

## \* CHAPTER 3

# Immunogenetics of allogeneic HSCT

---

## \* 3.1

# The role of HLA in HSCT

J.M. Tiercy

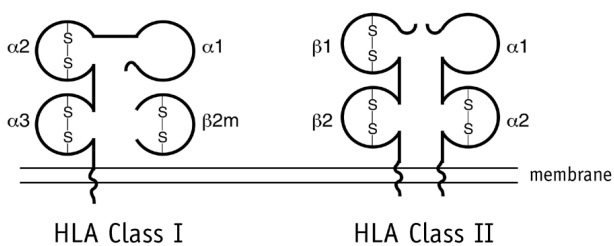
## 1. Introduction

Tissue compatibility is determined by genes of the major histocompatibility complex (MHC), known as the HLA system in man, that are clustered on the short arm of chromosome 6. The HLA region is a multigenic system that encodes structurally homologous cell surface glycoproteins characterised by a high degree of allelic polymorphism in human populations. Immune responses against incompatible HLA antigens represent a major barrier to haematopoietic stem cell transplantation (HSCT). The accuracy of histocompatibility testing and matching criteria will therefore have important consequences on transplant outcome. This is particularly true in the case of transplantation with HSC from unrelated donors, where serologically hidden incompatibilities may account for the increased rate of post-transplant complications.

## 2. HLA antigens

The homologous HLA Class I (HLA-A, -B, -C) and Class II (HLA-DR, -DQ, -DP) antigens are codominantly expressed and differ in their structure (Figure 1), tissue distribution and characteristics in peptide presentation to T-cells (1). The biological function of HLA molecules is to present peptide antigens to T-cells, thereby playing a central role in T-cell-mediated adaptive immunity. HLA Class I molecules, which are expressed on most nucleated cells, are composed of an  $\alpha$ -chain (encoded in the MHC) non covalently associated with  $\beta$ 2-microglobulin (encoded on chromosome 15) (Figure 1). The two outermost  $\alpha$ 1 and  $\alpha$ 2 domains of the heavy chain form the peptide binding site. Peptides (usually of 8–10 amino acids) presented by Class I

Figure 1: Schematic representation of HLA Class I and Class II molecules



*The two polymorphic domains  $\alpha$ 1/ $\alpha$ 2 of HLA Class I are encoded by exons 2 and 3, respectively, and exon 3 codes for the most proximal  $\alpha$ 3-domain that interacts with  $\beta$ 2-microglobulin ( $\beta$ 2m) and CD8. The HLA Class I  $\beta$ -chain is non covalently associated with  $\beta$ 2m. The Class II molecules are heterodimers composed of an  $\alpha$  and a  $\beta$ -chain. The most distal domains of each of the two chains form the peptide-binding site*

molecules are derived from proteolytic degradation of cytoplasmic proteins by the proteasome. These are transported across the endoplasmic reticulum where they bind to Class I antigens. Pathogen-derived peptides presented to Class I antigens are usually recognised by CD8+ cytotoxic T-lymphocytes (CTL) (1).

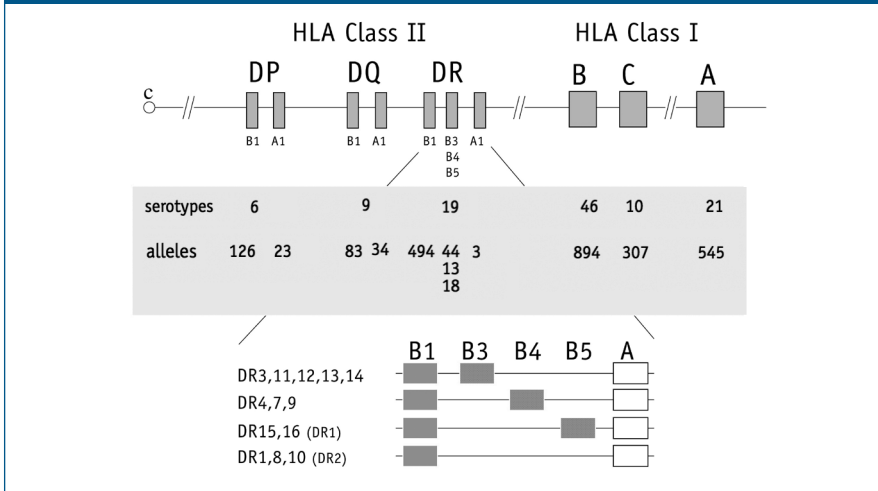
HLA Class II antigens are expressed on a subset of cells of the immune system comprising dendritic cells, B cells, activated T-cells, macrophages, collectively referred to as antigen presenting cells (APC). They are heterodimers composed of the two membrane-bound  $\alpha$ - and  $\beta$ -chains that are encoded by two genes that co-localise in the MHC. The peptide-binding pocket is formed by the most distal domains of the two chains. Extracellular antigens internalised by endocytosis/phagocytosis are degraded in an endocytic compartment into peptides of 10–30 amino acids that bind Class II molecules. HLA Class II-peptide complexes expressed on the membrane are usually recognised by CD4+ T-helper cells (1).

Peptide-HLA complexes are the ligands of clonally distributed T-cell receptors (TCRs). TCRs are also able to recognise allogeneic HLA molecules at a high frequency, so that 1–10% of the peripheral blood lymphocytes of a donor can respond to a given allo-MHC antigen (2). Immune responses against incompatible HLA antigens may be extreme, such as in the case of graft-versus-host disease (GvHD) mediated by alloreactive cytotoxic T-lymphocytes (CTL), and thus represent a major barrier to HSCT.

