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Review

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BSHI Guideline: HLA matching and donor selection for haematopoietic progenitor cell transplantation

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Summary

A review of the British Society for Histocompatibility and Immunogenetics (BSHI) "Guideline for selection and HLA matching of related, adult unrelated donors and umbilical cord units for haematopoietic progenitor cell transplantation" was undertaken by a BSHI appointed writing committee. Literature searches were performed, and the data extracted were presented as recommendations according to the GRADE nomenclature.

List of recommendations

- All laboratories performing H&I testing for allogeneic HPC transplantation should follow EFI standards and be accredited by EFI and UKAS/CPA (Grade 1A)
- HLA typing definitions as described by Nunes *et al.* (2011) and here should be used (Grade 1A)
- HLA typing results should use official WHO HLA Nomenclature (Grade 1A)
- The clinical urgency should be made available to the individual performing the related and unrelated donor search (Grade 1B)
- HLA high-resolution typing should be performed on potential matching; mismatching and haploidentical related donors when familial haplotypes cannot be fully assigned (Grade 1A)
- Patients and selected related donors should be typed for HLA-A, -B, -C, -DRB1 and -DQB1 (+/− DPB1) (Grade 1A)
- All patients and donors must have their HLA type confirmed on a second sample pretransplant (Grade 1A)
- The patient should be high-resolution typed prior to submitting the HLA type for an unrelated donor search (Grade 1A)
- A 10/10 high-resolution HLA-A, -B, -C, -DRB1 and -DQB1-matched unrelated PBSC or bone marrow donor should be used where possible (Grade 1A)
- Where a 10/10 matched PBSC or bone marrow donor is not available a single mismatch at HLA-A, -B, -C, -DRB1 or -DQB1 is acceptable (Grade 1A)
- Alternative progenitor cell donors (cord blood or haploidentical) should be considered early in the donor search when a patient is unlikely to have an HLA-matched unrelated donor (Grade 1A)
- HLA-DRB3, -DRB4, -DRB5 typing should be performed and, when a choice of otherwise equally matched and appropriate (e.g. CMV status) donors is available, mismatches for these should be minimized (Grade 2A)
- For unrelated donor selection, HLA-DPB1 typing should be performed and when a choice of otherwise equally matched and appropriate (e.g. CMV status) donors is available, nonpermissive mismatches should be minimized (Grade 2C)
- For mismatched related and unrelated donor selection, HVG mismatches are favoured over bidirectional and GVH mismatches (Grade 2C)
- UCB units should be HLA typed to high-resolution HLA-A, -B, -C, -DRB1, -DQB1 (Grade 1B)
- Selection of UCB units should follow national consensus guidelines published by Hough *et al.* (2016) (Grade 1A)
- HLA alloantibody testing of the recipient should be performed at the time of donor search and should be repeated at the time of donor work-up request if an HLA-mismatched donor is selected (Grade 1A)

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- The clinical team must be made aware of any HLA alloantibody incompatibility for a selected donor (Grade 1A)
- When a choice of equally well-matched donors is available, avoid selection of donors against which the patient has HLA alloantibodies (Grade 1A)
- HLA alloantibody testing should be performed in cases of failed engraftment if the donor is HLA mismatched (Grade 1B)
- The guideline published by Emery *et al.* (2013) recommending CMV matching between patient and donor should be followed (Grade 1A)
- Major ABO incompatibilities should be avoided when there is a choice of HLA- and CMV-matched donors (Grade 1A)
- Male donors should be preferentially chosen where the patient has multiple HLA-, CMV- and ABO-matched donors (Grade 1C)
- Younger donors should be preferentially selected (Grade 1B)
- Homozygosity and novel HLA alleles identified within DNA extracted from patients with a high frequency of circulating tumour cells should be confirmed by family studies or using DNA extracted from nondiseased cells (Grade 2A)
- Individuals actively involved in the provision of a donor selection service should undertake CPD, and the service should be directed by a RCPath Fellow and Consultant in H&I (Grade 1A)

Scope

These evidence-based recommendations expand and adapt previous guidance (Harvey *et al.*, 2012).

Method

This guideline was produced by the following actions:

1 A writing committee (authors of this manuscript) comprising Histocompatibility and Immunogenetics (H&I) scientists providing an H&I clinical service for related and unrelated donor haematopoietic progenitor cell transplantation was established. Ann-Margaret Little was appointed as the chair of the committee.
2 A search of peer-reviewed literature to 31 June 2015 was undertaken.
3 Recommendations were produced from evidence obtained from the literature search. Due to the specialist nature of histocompatibility testing in the context of haematopoietic progenitor cell allotransplantation, there are few large and/or multicentre studies in this field and meta-analyses are not available. Some recommendations are based on both literature review and consensus of expert opinion.
4 The GRADE nomenclature was used to evaluate the impact of evidence and to define the strength of the recommendations [http://www.gradeworkinggroup.org/intro.htm#criteria].

For each recommendation the quality of evidence has been graded as:

A (high)
B (moderate)
C (low)
D (very low)

For each recommendation, the strength of recommendation has been indicated as one of the following:

Level 1 (we recommend)
Level 2 (we suggest)
Not graded (where there is not enough evidence to allow formal grading)

Disclaimer

These recommendations represent consensus opinion from experts in the field of H&I within the UK. They represent a snapshot of the evidence available at the time of writing. This evidence may become superseded with time. It is recognized that recommendations have been made even when the evidence is weak. The BSHI cannot attest to the accuracy, completeness or currency of the opinions and information contained herein and does not accept any responsibility or liability for any loss or damage caused to any practitioner or any third party as a result of any reliance being placed on this guideline or as a result of any inaccurate or misleading opinion contained in the guideline.

Background

The infusion (transplantation) of haematopoietic progenitor stem cells (HPC) into a patient with haematological failure due to malignant or nonmalignant causes can result in successful engraftment of donor-derived HPC which undergo haemopoiesis to replace the malfunctioning cells of the patient's immune system. HPC transplantation is also referred to as bone marrow transplantation (as the HPCs may be taken from the bone marrow of the donor) and stem cell transplantation. HPC transplants have been successfully performed since the late 1960s. The effectiveness of these transplants in terms of patient overall survival and disease-free survival has improved with each decade due to more accurate histocompatibility matching between donor and patient; improved patient conditioning protocols; use of therapeutic agents; prevention and treatment of infections; and post-transplant supportive care.

The HPCs that are transplanted are derived from the following sources:

Autologous HPC: the cells are taken from the patient

Syngeneic HPC: the donor is genetically identical to the patient, for example an identical twin

Allogeneic HPC: the donor is not genetically identical to the patient and can be related or unrelated.

This guideline describes the selection of donors for allogeneic HPC

HLA matching donors and patients in HPC transplantation

Amongst the many factors that contribute to successful transplantation, the most significant is the degree of histocompatibility between donor and patient. Compatibility is primarily assessed by the degree of sharing of genes that encode human leucocyte antigen (HLA) proteins and secondarily by additional genetic factors such as blood group and nongenetic factors including cytomegalovirus (CMV) infection status.

HLA proteins are found on the cell surface of most cells within the human body. There are two different classes of HLA proteins: HLA class I (includes HLA-A, HLA-B and HLA-C proteins) which are found on the surface of all nucleated cells and platelets, and HLA class II (including HLA-DR, HLA-DQ and HLA-DP proteins) which are normally expressed on cells involved in antigen presentation such as dendritic cells and B cells. The expression of HLA class II molecules can be induced on other cell types such as T cells following activation. HLA proteins play an important role in the development of immune responses against non-self-antigens. These non-self-antigens are derived from viruses; bacteria; abnormal proteins expressed by malignant cells; and also non-self-proteins, including HLA proteins expressed on transplanted cells and organs. Peptides derived from both self- and non-self-antigens are bound to HLA proteins within the antigen processing pathways, and the bound peptides are presented to receptors expressed by CD4+ and CD8+ T cells. The responding T cells are able to distinguish between self- and non-self-peptides due to T-cell education pathways occurring during T-cell development in the thymus, resulting in an immune response being initiated specifically to non-self-peptides and not to self-peptides. HLA proteins also interact with receptors on natural killer (NK) cells, and this interaction plays an important role in the generation of immune responses to virally infected cells and malignant cells.

The genes encoding HLA proteins are located on the short arm of chromosome six within a gene dense region of the genome entitled the major histocompatibility complex (MHC), so named due to the role the genes found in this region have in determining compatibility between donor and patient in transplantation. Unlike most other genes in the human genome, HLA genes are hyperpolymorphic, that is there are many variations within the HLA gene sequences and each variant is called an allele. Most allele differences are nonsynonymous (change the amino acid composition of the HLA proteins). Variations in the amino acid sequence of HLA proteins can affect the way in which self- and non-self-peptides are presented to T cells and how they are recognized by NK cells.

Each human expresses at least six HLA proteins (HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ and HLA-DP), and usually twelve different proteins as most individuals inherit different alleles from their mother and father (2xHLA-A, 2xHLA-B, 2xHLA-C, 2xHLA-DR, 2xHLA-DQ and 2xHLA-DP). Possessing multiple different HLA proteins that are able to interact individually with immune cells enables our immune systems to respond rapidly and effectively to unwanted pathogens and malignant changes. At the population level, the existence of multiple individuals with different variants of HLA proteins increases the chances that an individual will exist with a functional HLA phenotype able to initiate an immune response against a particular pathogen. Therefore, multiple polymorphic HLA genes are essential for the immune system to be able to defend its host against a plethora of non-self-antigens. However, this polygenic and polymorphic HLA system confers a significant obstacle when cells from one individual are transplanted into another.

In a HPC transplant, alloreactive cells of the patient can initiate an immune response against non-self-antigens expressed by the donor cells causing rejection of the donor cells seen clinically as failed engraftment. Pretransplant conditioning of the patient with chemotherapy and/or irradiation reduces this host-versus-graft (HVG) response allowing the donor cells to engraft. Conversely, immune reactive cells from the donor can initiate an attack against non-self-antigens expressed by tissues of the recipient causing a graft-versus-host (GVH) immune response. This 'acute graft-versus-host disease' (aGVHD) is graded from I to IV (mild to severe) and involves multiple organs of the patient including skin, gut and liver with grade IV aGVHD being life-threatening. The GVH response can also be beneficial when directed specifically to malignant antigens, that is graft versus leukaemia (GVL).

The impact of matching the HLA alleles of patient and donor, as is the case when the donor is a HLA-matched sibling, contributes significantly to optimal outcome by reducing the alloreactions that contribute to HVG and GVHD responses.

Early data demonstrated better outcomes for patients receiving a transplant from a HLA-matched sibling donor compared with an unrelated donor. HLA typing to high and allele level resolution has resulted in improved matching between unrelated donors and patients leading to increased survival for patients receiving unrelated donor transplants and giving comparable outcomes for some disease, for example no effect of donor type on overall survival was observed in a cohort of 108 patients transplanted for haematological malignancies within a reduced intensity conditioning (RIC) regimen (Robin *et al.*, 2013). An earlier study comparing transplant outcomes for 226 adult acute myeloid leukaemia (AML) patients in first complete remission receiving myeloablative

unrelated and related donor transplants during 1996–2007 demonstrated the probability of an unfavourable outcome in terms of overall survival, relapse and non-relapse mortality (NRM) was higher for patients receiving a unrelated donor transplant although not statistically significant, with patients receiving a 9/10 matched unrelated donor having similar outcomes to patients receiving a 10/10 matched unrelated donor transplant (Walter *et al.*, 2010). Thus, selection of optimum related and unrelated donors both provide good outcomes for transplanted patients.

