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Haplo-PtCy: adjusting the HLA barrier

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In this issue of *Blood*, Fuchs et al¹ unraveled the role of patient-donor HLA matching in the outcome of haploidentical hematopoietic cell transplantation (HCT) as treatment for acute leukemia (AL) or myelodysplastic syndrome (MDS).

Since its description in the 1990s, successful HCT from related donors mismatched for an entire HLA haplotype (haplo-HCT) has challenged the dogma of complete patient-donor HLA matching as a prerequisite for engraftment and prevention of lethal graft-versus-host disease (GvHD).² The introduction of post-transplant cyclophosphamide (PtCy)-based GvHD prophylaxis initiated the era of T-cell replete haplo-HCT (haplo-PtCy). This resulted in the continuously increasing clinical use this treatment modality has witnessed over the last decade, and further fueled discussions about the relevance of the HLA barrier in HCT. This question is of considerable practical importance because many patients have several haploidentical family donors. Based on currently available evidence, donor-selection guidelines for haplo-HCT consider HLA only by recommending avoidance of alloantibodies in the patient against donor-specific HLA allotypes.³

By definition, patients share 1 entire HLA haplotype (ie, 1 allele each of the 6 loci HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, HLA-DPB1) with their haploidentical family donors, whereas the other haplotype is unshared (see figure). However, the unshared haplotypes of patient and donor can, by chance, carry the same allele at ≥ 1 of the 6 HLA loci, resulting in a minimum of 0 and a maximum of 6 mismatched HLA alleles. It is unknown whether the number and/or the locus of these accidentally shared HLA alleles has any impact on HCT outcome. Moreover, qualitative differences in HLA mismatches, such as the B leader⁴ or DPB1 T-cell epitope (TCE) nonpermissiveness,⁵ relevant in unrelated HCT, have not

been comprehensively studied in haplo-HCT.

