How to select the best available related or unrelated donor of hematopoietic stem cells?

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ABSTRACT

Recognition of HLA incompatibilities by the immune system represents a major barrier to allogeneic hematopoietic stem cell transplantation. HLA genotypically identical sibling donors are, therefore, the gold standard for transplantation purposes, but only 30% patients have such a donor. For the remaining 70% patients alternative sources of stem cells are a matched unrelated adult volunteer donor, a haploidentical donor or a cord blood unit. The definition of 'HLA matching' depends on the level of resolution and on which loci are tested. The development of HLA molecular typing technologies and the availability of more than 27 million donors in the international database has greatly facilitated unrelated donor searches. The gold standard is high resolution typing at the HLA-A, -B, -C, -DRB1, and -DQB1 loci (10/10 match). Single disparities for HLA-A, -B, - C, or -DRB1 are associated with increased risk of post-transplant complications, but less so in patients with advanced disease, and in those undergoing T-cell-depleted allografting. HLA-DQB1 mismatches seem to be better tolerated and some HLA-C, -DRB1 and -DPB1 disparities are potentially less immunogenic. HLA typing by next-generation sequencing methods is likely to change matching algorithms by providing full sequence information on all HLA loci in a single step. In most European populations a 10/10 matched donor can be found for at least 50% of patients and an additional 20-30% patients may have a 9/10 matched donor. Genetic factors that help in identifying donors with less immunogenic mismatches are discussed. Haploidentical donors are increasingly used as an alternative source of stem cells for those patients lacking a matched unrelated donor.

Introduction

Among the many factors that influence the outcome of hematopoietic stem cell transplantation (HSCT) polymorphism of the classical human leukocyte antigen (HLA) genes represents the most important barrier.[1,2] The number of known HLA alleles is still growing and this trend will become even more pronounced with the wider use of high throughput sequencing methods[3] in clinical laboratories that perform histocompatibility testing. Allorecognition of HLA allelic differences by T lymphocytes confers a higher risk of acute graft-*versus*-host disease (GVHD) and mortality. HLA genotypically matched sibling donors are, therefore, the gold standard source of stem cells for allogeneic HSCT. However, since about 70% of patients do not have an available HLA-identical sibling, at least in Western countries, alternative sources have to be considered, such as HLA-'matched' unrelated adult donors, cord blood units, or haploidentical donors. Since 2007, the number of transplants with stem cells from an unrelated donor has been higher than the number from matched sibling donors, reaching 53% in 2013.[4]

The developments of molecular typing technologies and the continuous increase in the number of volunteer donors in the Bone Marrow Donor Worldwide (BMDW) database have undoubtedly improved the identification of well-matched, unrelated donors and contributed to the impressive expansion of HSCT programs worldwide.[1,4-7] Over 27 million donors are now registered in the international database (*www.bmdw.org*) and an increasing proportion of these donors are typed by

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molecular techniques at all HLA loci and at the allele level. Nevertheless, despite these achievements, many patients will still not have a fully matched donor because of the extremely great diversity of HLA alleles and haplotypes.[1,4-7] As of January 2016 more than 14,000 HLA alleles have been assigned, accounting for more than 10,000 different HLA proteins (*www.ebi.ac.uk/ipd/imgt/hla*) (Figure 1). This increasing level of complexity has negative consequences for patient/unrelated donor matching. Thus for many patients a challenge for the histocompatibility laboratory is to identify mismatched donors or cord blood units with the lowest potential for recognition by the immune system, in particular by the direct T-cell allorecognition mode. A better characterization of 'permissive' mismatches would undoubtedly allow increased access to HSCT for many patients. These strategies should be weighted for by donor-associated non-HLA criteria that also affect clinical outcome. This review focuses on essential immunogenetic parameters that have been reported to be relevant in unrelated and haploidentical donor search algorithms. The selection of cord blood units is not discussed here. Issues that are less familiar to the clinicians are reviewed, such as the impact of HLA typing resolution on matching criteria, the clinical relevance of so-called 'permissive' mismatches, and the impact of high throughput sequencing techniques on donor selection. A few simple recommendations for unrelated donor search algorithms are summarized in the conclusion.

