSTRACT: This report describes an algorithm for identifying acceptable HLA antigens for highly alloimmunized patients without the need for extensive serum screening. This algorithm is based on the concept that immunogenic epitopes are represented by amino acid triplets on exposed parts of protein sequences of human leukocyte antigen chains (HLA-A, HLA-B, and HLA-C) accessible to alloantibodies. A computer program (HLA-Matchmaker) has been developed to determine class I HLA compatibility at the molecular level. It makes intralocus and interlocus comparisons of polymorphic triplets in sequence positions to determine the spectrum of non-shared triplets on donor HLA antigens. In most cases it is possible to identify certain mismatched HLA antigens that share all their polymorphic triplets with the patient's HLA antigens and could therefore, be considered fully compatible. HLAMatchmaker permits also the iden-

tification of additional mismatches that are acceptable as determined from the triplet information on HLA-typed panel cells that do not react with patient's serum.

HLAMatchmaker provides an assessment of donor-recipient HLA compatibility at the structural level and this algorithm is different from conventional methods based on the mere counting of numbers of mismatched HLA antigens or CREGs. This donor selection strategy is suitable especially for allosensitized patients in need of a compatible transplant or platelet transfusion. *Human Immunology* 63, 339–352 (2002). © American Society for Histocompatibility and Immunogenetics, 2002. Published by Elsevier Science Inc.

KEYWORDS: histocompatibility; HLAMatchmaker; triplet; PRA; highly sensitized patients

INTRODUCTION

Transplant candidates are generally considered highly alloimmunized if their serum panel reactive antibody (PRA) activity exceeds 85%. Such patients can be successfully transplanted provided that the donor has no mismatched human leukocyte antigens (HLA) that react with the patient's alloantibodies. Serum screening against HLA-typed panels must be done to determine the overall antibody specificity spectrum so that unacceptable HLA antigen mismatches can be avoided. Many screening protocols are based on complement-dependent lymphocytotoxicity determined by direct testing, such as the NIH standard and Amos modified tests [1–3], and

by the more sensitive anti-human globulin (AHG) augmentation technique [4–6]. Newer methodologies for serum screening include enzymeimmunoabsorbent assays (ELISA) [7–10] and flow cytometric analysis [11–13].

Most clinical laboratories screen sera against HLA-typed panels ranging generally from 30 to 60 in size. Serum reactivity patterns are analyzed by 2×2 table statistics, such as the Chi-square test, to determine significant correlations between positive reactions and the specificity of HLA antigens in the panel. Besides the WHO-designated HLA antigens, correlations have been determined for public epitopes assigned from so-called cross-reacting groups (CREGs) of HLA antigens [14–21], and for amino acid polymorphisms defined from sequence information of HLA molecules [22–24]. Significant correlations provide an assessment of the alloantibody specificity patterns of sera and this information permits a determination whether mismatched HLA antigens of a potential donor might be acceptable or unacceptable to the patient.

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This approach works generally quite well if the patient's serum is not overly reactive and its PRA is <80%. However, the 2×2 table statistics becomes unreliable for highly reactive sera. For instance, a serum with a 96% PRA against a 50-cell panel, manifests negative reactions with only two panel cells. This number is too small for meaningful interpretations of Chi-square statistics, especially if the serum has two or more HLA-antibody specificities. This problem might be overcome by screening high PRA sera against large cell panels [17, 24], and/or perform absorption/elution studies with selected cells and then rescreen the absorbed sera and the eluates [25–28]. However, such methods for alloantibody identification are very labor intensive, and they require considerable resources not readily available in a clinical laboratory setting. Often enough, many highly sensitized patients remain on the waiting list with little prospect of a transplant because no information is available about acceptable HLA mismatches and the probability of finding a zero antigen mismatch is very low.

Another method for analyzing screening results focuses on the identification of acceptable antigen mismatches expressed by panel cells that give negative reactions with patient's serum [29–31]. It can also be applied to patients with extremely reactive sera (*i.e.*, 100% PRA by routine panel screening) if panel cells are used that are selectively mismatched for a single HLA antigen. The acceptable mismatch approach works generally quite well for patients with common HLA antigens in their phenotypes, but it requires access to a very large pool of HLA typed panel cell donors.

This report describes an alternative strategy for identifying potential donors for highly sensitized patients. HLAMatchmaker is a computer-based algorithm that focuses on the structural basis of HLA class I polymorphisms so that compatible HLA mismatches can be determined for each patient without the need for extensive serum screening.

HLAMatchmaker considers 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 α -helices and β -loops (also referred to as β -bulges or β -turns between the secondary structures) of the protein chain structure. Each HLA molecule expresses on its surface multiple amino acid defined antigenic determinants recognized by distinct alloantibodies [18, 22, 25, 32–46]. The residues in the strands of the β -pleated sheets of the peptide-binding groove are excluded from this matching algorithm because they cannot make direct contact with alloantibodies.

This HLA matching algorithm is based on comparisons of linear sequences of amino acid triplets as motifs

for potentially immunogenic epitopes. Each HLA antigen represents a distinct string of polymorphic triplets and an HLA mismatch is assessed by determining the number of triplets not shared with the recipient's HLA antigens. This report describes the logistics of HLA-Matchmaker and how this algorithm permits the identification of HLA-compatible donors for highly sensitized patients without the need for extensive serum screening.

METHODS AND RESULTS

Amino Acid Triplet Polymorphisms of 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) [47–50]. The definition of the repertoire of triplets considers published amino acid sequences of serologically defined HLA antigens (see the IMGT/HLA database at <http://www.anthonynolan.com/HIG/index.html>).

Table 1 represents the total repertoire of polymorphic triplets in the antibody-accessible positions of amino acid sequences of serologically defined HLA-A, HLA-B, and HLA-C antigens. This list resulted from a comparative analysis of the amino acid sequences of molecular equivalents of serologically defined HLA antigens. 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 standard letter code; an uppercase letter corresponds to the residue in the numbered position, whereas the lowercase letters describe the nearest neighboring residues. 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 the same on all HLA class I chains and, therefore, they are not listed. For instance, b12aM represents an alanine residue in position 11 and a methionine residue in position 12 on HLA-B chains. The triplet b41T has a threonine in position 41 and the two neighboring monomorphic residues are not listed.

