Histocompatibility assessment in hematopoietic stem cell transplantation: recommendations from the Italian Association of Immunogenetics and Biology of Transplantation (Associazione Italiana di Immunogenetica e Biologia dei Trapianti - AIBT)

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The outcome of allogeneic hematopoietic stem cell transplantation (HSCT) is significantly influenced by the degree of HLA histocompatibility between donor and recipient. To provide shared indications for required histocompatibility testing and interpretation before HSCT, the Italian Society for Immunogenetics and Transplantation Biology (*Associazione Italiana di Immunogenetica e Biologia dei Trapianti* [AIBT]) gathered members and created a working group to discuss and develop recommendations for histocompatibility assessment in HSCT.

After a review of the literature and multiple panel discussions, AIBT developed up-to-date recommendations for the resolution levels of HLA typing, histocompatibility definitions of patients and donors, importance of anti-HLA antibodies, and significance of NK alloreactivity, which are reported in this document. These recommendations have been shared with the Italian Group for Bone Marrow Transplantation (*Gruppo Italiano per il Trapianto di Midollo Osseo, cellule staminali emopoietiche e terapia cellulare* [GITMO]) and the Italian National Center for Transplantation (*Centro Nazionale Trapianti* [CNT]). Notably, the increased use of HLA-mismatched transplantation (i.e., mismatched unrelated, haploidentical) in recent years has made these indications even more relevant for the standardization and improvement of quality of care.

This document represents a useful instrument for health care workers involved in the field of HSCT, enhancing synergy with transplant physicians and enabling greater optimization of the available resources.

Keywords: *HLA, histocompatibility, stem cell transplantation, anti-HLA antibodies, natural killer.*

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INTRODUCTION

In recent years, the growth of indications for hematopoietic stem cell transplantation (HSCT), increasing availability of donors from international registries, and continuous therapeutic advances have made HSCT an established therapeutic option for a growing number of patients, despite the complexity of the procedure and the risk of severe

post-transplant complications. The outcome of allogeneic HSCT is significantly influenced by the degree of HLA histocompatibility between donor and recipient. Indeed, antigenic and/or allelic differences (mismatches) at the second and third exon of HLA class I genes and the second exon for HLA class II genes are significantly associated with reduced overall survival (OS) after both myeloablative and reduced-intensity regimens[1-5]. Recent data indicate that the transplantation outcome may also be negatively affected by anti-HLA-specific antibodies in the recipient that are directed against the donor's HLA epitopes (donor-specific antibodies, DSA)[6], particularly in transplantation from a haploidentical donor[7,8]. New evidence is emerging on additional factors that may influence transplant outcome, such as DPB1 matching[9-12] and KIR gene analysis in unrelated and haploidentical donor transplants[13-15]. At the 88 transplantation centers in Italy at the time of writing[16], approximately 2,000 allogeneic HSCTs are performed per year (1,911 in 2020, the Italian Group for Bone Marrow Transplantation (*Gruppo Italiano per il Trapianto di Midollo Osseo, cellule staminali emopoietiche e terapia cellulare* [GITMO] data), with an average annual increase of 5-10%. Unlike other countries where immunogenetics and histocompatibility activities are centralized, Italy has several histocompatibility laboratories. As a result, significant differences in the volume of services and resources may occur, with subsequent heterogeneity in care processes and outcomes. In Italy, accreditation programs developed by European Federation for Immunogenetics (EFI) and the American Society for Histocompatibility and Immunogenetics (ASHI) are mandatory to fulfill minimum quality standards for histocompatibility laboratories involved in transplantation activities. However, new scientific evidence supports the need for unambiguous, shared indications and well-defined HLA compatibility standards in different types of HSCT.

Based on international guidelines and the most recent HSCT literature, the "Italian Society for Immunogenetics and Transplantation Biology (*Associazione Italiana di Immunogenetica e Biologia dei Trapianti* [AIBT]) Working Group on HSCT" has drawn up these recommendations in the hope of providing a useful tool to improve the quality of care, enhancing synergy with transplant physicians and enabling optimization of the available resources.

METHODOLOGY AND DEGREES OF RECOMMENDATIONS

Eight AIBT members (M.A., R.C., M.F., C.F., L.G., F.P., G.R., C.V.) joined this "AIBT working group" on a voluntary basis and performed a narrative review of the literature focused on the following topics, divided into chapters: 1) terms and levels of resolution in HLA typing; 2) first, extended and verification typing, anti-HLA antibody testing and Natural Killer (NK)-mediated alloreactivity; 3) role of anti-HLA antibodies; and 4) KIR gene repertoire analysis.