Searching for 'HLA-compatible' unrelated donors

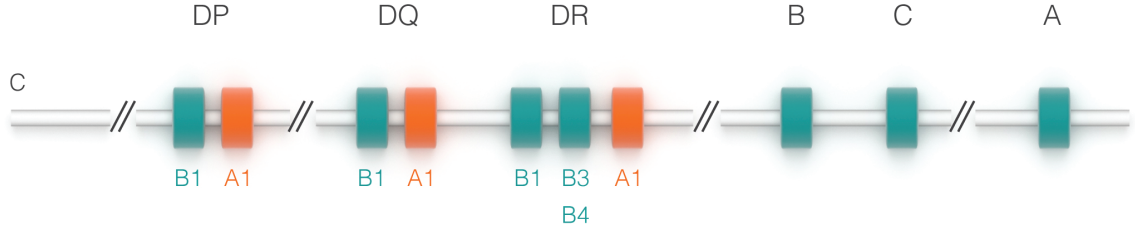
What do we mean by 'HLA-compatible'?

The compatibility status of each patient/donor pair depends on the level of resolution of HLA typing and on which loci are or are not tested. 'High resolution' typing is often described as 'four-digit typing' or 'allele-level typing' and may therefore not have exactly the same meaning in all centers. In order to establish a common language for histocompatibility terms a group of experts defined the following levels of resolution:[8]

(i) low resolution, or 'first-field level' typing, by reference to the two digits preceding the first separator, or antigen level typing, e.g. A*02;

(ii) high resolution typing, which is defined by one or a set of alleles that share the same antigen binding site formed by the α1/α2 domains of class I alleles (encoded by exons 2+3), and by the α1 domain of class II alleles (encoded by exon 2), *and that exclude null alleles* (i.e. alleles not expressed at the cell surface). For example A*02:01:01G includes all the alleles (n=52, based on the IMGT/HLA 3.23.0 release, January 2016) sharing the same exons 2+3 nucleotide sequence as A*02:01:01). The alleles or groups of alleles are designated by 'second- and third-field level' names, referring to the ≥2 digit numbers preceding, respectively, the second and third separators. Alleles with nucleotide sequences encoding the same protein sequence for the antigen binding site are designated by the suffix 'P', e.g. A*02:01P.

(iii) allele level typing, which corresponds to a unique nucleotide sequence for an HLA gene, as defined by using all digits in the first, second, third and fourth fields, e.g. A*02:01:01:01. Functionally the third and fourth fields which characterize alleles that differ, respectively, by silent substitutions in the coding sequence and by substitutions in the non-coding sequence, are irrelevant, except when substitutions prevent the expression of HLA alleles (e.g. the null allele B*15:01:01:02N). Missing a null allele



serotypes (~100)	6	9	19	46	10	21
alleles (>14,000)	673	955	1,977	4,179	2,902	3,356
proteins (>10,000)	539	648	1,444	3,095	2,067	2,372

Figure 1. Schematic map of the 4 Mb human major histocompatibility complex. The map is not drawn to scale, double separators (//) indicate larger distances and correspond to the regions where recombinations occur most frequently. C: centromere. The first row below the map indicates the number of well-defined serotypes for each locus. The DR serotypes include the antigens (heterodimers) encoded by the DRA/DRB1 (DR1-DR18), DRA/DRB3 (DR52), DRA/DRB4 (DR53) and DRA/DRB5 (DR51) genes. In the second row the total number of alleles is given (IMGT/HLA database version 3.23.0). The third row indicates the total number of different proteins. A total of 387 class I alleles with no surface expression (null alleles) and 90 class II null alleles have been described. Alleles that share identical sequences in the peptide-binding region represent 9% of the A, 6% of the B, 7.7% of the C, 1.7% of the DRB1, 17.2% of the DQB1 and 6.2% of the DPB1 alleles. Matching for the HLA-A, -B,- C,- DRB1 and -DQB1 loci is referred to as a 10/10 match, when HLA-DPB1 is included it becomes a 12/12 match. Matching for HLA-A, -B, -C, and -DRB1 loci is an 8/8 match. There is still no international standard for reporting DRB3/4/5 as well as DQA1 and DPA1 mismatches. Donor search algorithms do not include DQA1 and DPA1 testing because of strong linkage disequilibrium with the corresponding DQB1 and DPB1 loci.

