HLA matching in unrelated stem cell transplantation: what to type for?

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RESUMEN
El tipaje HLA es el factor más importante cuando se busca un donante no relacionado en trasplante de médula ósea. Desde que se descubrió el sistema HLA, las técnicas de tipaje se han desarrollado significativamente, desde los iniciales tipajes serológicos hasta las técnicas moleculares que se utilizan hoy rutinariamente. El descubrimiento de la reacción en cadena de la polimerasa (PCR) y su ulterior aplicación al campo del HLA, nos ha permitido conocer mejor la región HLA, descubrir nuevos genes y un número aparentemente ilimitado de nuevos alelos HLA en los diferentes loci. Más de mil alelos HLA de clase I y II se han descrito hasta la fecha mediante secuenciación de DNA. Este gran polimorfismo demanda nuevos métodos moleculares (más rápidos, mejores y precisos) para identificar dichos alelos en los diferentes loci. Debido al hecho, bien conocido, de que una mayor identidad HLA entre donante y receptor es crucial para determinar el éxito de un trasplante, es preciso que en unos pocos años solamente se realizará tipaje HLA a la más alta resolución (resolución alélica) cuando se busque el mejor donante no relacionado para un receptor a la espera de trasplante de células hematopoyéticas.

PALABRAS CLAVE: HLA/ Identidades/ Tipaje/ Donantes no emparentados/ Trasplante de células hematopoyéticas.

ABSTRACT
HLA typing is the most important factor when selecting unrelated bone marrow donors for transplantation. Since the discovery of the HLA system, the typing techniques have evolved tremendously from the initial serology methods to the DNA techniques used routinely today. Polymerase Chain Reaction (PCR) applications to the HLA field have allowed us to have a greater knowledge of the HLA region, aiding in the discovery of novel HLA genes and an increasing number of HLA alleles at the different loci. To date more than a thousand HLA class I and class II alleles have been identified by sequencing analysis. This tremendous polymorphism demands quicker, better and more accurate molecular methods to precisely identify these alleles at the more polymorphic loci. Due to the fact that good HLA matching between donor and recipient is crucial in determining the transplant outcome, one can imagine that in a few years time, only high (allelic) level resolution will be used for HLA typing when looking for the best donor for a recipient requiring stem cell transplantation.

KEY WORDS: HLA/ Matching/ Typing/ Unrelated donors/ Stem cell transplantation.
INTRODUCTION

Haematopoietic stem cell transplantation (HSCT) was first used successfully 34 years ago in children suffering of congenital immune deficiencies (1). Nowadays, HSCT is becoming increasingly utilised as a component of curative treatment in the field of haematological malignancy. However, only 25-30% of patients worldwide eligible for a HSCT have HLA identical sibling donors (2). It is therefore, necessary to find an HLA matched unrelated donor (UD). To overcome this problem, potential stem cell donors, now numbering over 7 million, have been recruited by more than 54 registries worldwide (3).

The main cause of post-transplant complication in the unrelated donor HSCT situation is graft versus host disease (GvHD), which is generally not encountered when transplanting siblings which have been HLA matched at the same level as an unrelated donor and patient. GvHD occurs when donor-derived cells stimulate an immune response against the patient’s tissue (4).

The spectrum of diseases treatable by allogeneic haematopoietic stem cell transplantation is getting steadily larger, although transplants are still performed most frequently for acute leukaemia and chronic myeloid leukaemia (CML) (5). The therapeutic use of HSCT for conditions such as breast carcinoma and autoimmune disease is more recent (6,7) (Table I). Annually, more than 30,000 patients undergo HSCT with stem cells derived from a related or an unrelated donor (allogeneic transplantation) or with the patient’s own, previously stored marrow (autologous transplantation). Historically, only bone marrow tissue obtained by aspiration was used (rich in haematopoietic stem cells). However, with medicine advances and research there are now alternative sources of these stem cells, these being mainly cord blood or adult peripheral blood (after stem cell mobilisation with the use of chemotherapy and granulocyte-colony stimulating factor, G-CSF) (8). Table II summarises the different names of haematopoietic stem cell transplantation depending on the different transplant criteria.