Testing for HLA typing and alloantibody identification

HLA testing methodologies

HLA typing and alloantibody identification can be undertaken by different methods. It is beyond the scope of this guideline to advocate which methods should be utilized. All methods used to determine the HLA type of a patient and donor and to determine the HLA alloantibody status of a patient undergoing transplantation must be validated within the laboratory where it is used. Within the UK, HLA typing and alloantibody identification for HPC transplantation must be performed according to the standards for histocompatibility testing produced by the European Federation for Immunogenetics (EFI) and must be undertaken by a laboratory accredited by the United Kingdom Accreditation Service (UKAS)/Clinical Pathology Accreditation (CPA), Ltd and EFI.

Recommendation

All laboratories performing H&I testing for allogeneic HPC transplantation should follow EFI standards and be accredited by EFI and UKAS/CPA (Grade 1A).

HLA typing resolution definitions

Definitions of low, intermediate, high and allelic

HLA alleles can be identified to varying degrees of resolution depending on the methodology and interpretation used. The definitions for low, high and allele resolution typing as compiled by a joint international working party: the Harmonization of Histocompatibility Typing Terms Working Group, to define a consensual language for laboratories, physicians and registries to communicate histocompatibility typing information are used within this guideline (Nunes *et al.*, 2011). In addition, we accept the following definition for intermediate resolution.

Intermediate resolution

The term intermediate resolution can be applied when high resolution cannot be achieved, and the provided HLA type includes a subset of alleles sharing the digits in the first field of their allele name and excludes some alleles sharing those digits, for example A*02:01 or A*02:02 or A*02:07 or A*02:20 but not other A*02 alleles. There may be cases in which the subset of alleles includes one or more alleles within a group beginning with different digits, but these alleles should be the exception, for example A*01:01 or A*01:02 or A*01:14 or A:36:04 (Fig. 1).

Reporting HLA typing results

Due to the variety of assays available to a clinical H&I laboratory and the variation in resolution of HLA typing results generated, a standard format for reporting is encouraged to ensure that data can be communicated between laboratories and their users. The EFI standards provide instruction on the reporting of homozygosity, heterozygosity and haplotype assignments. When reporting high-resolution results containing ambiguous allele combinations, all ambiguities must be stated on the report, or alternatively a comment made that ambiguities have not been excluded and are available to the user upon request. It is necessary for laboratories performing HLA typing to define the level of resolution typing that they are undertaking, for example reporting intermediate-resolution results as high resolution is inaccurate and could lead to in appropriate selection of optimum donor by missing rare and novel alleles.

Official names are assigned to the HLA genes, antigens and alleles by the WHO Nomenclature Committee for Factors of the HLA System (Marsh *et al.*, 2010). The IPD-IMGT/HLA Database is the official repository of the HLA allele sequences (Robinson *et al.*, 2015).

Recommendations

1 HLA typing definitions as described by Nunes *et al.* (2011) and here should be used (Grade 1A).
2 HLA typing results should use official WHO HLA Nomenclature (Grade 1A).

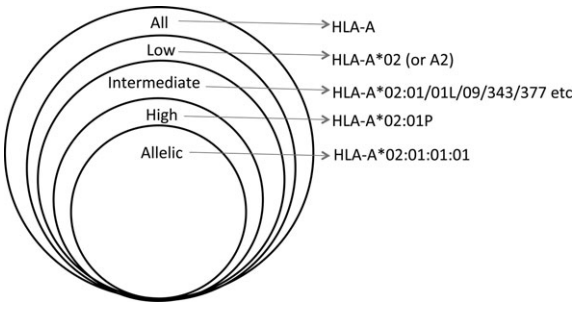

Figure 1. Diagram representing the interaction with between different levels of HLA typing resolution and their nomenclature. Modified from Nunes *et al.* (2011).

Stage of disease, time to transplant and HLA matching

One of the earliest steps in donor selection is to consider the disease status of the patient. Patients with a slowly progressing disease such as a myelodysplastic syndrome (MDS) allocated to international prognostic score system (IPSS) risk groups low and intermediate-1 will have time to allow a search for the best matched related or unrelated donor. In these cases, delayed transplantation to source the optimum donor can maximize overall survival. However, for patients with acute leukaemias where the patient's condition can rapidly deteriorate, there may only be a limited window of opportunity to transplant when the patient is in clinical remission, thus limiting the time available for an extended related or unrelated donor search (Cutler *et al.*, 2004). A patient progressing to an advanced disease usually has a higher mortality risk from the disease than the added risk of a transplant from a single allele mismatch donor or alternative donor therapy such as umbilical cord blood (UCB) transplantation. The impact of the time required to identify an optimum matching donor has to be offset against the potential negative impact of the disease stage and progression and will determine the source of progenitor cells selected for treatment (Weisdorf, 2008). The transplant team must advise the H&I laboratory on the stage of the patient's disease giving an indication of the clinical urgency, and the H&I specialist must advise on the likelihood of finding an optimum high-resolution matched donor within the time frame defined by the transplant team.

Recommendation

The clinical urgency should be made available to the individual performing the related and unrelated donor search (Grade 1B).

Histocompatibility matching for related donor selection

The initial search for an HLA-matched donor is usually within the patient's family although for certain genetic diseases a related donor may not be appropriate if they are a carrier of the same genetic mutation. Although recent studies show good outcomes with both related and unrelated donors (Robin *et al.*, 2013), there is still an advantage in selecting a related donor over and above genetic compatibility. Related donors are usually quick to identify and are flexible in terms of timing the transplant; thus, transplants can be expedited to suit the patient's clinical condition.

HLA-matched related donor selection

There is a 25% theoretical chance of finding an HLA-identical sibling for a patient. However, due to parents sharing common haplotypes, the actual number of patients identifying an HLA-A-, -B-, -C-, -DRB1- and -DQB1-matched sibling donor is closer to 30%. The selection of mismatched and haploidentical relatives increases the options of finding a donor within the patient's family.

The availability of parental HLA typing data is useful for assignment of haplotypes. These data can usually be derived for paediatric patients but are rarely available for adult patients. The unequivocal determination of familial haplotypes enables the identification of sibling donors that are HLA matches, without having to perform high/allele resolution HLA typing. This practice is referred to as 'identity by descent' (Nunes *et al.*, 2011).

Although meeting current EFI standards, HLA-A, -B and -DRB1 typing of siblings (without parents) to identify potential matches does not allow accurate determination of haplotypes and can lead to wrongly establishing presumptive matches. This is a particular concern when there is haplotype sharing between parents or apparent homozygosity (at low-/intermediate-resolution HLA typing) within a parent. This is illustrated in Fig. 2.

When haplotypes cannot be established, and an apparent HLA matching related donor has been identified, or in the case of families where the parents share a haplotype or a parent is apparently homozygous for an HLA haplotype, then the recipient and selected donor(s) should be high/allele resolution typed at HLA-A, -B, -C, -DRB1 and -DQB1 loci (Fig. 3). HLA-DPB1 typing can also be used to aid identification of genotype-matched donor particularly when a common haplotype is within the family.

All potential recombination events within the HLA region, identified within a patient, should be investigated and clarified by performing extended HLA typing on available family members.

HLA-mismatched related donor selection

A single HLA antigen or allele-mismatched related donor may be identified within a family, due to the parents sharing a closely matched haplotype or in the infrequent occurrence of genetic recombination. This related donor could be an acceptable mismatched donor in the absence of a fully matched related donor. Comparison of outcome data for patients receiving 10/10 matched unrelated donor transplants vs. patients receiving 9/10 related donor transplants demonstrated no statistical differences in overall survival, disease-free survival, transplant-related mortality, relapse and grades III-IV aGVHD. A lower incidence of cGVHD was observed in the 9/10 matched related donor transplants (Valcárcel *et al.*, 2011).

If HLA-mismatched related donors are selected, the degree of mismatching must be accurately determined by performing high-resolution typing for HLA-A, -B, -C, -DRB1 and -DQB1 as low-/intermediate-resolution

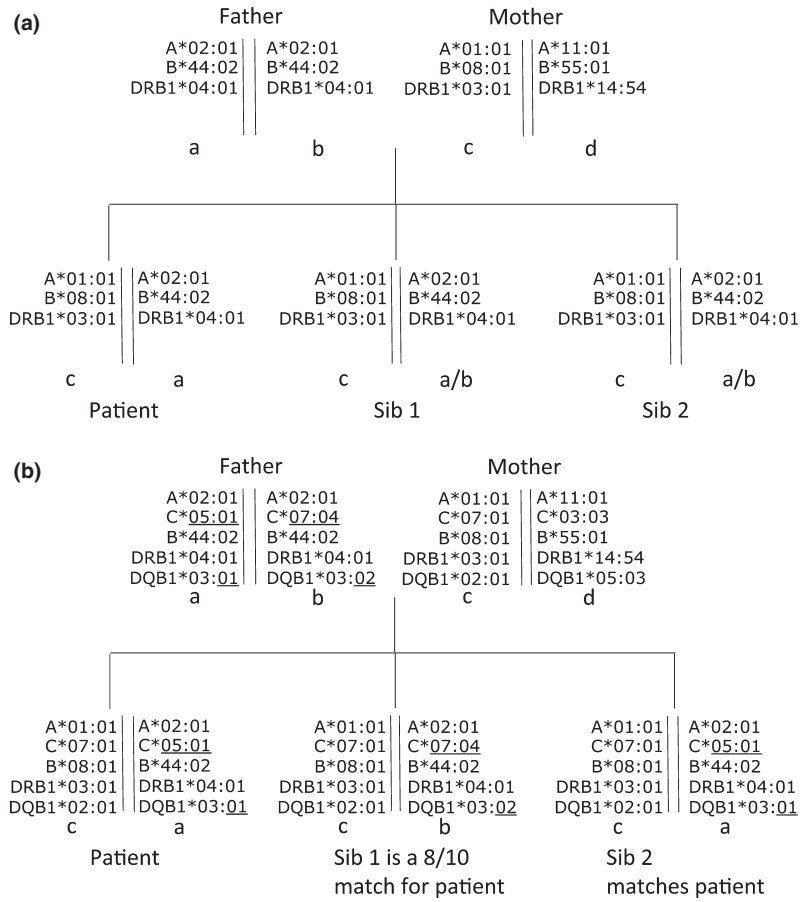

Figure 2. (a) High-resolution typing for HLA-A, -B and -DRB1 identifies both sibling 1 and sibling 2 as having the same HLA type as the patient. The father appears homozygous for all HLA loci tested. (b) Extended typing for HLA-C and DQB1 identifies sibling 1 as being mismatched. The father is heterozygous for HLA-C and DQB1.

typing could mask additional mismatches (Hansen, 2012; Kanda *et al.*, 2012).

Haploidentical family donors

A number of clinical protocols include the use of a single haplotype identical family member (haploidentical). This transplant format was pioneered by the Perugia and Frankfurt groups combined with a 'mega-dose' of selected stem cells (Aversa *et al.*, 2001; Rizzieri *et al.*, 2007). Current protocols include the post-transplant administration of cyclophosphamide to actively destroy proliferating alloreactive lymphocytes thus reducing the risk of severe GVHD caused by the HLA mismatches (Reisner *et al.*, 2011; Chang & Huang, 2014).