will lead to a mismatch that is very likely to be recognized by alloreactive T cells and have a deleterious clinical impact.[9,10] It should be mentioned here that substitutions in non-coding sequences may influence the level of expression (e.g. the A24low allele A*24:02:01:02L). Such variability may also have an impact on anti-HLA allorecognition;

(iv) other levels of resolution, usually referred to as intermediate-level resolution, include any typing results that fall between low and high resolution. This term is used when the technique resolves a group of alleles, usually defined at the second-field level and irrespectively of the site of the polymorphisms. For example, DRB1*11:01/11:09/11:28, a string of three alleles that are depicted by the National Marrow Donor Program (NMDP) code DRB1*11:BYCC. Depending on the number and the nature of the unresolved ambiguities, the 'intermediate' level of resolution can be quite heterogeneous. It has practical relevance for donor selection when it allows discrimination between frequent alleles, as shown in the example given in Table 1 (DRB1*04:04 absent in the string of alleles assigned in the donor under the code DRB1*04:VN).

Examples with these different levels of resolution and their impact on matching status are presented in Table 1.

In most European centers the gold standard is to look for an HLA-A, -B, -C, -DRB1, and -DQB1-matched donor, a so-called 10/10 match. An alternative matching algorithm, which is recommended by the NMDP, is to look for an HLA-A, -B, -C, and -DRB1-compatible donor (8/8 match). When HLA-DPB1 typing is included, donors with a 12/12 match are sought. Although less polymorphic the DRB3/B4/B5 loci may lead to additional HLA class II mismatches. However, there is neither a common practice nor any international recommendation on how to count these mismatches in the 10/10 matching algorithm.

In clinical studies, there is still some confusion in using the histocompatibility terms for reporting HLA compatibility. For example, the German[5] and the Center for International Blood and Marrow Transplant Research (CIBMTR) studies[11,12] refer to high-resolution typing, whereas the International Histocompatibility Working Group (IHWG)[13] and a recent CIBMTR[14] study refer to allele-level matching and Japanese[15] and Swiss[16] studies refer to allele-level typing at the second-field level.

Probability of identifying an HLA-identical sibling donor or a highly matched unrelated donor

The probability of identifying an HLA-identical sibling donor depends only on the number of siblings and is 25% for patients with one sibling, 44% for those with two, 58% for those with three, 68% for those with four, and up to 90% for patients with eight siblings. On the other hand the probability of identifying a highly matched unrelated donor depends on the frequency of the patient's HLA haplotypes. As shown in Table 2, the *average* probability of identifying a matched unrelated donor differs greatly depending on the ethnic origin of the patients and on the matching grade required by the transplant center (8/8 or 10/10). Indeed, depending on ethnic origin, 1-5% of patients do not have a single potentially matched donor upon direct interrogation of the BMDW database,[7,17] because the large majority of donors registered in the database are of Western European ancestry. The ethnic origin of the patient strongly influences the probability of finding a matched donor,[18] with the lowest probability assigned to patients of African ancestry. In European countries 45-65% patients will eventually have a 10/10 matched donor, and a 9/10 matched donor may be identified for an additional 20-30% of patients (Table 2).

Impact of single mismatches

There is now a general consensus that single HLA mismatches at the HLA-A, -B, -C and -DRB1 loci are clinically relevant.[1,5-7,11,19,20] A comprehensive review of the impact of HLA-C incompatibilities on clinical outcome has been published recently.[21]

In a large CIBMTR study on patients with chronic myeloid leukemia no significant difference in overall survival was noted between patients transplanted with HLA class I or class II mismatched grafts.[22] With regards to HLA class II disparities, several studies indicated that HLA-DQB1 disparities are not associated with mortality.[5,11,20] Because of the high priority given to HLA-DRB1 matching and the strong DRB1-DQB1 linkage disequilibrium, studies are often underpowered to reveal the clinical relevance of DQB1 disparities. Evidence for a role of HLA-DPB1 mismatches is now well documented.[6,13,23-25] In 10/10-matched HSCT, DRB3 and DRB4 mismatches were associated with poorer outcome, although most donor-recipient pairs were also incompatible for the DPB1 locus.[16,26] In

Table 1. Examples of patient/donor matching status as a function of HLA typing resolution levels.