### 3. Genomic organisation of the HLA system

The MHC comprises 12 classical HLA genes located on a 3.6 Mb segment of the short arm of chromosome 6. Three HLA Class I genes (A, B, C) (Figure 2) encode for the heavy chains of HLA-A, B and -C antigens. Polymorphic residues are essentially located in the  $\alpha$ 1- and  $\alpha$ 2-domains encoded by exons 2 and 3, respectively, which form the peptide binding site. HLA Class II antigens (DR, DQ, DP) are heterodimers encoded by an  $\alpha$ -chain and a  $\beta$ -chain gene (e.g. DRA/DRB1 or DQA1/DQB1) that co-localise at the centromeric part of the MHC (Figure 2) (1, 3). Essentially all of the polymorphism is located on exon 2 of  $\beta$ -chain genes, whereas the DRA gene is non polymorphic, and DQA1 and DPA1 loci exhibit a lower level of polymorphism (Figure 1). The HLA-DR sub-region presents an additional level of complexity since a second polymorphic DRB gene may be present, i.e. DRB3 in DR11/DR12/DR13/DR14/DR17/DR18 haplotypes, DRB4 in DR4/DR7/DR9 haplotypes, and DRB5 in DR15/DR16 haplotypes (Figure 2). Because of the co-dominant expression of HLA genes, a heterozygous individual may therefore express up to 12 different HLA antigens.

Figure 2: HLA as a multigenic and polymorphic system



Schematic representation of the 12 HLA Class I and II loci in the MHC that comprises >200 genes on the short arm of chromosome 6. The corresponding number of antigens (as defined by serology) and alleles (as defined by nucleotide sequence) are indicated for each locus (3). About 10% of the alleles assigned by the HLA Nomenclature Committee are characterised by silent substitutions, and 2% are null alleles (3). Lower part of the figure: the HLA-DR subregion presents an additional level of complexity, with the presence of a second DRB gene in most haplotypes: DRB3, DRB4 or DRB5, which encode respectively the DR52, DR53, and DR51 antigens. For the nomenclature: see Appendix 1

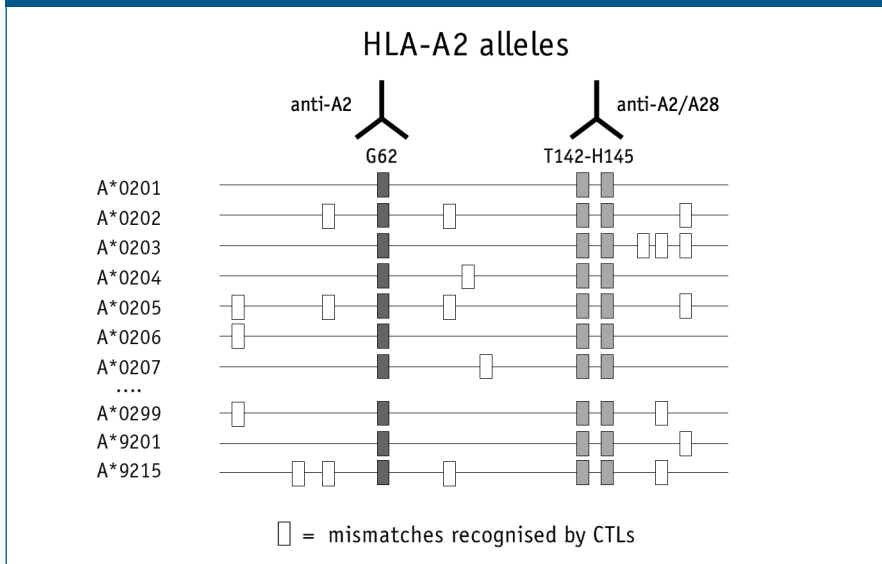
#### 4. Allelic polymorphism

HLA genes are the most polymorphic loci of the human genome accounting for a total of 2584 alleles currently identified in worldwide populations (Figure 2) (3). Polymorphism of the HLA system was initially detected by serology, i.e. by using typing reagents derived from sera of multiparous women, or individuals who have been immunised by multiple transfusions. In the early 1980's molecular cloning of the first HLA genes opened the way for a complete understanding of the molecular basis of HLA diversity and for the development of a variety of DNA-based typing techniques. Most of the serologically defined specificities are now subdivided into numerous alleles, and this number is still continuously growing (3). A regular update of the new alleles at the various HLA loci is available on the web ([www.ebi.ac/imgt/hla](http://www.ebi.ac/imgt/hla)) (Figure 2). Appendix 1 lists the HLA-A/B/C/DR/DQ serotypes and the corresponding groups of alleles detected by high resolution typing. The variability within a given serotype was recognised by cellular assays long before the DNA sequencing era. For example CTLs were shown to discriminate between two

HLA-A2 serologically identical individuals. Figure 3 shows a schematic alignment of the  $\alpha 1/\alpha 2$  domain sequences with unique residues shared by all A2 alleles (G62, T142-H145). These residues are recognised by the monospecific and/or polyspecific allo-antisera used as typing reagents. Each of the 103 different A2 alleles expressed at the cell surface differs by a very limited number of residues, usually 1–4, and such allele mismatches can be efficiently recognised by CTLs (4–7). Although many of the Class I and II antigens comprise a large number of alleles worldwide, a limited number of alleles are found in any given population at a gene frequency >0.1% (8, 9).