For the majority of paediatric patients, the donor will either be the patient's mother or father. For adult patients this choice is often impracticable, but siblings or even children may be considered.

Haploidentical transplantation by definition includes HLA mismatching. Mismatching for HLA proteins that interact with different NK cell inhibitory receptors (killer-cell Immunoglobulin-like Receptors, KIR) such as HLA-C can initiate GVH NK cell-mediated alloreactions. Post-transplantation, NK cells will be generated with the KIR repertoire of the donor. Included within this NK cell population will exist alloreactive NK cells defined by their killing ability not being inhibited by the HLA proteins expressed by host cells including dendritic cells, T cells and leukaemic cells resulting in a reduction in GVHD, prevention of graft rejection and destruction of residual leukaemic cells, respectively.

The latter graft-versus-leukaemia (GVL) effect could be enhanced if haploidentical donors are selected to encourage NK cell mediated alloreactivity. The role of KIR and KIR ligands is discussed later.

As with all related donor transplants, identification of haplotypes within the family and/or high-resolution typing of the potential haplotype-matched donor is required. All haploidentical donors will be at least a 5/10 match to the patient. Additional matching on the mismatched haplotype may be observed; however, a beneficial impact of additional matching alleles was not demonstrated in a retrospective study of 185 recipients of nonmyeloablative HLA-haploidentical transplants for haematological malignancies (Kasamon *et al.*, 2010). Thus, when multiple haploidentical donors are

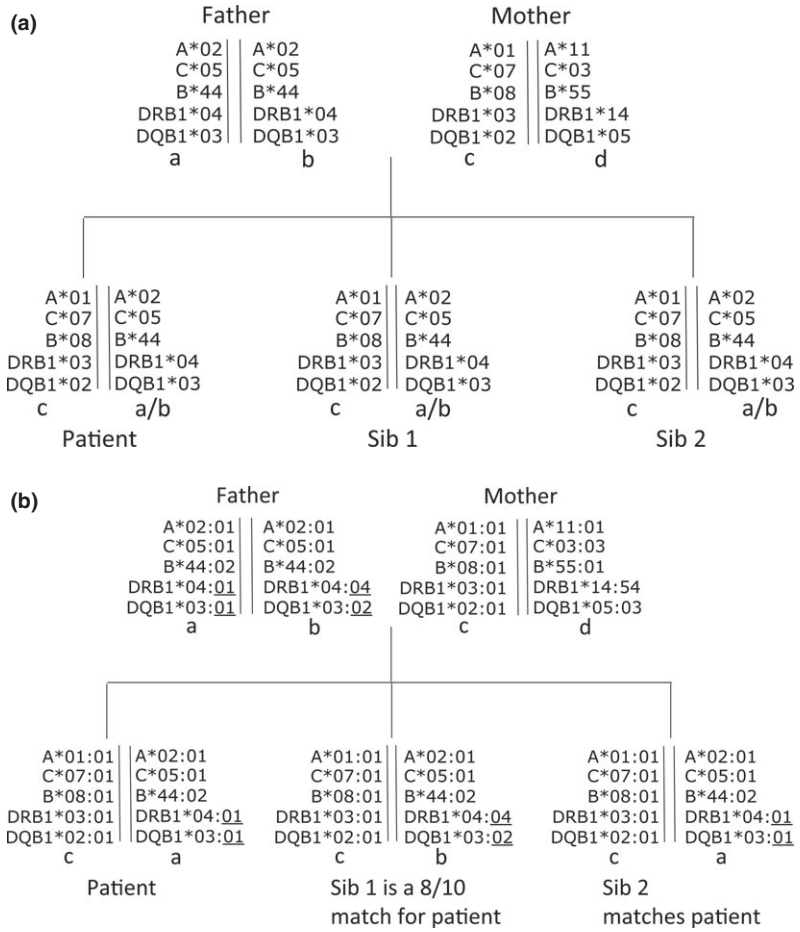

Figure 3. (a) Low-resolution typing at five HLA loci identifies both sibling 1 and sibling 2 as having the same HLA type. (b) HLA allele level typing identifies the father as being heterozygous for HLA-DRB1 and DQB1, and sibling 1 and sibling 2 have inherited different haplotypes from the father resulting in sibling 1 being an 8/10 match and sibling 2 being a 10/10 match for the patient.

available, the donor may be selected based on non-HLA donor characteristics such as CMV status.

Other haploidentical donor factors

Exposure of the patient to noninherited maternal antigens (NIMA) via transplacental trafficking of maternal and foetal cells during pregnancy may result in tolerance to these antigens when present on cells transplanted to the patient thus reducing HVG immune responses. Additionally, GVL reactions may be enhanced resulting in lower relapse rates. In a study of 118 patients receiving haploidentical transplants, an increase in 5-year event-free survival ($50.6 \pm 7.6\%$ vs. $11.1 \pm 4.2\%$; $P < 0.001$) due to reduced relapse and reduced transplantation related mortality was observed when the donor was the mother, compared with the donor being the father (Stern *et al.*, 2008). This reduced relapse rate in recipients of maternal HPC is independent of NK cell alloreactions and supports an earlier study (Kolb *et al.*, 2005). In contrast, multivariate analyses of 1210 haploidentical transplants in China have shown that transplants where the mother is the donor have increased NRM, aGVHD and decreased survival compared with transplants where the father is the donor. In this study, a sibling donor with NIMA mismatches was concluded to be the optimum donor (Wang *et al.*, 2014). Thus, there is currently insufficient evidence in the literature to support the use of particular haploidentical donors from within the family and optimum donor selection should take into account non-HLA factors.

Related cord blood donor

HLA typing of potential related cord blood donors must be performed to the same resolution as undertaken for another related donor.

Final donor selection

Both patient and the selected related donor must be HLA typed using a second independent sample. This should happen as early as possible to exclude any sampling or laboratory errors and must take place prior to the initiation of the patient's conditioning protocol.

The second HLA type may be performed by low-/intermediate-resolution methods and must cover at a minimum HLA-A, -B and -DRB1 loci.

Recommendations

1 HLA high-resolution typing should be performed on potential matching, mismatching and haploidentical related donors when familial haplotypes cannot be fully assigned (Grade 1A).
2 Patients and selected related donors should be typed for HLA-A, -B, -C, -DRB1 and -DQB1 (Grade 1A).
3 All patients and related donors must have their HLA type confirmed on a second sample pretransplant (Grade 1A).

Unrelated adult donor selection

For those patients without an identified matched/mismatched related donor, a search for an unrelated donor should be undertaken. European Society for Blood and Marrow Transplantation (EBMT) data show that more unrelated donor allogeneic transplants (54%) than HLA-matched and HLA-mismatched sibling donor transplants (46%) took place in 2012 (Passweg *et al.*, 2014).

The search process

For some patients with a short time frame to transplant, it may be necessary to embark on the unrelated donor search simultaneously whilst searching for a related donor. The decision to initiate an unrelated donor search must be made in conjunction with the transplant team and with the authorization (Scotland) or consent (rest of UK) of the patient to permit the sharing of patient personal identification details with the national and international registries.

Within the UK, there is an aligned unrelated donor registry, operated by the Anthony Nolan, providing search reports with details of donors from the Anthony Nolan; the British Bone Marrow Register (BBMR); the Welsh Bone Marrow Donor Register (WBMDR); and Delete Blood Cancer UK. Search requests are sent to the Anthony Nolan and a search report of UK donors, together with a summary report of the results from a search of the international database of donor registries, Bone Marrow Donors Worldwide (BMDW) is provided. The number of potential matching UK donors identified will determine whether a detailed search of international registries is required. It is the responsibility of the search requester to establish with the Anthony Nolan search team, cut-off criteria in terms of minimum number of potential matching UK donors before initiating an international donor search.

The most useful results from the unrelated donor search are obtained when the patient has been HLA typed to high/allele level resolution, as this allows elimination of mismatching donors from the search summary.

The search algorithm

The World Marrow Donor Association (WMDA) has published a 'framework for the implementation of HLA matching programmes in haematopoietic stem cell donor registries and cord blood banks', which outlines the minimum requirements for a search programme (Bochtler *et al.*, 2011). Essentially, the registry's matching programme must be capable of comparing donor–patient pairs irrespective of whether their HLA typing derives from serologic or DNA based methods. These comparisons are based around the assignment of a search determinant to each HLA allele or National Marrow Donor Program (NMDP) code based on files which are published on the HLA Nomenclature website: http://hla.alleles.org/wmda.

Once the patient's HLA type has been run against the registry's database, the first step in processing returned donors is to assign a match grade for each locus, where allelic match > serology match > allelic mismatch > serology mismatch.

For two allelic assignments a1 and a2, there are three possible allelic grades, all of which can be distinguished by the algorithm:

- *Nucleotide Sequence Match* – a1 and a2 have identical nucleotide sequences in all regions (exons and introns) underlying the current definition of the allele name, for example *A*02:01:01:01* vs. *A*02:01:01:01*
- *Amino Acid Sequence Match* – a1 and a2 have identical amino acid sequences but differ within their nucleotide sequences, for example *A*01:01:02* vs. *A*01:01:03*, *B*40:06:01:01* vs. *B*40:06:01:02*
- *Antigen Recognition Site Match* – a1 and a2 differ within their amino acid sequence but not within the regions interacting with the presented antigen and the T-cell receptor (encoded by exons 2–3 for class I and exon 2 for class II). For example, *A*74:01* vs. *A*74:02*

Once the scoring is complete, the list of donors is sorted by descending match count and total score; additional sort parameters such as age, gender and locus score are determined by the user.

UCB unit searching is based on a similar algorithm, with the added ability to match on the UCB unit's NIMA type.

Currently, the algorithm used in the UK by the Anthony Nolan shortlists potential matching donors when HLA-A, -B and -DRB1 potentially match but does not consider matching beyond the first field of the HLA type for HLA-C and -DQB1, when typing for these loci are available. The search requester must check the true level of matching which involves

decoding NMDP HLA typing codes to determine whether the shortlisted potential matching donors have mismatches at HLA-C and -DQB1.

Other registries such as the NMDP (USA registry) and ZKRD (German registry) use algorithms that match to high resolution for HLA-A, -B, -C, -DRB1 and give a probability value on the likelihood of a donor being a match for a patient based on HLA allele and haplotype frequencies in the donor populations.

It is essential that the person interpreting search reports understands the algorithm used by the various different registries to ensure optimum donor selection.

Genetic factors impacting on the identification of a HLA-matched unrelated progenitor cell donor

The number of HLA-A, -B and -DRB1 low-resolution matched donors available for a patient following a BMDW search often reflects the likelihood of finding a high-resolution matched unrelated donor. It has been reported that Caucasoid patients have a 40–75% chance of having a high-resolution matched donor at HLA-A, -B, -C, -DRB1 and -DQB1 (10/10 match) and that the probability of finding a 10/10 high-resolution match is highly predictable (Tiercy *et al.*, 2000, 2007; Hirv *et al.*, 2009; Gragert *et al.*, 2014). The chance of a 10/10 match in other ethnic groupings, with HLA haplotypes that are less well represented on the unrelated donor registries, is significantly lower (Heemskerk *et al.*, 2005; Schmidt *et al.*, 2009; Gragert *et al.*, 2014). Hence, patients are less likely to find a matched donor from an ethnic group differing from their own and patients with parents coming from differing ethnic groups (mixed race) are at increased risk of not finding any match.