### Table I

Therapeutical uses of haematopoietic stem cell transplantation

<table>
<thead>
<tr>
<th>Haematological malignancies</th>
<th>Other diseases</th>
<th>Congenital diseases</th>
<th>Autoimmune diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemias</td>
<td>Solid tumours</td>
<td>Immune deficiencies*</td>
<td>In humans#</td>
</tr>
<tr>
<td>Acute myelogenous</td>
<td>Neuroblastoma</td>
<td>Severe combined</td>
<td>Crohn’s disease</td>
</tr>
<tr>
<td>Chronic myelogenous</td>
<td>Glioma</td>
<td>Hemophagocytic</td>
<td>Dermatitis herpetiformis</td>
</tr>
<tr>
<td>Acute lymphoblastic</td>
<td>Soft-tissue sarcoma</td>
<td>lymphohistiocytosis</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Chronic lymphoblastic</td>
<td>Testicular cancer</td>
<td>Wiskott-Aldrich syndrome</td>
<td>Lupus erythematosus</td>
</tr>
<tr>
<td>Myelodysplasia</td>
<td>Ovarian cancer</td>
<td>HLA class I or II deficiency</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td></td>
<td>Breast carcinoma</td>
<td>Leukocyte adhesion deficiency</td>
<td>Psoriasis</td>
</tr>
<tr>
<td></td>
<td>Ewing’s sarcoma</td>
<td>X-linked lymphoproliferative disease</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Lymphoproliferative diseases</td>
<td>Anaemias</td>
<td>Chronic granulomatous disease</td>
<td>Vasculitis</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Severe aplasia</td>
<td>Omenn syndrome</td>
<td></td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>Paroxysmal nocturnal haemoglobinuria</td>
<td>Chediak-Higashi syndrome</td>
<td></td>
</tr>
<tr>
<td>Non Hodgkin’s lymphoma</td>
<td>Fanconi’s anaemia</td>
<td>Other diseases</td>
<td></td>
</tr>
<tr>
<td>Langerhans’s histiocytosis</td>
<td>Thalassemia</td>
<td>Thalassemia</td>
<td>Adjuvant arthritis</td>
</tr>
<tr>
<td></td>
<td>Hurler’s syndrome</td>
<td>Hunter’s syndrome</td>
<td>Antiphospholipid syndrome</td>
</tr>
<tr>
<td></td>
<td>Hunter’s syndrome</td>
<td>Adrenoleukodystrophy</td>
<td>Autoimmune arthritis</td>
</tr>
<tr>
<td></td>
<td>Adrenoleukodystrophy</td>
<td>Batten disease (neuronal ceroid lipofuscinosis)</td>
<td>Autoimmune nephritis</td>
</tr>
<tr>
<td></td>
<td>Batten disease (neuronal ceroid lipofuscinosis)</td>
<td>Gunther’s erythropoietic porphyria</td>
<td>Diabetes mellitus</td>
</tr>
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<td></td>
<td>Gunther’s erythropoietic porphyria</td>
<td>Osteopetrosis</td>
<td>Myasthenia gravis</td>
</tr>
<tr>
<td></td>
<td>Osteopetrosis</td>
<td>Other diseases</td>
<td>Relapsing experimental autoimmune encephalomyelitis</td>
</tr>
</tbody>
</table>

*Few individuals with autoimmune disease who developed a coincident haematological disorder and underwent myeloablative conditioning and HSCT. With rare exceptions, allogeneic HSCT has led to long-term resolution of the autoimmune disease.

#Immune deficiency diseases in which more than 10 patients have been treated by HSCT.
HLA POLYMORPHISM: THE LIMITS FOR UDHSCT

To date, more than 20,000 patients have received a UDHSCT, but there is still no consensus about how precisely an unrelated donor’s HLA antigens (or alleles) should match those of the recipient (2,9). Most donors have been selected on the basis of minimum identity for HLA-A and -B (determined by serology) and for HLA-DR (often typed by DNA analysis).