The combination of HLA alleles inherited on the same segment of chromosome 6 is referred to as a *haplotype*, for example the A1-B8-DR3 or the DRB1\*1501-DQB1\*0602 haplotypes. Because of linkage disequilibrium HLA disparities at a given locus will frequently be associated with incompatibilities at an adjacent locus, as observed in many instances for B-Cw and DRB1-DQB1 bi-locus groups.

Figure 3: Schematic representation of HLA-A2 alleles showing common residues on the  $\alpha 1/\alpha 2$ -domains that are recognised by alloantisera



Open boxes mark residues that differ between the 0201-0207, 9201, and 9215 alleles. For example 0201 and 0206 alleles differ at one single position (Phe9 vs. Tyr9). Such a serologically silent mismatch is recognised by CTLs (see Figure 4)

## 5. HLA typing: Methods and resolution levels

**Serology** (microlymphocytotoxicity) is still a method of choice for low resolution typing in most laboratories, at least for HLA-AB, due to its simplicity and low cost. However the lack of monospecific alloantisera, the low-resolution power of the method and the requirement for viable lymphocytes, all contributed to the development of genomic DNA typing techniques, initially for Class II, later on for Class I typing.

**DNA typing** techniques are based on the nucleotide sequence information of the polymorphic DNA segments, using PCR technology. A number of HLA typing methods based on DNA sequence variations have been developed, mainly using PCR-SSP (sequence-specific primers) amplification, or reverse PCR-SSOP (sequence-specific oligonucleotide probes) hybridisation on solid support (e.g. microbead arrays), or direct sequencing (8, 10).

Three different **levels of resolution** for HLA DNA typing are usually recognised (Table 1). Low-resolution, also referred to as *generic* typing or *2-digit* typing, corresponds to the identification of broad families of alleles that cluster into serotypes (e.g. A\*02), and is thus the equivalent of serological typing (A2). High resolution, or 4-digit

**Table 1: HLA nomenclature and levels of resolution**

HLA (a)	Definition	Resolution
A2	Refers to the A2 antigen defined by monospecific/polyspecific antisera	low
A*02	Any of the A*02 alleles (A*0201-0299 and A*9201-9215)	low
DRB1*1102/1103/1111/1114	Either one of these 4 alleles, other DRB1*11 alleles are excluded	intermediate
A*0201	Allele defined	high
A*0232N	Allele with a substitution in the coding sequence that leads to a stop codon (null allele), this allele is not expressed	high
DRB1*030101 and DRB1*030102	2 DRB1*0301 alleles that differ by a silent substitution in exon 2, this difference is functionally silent, but may influence DNA typing	high
A*24020102L	A*2402 allele with low expression due to a mutation in intron 2 sequence, this allele is expressed at a level that is undetectable by serological typing	high

(a) DNA nomenclature: the first 2 digits refer to the serotype (A\*02), the 3<sup>rd</sup> and 4<sup>th</sup> digits define substitution(s) in the coding sequence (A\*0201), the 5<sup>th</sup> and 6<sup>th</sup> digits describe synonymous substitution(s), and the 7<sup>th</sup> and 8<sup>th</sup> 2 digits refer to substitution(s) in intron or 5' or 3' sequences. N and L mark alleles with respectively no or low surface expression. The suffix S means an allele encoding a protein that is expressed as a secreted molecule only and the suffix Q means an allele with a mutation that has been shown previously to have a significant effect on cell surface expression but without confirmation, and therefore with a questionable expression status

typing, allows the discrimination of the individual alleles within each serotype (e.g. A\*0201). Intermediate resolution HLA typing of a potential donor for this patient might give as a result A\*0205/08/22: that means that the donor can be either A\*0205, or A\*0208, or A\*0222, but definitively not A\*0201 or any of the other A2 alleles. This level of typing results from the fact that these 3 alleles share common sequence determinants and are therefore identified in the same hybridisation group pattern. It is obviously very practical for rapid donor selection. In the example given above, such a donor typed as A\*0205/08/22 would be disregarded for further analysis, whereas a parallel donor with the HLA type A\*0201/01L/09/43N/66/75 would be selected and tested further in order to disclose compatibility at the allele level with the A\*0201-positive patient.

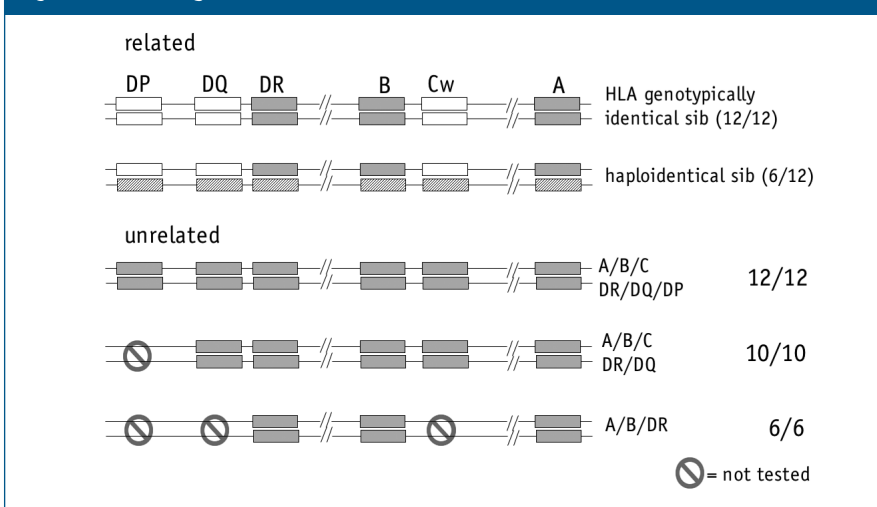
The accreditation program for the histocompatibility laboratories set up by the European Federation for Immunogenetics (EFI) has defined minimal criteria for HLA typing in related and unrelated HSCT ([www.efiweb.org/standards.html](http://www.efiweb.org/standards.html)).