Most polymorphic triplets reside in the membrane-terminal $\alpha 1$ (positions 1–90) and $\alpha 2$ (positions 91–182) domains of the HLA chains. The locations of the triplets on the α helices and the β loops of the molecular structure are described according to the helix (H1, H2, etc.), strand (S1, S2, etc.), and loop (S1 → S2, S2 → S3, etc.) annotations as previously reported [48]. On each domain, a very short nearly vertical helix (H1) precedes a long curved (H2 in $\alpha 1$) or kinked (H2a, H2b, and H3

TABLE 1 Polymorphic triplets in antibody-accessible locations on HLA-A, HLA-B, and HLA-C molecules

Location	HLA-A	HLA-B	HLA-C
$\alpha 1S1 \rightarrow S2$	a9 F* S* <u>T</u> Y*	b9 D* H Y*	c9 D* F* S* Y*
$\alpha 1S1 \rightarrow S2$	a12 sV	b12 sV ^o aO aV*	c12 sV ^o aV*
$\alpha 1S1 \rightarrow S2$	a14 R	b14 R	c14 R ^o <u>W</u>
$\alpha 1S1 \rightarrow S2$	a17 gR ^o gS	b17 gR	c17 gR ^o sR
$\alpha 1S3 \rightarrow S4$	a41 A	b41 A ^o T	c41 A
$\alpha 1S3 \rightarrow S4$	a45 Me kMe	b45 Ee Te Ge ^o Ke Ma GeV	c45 Ge
$\alpha 1H2$	a56 G ^o R E	b56 G	c56 G
$\alpha 1H2$	a62 Rn* Qe Ee Lq Ge*	b62 Rn* Re ^o Ge*	c62 Re
$\alpha 1H2$	a66 rKv rNv gKv rNm*	b66 qKy* Qlc qls qly qlf rNm*	c66 qKy* gNy
$\alpha 1H2$	a70 aQs aHs	b70 aQa tNt aKa aSa rQa ^o	c70 rQa
$\alpha 1H2$	a74 D* N H iD	b74 D* Y	c74 D* aD
$\alpha 1H2$	a76 An Vd En* Es*	b76 Es* En* Ed Vs* Vg	c76 Vs* Vn
$\alpha 1H2$	a80 gTI rla*	b80 rla* rNI* rTI rTa	c80 rNI* rKi
$\alpha 1H2$	a82 aLr* IRg ^o	b82 aLr* IRg ^o ILr	c82 IRg
$\alpha 1H2 \rightarrow \alpha 2S1$	a90 D* A*	b90 A* D*	c90 A* D*
$\alpha 2S1 \rightarrow S2$	a105 P ^o S	b105 P	c105 P
$\alpha 2S1 \rightarrow S2$	a107 G ^o W Grl	b107 G	c107 G
$\alpha 2S3 \rightarrow S4$	a127 N ^o K	b127 N	c127 N
$\alpha 2S3 \rightarrow S4$	a131 R	b131 R ^o S	c131 R
$\alpha 2S4 \rightarrow H1$	a138 T	b138 T	c138 T ^o K
$\alpha 2H1$	a142 I ^o T	b142 I	c142 I
$\alpha 2H1$	a144 tKr tKh tQr ^o	b144 tQr ^o tQI sQr	c144 tQr
$\alpha 2H1$	a147 W	b147 W ^o L*	c147 W ^o L*
$\alpha 2H1$	a149 aVh aAh aAr ^o tAh	b149 aAr	c149 aAr
$\alpha 2H2a$	a151 vHa aHv aHe aRv* aRw aRr aHa	b151 aRv* aRe*	c151 aRt aRe* aRa
$\alpha 2H2a$	a156 L* W* R* Q	b156 W* L* D R*	c156 L* R* W*
$\alpha 2H2a$	a158 A ^o V	b158 A ^o T	c158 A
$\alpha 2H2b$	a156 R T* dT E*	b163 L* E* T* dL	c163 T* E* L*
$\alpha 2H2b$	a166 Dg Ew ^o	b166 Ew ^o Es	c166 Ew
$\alpha 2H2b$	a171 Y ^o H*	b171 Y ^o H*	c171 Y
$\alpha 2H3$	a177 Et	b177 Dt Et ^o Dk	c177 Kt Et
$\alpha 2H3$	a180 Q	b180 Q ^o E	c180 Q
$\alpha 2H3 \rightarrow \alpha 3S1$	a184 dP ^o dA	b184 dP	c184 eH eP
$\alpha 2H3 \rightarrow \alpha 3S1$	a186 K ^o R	b186 K	c186 K
$\alpha 3S1 \rightarrow S2$	a193 Av Pi*	b193 Pi* Pv*	c193 Pv* PI
$\alpha 3S1 \rightarrow S2$	a199 A	b199 A ^o V	c199 A
$\alpha 3S1 \rightarrow S2$	a207 G ^o S	b207 G	c207 G
$\alpha 3S5$ strand	a246 A ^o S Va	b246 A ^o E	c246 A
$\alpha 3S5$ strand	a248 V	b248 V ^o G	c248 V ^o O
$\alpha 3S5 \rightarrow S6$	a253 Ee ^o Kc Qe	b253 Ee	c253 Ee ^o Eq

Triplets in underlined bold font are uniquely present on one (or two) HLA antigens; triplets in bold font (not underlined) are found on groups of three or more cross-reacting antigens within the same locus; triplets marked by a superscript (°) are polymorphic at one locus but monomorphic at another locus; triplets marked by an asterisk (*) are polymorphic at two or three loci.

