

The impact of HLA matching on outcomes of unmanipulated haploidentical HSCT is modulated by GVHD prophylaxis

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Key Points

- In unmanipulated haplo-HSCT, antigenic HLA-DRB1 match, stem cell source, conditioning, and donor sex are associated with GVHD.
- The role of HLA-matching status and other factors influencing alloreactivity is more prominent with PTCy compared to ATG GVHD prophylaxis.

Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) with unmanipulated grafts is increasingly adopted for high-risk acute leukemia, with acute graft-versus-host disease (aGVHD) prophylaxis based on antithymocyte globulin (ATG) or posttransplant cyclophosphamide (PTCy) as main platforms. No consensus exists on selection criteria over several haploidentical donors. We evaluated the impact of donor-recipient antigenic and allelic HLA-A, -B, -C, and -DRB1 mismatches on mismatched haplotype on outcomes of 509 unmanipulated haplo-HSCTs performed for acute leukemia under a PTCy (N = 313) or ATG (N = 196) regimen. An antigenic but not allelic mismatch at the HLA-DRB1 locus was an independent risk factor for grade ≥ 2 aGVHD in PTCy (hazard ratio [HR], 2.0; 95% confidence interval [CI], 1.2-4.0; $P = .02$) but not in ATG regimens (HR, 1.3; 95% CI, 0.4-3.4; $P = .6$). Moreover, the hazards of aGVHD were significantly associated with other factors influencing alloreactivity, including peripheral blood as stem cell source (HR, 2.2; 95% CI, 1.4-3; $P < .01$), reduced-intensity conditioning (HR, 0.6; 95% CI, 0.4-0.9; $P = .04$), and female donors (HR, 1.8; 95% CI, 1-3.2; $P = .05$), in PTCy but not ATG regimens. No significant associations were found between cumulative number of HLA mismatches and GVHD, or between HLA-matching status and other study end points including transplant-related mortality, disease-free survival, and relapse. Based on these data, the role of HLA mismatching on unshared haplotype appears not to be sufficiently prominent to justify its consideration in haploidentical donor selection. However, the role of HLA matching in haploidentical HSCT might be modulated by GVHD prophylaxis, calling for further investigations in this increasingly relevant field.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a central component of treatment algorithms for patients with high-risk acute leukemia.^{1,2} Donor-recipient HLA compatibility plays a critical role for successful HSCT. The increasing experience with unrelated donors and cord blood transplantation highlights the importance of ascertaining matching at least at HLA-A, -B, -C, and -DRB1 loci between patient and donor, in order to minimize the number of mismatches.³⁻¹⁰ Moreover, when only HLA-mismatched donors are available, clinical data suggest that risks associated with HLA mismatching are not equivalent across all HLA loci.¹¹⁻¹⁹

HSCT from haploidentical donors, namely family-related donors who share a 1-haplotype only genotypical identity with the patient, is a valid option for the sizeable population of patients who lack a matched related or unrelated donor.²⁰ Haploidentical HSCT (haplo-HSCT) presents a major immunological barrier because recognition of the recipient's mismatched HLA by the donor's cells provides a potent stimulus for graft-versus-host disease (GVHD), whereas recognition of the donor's mismatched HLA by the recipient's immune system leads to graft rejection. Despite this, over recent years, clinical protocols for unmanipulated T-cell-replete haplo-HSCT have been successfully developed, using either posttransplant cyclophosphamide (PTCy) or antithymocyte globulin (ATG) as alternative platforms to harness alloreactivity to the mismatched HLA haplotype.²¹⁻²⁷

Although haploidentical donors are referred to as HLA-haplotype-mismatched, some recipients who share an HLA haplotype with their donor are also matched for 1 or more HLA antigens on the unshared haplotype. Until now, scarce evidence existed on the relative role of optimal HLA mismatching on the unshared haplotype in haplo-HSCT. Kasamon et al reported no association between the degree of HLA disparity on the mismatched haplotype and survival after nonmyeloablative transplants with PTCy.²⁸ Huo et al reported that a HLA-B mismatch is associated with an increased risk of acute GVHD (aGVHD) and transplant-related mortality (TRM), as well as worse disease-free survival and overall survival (OS) after T-repleted haplo-HSCT.²⁹ These results were, however, not confirmed in a more recent study by the same group.³⁰

In order to overcome some limitations of previous single-center experiences and heterogeneity of included diseases, we performed a registry-based retrospective study on adult acute leukemia patients who received an unmanipulated haplo-HSCT, and who were reported to the Acute Leukemia Working Party (ALWP) registry of the European Society for Blood and Marrow Transplantation (EBMT).

The aim of the study was to evaluate whether HLA mismatches at specific loci on the unshared haplotype defined at the antigenic or the allelic level are associated with divergent clinical risks after unmanipulated haplo-HSCT.

Methods

Collection of data and inclusion criteria

We included adult patients with de novo acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) who underwent an unmanipulated haploidentical bone marrow (BM) or peripheral

blood (PB) transplantation as their first HSCT between January 2007 and December 2014. EBMT centers were asked to provide HLA typing results for patients and donors at the best available resolution level, and to provide an update of transplantation outcomes through specific clinical forms. We selected 509 donor-recipient pairs for whom we had molecular HLA-A, -B, -C, and -DRB1 typing at an antigen (ie, first field; N = 335) or allelic (ie, second field; N = 174) resolution level.³¹ Haploidentical donors were defined as first-degree relatives who shared 1 HLA haplotype and were mismatched at 1 or more loci on the unshared haplotype.

The research was approved and conducted within the ALWP of EBMT. Patients gave informed consent for data entry into the EBMT registry and for its analysis in accordance with the Declaration of Helsinki.

HLA-matching status

High-resolution HLA allele typing was available for 174 of 509 pairs (34%), while it was missing in the remaining 335 of 509 pairs (66%).