## 6. HLA matching in related HSCT

The **best donor** is a HLA genotypically matched sibling as identified by family typing. The family study allows not only the identification of a potential related donor, but also the confirmation of the patient's genotype, which is important if an unrelated donor search is initiated. HLA-A, B, DR low resolution typing (serology or 2-digit DNA typing) is able in most cases to determine the paternal and maternal haplotypes present in the patient and a potential sibling donor. Thus ABDR typing can confirm genotypic identity for the whole set of HLA genes, i.e. a 12/12 match (Figure 4). Because of weak linkage disequilibrium between DP and the DR/DQ loci, a low level of DP-mismatched sibling donors (1–2%) are identified due to recombination. An HLA-A/B or B/DRB1 recombination event is detected by routine HLA-A/B/DR typing in about 2% of families. For a given patient the probability of having a genotypically identical sibling donor is 25% for each sibling, whereas the probability of having a haploidentical donor (i.e. with one shared haplotype) is 50%. When the patient has a very frequent ABDR haplotype it may be worth searching for a phenotypically identical donor in the blood-related members of the extended family, particularly when information on consanguinity is provided, for example when there is sound information that there has been intermarrying between aunts/uncles on paternal and aunts/uncles on maternal side, so that cousins may be HLA genotypically identical.

In some families, apparent homozygosity of one of the parents may prevent formal identification of genotypic identity. As illustrated in the example shown in Table 2, both siblings 1 and 2 are phenotypically identical to the patient, based on ABDR

Figure 4: Matching criteria in related and unrelated HSCT



By definition an HLA-genotypically identical sibling donor is compatible at the allele level at all loci on both chromosomes (12/12 match). In unrelated HSCT, matching for A/B/C/DRB1/DQB1 loci is usually searched for (10/10 match). In addition to DRB1 compatibility, some centres also consider DRB3/DRB5 polymorphism. DRB3 mismatches occur frequently in DR13 haplotypes. Because of strong linkage disequilibrium with DRB1 (at least in Caucasoids), the DRB5 locus is usually not tested. In DR15/16 haplotypes, DRB5 mismatches usually co-occur with DRB1 disparities. Searching for a 12/12 match implies DPB1 typing. Donors with an 8/8 match (not shown) or a 6/6 match apply, respectively, when HLA-C/DP, or HLA-C/DQ/DP are not tested

low resolution typing. However typing for HLA-C and HLA-DRB1 at the allele level shows that sibling 1 is actually compatible with the patient, whereas sibling 2 has inherited a different maternal haplotype with 2 mismatches.

In case of mismatched related HSCT, the risk of GvHD (recipient alleles absent in the donor) and graft failure (donor alleles absent in the recipient) increase with the number of HLA disparities on the non-shared haplotype (reviewed in ref. 11). A differential effect of Class I and Class II mismatches has been described, in which aGvHD risk was associated with Class II disparities (12).

## 7. HLA matching in unrelated HSC transplantation

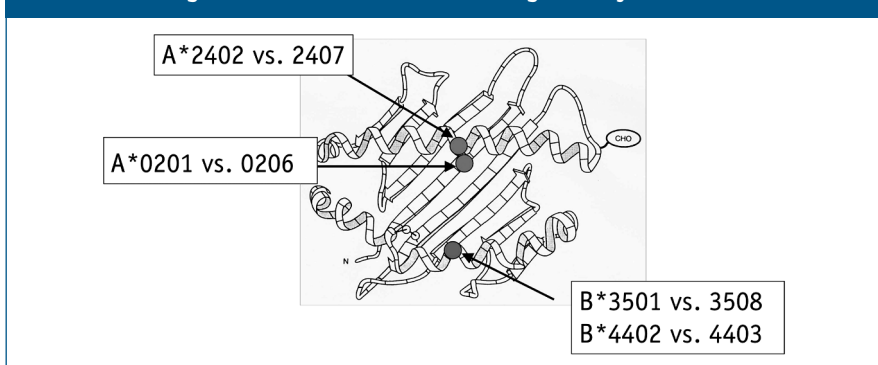
When no HLA-genotypically identical sibling donor is available, transplantation with HSC from HLA-ABCD/DRB1/DQB1-allele matched unrelated donors can result in comparable disease-free survival rates, notably for good-risk patients (11). Donor

**Table 2: Low versus high resolution HLA typing in homozygous haplotypes**

	Haplotype	Low resolution typing	High resolution typing
father	a	A1-B8-DR3	A*0101-B*0801-Cw*0701-DRB1*0301
	b	A31-B18-DR15	A*3101-B*1801-Cw*1203-DRB1*1501
mother	c	A2-B51-DR11	A*0201-B*5101-Cw*0501-DRB1*1101
	d	A2-B51-DR11	A*0201-B*5101-Cw*1203-DRB1*1104
patient	a	A1-B8-DR3	A*0101-B*0801-Cw*0701-DRB1*0301
	c	A2-B51-DR11	A*0201-B*5101-Cw*0501-DRB1*1101
sibling 1	a	A1-B8-DR3	A*0101-B*0801-Cw*0701-DRB1*0301
	c	A2-B51-DR11	A*0201-B*5101-Cw*0501-DRB1*1101
sibling 2	a	A1-B8-DR3	A*0101-B*0801-Cw*0701-DRB1*0301
	d	A2-B51-DR11	A*0201-B*5101-Cw*1203-DRB1*1104

