Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation
René J Duquesnoy

HLAMatchmaker is a computer algorithm that determines HLA compatibility at the structural level. Donor-recipient histocompatibility is assessed with polymorphic amino acid configurations that represent structurally defined elements of HLA epitopes originally assigned as triplets and more recently as eplets. For many patients, HLAMatchmaker can identify mismatched HLA antigens that can be considered compatible at the structural level. Structurally based HLA matching reduces humoral allosensitization and correlates with good transplant outcome. Moreover, HLAMatchmaker is useful in the analysis of serum antibody reactivity and benefits the strategy of identifying acceptable mismatches for highly sensitized patients.

Addresses
Division of Transplantation Pathology, University of Pittsburgh Medical Center, Thomas E. Starzl Biomedical Science Tower, Room W1552, Pittsburgh, PA 15261, United States

Corresponding author: Duquesnoy, René J (Duquesnoyr@upmc.edu)

Current Opinion in Immunology 2008, 20:594–601
This review comes from a themed issue on Immunogenetics and Transplantation
Edited by Frans H. J. Claas and Rene Duquesnoy
Available online 7th August 2008
0952-7915/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
DOI 10.1016/j.coi.2008.06.010

Introduction
In organ transplantation, the degree of HLA compatibility is generally determined by counting the number of mismatched HLA-A, B, DR antigens of the donor. It is well known that the zero-antigen mismatches have the highest success rates but why do so many mismatched transplants do so well? The answer to this question seems related to the antibody responses to HLA mismatches. Many studies have demonstrated that HLA class I and class II antibodies are significant risk factors for transplant failure.

An important consideration is that HLA antigens have multiple epitopes that can be recognized by specific antibodies. The original description of the epitope repertoire was based on serological cross-reactivity between HLA antigens and antibody specificities against so-called private and public determinants. During the eighties and nineties, many studies with HLA-specific monoclonal antibodies provided evidence that private and public epitopes correspond to distinct amino acid residues or short sequences in HLA. These findings have been applied to the serum antibody analysis of sensitized patients and amino acid residue matching for kidney transplantation. Most recently, Terasaki’s group has conducted extensive studies on antibody reactivity patterns with single HLA alleles in antigen-binding assays on a Luminex platform [1–3] As described elsewhere in this issue, these analyses have identified 103 class I and 83 class II epitopes defined by polymorphic amino acid residues [4]. This review describes how HLAMatchmaker approaches HLA compatibility at the epitope level.

Amino acid residue polymorphisms on the HLA molecular surface
HLA epitopes are determined by antibody-accessible polymorphic amino acid residues on the molecular surface. Three-dimensional modeling of HLA molecules has revealed a broad array of polymorphic residues. Figure 1 shows the polymorphic residues on three crystallized class I molecules, HLA-A2, HLA-B27 and HLA-Cw3. The molecular surface around the bound peptide (see top view) has similar numbers of exposed polymorphic positions on the α1 helices of HLA-A and HLA-B antigens but more polymorphic positions are visible on the α2 helices of HLA-A antigens. The α helices of HLA-C antigens have much fewer polymorphic positions.

By contrast, HLA-C antigens have many polymorphic positions in the membrane-proximal domain. HLA-A antigens have also more surface-exposed polymorphic positions in that region and it should be noted that the sequence positions in the membrane-proximal domain of HLA-B are all monomorphic.

HLA-DR and HLA-DQ molecules have different patterns of surface expression of polymorphic residues (Figure 2). The structural polymorphism of HLA-DR is restricted to the β chain, the α chain is monomorphic. Polymorphic residues are readily visible on the top of the molecule adjacent to the bound peptide and many of them involve contiguous sequences. Polymorphic residues on the side of the molecule generally comprise distinct clusters in both β1 and β2 domains. A few polymorphisms are visible at the bottom part of the molecule nearby the cell membrane. DRB and DQβ
seem to show similar numbers of polymorphic positions. DQA displays somewhat contiguous polymorphic positions on the top of the molecule nearby the bound peptide and on the side of the \(\alpha_1\) domain. The polymorphic positions in the \(\alpha_2\) domain seem to be more in distinct clusters.

