

Donor selection: Histocompatibility and immunogenetics

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ABSTRACT

Background: Histocompatibility factors have long influenced donor selection, a key component of allogeneic hematopoietic cell transplantation (HCT). **Objective:** To update the 2021 consensus recommendations on histocompatibility and donor selection by the Sociedade Brasileira de Terapia Celular e Transplante de Medula Óssea (SBTMO). **Methods:** We conducted a thorough literature review and compiled current evidence on HLA factors in contemporary HCT. **Results:** This review highlights the current impact of histocompatibility factors in allogeneic HCT, including donor-specific anti-HLA antibodies (DSA), high-resolution HLA mismatching, and HLA-DPB1 permissiveness on HCT outcomes. Additionally, it highlights how non-HLA factors, including donor age, the use of post-transplant cyclophosphamide, the timing of HCT, and donor availability, influence histocompatibility and affect donor prioritization. It also presents current alternative donor selection algorithms and addresses emerging topics, such as the significance of HLA loss of heterozygosity following relapse post-HCT. **Conclusion:** The present article provides a comprehensive overview of the current role of HLA factors in modern allogeneic HCT.

Keywords: Histocompatibility Testing. Donor Selection. Hematopoietic Stem Cell Transplantation. Graft Rejection. Graft vs Host Disease.

INTRODUCTION

Donor selection is a crucial component of allogeneic hematopoietic cell transplantation (HCT) and significantly affects the procedure's success¹. The traditional hierarchy for selecting donors in allogeneic HCT has primarily focused on HLA matching². As such, the first option was a 12/12 HLA-matched sibling donor (MSD). If a suitable MSD was unavailable, the next option was an 8/8- or 10/10-matched unrelated donor (MUD). If MUDs were also unavailable, alternative donor sources were the third option and included mismatched unrelated donors (MMUD) with 7/8 or 9/10 matching, haploidentical donors (HAPLO), or unrelated umbilical cord blood (UCB) units^{2,3}.

However, to minimize time-to-transplant and mitigate the risk of disease progression, it is currently recommended to initiate searches for all potential donor sources (MSD, MUD, MMUD, HAPLO, and UCB) simultaneously at the time of HCT evaluation⁴⁻⁶. Accordingly, for patients lacking a suitable MSD, the donor search should move

beyond the traditional sequential hierarchy and, at search initiation, use search prognosis tools (<https://search-prognosis.b12x.org/>) to identify patients unlikely to find an 8/8- or 10/10-MUD⁵. For these patients, the immediate prioritization of alternative donor sources is essential to ensure timely and efficient access to HCT^{5,6}.

RECOMMENDATIONS FOR HLA TYPING

High-resolution HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 typing is recommended as the minimum criterion for patients undergoing related or unrelated donor searches⁷. For pre-transplant patients, both peripheral blood and buccal swab samples should be collected; the oral swab must be used to confirm homozygosity and detect eventual somatic mutations. In addition, HLA confirmatory typing (CT) using a new sample is mandatory for all patients and donors. This must be performed prior to starting the conditioning regimen to rule out any potential identification or laboratory errors⁷. Medium-resolution CT is acceptable if initial typing was performed at high resolution. Given the potential need for a second HCT, complete HLA genotyping of the patient is essential to support subsequent donor searches. Complete patient genotyping and DNA cryopreservation are required to facilitate potential future searches and ensure readiness for subsequent HCT⁸.

In HAPLO evaluation, initial HLA typing of relatives may be conducted at intermediate resolution for HLA-A, -B, -DRB1, and -DQB1. Afterward, it is mandatory to obtain a high-resolution confirmatory typing for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 in a new sample of the selected HAPLO. More details on the sample collection for HLA typing have been described elsewhere⁸.