High resolution typing may disclose hidden incompatibilities in families where the 4 parental genotypes cannot be unambiguously determined. In this example the mother is ABDR homozygous by serology, but in fact has 2 different haplotypes on the basis of high resolution typing

identification has been largely facilitated by the Bone Marrow Donor Worldwide (BMDW) Registry ([www.bmdw.org](http://www.bmdw.org)) which provides access to >11 million HLA-typed donors and cord blood units available in the national registries. Compared to HSCT from HLA genotypically identical sibling donors, unrelated HSCT is associated with an increased frequency of post-transplant complications, which are mainly due to undisclosed HLA incompatibilities not detected by serology (8, 13). Allele mismatches usually involve difference(s) in the peptide-binding site (Figure 5) that influence

**Figure 5: Examples of common serologically hidden HLA Class I incompatibilities that involve single amino acid mismatches recognised by CTLs**

T-cell recognition either by direct contact with TCR or indirectly by modulating the repertoire of peptides bound by the HLA molecule.

### 7.1. Matching criteria for unrelated donors

Whereas genotypically identical siblings share by definition the same alleles at all loci, the degree of allele matching in two unrelated individuals strictly depends on how many loci are considered for high resolution typing. Figure 4 illustrates the three most common situations: the gold standard matching comprises the analysis of HLA-A, -B, -C, -DRB1, and -DQB1 loci. When a patient and a donor share the same 5 alleles on both haplotypes the situation is referred to as a 10/10 match. If HLA-C or -DQB1 typing is omitted, this will be an 8/8 match, and if only the A/B/DRB1 loci are considered, it becomes a 6/6 match. Obviously because of the multiple possible B-C and DRB1-DQB1 associations, a 6/6 match may in fact correspond to a 6/10 match, i.e. to an incompatible combination (Table 3).

In the case of non-malignant diseases the graft versus leukaemia effect that may be mediated by DP incompatibilities is not necessary and DPB1 matching might be considered when several HLA-A/B/C/DR/DQ-compatible donors are available (12/12 match).

**Table 3: Hidden incompatibilities in HLA-ABDRB1 allele-matched donors**

Matching		A*	B*	Cw*	DRB1*	DQB1*
	patient	0201/1101	0801/3503	NT	0301/1101	NT
6/6	donor	0201/1101	0801/3503	NT	0301/1101	NT
	patient	0201/1101	0801/3503	0701/0401	0301/1101	0201/0301
8/10	donor	0201/1101	0801/3503	0701/ <b>1203</b>	0301/1101	0201/ <b>0501</b>

### 7.2. Allele matching: Relative importance of individual loci

In the early 1990's the role of HLA matching was hampered by the poor resolution achieved by HLA typing, particularly for HLA Class I alleles. Based on high resolution typing methods recent studies (14–18) have reached the almost general consensus that allele-level matching does improve transplant outcome after both myeloablative and reduced-intensity conditioning regimen (reviewed in ref. 11). The HLA effect on transplant outcome is modulated by the disease risk with an effect of single disparities reported to be significant for low risk disease patients (16).

However the relative importance of individual loci still remains an open issue. Whereas

the effect of A/B/C/DRB1 mismatches have been well documented by most retrospective large scale studies, the role of DQ and DP incompatibilities appear more controversial (reviewed in ref. 11). The National Marrow Donor program (NMDP) Histocompatibility Committee has recommended allele-level typing for HLA-A/B/C/DRB1 (8/8 match) (13). There is however some evidence suggesting a trend of an additive HLA-DQ effect in HSCT from donors with multiple mismatches (16). Recent data on T-cell-depleted unrelated HSCT have documented that HLA-DPB1 matching was associated with increased risk of relapse, irrespective of the compatibility status for the other loci (19). A comparison of serological versus allele class I mismatches in CML patients suggested that qualitative differences may influence the risk of graft failure, with a higher risk in serotype-mismatched patients (15, 20).

Contrasting outcomes have been reported in studies with patients from different ethnic backgrounds: in the Japanese Marrow Donor Program (JM DP) study (14), HLA-A/B/C/DRB1 mismatches were found to be significant risk factors for grades III–IV acute GvHD, whereas the U.S. National Marrow Donor program (NMDP) data revealed a DRB1 effect with no contribution of HLA-DQ/DP or HLA-B/C (15) mismatches. HLA-A/B/C/DRB1, but not DQ/DP mismatches decreased survival in the NMDP study (15), whereas in the JM DP study only A/B/DRB1 disparities were associated with mortality (14). Differences between studies may involve selection criteria of each transplant centre, patients age or other pre-transplant risk factors, experience in treating GvHD, as well as the relevance of the GvL effect in CML patients. Also when comparing studies from varying population groups, major differences between mismatched allele combinations may contribute to contrasting clinical outcomes.

Selection of cord blood units is generally based on HLA-A/B-low and -DRB1-high resolution matching, taking into account that cell dose is the most important parameter affecting outcome. Data on the effect of HLA matching in cord blood transplantation are difficult to interpret, since most reports lack HLA-C/DQ and high resolution HLA-AB typing results. Nevertheless cord blood appears less HLA restricted than adult HSCT, in other words HLA incompatibilities may be less detrimental (21).