The HLA system, encoded within the human Major Histocompatibility Complex (MHC) on chromosome 6 consists of the most complex genetic loci within the human genome. The human MHC, containing over 300 genes, can be divided into three regions: class I and class II (coding for the HLA class I and class II proteins, respectively) and a central class III region that contains no HLA genes (10). To date, ten HLA class I genes have been described: HLA-A, -B, -C, -E, -F, -G, -H, -J, -K, and –L. Of these, HLA-H, -J, -K, and –L have been shown to be pseudogenes. The expressed genes encode the heavy chain of the HLA class I molecule which associates non-covalently with β₂-microglobulin and an endogenously derived peptide (8-10 amino acids in length) before being transported to the cell surface. At the cell surface, HLA class I molecules are recognised by CD8 positive T cells through interaction with the T cell receptor and the CD8 molecule (11,12). These HLA class I antigens are also recognised by Killer Immunoglobulin-like Receptors (KIR) expressed on Natural Killer (NK) cells and some T cells (13).

Nucleotide and protein sequence analysis of the classical HLA class I molecules, HLA-A, -B and, -C, has demonstrated extensive polymorphism for these genes and the encoded proteins which are expressed on all nucleated cells. The non-classical HLA class I genes, HLA-E, -F and -G show both a reduced level of polymorphism and a much more restricted expression (14). Thus, in terms of matching individuals for transplantation, only HLA-A, -B and -C genes have been considered.

In the case of HLA class II antigens, both the α and β chains are encoded within the MHC. Five families of HLA class II genes have been described so far: HLA-DR, -DQ, -DP, -DM, and -DO. For each of these families there are at least two genes encoding an α and β chain (DPA1 and DPB1 for example). HLA class II αβ heterodimers also associate with exogenously derived peptides for presentation at the cell surface, but the expression of class II molecules is mainly restricted to antigen presenting cells (dendritic cells, macrophages and B-cells) or to other cell types after appropriate stimulation. The most polymorphic genes within the class II region are HLA-DR, -DQ, and –DP, and the complete trimolecular complexes are recognised by CD4 T cells.

The polymorphism found in class I and class II molecules is mainly restricted within and around the peptide binding site of HLA proteins. Thus the polymorphism is defining the particular structure of the peptides which can bind to HLA molecules and ultimately determines the immune response carried by a specific T cell receptor. In transplantation, HLA mismatches between donor and recipient can lead to the activation of T and NK cells, resulting in graft rejection or GvHD. Thus, for successful transplants of unrelated donor tissues, it is necessary to precisely determine the HLA type of both donor and recipient.

HLA TYPING STRATEGIES

Serology

HLA polymorphism was first described by agglutination assays using sera from patients whom had received multiple blood transfusions or multiparous women; but soon afterwards the classical complement dependent cytotoxicity technique was described (15) and has been widely used in the past 37 years with only minor modifications.

There were two major limitations with serology as a typing technique. Firstly, the ability to obtain antisera capable of distinguishing the large number of different HLA specificities, and, secondly, that as the antisera were polyclonal they contained antibodies recognising both multiple HLA
specificities and the products from multiple HLA genes. Sera were mainly obtained from multipa-
rous women or pre-sensitised patients (after mul-
tiple transfusions), but obviously the source of
such antisera is very limited. To avoid these limi-
tations, monoclonal antibodies against HLA spe-
cificities were developed. As such an infinite
supply of monoclonal antibodies could be obtai-
ned and it has been able to thoroughly characte-
rise these antibodies and determine exactly which
sequence motifs or epitopes are recognised on the
different HLA molecules (16).

However, despite the number of serological re-
gents which have been made available, there are
many HLA class I and II polymorphisms which
remain undetectable by serology. For example, al-
though there are currently 250 different HLA-A
alleles recognised, it is only possible to determine
24 serologically defined HLA-A locus antigens
(17,18) (Table III). Many of these undetected poly-
morphisms are crucial for peptide binding and anti-
gen presentation and, thus, important for the suc-
cessful outcome of stem cell transplantation.

### Molecular matching techniques

The development of PCR methodologies for
amplifying and cloning genes and gene fragments
has had a profound effect on the understanding of
HLA polymorphism and its extent. Also, with the
evolution of the PCR assay itself, molecular methods
for HLA typing have undergone a huge revolu-
tion (19). In the last 15 years, the nucleotide sequences
of alleles encoding all the recognised serological spe-
cificities have been described. It is well known now
that a given serological allele has multiple molecu-
lar alleles or sequences. For example, HLA-A2 is
recognised as a single antigen; however, 61 different
molecular variants are defined at the DNA level (20).