Altogether, these models illustrate the rather extensive degree of structural polymorphism and they suggest that epitopes have a widespread distribution over the molecular surface. Depending on their location, epitopes may have different degrees of antibody accessibility. Some antibodies may react with epitopes on solubilized but not with membrane-bound HLA molecules.

The concept of HLAMatchmaker
HLAMatchmaker was originally introduced as a matching algorithm whereby each HLA antigen is viewed as a string of short linear sequences (triplets) involving polymorphic amino acid residues in antibody-accessible positions; they are considered key elements of epitopes that can induce the formation of specific antibodies [6*]. Although, as shown below, the triplet version of HLAMatchmaker has proven to be clinically useful, it provides an incomplete description of the structural HLA epitope repertoire. Expanded criteria including longer sequences and polymorphic residues in discontinuous positions have been applied to a new version of HLAMatchmaker developed from stereochemical modeling of crystallized complexes of antibodies with different protein antigens and published data about the contributions of critical amino acid residues to antigen–antibody binding energy [7**]. Antigenic proteins have structural epitopes consisting of 15–22 residues that constitute the binding face with antibody. The surface of a structural epitope varies between 700 and 850 Å\(^2\) and is about the same as the surface around the bound peptide-binding groove of an HLA molecule. Most structural epitopes have a patch

---

**Figure 1**

<table>
<thead>
<tr>
<th>Top view</th>
<th>Side View</th>
<th>Side View</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A2</td>
<td><img src="image1" alt="HLA-A2 Top View" /></td>
<td><img src="image2" alt="HLA-A2 Side View Left" /></td>
</tr>
<tr>
<td>HLA-B27</td>
<td><img src="image3" alt="HLA-B27 Top View" /></td>
<td><img src="image4" alt="HLA-B27 Side View Left" /></td>
</tr>
<tr>
<td>HLA-Cw3</td>
<td><img src="image5" alt="HLA-Cw3 Top View" /></td>
<td><img src="image6" alt="HLA-Cw3 Side View Left" /></td>
</tr>
</tbody>
</table>

Polymorphic residue positions on HLA-A, B and C molecules. The following crystalline models were downloaded from the National Center for Biotechnology Information (NCBI) website [http://www.ncbi.nlm.nih.gov/Structure](http://www.ncbi.nlm.nih.gov/Structure): A*0201 (PDB #1JF1), B*2705 (PDB #1HSA) and Cw*0202 (PDB #1EFX) and viewed with the Cn3D software program [5].

---

Usefulness of HLAMatchmaker in HLA epitope matching Duquesney 595

of about 2–5 so-called highly energetic residues (sometimes referred to as ‘hot spots’) that dominate the strength and specificity of binding with antibody. The residues of such functional epitope are about \(3\) Å apart from each other and at least one of them is non-self. The remaining residues of a structural epitope contribute supplementary interactions that increase the stability of the antigen–antibody complex. These concepts have been applied to the new version of HLAMatchmaker [7**].

Class I HLA molecules have 75 polymorphic positions and a determination of their residue compositions within a 3-Å radius has yielded a total of 199 so-called eplets on HLA-A, B, C antigens, 110 are on the \(\alpha\) helices, 60 are on the side surface and 29 are in less accessible positions at the bottom and under the peptide-binding groove [7**].

Many eplets are identical to triplets but others have residues in discontinuous sequence positions that cluster together on the molecular surface. Serologically defined HLA determinants correspond well to eplets. The eplet version of HLAMatchmaker represents therefore a more complete repertoire of structurally defined HLA epitopes and provides a more detailed assessment of HLA compatibility.