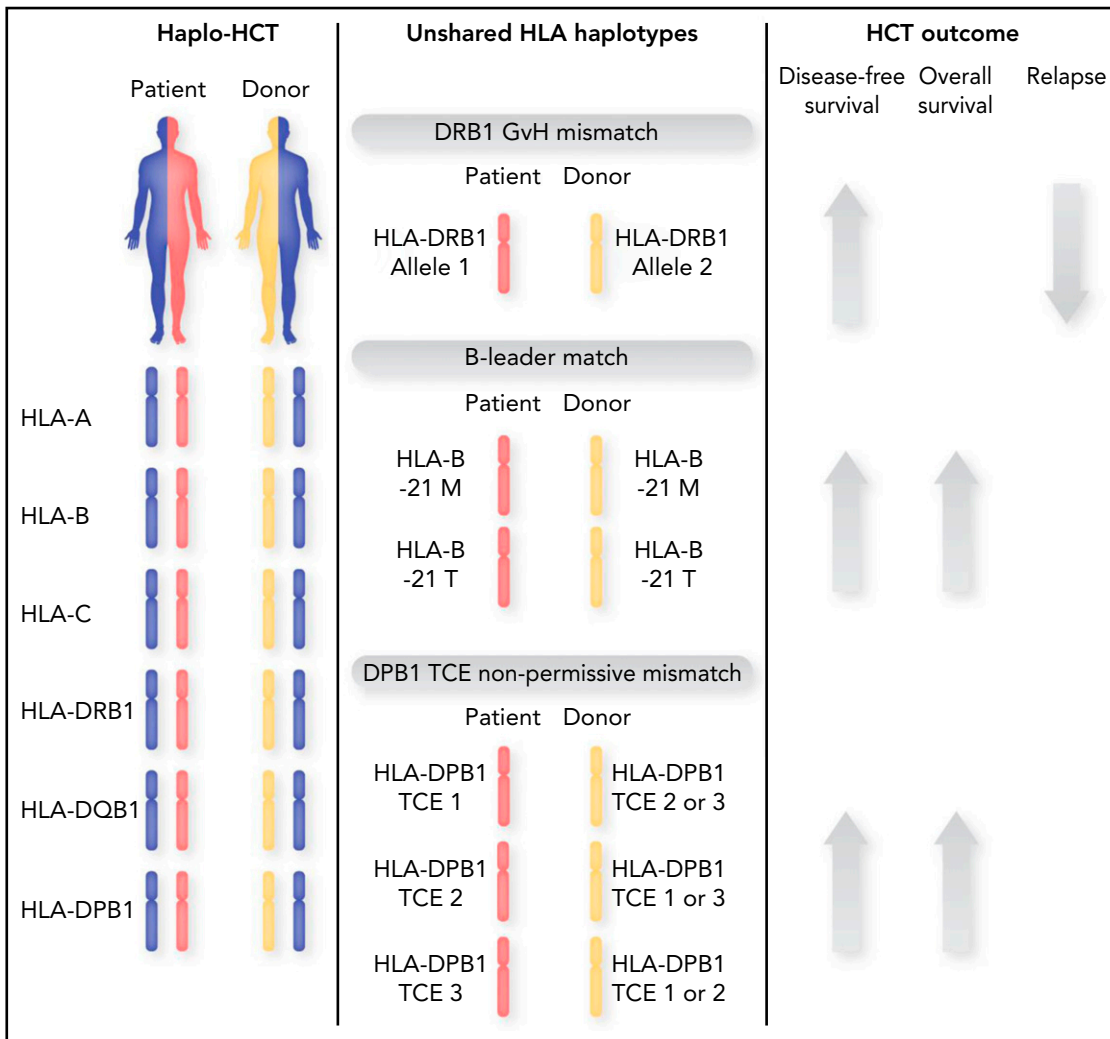
Fuchs et al¹ systematically addressed these questions in a cohort of 1434 patients, who were treated for AL or MDS by haplo-PtCy, reported to the Center for International Blood and Marrow Transplant Research. They found that the number of HLA mismatches on the unshared haplotype did not have any significant impact on outcome. In contrast, HLA-DRB1 graft-versus-host (GvH) mismatches were associated with lower relapse rates and, when combined with a match at HLA-DQB1, better disease-free survival (DFS). Moreover, B leader matches and DPB1 TCE nonpermissive GvH (DPB1-NP) mismatches were associated with improved DFS and overall survival (see figure). This reflected lower transplant-related mortality (TRM) for the B leader. For DPB1-NP, statistical power was more limited than for the other models, because DPB1 typing was available for less than half of the cohort. It is tempting to speculate that the survival advantage for DPB1-NP might result from lower relapse and lower TRM, as evidenced by the lower hazard ratios for these 2 endpoints, although the differences were not statistically significant.

The findings from Fuchs et al¹ elegantly demonstrate how the HLA barrier has not been erased but needs to be adjusted in the setting of haplo-PtCy. Locus-specific effects for HLA class II and biological matching models, rather than the number of HLA mismatches, turned out to be the key to pinpointing the impact of patient-donor HLA matching status on outcome. Interestingly, HLA class II DRB1 (and possibly DPB1-NP) mismatches were associated with lower

relapse, but not higher acute GvHD, in this setting. Targeted genomic and/or global transcriptional downregulation of HLA class II is a mechanism of immune evasion in leukemia relapse after HCT, hinting at its importance in graft-versus-leukemia (GvL).⁶⁻⁸ The observation that the benefits of defined HLA class II mismatches on relapse were not negated by increased GvHD might be explained by the immunological effects of PtCy, which selectively eliminates activated (ie, in the early transplant phase alloreactive) effector T cells while sparing regulatory T cells.² This mode of action might classify PtCy as a game changer for T-cell alloreactivity, which raises the bar of HLA disparity needed for severe toxic GvHD but not for GvL. Of note, permissive, rather than nonpermissive, DPB1 TCE mismatches are associated with improved survival in unrelated HCT with calcineurin inhibitor-based immune prophylaxis.⁵ In the context of PtCy, stronger T-cell alloreactivity against DPB1 TCE nonpermissive mismatches, mechanistically based on the degree of immunopeptidome divergence between HLA-DP allotypes determining the number and diversity of alloreactive T cells,⁹ might be required for efficient GvL without excessive GvHD. Further research is needed to understand whether these effects of PtCy also apply to its use in unrelated HCT, in which relevant clinical trials are under way.