The use of 'minimally' mismatched adult volunteer donors and cord blood units increases significantly the likelihood of finding a usable donor (Gragert *et al.*, 2014). The frequencies of HLA-B and -C and/or HLA-DRB1 and -DQB1 associations in differing ethnic groups are available for some donor registries for comparison with the HLA type of the patient (Cano *et al.*, 2007; Kollman *et al.*, 2007; Maiers *et al.*, 2007; Gragert *et al.*, 2013; Mack *et al.*, 2013), and these tools can be used to help predict how likely it will be to find a match for a patient.

The following factors must be considered when searching for a high-resolution matched unrelated donor.

- Commonly found HLA-B and -C and/or HLA-DRB1 and -DQB1 associations within the patient's HLA type will have a positive impact on the likelihood of finding a donor
- Uncommon haplotypes present in the patient where the allele of one locus is not in linkage disequilibrium with alleles of the neighbouring locus, such as uncommon HLA-B and -C and/or HLA-DRB1 and -DQB1 associations have a negative impact on likely donor identification
- The presence of an allele (in the patient) with a frequency of <5% within the low-resolution typed potential donors (e.g. B*44:05) and the presence of an allele (in the patient) that is a possible match for low-resolution donor types where other alleles having frequencies of >10% are the alternative possible mismatches (e.g. B*35, B*44, DRB1*04, DRB1*11, DRB1*13) will negatively impact on the identification of a matched donor
- The presence of alleles from the low-resolution typing group HLA-B*51 and B*18 and the presence of alleles B*27:05, B*44:02 and B*44:03 in the patient have an increased risk of a HLA-C mismatch
- The presence of alleles from the low-resolution typing group HLA-DRB1*04 and DRB1*07 in the patient has a raised risk of a HLA-DQB1 mismatch

HLA matching requirements for unrelated donor transplants

Multiple studies have reported optimum transplant outcome is achieved when the patient and donor are matched for HLA-A, -B, -C and -DRB1 alleles (Petersdorf *et al.*, 2001, 2004; Morishima *et al.*, 2002; Flomenberg *et al.*, 2004; Lee *et al.*, 2007; Woolfrey *et al.*, 2011).

The role of HLA-DQ matching is less well supported, and NMDP data from 3857 transplants for malignant disease (Lee *et al.*, 2007) showed that individual mismatches for HLA-DQB1 had no impact on survival. However, if the DQB1 mismatch was associated with an additional mismatch, for example 7/8 or 6/8 HLA-A, -B, -C, -DRB1 mismatch, then there was an association with poorer survival albeit not statistically significant. Although HLA-DQB1 mismatching did not reach significance in relation to survival, in a recent German study (Fürst *et al.*, 2013), HLA-DQ antigen mismatching achieved a higher hazards risk for survival compared with HLA-DQ antigen matches.

Overall, single mismatches are deemed acceptable, with multiple mismatches conferring significantly worse outcomes (Crocchiolo *et al.*, 2009a) with the effect observed greater when the patient had acute leukaemia and was transplanted early during first remission compared to patients with advanced disease.

Impact of mismatches at individual HLA loci on transplant outcome

There is no consensus regarding which of the HLA-A, -B, -C, -DRB1 loci are more detrimental to mismatch. HLA-A mismatching and HLA-DRB1 mismatching were reported as being less well tolerated compared with HLA-B and -C mismatches in a NMDP study with all mismatches reducing overall survival at 1 year by 9–10% (Lee *et al.*, 2007). In contrast, the Japanese

registry reported transplants with HLA-A and -B mismatches had worse survival than HLA-C and -DRB1 mismatches (Morishima *et al.*, 2002). HLA-B mismatches were associated with a higher risk of aGVHD II-IV in an Italian study of 805 patients transplanted for haematological malignancies (Crocchiolo *et al.*, 2009a) whereas HLA-C antigen mismatches were associated with: lower leukaemia-free survival, increased risk for mortality and grade III-IV GvHD in an NMDP/Centre for International Blood and Marrow Transplant Research (CIBMTR) study of 1933 patients transplanted with haematological malignancies (Woolfrey *et al.*, 2011). The variability in the outcomes of these studies can be attributed to: differences in study design; patient demographics; source of stem cells, including T-cell depletion; differences in HLA polymorphism within groups of patients of different ethnicities; use of serotherapy; and GvHD prophylaxis.

A retrospective analysis of 2646 T-cell replete transplants performed for haematological malignancies in Germany has been performed (Fürst *et al.*, 2013). All patients and donors were HLA typed to high resolution. The impact of mismatches, defined at both high (allele) and low (antigen) resolution, on overall survival, disease-free survival, relapse, transplant-related mortality and primary graft failure was analysed. Overall allele and antigen mismatches did not correlate with a differential impact on transplant outcome. This finding supports previous work published by Lee *et al.* (2007) (except for HLA-C where allele mismatches were not associated with poorer outcome by Lee *et al.*) and Crocchiolo *et al.* (2009a). Thus, any type of mismatch can have a negative impact on outcome.

Mismatches of alleles at either of HLA-A, -B, -C, -DRB1 and -DQB1 were associated with a decrease in overall survival (only significant for HLA-A, -B, -C and -DRB1), a decrease in disease-free survival (significant for HLA-C) and an increase in transplant-related mortality (significant for HLA-A, -B, -C and -DRB1). Of all associations observed, the most significant were with HLA-C antigen mismatches, affecting overall survival and disease-free survival, with HLA-B allele mismatching being the most significant affecting transplant-related mortality. HLA-C allele mismatches gave consistently lower hazard risks for the outcomes studied suggesting that there may be permissive mismatching at the HLA-C allele level (Fürst *et al.*, 2013). Permissive HLA-C mismatching has also been described for HLA-C*03:03 and C*03:04. The transplant outcome for patients receiving a 7/8 (C*03:03/C*03:04) mismatched transplant was not significantly different from patients receiving a 8/8 matched transplant (Fernandez-Vina *et al.*, 2014).

The impact of HLA-C antigen vs. allele mismatches has been further elucidated in a study of 1975 HLA-C only mismatched (matched HLA-A, -B, and -DRB1) transplants (Petersdorf *et al.*, 2014). The level of expression of the mismatched HLA-C proteins was assessed using median fluorescence intensity (MFI) data procured from previous study of healthy and human immunodeficiency virus (HIV)-infected individuals (Apps *et al.*, 2013).

Increasing expression level of the patient's mismatched (nonshared) HLA-C was significantly associated with increased risks of aGVHD III-IV, NRM and overall mortality but with no impact on relapse. Increasing expression level of mismatched (nonshared) HLA-C in the donor was also associated with an increase in NRM and mortality, but with no effect on aGvHD or relapse. The allele mismatches were predominantly C*07 and C*03, which have been shown to have low levels of protein expression, and these mismatches may account for the previously reported permissive HLA-C mismatches. Mismatching for lower expressing HLA-C alleles of the patient, compared with higher expressing HLA-C alleles may lower the GVH immune response supporting selection of mismatches for lower expressed over mismatches for high expressed alleles, and avoidance of HLA-C-mismatched donors for patients with two highly expressed HLA-C. This study also demonstrated a higher risk in double mismatch (8/10) transplants involving a class I and class II mismatch compared with double class I or double class II. Other research has highlighted the impact of the HLA-C bearing haplotype on HLA-C expression which could differ in HLA-C-mismatched transplants (Bettens *et al.*, 2014).

One of the problems making it difficult to define an accurate risk estimate for single loci mismatches is the extensive polymorphism exhibited at HLA loci. Mismatches at any loci may involve 1 vs. 10 vs. 20 amino acid mismatches, for example A*01:01 v A*02:01; A*01:01 v A*03:01; A*01:01 v A*23:01 and not all mismatches at a given loci will have equal effects on GVL and GVH immune responses post-transplant. It has been calculated that a database of 11 000–1.3 million transplants would be required to provide sufficient statistical power to detect an association between particular HLA allele mismatches and survival (Baxter-Lowe *et al.*, 2009).

Impact of individual amino acid substitutions on transplant outcome

The impact on transplant outcomes (acute, chronic GVHD, transplant-related mortality (TRM), relapse and overall survival) of amino acid substitution at peptide binding positions 9, 99, 116 and 156, and KIR binding position at amino acid 77 was studied in a multivariate analysis of a heterogenous cohort of patients transplanted for haematological malignancies (Pidala *et al.*, 2013). Individual mismatches at residues 99 and 116 within HLA-C were associated with an increased TRM and severe acute GVHD, respectively. A mismatch at residue 9 within HLA-B was associated with an increase in chronic GVHD. None of the

mismatches studied had an affect on outcome when located within HLA-A.

In the study of Petersdorf *et al.* (2014), patients with HLA-C mismatches at residue 116 had an increased risk of aGVHD compared with residue 116 matched patients and a slightly higher risk of overall mortality. These findings were not statistically significant. However, the incidence of NRM was found to increased in patients that were mismatched for residue 116 as the expression of the HLA-C mismatch also increased (HR, 1.31; 95%CI, 1.09–1.58; $P = 0.004$). This was not observed in patients mismatched for higher expressing HLA-C alleles that were residue 116 matched (HR, 0.98; 95% CI, 0.78–1.23; $P = 0.88$). Patients receiving HLA-C mismatches with specific amino acid differences at KIR interacting residues 77 and 80 had HLA-C mismatches with higher expression compared to patients with matching residues 77 and 80. No statistical significant associations with aGVHD, overall mortality or NRM were identified, although similar to mismatching at residue 116, the risk of NRM increased as the HLA-C mismatch expression levels increased for the patients receiving residue 77 and 80 mismatched transplants (HR, 1.38; 95% CI, 1.14–1.67; $P = 0.0009$). This was not observed for patients receiving residue 77 and 80 matched transplants (HR, 1.01; 95% CI, 0.82–1.24; $P = 0.91$). Thus, mismatching for allotypes that are highly expressed and contain mismatches at residues 77 and 80 could initiate donor NK cell alloreactivity.

Therefore, if a choice of mismatched donors is available, consideration of the location of the mismatched amino acid residues and avoidance of the described nonpermissive mismatches may contribute to better outcome. For HLA-C mismatches, apparent expression levels may also be considered.

Impact of HLA-DPB1 mismatching

Recombination hotspots are located between HLA-DQB1 and -DPB1 genes (Cullen *et al.*, 1997); therefore, matching patients and unrelated donors with common HLA-A, -C, -B, -DRB1, -DQB1 haplotypes does not necessarily implicate matching for HLA-DPB1 and -DPA1 alleles. It is reported that within families up to 5% of otherwise 10/10 matched siblings will also be HLA-DPB1 mismatched attributed to recombination between HLA-DQ and -DP genes (Büchler *et al.*, 2002). HLA-DP-specific T cells have been detected and associated with both GVL (Rutten *et al.*, 2008, 2013) and GVHD (Stevanovic *et al.*, 2013) supporting the direct role of HLA-DP proteins in the immune responses occurring between patient and donor cells post-transplant.