In order to impute high-resolution matching in these pairs, we performed an *in silico* simulation for all donors and patients with the list of possible diplotypes generated using the German National Bone Marrow Donor Registry haplotype frequency data set. For 10 pairs, 1 or both of those diplotypes lists were empty. For the rest, the 2 lists were compared (checking up to 800 000 combinations per pair) to find all possible haploidentical combinations and to calculate the conditional probability of each haplotype triplet according to an established algorithm.^{32,33} Mismatches at low resolution were directly observable and counted as allele mismatches. For each HLA match defined only at low resolution, we used the respective probability to be matched at high resolution: if it was $\geq 95\%$, we classified the respective locus to be matched, if it was $\leq 5\%$, we classified the locus to be mismatched. Donor-recipient pairs for whom the probability of each locus to be matched at high resolution was between 6% and 94% were excluded from the analysis, for a total of 418 pairs included to explore the role of high-resolution matching on outcomes.

Among the 509 donor-recipient pairs for whom we considered antigenic HLA matching, 16% were matched at HLA locus A, 11% at locus B, 18% at locus C, and 18% at locus DRB1 (supplemental Table 1). The subgroup of 418 donor-recipient pairs for whom we could impute high-resolution HLA matching reflected the overall characteristics of the entire population (supplemental Table 1). Of them, among pairs with < 4 allelic mismatches, 10% were matched at HLA locus A, 9% at locus B, 11% at locus C, and 9% at locus DRB1 (supplemental Table 1). The percentages of antigenic and allelic mismatches at each specific HLA locus in the 2 groups of adopted GVHD-prophylaxis regimens are shown in Table 1.

In order to evaluate the possible differences on haplo-HSCT outcomes arising from the direction of HLA mismatches, we defined HLA mismatches as bidirectional, as host-versus-graft (HVG) direction only (that means that donor's alleles were not shared by the recipient) or graft-versus-host (GVH) direction only (that means that recipient's alleles were not shared by the donor). The numbers of bidirectional, HVG-, or GVH-mismatched pairs for each HLA locus,

Table 1. Donor-recipient HLA-matching status

| | PTCy regimen, n (%) | ATG regimen, n (%) | P |
|--|------------------------|-----------------------|-----|
| HLA-matching status in the entire population | | | |
| HLA-A | | | |
| Antigen mismatch | 260 (83) | 170 (87) | .26 |
| Antigen match | 53 (17) | 26 (13) | |
| HLA-B | | | |
| Antigen mismatch | 277 (88) | 177 (90) | .52 |
| Antigen match | 36 (12) | 19 (10) | |
| HLA-C | | | |
| Antigen mismatch | 260 (83) | 157 (80) | .40 |
| Antigen match | 53 (17) | 39 (20) | |
| HLA-DRB1 | | | |
| Antigen mismatch | 260 (83) | 158 (81) | .48 |
| Antigen match | 53 (17) | 38 (19) | |
| HLA-matching status in subgroup with HLA high-resolution imputation | | | |
| HLA-A | | | |
| Antigen* mismatch | 222 (86) | 149 (93) | .03 |
| Antigen match | 36 (14) | 11 (7) | |
| Allele† mismatch | 229 (88) | 151 (94) | .07 |
| Allele match | 29 (12) | 9 (6) | |
| HLA-B | | | |
| Antigen mismatch | 235 (91) | 146 (91) | .70 |
| Antigen match | 23 (9) | 14 (9) | |
| Allele mismatch | 239 (93) | 145 (91) | .46 |
| Allele match | 19 (7) | 15 (9) | |
| HLA-C | | | |
| Antigen mismatch | 226 (88) | 133 (83) | .19 |
| Antigen match | 32 (12) | 27 (17) | |
| Allele mismatch | 236 (91) | 139 (87) | .13 |
| Allele match | 22 (9) | 21 (13) | |
| HLA-DRB1 | | | |
| Antigen mismatch | 221 (86) | 140 (87) | .96 |
| Antigen match | 37 (14) | 20 (13) | |
| Allele mismatch | 236 (91) | 148 (92) | .71 |
| Allele match | 22 (9) | 12 (8) | |
| NK alloreactivity in GVL vector | | | |
| Yes | 97 (38) | 67 (42) | .38 |
| No | 161 (62) | 93 (58) | |

*First field HLA typing as reported by the transplant centers.

†Second field HLA typing was reported by the transplant centers in 174 pairs (134 PTCy, 40 ATG). For the remaining 244 pairs, allele level matching was imputed by in silico simulation as described in "Methods."

either in the group with antigenic HLA matching, and in the group with allelic HLA matching, are shown in supplemental Tables 3 and 4.

Clinical end points and statistical methods

Neutrophil engraftment was defined as achievement of an absolute polymorphonuclear leukocyte count >500 cells/mm³ for

3 consecutive days. aGVHD and chronic GVHD (cGVHD) were defined and scored according to the Glucksberg and Seattle criteria, respectively.^{34,35} TRM was defined as death from any cause while in continuous remission of the primary disease. Relapse was defined as hematological relapse with over 5% morphological blast counts in PB or BM. Leukemia-free survival (LFS) was defined as the interval from HSCT to either relapse or death in remission. OS was defined as the interval from HSCT to death from any cause.

In order to analyze the association between HLA matching and transplant end points taking into account the 2 main currently used GVHD-prophylaxis platforms (ie, high-dose PTCy and anti-human thymocyte immunoglobulin), we conducted our analyses separately for each of these platforms.

The χ^2 test was adopted for the comparison of categorical variables between the 2 GVHD prophylaxis regimens; the Kruskal-Wallis test was adopted to compare median values of continuous variables between the 2 groups.

The probabilities of LFS and OS were estimated using the Kaplan-Meier estimator.³⁶ Cumulative incidences were estimated for engraftment, GVHD, TRM, and relapse to accommodate competing risks.³⁷ Relapse was a competing risk for TRM; death from any cause was a competing risk for engraftment and relapse. Both relapse and death from any causes were competing risks for GVHD.