Data from the literature were retrieved using PubMed, Cochrane and EMBASE. Assignment of the degree of recommendations was made according to the following scale:

Strength of recommendation:

- Level **1**, high clinical impact. Recommended, *"Must"*.
- Level **2**, low clinical impact. Suggested, "*Should*".

Degree of evidence:

- Grade **A**: consensus in the literature or the standards or guidelines of reference institutions or scientific societies (EFI, IBMDR, EBMT, NMDP, BSHI, etc.).
- Grade **B**: indication from extensive data in the literature.
- Grade **C**: opinion based on AIBT Working Group expertise.

Individual opinions, based on literature review and personal experience, were discussed, and the text was modified until a consensus was reached. The standards or guidelines of reference institutions and scientific societies (see above) represented a starting common ground, and all final recommendations were made by consensus.

Once finalized, the document was submitted to the GITMO board and to the Italian National Center for Transplantation (*Centro Nazionale Trapianti* [CNT]) for approval. Possible corrections or minor modifications proposed by either GITMO and CNT were introduced upon re-approval by AIBT working group members. The final version has been approved by all the co-Authors.

SCOPE OF APPLICATION

These recommendations are intended for transplant programs performing the following types of HSCT:

- Transplantation from an HLA-identical or single-locus mismatched family donor.
- Transplantation from an HLA-haploidentical family donor.

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• Transplantation from an unrelated donor.

These activities *must* be performed by internationally accredited laboratories (EFI, ASHI) and by personnel fulfilling the requirements of the accreditation bodies' standards **(1A)**.

TERMS AND LEVELS OF RESOLUTION IN HLA TYPING

HLA alleles can be identified at various degrees of resolution depending on the typing methodologies used. To standardize communication between laboratories, clinicians, and registries, it is necessary to use shared typing level definitions as reported by the Harmonization of Histocompatibility Typing Terms Working Group[17].

Allelic resolution

The typing result is a single allele among those identified in the most recent version of the IPD-IMGT/HLA database[18].

High resolution

The typing result is a set of alleles coding for the same amino acid sequence in the region of the molecule that corresponds to the Antigen Recognition Domain (ARD, includes the second and third exon for HLA class I molecules and the second exon for HLA class II molecules), excluding alleles that are not expressed (Null) or have doubtful expression (Q). World Health Organization (WHO) nomenclature can be used for P-group alleles.

Low resolution

The typing result is the characterization of HLA antigens or a group of HLA antigens and is limited to the first field (two digits) of the HLA DNA-based nomenclature.

Other resolution levels

If high-resolution typing is not feasible in the laboratory, the results can be reported at intermediate resolution, such as typing considering only Common/Well Defined alleles or the designation of alleles in G-groups. Despite their ambiguity with Null alleles (i.e., A*01:01:01G) or Q, G-groups are generally preferred over NMDP codes since the former allows for a more straightforward subsequent designation of the high-resolution typing and are thus regularly used by all laboratories across the country.

Extended typing

This typing is performed to extend the results of existing typing to additional loci not yet typed or to a higher resolution level. If a different sample is used and includes verification of already typed loci, it can also be considered verification typing.

Verification typing

This typing is performed on a different sample to check the concordance with the results obtained with the first sample. Verification typing does not require the same level of resolution as the first typing, only concordance between the two.

FIRST, EXTENDED AND VERIFICATION TYPING, ANTI-HLA ANTIBODY TESTING AND NK-MEDIATED ALLOREACTIVITY

Transplantation from an HLA-identical or single-locus mismatched family donor

The search for a donor for a patient awaiting HSCT is generally performed first within the household. Among siblings, the probability of finding an identical donor is approximately 25 to 30%.

First typing

To assess the compatibility of a potential donor within the family group, typing of the HLA-A, -B, -C and -DRB1 loci *must* be performed at low resolution on the patient and on all available family members, including siblings, parents and children. Family members who are clinically unsuitable for donation, based on an anamnestic assessment performed by a hematologist at the time of the family study, may also be typed to correctly define haplotype segregation. In cases where parental haplotypes are unequivocally segregated, it is possible to identify a genotypically identical HLA donor, even without performing extended typing[17]. The correct segregation of parental haplotypes is also relevant for the selection of a potential haploidentical donor (**1A**).