The 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*1302 B*1801 B*2705 B*3501 B*3701 B*3801 B*3906 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 (B60) B*4002 (B61) B*1501 (B62) B*1516 (B63) B*1401 (B64) B*1402 (B65) B*6701 B*1509 (B70) B*1510 (B71) B*1503 (B72) B*7301 B*b1502 (B75) B*1511 (B76) B*1513 (B77) B*7801 B*8101 B*8201 Cw*0102 Cw*0202 Cw*0302 Cw*0401 Cw*0501 Cw*0602 Cw*0701 Cw*0801. Note these alleles were selected because of their relatively high frequencies in our local population; they are used in the triplet matching examples described in this article. Although other alleles might be more frequent in different populations, they have the same triplets as those shown in this table.

in $\alpha 2$ helix. As depicted in Table 1, triplet polymorphisms can be found in 8 locations of the $\alpha 1$ helix ($\alpha 1$ 72–2) and 12 locations in the $\alpha 2$ helix ($\alpha 2H1$, $\alpha 2H2a$, $\alpha 2H2b$ and $\alpha 2H3$).

There are four β -loops in the $\alpha 1$ domain and the polymorphic triplets are defined within the following sequences: $\alpha 1S1 \rightarrow S2$ (residues 9–21), $\alpha 1S2 \rightarrow S3$

(28–31), $\alpha 1S3 \rightarrow S4$ (37–46), and $\alpha 1S4 \rightarrow H1$ (47–50). Similarly, the $\alpha 2$ domains have four β -loops: $\alpha 2$ S1 \rightarrow S2 (residues 103–109), $\alpha 2$ S2 \rightarrow S3 (118–121), $\alpha 2$ S3 \rightarrow S4 (126–133), and $\alpha 2$ S4 \rightarrow H1 (135–138). The $\alpha 1$ S2 \rightarrow S3 and $\alpha 2$ S2 \rightarrow S3 loops extend below the β -sheets and interact with the $\alpha 3$ domain and the β_2 -microglobulin part of the HLA molecule, respec-

tively. These β -loops do not exhibit any triplet polymorphisms and they seem inaccessible to antibodies. The remaining β -loops extend above the β -sheet and pack against the outer faces of the α -helices and all appear to be antibody accessible. The $\alpha 1H2 \rightarrow \alpha 2S1$ loop between the $\alpha 1$ and $\alpha 2$ domains (residues 84–94) forms a flat open structure on the top surface of HLA adjacent to the antigen-binding cleft. Triplet polymorphisms can be found in a total of 12 locations of the β -loops of the $\alpha 1$ and $\alpha 2$ domains and the interdomain loop.

The $\alpha 3$ domain of the HLA molecule has a fold, like an immunoglobulin constant domain and its seven β -strands reveal considerable structural homologies with $\beta 2$ -microglobulin and the CH3 domain of IgG [48]. There are six loops between the β -strands. The $\alpha 3$ domain is generally much less polymorphic than the $\alpha 1$ and $\alpha 2$ domains and triplet polymorphisms may be found in two locations of the β -loops ($\alpha 3S1 \rightarrow S2$ and $\alpha 3S5 \rightarrow S6$). The loop between the $\alpha 2$ and $\alpha 3$ domains has two locations where triplet polymorphism may occur.

Are there other polymorphic locations in the $\alpha 3$ -domain that might be accessible to antibodies? The $\alpha 3$ -domain can bind to the T-cell accessory molecule CD8 and the contact region involves the $\alpha 3S3 \rightarrow S4$ β -loop (residues 224–228) and other residues near position 245 in the β -strand $\alpha 3S5$ [51, 52]. This part of the $\alpha 3$ -domain may also serve as a contact region for immunoglobulin variable domains [48] and, therefore, might be accessible to antibodies. Whereas the $\alpha 3S3 \rightarrow S4$ β -loop is highly conserved, the β -strand $\alpha 3S5$ has two locations where triplet polymorphisms may occur.

A total of 142 different polymorphic triplets have been designated to the serologically defined HLA-A, -B, and -C antigens (Table 1). Triplet polymorphisms occur at 30 locations on HLA-A chains, 27 locations on HLA-B chains, and 19 locations on HLA-C chains. The polymorphic triplets have been categorized into four groups according to the potential of being recognized as non-self or self when the patient is exposed to an HLA mismatch. The first group consists of triplets that are present on one or two HLA antigens. They are depicted in bold underlined font in Table 1. Several private HLA antigens can be distinguished by unique triplets. For instance, a163dT is found only on HLA-A3 molecules and a monospecific antibody to HLA-A3 seems to react with an a163dT-based epitope. Other HLA antigens have unique triplets and their serologic splits can be distinguished with other triplets. For instance, HLA-B16 has a unique b158T triplet and its splits HLA-B38 and HLA-B39 can be distinguished by triplets b80rIa and b82aLr versus b80rNl and b82lRg, respectively. These triplets correspond to the Bw4 and Bw6 epitopes.

Some HLA antigens have two or more unique triplets in their sequence. For instance, HLA-A30 has two unique triplets: a17S and a151aRw, and it seems possible that HLA-A30 specific antibodies recognize two structurally distinct epitopes. The uncommon antigen HLA-A80 has five unique triplets and HLA-Cw7 has four unique triplets. It should be noted that many private HLA-A and HLA-B antigens do not have corresponding unique triplets in the antibody-accessible sites of their sequence.

Several triplets can be uniquely found on pairs of HLA antigens. Examples are a56R on HLA-A30+A31 and a76Es on HLA-A25+A32. The serologic cross-reactivity of these antigen pairs is well-known and it seems likely that these triplets are critical components of the antigenic epitopes recognized by cross-reacting antibodies.

The second group consists of polymorphic triplets that are shared between three or more HLA antigens encoded by the same class I locus; they are depicted in bold font in Table 1. Several of them seem to correspond with public epitopes or CREGs. For instance, the members of the CREG HLA-A2, HLA-A23, HLA-A24, HLA-A68, and HLA-A69 antigens share a127K, which may represent a distinct public epitope recognized by alloantibodies in sensitized patients. Similarly, the CREG HLA-B7, HLA-B40, and HLA-B48 antigens have a distinct b177Dk triplet.