Each eplet is assigned a position number in the amino acid sequence and the polymorphic residues within a radius of about 3 Å; this notation does not use monomorphic residues. Amino acid residues are marked with the standard letter code. For instance, the class I eplet 11AMR is in sequence position 11 and has three polymorphic residues: alanine (A), methionine (M) and arginine (R). Many eplets are listed with one or two residues (for instance 9H and 193PV) because their neighboring residues are the same on all HLA Class I chains and they are therefore not shown.

HLAMatchmaker applies two principles: (1) each HLA antigen represents a distinct string of structurally defined epitopes as potential immunogens that can induce specific antibodies and, (2) patients cannot make antibodies against epitopes that are expressed by their own HLA molecules [6*]. The algorithm assesses donor–recipient compatibility through intralocus and interlocus comparisons, and determines what epitopes on mismatched HLA molecules are

---

**Figure 2**

<table>
<thead>
<tr>
<th>Top view</th>
<th>(\beta)-chain side view</th>
<th>(\alpha)-chain side view</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR</td>
<td>HLA-DR</td>
<td>HLA-DR</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td>HLA-DQ</td>
<td>HLA-DQ</td>
</tr>
</tbody>
</table>

Polymorphic amino residue positions on HLA-DRB and HLA-DQ molecules. The following crystalline models were downloaded from the NCBI website http://www.ncbi.nlm.nih.gov/Structure: DRA1*0101, DRB1*0101 (PDB # 1KG0) and DQA1*0301, DQB1*0302 (PDB # 1JK8) and viewed with the Cn3D software program [5].
different or shared between donor and patient. This analysis considers each donor HLA antigen mismatch towards the entire HLA-A, B, C phenotype of the recipient.

Table 1 shows examples with three mismatched antigens B51 (B*5101), B27 (B*2705) and B61 (B*4002) for eight different HLA-A, B, C phenotypes. Although they are all considered one-antigen mismatches by conventional matching criteria, they display marked differences in structural epitope compatibility. For certain HLA phenotypes a given mismatch has no or few mismatched epitopes (B51 for cases 7 and 8; B61 for cases 3, 5, 6 and 8) but for other phenotypes, the same HLA antigen has many mismatched epitopes (B51 for cases 1, 2 and 3; B61 for case 5) and is therefore, structurally highly incompatible. Table 1 also illustrates that certain antigens such as B27 do not often have zero or few mismatched epitopes. Altogether, the epitope load of a donor HLA mismatch is influenced by the recipient’s HLA type representing a repertoire of self-epitopes to which no antibodies can be made.

The class II eplet version of HLAMatchmaker is based on 44 DRB, 33 DQB, 29 DQA, 20 DPB and 9 DPA polymorphic positions that contribute a repertoire of 146 DRB, 74 DQB, 56 DQA, 45 DPB and 19 DPA eplets [8]. Conventional matching criteria for organ transplantation have always emphasized the importance of DR antigens. However, many studies have shown that the immunogenic products of other class II loci can elicit antibody responses that are detrimental to the allograft. Thus we must consider that each DR antigen mismatch (and often enough a match) will have an extra epitope load because of additional incompatibilities at the DRB3/4/5, DQ and DP loci. HLAMatchmaker can determine the extent of the class II epitope load. As an example, a patient who types as DR15, DR18 may have the following genotype: DRB1*1501, DRB5*0101, DQB1*0502, DQA1*0102/DRB1*0302, DRB3*0101, DQB1*0402, DQA1*0401. Table 2 shows most common DR-DQ haplotypes of fourteen DR antigens and the numbers of corresponding mismatched eplets. Five antigens DR4, DR7, DR9, DR11 and DR12 have more than twenty mismatched eplets, a relatively high epitope load. Conversely, DR1, DR8 and DR16 have fewer than ten mismatched eplets. DR17 which is serologically similar to DR18 has no mismatched DRB eplets but there are nine mismatched DQB1 and eight DQA1 eplets. The ‘self’ DR15 antigen has a DQB1*0602 mismatch with six eplets. Although this example does not include HLA-DP, it illustrates how HLAMatchmaker can determine different epitope loads among the class II mismatches.