Patient	Donor	Compatibility high resolution exons 2+3 (cl.I) exon 2 (cl. II)	Compatibility allele level/ 2nd field
A*02	A*02	potential match	potential match
A*02:01:01G[a]	A*02:01:01G	potential match	potential match
A*02:01P[b]	A*02:01P	match	potential match
A*02:01	A*02:06	mismatch	mismatch
A*02:06	A*02:126[c]	match	mismatch
A*02:01:01G	A*02:09	potential match	potential match
A*02:01	A*02:09	match	mismatch
DRB1*14:01:01	DRB1*14:54:01	match	mismatch
A*02:01:01:01	A*02:01:01:01	match	match
A*02:01:01:01	A*02:26	mismatch	mismatch
A*02:01:01:01	A*02:01:01:02N	mismatch	mismatch
DRB1*11:BYCC (11:01/11:09/11:28)	DRB1*11:RDPB (11:01/11:95/11:97/11:100/11:117)[d]	potential match	potential match
DRB1*04:04	DRB1*04:VN (04:01/04:13/04:16/04:21)[e]	mismatch	mismatch
C*07:02:01G	C*07:02	match	potential match
C*07:02:01G	C*07:FEAU (07:02/07:50/07:66/07:74)[f]	match	potential match

*[a]Nomenclature: G marks all the alleles with the same nucleotide sequence in the peptide binding site (including null alleles). Since A*02:01:01G includes five null alleles (A*02:43N/02:83N/02:305N/02:356N/02:608N) the patient and donor should be tested for these alleles -unless cell surface expression has been established by serological typing- before being categorized as matched at a high resolution level. [b]P denotes a string of alleles that encode the same protein sequence in the peptide binding site (α1/α2 domains for class I and α1 for class II alleles) as the first numbered allele in the group: [c]A*02:126 differs from A*02:06 by a residue outside the peptide binding site. [d]the DRB1*11:01, *11:95, *11:97 and *11:100 share the same α1 domain but not DRB1 *11:117. [e]this string of four alleles does not include DRB1*04:04, this donor is therefore incompatible at locus DRB1. [f]the C*07:02, *07:50, *07:66 and *07:74 alleles share the same α1/α2 domains protein sequence, as does C*07:02:01G, these four alleles are included in the C*07:02:01G group of alleles.*

7/8-matched HSCT more than two mismatches at the DP, DQ and DRB3/4/5 loci were associated with an increased risk of mortality.[27]

The Japan Marrow Donor Program (JMDP) studies showed that the impact of single HLA incompatibilities has changed over time because of multiple factors, such as varying clinical protocols (GVHD prophylaxis, treatments for infections), HLA mismatches readily available in the latest period, and more intensive GVHD prophylaxis in patients with HLA-DRB1 mismatches in the earlier period.[28] Furthermore, an initial observation that HLA-B and -C incompatibilities were better tolerated than -A or -DRB1 mismatches[11] has not been confirmed in more recent studies.[5,29] In their latest report Morishima *et al.*[15] provided evidence that single HLA-A, -B or -C allele mismatches and double HLA-DRB1/DQB1 mismatches are associated with increased mortality in non-T-cell-depleted bone marrow transplantation. Interestingly single HLA-DRB1, -DQB1 or -DPB1 mismatches did not significantly affect overall survival rate. Indeed HLA-DPB1 incompatibilities were associated with both increased acute GVHD and lower relapse rates.[15]

The impact of HLA mismatches on overall mortality has been reported to be most apparent in patients with early disease.[12] In HSCT for non-malignant disorders single HLA-A, -B, -C or -DRB1 mismatches were not associated with acute or chronic GVHD but were associated with graft failure.[19]

A large study of unrelated donor reduced-intensity conditioning HSCT based on 8/8 matching recently found that single HLA-A, -B, -C or -DRB1 mismatches were associated with a higher incidence of acute GVHD and a lower disease-free survival rate without differences in relapse rate or chronic GVHD.[30] In this study HLA-C*03:03/03:04[28] and HLA-DPB1[31] permissive mismatches were not associated with better outcome. The key findings reported in recent publications (2013-2016) are summarized in Table 3.