The third group consists of triplets that are polymorphic for one class I locus but monomorphic for another class I locus; they are marked with a \circ symbol in Table 1. Such triplets cannot represent immunogenic epitopes because they are always present on the patient's own HLA antigens. For instance, the a56G polymorphic triplet is found on all HLA-A molecules except HLA-A30 and HLA-A31, but the corresponding triplet at position 56 on HLA-B (b56G) or HLA-C (c56G) is monomorphic. This means that a56G is always a self-triplet and, therefore, cannot be immunogenic. Similarly, b12sV is polymorphic for HLA-B but this triplet cannot be immunogenic because all patients have the monomorphic a12sV triplet on their HLA-A molecules.

The fourth group of triplets are polymorphic for two (or all three loci) and they are marked with a * symbol in Table 1. Several of them represent well-known interlocus public epitopes. For instance, HLA-A2 and HLA-B17 share a public epitope [53, 54] that corresponds to the Ge triplet in position 62. This triplet is called a62Ge on HLA-A2 and b62Ge on HLA-B17 chains and monoclonal antibodies specific for HLA-A2+B17 recognize an epitope that corresponds to 62Ge [55]. Another example is the interlocus public epitope shared between the Bw4 group of HLA-B antigens and the A locus antigens HLA-A23, HLA-A24, HLA-A25, and HLA-A32 [56, 57]. This epitope corresponds to sequences marked by

TABLE 2 Summary of HLA-A, B, and C triplet polymorphisms

Triplet polymorphisms	HLA-A	HLA-B	HLA-C	Total number of unique triplets
1. Triplets on one or two HLA antigens encoded by the same locus	25	11	8	44
2. Triplets shared between three or more HLA antigens encoded by the same locus	24	24	8	56
3. Polymorphic triplets that are monomorphic for another locus (loci)	17	17	7	29
4. Polymorphic triplets that are also polymorphic for another class I locus	21	28	20	33
Total number of triplets:	87	80	42	142

Abbreviation: HLA = human leukocyte antigen.

the 76En, 80rIa, and 82aLr triplets. The HLAMatchmaker algorithm includes interlocus comparisons between polymorphic triplets at the different HLA loci to determine whether a triplet on a mismatched HLA molecule must be considered as non-self. For instance, patients who type for HLA-B17 should not recognize a62G on an HLA-A2 mismatch as non-self because HLA-B17 molecules carry the same triplet b62G in the same sequence position as a62G.

Table 2 illustrates an enumeration of the four groups of polymorphic triplets on HLA-A, HLA-B, and HLA-C molecules. In the overall repertoire of 142 polymorphic triplets, a total of 29 triplets (group 3) cannot be considered as immunogenic, whereas the remaining 113 triplets may have immunogenic potential. An accompanying article addresses the relative immunogenicity of polymorphic triplets on class I HLA molecules [58].

Determination of HLA Class I Compatibility at the Amino Acid Triplet Level

HLAMatchmaker applies two principles: (1) each HLA antigen represents a distinct string of polymorphic triplets as potential immunogens that can induce specific alloantibodies; and (2) sensitized patients do not have alloantibodies against triplets present on their own HLA molecules. The algorithm assesses donor-recipient compatibility through intralocus and interlocus comparisons,

and determines what triplets on mismatched HLA molecules are different or shared between donor and patient. This analysis considers each donor HLA antigen mismatch towards the entire HLA-A, HLA-B, HLA-C phenotype of the recipient.

As an example, a mismatch between HLA-B18 (donor) and HLA-B7 (recipient) can be characterized by triplet differences in 12 sequence positions: 9, 45, 66, 70, 74, 131, 151, 156, 163, 171, 177, and 180 (Figure 1a). The structural nature of an HLA mismatch is greatly influenced by the recipient's own HLA antigens. For instance, an HLA-B18 mismatch for HLA-B37 represents triplet differences in six sequence positions: 62, 76, 80, 82, 156, and 171, and this triplet mismatch pattern is very different than that seen for HLA-B7 (Figure 1b). For a recipient with the HLA-B7,B37 phenotype, a HLA-B18 mismatch would represent only two triplets, b156L and b171H, whereas all the other triplets of HLA-B18 are shared with HLA-B7 and/or HLA-B37 (Figure 1c). This approach of intralocus triplet sharing is similar to the concept of CREG (or public epitope) matching except that this algorithm considers HLA compatibility in more precise structural detail.

Of course each patient types for HLA-A (and HLA-C) antigens, and HLAMatchmaker incorporates interlocus comparisons of triplet sharing. In this example, the HLA-B7,B37 recipient types also as HLA-A33. Figure 1d reveals that HLA-A33 has the same two triplets, 156L and 171H, in identical positions as the two triplets of HLA-B18 that are different from HLA-B7,37. This

FIGURE 1 Example of human leukocyte antigen (HLA) matching at the triplet level.

	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
a:																																								
Recipient	HLA-B7	Y	sV	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNI	IRg	A	P	G	N	R	I	tQr	W	aAr	aRe	R	A	E	Ew	Y	Dk	E	dP	K	Pi	A	G	A	V	E
Donor	HLA-B18	H	sV	R	gR	A	Te	G	Rn	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	E
b:																																								
Recipient	HLA-B37	H	sV	R	gR	A	Te	G	Re	qls	tNt	Y	Ed	rTI	ILr	A	P	G	N	S	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
Donor	HLA-B18	H	sV	R	gR	A	Te	G	Rn	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	E
c:																																								
Recipient	HLA-B7	Y	sV	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNI	IRg	A	P	G	N	R	I	tQr	W	aAr	aRe	R	A	E	Ew	Y	Dk	E	dP	K	Pi	A	G	A	V	E
Recipient	HLA-B37	H	sV	R	gR	A	Te	G	Re	qls	tNt	Y	Ed	rTI	ILr	A	P	G	N	S	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
Donor	HLA-B18	H	sV	R	gR	A	Te	G	Rn	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	E
d:																																								
Recipient	HLA-A33	T	sV	R	gR	A	Me	G	Rn	rNv	aHs	iD	Vd	gTL	IRg	A	S	G	N	R	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	R	Av	A	S	S	V	Q
Recipient	HLA-B7	Y	sV	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNI	IRg	A	P	G	N	R	I	tQr	W	aAr	aRe	R	A	E	Ew	Y	Dk	E	dP	K	Pi	A	G	A	V	E
Recipient	HLA-B37	H	sV	R	gR	A	Te	G	Re	qls	tNt	Y	Ed	rTI	ILr	A	P	G	N	S	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
Donor	HLA-B18	H	sV	R	gR	A	Te	G	Rn	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	E