### Effect of epitope load on the HLA antibody response

Early studies by Lobashevsky et al. [9] have shown that the number of immunogenic triplet mismatches offers a reliable predictive value for flow cytometry crossmatches with sera from highly sensitized renal patients. Dankers et al. [10] demonstrated a strong correlation between the number of mismatched triplets and the incidence of

<table>
<thead>
<tr>
<th>Case</th>
<th>Phenotype</th>
<th>#Ep B51 (B*5101), mismatched eplets</th>
<th>#Ep B27 (B*2705), mismatched eplets</th>
<th>#Ep B61 (B*4002), mismatched eplets</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A<em>0101 A</em>0201 B<em>1402 B</em>0702 Cw<em>0701 Cw</em>0702</td>
<td>11AMR, 44RTE, 76ERI, 82ALR, 113HN, 193PV</td>
<td>5</td>
<td>9H, 70AKA, 76ERT, 82LLR, 151RV</td>
</tr>
<tr>
<td>2</td>
<td>A<em>0101 A</em>0201 B<em>0702 B</em>0801 Cw<em>0701 Cw</em>0702</td>
<td>44RTE, 76ERI, 82ALR, 131S, 163L, 193PV</td>
<td>5</td>
<td>9H, 70AKA, 76ERT, 82LLR, 131S</td>
</tr>
<tr>
<td>3</td>
<td>A<em>0101 A</em>0201 B<em>0702 B</em>4501 Cw<em>0701 Cw</em>0702</td>
<td>44RTE, 76ERI, 82ALR, 113HN, 193PV</td>
<td>3</td>
<td>9H, 70AKA, 76ERT, 82LLR</td>
</tr>
<tr>
<td>4</td>
<td>A<em>0101 A</em>2501 B<em>0702 B</em>0801 Cw<em>0701 Cw</em>0702</td>
<td>4</td>
<td>9H, 70AKA, 73TD, 76ERT, 82LLR, 113YH, 131S</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>A<em>0101 A</em>0201 B<em>0702 B</em>4403 Cw<em>0501 Cw</em>0702</td>
<td>44RTE, 76ERI, 113HN</td>
<td>3</td>
<td>9H, 70AKA, 82LLR</td>
</tr>
<tr>
<td>6</td>
<td>A<em>0101 A</em>0201 B<em>4501 B</em>3901 Cw<em>0501 Cw</em>1701</td>
<td>4</td>
<td>65QIA, 70AKA, 76ERT, 82LLR</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>A<em>0101 A</em>2501 B<em>3501 B</em>4101 Cw<em>0602 Cw</em>0401</td>
<td>None</td>
<td>8</td>
<td>44REE, 65QIA, 70AKA, 73TD, 76ERT, 82LLR</td>
</tr>
</tbody>
</table>
humoral sensitization induced by a kidney transplant or developed during pregnancy. By contrast, there seems no significant association between triplet mismatching and cytotoxic T-cell precursor frequencies, an indicator of cellular alloimmune responsiveness [11]. Taylor’s group at Cambridge University Hospitals in the United Kingdom have also reported a correlation between the number of mismatched triplets and eplets and the presence of HLA antibodies detected in Luminex assays with single class I alleles [12,13].

Epitope loads also affect anti-class II antibody responses. Donor-specific, DRB1-reactive antibodies are less often detectable than antibodies against other class II epitopes [14]. Antibody absence correlates with low numbers of mismatched DRB1 eplets. By contrast, donor-specific DRB3, 4 and 5 mismatches induce more antibody responses and they have higher numbers of incompatible eplets. Especially striking is the high incidence of antibodies against DRB5 especially at the level of structurally defined DPB epitopes has an adverse effect on kidney transplant survival [22]. Class I triplet-based matching is also associated with a better prognosis of penetrating keratoplasty and reduces the time on the waiting list for most patients awaiting a corneal transplant [23]. There is also an association between eplet numbers of donor HLA class I antigen mismatches and pediatric cardiac transplant rejection [24].