Analysis of the impact of HLA-DPB1 matching and mismatching on transplant outcome has been studied in both single-centre and multicentre studies. In an analysis of a heterogenous international cohort of transplant recipients, allelic DPB1 mismatches were shown to offer a GVL advantage via a reduction in relapse, but this was also associated with increased aGVHD and a suggestive increase in mortality (Shaw *et al.*, 2007). In an NMDP study, there was no significant association of single or double HLA-DPB1 allele mismatches with survival compared with no DPB1 mismatches in an otherwise HLA-A-, -B-, -C-, -DRB1-matched group of recipients. An increased risk of TRM and decreased risk of relapse was suggestive in this study albeit not significant (Lee *et al.*, 2007).

In a UK multicentre study, the impact of DPB1 allele matching was associated with better overall survival in patients transplanted with early leukaemia but not in patients transplanted with late stage disease (Shaw *et al.*, 2010), supporting other studies where the effect of HLA matching is not as strong in patients transplanted at late stage. This effect was restricted to patients receiving 10/10 matched transplants, as a survival advantage was observed in patients receiving 9/10 matched transplants which also had a single DPB1 mismatch. This finding also held in multivariate analysis (OR 0.478; 95%CI 0.30–0.75; $P = 0.001$).

A single-centre UK study of 130 patients transplanted for malignancies demonstrated a trend towards a negative impact of double HLA-DPB1 mismatching on overall survival in patients otherwise matched for 10/10 HLA loci (HLA-A, -B, -C, -DRB1, -DQB1). This finding was more evident in patients receiving myeloablative conditioning compared with those receiving RIC (Burt *et al.*, 2014).

HLA-DPB1 mismatches have been assigned as either permissive or nonpermissive based on observed immunogenicity to T-cell epitopes (Zino *et al.*, 2004; Crocchiolo *et al.*, 2009b). The effect of dividing DPB1 mismatches into these two groups has provided evidence of DPB1 mismatching impacting on survival. In a study of 621 unrelated donor HPC transplants, recipients with permissive DPB1 mismatches had a significantly higher 2-year survival than those with nonpermissive DPB1 mismatches (55% vs. 39%, $P = 0.005$). This improved survival was due to a decrease in NRM (Crocchiolo *et al.*, 2009b). Overall nonpermissive DPB1 mismatches (unlike earlier studies of DPB1 allele mismatches) were not associated with an increase in aGVHD and concomitant GVL.

The assignment of permissive and nonpermissive DPB1 mismatches was included in a large multicentre study (8539 transplants) conducted by the International Histocompatiblity Workshop Working Group in HPC transplantation (Fleischhauer *et al.*, 2012). Of the patients receiving a 10/10 matched transplant (HLA-A, -B, -C, -DRB1, -DQB1), HLA-DPB1 nonpermissive mismatches were associated with a significant increased risk of overall mortality (HR 1.15, 95% CI 1.05–1.25; $P = 0.002$), NRM (HR 1.28, 1.14–1.42; $P < 0.0001$), and in this study, an association was observed with severe GVHD (OR 1.31, 95% CI 1.11–1.54; $P = 0.001$) but not relapse (HR 0.89, 95% CI

0.77–1.02; $P = 0.10$) compared to permissive mismatches. Although differences in outcome were observed between the DPB1-matched and DPB1-permissive-mismatched patients, this did not affect overall mortality.

A CIBMTR study of a patient cohort considered more contemporaneous based on, patient disease, transplant conditioning protocol and HPC source (peripheral blood stem cell, PBSC vs. bone marrow), has addressed the impact of DPB1 matching vs. permissible and nonpermissive mismatches (Pidala *et al.*, 2014). An increase in aGVHD grades II–IV and III–IV and a decrease risk of relapse were observed in patients receiving DPB1-mismatched donors. Dividing the DPB1-mismatched donors into permissive and nonpermissive mismatches identified the nonpermissive mismatches as having an increase in transplant-related mortality and an increase in overall mortality compared to both the permissive mismatched and the matched patients. The DPB1 mismatching outcome was only significant within patients receiving an 8/8 and a 10/10 matched donor.

HLA-DPA1 is significantly less polymorphic than HLA-DPB1 and certain alleles encoded by the two loci are in linkage disequilibrium. Analysis of the role of HLA-DPA1 mismatches had no effect on transplant outcome observed for the DPB1 permissive and nonpermissive mismatches in an NMDP study of 1281 10/10 matched unrelated donor transplants (Fleischhauer *et al.*, 2014).

Although there are variations in the clinical outcomes for HLA-DPB1 allele and nonpermissive mismatched transplants, overall, matching for HLA-DPB1 and avoidance of nonpermissive mismatches is associated with better overall survival. Therefore, matching/mismatching at the HLA-DPB1 locus should be considered on an individual basis, taking into account matching at other loci and following the transplant physician's evaluation of the patient's transplant-related risks. An online tool is available for assignment of permissive and nonpermissive DPB1 mismatches based on the T-cell epitope analyses performed (Shaw *et al.*, 2013).

Matching for HLA-DRB3, -DRB4 and -DRB5 (with DQB1 and DPB1)

Further analysis of the NMDP data set of 3853 unrelated donor transplants has demonstrated that whilst not significant in isolation, mismatching for DRB3/4/5, DQB1 and DPB1 (defined as 'lesser expressed HLA loci', LEL) increased the risk associated with the presence of a mismatch at HLA-A, -B, -C or -DRB1 (Fernandez-Vina *et al.*, 2013). Transplants matched for 7/8 HLA-A, -B, -C, -DRB1 with three or more LEL mismatches in the GvH direction had a higher risk of mortality and transplant-related mortality than 7/8 transplants with 0 or 1 LEL mismatches. Thus, HLA typing donors and patients for HLA-DRB3, -DRB4 -DRB5, -DQB1 and -DPB1 is warranted when mismatched donor selection is occurring to minimize the number of mismatches at the LEL.

Direction of HLA mismatch

Donor and patient HLA mismatches may be bidirectional, that is GVH and HVG or unidirectional, for example GVH (when the donor is homozygous for a particular locus) or HVG, when the patient is homozygous at a given HLA locus. The effect of direction of HLA mismatch has been investigated within a cohort of 2687 unrelated donor transplants in patients with malignant disease (Hurley *et al.*, 2013). In multivariate analyses, patients receiving a 7/8 (HLA-A, -B, -C, DRB1) matching graft with unidirectional GVH mismatch and patients receiving a 7/8 bidirectional mismatch had significantly worse transplant-related mortality, overall survival and disease-free survival compared to patients receiving a 8/8 matched transplant. This worse transplant outcome (compared with 8/8 transplants) was not shared with patients receiving a 7/8 matching graft with unidirectional HVG mismatch.

This difference in outcome observed for the 7/8 HVG mismatches is likely caused by the observed reduction in probability of acute GVHD observed in this group, which was significantly less than the 7/8 bidirectional mm and 7/8 GVH mm ($P = 0.003$) and not significantly different from the 8/8 group. No differences were observed between the three 7/8 mismatched groups and the 8/8 matched transplants with other clinical outcomes including engraftment, relapse and chronic GVHD.

These findings support selection of a 7/8 HVG mismatch over a 7/8 bidirectional or 7/8 GVH mismatch donor.

This study did not confirm previous data reported by the Seattle group in 2001 where transplants mismatched in the HVG direction were associated with lower neutrophil engraftment and secondary graft failure. However, the demographics of the transplant group in this latter study differ in that the transplants were bone marrow and HLA matching was not completely at high resolution (Petersdorf *et al.*, 2001).

HLA typing requirements for unrelated donor transplantation

EFI standards v6.2 recommend high-resolution HLA-A, -B, -C and -DRB1 typing to be performed for unrelated progenitor cell transplantation, and this minimum requirement is increasingly enforced by the unrelated donor registries (Bray *et al.*, 2008; Spellman *et al.*, 2012; NMDP, 2015). High-resolution typing of the patient helps to determine the likelihood of finding a potential 10/10 matched donor. The optimal number of donors to select for further HLA typing should be decided on a patient by patient basis taking into account all the factors that can influence the likelihood of

finding a suitable donor and the clinical urgency. Acceptable levels of matching and mismatching (i.e. which loci must be matched and which may be mismatched) must be determined and agreed by local transplant policies. If the patient is to be entered onto a clinical trial that requires a 10/10 matching donor, then the H&I specialist must be informed to avoid wasting time searching for mismatched donors if there are no fully matched donors available and the transplant team should be notified as soon as it is known that a fully matching donor search has been unsuccessful.

Recommendations

1 The patient should be high-resolution typed prior to submitting the HLA type for an unrelated donor search (Grade 1A).
2 A 10/10 high-resolution HLA-A-, -B-, -C-, -DRB1- and –DQB1-matched unrelated PBSC or bone marrow donor should be used where possible (Grade 1A).
3 Where a 10/10 matched PBSC or bone marrow donor is not available a single mismatch at HLA-A, -B, -C, -DRB1 or -DQB1 is acceptable (Grade 1A).
4 Alternative progenitor cell donors (cord blood or haploidentical) should be considered early in the donor search when a patient is unlikely to have an HLA-matched unrelated donor (Grade 1A).
5 HLA-DRB3, -DRB4, -DRB5 typing should be performed, and when a choice of otherwise equally matched and appropriate (e.g. CMV status) donors is available, mismatches for these should be minimized (Grade 2A).
6 For unrelated donor selection, HLA-DPB1 typing should be performed and when a choice of otherwise equally matched and appropriate (e.g. CMV status) donors is available, nonpermissive mismatches should be minimized (Grade 2C).
7 For mismatched related and unrelated donor selection, HVG mismatches are favoured over bidirectional and GVH mismatches (Grade 2C).
8 All patients and unrelated donors must have their HLA type confirmed on a second sample (Grade 1A).

Selection of unrelated umbilical cord blood units

Umbilical cord blood is an alternative source of HPCs that can be used to treat patients with both malignant and nonmalignant disorders (reviewed in Ballen *et al.*, 2013). An early study undertaken by the CIBMTR-Eurocord showed comparable survival outcomes comparing patients receiving HLA-identical cord blood transplants with patients receiving HLA-identical sibling donor transplants. This study highlighted delayed granulocyte and platelet engraftment in UCB transplant recipients but also demonstrated a reduction in both acute and chronic GVHD (Rocha *et al.*, 2000).

Similarly, a comparison of unrelated HLA mismatched UCB transplants with matched unrelated adult donors transplants demonstrated recipients of the UCB transplants experienced delayed engraftment, less acute and chronic GVHD with a similar relapse rate, overall survival (OS) and leukaemia-free survival (LFS), (Rocha *et al.*, 2001).

The use of UCB was initially restricted to children due to the low cell doses obtained and poorer results obtained with adult recipients (Laughlin *et al.*, 2001). However, the selection of UCBs with higher cell doses and the success with infusion of two UCBs to adult recipients (double cord blood transplants), together with improved conditioning protocols has led to UCB being a source of HPCs for both children and adults (Barker *et al.*, 2003; Scaradavou *et al.*, 2013).

The reduced incidence and severity of acute and chronic GVHD observed following UCB transplantation has allowed the use of mismatched UCB donors which would exceed the mismatches accepted in related or unrelated adult donor transplants. This has led to the use of mismatched UCB donations to enable transplantation of patients that do not have an appropriately matched or mismatched adult donor.