Univariate comparisons of survival curves were made using the log-rank test³⁸; the Gray test was used for univariate comparisons of cumulative incidence functions.³⁹ Multivariate Cox proportional hazard models⁴⁰ were built to adjust for clinical factors associated with a univariate analysis *P* value $<.15$. Covariates included in each model were: patient age (continuous) and sex, diagnosis (ALL vs AML), disease status at HSCT, donor sex, host-donor cytomegalovirus (CMV) status, intensity of conditioning regimen, stem cell source (PB vs BM), and transplant center. Interactions between each covariate and match at each HLA locus were tested and were not found.

Affirmation of the proportional hazard assumption was met for all variables. All tests were 2-sided. The type I error rate was fixed at 0.05 for determination of factors associated with time to event. Adjustments for multiple comparisons were performed. Statistical analyses were performed with SPSS (SPSS Inc/IBM, Armonk, NY) and R (R Development Core Team, Vienna, Austria) software.

Results

Patient population and transplant procedures

The main population characteristics are summarized in Table 2. AML was the main indication for haplo-HSCT in both GVHD-prophylaxis platforms. At the time of transplant, more than half of the patients were in complete hematological remission.

Sixty-one percent of patients received high-dose PTCy as selective alloreactive T-cell depletion, majorly associated with calcineurin inhibitors and mycophenolate mofetil. Thirty-nine percent of patients received an ATG-based GVHD prophylaxis (median dose, 30 mg/kg; range, 2.5-80 mg/kg), mostly combining calcineurin inhibitors or sirolimus and mycophenolate mofetil.

A RIC regimen was more likely adopted in PTCy-based than ATG-based regimens (59% vs 36%, respectively; *P* $<.01$). The applied

Table 2. Patients, donors, and transplant characteristics

| | PTCy-based GVHD prophylaxis, N = 313 | ATG-based GVHD prophylaxis, N = 196 | P |
|--|--------------------------------------|-------------------------------------|-----|
| Patient age, median (range), y | 46 (18-77) | 44 (18-76) | .52 |
| Patient sex, male (%) | 182 (58) | 110 (56) | .62 |
| Time from diagnosis to transplant, median (range), mo | 9 (2-121) | 9 (2-192) | .77 |
| AML/ALL, n (%) | 233 (74)/80 (26) | 140 (71)/56 (29) | .45 |
| Disease status at transplant, n (%) | | | |
| CR1 | 117 (37) | 65 (33) | |
| CR ≥2 | 90 (29) | 47 (24) | .12 |
| Advanced (primary induction failure, relapse) | 106 (34) | 84 (43) | |
| Cytogenetic risk stratification, n (%)* | | | |
| AML | | | |
| Favorable | 22 (13) | 12 (10) | .78 |
| t(8;21)(q22;q22); RUNX1-RUNX1T1 | | | |
| inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFβ-MYH11 | | | |
| Mutated NPM1 without FLT3-ITD (normal karyotype) | | | |
| Mutated CEBPA (normal karyotype) | | | |
| Intermediate I | 82 (48) | 56 (46) | |
| Mutated NPM1 and FLT3-ITD (normal karyotype) | | | |
| Wild-type NPM1 and FLT3-ITD (normal karyotype) | | | |
| Wild-type NPM1 without FLT3-ITD (normal karyotype) | | | |
| Intermediate II | 30 (18) | 22 (18) | |
| t(9;11)(p22;q23); MLLT3-MLL | | | |
| Cytogenetic abnormalities not classified as favorable or adverse | | | |
| Adverse | 37 (21) | 31 (26) | |
| inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1 | | | |
| t(6;9)(p23;q34); DEK-NUP214 | | | |
| t(v;11)(v;q23); MLL rearranged | | | |
| 5 or del(5q); -7; abn(17p); complex karyotype | | | |

Table 2. (continued)

| | PTCy-based GVHD prophylaxis, N = 313 | ATG-based GVHD prophylaxis, N = 196 | P |
|--|--------------------------------------|-------------------------------------|------|
| ALL | | | |
| Standard | 18 (41) | 20 (43) | .80 |
| Poor | 26 (59) | 26 (57) | |
| Complex (>5 abn, t(9;22), t(4;11), t(8;14), hypodiploid) | | | |
| Donor age, median (range), y | 38 (13-70) | 39 (12-74) | .17 |
| Donor sex, male (%) | 170 (54) | 101 (52) | .54 |
| Female donor/male recipient, n (%) | 79 (25) | 55 (28) | .49 |
| CMV serostatus, donor/patient, n (%) | | | |
| Negative/negative | 29 (9) | 28 (14) | .07 |
| Other combinations | 284 (91) | 168 (86) | |
| Intensity of conditioning regimen, n (%) | | | |
| RIC | 140 (59) | 70 (36) | |
| MAC | 173 (41) | 126 (64) | <.01 |
| Donor stem cell source, n (%) | | | |
| PB | 167 (53) | 142 (72) | |
| BM | 146 (47) | 54 (28) | <.01 |

CR, complement receptor; MAC, membrane attack complex; MLL, mixed lineage leukemia. *Cytogenetic analysis was available for 75% of the total population; cytogenetic risk classification was assigned according to published criteria.^{47,48}

regimen showed a broad variety, with busulfan- and fludarabine-based protocols being the most frequently used. Total-body irradiation was part of the conditioning in 11% of patients treated with a RIC and 19% of patients treated with a myeloablative regimen in the PTCy group, whereas it was used in 5% of patients treated with a RIC and 17% of patients treated with a myeloablative regimen in the ATG group. The stem cell source was BM for 47% of patients in the PTCy-based regimens, whereas in the ATG-based regimens only 28% of patients received BM-derived stem cells ($P < .01$).