Interestingly, the survival advantage for B leader matches was associated with lower TRM but not with reduced relapse. In B leader matched haplo-HCT, the HLA-B alleles on the unshared haplotype of patient and donor carry the same amino acid at position -21 (ie, threonine [T] or methionine [M]) (see figure). M leaders, but not T leaders, are able to supply peptides binding to HLA-E, thereby impacting the function of CD94/NKG2A-bearing natural killer cells¹⁰ and, possibly, HLA-E-restricted T cells. It remains to be elucidated if and how these immune cells are involved in the beneficial effects of B-leader matching.

Based on their findings, Fuchs et al¹ developed a webtool to predict DFS for a given patient with defined relevant clinical characteristics, after HCT from haploidentical donors, according to their HLA-B leader, DRB1/DQB1, and DPB1 TCE matching status (<http://haplodonorselector.b12x.org/v1.0/>). This



In HCT from haploidentical family donors, 1 HLA haplotype is shared (blue) and the other is unshared (ie, patient-specific [red] or donor-specific [yellow]). DRB1 GvH mismatches are present in HLA-DRB1 heterozygous patients carrying a different HLA-DRB1 allele than the donor on their unshared haplotypes (ie, patient allele 1 and donor allele 2). B-leader matches are present when the HLA-B alleles on the unshared haplotypes of patient and donor carry an identical leader peptide sequence at position -21 (ie, methionine [M] or threonine [T]).⁴ DPB1 TCE nonpermissive mismatches are present when the HLA-DPB1 alleles on the unshared haplotypes of patient and donor belong to different TCE groups (ie, patient TCE 1 and donor TCE 2 or 3, patient TCE 2 and donor TCE 1 or 3, or patient TCE 3 and donor TCE 1 or 2), with the relevant TCE group in the patient also not present on the shared haplotype.⁵ Arrows indicate a significant association with better DFS and better overall survival or lower relapse after haplo-HCT in the presence of the specific HLA matching status compared with the reference, as per multivariable analyses presented in Fuchs et al.¹

freely accessible tool will prove useful in guiding clinicians in the frequent need to select the optimal donor for haplo-HCT. Of note, the tool does not take into account the patient's alloantibody status, which was not investigated here because of the unavailability of the relevant data but has been identified as critical in numerous other studies³; hence, it should be integrated into the decision making. It will also be important to update the webtool once evidence on additional relevant variables, such as killer immunoglobulin-like receptors, emerges.

In summary, Fuchs et al provide long-awaited comprehensive evidence on the

role of HLA matching in haplo-HCT. Apart from their practical relevance, these data remind us that qualitative, rather than quantitative, effects are the key to understanding the role of patient-donor HLA disparity in HCT.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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HEMATOPOIESIS AND STEM CELLS

Comment on Yu et al, page 1529

Angiocrine ANGPTL2 executes HSC functions in endothelial niche

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Niche cells within the bone marrow (BM) microenvironment support the maintenance and reconstitution of hematopoiesis. In this issue of *Blood*, Yu et al demonstrate that angiotensin-like 2 (ANGPTL2) secreted by the vascular niche endothelial cells serves as the seminal paracrine angiocrine factor, orchestrating hematopoietic stem cell (HSC) homeostasis and regeneration after myelosuppression.¹