In an analysis of 1061 single UCB transplants for malignant disease, the lowest TRM was observed in recipients of 0 mismatched units (HLA-A, -B low/intermediate; -DRB1 high resolution) regardless of cell dose. For transplants performed with HLA mismatches, there was a positive association with survival outcomes for patients with 1 mismatch with a Total Nucleated cell (TNC) dose $\geq 2.5 \times 10^7$ kg^{-1} and patients with two mismatches and a TNC dose $\geq 5 \times 10^7$ kg^{-1}, with no difference in survival outcomes between 1 mismatched units with a TNC dose 2.5–4.9 $\times 10^7$ kg^{-1} and two mismatched units with a dose of $\geq 5 \times 10^7$. Therefore, the greater the HLA mismatch between UCB donor and patient, the greater is the requirement for a higher TNC dose (Barker *et al.*, 2010).

An investigation into the impact of HLA allele level matching (Eapen *et al.*, 2014) found that independent to HLA matching, UCB TNC dose was the only other donor factor associated with NRM. Transplants performed with single UCB units with $<3 \times 10^7$TNC kg^{-1} had NRM rates that were 15–20% higher than transplants performed with UCB units with a higher TNC.

Thus, the evidence supports HLA matching being critical above a minimum cell dose threshold. HLA matching can compensate for lower TNC dose but a minimum TNC has to be achieved.

Role of HLA matching in unrelated umbilical cord blood transplantation

As successful outcomes can be achieved using mismatched UCB transplants, the requirement for high/

allele level matching between UCB unit and patient has not been as rigorous with matching defined using HLA-A, -B low-/intermediate-resolution and HLA-DRB1 high-resolution typing, that is 6/6, 5/6, 4/6.

Matching for HLA-C was not considered originally in UCB selection algorithms; however, a multicentre study of single UCB transplants identified a beneficial impact of HLA-C matching as HLA-C mismatches were associated with an increase in TRM. The negative impact of HLA-C mismatching was observed in both HLA-A, -B (intermediate resolution), -DRB1 (high resolution) matched and mismatched transplants (Eapen *et al.*, 2011).

The role of allele level HLA matching between UCB and patient was investigated in a cohort of 1568 recipients of single cord blood transplants for haematological malignancies (Eapen *et al.*, 2014). The patients were predominantly paediatric. Analysis of allele level HLA matching demonstrated that only 54% of patient/donor pairs considered a match for HLA-A, -B low, -DRB1 high resolution were matched for HLA-A, -B, -C, -DRB1 at allele level resolution.

HLA allele matching impacted on the risk of NRM with pairs mismatched for 0, 1, or 2 alleles having reduced risk compared with transplants performed with 3, 4 or 5 allele mismatches. The results suggest that isolated mismatches at HLA-A, -C or -DRB1 but not HLA-B are associated with a threefold increase in NRM risk suggesting an isolated HLA-B mismatch may be better tolerated, although this finding has to be considered with caution as there were only 31 donor/patient pairs with isolated HLA-B mismatches within this transplant cohort. These results support HLA typing of UCB units to high/allele level resolution for accurate selection of the best matching unit. Neutrophil recovery was lower for transplants mismatched for 3 or more alleles. A significant effect of HLA allele level matching on the incidence of aGVHD II-IV and cGVHD and on relapse was not observed, only UCB units mismatched for 4 or more alleles had increased incidence of relapse. No significant association between HLA allele mismatching and overall survival for transplants performed with 0–4 allele mismatches was found.

There was no benefit in the NRM rates by increasing the TNC to $>3 \times 10^7$ kg^{-1} in transplants performed with 0, 1, 2, 3 and 4 HLA allele mismatches. Thus, when there are multiple UCB units available with TNC $\geq 3 \times 10^7$ kg^{-1}, it is not necessary to select the UCB with the largest TNC. Instead consideration should be made of the HLA matching available. Consideration of high-resolution typing together with cell dose has demonstrated that better matching UCB units may be selected without impeding on an acceptable cell dose (Dahi *et al.*, 2014).

The impact of allele level mismatching on outcome in double cord transplants has recently been described in a single-centre cohort of 133 patients transplanted for haematological malignancies (Oran *et al.*, 2015). This study supports matching at least 5/8 HLA-A, -B, -C, -DRB1 alleles to reduce transplant-related mortality.

Direction of HLA mismatch

The effect of direction of HLA mismatch was investigated in a cohort of 1202 single UCB unit transplants. Unidirectional mismatches were identified and classified as either GvH or HVG (rejection) mismatches. Engraftment was faster in patients with GVH unidirectional mismatches compared with patients with single bidirectional mismatches HR = 1.6, $P = 0.003$). Other benefits to unidirectional mismatches included lower TRM, lower overall mortality and treatment failures. The HVG unidirectional mismatches exhibited slower engraftment, higher graft failure and higher relapse rates.

The outcome of this study is that the direction of the mismatch should be calculated and priority should be given to unidirectional GVH mismatches over HVG unidirectional mismatches (Stevens *et al.*, 2011).

However, these findings were not confirmed in a Eurocord study of 1565 single UCB unit transplants for malignant disease. In this cohort, one or two HLA mismatches in the GVH or HVG direction were not associated with NRM and survival (Cunha *et al.*, 2014).

A Japanese study of 2977 single UCB transplants for malignant disease did not find any significant association with overall mortality for transplants performed with unidirectional mismatches in either GVH or HVG direction (Kanda *et al.*, 2013). GVH mismatches were associated with a lower incidence of NRM for paediatric recipients only which were also associated with a higher incidence of relapse.

The HLA data included in these three studies was not at high resolution; therefore, additional mismatches not accounted for in the analysis are likely. HLA-C, -DQ and -DP matching was not considered. The role of HLA alloantibodies was not addressed. The impact of NIMA matching was included in the study of Stevens *et al.* (2011), but not in the others. These studies are also complicated as multiple mismatches are present and not all mismatches (in the same direction) will impact the same biological effect. Further work is required to elucidate the impact of unidirectional mismatches.

Impact of CD34+ cell dose

Although the TNC count is used at the time of unit selection as a measure of the potency of the UCB unit, this may not be the most accurate predictor of biological engraftment. Prediction of engraftment potential of transplanted cord blood units has been described based on precryopreservation Colony Forming Unit (CFU) $\geq 50 \times 10^3$ kg^{-1} (Migliaccio *et al.*, 2000) and

CD34+ cell count $\geq 1.5 \times 10^5$ kg^{-1} (Sanz *et al.*, 2010). Post-thaw counts are also valid predictors; however, these are only available after the UCB unit has been selected.

A study of 128 double UCB transplants performed in patients with malignant disease identified the cord blood bank precryopreservation CD34+ cell dose of the dominant UCB unit as the only independent predictor of neutrophil engraftment (HR, 1.95; 95%CI: 1.30–2.90; $P \leq 0.001$) (Purtill *et al.*, 2014). The precryopreservation CD34+ cell dose correlated with the transplant centres post-thaw CD34+ cell count, with better correlation for: (i) UCB units from cord blood banks that were FACT-Netcord accredited compared with banks that did not have this accreditation, and (ii) UCB units banked during 2005–2012, compared with 1997–2004.

Cryopreserved UCB units with volumes <24.5 and >26.0 mL and UCB units from nonaccredited banks were associated with low post-thaw CD34+ cell recovery (<65%) and post-thaw CD34+ cell viability. The year of cryopreservation and processing method did not impact on CD34+ cell recovery and viability. Compared with the precryopreservation TNC count, the precryopreservation CD34+ cell dose correlated better with post-thaw CD34+ cell count, and therefore, this measurement was deemed a better overall predictor of successful neutrophil engraftment with an arbitrary CD34+ cell threshold of 0.7×10^5 kg^{-1}.

The UCB unit search process

A search for an UCB unit can be undertaken simultaneously with an adult unrelated donor search. This is essential if the patient has a rare HLA type or if the patient requires a speedy transplant. Within the UK, a joint Anthony Nolan and BBMR cord blood search report is produced when requested. International cord blood units are listed on the BMDW website and a search report of these will be provided by the Anthony Nolan. Additional information on UCB units stored within cord blood banks in the USA can be obtained via searching the NMDP.

A shortlist of up to ten cords should be produced for each patient. These cords are selected based on the following information available on the search reports:

1 TNC: ensure cords shortlisted meet the minimum threshold required for a single (3×10^7 kg^{-1}) and for a double UCB transplant ($2 \times 1.5 \times 10^7$ kg^{-1}) as defined in the local transplant protocol.
2 HLA match: high-resolution matching for HLA-A, -B, -C and -DRB1 at a minimum should be considered and aim to select UCB with ≤2 allele mismatches where possible.
3 CD34 cell count: indication of viability of cord.
4 Cord blood bank accreditation.

For each of the UCB shortlisted, a request should be made to the cord blood bank for a detailed unit report. This will give additional information including viability testing performed; method for volume reduction and volume cryopreserved; and age of cord etc.

Confirmatory HLA typing of UCB units should be performed according to FACT/NETCORD and EFI standards.

Within the UK, the Cord Blood Unit Selection Advisory Panel has been established under the auspices of the British Society of Blood and Marrow transplantation (BSBMT) and BSHI. The role of this panel is to provide an independent advisory service for transplant physicians, H&I scientists and referring haematologists in the final selection of UCB units for transplantation. Guidance for using this service is available at the BSHI and BSBMT websites: http://www.bshi.org.uk and http://bsbmt.org.

Recommendations

1 UCB units should be HLA typed to high-resolution HLA-A, -B, -C, -DRB1, -DQB1 (Grade 1B).
2 Selection of UCB units should follow national consensus guidelines published by Hough *et al.* (2016) (Grade 1A).

HLA alloantibodies

Until recently, the impact of HLA antibodies on HPC engraftment has been unclear. Opinion was formed from contradictory case study reports in the literature with few cases available for analysis because of the matching criteria inherent in HLA-matched related and unrelated donor transplants. The use of HLA-mismatched cord blood and related haploidentical donors has led to more transplants being performed where the patient has antibodies directed against HLA specificities present in the donor (donor-specific antibodies, DSA). Recent studies indicate that DSA in the recipient is a significant risk factor for transplant nonengraftment (Ciurea *et al.*, 2011; Taniguchi *et al.*, 2012).

In a Japanese study of 374 cord blood transplants, 16.4% (41/250) of patients aged between 16 and 74 years transplanted for malignancies had HLA antibodies (Takanashi *et al.*, 2010). Of those patients, eight had antibodies against HLA antigens present in the transplanted cord blood. Engraftment for patients with HLA antibodies but not against antigens present in the transplanted cord blood unit was 93.6% with a median time to engraftment of 21 days. However, when the HLA antibody was directed at donor antigen, engraftment fell to 58% ($P = 0.017$) with a median time to engraftment of 46 days.

A National Marrow Donor Program study looking at failed unrelated adult donor HPC transplants found that the presence of recipient HLA antibodies reactive to donor HLA antigens was associated with an increased risk of nonengraftment (OR 22.8, $P = 0.0002$) (Spellman *et al.*, 2010).