Direct allorecognition of mismatched HLA antigens is mediated at least partly by cross-reactive viral peptide-specific memory T cells.[32] Mismatches that are characterized by changes in amino acid residues not seen by the T-cell receptor, i.e. outside the $\alpha1/\alpha2$ domains for class I antigens, are not expected to be recognized and could, therefore, be considered as acceptable mismatches. However indirect allorecognition of HLA allopeptides could also play a role. Indeed the number of peptides derived from incompatible HLA molecules presented by HLA antigens shared between the recipient and the donor can be predicted using the PIRCHE (predicted indirectly recognizable HLA epitopes) model.[33,34] In unrelated HSCT with HLA-C or -DPB1 mismatches, the number of PIRCHE has been reported to correlate with clinical outcome.[33,34]

First-field versus *second-field (antigen* versus *allele, or low* versus *high) mismatches*

Comparisons of the impact of single allele and single antigen mismatches on clinical outcome did not reveal significant differences,[5,11,12] except, possibly, for the HLA-C locus for which allele mismatches have been reported to be less detrimental than antigen mismatches.[18,20] However this could possibly be explained by the very high frequency (68.7%) of C*03:03/03:04 mismatches in the NMDP study.[29] This incompatibility had previously been reported to be more permissive, on the basis of *in vitro* assays measuring direct cytotoxic T-lymphocyte alloreactivity.[35,36]

Searching for 'permissive' mismatches

The identification of so-called permissive mismatches has been a long-lasting challenge. As a first approach, determination of the frequency of cytotoxic T-lymphocyte precursors revealed a number of HLA class I incompatibilities that had not previously been recognized and that could be considered as more permissive.[36,37] However it was not possible to reliably predict this lack of recognition by looking at the structural differences between the mismatched alleles.[38] It seems reasonable to predict that HLA disparities characterized by substitutions in the peptide binding site which significantly alter the set of pep-

Table 2. Overall probabilities of identifying a 7/8, 8/8, 9/10 and 10/10 matched unrelated donor.

Ethnic origin (country)[a]	Match 8/8	Match ≥7/8	Match 9/10	Match 10/10	Match 9-10/10	Reference
European (NL)					69%[e]	62
European (UK)					72%	63
European (A)					80%[f]	64
European (D)			20%	61%		17
European (CH)			24%	58%		7
European (NL)			31%	48%		46
European (IT)			32%	43%		65
European (HR)			30%	65%		66
European (USA)	75%	97%				18
African (USA)	18%	71%				18
ME/ NA (USA)[b]	46%	90%				18
Asian (USA)[c]	27-42%	76-88%				18
Hispanic (USA)[d]	34%	80%				18

[a]*NL: the Netherlands; UK: United Kingdom; A: Austria; D: Germany; CH: Switzerland; HR: Croatia; USA: United States of America;* [b]*ME: Middle Eastern; NA: North African;* [c]*Asian: Chinese, Korean, South Asian, Japanese, Southeast Asian, Vietnamese;* [d]*Hispanic: South/Central American;* [e]*<9/10 in 13% patients;* [f]*exceptionally 8/10 matched donors.*

Table 3. Impact of specific HLA locus or allele mismatches as reported in recent (2013-2016) multicenter studies of unrelated HSCT.

Ref.	N. of patients	Main conclusions
5	2,646	Single HLA-A,B,C,DRB1 MM (either antigen or allele) associated with increased mortality, additional risk with <9/10 matched (including DQB1) donors
13	8,539	Non-permissive DPB1 MM associated with increased mortality in 9-10/10 matched HSCT
30	3,853	In 7/8 matched HSCT : >2 MM at DRB3/4/5, DQB1 or DPB1 loci associated with lower survival
29	7,349	C*03:03/03:04 MM better tolerated, lower impact of C-locus MM explained by the high frequency of C*03:03/03:04 MM in the 7/8 matched group
12	8,003	Single HLA-A,B,C,DRB1 MM associated with increased mortality, DQB1 MM associated with increased acute GVHD, non-permissive DPB1 MM associated with increased mortality in 10/10 or 8/8 matched cases
15	7,898	Single HLA-A,B,C and double HLA-DRB1-DQB1 MM associated with increased mortality, HLA-A,B,C,DPB1 MM associated with higher risk of acute GVHD, reduced relapse only with C,DPB1 MM
30	2,588	Reduced intensity conditioning HSCT: increased mortality in 7/8 matched HSCT, no impact of C*03:03/03:04 or permissive DPB1 MM
16	803	Single HLA-A,B,C MM (9/10) associated with higher mortality, HLA-DRB1/DQB1 MM more permissive (high ratio of DRB1*11:01/11:04 and DQB1*03:01/03:02 MM)
50	2,029	In 11/12 matched HSCT: single nucleotide polymorphism in the regulatory region of DPB1 locus associated with acute GVHD
44	6,967	Patient and/or donor B*51:01 and patient C*14:02 associated with increased acute GVHD and mortality
16	11,039	Donor age (>32 years) and 7/8, 6/8 mismatched donors associated with lower overall survival