In families where segregation of parental haplotypes is not possible or not unique (i.e., in cases where parents share a haplotype or one parent is homozygous at one or more loci), the apparent HLA-identical donor is defined as phenotypically identical.

The chance of identifying a genotypically or phenotypically HLA-compatible donor within the household is relatively low, and it may be necessary to rapidly refer the patient to a transplant from an unrelated (matched unrelated donor, MUD) or haploidentical donor. Thus, at this stage, it is *recommended* to perform genomic typing for the HLA-A,

-B, -C, -DRB1, -DQB1 and -DPB1 loci at high resolution on the candidate, with optional typing for the HLA-DRB3/4/5, -DQA1 and -DPA1 loci[19] (**1C**).

Any cases of homozygosity detected when typing the blood samples of patients with a high concentration of circulating neoplastic cells *must* be confirmed by family studies or by repetition of the typing, possibly by another method and/or on biological samples with a reduced number of neoplastic cells (i.e., salivary swabs or peripheral blood samples after disease remission) (**1A**).

Particular attention must be paid to patients who have already undergone HSCT, in whom the persistence of donor cells could yield erroneous results. In these cases, pre-transplant samples or tissue samples lacking leukocyte infiltration (i.e., salivary swabs) *should* be used **(2B)**.

Extended typing

Extended typing for genotypically identical HLA family donors

When a genotypically identical donor has been identified within the household based on the haplotype segregation, it is *suggested* to perform typing of the DPB1 locus on the donor-recipient pair to exclude possible genetic recombination events, which are relatively frequent due to the low linkage disequilibrium between the DPB1 locus and other class II regions[20] **(2B)**.

Clinically, mismatches at the DPB1 locus can be "permissive" or "non-permissive", a distinction based on the decreased or increased ability of specific DP allotypes to induce alloreactive responses by T lymphocytes[21]. Identifying a donor with DPB1 T-cell epitope (TCE) permissive mismatches is associated with significant improvement in the outcome of HSCT[9].

Extended typing for phenotypically identical HLA family donors

If haplotype segregation cannot be unambiguously identified within the household, high-resolution typing of the HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 loci (or at least those loci where segregation ambiguity is found) *must* be performed on both the phenotypically identical patient and donor. Typing of other loci can be performed if required by local transplant protocols (**1A**).

If one or more mismatches are detected in the donor-recipient pair, i.e., in case of crossing over, anti-HLA antibody screening *must* be performed on the recipient **(1C)**.

Verification typing

Prior to transplantation, HLA typing *must* be repeated on a second biological sample from both the patient and the identified donor with the purpose of checking the concordance with the results obtained with the first sample. In this case, it is sufficient to perform low-resolution typing, at least for the HLA-A, -B, and -DRB1 loci (**1A**).

Transplantation from HLA-haploidentical family donor

Alternative transplantation and graft-versus-host disease prophylaxis strategies have been developed over the years to address situations such as the absence of a genotypically or phenotypically identical HLA donor within the household or the lack of a suitable MUD from international registries (algorithms available for prognostic research are useful for this purpose[22]). Alternative solutions for HSCT include the use of cord blood or the possibility of selecting a partially compatible family donor. Among the patient's relatives (parent, child, sibling, cousin, etc.), a haploidentical donor sharing an HLA haplotype with the patient, can be identified, allowing the patient to access transplant therapy with satisfactory results for certain types of patients/diseases[8,23-25]. Unlike transplantation from a phenotypically identical HLA donor, transplantation from haploidentical and unrelated donors requires anti-HLA antibody testing and NK-mediated alloreactivity (see below).

First typing

The laboratory should already have the HLA data from the first typing performed on all members of the patient's family, as described in section "Transplantation from an HLA-identical or single-locus mismatched family donor". Additional tests required before haploidentical donor transplantation are described below.

Extended typing

If the study of familial segregation allows for the identification of a donor who shares an entire uniquely identified HLA haplotype, this donor is defined as genotypically haploidentical. In such a case, typing *must* be extended in the same manner as for the genotypically identical donor (section "Transplantation from an HLA-identical or single-locus mismatched family donor", subparagraph "Extended typing for genotypically identical HLA family donors") (**1A**).

In the absence of unequivocal haplotype segregation, the "haplo-identity" of the donor (phenotypically haploidentical donor) *must* be confirmed by

high-resolution typing of at least the shared haplotype for the HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 loci **(1C)**.