A further American study of 73 double cord blood transplants revealed that 18 of the patients had donor-specific antibodies (Cutler *et al.*, 2011). Nine patients had DSA directed at the first infused CBU, and two had DSA against the second infused CBU. Seven patients had DSA against both cord units infused, and four patients had multiple DSA antibodies also reactive with both cords infused. The study links important clinical consequences to DSA.

The clinical-associated complications were as follows:

- An increased incidence of graft failure (5.5% vs. 18.2% vs. 57.1% for none, single or dual DSA positivity, $P = 0.0001$)
- prolongation of the time to neutrophil engraftment (21 vs. 29 days for none vs. any DSA, $P = 0.04$)
- excess 100-day mortality or relapse (23.6% vs. 36.4% vs. 71.4% for none, single or dual DSA positivity, $P = 0.01$)
- The intensity of DSA reactivity was correlated with graft failure (median of mean fluorescent intensity (MFI) 17 650 vs. 1850, $P = 0.039$)

Similar results emerged from a Eurocord registry analysis of UCB transplants (60% double cord) performed after RIC regimen (Ruggeri *et al.*, 2013). Of 294 patients, 62 patients (21%) had detectable HLA alloantibodies and 14 with DSA (seven double cord recipients and seven single cord recipients). Cumulative incidence of day 60 neutrophil engraftment was reduced in those patients with DSA compared with patients without DSA (44% vs. 77%, $P = 0.003$). In multivariate analysis, the pretransplant presence of DSA was the only factor that independently associated with neutrophil recovery (HR 1.69, 95%CI: 1.2–12.6; $P = 0.002$). There was also an association with the median MFI values, with those patients engrafting having DSA with lower MFI values compared with patients that did not engraft. Graft failure was associated with a higher risk of TRM and lower OS.

These studies indicate that in HLA-mismatched HPC transplants, HLA-DSA in the recipient should be considered as a significant risk factor for nonengraftment and that HLA antibody screening of patients prior to and following transplantation may be a useful tool to follow-up the outcome of these transplants.

Recommendations

1 HLA alloantibody testing of the recipient should be performed at the time of donor search and should be repeated at the time of donor work-up request if an HLA-mismatched donor is selected (Grade 1A).
2 The clinical team must be made aware of any HLA alloantibody incompatibility detected in the recipient (Grade 1A).
3 When a choice of equally well-matched donors is available, avoid selection of donors against which the patient has HLA alloantibodies (Grade 1A).
4 HLA alloantibody testing should be performed in cases of failed engraftment if the donor is HLA mismatched (Grade 1B).

Non-HLA factors to be considered for related and unrelated donor selection

Cytomegalovirus

Cytomegalovirus infection can cause significant complications post-transplantation. CMV disease affects different organs including, lung (pneumonia), liver (hepatitis), gut (gastroenteritis), eye (retinitis) and the brain (encephalitis). Even with recent improvements in antiviral prophylactic therapies, CMV seropositivity remains associated with an adverse prognosis and is still a major cause of morbidity and mortality in allogeneic SCT (reviewed in Ljungman, 2014a). CMV positivity can be transmitted from a positive donor to a negative patient and patients who are CMV positive pretransplant are susceptible to CMV reactivation post-transplant.

A large study undertaken by EBMT of 16 628 allotransplanted acute leukaemia patients (Schmidt-Hieber *et al.*, 2013) reported that donor or recipient CMV seropositivity (versus donor and recipient CMV seronegativity) was associated with a significant decrease in leukaemia-free survival and overall survival with an increase in NRM and a small increase in relapse incidence. The negative impact on disease-free survival (DFS) and OS held up in multivariate analyses. OS was reduced in ALL (46% for CMV seropositive donor and/or patient vs. 55% for CMV seronegative donor and patient) compared with AML (52% for CMV seropositive donor or patient vs. 56% for CMV seronegative donor and patient). This was despite the use of strategies for prophylaxis, monitoring and pre-emptive treatment of CMV. There was no effect of CMV status on acute and chronic GVHD.

The negative effect of CMV seronegative patients receiving CMV seropositive donations from unrelated donors was confirmed in a second EBMT study of transplants performed in 20 193 CMV seronegative patients. However, no effect was seen in patients receiving transplants from related donors (Ljungman *et al.*, 2014b).

Two smaller studies of patients transplanted for B-cell lymphoma (Mariotti *et al.*, 2014) and myeloma (El-Cheikh *et al.*, 2013) did not identify any impact of CMV serostatus on outcome.

The impact of multiple donor factors including CMV on survival was investigated in a multicentre study of 1271 UK patients transplanted with an unrelated donor for malignant disease (Shaw *et al.*, 2014). Patients who were CMV seropositive at the time of transplant had a reduced median survival (1.7 years) compared with CMV seronegative patients (2.5 years, $P = 0.013$). The donor and patient CMV matching

status had a significant effect with median survivals of 2.8, 2.2, 1.5 and 1.1 years in the categories of neg/neg, pos/pos, neg/pos and pos/neg ($P = 0.001$). A relationship between HLA matching and CMV status on outcome was not observed for CMV seronegative patients regardless of whether the donor was CMV matched or mismatched ($P = 0.061$), either in the 10/10 ($P = 0.61$) or 9/10 matched setting ($P = 0.13$). However, there was a difference in median survival for CMV seropositive patients with patients receiving a 10/10 HLA-matched CMV seronegative donor having a median survival of 1.8 years compared with 2.4 years for patients receiving a 10/10 HLA-matched CMV seropositive donor ($P = 0.23$). When the donor was a 9/10 HLA match, the median survival for seropositive patients was 0.7 years with a CMV seronegative donor compared with 2.2 years with a CMV seropositive donor ($P = 0.004$). The difference in survival was attributed to an increase in GVHD and the morbidity and mortality associated with recurrent CMV reactivations. This study supports better outcomes are achieved when donor and patient are matched for CMV status.

CMV seropositive patients receiving transplants from CMV seropositive donors have been reported to have better survival and reduced TRM compared with seropositive patients receiving seronegative donations (Ljungman *et al.*, 2003). The study of Schmidt-Hieber *et al.* (2013), also demonstrated that CMV seropositive recipients of a CMV seropositive graft had a significantly ($P = 0.04$) better OS compared with CMV seropositive recipients of a CMV seronegative graft, with no difference in LFS observed ($P = 0.24$).

Analysis of 29 349 CMV seropositive transplant recipients identified within the EBMT registry found no effect of donor CMV status on outcome for transplants performed with a matched or mismatched family donor (Ljungman *et al.*, 2014b). An effect was observed for CMV seropositive patients receiving transplants from unrelated donors with lower relapse mortality observed albeit at borderline significance (HR 0.94; $P = 0.05$) when a CMV seropositive donor was used. For patients receiving myeloablative conditioning better overall survival; lower NRM and improved relapse-free survival outcomes were observed when the donors were CMV seropositive. Similar effects were not observed for patients transplanted with RIC. The retention of host CMV-specific T-cell function in patients receiving RIC is an explanation for the lack of an association observed (Ljungman *et al.*, 2014b).

The British Committee for Standards in Haematology, the British Society of Blood and Marrow Transplantation and the UK Virology Network guideline for Management of cytomegalovirus infection in haematopoietic stem cell transplantation recommend CMV matching between patient and donor (Emery *et al.*, 2013).

ABO blood group incompatibility

ABO incompatibility (ABOi) between patient and donor is a common feature of HPC transplantation. The ABOi can be major, minor or bidirectional (Table 1). Major ABOi transplants, in particular for blood group O patients, can cause delayed red cell engraftment, and infrequently pure red cell aplasia (PRCA). Reduced-toxicity regimes such as low intensity conditioning and graft-versus-host prophylactics are associated with extended host isohaemagglutinin production and PRCA (Bolan *et al.*, 2001).

Major and minor ABO incompatibilities do not have a significant effect on overall survival and incidence of GVHD (reviewed in Booth *et al.*, 2013) and do not constitute a major contraindication to donor selection. However, there are several single-centre studies that indicate ABOi having an impact on clinical outcome.

Recipients of major and minor ABOi RIC transplants are dependent on red blood cell transfusions for longer compared to ABO compatible RIC transplants (Watz *et al.*, 2014). Patients receiving major ABOi RIC transplants who then developed persistent recipient type ABO (PRABO) antibodies had a poorer overall survival (17% vs. 73%, $P = 0.002$) and transplant-related mortality (50% vs. 21%, $P = 0.03$) compared to patients receiving major ABO incompatible RIC transplants without PRABO (Watz *et al.*, 2014).

An investigation into red cell aplasia, for transplants performed between 2007 and 2008, identified 27% of patients received major ABOi transplants and 7.5% of these major ABOi transplanted patients developed PRCA (Aung *et al.*, 2013). Chimerism studies for T-cell and myeloid cell lineage and time to engraftment for neutrophil and platelets did not differ significantly for the major ABOi patients that did, and did not develop PRCA. All patients with PRCA required red cell transfusion support for several months and suffered from significant iron overload. These complications can be reduced where major ABO mismatches are avoided in donor selection.

Therefore, in selection of HPC donors, avoidance of major ABOi is preferred, but in the absence of a minor ABOi or ABO compatible donor, major ABO incompatibilities can be selected. When a blood group O

Table 1. Types of donor–recipient ABO incompatibilities

	ABO blood type	
Mismatch type	Recipient	Donor
Major	O	A, B, AB
Major	A	AB
Major	B	AB
Minor	A	O
Minor	B	O
Minor	AB	O, A, B
Bidirectional	A	B
Bidirectional	B	A

patient is being considered for ABOi transplant, it is useful to determine the patient's anti-A and anti-B titres as this may help the selection when there is a choice of ABOi donors.

Donor sex

Using a male donor has been reported in some studies as having a positive effect on long term survival regardless of the sex of the recipient (Gustafsson *et al.*, 2004; Pond *et al.*, 2006) but not in others (Lee *et al.*, 2007). Regardless, donor sex selection priority is usually given to male donors due of their usually larger size associated with higher HPC counts obtained and also because of the increase in GVHD that has been reported with female multiparous donors (Kollman *et al.*, 2001). A recent German study of 2646 transplants performed in patients with haematological malignancies found that transplants performed with international donors had a worse outcome compared to transplants with national donors and male patients transplanted with female international donors showed an even higher hazard ratio in analysis of OS (HR 1.51, CI 1.18–1.92, $P < 0.001$) than other sex-matched groups (Fürst *et al.*, 2013).

In contrast, a multicentre analysis of the effect of donor characteristics on the outcome of 709 RIC transplants (Passweg *et al.*, 2011) demonstrated no association between donor age, parity and sex matching with transplant outcome, with only HLA matching being predictive for survival.

An investigation into the impact of cord blood donor sex compatibility has demonstrated no impact on survival in adults with haematological malignancies receiving a myeloablative single unit cord blood transplant. However, a higher incidence of chronic GVHD (HR 2.97, $P = 0.02$) was observed in male recipients of female cord blood donors and a lower incidence of platelet engraftment (HR 0.56, $P = 0.02$) in female recipients with male cord blood donors (Konuma *et al.*, 2014). These findings require confirmation in further studies.