Molecular polymorphism of HLA alleles is
mostly confined to exons 2 and 3 in class I anti-
gens (encoding for α1 and α2 domains) and to
exon 2 in class II antigens (encoding for α1 or β1
protein domains). Thus, DNA methods for HLA
typing are focussed on those polymorphic exons.

Table IV summarises the most widely used me-
thods for HLA typing.

### Restriction based methods

**RFLP**

The first molecular method used for HLA
typing was restriction fragment length polymor-
phism (RFLP), which was only used for HLA class
II genotyping (21) and after the 10th International
Histocompatibility Workshop, was widely adop-
ted by most HLA typing laboratories. Nowadays it
is no longer used in HLA typing laboratories, as it
yields only a low resolution typing (similar to sero-
logy), it is time consuming, and requires large
amounts of DNA for typing.

**PCR-RFLP**

With the introduction of PCR techniques, RFLP
was adapted to PCR amplified DNA fragments
(22). PCR-RFLP utilizes a panel of restriction
endonucleases to digest PCR products prior to
RFLP analysis on agarose or acrylamide gels.
Assignment of HLA specificities is derived from
patterns of endonucleolytic fragments, resulting
from the allele-specific distribution of restriction
sites. Reliability and resolution of this method
were low. These problems could be overcome by
the adoption of internal control restriction
sites in PCR primers and by using single restric-
tion sites. However, there are more simple and
cheaper methods for low resolution typing and
PCR-RFLP is not the method of choice.

### Sequence-specific based methods

**PCR-SSP**

This consists of a one-step PCR using sequence-
specific primers; multiple PCR primers pairs must
be used independently on the same DNA to be
The primers pairs are designed such that the 3' end defines the specificity of the primer with the target sequence. Originally described for class II typing, PCR-SSP has also been adapted to class I typing (24). PCR-SSP can be used to obtain from low to high resolution HLA typing. However, when increasing the resolution, the number of primer pairs increases exponentially and handling of samples and manipulation of the data (in absence of automation) results in an unbearable workload for high throughput HLA typing. Additionally, new allele variants may be missed by this technique unless the novel allele shows variation in the same region as the primer binding sites.

<table>
<thead>
<tr>
<th>Technique</th>
<th>SAM/PCR/DIG</th>
<th>N/RES</th>
<th>Problems</th>
<th>Time (hr)</th>
<th>Specificity given by</th>
<th>REV/DET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-lymphocytotoxicity</td>
<td>Cells/No/No</td>
<td>10/L</td>
<td>Lack of antibodies for all molecular defined specificities</td>
<td>4-6</td>
<td>Polyclonal (antisera) or monoclonal antibodies</td>
<td>Col Fluor</td>
</tr>
<tr>
<td>RFLP (Restriction fragment length polymorphism)</td>
<td>DNA/No/Yes</td>
<td>40/L-M</td>
<td>Not all the known polymorphic sites define or destroy a restriction sequence</td>
<td>24-36</td>
<td>Restriction enzymes recognise specific DNA sequences</td>
<td>Rrad Chemi Col</td>
</tr>
<tr>
<td>PCR-RFLP (identical to previous but after DNA amplification)</td>
<td>DNA/Yes/Yes</td>
<td>40/L-M</td>
<td>Not all the known polymorphic sites define or destroy a restriction sequence</td>
<td>12-24</td>
<td>Restriction enzymes recognise specific DNA sequences</td>
<td>Gel Agar + EtBr</td>
</tr>
<tr>
<td>SSP (sequence-specific PCR using &quot;allele&quot; derived primers)</td>
<td>DNA/Yes/No</td>
<td>5/L-M-H</td>
<td>For more resolution more primers needed (more labour intensive)</td>
<td>2-4</td>
<td>PCR primers are allele-specific</td>
<td>Gel Agar + EtBr</td>
</tr>
<tr>
<td>SSO (sequence-specific oligotyping using &quot;motif&quot; specific oligonucleotides and generic PCR)</td>
<td>DNA/Yes/No</td>
<td>96/M-H</td>
<td>&quot;cis&quot; and &quot;trans&quot; polymorphic motives combinations are indistinguishable</td>
<td>4-6</td>
<td>Specific hybridisation between oligonucleotides and the amplified product</td>
<td>Rad Chemi Col</td>
</tr>
<tr>
<td>SBT (sequence based typing or direct sequencing)</td>
<td>DNA or RNA/Yes/No</td>
<td>96/H</td>
<td>&quot;cis&quot; and &quot;trans&quot; polymorphic motives combinations are indistinguishable</td>
<td>12</td>
<td>DNA sequence itself</td>
<td>Fluor</td>
</tr>
<tr>
<td>RSCA (reference strand mediated conformation analysis after heteroduplex formation)</td>
<td>DNA/Yes/No</td>
<td>96/H</td>
<td>Reference DNAs needed for all known alleles</td>
<td>12</td>
<td>Heteroduplex conformation on non-denaturing acrylamide gels</td>
<td>Fluor</td>
</tr>
<tr>
<td>SSCP (single strand conformation polymorphism)</td>
<td>DNA/Yes/No</td>
<td>40/M-H</td>
<td>Maximum length of PCR product: 300 bp</td>
<td>2-4</td>
<td>Conformation of single strand DNA on acrylamide gels</td>
<td>Gel Acryl + silver Fluor</td>
</tr>
</tbody>
</table>