HISTOCOMPATIBILITY FACTORS IN DONOR SELECTION

Donor-specific anti-HLA antibodies

Patients eligible for HCT may become allosensitized against mismatched HLA antigens through blood transfusions and previous pregnancies, and eventually through prior HLA-mismatched transplants⁹. In the HLA-mismatched HCT setting, donor-specific anti-HLA antibodies (DSA) refer to antibodies that target the mismatched HLA antigens of the donor¹⁰.

Clinically, the presence of DSAs has been consistently associated with increased risks of graft failure, higher non-relapse mortality, and lower overall survival after HLA-mismatched allo-HCT^{11–13}. The detrimental impact of DSAs on transplant outcomes has been described in all HLA-mismatched donor sources, for both malignant and nonmalignant indications^{14–18}. Likewise, DSAs have also been associated with poorer outcomes following salvage HCT for not only malignancies but also nonmalignant diseases^{19,20}. Accordingly, given DSA's substantial impact on HLA-mismatched HCT, avoiding DSA has been currently considered a major criterion for alternative donor prioritization^{1,4,6}. Moreover, when only DSA-positive donors are available, desensitization therapy should be performed to overcome the DSA barrier and facilitate engraftment. Details on DSA testing, DSA's clinical impact, and current desensitization protocols in allo-HCT are provided elsewhere¹⁰.

In practice, DSA assessment in allo-HCT largely relies on the concept of a virtual crossmatch (VXM), in which the patient's antibody profile, determined by single antigen bead (SAB) assays, is compared with the donor's HLA-mismatched antigens^{21,22}. Although VXM appears simple in concept, this *in-silico* assessment is complex and should account for the well-known SAB caveats, such as false-positive and false-negative reactions²³. In some instances, VXM should be complemented with cell-based assays, such as flow cytometry crossmatch or adsorption/elution, to ensure an accurate DSA assignment^{9,24}. More recently, VXM accuracy has been significantly impacted by the new serologic nomenclature of HLA associated antigens²⁵. While this updated nomenclature has enhanced VXM analysis by providing a serological equivalent for thousands of novel HLA alleles identified by next-generation sequencing, several new associated antigens common in the Brazilian population are not represented in currently available commercial SAB panels, potentially compromising DSA assignment. In this regard, a discussion between the HCT unit and the HLA laboratory should be carried out to address the caveats of DSA analysis under the novel associated antigens serological designation^{26–28}.

High-resolution HLA mismatching

Donor/recipient high-resolution HLA mismatching has been a paramount factor in unrelated donor HCT²⁹. When present, increasing degrees of HLA mismatching are associated with higher risks of severe acute graft-versus-host disease (GVHD), leading to increased non-relapse mortality (NRM) and worse overall survival^{30–32}. Indeed, a previous meta-analysis showed that, when compared to 10/10 MUDs, class I HLA mismatches were associated with higher mortality risks than class II disparities²⁹. Notably, this finding has been recently validated in two large, multicenter studies^{33,34}.

Various models of HLA mismatch permissiveness have been evaluated in allogeneic HCT^{35–38}. However, none of these models has demonstrated significant clinical benefits or have been validated in independent cohorts. More recently, the class I peptide-motif binding (PBM) model, as a proxy for immunopeptidome divergence, has been tested and validated in two large, multicenter studies with independent cohorts^{34,39}. These studies showed that PBM mismatches in the graft-versus-host (GvH) direction were associated with inferior survival. Therefore, when multiple young, DSA-negative, MMUDs are available, it is advisable to avoid class I PBM GvH mismatches in MMUD HCT using conventional GVHD prophylaxis. In HLA-B mismatched HCT, the B-leader model has been proposed in an international multicenter study, which showed that B-leader mismatching increased acute GVHD risks compared to B-leader-matched MMUD transplants³³. However, a recent European multicenter study failed to replicate the clinical benefits of the B-leader model³⁴. Therefore, further data is necessary to clarify these conflicting results.