Although the group of Opelz concluded from their analysis of the Collaborative Transplant Database that triplet matching had no significant association with kidney graft survival [20], their data showed clearly similar five-year graft survivals for the zero-antigen mismatches and groups with zero or few triplet mismatches [21].

Altogether, these findings suggest that HLA-Matchmaker has the potential of optimizing donor kidney allocation to reduce the problem of humoral alloimmunization.

**Effect of HLA epitope load on transplant outcome**

The triplet matching concept has clinical relevance as suggested by an analysis of the UNOS and Eurotransplant kidney transplant databases showing that HLA-A,B mismatched kidneys that are compatible at the triplet level exhibit almost identical graft survival rates as the zero HLA-A,B antigen mismatches defined by conventional criteria [16]. This beneficial effect of triplet matching applies to both non-sensitized and sensitized patients and also to white and non-white patients. Haririan *et al.* [17] have also shown that triplet matching can provide useful prognostic information about kidney transplantation in African-Americans. Very recently, Valentini *et al.* applied HLA-Matchmaker to the selection of successful kidney transplants for two highly sensitized pediatric patients following desensitization treatment with intravenous immunoglobulin [18,19].

Although the group of Opelz concluded from their analysis of the Collaborative Transplant Database that triplet matching had no significant association with kidney graft survival [20], their data showed clearly similar five-year graft survivals for the zero-antigen mismatches and groups with zero or few triplet mismatches [21].

<table>
<thead>
<tr>
<th>Patient</th>
<th>DR antigen</th>
<th>DRB1</th>
<th>DRB3/4/5</th>
<th>DQB1</th>
<th>DQA1</th>
<th>Eplet total</th>
<th>DRB1 eplets</th>
<th>DRB3/4/5 eplets</th>
<th>DQB1 eplets</th>
<th>DQA1 eplets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>DR1</td>
<td>DRB1*0101</td>
<td>None</td>
<td>DQB1*0501</td>
<td>DQA1*0101</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>DR4</td>
<td>DRB1*0401</td>
<td>DRB4*0101</td>
<td>DQB1*0301</td>
<td>DQA1*0302</td>
<td>42</td>
<td>8</td>
<td>14</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>DR7</td>
<td>DRB1*0701</td>
<td>DRB4*0101</td>
<td>DQB1*0202</td>
<td>DQA1*0201</td>
<td>41</td>
<td>10</td>
<td>14</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>DR8</td>
<td>DRB1*0801</td>
<td>None</td>
<td>DQB1*0402</td>
<td>DQA1*0401</td>
<td>42</td>
<td>8</td>
<td>14</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>DR9</td>
<td>DRB1*0901</td>
<td>None</td>
<td>DQB1*0302</td>
<td>DQA1*0302</td>
<td>36</td>
<td>8</td>
<td>14</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>DR10</td>
<td>DRB1*1001</td>
<td>None</td>
<td>DQB1*0501</td>
<td>DQA1*0101</td>
<td>12</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>DR11</td>
<td>DRB1*1101</td>
<td>DRB3*0202</td>
<td>DQB1*0301</td>
<td>DQA1*0501</td>
<td>22</td>
<td>3</td>
<td>2</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>DR12</td>
<td>DRB1*1201</td>
<td>DRB3*0202</td>
<td>DQB1*0301</td>
<td>DQA1*0501</td>
<td>26</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>DR13</td>
<td>DRB1*1301</td>
<td>DRB3*0101</td>
<td>DQB1*0603</td>
<td>DQA1*0103</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>DR14</td>
<td>DRB1*1401</td>
<td>DRB3*0202</td>
<td>DQB1*0503</td>
<td>DQA1*0104</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>DR15 (self)</td>
<td>DRB1*1501</td>
<td>DRB3*0101</td>
<td>DQB1*0602</td>
<td>DQA1*0102</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>DR16</td>
<td>DRB1*1601</td>
<td>DRB3*0202</td>
<td>DQB1*0502</td>
<td>DQA1*0102</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>DR17</td>
<td>DRB1*0301</td>
<td>DRB3*0101</td>
<td>DQB1*0201</td>
<td>DQA1*0501</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>DR18 (self)</td>
<td>DRB1*0302</td>
<td>DRB3*0101</td>
<td>DQB1*0402</td>
<td>DQA1*0401</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
alloimmunized to HLA. Two studies have shown an association between successful transfusions and low numbers of HLAMatchmaker-determined triplets or eplets on donor platelets [25,26]. A recent report describes an HLAMatchmaker-based strategy for platelet transfusions in thrombocytopenic patients [27]. By contrast, triplet matching does not benefit patient survival after unrelated donor bone marrow transplantation although there is a slight effect on engraftment and acute graft-versus-host disease [28].