Table IV: Widely used HLA typing techniques

SAM: sample used; RFLP, on average, consumes more DNA than PCR; PCR: denotes if amplifications is needed; DIG: denotes if enzymatic digestion is needed; N: number of samples that a single technician could process per working day in a small-medium laboratory; RES: resolution level obtained ("L"low, "M"edium or "H"igh; TIME: hours needed to see the results (on average); DET: Detection method: "rad"ioactive, "chemi"luminescent, "col"orimetric, "fluor"escence (a proper microscope or an automatic DNA sequencer is needed), on "gel" (either "Agar"ose or "Acryl"amide gels) stained with "Et"idium "Br"omide or "silver".

typed (23).
SSO (SSOP)

Sequence-Specific Oligonucleotide (SSO) typing was the first PCR-based method to offer substantial improvements on the classic RFLP. It consists of hybridisation of a panel of SSO probes to PCR-amplified target HLA sequences. The target DNA is amplified by locus or group specific PCR. Then, the hybridisation reaction was carried out on a membrane-based solid phase dot (or slot) blot. Each SSO probe is complementary to a different sequence motif within the hypervariable HLA sequences, and thus specific patterns of probe hybridisation can be used to identify individual HLA alleles and combinations of alleles (25). This method can vary in the level of typing resolution required, which is again proportional to the number of probes utilised. To perform high resolution typing with SSO a large number of SSO primers is required, thus decreasing the throughput of the technique due to the high degree of manipulation. To partially avoid this problem, PCR-SSO has been adapted to a different format, named reverse dot-blot. In this assay the probes are pre-immobilised onto the membrane and the PCR product is hybridised to probes on the membrane, this is more suitable for automation and for high-throughput analysis.

Recently, a new adaptation of PCR-SSO has been described (26). It consists on liquid-phase hybridisation of the amplified DNA with allele-specific oligodeoxynucleotides coupled to an array of fluorescent micro spheres. The hybridisation step is done in a single tube and the mixture is analysed in a 96-well format special flow cytometer. This technique allows for a fast-automated analysis. As with SSP the detection of new HLA alleles still poses a problem with SSO typing, if the new variant positions are located out of the regions covered by the probes.

SBT

Sequence-Based Typing (SBT) is now an affordable alternative for HLA typing thanks to the improvements with automated DNA sequencers. SBT involves PCR amplification of the HLA region of interest followed by direct sequencing of the PCR product (27). Either genomic DNA or cDNA (RNA) can be used for SBT. The SBT method relies on the knowledge and manipulation of a database containing all known HLA class I and class II sequences (28, 29).

SBT is a high resolution typing method, but has the problem that the sequences obtained from some particular heterozygous allele combinations can give ambiguous results as both alleles are sequenced and analysed together. For example the heterozygous sequence obtained from an individual who has both DRB1*04011 and DRB1*0403 alleles, will be identical to that of a second individual who has the DRB1*0407 and DRB1*0413 alleles. Such ambiguous results are also a problem for SSO typing and remain unresolved by this technique also. As such these types of ambiguities are often resolved by either specific amplification of alleles using an SSP reaction or by conformational methods such as Single Strand Conformation Polymorphism (SSCP) or Reference Strand mediated Conformation Analysis (RSCA) (30).