MM: mismatch.

tides presented by the HLA molecules will be more efficiently recognized by alloreactive T cells, whereas mismatches involving residues outside the peptide binding site are not expected to be recognized. Indeed a semi-quantitative, *in vitro* measurement of $CD8^+CD137^+$ alloreactive T cells in mixed lymphocyte reactions demonstrated that such a mismatch in the B44 serotype (i.e. B*44:02/44:27) was not recognized by cytotoxic T-lymphocytes and could possibly be considered as permissive.[39]

Based on *in vitro* assays set up to detect anti-HLA-DP alloreactive T cells, a new algorithm has been proposed for the identification of *non-permissive* HLA-DPB1 disparities, as defined by the presence of T-cell epitope mismatching.[31,40] Two groups of alleles with high (DPB1*09:01, 10:01, 17:01) and intermediate (DPB1*03:01, 14:01, 45:01, 86:01) immunogenicity have been assigned, whereas all the remaining most frequent HLA-DPB1 alleles are classified in a third group. Each patient/donor pair in which an HLA-DPB1 allele of the high or intermediate immunogenicity groups is present in the patient or the donor only is classified as a non-permissive mismatch (graft-*versus*-host or host-*versus*-graft direction). Of the total donor pool 70% consisted of either HLA-DPB1-matched donors or donors with a permissive HLA-DPB1 mismatch. Non-permissive HLA-DPB1 mismatches were associated with increased hazards for acute GVHD and transplant-related mortality, but not for relapse.[31] In the IHWG study,[13] HLA-DPB1 non-permissive mismatches were associated with increased risks of overall mortality in both 10/10- and 9/10-matched transplants. In contrast to these findings, a recent study[12] found that *any* HLA-DPB1 mismatch was associated with acute GVHD. However the adverse impact of non-permissive HLA-DPB1 mismatches on transplant-related mortality and overall mortality was confirmed in 8/8- and in 10/10-, but not in 7/8- or in 9/10-matched cases.

Based on a retrospective clinical study, HLA-DRB1/DQB1 mismatches have recently been reported to be more permissive than HLA-A, -B or -C disparities in 9/10-matched HSCT.[16] This was correlated with a preferential selection of donors with DRB1*11:01/11:04, DRB1*14:01/14:54 and DQB1*03:01/03:02 mismatches, which might be associated with weaker immunogenicity, as suggested by the results of previous *in vitro* assays.[16]

A few reports have described the role of individual amino acids on clinical outcome. The impact of individual HLA amino acid mismatches, such as those reported in the JMDP study[41] may not be applicable in other populations which show greater heterogeneity in HLA disparities and, therefore, fewer mismatches of similar nature. A large scale analysis evaluated the clinical impact of specific amino acid substitutions in HSCT patients with single class I mismatches.[42] It was found that patients with mismatched donors lacking an amino acid (aa) substitution at aa116 and aa99 of HLA-C and aa9 of HLA-B alleles had outcomes similar to those of patients grafted with hematopoietic stem cells from 8/8-matched donors. In particular substitutions at aa116 and aa99 were both associated with increased transplant-related mortality in the multivariate analysis.[42] The importance of aa116 of HLA-C had been observed previously.[43] Supporting the hypothesis that levels of expression of target antigens in HLA-incompatible combinations might also affect allorecognition, two studies found that patients with HLA-C*14:02 (high expression allele) had increased risks of acute GVHD and mortality.[44,45]

Immunogenetics of HLA haplotypes

There is growing evidence that, in addition to individual HLA allele disparities, non-HLA polymorphisms in the major histocompatibility complex have an impact on clinical outcome. Indeed, a haplotype effect has been reported by several groups.[23,46-50] In one study[5] the survival of patients transplanted with 10/10-matched donors from the *national* registry was better than that of patients transplanted with 10/10-matched donors from the *international* registry. This could reflect the impact of non-HLA polymorphisms which may vary in populations from different origins and may be linked to different HLA-A, -B, and -DRB1 haplotypes. The translation of major histocompatibility-resident DNA variations into clinical practice still requires careful assessment of the relative importance of

such polymorphisms on outcome. A promising candidate non-HLA polymorphism is rs9277534, a single nucleotide polymorphism associated with HLA-DPB1 expression and reported to be correlated with increased risk of acute GVHD.[50] Appropriate selection of donors based on rs9277534 typing could potentially lead to a decrease in the incidence of acute GVHD.