## 8. Probability estimates of finding a matched unrelated donor

Identification of an unrelated HLA allele-matched HSC donor is a costly and time consuming procedure. To improve search efficiency, an estimate of the probability of identifying a 10/10 HLA matched donor allows an early decision to transplant with HSC from an alternative donor (haploidentical, cord blood, autologous), or to accept varying degrees of allele-mismatches for searches with a low probability of success, depending on the clinical context and local guidelines. Based on the current

knowledge of allele and haplotype frequencies in different populations the probability of identifying a 10/10 matched donor at the start of the search is highly predictable (22). The number of ABDR-matched (low resolution) donors available in the Registry for a given patient often reflects the chance of finding a donor compatible at the allele level. However, the following parameters have a negative impact on this probability:

- presence of a rare allele in the patient (<5% within a given serotype, e.g. B\*4405)
- HLA-ABDR haplotype that does not belong to the 10 most frequent haplotypes in Caucasoids
- unusual B-DR association (e.g. B65-DRB1\*0101)
- unusual DR-DQ association, e.g. DRB1\*1501-DQB1\*0603
- presence of an antigen that is split into >2 alleles occurring at a frequency >10% of a given serotype in the population, e.g. B35, B44, DR4, DR11, DR13
- presence of B\*2705, B18, B\*4402, B\*4403, B51 (higher risk of HLA-C mismatch)
- <3 donors available in the BMDW registry.

Because of different distribution of alleles and HLA haplotypic associations, allele matching will also be more difficult when patients and potential donors belong to different ethnic groups. For European Caucasoid patients a 40–50% probability of identifying a 10/10 matched donor has been reported (22, 23).

## 9. Choice of donor based on HLA typing

Vigorous T-cell responses are generated against allogeneic tissues when the graft and the host express different MHC molecules. Single amino acid differences in the antigen recognition site of a HLA molecule can initiate graft rejection or GvHD. The choice of the donor will therefore depend on the degree of HLA identity between donor and recipient.

### Looking for a related donor

1. HLA-A/B/DR typing (serology or low resolution DNA typing) of patient, the sibling(s) and both parents to determine the haplotypes.
2. If a sibling is **geno-identical** the transplant can be performed after a confirmatory typing on a second blood sample from patient and donor but without further HLA investigations.
3. If one sibling is a monozygotic twin this is a **syngeneic transplant**, this donor can be chosen but some of the GvL effect might be lost.
4. If both parents share a common haplotype or if one parent is HLA-ABDR homozygous, this is considered to be a **pheno-identical** transplant. High

resolution Class I and II typing is recommended to define the exact degree of compatibility.

5. If a family donor with only one HLA difference (5/6 match) is found, the transplant can be performed despite the possibility of an increased risk of GVHD. This is a related one **antigen mismatched** donor transplant. High resolution Class I and II typing is recommended to define the exact degree of compatibility.
6. Parents and/or siblings sharing only one haplotype with the recipient are considered as a **haploidentical-related** donor transplant.

### Looking for an unrelated donor

1. One should obtain a complete Class I and Class II high resolution typing for the patient (HLA-A, -B, -C, -DRB1 and -DQB1). Several levels of compatibility are defined: 12 out of 12 (A/B/C/DRB1/DQB1/DPB1) 10 out of 10 (A/B/C/DRB1/DQB1) or 8 out of 8 (A/B/C/DRB1) (see section 7). The level of HLA incompatibility (low or high resolution typing) accepted can vary from centre to centre but the priority is to look for the highest degree of compatibility at the allele level. DPB1 typing should be included in case several 10/10 matched donors are available.
2. Look at the BMDW (Bone Marrow Donor Worldwide) database to evaluate the probability of finding a donor, and determine which registries have suitable donors.
3. Send a search request to the bone marrow donor registries which have suitable donors in order to perform a confirmatory typing (high resolution) to find the best HLA matched unrelated donor.
4. Search simultaneously for a cord blood unit through BMDW and Netcord in order to find an unrelated cord blood donor.

If several HLA identical bone marrow donors are found, choose the male, ABO identical, and/or CMV-negative donor.

If there is no well matched (10/10, 9/10, or possibly 8/10) unrelated donor or if the time frame is too short, choose a cord blood unit, as long as the number of nucleated cells is  $>2 \times 10^7/\text{kg}$  and there are no more than 2 HLA mismatches (4/6).

## 10. Conclusions

The HLA system, with 100 serologically defined specificities and over 2500 alleles, represents a major barrier to HSC transplantation. There is now a broad consensus that selection of unrelated HSC donor by high resolution molecular typing technology contributes to a better clinical outcome. However the relative importance of individual loci still remain to be better defined, and multicentre studies should contribute to resolving this issue. The difficulty in reaching clear consensus among

the clinical studies, particularly with respect to the role of single locus mismatches, possibly results from subtle differences in patients groups with respect to patient and donor selection criteria, pre-transplant risk factors or GvHD prophylaxis. Additional immunogenetic factors such as minor histocompatibility antigens, cytokine and chemokine genes, or activating/inhibitory killer immunoglobulin-like receptor (KIR) genes, may play a role in determining transplant outcome. Donor HLA matching criteria should take into account parameters such as the time frame allowed by the patient's disease and the probability of identifying a well matched donor based on the patient's HLA phenotype.