Overall outcomes

The end points in this study were engraftment, 100-day aGVHD, and 2-year cGVHD, TRM, relapse, LFS, and OS. The cumulative incidence of neutrophil engraftment at 60 days was $95\% \pm 2\%$, with a median time to engraftment of 17 (range, 3-94) days. The overall cumulative incidences of grade ≥ 2 aGVHD and grade ≥ 3 aGVHD at 100 days were $31\% \pm 5\%$ and $12\% \pm 3\%$, respectively, in PTCy-based regimens, whereas they were $34\% \pm 7\%$ and $14\% \pm 4\%$, respectively, in ATG-based regimens.

The 2-year cumulative incidence of cGVHD was $27\% \pm 5\%$ and $32\% \pm 7\%$ in PTCy and ATG-based regimens, respectively. The 2-year cumulative incidence of extensive cGVHD was $11\% \pm 4\%$ and $13\% \pm 4\%$ in PTCy and ATG-based regimens, respectively.

The cumulative incidence of 2-year TRM was $27\% \pm 5\%$ in PTCy regimens and $29\% \pm 6\%$ in ATG regimens. Death was mainly due

to infections (53% of patients: bacterial in 54% of cases, viral and fungal in 28% and 18% of cases, respectively), followed by GVHD (30%, in half of the cases GVHD was associated with infections). In the remaining 17% of patients, death was due to other causes including pulmonary toxicity, sinusoidal obstruction syndrome, hemorrhage, graft failure, secondary malignancies, and cardiac toxicity in decreasing order of frequency.

The 2-year cumulative incidence of hematological relapse was $32\% \pm 5\%$ in PTCy regimens and $38\% \pm 7\%$ in ATG regimens.

Median follow-up for survivors was 23 (range, 1.5-61) months in patients receiving PTCy regimens, and 36 (range, 1.3-93) months in those receiving ATG. The probabilities of OS and LFS at 2 years after transplantation were $44\% \pm 6\%$ and $39\% \pm 6\%$, respectively, in the PTCy group, and $40\% \pm 7\%$ and $33\% \pm 7\%$, respectively, in the ATG group.

Effect of HLA disparities on clinical outcome

Association between matching at each of the 4 HLA loci (A, B, C, DRB1) and clinical outcome was analyzed separately in patients receiving GVHD prophylaxis with PTCy and ATG.

In univariate models, the only significant association found was for aGVHD, which was significantly higher in the presence of an antigenic ($P = .02$) but not an allelic ($P = .33$) HLA-DRB1 mismatch in PTCy regimens (Figure 1; supplemental Table 2). This was reflected also examining the cumulative incidence of aGVHD selectively on the cohort of patients with high-resolution HLA imputation, where donor-recipient pairs with an antigen mismatch at the HLA-DRB1 locus had a trend to higher incidence of grade 2-4 aGVHD compared with HLA-DRB1 antigen-matched pairs ($33\% \pm 7\%$ vs $19\% \pm 10\%$, respectively; $P = .1$).

Consistently, this association was dependent on the directionality of the mismatch, with significantly higher incidence of aGVHD in bidirectional or GVH-directed antigenic HLA-DRB1 mismatches compared with others ($P = .05$; supplemental Table 4). In contrast, the association between HLA-DRB1 mismatches and aGVHD could not be appreciated in ATG-based transplants (antigenic mismatches, $P = .54$; allelic mismatches, $P = .08$) (supplemental Table 2). The association with HLA-DRB1 locus matching was not reflective of the total number of HLA mismatches because no association was found between the incidence of aGVHD and the cumulative number of antigenic or allelic HLA mismatches on the unshared haplotype (Figure 2).

For the other clinical end points including engraftment, severe aGVHD, cGVHD, TRM, relapse, LFS, and OS, no associations with mismatching at any of the HLA loci could be found, neither in PTCy nor in ATG regimen (supplemental Table 2). Likewise, no associations with any outcome end point were found with the cumulative number of HLA locus mismatches on the unshared haplotype (supplemental Table 5).

The association between antigenic HLA-DRB1 mismatching and aGVHD in the PTCy but not in the ATG regimen was confirmed after adjusting for the significant clinical factors including diagnosis, disease status at transplant, stem cell source, donor sex, conditioning regimen, and recipient age (Table 3). Antigenic HLA-DRB1 mismatching was an independent predictive factor for aGVHD after haplo-HSCT with PTCy GVHD prophylaxis, with significantly higher adjusted hazards compared with HLA-DRB1

matching (hazard ratio [HR], 2.0; 95% confidence interval [CI], 1.2-4.0; $P = .03$). In contrast, allele level mismatches had no effect.

Consistent with the univariate results, this association was not observed in patients receiving ATG (Table 4). Similarly, antigenic or allelic HLA mismatches at the other loci were not independently associated with any of the other outcome end points regardless of the GVHD prophylaxis (Tables 3 and 4).

Predicted natural killer (NK) alloreactivity in the graft-versus-leukemia (GVL) vector according to the "ligand-ligand incompatibility" model was tested both in PTCy and in ATG regimens. The frequencies of pairs with predicted NK alloreactivity are shown in Table 1. Apparently, pairs with predicted NK alloreactivity in the PTCy setting seemed to suffer from a higher relapse rate in univariate analysis (supplemental Table 6); however, this did not translate into a worse progression-free survival or OS. A subanalysis performed only on AML patients did not differ from the above-mentioned results.

Other clinical factors and transplant outcomes

The GVHD prophylaxis regimen modulated the influence of variables other than HLA-matching status as well. Of note, PB as stem cell source and myeloablative conditioning were both independently associated with a higher HR for grade 2-4 aGVHD in the PTCy but not in the ATG regimens (Tables 3 and 4). Moreover, patients receiving haploidentical transplantation from female donors (irrespective of the patients' sex) had a higher incidence of grade 2-4 aGVHD in the PTCy but not in the ATG regimens, and experienced a lower incidence of relapse together with a better OS and LFS in this group (Figure 3).

In contrast, advanced disease status was an independent factor for aGVHD in the ATG but not in the PTCy regimens.

Consistent with previous reports,²⁰ advanced disease status at transplantation was correlated with worse LFS and OS, as well as with higher rates of relapse and TRM. Finally, a diagnosis of ALL was associated with worse LFS and OS. Patients suffering from ALL showed a higher risk of relapse and TRM especially with ATG-based regimens (Table 3).