HLA-C mismatching has unique features that may affect MMUD prioritization^{40,41}. A previous multicenter registry study found that 7/8 MMUD HCT with a donor/recipient HLA-C*03:03/C*03:04 mismatch had outcomes comparable to those of 8/8 MUD HCT with standard GVHD prophylaxis⁴⁰. Notably, the HLA-C*03:03/C*03:04 mismatch permissiveness has been validated in an independent cohort³². In turn, previous studies indicate that differential HLA-C expression may affect MMUD HCT outcomes, with highly expressed HLA-C alleles (e.g., C*14:02) in the GvH vector associated with a higher incidence of severe acute GVHD and NRM^{41,42}.

The role of HLA mismatching in unrelated donor HCT for nonmalignant disorders has been evaluated in a previous multicenter study⁴³. Compared with 8/8 MUD, 7/8 and 6/8 MMUDs were strongly associated with higher odds of graft failure, while 6/8 MMUDs were associated with increased mortality. Interestingly, mismatches at HLA-DQ and HLA-DP were not correlated with any outcomes after unrelated donor HCT for nonmalignant disorders⁴³. More recently, the impact of HLA disparities in pediatric unrelated donor HCT for hematological malignancies has also been investigated in a large multicenter cohort. This study found that both single and double HLA mismatches significantly increased the risks of mortality, NRM, and grade III–IV acute GVHD, although with lower relapse rates⁴⁴. Regarding specific HLA loci, HLA-A and -C mismatches were associated with poorer outcomes, whereas DRB1 mismatches were not associated with worse survival⁴⁴.

Anti-thymocyte globulin (ATG) has been historically used to mitigate the deleterious impact of HLA mismatching in unrelated donor HCT⁴⁵. Early evidence suggests that the risk of a single HLA mismatch is effectively reduced when ATG is used^{46,47}, a finding that has been confirmed in a recent cohort of 9/10 MMUD HCT³². In contrast, unrelated donor transplantation with ATG performed across two or more HLA mismatches is associated with poor overall survival and increased NRM^{32,48}.

Prospective studies with HLA-mismatched unrelated donors (7/8 to 4/8 HLA matching) under post-transplant cyclophosphamide (PTCy)-based GVHD prophylaxis, using either bone marrow or peripheral blood stem cells, showed promising survival^{49,50}. Recent multicenter registry studies have examined the impact of post-PTCy on HLA mismatching following unrelated donor HCT. These studies have produced conflicting results^{34,50–52}, with the majority suggesting that PTCy effectively reduces the negative effects of HLA disparities on survival outcomes after MMUD transplantation^{50–52}.

While high-resolution mismatches at HLA-A, -B, -C, and -DRB1 loci also significantly affect UCB transplants using single or double cord blood units⁵³, the overall degree of HLA matching has not been correlated with GVHD and survival outcomes after haploidentical HCT with PTCy^{54,55}; consequently, HLA matching is not prioritized as a selection parameter for HAPLO transplantation^{4,56}.

HLA-DPB1 permissiveness

The clinical utility of HLA-DPB1 permissiveness, as assessed by the T-cell epitope (TCE) model, has been extensively tested and independently validated in multiple high-quality, multicenter observational studies in unrelated donor HCT^{30,57–60}. In contrast, the role of HLA-DPB1 TCE permissiveness is less explored in HAPLO⁶¹ and UCB settings⁶².

Additionally, HLA-DP expression has been reported to influence the risk of acute GVHD following 10/10 MUD transplants⁶³. The clinical impact of the HLA-DPB1 expression model has been replicated in other multicenter study⁶⁴. Moreover, the combined model of TCE and expression, named TCE-permissive/high expression, has been proposed and was associated with improved relapse-free survival and reduced relapse, without concomitant increase of NRM⁶⁰.

The core/non-core model refines the TCE3 group and has been validated in two large registry studies^{65,66}. In high-risk malignancies, a non-core GvH-permissive mismatch may reduce relapse without increasing NRM, thereby improving disease-free survival after MUD HCT with conventional GVHD prophylaxis. In contrast, in 10/10 MUD HCT using PTCy-based GVHD prophylaxis, the negative impact of TCE non-permissive mismatches appears to be abrogated, thus resembling the outcomes of TCE permissive-mismatched and 12/12 MUD transplants^{34,67}.