TABLE 3 Examples of zero-, one-, and two-triplet mismatches for different HLA phenotypes

	HLA type of patient*	Zero-triplet mismatch	One-triplet mismatch	Two-triplet mismatch
Case 1	A2,A30; B42,B53; Cw4,Cw7	A69, B8, B35, B55, B56, B59	B51 (b171H) B54 (b45Gev) B67 (b158T) B70 (b66qIc) B71 (b66qIc) B72 (b66qIc) B78 (b171H)	A68 (a156W,a246Va) A32 (a107Gr1,a246S) A74 (a107Gr1,a246S) B7 (b163E,b177Dk) B38 (b66qIc,b158T) B39 (b66qIc,b158T) B46 (b45Ma,b156W) B52 (b66qIs,b171H) B58 (b66rNm,b70aSa) B64 (b66qIc,b171H) B65 (b66qIc,b171H) B75 (b45Ma,b66qIs) B76 (b45Ma,b156W) B77 (b45Ma,b66qIs) B78 (b151aRe,b171H) B82 (b163dL,b166Es) A68 (a156W,a246Va) A74 (a107Gr1,a246S) B54 (b45Ge1,b131S) B55 (b131S,b151aRe) B56 (b131S,b163L), B67 (b131S,b158T)
Case 2	A2,A30; B8,B42; Cw7,-	A69	None	A32 (a107Gr1,246S) A68 (a156W,a246Va) A74 (a107Gr1,a246S) B41 (b156D,B177Dt) B45 (b156D,b166Es) B46 (b45Ma,b156W) B58 (b66rNo,b70aSa) B62 (b45Ma,b156W) B70 (b45Ee,b66qIc) B71 (b45Ee,b66qIc)
Case 3	A2,A30; B53,B60; Cw3,Cw5	A69, B35, B49, B50, B61	B18 (b171H) B48 (b45Ee) B51 (b171H) B52 (b171H) B59 (b45Ee) B72 (b45Ee) B75 (b45Ma) B77 (b45Ma) B78 (b171H)	B62 (b45Ma,b156W) B67 (b131S,b158T) B70 (b45Ee,b66qIc) B71 (b45Ee,b66qIc) B78 (b171H)
Case 4	A3,A11; B8,-;Cw2,-	none	none	B42 (b66qIy,b70aQa)
Case 5	A2,-;B35,-;Cw4,CW8	none	B78 (b171H)	A69 (a66rNv,a70aQs)

*Triplet assignments were made from molecular equivalents of serological antigens. These alleles are listed in Table 1.

means that all polymorphic triplets of HLA-B18 can be found on one or more of the recipient's HLA-A33, HLA-B7, and HLA-B37 antigens. Thus, HLA-B18 must be fully histocompatible to the HLA-A33,X; B7,B37 phenotype, at least from the perspective of antibody-accessible triplets (X can be any A locus antigen).

Table 3 lists five examples of HLA matching at the triplet level; these cases have been selected to illustrate how the HLA phenotype of the patient can influence the number of HLA antigens with zero or few triplet mismatches. Case 1 deals with the HLA-A2,A30; B42,B53; Cw4,Cw7 phenotype that has several antigens that are more common to African-Americans. Six HLA-A, HLA-B antigens are zero-triplet mismatches, namely the cross-reactive HLA-A69, HLA-B35, HLA-B55, and HLA-B56 as well as HLA-B8 and HLA-B59, which are generally not considered to be crossreactive with any

antigen in this phenotype. Seven HLA antigens are one-triplet mismatches and 15 HLA antigens are two-triplet mismatches (Table 3) and many of them, but not all (*e.g.*, HLA-B38 and B39), crossreact with patient HLA antigens.

Case 2 illustrates another example: the HLA-A2,A30; B8,B42; Cw7, phenotype differs from Case 1 by only one antigen, HLA-B8 (this antigen is a zero-triplet mismatch for Case 1), and is negative for HLA-Cw4. Only one HLA antigen (HLA-A69) is a zero-triplet mismatch for Case 2. None are one-triplet mismatches and only six HLA antigens (A68, A74, B54, B55, B56, and B67) are two-triplet mismatches (Table 3). Although the HLA phenotypes of Case 1 and Case 2 are very similar, the numbers of HLA antigens with zero or few triplet mismatches are very different.

Case 3 types as HLA-A2,A30; B53,B60; Cw3,Cw5,

and the HLA-A,B phenotype is different from Case 1 in that it has HLA-B60 instead of HLA-B42 (both antigens are members of the B7 CREG). Five HLA antigens are fully matched at the triplet level, nine HLA antigens are one-triplet mismatches, and ten HLA antigens are two-triplet mismatches (Table 3). This group of zero and few triplet mismatches has different HLA antigens than seen for Case 1. For instance, HLA-B49 and HLA-B50 are members of the B5 CREG that includes HLA-B53. These antigens are zero-triplet mismatches for HLA-A2,A30; B53,B60; Cw3,Cw4 (Case 3), but for HLA-A2,A30; B42,B53; Cw4,Cw7 (Case 1) they have four mismatched triplets: b9H, b41T, b45Ke, and b66qIc. On the other hand, the crossreactive HLA-B51 is a one-triplet mismatch for both cases. Thus, depending on the HLA phenotype of the patient, HLA mismatching within crossreactive groups may indicate considerable differences at the triplet level.