Discussion

The present study investigates, for the first time, the role of antigen and allele HLA mismatches on the clinical outcome of haplo-HSCT in the context of the 2 majorly used platforms for GVHD prophylaxis, PTCy and ATG. We show that HLA matching on the unshared haplotype has a limited impact, with only antigenic HLA-DRB1 mismatches significantly associated with the single end point grade ≥ 2 aGVHD in transplants performed under the PTCy regimen, and no association between the cumulative number of HLA mismatches and outcome. This finding has important practical implications because several haploidentical donors are available for most patients and therefore selection based on optimal HLA mismatching on the unshared haplotype is a tempting concept. Our data do not provide support for this notion, but rather suggest that HLA mismatching on the unshared haplotype should not be used as a major factor in haploidentical donor selection. A possible explanation for this observation could be that the presence of mismatches at the other loci on the unshared haplotype might blur subtle effects from matches at individual loci. In line with this concept, the only

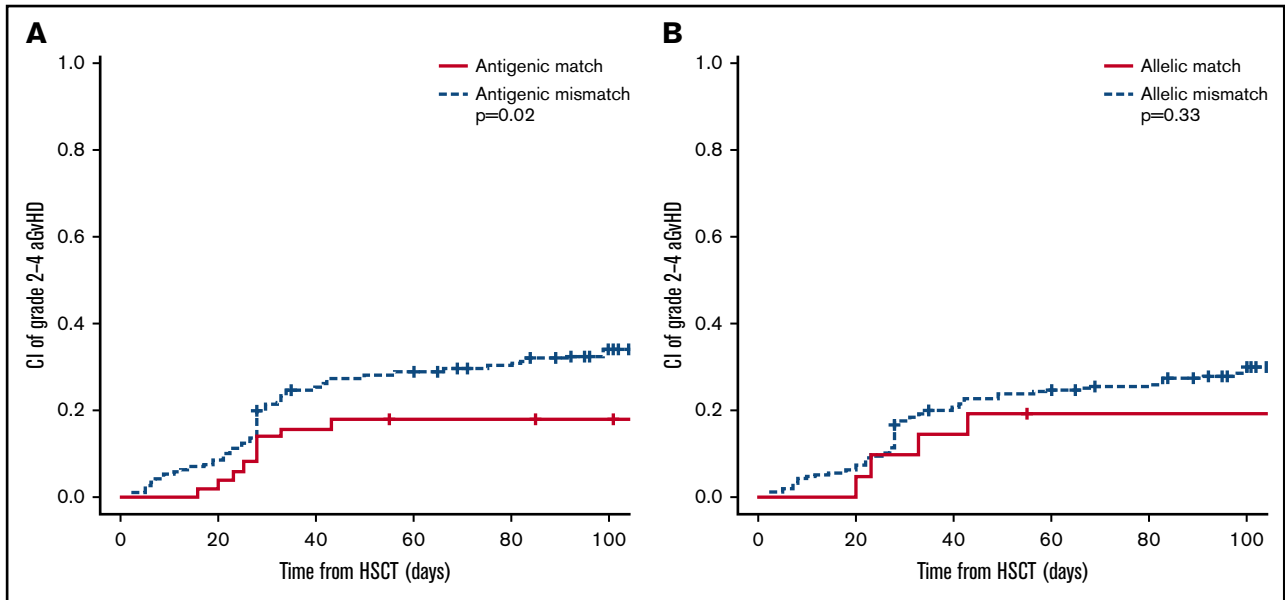


Figure 1. Influence of HLA-DRB1 matching status on aGVHD 2-4 in PTCy regimens. (A) Stratification according to antigenic HLA-DRB1 matching (N = 313). (B) Stratification according to allelic HLA-DRB1 matching (N = 258).

significant association we observed in this study between HLA-DRB1 mismatching and aGVHD was present at the antigenic but not at the allelic level, further suggesting that subtle molecular differences in HLA polymorphism are probably too weak to make a difference in the clinical setting of haplo-HSCT. Further biological investigations regarding T-cell alloreactivity in the haploidentical context are warranted to clarify this point.

Our finding is in contrast with the report by Kasamon et al, in which no significantly increased risk of aGVHD was associated with a HLA-DRB1 antigen mismatch on the unshared haplotype in

patients undergoing unmanipulated BM haplo-HSCT with a non-myeloablative regimen and PTCy as GVHD prophylaxis. This difference may be dictated by differences in the intensity of the conditioning regimen and graft source in the study by Kasamon et al²⁸: as shown in our current analysis, RIC regimens and BM transplants were both associated with a significant reduction of aGVHD incidence.

Perhaps the most important finding from our study is that the GVHD prophylaxis modulates the influence of different variables, including but not limited to HLA matching on outcome, in particular aGVHD.