CURRENT CRITERIA FOR SELECTING DONORS FOR ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

The classical donor hierarchy has been challenged by novel data that account for the use of PTCy as GVHD prophylaxis⁵⁰, the HCT timing and donor availability⁵, and donor age^{51,68}. Accordingly, current donor selection strategies are tailored to both non-HLA and classical HLA factors, such as high-resolution HLA mismatching, HLA-DPB1 TCE permissiveness, and the presence of DSAs^{4,6}. The relative impact of HLA and non-HLA factors on donor prioritization for each donor type is detailed below and summarized in Table 1.

Table 1. Contemporary criteria for selecting donors for patients without a matched sibling donor.

MUD/MMUD (non-PTCy)	MUD/MMUD (PTCy)	Haploidentical donors	UCB
• Avoid HR MM at HLA-A, -B, -C, and DRB1; class I MM is more detrimental than DRB1 MM; • Avoid older donors*; • Avoid HLA-DPB1 TCE nonpermissive MM; • Avoid DSA; • For class I HLA MM, avoid PBM GvH MM	• Avoid DSA; • Avoid older donors*; • Number, quality and loci of the HLA MM: currently not considered; • HLA-DPB1 TCE permissiveness: currently not considered	• Avoid DSA; • Avoid older donors*; • Prioritize other non-HLA factors (ABO, sex match, cytomegalovirus, and others)*; • B-leader/DRB1 MM/HLA-DPB1 nonpermissive MM: conflicting results	• Prioritize units with higher cell doses (CD34⁺ and TNC) and unit quality; • Avoid HR MM at HLA-A, -B, -C, and -DRB1 (≥ 6/8 matching: optimal); • Avoid DSA

MUD: matched unrelated donor; MMUD: mismatched unrelated donor; PTCy: post-transplant cyclophosphamide; UCB: unrelated umbilical cord blood; HR: high-resolution; MM: HLA mismatch; TCE: T-cell epitope; DSA: donor-specific anti-HLA antibodies; PBM: peptide-motif binding; GvH: graft-versus-host; *details on the role of donor age and other non-HLA factors in allogeneic donor selection will be included in a dedicated article of the present SBTMO's Consensus Guidelines.

Source: Elaborated by the authors.

Unrelated donors

Currently, unrelated donor selection depends on whether PTCy is used or not for GVHD prophylaxis^{4,6}.

In a non-PTCy setting, HLA mismatches with high immunopeptidome divergence (*i.e.*, class I PBM GvH and HLA-DPB1 TCE non-permissive mismatches) significantly impact survival after unrelated donor HCT and

should be carefully evaluated and avoided whenever possible^{34,39}. In the 10/10 MUD HCT, the TCE-3 core/non-core model can be applied to yield a strong graft-versus-leukemia (GvL) effect without increasing the risk of severe acute GVHD or NRM. In addition, donor age is the most important non-HLA factor, and the presence of DSA should be avoided whenever possible^{4,6}.

In contrast, under PTCy-based GVHD prophylaxis, the deleterious effects of high-resolution HLA disparities and HLA DPB1 TCE non-permissive mismatches are largely minimized. As such, allo-HCT using highly mismatched unrelated donors appears to be feasible and associated with promising outcomes. Furthermore, while both the number and quality of HLA incompatibilities are not currently included in MMUD prioritization, the presence of DSAs and donor age emerge as the most important factors to consider in MUD/MMUD selection under PTCy^{4,6}.