Verification typing

Prior to transplantation, HLA typing *must* be repeated on a second biological sample of both the patient and the identified donor with the purpose of checking the concordance with the results obtained with the first sample. In this case, it is sufficient to perform low-resolution typing, at least for the HLA-A, -B, and -DRB1 loci (**1A**).

Anti-HLA antibody testing

In haploidentical donor selection, due to the presence of multiple donor-recipient mismatches, anti-HLA antibody screening *must* be performed in the recipient to detect the presence of DSA, which are known to affect transplant outcome[6-8] (**1A**).

Performing anti-HLA antibody screening is *recommended* in the early stages of the donor search. This enables selection of the best available donor and allows for repeat analysis at the time of work-up to define the clinical risk and the possible need to apply desensitization protocols (**2B**).

A more detailed discussion with a list of recommendations for the detection of anti-HLA antibodies in HSCT can be found in chapter "Role of anti-HLA antibodies".

NK-mediated alloreactivity

Several studies have shown that the presence of an alloreactive NK population in the donor is associated with a better clinical course after haploidentical transplantation[26,27], as well as the presence of specific killer-cell immunoglobulin-like receptor (KIR) genotypes (B/X and B content value analysis)[28,29] and certain activating KIR genes[24,30]. These studies have led the European Society for Blood and Marrow Transplantation (EBMT) to include KIR gene analysis in their recommendations for haploidentical donor testing[8].

In cases where assessment of NK-mediated alloreactivity is required, the haploidentical recipient-donor pair *must* be evaluated at low resolution for the A locus and at high resolution for the HLA-B and -C loci **(1C)**. The donor's KIR gene pool study *should* also be performed **(2C)**.

Applications are available to detect the possibility of NK-mediated alloreactivity in the donor-recipient pair, such as the KIR ligand calculator software on the IPD-IMGT site[31].

An in-depth study of NK-mediated alloreactivity in HSCT is reported in chapter "KIR gene repertoire analysis".

Transplantation from unrelated donor

Unrelated donors are recruited through the national donor registries, which are collected by the World Marrow Donor Association (WMDA). The national registry in Italy is the Italian Bone Marrow Donor Registry (IBMDR), whose activity is managed by operating standards that are updated annually and adapted according to technological and scientific advances. These standards state that "the recruited adult donor is typed at the HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 loci by molecular biology methods with medium/high resolution - two fields - with the possibility of using the WHO nomenclature with the G/P and possible indication of rare ambiguities"[32], preferably by allelic resolution technologies (next generation sequencing, NGS), starting from a peripheral blood or salivary sample **(1A)**.

The probability of finding a compatible donor for the HLA-A, -B, -C, -DRB1, and -DQB1 loci (namely, a 10/10-matched donor) is currently ~80% for Caucasian individuals but decreases significantly for different ethnic groups[33]. In Italy, the chance of finding an 8/8-matched (loci A, B, C and DRB1) donor has improved over time from 54% in 2012 to 67% in 2017 and 72% in 2018[34].

First typing

The unrelated donor transplant candidate *must* be typed at high resolution for the HLA-A, -B, -C, -DRB1, and -DQB1 loci, with optional typing for the HLA-DRB3/4/5, -DQA1, -DPB1, and -DPA1 loci (**1A**).

This typing is referred to as "MUD search activation" for the patient.

The immunogenetic data obtained *must* be entered by the laboratory that performed the typing into IBMDR management software to activate the search **(1C)**.

Given the clinical relevance of potential anti-HLA antibodies in the patient, particularly those specifically directed against the donor (DSA), it is *recommended* to perform anti-HLA antibody screening at the beginning of the search and to repeat the analysis at the time of work-up **(1C)**.

Extended typing

Extended typing for adult unrelated donors

Following the identification of a potential donor in the international registries, IBMDR standards require that the histocompatibility reference laboratory of the transplant center (or Transplant Program, TP) receives a biological sample of the selected donor on which high-resolution genomic typing of the HLA-A, -B, -C,

-DRB1, -DQB1 and -DPB1 loci *must* be performed; typing of the HLA-DRB3/4/5, -DQA1 and -DPA1 loci is optional according to the local transplant protocol (**1A**).