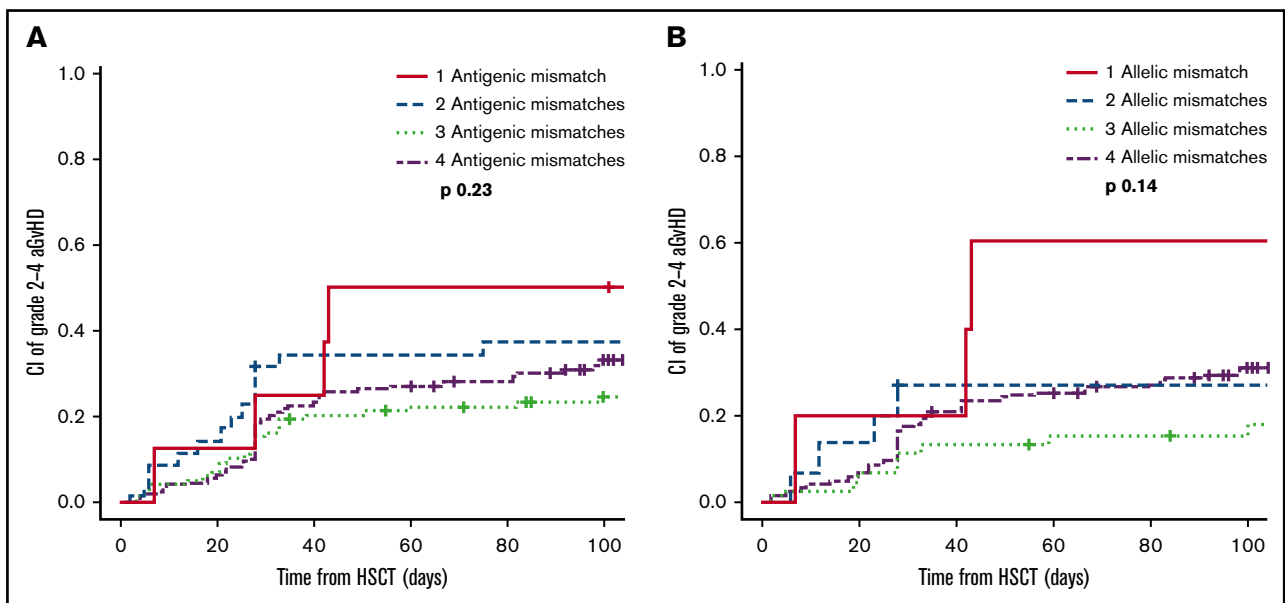


Figure 2. Influence of the number of HLA-A, B, C, DRB1 mismatches on aGVHD 2-4 in PTCy regimens. (A) Stratification according to the number of antigenic mismatches on the unshared haplotype (N = 313). (B) Stratification according to the number of allelic mismatches on the unshared haplotype (N = 258).

Table 3. Multivariate regression models for associations between HLA-matching status and clinical outcome in PTCy regimens

| | aGVHD ≥2 | | cGVHD | | TRM | | Relapse | | OS | | LFS | |
|---|---------------|--------------------|---------------|-----|---------------|------|---------------|--------------------|---------------|--------------------|---------------|--------------------|
| | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P |
| HLA-matching status at the antigenic level | | | | | | | | | | | | |
| HLA-A, mismatch vs match | 0.7 (0.4-1.1) | .14 | 0.9 (0.5-1.7) | .80 | 0.7 (0.4-1.1) | .14 | 1.3 (0.7-2.3) | .38 | 0.9 (0.6-1.3) | .48 | 0.9 (0.6-1.4) | .65 |
| HLA-B, mismatch vs match | 0.8 (0.4-1.5) | .51 | 1.4 (0.6-3.4) | .45 | 1.9 (0.7-4.9) | .20 | 0.7 (0.4-1.5) | .39 | 1.2 (0.6-2.1) | .62 | 1.1 (0.6-1.9) | .79 |
| HLA-C, mismatch vs match | 1.1 (0.6-2) | .70 | 1.6 (0.8-3.4) | .21 | 0.9 (0.5-1.9) | .87 | 1.5 (0.8-2.8) | .25 | 1.2 (0.7-2) | .49 | 1.2 (0.7-1.9) | .47 |
| HLA-DRB1, mismatch vs match | 2 (1.2-4) | .03* | 0.8 (0.5-1.4) | .40 | 0.7 (0.4-1.2) | .18 | 1.4 (0.8-2.6) | .25 | 1 (0.6-1.5) | .89 | 1 (0.7-1.6) | .84 |
| Leukemia diagnosis, ALL vs AML | - | - | - | - | 1.7 (1-2.8) | .06 | 1.5 (0.9-2.5) | .10 | 1.8 (1.2-2.6) | <10 ^{-2*} | 1.5 (1.1-2.2) | .03* |
| Disease status, advanced vs CR1 | - | - | - | - | 1.7 (1.1-2.9) | .05* | 5.2 (3-9.1) | <10 ^{-5*} | 3.4 (2.3-5.1) | <10 ^{-5*} | 3 (2-4.4) | <10 ^{-5*} |
| Stem cell source, PB vs BM | 2.2 (1.4-3) | <10 ^{-2*} | - | - | - | - | - | - | - | - | - | - |
| Donor sex, female vs male | 1.7 (1.1-2.8) | .04* | - | - | - | - | 0.6 (0.4-0.9) | .04* | 0.7 (0.5-0.9) | .04* | 0.7 (0.5-0.9) | .03* |
| CMV status, neg/neg vs other | - | - | - | - | - | - | - | - | - | - | - | - |
| Conditioning intensity, RIC vs MAC | 0.6 (0.4-0.9) | .04* | - | - | - | - | - | - | - | - | - | - |
| Recipient age, 10-y intervals | - | - | - | - | 1.2 (1-1.4) | .05 | - | - | - | - | - | - |
| HLA-matching status at the allelic level | | | | | | | | | | | | |
| HLA-A, mismatch vs match | 0.7 (0.4-1.5) | .42 | 0.9 (0.4-2.2) | .87 | 1.3 (0.5-3.2) | .54 | 1.3 (0.6-3) | .48 | 1.4 (0.7-2.7) | .31 | 1.3 (0.7-2.3) | .45 |
| HLA-B, mismatch vs match | 0.6 (0.2-1.5) | .26 | 2.2 (0.5-9.3) | .26 | 1.1 (0.3-4) | .99 | 1.1 (0.4-3.5) | .80 | 0.9 (0.4-2.2) | .86 | 1.2 (0.5-2.7) | .73 |
| HLA-C, mismatch vs match | 2.8 (0.9-8.8) | .06 | 1.4 (0.4-4.3) | .58 | 1.8 (0.5-6) | .35 | 1.4 (0.6-3.6) | .44 | 1.6 (0.8-3.5) | .22 | 1.6 (0.8-3.2) | .22 |
| HLA-DRB1, mismatch vs match | 1.3 (0.4-3.4) | .60 | 1.4 (0.5-3.8) | .54 | 0.6 (0.3-1.2) | .13 | 1.2 (0.5-3.1) | .68 | 0.7 (0.4-1.4) | .35 | 0.9 (0.5-1.5) | .54 |
| Leukemia diagnosis, ALL vs AML | - | - | - | - | 1.9 (1-3.4) | .05* | 1.5 (0.8-3.1) | .13 | 1.9 (1.2-3) | <10 ^{-2*} | 1.7 (1.1-2.5) | .02* |
| Disease status, advanced vs CR1 | - | - | - | - | 1.3 (0.7-2.4) | .36 | 5.2 (2.7-9.7) | <10 ^{-4*} | 2.9 (1.9-4.5) | <10 ^{-4*} | 2.6 (1.7-4) | <10 ^{-4*} |
| Stem cell source, PB vs BM | 2.8 (1.7-4.7) | <10 ^{-2*} | - | - | - | - | - | - | - | - | - | - |
| Donor sex, female vs male | 1.8 (1-3.2) | .05* | - | - | - | - | 0.6 (0.4-0.9) | .03* | 0.6 (0.4-0.8) | .02* | 0.6 (0.4-0.8) | .02* |
| CMV status, neg/neg vs other | - | - | - | - | - | - | - | - | - | - | - | - |
| Conditioning intensity, RIC vs MAC | 0.6 (0.3-0.9) | .04* | - | - | - | - | - | - | - | - | - | - |
| Recipient age, 10-y intervals | - | - | - | - | 1.3 (1-1.4) | .05 | - | - | - | - | - | - |