Haploidentical donors

For patients lacking a highly matched unrelated donor the choice between a mismatched unrelated donor, a haploidentical donor, or a cord blood unit largely depends on the centers' expertise.[51] These different graft sources have not yet been compared directly in a randomized trial. High-dose cyclophosphamide treatment after non-myeloablative conditioning and T-cell-replete haploidentical HSCT has been shown to result in acceptable rates of graft rejection and acute GVHD.[52] This new protocol has provided a valuable alternative for adult patients with hematologic malignancies who lack a matched related or unrelated donor and has drastically increased patients' chance of access to allogeneic transplantation. Indeed, a trend towards increased use of haploidentical donors and a concomitant decrease of cord blood unit transplantation have been confirmed recently.[4]

In selecting the best haploidentical donor, the number of mismatched HLA antigens on the non-shared haplotype does not seem to play a role.[53] On the other hand, evaluation of the anti-HLA immunization of the patient should be performed systematically. Indeed the presence of donor-specific antibodies has been shown to be associated with an increased risk of primary graft failure[54,55]

The anti-tumor effect of alloreactive natural killer cells has been well documented for more than a decade.[56,57] Ligands for the inhibitory killer-cell immunoglobulin-like receptors (KIR) are HLA-Bw4, HLA-C alleles with a Lys at position 80 (HLA-C1), or an Arg at position 80 (HLA-C2). Natural killer cells are defined as alloreactive when they express inhibitory KIR specific for HLA class I epitopes (Bw4, C1, C2) not expressed on the patient's cells,[56,57] and/or activating KIR that recognize ligands expressed by the recipient's cells, for example KIR2DS1-positive natural killer cells and an HLA-C2-positive recipient.[58] In its simplest form, the search for a haploidentical donor is based on incompatibility between the donor's and recipient's HLA ligands. For example, when a patient has a C1/C1 genotype and the donor a C1/C2 genotype, the inhibitory signal provided by the C2 epitope is lacking in the patient. The absence on patient's cells of one HLA ligand (Bw4, C1 or C2) recognized by inhibitory KIR can thus lead to potential alloreactivity mediated by 'licensed' natural killer cells from donors who are positive for this ligand. The role of activating KIR might also be considered in donor selection, i.e. by identifying the presence of the KIR2DS1 locus[58] or by identifying KIR B haplotype-positive donors.[59,60] An even more sophisticated approach would take into account the KIR2DL1 allelic polymorphism affecting the strength of the inhibitory receptor.[61]

Influence of next-generation sequencing methods on unrelated donor selection

The recent developments in next-generation sequencing technologies based on single molecule sequencing enable high-quality resolution of full-length HLA sequences up to the fourth-field level (intron and untranslated sequences). Next-generation sequencing is rapidly entering clinical HLA laboratories because it provides powerful and efficient HLA typing that also meets the turn-around-time requirements of the HSCT field. The advantages of next-generation sequencing technology in the selection of unrelated donors are the following:

(i) HLA typing results will be available without any ambiguity on the level of resolution, i.e. there will be no confusion on the concept of high resolution/allele level typing, especially with the identification of the null alleles;

(ii) complete, or almost complete sequence information will be available for all loci, meaning that all loci can be taken into account simultaneously for donor selection. This could be particularly relevant when only donors with multiple mismatches are available. For example donors with single class I mismatches could be sorted out on the basis of additional HLA-DRB3/DRB4/DPB1 compatibility;

(iii) not only will typing by next-generation sequencing enable matching at the protein level (corresponding to second-field typing, or to the former 'four-digit typing'), but it will also provide information on non-coding variations that can potentially affect the level of expression of a given HLA antigen.[49] The potential impact of non-coding polymorphisms is presently only speculative and will eventually be determined by large, retrospective collaborative studies. Some polymorphisms may be surrogate markers of HLA haplotypes associated with higher risks of post-transplant complications. A promising example is the rs9277534 single nucleotide polymorphism in the HLA-DPB1 regulatory region which has been shown to be correlated with acute GVHD risk.[50] Patient/donor matching algorithms should still be based on second-field level allele typing, with the exception of the null alleles defined by fourth-field level typing. It is, however, highly recommended that the full length HLA sequence information is recorded for each transplant pair.