## References

1. Klein J, Sato A. The HLA system. First of two parts. *New Engl J Med* 2000; 343: 702-709.
2. Afzali B, Lechler RI, Hernandez-Fuentes MP. Allorecognition and the alloresponse: Clinical implications. *Tissue Antigens* 2007; 69: 545-556.
3. Marsh SGE, Albert ED, Bodmer WF, et al. Nomenclature for factors of the HLA system, 2002. *Tissue Antigens* 2002; 60: 407-464.
4. Fleischhauer K, Kernan NA, O'Reilly RJ, et al. Bone marrow-allograft rejection by T lymphocytes recognizing a single amino acid difference in HLA-B44. *N Engl J Med* 1990; 323: 1818-1822.
5. Rufer N, Tiercy J-M, Breur-Vriesendorp B, et al. Histo incompatibilities in ABDR-matched unrelated donor recipient combinations. *Bone Marrow Transplantation* 1995; 16: 641-646.
6. Oudshoorn M, Doxiadis II, van den Berg-Loonen PM, et al. Functional versus structural matching: Can the CTLp test be replaced by HLA allele typing? *Hum Immunol* 2002; 63: 176-184.
7. Scott I, O'Shea J, Bunce M, et al. Molecular typing reveals a high level of HLA class I incompatibility in serologically well matched donor/recipient pairs - Implications for unrelated bone marrow donor selection. *Blood* 1988; 92: 4864-4871.
8. Tiercy JM, Villard J, Roosnek E. Selection of unrelated bone marrow donors by serology, molecular typing and cellular assays. *Transpl Immunol* 2002; 10: 215-221.
9. Hurley CK, Fernandez-Vina M, Hildebrand WH, et al. A high degree of HLA disparity arises from limited allelic diversity: Analysis of 1775 unrelated bone marrow transplant donor-recipient pairs. *Human Immunol* 2007; 68: 30-40.
10. Little AM, Marsh SG, Madrigal JA. Current methodologies of human leukocyte antigen typing utilized for bone marrow donor selection. *Curr Opin Hematol* 1998; 5: 419-428.
11. Petersdorf EW. Risk assessment in hematopoietic stem cell transplantation. *Best Pract Res Clin Haematol* 2007; 20: 155-170.
12. Ottinger HD, Ferencik S, Beelen DW, et al. Hematopoietic stem cell transplantation: Contrasting the outcome of transplantations from HLA-identical siblings, partially HLA-mismatched related donors, and HLA-matched unrelated donors. *Blood* 2003; 102: 1131-1137.
13. Hurley CK, Wagner JE, Setterholm MI, Confer DL. Advances in HLA: Practical implications for selecting adult donors and cord blood units. *Biol Blood Marrow Transplant* 2006; 12

- (1 Suppl 1): 28-33.
14. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. *Blood* 2002; 99: 4200-4206.
  15. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high resolution matching on outcomes of unrelated donor bone marrow transplantation. *Blood* 2004; 104: 1923-1930.
  16. Petersdorf EW, Anasetti C, Martin PJ, et al. Limits of HLA mismatching in unrelated hematopoietic cell transplantation. *Blood* 2004; 104: 2976-2980.
  17. Chalandon Y, Tiercy J-M, Schanz U, et al. Impact of high resolution matching in allogeneic unrelated donor stem cell transplantation in Switzerland. *Bone Marrow Transplant* 2006; 37: 906-916.
  18. Carreras E, Jimenez M, Gomez-Garcia V, et al. Donor age and degree of HLA matching have a major impact on the outcome of unrelated donor hematopoietic cell transplantation for chronic myeloid leukemia. *Bone Marrow Transplant* 2006; 37: 33-40.
  19. Shaw BE, Marsh SG, Mayor NP, et al. HLA-DPB1 matching status has significant implications for recipients of unrelated donor stem cell transplants. *Blood* 2006; 107: 1220-1226.
  20. Petersdorf EW, Hansen JA, Martin PJ, et al. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. *New Engl J Med* 2001; 345: 1794-1800.
  21. Gluckman E, Rocha V. Donor selection for unrelated cord blood transplants. *Curr Op Immunol* 2006; 18: 565-570.
  22. Tiercy J-M, Nicoloso de Faveri G, Passweg J, et al. The probability to identify a 10/10 HLA allele-matched unrelated donor is highly predictable. *Bone Marrow Transplantation*, 2007; 40: 515-522.
  23. Tiercy J-M, Bujan-Lose M, Chapuis B, et al. Bone marrow transplantation with unrelated donors: What is the probability of identifying an HLA-A/B/Cw/DRB1/B3/B5/DQB1-matched donor? *Bone Marrow Transpl* 2000; 26: 437-441.