HLAMatchmaker-based analysis of serum antibody reactivity
HLAMatchmaker has primarily been used to analyze the reactivity patterns of sera from highly sensitized patients and the identification of potential donors with acceptable mismatches. The group of Frans Claas at Leiden University Medical Center in The Netherlands has convincingly demonstrated that the primary purpose of serum screening must focus on the identification of acceptable mismatches for highly sensitized patients [29**,30*,31,32]. This approach shortens the waiting time for a suitable kidney donor and leads to excellent graft survival comparable to those seen with non-sensitized recipients [29**]. The application of HLAMatchmaker has enhanced the Acceptable Mismatch program [30*] and is now routinely used in Eurotransplant [32]. The cumulative frequencies of self-antigens and acceptable mismatches can be used to calculate the probability of finding a donor as an assessment of the transplantability of a sensitized patient [33].

Goodman et al. [12] have also used HLAMatchmaker in the analysis of sera from highly sensitized patients in single class I allele Luminex assays to determine acceptable mismatches. Other investigators have also demonstrated the usefulness of HLAMatchmaker to analyze antibody reactivity in different serum screening assays to identify suitable donors for retransplantation [34–38].

A recent study addressed the use of HLAMatchmaker to analyze the reactivity patterns of class II specific antibodies in transplant patients [14]. Sera from 75 sensitized patients were screened with single DR, DQ and DP heterodimers on a Luminex platform. About one-quarter of the patients had donor-specific antibodies induced by DRB1 eplets versus a 50% frequency of antibodies to DRB3 and DRB5 eplets and more than 80% frequency of antibodies to DRB4 eplets. Donor-specific antibodies to DQB eplets were found in more than 80% of the sera and anti-DQA eplet antibodies were detected with a frequency of about 60%. About one-third of the sera had anti-DP antibodies; they reacted predominantly with two DPB eplets and an allelic pair of DPA eplets.

The HLAMatchmaker analysis of serum screening results with single allele panels should consider high-resolution (four-digit) molecular typing of both antibody producer and immunizing donor to determine their exact immunogenetic relationship. Also, very helpful is the categorization of HLA sensitized patients according to the presence of sensitizing tissue: (1) sensitizing tissue is absent such as a previous transplant has been removed, or prior transfusion or pregnancy, (2) sensitizing tissue is present and may absorb donor-specific HLA antibodies especially class I and (3) the combination of both conditions.