Tissue-specific endothelium, such as BM endothelial cells (BMECs), is endowed with unique organotypic specialized functions, including modulation of metabolism, immune cell trafficking, inflammatory response, vascular tone, regeneration, and repair.^{2,3} It is well documented that blood vessels in the BM are not just passive conduits delivering nutrients and oxygen to hematopoietic cells. BMECs execute specialized hematopoietic-supportive functions that promote hematopoietic stem and progenitor cell (HSPC) proliferation and self-renewal through a coordinated angiocrine supply of stimulatory and inhibitory membrane-bound and secreted factors.^{4,5} Direct cellular contact between BMECs and hematopoietic cells is essential for balanced self-renewal and differentiation of HSCs and HSPCs⁶ in homeostatic and regenerative states (see figure). Although numerous angiocrine factors, including bone morphogenetic proteins (BMP2 and BMP4), Notch ligands, CXCL12, and Kit ligand, could be supplied by other BM niche cells, certain

factors presented specifically by BMECs might exert dominant functions in HSC homeostasis. However, the identity of these unique vascular niche-derived HSC-dominant angiocrine factors are not well defined. Notably, growth factors, such as ANGPTL2, which have been shown to regulate HSC metabolic, inflammatory, and angiogenic functions,⁷ could also be secreted by diverse BM niche cells, including BMECs, stromal cells, and hematopoietic cells themselves. Whether preferential secretion of ANGPTL2 by a specific niche cell drives hematopoiesis has not been formally studied.

To address this, Yu et al used sophisticated gene knockdown in each of the niches to demonstrate that angiocrine-derived ANGPTL2 selectively secreted by BMECs, but not other stromal niche cells, serves as a critical factor that maintains and re-establishes hematopoietic cell stemness. To demonstrate the specific angiocrine ANGPTL2 function, Yu et al leveraged a multiprong lineage-specific

strategy in mice, whereby the *Angptl2* gene was silenced in different niche components, including vascular cells, mesenchymal cells, and megakaryocytes, to provide a unique insight into how niche-specific factors determine the biological behavior of HSCs. Although other studies have demonstrated a functional overlap among mesenchymal, osteoblastic, perivascular, and vascular niches, Yu et al revealed the cardinal role of only endothelial cell-derived ANGPTL2 in maintaining HSC functions at steady-state conditions and during hematopoietic recovery following 5-fluorouracil-induced hematopoietic ablation. ANGPTL2 deletion in BMECs, but not in stromal cells, resulted in impairment of HSC sustenance and reconstitution after hematopoietic ablation.

Mechanistically, Yu et al showed that, through activation of its receptor human leukocyte immunoglobulin-like receptor B2,⁸ ANGPTL2 enhances peroxisome proliferator activated receptor δ (PPAR δ) expression to directly transactivate G α /G β switch 2 (G α s2) levels to maintain the quiescent status of HSCs (see figure). PPARs are nuclear receptors that play pivotal roles in the regulation of the metabolic status of various cell lineages. The most dramatic change observed in the BM niche during aging is excessive adipocyte expansion that presumably has a negative impact on HSC function.⁹ An altered metabolic microenvironment in the stressed and aged BM might induce the defects observed in HSCs, setting the stage for myelodysplasia and hematopoietic failure during recovery from myeloablation. Because endothelial cells act as critical gatekeepers of tissue-specific metabolites, including free fatty acids, it is plausible that imbalanced metabolic fluxes and inflammatory stress within BMECs can result in aging-induced damage within the BM niche, thereby impairing HSC functions. Based on the result illustrated in the article by Yu et al, aberrant secretion of endothelial cell-derived ANGPTL2 might instigate metabolic disruptions that contribute to HSC dysfunctions manifested in myelodysplastic maladies, induction of clonal hematopoiesis, and aging, as well.

Within the hematopoietic microenvironment, BMECs manifest remarkable intra-organ vascular heterogeneity,^{2,3} in which specialized arterial, sinusoidal, and periosteal endothelial cells provide unique