As previously described, the weak linkage disequilibrium of the DPB1 locus implies that mismatches between patient and donor are frequent, even in matched pairs for all other loci (about 80%)[35]. Therefore, it is relevant to the transplantation outcome to identify permissive and non-permissive TCE mismatches; for this purpose, online tools are available to predict the effects of any mismatch(es) at the DPB1 locus based on updated algorithms, such as the DPB1 TCE algorithms[36] (**2A**).

Other online algorithms that estimate the risk of developing alloreactive responses related to specific HLA mismatches are also available (PIRCHE [Berlin, Germany], HLA-Matchmaker).

In addition to the number of HLA mismatches, the directionality detected in the donor-recipient pair may influence the outcome of HSCT. Interestingly, host-versus-graft (HvG) mismatches appear to have a better prognosis than graft-versus-host (GvH) and bidirectional mismatches[37].

The description of mismatches and their directionality in the typing report is therefore *suggested* (**2B**).

Extended typing for umbilical cord units

Umbilical cord HSCT is an established therapeutic option for both pediatric and adult patients. Independent factors that significantly influence the outcome of transplantation are the dose of cryopreserved cells per unit (total nucleated cells: 3.0×10^7/kg or CD34+ 1.5×10^5/kg) and the degree of HLA matching, which is why the current selection of umbilical cord units for transplantation involves high-resolution typing of at least the HLA-A, HLA-B, HLA-C and HLA-DRB1 loci[38] (**1A**).

In the literature, the influence of sharing the Non Inherited Maternal Antigens (NIMAs) and Inherited Paternal Antigens (IPAs), already known from organ transplants[39], is particularly evident in the case of cord blood transplantation[40,41] (**2C**).

Verification typing

The same guidelines in paragraphs "Verification typing" apply to transplantation from adult unrelated donors, considering the first typing of the patient and donor genetic data have already been entered in the registry **(1A)**.

As for the other transplantation types, the patient's verification typing *must* be performed on a second sample to check the previous typing, at least for HLA-A and -B loci at low resolution to discern serological split equivalents and HLA-DRB1 at high resolution **(1A)**.

Concerning HSCT from umbilical cord units, once a candidate unit has been identified, verification typing *must* be performed on the attached segment prior to the conditioning regimen (**1C**). This typing will include high-resolution HLA-A, HLA-B, HLA-C and HLA-DRB1 loci as well as other loci, if required by the TP. If no attached segments are available, typing can be performed on a satellite (i.e., unattached) sample, in agreement with the TP. In this case, the typing *must* be repeated by the TP reference histocompatibility laboratory on a sample taken directly from the unit immediately after thawing, providing results as quickly as possible for at least the HLA-A, HLA-B and HLA-DRB1 loci at low resolution (**1A**).

Anti-HLA antibody testing

The same haploidentical transplantation setting (paragraph "Anti-HLA antibody testing") applies for unrelated donors.

NK-mediated alloreactivity

As stated in the current IBMDR operating standards at the time of writing, typing of KIR genes can also be requested by the TP during the unrelated donor selection process.

These indications need to be updated in connection with the annual review of the IBMDR standards. Indeed, certain categories of patients who receive transplantation from donors characterized by particular KIR genotypes (B/X) and a high B content value (≥2) have a better clinical course[42-44].

ROLE OF ANTI-HLA ANTIBODIES

Rejection is rare in HSCT from an HLA-identical, sibling or MUD donor (<3%). It is more frequent in transplantation from an alternative, haploidentical or cord donor (>10%) due to HLA mismatches and reduced immunological competence of the graft. Clinical studies indicate that DSA are the most important risk factor for rejection. Rejection clinically manifests as failed or delayed engraftment (graft failure) or impaired bone marrow function (poor graft function). Both conditions have a strong impact on transplant-related mortality and overall survival[45-48].

Anti-HLA antibodies, especially DSA, may be present in the HSCT candidate due to previous immunizations.

In addition, more than 40% of women with previous pregnancies present with DSA, often at a high titer, due to the synergistic effect of transfusions on pregnancy immunization[49].

When mismatches in the donor-recipient pair are present, screening for anti-HLA antibodies is recommended. This is particularly true in haploidentical transplantation characterized by the presence of several mismatches between donor and recipient due to the presence of a whole unshared HLA haplotype.

In a recent multicenter study, multivariate analysis revealed that independent risk factors for antibody formation in the recipient awaiting HSCT are, other than the number of pregnancies and transfusions, female gender and underlying disease (myelodysplastic syndrome)[50]. Therefore, in the case of haploidentical transplantation, it is relevant to perform screening for anti-HLA antibodies in the recipient to allow for the selection among multiple potential haploidentical donors and to repeat the screening at the time of work-up for a clinical risk definition and possible desensitization. The screening has to be extended to antibodies directed against all HLA class I and II specificities.