Donor age

In a 2001 NMDP study of 6978 unrelated donor transplants performed from 1987 to 1999, recipients receiving transplants from younger aged donors were associated with lower levels of aGvHD III-IV (30% 18–30 years; 34% 31–45 years and 34% >45 years, $P = 0.005$) and cGvHD (44% 18–30 years; 48% 31–45 years and 49% >45 years, $P = 0.02$) and improved 5-year overall survival (33% 18–30 years; 29% 31–45 years and 25% >45 years, $P = 0.0002$) (Kollman *et al.*, 2001). The transplants included in this study were not all matched for high-resolution HLA typing and included both HLA-matched and HLA-mismatched donors. However another large NMDP study of 3857 transplants where high-resolution matching was included was unable to confirm this donor age effect (Lee *et al.*, 2007).

A recent study of donor age in both matched sibling transplants and high-resolution matched unrelated transplants found no significant difference in overall survival in recipients of transplants from matched unrelated donors in three age groups: 19–29 years ($n = 254$) vs. 30–39 years ($n = 306$) vs. 40–49 years ($n = 194$); $P = 0.5$ (Alouis *et al.*, 2013). This study only included patients over the age of 50 which may have impacted the results obtained.

A CIBMTR study (GS08-01) of 6349 transplants examining the effect of donor characteristics on GVHD and survival following unrelated donor HPC transplantation identified: donor age, high-resolution HLA matching and blood group matching associated with overall survival. Specifically patients transplanted from older donors had lower overall survival at 5 years compared with younger donors: Donors aged 33–50 years (HR 1.13, $P = 0.0004$) and >50 years (HR 1.29, $P < 0.0001$) compared with donors aged 18–32 years (Kollman *et al.*, 2013).

NK cell receptors

NK cells participate in the defence against infection, malignancy and allo-antigens. These reactions are mediated by receptors on the NK cells and ligands on the target cells including killer-cell immunoglobulin-like receptors (KIR) and HLA, respectively. Mismatching for HLA proteins that interact with different KIR such as HLA-C can initiate GVH NK cell mediated alloreactions. Post-transplantation, NK cells will be generated with the KIR repertoire of the donor. Included within this NK cell population will exist alloreactive NK cells defined by their killing ability not being inhibited by any mismatched HLA proteins expressed by host cells including dendritic cells, T cells and leukaemic cells resulting in a reduction in GVHD, prevention of graft rejection and destruction of residual leukaemic cells, respectively.

The latter graft-versus-leukaemia (GVL) effect could be enhanced if donors are selected to encourage NK cell mediated alloreactivity. This was first demonstrated in the study of Ruggeri *et al.* (2002) who demonstrated improved survival attributed to decreased relapse, GVHD and rejection in AML patients receiving KIR ligand (HLA-C) mismatched transplants. The mechanism for this effect was attributed to the donor possessing allo-reactive NK cells that were not inhibited by the patient's mismatched HLA-C allele. However, the results observed in this study have not been extensively reproduced with some studies reporting worse overall survival (Leung, 2011). This is attributed to heterogeneity in the patient and donor demographics; the diagnoses, with AML recognized as being the main disease affected by NK cell alloreactivity; and also the T-cell content of the transplanted graft. Additionally, analysis of the KIR ligand

mismatch alone does not account for the variability of KIR proteins caused by differences in gene content and polymorphisms affecting expression and functionality.

KIR genes are located on chromosome 19, and as such are inherited independently of HLA genes. Two groups of KIR haplotypes have been described. Haplotype 'KIR A' possesses one activating gene whereas haplotype 'KIR B' possesses multiple activating genes and as such KIR B is considered to effect greater alloreactivity than KIR A. Analysis of the impact of KIR haplotypes on transplant outcome has shown that transplants (T-replete) with donors possessing at least one KIR B haplotype had a 3-year overall survival that was significantly higher that transplants performed with donors that possessed two KIR A haplotypes (31% vs. 20%, $P = 0.007$) (Cooley *et al.*, 2009). Further studies have associated the presence of KIR B in the donor with relapse protection and improved survival for patients with AML but not ALL, with homozygosity for KIR B haplotype centromeric motifs having the strongest independent effect (Cooley *et al.*, 2010).

Further study (Venstrom *et al.*, 2012) of a CIBMTR cohort of patients with both AML and ALL demonstrated decreased rate of relapse for AML but not ALL patients when the donor possessed the KIR B haplotype gene *KIR2DS1* in association with the donor possessing one or two HLA-C allotypes with the C1 epitope (not ligands for *KIR2DS1*) compared to donors that were HLA-C homozygous for the C2 epitope (ligands for *KIR2DS1*) or negative for KIR2DS1. Mismatching for HLA-C in the absence of donor KIR2DS1 did not impact on relapse. Individuals that are KIR2DS1 and HLA-C C2 epitope homozygotes have previously been shown to be hyporesponsive to target cells due to a tolerogenic effect of the interaction between KIR2DS1 and its self-ligand (Sun & Lanier, 2008; Tripathy *et al.*, 2008) supporting an alloreactive response mediated by NK cells expressing KIR2DS1 and HLA-C with the C1 epitope. The presence of HLA-C with C1 epitope within the patient also associated with reduced relapse when the donor expressed KIR2DS1. This was not observed when the patient was homozygous for HLA-C with C2 epitope (Venstrom *et al.*, 2012). Therefore, genotyping donors for KIR2DS1 are required to assess this effect on outcome.

The impact of KIR B haplotype-specific motifs, as defined by variable gene content at the centromeric and telomeric ends of KIR B haplotypes was investigated in a cohort of 1532 T-cell replete transplants for AML (Cooley *et al.*, 2014). 56% of the transplants were matched for HLA-A, -B, -C, -DRB1 and -DQB1 with the remainder having 1, 2, 3 or more mismatches. Confirming previous studies, transplants performed with donors possessing two or more KIR B motifs experienced an increase in LFS and protection from relapse, compared to transplants performed with donors possessing 0 or 1 KIRB motif.

Patients possessing one or two HLA-C allotypes with the C1 epitope were associated with improved LFS attributed to a reduction in relapse when the donor possesses 2 or more KIR B motifs, but not when the donor possessed 1 or 0 B motifs. This effect was most significant for the HLA-C-mismatched transplants with enhanced LFS (RR 0.57 [0.40–0.79], $P = 0.001$) and reduced relapse (RR 0.54 [0.33–0.88], $P = 0.013$). Comparison of the HLA-C mismatches, within this subset of transplants, did not show a benefit for KIR ligand (C1 and C2 epitope) mismatching. These data support a benefit of HLA-C mismatching, in terms of improved LFS due to a reduction in relapse, when the donor possesses 2 or more KIR B motifs and the patient possesses at least one HLA-C allotype with the C1 epitope. In contrast, no significant associations with donor KIR was observed for the Bw4 and C2 epitopes when present in the patient or the Bw4, C1 and C2 epitopes present in the donor.

Although the data supporting donor KIR B haplotype and/or B motif together with patient KIR ligand HLA-C epitope having an impact on transplant outcome are impressive, there currently is no large scale study described with patients transplanted in a T-cell deplete protocol receiving predominantly PBSC donations to verify the data described that have been procured from predominantly north American cohorts with T-cell replete protocols and bone marrow donations.

Recommendations

1 The guideline published by Emery *et al.* (2013) recommending CMV matching between patient and donor should be followed (Grade 1A).
2 Major ABO incompatibilities should be avoided when there is a choice of HLA- and CMV-matched donors (Grade 1A).
3 Male donors should be preferentially chosen where the patient has multiple HLA-, CMV- and ABO-matched donors (Grade 1C).
4 Younger donors should be preferentially selected (Grade 1B).

Tumour-specific mutations

The improvement and wide scale application of high-resolution HLA typing methods has led to a significant increase in the number of mutations identified when HLA typing patients using DNA extracted from peripheral blood. These mutations can be attributed to a novel HLA allele expressed in all tissues or could be specific to the patient's tumour (Mrazek *et al.*, 2014). If discovered, effort must be made via HLA typing of relatives and HLA typing of DNA extracted from patient tissue not affected by disease (e.g. skin plug) to determine whether the allele is novel or tumour specific. Only novel alleles will be assigned an official HLA allele name (Marsh *et al.*, 2010; Robinson *et al.*, 2015).

The expression of HLA proteins can be reduced within tumours due to deletion or mutations within genes encoding HLA proteins. Loss of heterozygosity at HLA loci can also occur. Care must be taken when patients are HLA typed from DNA extracted from peripheral blood with a high frequency of tumour cells in circulation. Homozygosity at HLA loci must be confirmed via family studies or repeat HLA testing when the patient is in remission or by testing DNA extracted from a nondiseased cells such as buccal swab or skin plug.

Recommendation

Homozygosity and novel HLA alleles identified within DNA extracted from patients with a high frequency of circulating tumour cells should be confirmed by family studies or using DNA extracted from nondiseased cells (Grade 2A).

Graft identification advisory service

The provision of a professional Graft identification advisory service (GIAS) to a transplant centre requires trained staff able to undertake both straightforward and complex donor selection. GIAS may be delivered from an H&I laboratory supporting the transplant centre, from a donor registry or from within the transplant team. Key to the successful selection of optimum donors from related and unrelated sources is expert knowledge of the HLA system including: polymorphism, linkage disequilibrium, impact of recombination, ethnic variation and HLA serology. A GIAS service must be directed by a consultant grade RCPath Fellow trained in H&I, with adequate cover provide during absences. This may be achieved via network arrangements with other organizations.

H&I scientists who have completed the British Society for Histocompatibility and Immunogenetics (BSHI) diploma and are Health and Care Professions Council (HCPC) registered will have achieved an adequate level of education to enable active participation within a GIAS structure. Other 'non-H&I laboratory' training pathways such as that provided by the WMDA should be documented for members of a GIAS team.

Regular interactions between the GIAS and transplant teams must take place via multidisciplinary team meetings, to ensure the needs of individual patients are being delivered according to local, national and international transplant protocols.

All GIAS team members must participate in continual professional development (CPD) to ensure maintenance of current knowledge and to be aware of future developments.

Recommendation

Individuals actively involved in the provision of a donor selection service should undertake CPD and the service should be directed by a RCPath Fellow and Consultant in H&I (Grade 1A).

Useful websites

Guidance and tools to assist in donor selection and allele frequencies are freely available at the following sites:

NMDP bioinformatics resources https://bioinformatics.bethematchclinical.org/ include tools such as haplostats for aiding haplotype prediction and an on line tool for deciphering NMDP codes. Tables with haplotype frequencies in different ethnic groups are also available.

Bone Marrow Donors Worldwide, http://bmdw.org, is the continuing effort to collect the HLA phenotypes and other relevant data of volunteer stem cell donors and cord blood units and is responsible for the coordination of their worldwide distribution.

HLA Nomenclature information: http://hla.alleles.org, houses up-to-date HLA nomenclature including easy to use tables of alleles and lists of alleles in G and P groups.

IPD-IMGT/HLA Database permits access to HLA DNA and protein sequences, http://www.ebi.ac.uk/ipd/imgt/hla/ and includes the official sequences for the WHO Nomenclature Committee for Factors of the HLA System. The IPD-IMGT/HLA Database is part of the Immuno Polymorphism Database (IPD).

Allele Frequencies including information on frequency and population distribution of HLA alleles http://www.allelefrequencies.net (Gonzalez-Galarza *et al.*, 2015)

Disclosures

This study did not involve human subjects or animals, and therefore, no ethical approval was required. The study was funded by the authors own departmental resources. The authors declare that they have no conflict of interests.

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