## Appendix 1. List of HLA-A, -B, -Cw, -DR and -DQ serotypes with their corresponding groups of alleles (June 2007)

<b>serotype</b>	<b>alleles A*</b>	<b>serotype</b>	<b>alleles A*</b>
A1	0101-0125	A30 (19)	3001-3021
A2	0201-0299, 9201-9215	A31 (19)	3101-3117
A203	0203	A32 (19)	3201-3215
A210	0210	A33 (19)	3301-3310
A3	0301-0329	A34 (10)	3401-3408
A11	1101-1130	A36	3601-3604
A23 (9)	2301-2315	A43 (10)	4301
A24 (9)	2402-2476	A66 (10)	6601-6606
A2403	2403, 2410	A68 (28)	6801-6838
A25 (10)	2501-2506	A69 (28)	6901
A26 (10)	2601-2634	A74 (19)	7401-7412N
A29 (19)	2901-2916	A80	8001
	<b>alleles B*</b>		<b>alleles B*</b>
B7	0702-0754	B53	5301-5312
B703	0703	B54 (22)	5401-5411
B8	0801-0833	B55 (22)	5501-5526
B13	1301-1317	B56 (22)	5601-5620
B14	1401-1407N	B57 (17)	5701-5712
B15	1501-1599, 9501-9529	B58 (17)	5801-5815
B18	1801-1826	B60 (40)	4001,4007,4010,4014, 4031,4034,4048,4054
B27	2701-2737	B61 (40)	4002-4004,4006,4009, 4016,4027,4029
B2708	2708	B62 (15)	1501,1504-07,1515, 1520,1524,1525,1527, 1528,1530,1532,1535, 1539,1545,1548,1570, 1571,1573,1582,1584,
B35	3501-3575	B63 (15)	1516-1517
B37	3701-3712	B64 (14)	1401
B38 (16)	3801-3816	B65 (14)	1402
B39 (16)	3901-3941	B67	6701-6702
B3901	3901	B70 (15)	1509,1537,1551
B3902	3902	B71 (70)	1510,1518,1580,1593
B40	4001-4074		
B4005	4005		
B41	4101-4108		
B42	4201-4209		
B44	4402-4453		
B45	4501-4507, 5002		

B46	4601-4610	B72 (70)	1503,1546
B47	4701-4705	B73	7301
B48	4801-4816	B75 (15)	1502,1508,1511,1521, 1531
B49 (21)	4901-4905		
B50 (21)	5001-5002,5004	B76 (15)	1512,1514
B51 (5)	5101-5148	B77 (15)	1513
B5102	5102	B78	7801-7805
B5102	5102	B81	8101-8102
B52 (5)	5201-5211	B82	8201-8202
		B83	8301

<b>serotype</b>	<b>alleles Cw*</b>	<b>serotype</b>	<b>alleles Cw*</b>
Cw1	0101-0118	Cw9	Cw*0303
Cw2	0202-0218	Cw10	Cw*0302,0304
Cw3	0302-0340	Cwx	1202-1221
Cw4	0401-0427	Cwx	1402-1408
Cw5	0501-0516	Cwx	1502-1520
Cw6	0602-0616N	Cwx	16011602,1604, 1606-1609
Cw7	0701-0748	Cwx	1701-1704
Cw8	0801-0814	Cwx	1801-1803

	<b>alleles DRB1*</b>		<b>alleles DRB1*</b>
DR1	0101-0110	DR9	0901-0906
DR1	0101-0116	DR10	1001
DR15 (2)	1501-1522	DR11 (5)	1101-1162
DR16 (2)	1601-1611	DR12 (5)	1201-1215
DR3	0302-0335	DR13 (6)	1301-1379
DR4	0401-0464	DR14 (6)	1401-1466
DR7	0701, 0703-0712		
DR8	0801-0832		
DR52	DRB3*0101-01 DRB3*0201-0218 DRB3*0301-0303	DR51	DRB5*0101-0112 DRB5*0201-0205
DR53	DRB4*0101-0106		
blank	DRB4*01030102N, 0201N,0301N		

	<b>alleles DQB1*</b>		<b>alleles DQB1*</b>
DQ2	0201-0205	DQ6 (1)	0601-0630
DQ3	0301-0320	DQ7 (3)	0301, 0304
DQ4	0401-0402	DQ8 (3)	0302, 0305, 0310
DQ5 (1)	0501-0505	DQ9 (3)	0303

## Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/ebmt-handbook2008answers.htm>

**1. The biological function of HLA molecules is to present peptide antigens to T-cells. The peptide binding site of HLA Class I molecules is composed of:**

- a) An  $\alpha$ -chain and a  $\beta$ -chain .....
- b) The proximal part of the  $\alpha$ -chain .....
- c) The distal part of the  $\alpha$ -chain .....
- d) The  $\alpha$ -chain and the  $\beta$ 2-microglobulin .....

**2. A heterozygous individual can express at the cell surface a maximum of:**

- a) 10 different HLA Class I and Class II antigens .....
- b) 12 different HLA Class I and Class II antigens .....
- c) 13 different HLA Class I and Class II antigens .....
- d) 14 different HLA Class I and Class II antigens .....

**3. A HLA-ABDR haplotype is:**

- a) A combination of alleles encoded on the same chromosome .....
- b) A combination of alleles shared by two unrelated individuals .....
- c) A combination of alleles that differ between a patient and a partially incompatible sibling .....
- d) A combination of alleles that are frequent in a given population .....

**4. In which patient/donor combination would you expect a higher risk of HLA-C mismatches?**

- a) Two HLA-ABDR phenotypically identical siblings .....
- b) Two HLA-ABDR phenotypically identical unrelated individuals .....
- c) Two HLA-ABDR phenotypically identical cousins .....
- d) Two HLA-ABDR genotypically identical siblings .....

**5. The HLA-ABDR typing of a patient's family leads to the identification of a potential sibling donor who differs from the patient by one single DR antigen. The father is homozygous for HLA-AB antigens and heterozygous for HLA-DR. The possible reason for the DR-incompatibility is:**

- a) A recombination event between HLA-A and -B in one paternal haplotype .....
- b) A recombination event between HLA-B and -DR in one paternal haplotype .....
- c) Inheritance of 2 different paternal HLA-ABDR haplotypes .....
- d) Either of the 3 possibilities .....