For certain HLA phenotypes it is impossible to identify zero-triplet mismatched HLA antigens. Two examples are HLA-A3,A11; B8,-; Cw2,- (Case 4), for which the most compatible HLA-B42 is a two-triplet mismatch and A2,-; B35,-; Cw4,Cw8 for which the best match is the one-triplet mismatched HLA-B78. Homozygosity for the HLA-A and HLA-B loci reduces the numbers of HLA antigens with zero or few triplet mismatches.

The examples in Table 3 illustrate how HLAMatchmaker can be used in the search of compatible donors for highly sensitized patients. The HLA phenotypes of such donors could constitute a combination of HLA antigens from the patient and other HLA antigens that are zero-triplet mismatches. For instance, an HLA-A30,A69; B8,B35 donor would be considered, by conventional criteria, a three-A,B antigen mismatch for an HLA-A2,A30; B42,B53; Cw4,Cw7 recipient (Case 1) but by this matching algorithm this phenotype would have no mismatched triplets and therefore, might be compatible at the HLA-A HLA-B loci.

Considering the numbers and frequencies of HLA antigens with zero-triplet mismatches, the chances of finding a histocompatible donor seem better for Cases 1 and 3 than for Cases 2, 4, and 5. Therefore, many donor searches may have to consider additional HLA antigens, preferably those with one or few triplet mismatches.

The proper selection of a triplet mismatch depends on the antibody specificity repertoire of the sensitized patient. Table 3 illustrates what triplets are involved in the one-triplet and two-triplet HLA antigen mismatches. For instance, in Case 1, the one-triplet mismatched antigens HLA-B51 and HLA-B78 involve the same triplet (b171H), whereas HLA-B70 and HLA-B71 are mismatched for b66qIc. HLA-B64 and HLA-B65 are mismatched for b66qIc and b171H, and HLA-B38 and

HLA-B39 are mismatched for b66qIc and b158T. A determination of antibody specificity to polymorphic triplets may identify unacceptable HLA antigens for a given patient. For instance, in Case 1, the presence of an antibody to b66qIc would render the one-triplet mismatches HLA-B70 and HLA-B71 and the two-triplet mismatches HLA-B38, HLA-B39, HLA-B64, HLA-B65 as unacceptable for this patient. An antibody to b171H would rule out HLA-B51, HLA-B64, HLA-B65, and HLA-B78 as potential donor antigens for Case 1. In a clinical setting it is generally not feasible, however, to perform a detailed analysis of antibody specificity patterns of high PRA sera.

Identification of Acceptable HLA Mismatches for High PRA Patients

Nevertheless, there is an additional approach to identify acceptable HLA antigen mismatches for high PRA patients. It considers the HLA types of panel cells that give negative reactions with the patient's serum. Such negative panel cells can be expected to share HLA antigens with the patient, whereas other HLA antigens may contain mismatched triplets that are apparently not recognized by the patient's antibodies. Such triplets would be acceptable even if they are present on other HLA antigens not expressed on the negative panel cells.

Case 6 represents a patient for whom it is difficult to identify HLA antigens with a zero-triplet mismatch. This patient has a 96% PRA with a 50-cell panel and he types as HLA-A3,A29; B18,B47; Cw7,-. The following HLAMatchmaker analysis yields information about HLA antigens with zero- to five-triplet mismatches:

Zero: B61

One: B37

Two: A74 B50

Three: A11 A31 A33 A34 A66 B27 B35 B39 B41 B45 B54 B60 B65 B72 B75

Four: A26 A30 A32 A43 B48 B55 B56 B62 B67 B70 B71 B73 B78

Five: A69 B8 B13 B46 B49 B59 B76

Because only HLA-B61 is a zero-triplet mismatch and HLA-B37 is a one-triplet mismatch, it would be difficult to find a compatible donor for this patient. However, the PRA information may permit the identification of additional HLA antigens with acceptable triplet mismatches. This patient's serum had a 96% PRA with a 50-cell panel and consistently negative reactions were seen with two panel cells with the following HLA types: A3,A33; B27,B64; Cw1,Cw8 and A3,A29; B44,B64; Cw7,-. If a high PRA was due to HLA-specific antibodies, one could expect that the negative panel cells would share some HLA antigens with the patient and this was apparently the case for this patient. The other HLA antigens on the

TABLE 4 Numbers of triplet mismatches for HLA-C antigens

	HLA type of patient	Cw1	Cw2	Cw3	Cw4	Cw5	Cw6	Cw7	Cw8
Case 1	A2,A30; B42,B53; Cw4,Cw7	1	3	0	—	2	1	—	2
Case 2	A2,A30; B8,B42; Cw7,-	6	9	5	8	9	7	—	5
Case 3	A2,A30; B53,B60; Cw3,Cw5	1	2	—	3	—	4	8	1
Case 4	A3,A11; B8,-;Cw2,-	3	—	2	4	3	1	8	3
Case 5	A2,-; B35,-; Cw4,Cw8	1	3	0	—	1	2	7	—
Case 6	A3,A29; B18,B47; Cw7,-	6	8	5	9	9	7	—	5

The triplet selection was based on the sequences of serologically defined HLA antigens corresponding to: Cw*0102, Cw*0202, Cw*0302, Cw*0401, Cw*0501, Cw*0602, Cw*0701, Cw*0801.

negative panel cells, in this case HLA-A33, HLA-B27, HLA-B44, and HLA-B64, carry unshared triplets but none of them were apparently recognized by the patient's antibodies. This information can be incorporated in the HLAMatchmaker analysis, which yields a new set of HLA antigens with zero/acceptable and few triplet mismatches:

Zero/Acceptable: A33 B27 **B37** B44 **B45** **B50** B61 B64 **B65** **B70** **B71** **B72**