Conclusion

When no HLA-identical sibling donor is available, an estimate of the probability of finding a fully matched unrelated donor, based on the frequency of the patient's haplotypes, will help the transplant center in taking a decision on whether to search for an unrelated donor or look for an alternative source of hematopoietic stem cells (haploidentical donor or cord blood unit). Various software programs based on high resolution HLA haplotype frequencies, such as HapLogic (NMDP), Optimatch (Germany) and Easymatch (France), can predict the number of potentially matched donors. When no 10/10-matched unrelated donor is found, prioritization of a 9/10 matched unrelated donor or an alternative donor remains difficult in the absence of randomized trials. Clearly the urgency of the transplantation and the transplant center's expertise will influence the algorithm. On the basis of published studies, some considerations and practical recommendations for the selection of optimally matched unrelated donors can be made, as summarized below:

(i) patient/donor HLA typing is mandatory for all loci taken into consideration by the transplant protocol: the minimal level is HLA-A, -B, -C, -DRB1, -DQB1 high resolution typing, i.e. exons 2 + 3 for class I and exon 2 for

class II, but second-field level typing (i.e. including polymorphisms outside the peptide binding site) is recommended;

(ii) single mismatches (first- or second-field level typing) at any of the four HLA-A, -B, -C, -DRB1 loci are associated with an increased risk of acute GVHD and mortality;

(iii) more than one mismatch among the HLA-A, -B, -C, -DRB1, and -DQB1 loci should be avoided;

(iv) before considering a mismatch, donor-specific antibodies must be identified in allosensitized patients;

(v) HLA-A, -B, -C, and -DRB1 mismatches involving residues located outside the peptide binding site (e.g. A*02:01/02:09), or residues that only fine tune the set of peptides bound to the HLA molecule (e.g. DRB1*11:01/11:04), or residues that are not seen by the T-cell receptor (e.g. C*03:03/03:04) could possibly be considered as weakly or non-immunogenic;

(vi) there is no evidence that allele mismatches should be preferred to antigen mismatches;

(vii) when no potentially HLA-A, -B, and -DRB1 compatible donor is available in the BMDW file, a potential 9/10-matched donor may be identified after selecting for HLA-A antigen mismatched donors;

(viii) HLA-DQB1 and -DRB3/4/5 mismatches should be preferred to other mismatches;

(ix) whenever two or more 10/10-matched donor are available, donor age[14] ABO blood group and HLA-DPB1 matching should be prioritized. Permissive HLA-DPB1 mismatches can be defined either by the T-cell epitope matching algorithm,[31] or by taking into account the level of DP expression tagged by the rs9277534 polymorphism.[50] On a simple, practical basis, mismatches among low expression HLA-DPB1 alleles (DPB1*02, 04, 17) should be prioritized over mismatches among high expression HLA-DPB1 alleles (DPB1*01, 03, 05, 06, 10, 11, 13, 15, 16, 19). In the case that HLA DPB1 incompatibilities are present, the patient should be tested for potential anti-DP donor-specific antibodies;

(x) the impact of mismatches may vary depending on the type and state of the underlying disease, the GVHD prophylaxis (T-cell depletion) used, and the conditioning regimen.

Although it is extremely difficult to predict the impact of any single HLA mismatch reliably, our current understanding of the immunogenetics of HSCT allows selection of mismatched donors whose cells are likely to induce a minimal alloresponse. Presently the choice between a mismatched unrelated donor, mismatched cord blood, or a haploidentical donor seems to depend on the transplant center's expertise whereas clear information on the optimal strategy awaits the results of randomized trials. Retrospective studies have nevertheless shown that it is possible to overcome the HLA barrier, to prioritize specific HLA disparities with potentially lower immunogenicity, and thus to increase the number of patients who can have access to HSCT.

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