Haploidentical donors

In Brazil, HAPLO HCT is performed only with the PTCy platform. In this setting, HAPLO selection relies primarily on avoiding DSA and prioritizing younger donors^{4,6}. While other non-HLA factors are also important, the degree of HLA matching in the mismatched haplotype is not currently considered for HAPLO selection^{4,6}. More recently, a model for qualitative assessment of HLA mismatches in haploidentical HCT with PTCy for malignancies has been proposed⁶¹. Although this model identified specific HLA (mis)matches, such as B-leader matching, DRB1 mismatching in the GvH vector, and non-permissive DPB1 mismatching in the GvH vector, as predictive of improved outcomes after haploidentical HCT with PTCy, it has not been consistently validated in other independent cohorts⁵⁵. Therefore, this predictive model should be used with caution when selecting HAPLO.

Unrelated umbilical cord blood units

While the use of UCB has been steadily declining in recent years, it remains a relevant donor source for adult patients with aggressive malignancies and pediatric patients with both malignant and non-malignant diseases⁶. UCB prioritization relies on unit quality and increased cell doses, mainly CD34⁺. Regarding HLA factors in UCB selection, avoiding both high-level DSAs and high-resolution HLA-A, -B, -C, and -DRB1 mismatches is associated with improved outcomes⁶. More details on the UCB selection can be found in other dedicated guidelines⁶⁹.

LABORATORY MONITORING OF ENGRAFTMENT AND IMMUNE ESCAPE: CHIMERISM AND HUMAN LEUKOCYTE ANTIGEN LOSS

Chimerism monitoring is a fundamental tool in the post-HCT setting⁷⁰. It allows donor engraftment assessment, early detection of graft failure or disease relapse, and helps guide clinical interventions, such as immunosuppression adjustment or donor lymphocyte infusion⁷⁰. Longitudinal monitoring is particularly important, as serial measurements enable the detection of dynamic changes over time and provide critical information for the timely management of HCT recipients⁷¹. A blood sample or DNA from both the recipient and the donor is essential to establish their genetic profiles and accurately quantify chimerism. Detailed information on collection, samples, and methodology is provided in the previous version of this consensus⁸.

Detection of HLA loss of heterozygosity at the time of relapse has important implications for clinical decision-making⁷². Leukemic cells that relapse after transplantation and lose the mismatched HLA haplotype may evade donor immune surveillance, ultimately becoming resistant to the GvL effect expected from the donor lymphocyte infusion⁷²⁻⁷⁴. Furthermore, the identification of HLA loss may influence donor selection for a second transplantation, favoring donors mismatched for the remaining recipient haplotype, which represents the residual target for the GvL response⁷²⁻⁷⁴.

CONCLUSION

Current evidence in allogeneic HCT highlights the importance of a personalized approach to donor selection. This approach considers HLA factors, as well as other significant non-HLA factors, including patient and donor ages, disease type and stage, donor availability, urgency of HCT, GVHD prophylaxis, graft source, and allosensitization level. Accordingly, integrating the HCT unit and the HLA laboratory is paramount to enhance immunologic risk stratification and optimize donor prioritization for patients eligible for an allogeneic HCT.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Not applicable.

AUTHORS' CONTRIBUTIONS

Substantive scientific and intellectual contributions to the study: Lima ACM, Oliveira DCM, Rampim GF, Fabreti-Oliveira RA and Torres MA. **Conception and design:** Lima ACM, Oliveira DCM, Rampim GF, Fabreti-Oliveira RA and Torres MA. **Acquisition of data:** Lima ACM, Oliveira DCM, Rampim GF, Fabreti-Oliveira RA and Torres MA. **Analysis and interpretation of data:** Lima ACM, Oliveira DCM, Rampim GF, Fabreti-Oliveira RA and Torres MA. **Manuscript preparation:** Lima ACM, Oliveira DCM, Rampim GF, Fabreti-Oliveira RA and Torres MA. **Manuscript writing:** Lima ACM, Oliveira DCM, Rampim GF, Fabreti-Oliveira RA and Torres MA. **Critical revision:** Lima ACM, Oliveira DCM, Rampim GF, Fabreti-Oliveira RA and Torres MA. **Final approval of the version to be published:** Lima ACM.

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DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

The authors declare that generative artificial intelligence tools were not used in the creation of the present manuscript.

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