The reference technique for antibody detection is the Bead-Based Immunoassay (Luminex), which allows for the identification of individual HLA specificities and the breadth or spectrum of immunization (panel reactive antibody), possibly supplemented with other techniques (complement-dependent cytotoxicity crossmatch, CDC-XM; flow cytometry crossmatch, FC-XM; C1q/C3d). Anti-HLA antibody testing is usually performed in other types of transplantation from non-HLA identical donors (HSCT from umbilical cord, mismatched unrelated donor, M-MUD)[51] and especially in the early stages of donor research to provide the opportunity to change the donor if DSA is detected. Furthermore, if the recipient has antibodies against epitopes expressed by loci that have not been typed in the donor (i.e., DQA1, DPA1), typing of these loci would be appropriate to distinguish between DSA and non-DSA.

The following detailed recommendations refer to the EBMT guidelines for the detection and identification of HLA antibodies in haploidentical HSCT[52].

HLA antibody screening is *recommended* at the beginning of the search, when the probability of finding an HLA-identical donor is low (**1A**).

For a proper definition of DSA, it is *recommended* that donor typing is extended to all HLA loci (**1B**).

It is *recommended* to include the presence of DSA in the selection algorithm for a mismatched donor from a family or from a registry. In the latter case, IBMDR standards already include DSA screening for the MUD search (**1A**).

If several equally compatible and similarly eligible donors are present, it is *recommended* to exclude donors with DSA, especially if they present high titers (**1B**).

In HSCT from 10/10-matched MUD, it is *suggested* to exclude donors if high-titer anti-DP DSA are present (**2C).**

In 7/8- or 9/10-matched unrelated HSCT, donors should be excluded if DSA against the mismatched allele is present[51] (**2B**).

In cord blood transplantation, it is *recommended* to exclude units for patients with non-malignant diseases and high-titer DSA (**1A**).

For malignant diseases, since the evidence is more controversial, it is *suggested* to exclude donors if patients have high-titer DSA (or desensitize, see later)[49] (**2B**).

Patient candidates for haploidentical transplantation often present with high-titer DSA, and this is particularly true for female recipients against the non-inherited haplotype in the offspring ("child-to-mother" HSCT)[53]. For these patients, it is *recommended* to exclude donors if high-titer DSA are present, especially in the case of child-to-mother; alternatively, desensitization is suggested[8] (**1A**).

It is *recommended* to use the cytometric/Luminex technique for screening and identification of anti-HLA antibodies (**1A**).

It is *suggested* to use the Mean Fluorescence Intensity (MFI) value as a reference for defining the immunological risk of DSAs. An MFI value >1,000 is normally indicated as the positive cut-off (**2B**).

It is *suggested* to define the immunological risk *on a case-by-case basis* in relation to the immunological study and the type of transplant, the donor and the clinical urgency (**2B**).

The study from which the EBMT Guidelines are derived indicates a graft failure rate of 9% and 54% for MFI <5,000 and MFI >5,000, respectively[47]. It is therefore *suggested* to consider MFI >5,000 as an immunological risk cut-off, particularly for high-expressing alleles, whereas low MFI levels (<3,000) probably do not represent risk factors for transplantation[48] (**2B**).

For correct immunological risk stratification, it is *suggested*

to supplement the standard Luminex test with FC- or CDC-XM and functional (Luminex C1q/C3d) tests. Positive FC-XM and C1q/C3d results are risk factors (**2B**).

It is *recommended* to exclude donors if the CDC XM test is positive[54] (**1B**).

It is *recommended* to repeat the antibody study, especially if DSA are present, at the donor work-up to define the immunological risk and the indication for possible desensitization (**1B**).

In the presence of DSA, desensitization is *recommended* on a case-by-case basis, particularly for MFI >5,000 or positive FC-XM[54] (**1B**).

If CDC-XM is positive or the DSA Luminex MFI is >20,000, desensitization may not be successful[55].

DSA monitoring in desensitization (target MFI <5,000 or FCXM neg) is *recommended*[56] (**1B**).

It is *suggested* to continue DSA monitoring after transplantation, until complete engraftment[57] (**2B**).