The first step of an HLAMatchmaker analysis of serum antibody reactivity is to identify those alleles that give negative reactions. Such alleles can be expected to have eplets that are not recognized by patient’s antibodies and from these eplets together with the patient’s own eplets we can identify alleles that are acceptable mismatches. Certain antibodies react only with a pair of eplets on the same allele. This was first observed in a study on human monoclonal antibodies derived from a woman who had become HLA sensitized during pregnancy [39]. As an example, an antibody generated against 62QE of the immunizing HLA-A3 antigen reacted with only 62QE-carrying antigens except HLA-A30 and HLA-A31. All reactive antigens share with HLA-A3 a glycine residue in sequence position 56 whereas the non-reactive 62QE-carrying antigens have an arginine residue. Positions 56 and 62 are about 11 Å apart; this distance is sufficient to permit contact with two different complement determining region (CDR) loops of antibody. One is the antibody specificity-mediating CDR loop which interacts with 62QE and the other CDR loop interacts with 56G which as been referred to as a Critical Contact Site necessary for sufficient antibody binding. In other words, the epitope recognized by this monoclonal antibody is represented by a pair of eplets. Thus, another explanation for a negative reaction with antibody could be that the allele has the specific eplet but lacks the Critical Contact Site. In such case, acceptable mismatches would more difficult to identify. This concept may also explain why antibodies react with certain antigens in binding assays but not in complement-dependent lymphocytotoxicity [40].

HLAMatchmaker-based matching: avoid immunogenic epitopes
An epitope has two characteristics namely antigenicity, that is its reactivity with antibody, and immunogenicity, that is its ability of inducing an antibody response. Immunogenicity depends on the structural difference between an immunizing protein and the antibody responder’s homologous proteins [41]. HLAMatchmaker can be used as a quantitative tool to determine the degree of a mismatch. As described above, the epitope load affects the HLA antibody response and allograft outcome. HLAMatchmaker seems also useful as a qualitative tool to assess epitope immunogenicity determined by the frequency of a specific antibody response [42]. High
immunogenicity epitope mismatches should be avoided whereas low-immunogenicity epitopes might be considered permissible mismatches.

There is no structurally based prediction model for determining epitope immunogenicity. Possible factors include location and exposure of an epitope on the molecular surface, the relative difference in amino acid residue composition and perhaps the (HLA) genetic make up of the antibody responder. At present, a practical approach is to collect information about the frequencies of epitope-specific antibody responses in context with the exposure rate to epitope mismatches [43]. One possibility is to analyze serum screenings on patients with rejected kidney transplants who have undergone allograft nephrectomy. HLAMatchmaker-based analysis reveals restricted antibody specificity patterns against certain structurally defined epitopes on immunizing donor HLA antigens whereas other donor epitopes do not react [44*]. Donor specific antibody reactivity may persist for long periods of time after allograft nephrectomy but often enough it diminishes and even become undetectable after a few months.

A preliminary study conducted under auspices of the 14th International HLA Workshop, has shown differences between class I epitope immunogenicity [43]. Serum screening was limited to lymphocytotoxicity assays and in some instances, antigen-binding assays such as Elisa and Flow beads. Almost no data were available about antibody responses to class II epitopes. This study is continuing as a 15th International HLA Workshop project. All serum screenings include Luminex assays with single HLA-A, B, C and HLA-DR, DQ and DP alleles so that HLA antibody reactivity patterns can be analyzed in more precise detail. We expect that informative allograft nephrectomy cases will generate reasonable estimates about epitope immunogenicity following kidney transplantation. Such information will be useful for the development of a donor-selection strategy based on permissible mismatching.

Conclusions
HLAMatchmaker is clinically useful in the management of transplant patients and in providing platelet transfusion support of thrombocytopenic patients. It permits a structural assessment of donor–recipient compatibility and the identification of antibodies against epitopes on HLA class I and class II alleles can determine mismatch acceptability for sensitized patients.

HLAMatchmaker programs, publications and tutorials can be downloaded free of charge from the website: http://www.HLAMatchmaker.net.

Acknowledgement
The HLAMatchmaker studies by the author are supported by Grant RO1-AI-55933 from the National Institutes of Health.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
* of special interest
** of outstanding interest


This paper describes the application of HLAMatchmaker in the acceptable mismatch program for successful kidney transplantation of highly sensitized patients.


This paper describes an HLAMatchmaker-based analysis of sera from highly sensitized patients and its role in the Acceptable mismatch program.


This report shows that donor-triplet reactive antibodies become readily detectable in sera following removal of a rejected kidney transplant.