Results for HLA matching were adjusted for the covariates listed.

-, Specific clinical variable was not significant in the multivariate model for the examined endpoint.

*Statistically significant values.

Table 4. Multivariate regression models for associations between HLA-matching status and clinical outcome in ATG regimens

| | aGVHD ≥2 | | | cGVHD | | | TRM | | | Relapse | | | OS | | | LFS | | |
|---|---------------|------|--|---------------|-----|--|---------------|--------------------|--|-----------------|--------------------|--|---------------|--------------------|--|---------------|--------------------|--|
| | HR (95% CI) | P | | HR (95% CI) | P | | HR (95% CI) | P | | HR (95% CI) | P | | HR (95% CI) | P | | HR (95% CI) | P | |
| HLA-matching status at the antigenic level | | | | | | | | | | | | | | | | | | |
| HLA-A, mismatch vs match | 1.2 (0.5-2.7) | .70 | | 1.7 (0.8-4) | .20 | | 1 (0.4-2.4) | .99 | | 0.8 (0.4-1.8) | .62 | | 0.9 (0.5-1.7) | .80 | | 0.9 (0.5-1.6) | .72 | |
| HLA-B, mismatch vs match | 1.2 (0.4-3.5) | .72 | | 0.8 (0.3-2.4) | .74 | | 1 (0.3-2.9) | .97 | | 0.6 (0.2-1.8) | .39 | | 0.9 (0.4-2) | .78 | | 0.8 (0.4-1.7) | .53 | |
| HLA-C, mismatch vs match | 1.2 (0.6-2.5) | .66 | | 1.2 (0.6-2.4) | .69 | | 1.4 (0.6-3.1) | .41 | | 1.3 (0.5-3.2) | .55 | | 1.4 (0.7-2.5) | .34 | | 1.3 (0.7-2.4) | .35 | |
| HLA-DRB1, mismatch vs match | 1.4 (0.7-2.8) | .36 | | 1.2 (0.6-2.5) | .55 | | 2.3 (0.9-6) | .08 | | 0.7 (0.0-4-1.2) | .18 | | 1.2 (0.7-1.9) | .55 | | 1 (0.6-1.6) | .95 | |
| Leukemia diagnosis, ALL vs AML | - | - | | - | - | | 1.9 (1.1-3.3) | .03* | | 1.7 (1-3.1) | .06 | | 2 (1.4-3.1) | <10 ^{-2*} | | 1.8 (1.2-2.7) | .04* | |
| Disease status, Advanced vs CR1 | 2 (1.1-3.7) | .03* | | - | - | | 2.2 (1.2-4.1) | .01* | | 8.6 (4.2-17) | <10 ^{-5*} | | 3.9 (2.4-6.3) | <10 ^{-5*} | | 4.3 (2.8-6.9) | <10 ^{-5*} | |
| Stem cell source, PB vs BM | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| Donor sex, female vs male | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| CMV status, neg/neg vs other | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| Conditioning intensity, RIC vs MAC | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| Recipient age, each 10 y | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| HLA-matching status at the allelic level | | | | | | | | | | | | | | | | | | |
| HLA-A, mismatch vs match | 2.3 (0.5-11) | .29 | | 2 (0.4-9.1) | .38 | | 0.6 (0.2-1.7) | .31 | | 0.5 (0.2-1.7) | .29 | | 0.7 (0.3-1.7) | .46 | | 0.5 (0.2-1.2) | .11 | |
| HLA-B, mismatch vs match | 3.3 (0.7-16) | .13 | | 1.8 (0.5-6.7) | .39 | | 1.4 (0.4-4.7) | .59 | | 0.5 (0.1-1.6) | .21 | | 1.1 (0.5-2.8) | .78 | | 0.9 (0.4-2) | .74 | |
| HLA-C, mismatch vs match | 0.7 (0.3-1.9) | .49 | | 1.3 (0.5-3.5) | .61 | | 1.1 (0.4-2.9) | .89 | | 1.8 (0.5-6.3) | .34 | | 1.1 (0.5-2.4) | .87 | | 1.3 (0.6-2.7) | .56 | |
| HLA-DRB1, mismatch vs match | 0.4 (0.2-1.1) | .06 | | 0.9 (0.3-2.6) | .78 | | 1.1 (0.4-3.3) | .89 | | 0.8 (0.3-2.4) | .74 | | 0.9 (0.4-2) | .81 | | 0.9 (0.4-2) | .89 | |
| Leukemia diagnosis, ALL vs AML | - | - | | - | - | | 1.8 (1-3.3) | .05* | | 1.9 (1-3.7) | .05* | | 1.9 (1.2-3) | <10 ^{-2*} | | 1.7 (1.1-2.5) | .02* | |
| Disease status, advanced vs CR1 | 2 (1-4.1) | .05* | | - | - | | 2.6 (1.3-5) | <10 ^{-2*} | | 6.5 (2.9-14) | <10 ^{-4*} | | 3.4 (2.5-7) | <10 ^{-4*} | | 1.8 (1.2-2.7) | <10 ^{-2*} | |
| Stem cell source, PB vs BM | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| Donor sex, female vs male | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| CMV status, neg/neg vs other | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| Conditioning intensity, RIC vs MAC | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |
| Recipient age, each 10 y | - | - | | - | - | | - | - | | - | - | | - | - | | - | - | |