KIR GENE REPERTOIRE ANALYSIS

The anti-leukemic activity pursued by donor NK cells is largely determined by the interaction between KIR receptors (expressed on NK cells) and HLA class I molecules (expressed on leukemic cells). The KIR locus comprises numerous genes (KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR3DL1/S1, KIR3DL2, KIR3DL3, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5) and two pseudogenes (KIR2DP1 and KIR3DP1) and is characterized by a high degree of both haplotype and allelic polymorphism. Consequently, the analysis of KIR genes is necessary because different individuals are often characterized by different KIR gene pools.

Several studies have suggested KIR gene analysis as an additional criterion in haploidentical donor selection. Various scientific evidence has prompted EBMT to include KIR gene analysis in its recommendations for haploidentical donor analysis[8]. However, it is important to remember that the disease heterogeneity (malignant hematological diseases or non-neoplastic diseases; acute lymphoblastic leukemia, ALL, or acute myeloid leukemia, AML; adult or child), disease status (complete or partial remission, CR or PR), differences in transplantation protocols (selection of hematopoietic system precursors, ex vivo depletion of T cells and B cells, in vivo depletion of alloreactive T cells), conditioning and post-transplant GvHD prophylaxis make it difficult to define an unambiguous algorithm for the study of KIR genes as part of haploidentical transplantation[58-60].

Several studies have shown that the presence of B/X KIR genotypes (especially if B content value is ≥2), an alloreactive NK population, or certain activating KIR genes in the donor are associated with a better clinical course for the patient[28,41,60-64].

Thus, if KIR gene characterization is required, the analysis of the KIR genotype, the B content score, and the presence/absence of an alloreactive NK population assessment are intended to be performed.

KIR genotype

KIR genes are inherited as haplotypes. Based on the presence/absence (P/A) of the different KIR genes, it is possible to define two different haplotypes termed "A" and "B". The A haplotypes have fixed gene content and include KIR3DL3, KIR2DL3, KIR2DP1, KIR2DL1, KIR3DP1, KIR2DL4, KIR3DL1, KIR2DS4 and KIR3DL2. By contrast, the B haplotypes are characterized by substantial gene content diversity and are positive for at least one of the following genes: KIR2DL2, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, and KIR3DS1. On the basis of the P/A of the various KIR genes, it is possible to assign the KIR genotype: A/A (i.e., genotypes characterized by two A haplotypes) or B/X (where X indicates the presence of either an A or a B haplotype)[65-67].

In different transplantation settings (MUD transplantation, HLA-matched or HLA-mismatched, and haploidentical transplantation), a better outcome was achieved in patients who received stem cells from donors with a B/X genotype[28,42,60].

B content

KIR haplotypes can be divided into two regions, termed centromeric (Cen) and telomeric (Tel), delimited by the presence of two framework genes and separated by a hot spot of recombination[65-67]. The main Cen regions are Cen-A, Cen-B1, and Cen-B2. In addition to the frame genes (namely, KIR3DL3 and KIR3DP1), the Cen-A regions include the KIR2DL3, KIR2DP1 and KIR2DL1 genes. The Cen-B regions are characterized by the presence of KIR2DS2 and KIR2DL2 and can be split into two subgroups. Cen-B1 regions include (in addition to the frame genes and KIR2DS2 and KIR2DL2) KIR2DL5B, KIR2DS3 (or KIR2DS5 in some donors from sub-Saharan Africa), KIR2DP1 and

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KIR2DL1. Cen-B2 regions are shorter, lacking KIR2DL5B, KIR2DS3 (or KIR 2DS5), KIR2DP1, and KIR2DL1. The main Tel regions are Tel-A and Tel-B. In addition to the framework genes (i.e., KIR2DL4 and KIR3DL2), the Tel-A regions include the KIR3DL1 and KIR2DS4 genes, while the Tel-B regions are characterized by the presence of KIR3DS1, KIR2DL5A, KIR2DS3 (or KIR2DS5), and KIR2DS1. Based on the analysis of the centromeric and telomeric regions, it is possible to assign the B content value, a number that ranges from 0 to 4 and is derived from the direct counting of the B-type regions present in the donor KIR genotype (**Table I**).

A B content value ≥2 has been described as a favorable factor in unrelated donor selection for adult AML patients and has been included in the selection criterion for the choice of haploidentical donors for pediatric patients with onco-hematological diseases[68,69]. The "Donor KIR B-content group calculator"[70] can be used for the analysis of the B-content value. In this case, the genotypes of the potential donors will be classified as "Neutral, Better and Best". As stated on the website, this analysis is performed upon request from the transplant physician in the case of MUD transplants and adult AML patients.