Conformational-based methods:

Conformational methods are based on the differential mobilities of a given allele in acrylamide gels. The differential mobility results from the conformation adopted by the DNA strands, either single or double strand molecules, while passing through the acrylamide gel. In both methods, the limiting conditions are the complexity of the banding patterns visualised and the dependence on gel variations. Automation of both techniques with the use of gel-based or capillary-based automated DNA sequencers allows intra and inter-gel normalisation as well as automated assignment of HLA alleles.

SSCP

Single Strand Conformation Polymorphism (31) has an important limitation as only DNA fragments of 200-400 nucleotides in length can be analysed. Thus, SSCP is unsuitable for high resolution typing of HLA class I alleles (the size of the PCR fragment including exon 2, intron 2, and exon 3 is around 1 kb).

RSCA

Reference Strand mediated Conformation Analysis (32) is a modification of the classical heteroduplex assay. Labelling only one of the strands of the reference DNA has diminished the complexity of heteroduplex banding patterns (Fig. 1). The inclusion of top and bottom markers in each lane and the use of a fluorescent-based automated DNA sequencer permit normalisation of results (avoiding intra- and inter-gel variations). It does not need an enormous array of reagents, unlike SSO and SSP, and allows analysis of DNA fragments bigger than 1 kb in length.

RSCA is one of the methods of choice when ambiguities have been obtained by other methods such as SBT (30). Moreover, RSCA allows the perfect match (between the donor and the recipient) of a given HLA locus without the need...
of the precise high resolution typing (Fig. 2), just by comparing RSCA profiles (electropherograms). A limitation of RSCA is that typing laboratories need to have a huge collection of different HLA types to precisely correlate a given mobility to a particular allele. Although experience has shown that novel alleles or alleles for which mobilities are unavailable, usually give new mobilities that have not previously been described. This limitation will be avoided when normalised mobility tables for the different loci become available to all RSCA typing laboratories. RSCA is being adapted to the most modern and more widely used, four colour-cassillac-based DNA sequencers.

**Cellular methods**

Cellular methods for estimating allo-reactivity between patient and donor cells are also used for the selection of the most appropriate HSCT donor. The most traditional method is Mixed Lymphocyte Culture (MLC), which has been used over the last 30 years as an indicator of HLA class II disparity between donor and recipient. With the move towards molecular DNA based HLA typing methods, MLC has become redundant and is less frequently used (33).

More sophisticated cellular methods, estimating (by limiting dilution assays) the frequency of receptor-reactivedonor CTL precursors (CTLp) or helper T-cells precursors (HTLp) responding to HLA class I and class II mismatches, respectively, have been developed and are more sensitive than MLC (34).

**IN SEARCH OF THE BEST MATCH**

Historically, HLA typing for related and unrelated HSCT has been reduced to HLA-A, -B, and -DR loci because these loci were easier to identify and were considered the most polymorphic. However, the development of molecular methods for HLA typing have shown that other loci (HLA-C, -DQ, and -DP) are also highly polymorphic and this fact has raised the question of their involvement in transplant outcome (2). Mismatches in these three loci have been involved, in less or high degree, in immune responses leading to the development of GvHD (35-37).

Other minor histocompatibility antigens (mHag) have been involved in allo-recognition and thus responsible of acute or chronic GvHD development in HLA-identical siblings. These mHag includes HA-1 and -2 antigens (38), CD31 (39) and MICA, MICB proteins (40) amongst others.
HLA MATCHING IN UNRELATED STEM CELL TRANSPLANTATION: WHAT TO TYPE FOR?

CONCLUSIONS

Serological methods have been used for HLA typing for more than three decades. However, the introduction of molecular genetic techniques to the HLA field has had a significant effect on the quality and reproducibility of HLA typing and also on the outcome of HSCT. Given the increasing extent of our knowledge regarding HLA polymorphism (Table III) and given the current development of technologies such as DNA-chips or DNA-arrays for mutation and polymorphism detection (41) it is likely that techniques providing low to medium level of resolution will be history in a few years and all HSCT typing will be performed at the highest level of resolution (allelic level). It is also likely that typing for HLA-C, -DQ, and -DP will be routinely included in the donor and recipient typing profile in most of the worldwide unrelated donor banks, in the near future.

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