One: A31 A74 B39 B49 B73 B75

Two: A32 B13 B35 B38 B41 B52 B55 B56 B59 B62 B77 B78

Three: A11 A26 A34 A43 A66 B8 B46 B48 B51 B53 B54 B60 B67 B76 B82

Four: A25 A30 B42 B63

Five: A69 B7 B57 B58 B81

As expected, HLA-A33, HLA-B27, HLA-B44, and HLA-B64 are now listed as zero/acceptable triplet mismatches (they are in italic font) because this patient had no antibodies against any triplet on these antigens. HLAMatchmaker identified seven additional HLA antigens (they are in bold and underlined) that became acceptable mismatches because their triplets were not recognized by patient's antibodies. Five HLA antigens became one-triplet mismatches for this patient. With this expanded list of HLA antigens with zero-triplet mismatches, a compatible donor might more readily found for this patient.

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 common private and public HLA epitopes [17, 19, 24]. Although most highly sensitized patients have been exposed to many mismatched triplets, their antibody reactivity patterns reveal specificity to a relatively small number of immunogenic triplets, whereas other triplets do not induce an antibody response and, therefore, must

be non-immunogenic for the patient. The generation and application of information about immunogenicity of triplets will be addressed in another report [58].

HLA-C Mismatching at the Triplet Level

The degree of HLA class I histocompatibility generally addresses the antigens encoded by the HLA-A and HLA-B loci. HLA-C is largely ignored and, from a matching perspective, this locus has remained an enigma. The serologic polymorphism of HLA-C is poorly defined and HLA-C molecules are expressed on the cell surface at much lower levels than HLA-A and HLA-B molecules [59]. HLA-C antigens appear not very immunogenic [60] and they do not seem to play a major role in the selection of compatible platelet donors for refractory thrombocytopenic patients [61]. Molecular typing has found that HLA-C alleles are more closely related to each other. In particular, the helix of the $\alpha 1$ domain of HLA-C molecules is unusually conserved, whereas the $\alpha 2$ domain is similar to that of HLA-B [62]. The serologically defined HLA-C antigens exhibit less amino acid polymorphism than HLA-A and HLA-B antigens, and this is reflected by the lower numbers of polymorphic triplets in fewer positions in the HLA-C sequences (Table 1).

HLAMatchmaker provides an opportunity to assess HLA-C antigen compatibility at the triplet level. Table 4 illustrates the results of six cases described above, including the five cases listed in Table 3. The numbers of HLA-C triplet mismatches were generally very low for combinations involving all HLA-C antigens except HLA-Cw7, which has four unique triplets. Incompatibility for HLA-Cw7 involves a higher number of mismatched triplets (6–8 triplets) than incompatibility for the other HLA-C antigens (0–3 triplets). Conversely, as Case 2 and Case 6 illustrate, the numbers of HLA-C triplet mismatches are highest for recipients who are homozygous for HLA-Cw7.

These findings provide some insight why, in many cases, HLA-C incompatibility does not seem to play a

major role in humoral HLA-specific alloimmunization. Often enough, an incompatible HLA-C antigen represents a zero or a few triplet mismatch. The exception is HLA-Cw7 (*i.e.*, both HLA-C*0701 and HLA-C*0702) for which the polymorphic triplet repertoire is different than for the other HLA-C antigens. This information is based on serologically defined HLA-C antigens. Molecular typing for HLA-C will permit a more precise assessment of HLA-C compatibility at the triplet level.

DISCUSSION

HLAMatchmaker is an easy to use computer program that determines donor HLA class I acceptability for highly sensitized patients, including kidney and heart transplant candidates, and for refractory thrombocytopenic patients requiring HLA-compatible platelet transfusions. This algorithm permits a determination of the structural basis of an HLA antigen mismatch and utilizes intralocus and interlocus comparisons of strings of amino acid triplets on antibody-accessible sites of HLA class I molecules. Considering the large repertoire of polymorphic triplets on class I HLA molecules, the surprising finding was made that certain HLA antigens, by conventional criteria, are mismatched for a given HLA type, but turn out to be fully compatible at the triplet level. This concept has clinical relevance because HLAMatchmaker can identify acceptable HLA mismatches for sensitized patients and appears to be useful predictor of a cross-match result [63, 64]. Moreover, recent studies have reported that in cadaver kidney transplantation, the zero-HLA-DR mismatched allografts with HLA-A,B mismatches, but no mismatched triplets, have the same survival rates as the zero-HLA-A, B, DR mismatches [65].

This matching algorithm applies the concept that each HLA antigen has multiple epitopes that can elicit specific alloantibodies. These antigenic determinants have been serologically defined as private and public (or CREG) epitopes [25], and many of them correspond to distinct amino acid residues or sequences in HLA molecules. The so-called epitope maps of the HLA-A2 and HLA-B7 CREGs are examples of the structural basis of private and public determinants [32, 33]. However, the information about the total repertoire of serologically defined HLA epitopes remains incomplete and this makes it difficult to apply HLA epitope-based matching strategies for identifying suitable donors for highly sensitized patients.

HLAMatchmaker addresses the total spectrum of antibody-accessible amino acid sequence polymorphisms as critical components of potentially immunogenic epitopes. It considers a linear sequence of three

amino acids as a minimal requirement for assessing HLA compatibility at the molecular level. Matching is assessed by determining whether or not a triplet in a given position of a mismatched HLA antigen is also found in the same position in any of the patient's own HLA-A, HLA-B, HLA-C molecules. A shared triplet in the same position on a mismatched HLA antigen cannot elicit a specific antibody response in the patient. This hypothesis has been verified experimentally in an accompanying article [58].