NK alloreactivity according to the KIR/KIR-L mismatch model in GvH direction

According to this model, the donor will be characterized by the presence of an alloreactive NK population in case there are NK cells expressing only an inhibitory KIR receptor capable of recognizing a "self" KIR ligand (enabling the education of the NK cell) that is absent in the recipient's genotype (enabling the elimination of residual leukemic cells, GvL effect)[71-73]. Therefore, the presence of NK alloreactivity can be predicted by analyzing the HLA Class I typing of both the donor and recipient and the KIR genotype of the donor.

Analysis of HLA class I/KIR ligand alleles

The free KIR ligand calculator tool[74] can be used to "convert" HLA class I alleles to KIR ligands (KIR-L). Notably, the results obtained can be integrated with information from some relevant publications. In particular, it should be noted that the program: i) does not detect the presence of the C1 epitope in the alleles HLA-B*46:01 and -B*73:01[75]; ii) classifies HLA-B*13:01 and -B*13:02 as ligands of KIR3DL1 (although it has been shown that these two alleles are not recognized by this KIR)[76]; and iii) does not take the HLA-A locus into account, thus it will be necessary to "manually" assign the presence of the Bw4 epitope if the donor and/or recipient are HLA-A*23, -A*24 or -A*32 positive[77,78]. **Table II** shows the main KIR inhibitors and their KIR-L.

Table I - *B content value*

	Cen-A/Cen-A	Cen-A/Cen-B	Cen-B/Cen-B
Tel-A/Tel-A	0	1	2
Tel-A/Tel-B	1	2	3
Tel-B/Tel-B	2	3	4

Analysis of the presence/absence of NK alloreactivity

The main types of alloreactivities (summarized in **Table III**) are indicated by the name of the KIR-ligand present in the donor and absent in the recipient.

The analysis of Bw4-type alloreactivity *must* consider the allelic polymorphism of the *KIR3DL1* locus **(1A)**. Some *KIR3DL1* alleles encode for receptors retained in the cytoplasm (*KIR3DL1*004*, *019*, *021*, *036*, *037*, *039*, *056*, *072)[23,24]. If the analysis performed does not allow for the identification of alleles encoding membrane receptors (thus functional), this information *must* be included in the report **(1C)**.

Non-classical KIR2DS1-mediated alloreactivity

It has been shown that KIR2DS1^{pos} NK cells can recognize and eliminate leukemic cells expressing HLA-C molecules

Table II - *Main KIR inhibitors and their KIR ligands*

KIR	Recognized HLA allotypes	KIR-L
KIR2DL1	HLA-C Lys80	C2
KIR2DL2/L3	HLA-C Asn80	
HLA-B*46:01, HLA-B*73:01	C1	
KIR3DL1	HLA-B and HLA-A Bw4	Bw4

Table III - *Main types of NK alloreactivities*

NK alloreactivity	KIR donor KIR ligands	Recipient KIR ligands	Relevant KIR (donor)
C1	C1^{pos}	C1^{neg}	KIR2DL2/L3
C2	C2^{pos}	C2^{neg}	KIR2DL1
Bw4	Bw4^{pos}	Bw4^{neg}	KIR3DL1
C1 + Bw4	C1^{pos} and Bw4^{pos}	C1^{neg} and Bw4^{neg}	KIR2DL2/L3 and KIR3DL1
C2 + Bw4	C2^{pos} and Bw4^{pos}	C2^{neg} and Bw4^{neg}	KIR2DL1 and KIR3DL1

carrying the C2 epitope. This NK subpopulation is "functional" only in HLA-C1/C1 and HLA-C1/C2 individuals[78]. Thus, KIR2DS1-mediated NK alloreactivity occurs if the donor is *KIR2DS1*^{pos}, HLA-C1/x, and the patient has at least one HLA-C2^{pos} allele.

CONCLUSIONS

A joint effort made within the Italian histocompatibility network allowed for the development of shared and updated recommendations for the histocompatibility assessment before HSCT. Given the increasing use of HLA-mismatched transplantation as well as the recognized importance of donor-specific anti-HLA antibodies and the role of NK-mediated alloreactivity, the present recommendations represent a relevant step forward in the standardization and improvement of quality of care in this setting.

The Authors declare no conflicts of interest.

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