# Matching inside and outside the HLA molecule in allogeneic hematopoietic stem cell transplantation

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Allogeneic hematopoietic stem cell transplantation (HSCT) remains the most effective cure for many patients suffering from hematologic disorders, and more than one million hematopoietic stem cell transplants have been performed worldwide. When a human leukocyte antigen (HLA)-matched sibling donor is not available, a search for an HLA-matched unrelated donor is initiated. Due to the international effort to establish registries of potential donors (currently almost 28 million), the success of unrelated donor HSCT has improved significantly.[1]

Matching for HLA is a critical factor in reducing the risk of the post-transplant complications of graft failure and graft-*versus*-host disease (GvHD). Ideally, HLA matching for all loci (12/12) should be the gold standard; however, the HLA system is highly polymorphic, with 7897 different HLA class I (HLA-A, -B and -C) proteins and 2768 different HLA class II (HLA-DRβ, -DQβ and -DPβ) proteins currently known (from http://www.ebi.ac.uk/ipd/imgt/hla/stats.html; accessed: July 2016). It is, therefore, often necessary to transplant patients using partially HLA-mismatched unrelated donors. The strategies adopted in an attempt to limit GvHD in the HLA-mismatched setting include the use of cord blood donor cells, where HLA mismatching is better tolerated,[2] or haplo-identical family members as donors with post-transplant cyclophosphamide to selectively eliminate the alloreactive T cells that cause GvHD.[3]

Nonetheless, standard practice remains the use of adult unrelated donors, often mismatched for one or more HLA loci. Studies of HSCT survival have provided the basis for the current recommendations from the National Marrow Donor Program (NMDP) and the Center for International Blood and Marrow Transplant Research (CIBMTR) for allele level matching unrelated donors with patients at the HLA-A, -B, -C and -DRB1 loci (8/8 match);[4,5] if unavoidable, a 7/8 match can be used.

Given the uncertainty regarding the impact of HLA mismatches on HSCT outcomes, strategies are being sought to help guide donor selection when several potential options are available. An algorithm has been developed for selecting favorable HLA-DPB1 mismatches based on clinical outcomes indicating a survival advantage from T-cell epitope matching.[6] HLA-A and HLA-B proteins can be segregated according to expression of shared antibody epitopes known as cross-reactive groups (CREG); however, a large retrospective study showed that an HLA allele mismatch within a CREG group does not result in better transplant outcomes than a mismatch outside GREG groups.[7] Similarly, HLA matchmaker is an algorithm for assessing compatibility at the antibody epitope level, but it also fails to predict outcomes after HSCT.[8] Antibody epitopes are typically located on the outer surface of proteins, but the polymorphic amino acids of HLA proteins are primarily concentrated at positions in the peptide-binding site. The HistoCheck scoring system was developed in an attempt to rank HLA-A, -B or -C mismatches taking into account all amino acid differences between allele mismatched pairs; however, retrospective review again showed that the strategy does not predict clinical outcomes after HSCT.[9,10]

In this issue of Haematologica, Lazaryan *et al.*[11] report a new approach to assessing the impact of HLA mismatches on the success of HSCT. They performed a retrospective analysis of outcomes of 1,934 patients after myeloablative HSCT for non-lymphoid malignancies using 7/8 HLA-matched unrelated donors. The single allele mismatches were grouped according to supertypes. The six HLA-A and six HLA-B groups were based on HLA class I peptide-binding motifs using the supertype classifications described by Sidney *et al.*[12] The polymorphic HLA residues lining the peptide-binding site determine the shape and therefore types of peptides bound. Although an HLA molecule can bind a diverse range of peptide sequences for surveillance by T cells, those bound by each allelic protein product share common motifs dictated by the shape of the peptide-binding site. Analysis of the sequences of peptides bound by HLA molecules led to identification of peptide-binding motifs and the realization that HLA molecules can be clustered into groups that bind overlapping peptide repertoires reflecting similarities in the structure of their peptide-binding sites. The five HLA-DR supertypes used by Lazaryan *et al.* are based on sequence and structural similarities in the peptide-binding sites defined using an algorithm developed by Doytchinova and Flower,[13] and the groupings agree well with known HLA-DR peptide-binding motifs. The classification of HLA-C into two groups was based on polymorphism at residue 77 in the peptide-binding site[14] that influences killer Ig-like receptor (KIR) binding. HLA-C supertypes based on peptide preferences have not been defined because less is known about the peptide-binding specificities of HLA-C.[15]

Of the 694 single HLA-A allele mismatches, 38% were supertype matched; of the 322 single HLA-B allele mismatches, 71% were supertype matched; of the 714 HLA-C single allele mismatches, 51% were supertype matched and of the 204 HLA-DRB1 single allele mismatches, 75% were supertype matched. Mismatching of HLA-B super-

**Table 1.** Number of protein polymorphisms at each HLA locus.

| HLA-CLASS I | | |
|---|---|---|
| HLA-A | HLA-B | HLA-C |
| 2480 | 3221 | 2196 |
| **HLA-CLASS II** | | |
| HLA-DRβ | HLA-DQβ | HLA-DPβ |
| 1569 | 647 | 552 |

types was found to be a significant independent risk factor for grade II-IV acute GvHD, with a cumulative incidence of 67% when HLA-B supertypes were mismatched compared to 47% when HLA-B supertypes were matched ($P$=0.007). HLA supertype mismatching was not significantly associated with any other major post-transplant outcome.

Grouping HLA alleles according to similarities in their peptide-binding motifs already has proven utility in identifying epitopes recognized by pathogen- and tumor-specific T cells, and understanding HLA associations with disease and protective immunity.[12] The association of HLA-B supertype mismatching with increased GvHD risk is the first evidence indicating that knowledge of peptide-binding motif supertypes might help guide prediction of the strength of allogeneic immune responses after transplantation. There has been uncertainty regarding the molecular basis of T-cell allorecognition. Some alloreactive T cells may recognize features on the outside surface of allogeneic HLA molecules independently of the peptide inside the binding site although most are specific for single peptides.[16] It is perhaps surprising that an association between peptide-binding motifs and GvHD was only seen for HLA-B supertype mismatches. The authors speculate that this may be due to higher polymorphism at the HLA-B locus (Table 1) driven by evolutionary pressures from infectious pathogens. Of note, HLA-B has diverse peptide-binding motifs covering preferences for proline or amino acids with basic, acidic, small or aliphatic properties at peptide position 2.[12] In contrast, HLA-A peptide-binding motifs have more limited preferences for peptides with small, aliphatic or aromatic amino acids at position 2[12] and HLA class II supertypes defined by peptide binding motifs have been shown to exhibit substantial repertoire overlap.[17] The peptides presented by HLA-B supertype mismatches may look more different and promote stronger alloreactive T-cell responses.

Despite the large size of the single-allele 7/8 HLA mismatched dataset used in this study (collated by the CIBMTR from multiple transplant centers), the extent of HLA diversity meant that numbers of individual HLA-B supertype mismatches were small. The capacity to detect specific combinations significantly associated with GvHD was limited to the HLA-B07-B44 supertype mismatch. Findings from this study indicate that HLA-B supertype matching is beneficial, but refinement to identification of specific mismatches to avoid was not achieved. Clustering HLA alleles into supertypes based on peptide-binding motifs is an encouraging beginning, but further development of reliable criteria for selecting optimal HLA mismatches in the unrelated donor HSCT setting will be challenging.

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