Why triplets? This algorithm considers the structural basis of the interaction between an antibody and a protein antigen. The antibody-binding sites of immunoglobulin molecules comprise six hypervariable loops that make contact with protein antigen [66, 67]. They are referred to as complementarity determining regions (CDR); three are on the heavy chain (CDR-H1, CDR-H2, and CDR-H3) and three are on the light chain (CDR-L1, CDR-L2, and CDR-L3). CDR-H3 has the highest sequence variability and conformational freedom [68], and this loop seems to play a primary role in the antibody specificity while the others enhance the specificity and the strength of binding to the antigen [69]. Three-dimensional structures of antigen-antibody complexes have revealed that the contact area between antibody and antigen is about 700 to 800 square angstroms and it involves about 15–22 pairs of amino acid residues [69–71]. It should be noted that the surface of the HLA molecule seen from above the peptide-binding region and the alpha helices is about 750 square angstroms [48]. With six CDRs on antibody and 15–22 amino acids on antigen as contact sites, one can estimate an average of three amino acids binding to each CDR. There is evidence that sequences of three residues can be recognized by certain antibodies with low affinity [72, 73]. For longer peptides of 4 to 8 residues that bind to an antibody, the replacement of each residue of the peptide by other amino acids will indicate that three contact residues are often essential for binding, whereas the other residues may be replaceable by virtually any amino acid [68, 74]. Thus, in the case of an HLA-specific antibody, one CDR may play a primary role in that it recognizes and binds the polymorphic triplet while the other CDRs interact with other sites on the HLA molecule. Such sites may have monomorphic and polymorphic residues. Site-directed mutagenesis studies have reported that amino acid substitutions in sequence positions distant from the epitope may influence the binding between HLA antigen and antibody [40, 75].

The selection of triplets for matching purposes does not imply that the structural basis of an epitope always involves exactly three amino acids. Many triplets have only one or two polymorphic residues, and some epitopes

might be defined by four or five polymorphic residues in adjacent positions. A typical example are the five amino acid sequence in positions 79–83 that are recognized by Bw4- and Bw6-specific antibodies. Serologic studies have also described a heterogeneity among the specificity patterns of Bw4-associated antibodies suggesting possible subtypes [42, 76, 77]. The application of triplet matching within the 79–83 sequence incorporates this Bw4 heterogeneity. A full mismatch for Bw4 is represented by two mismatched triplets and a partial Bw4 mismatch is indicated by a one-triplet mismatch in the 79–83 sequence. Triplets are also used to incorporate possible epitope heterogeneity in the 149–152 sequence, which has four polymorphic residues in adjacent positions.

For most patients, HLAMatchmaker can identify mismatched HLA antigens that are fully compatible at the triplet level. Many antigens crossreact with the HLA antigens of the patient and this finding is in accordance with the concept of CREG matching in donor selection strategies [21, 78, 79]. Often enough, the program identifies other cross-reacting antigens that are incompatible at the triplet level and should perhaps be avoided for matching purposes. Serologically based CREG matching strategies emphasize public epitopes over private epitopes. Depending on the HLA phenotype of the patient, certain private epitopes have considerable immunogenic potential and mismatching for them might be detrimental. This may explain why the beneficial effect of CREG matching on kidney transplant outcome remains controversial [78–83]. HLAMatchmaker permits a fine tuning of the CREG matching algorithm because it considers the structural organization of public and private epitopes.

The identification of HLA antigens with zero-triplet mismatches is greatly influenced by the patient's HLA phenotype. In several cases, especially if the HLA phenotype reveals homozygosity or has closely cross-reacting antigens, there are no or very few HLA antigens with zero-triplet mismatches. Acceptable HLA antigen mismatches can be identified from HLA-typed panel cells that give negative reactions with patient's serum [84]. It should be emphasized that such negative reactions are obtained with the most sensitive screening technique and that they can be reproduced with several serum samples, especially if the PRA is high. A negative reaction means that patient's antibodies do not recognize any triplet on the mismatched HLA antigen(s) of a given panel cell and this information can be incorporated in HLAMatchmaker for the identification of additional HLA antigens not expressed on the negative panel cells, but can be expected to be acceptable mismatches for the patient. How does one approach the analysis of a serum with a 100% PRA? Obviously, the HLA antigen composition

of the panel was such that the patient's antibodies always recognized one or more epitopes on each panel cell. The chances for a negative reaction will increase if panel cells are selected with HLA antigens shared with the patient whereas the other HLA antigens are mismatched for a few triplets. HLAMatchmaker can readily identify such antigens.

Several refinements are needed for an optimal application of the HLAMatchmaker algorithm. The assignment of triplets to HLA antigens may lack precision if the HLA typing information is based solely on serologic methods. DNA-based typing will permit the definition of HLA subtypes and, therefore, more accurate assignments of polymorphic triplets. Many molecular subtypes of serologically defined HLA antigens have different triplets in antibody-accessible positions. In such cases some serologically matched HLA antigens may have incompatible triplets recognized by the patient's antibodies.

Another limitation is the lack of sufficient HLA-C typing information. Serologic typing for HLA-C is often unreliable and only eight HLA-C antigens are considered in the version of HLAMatchmaker described in this report. Molecular methods are now routinely available for HLA-C typing and this permits a more detailed assessment of HLA class I compatibility at the triplet level. The HLAMatchmaker algorithm can also be expanded to class II HLA antigens encoded by the DR and DQ loci.

Although HLAMatchmaker can be used as a cross-match predictor algorithm to identify potential donors for sensitized patients, it may also offer a new approach of optimizing donor-recipient HLA compatibility with the goal of preventing or reducing antibody-mediated rejection of organ transplants. In a recent review, McKenna *et al.* [85] has emphasized the importance of HLA-specific antibody responses in transplant rejection. Any matching strategy for controlling humoral rejection should consider the overall repertoire of structurally defined epitopes and their immunogenicity relevant to antibody formation. Indeed, a recent study has reported that HLA matching at the triplet level has a beneficial effect on kidney transplant outcome [65].

Various versions of HLAMatchmaker can be downloaded free of charge from the website of the Transplantation Pathology Internet Service of the University of Pittsburgh Medical Center (<http://www.tpis.upmc.edu>).

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