Results for HLA matching were adjusted for the covariates listed.

- . Specific clinical variable was not significant in the multivariate model for the examined endpoint.

*Statistically significant values.

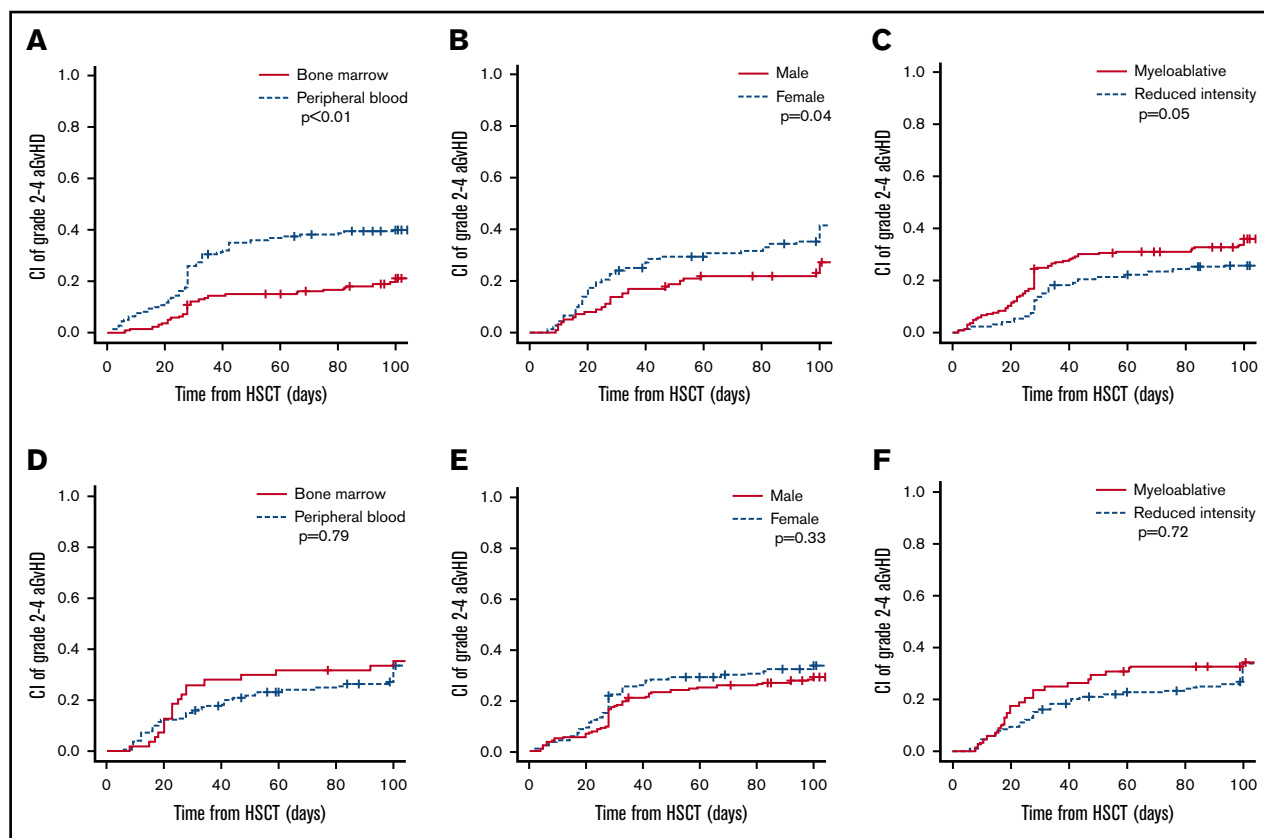


Figure 3. Influence of clinical variables on aGVHD 2-4 in unmanipulated HSCT. Stratification according to stem cell source (A,D), donor sex (B,E), and intensity of conditioning regimen (C,F) in PTCy regimens (A-C) or ATG regimens (D-F).

Indeed, our data suggest that the risk of aGVHD in PTCy but not ATG regimens is particularly sensitive to variables influencing T-cell alloreactivity including not only antigenic HLA-DRB1 matching status but also stem cell source (with peripheral blood stem cells likely containing a higher dose of administered T cells), conditioning intensity, and donor sex. This could reflect the different intrinsic mechanism by which T cells are eliminated globally or only after activation in the ATG and the PTCy regimen, respectively. It is interesting to note that of these variables, only donor sex also had an influence on relapse risk in PTCy regimen, which was not offset by a higher TRM and resulted in a disease-free survival advantage. This beneficial effect might imply peculiar biology of relapse after haploidentical transplantation which is frequently characterized by immune escape mechanisms including loss of the unshared HLA haplotype.⁴¹ This could suggest further investigation in the setting of PTCy platforms, where a globally reduced TRM could tilt the balance in favor of improved survival in patients grafted from female donors who could potentially exert a more potent GVL effect possibly being sensitized by previous pregnancies.

It should be noted that the population included in this study partially overlaps with the recently published data by Ruggeri et al,²⁷ in which haplo-HSCT using PTCy was compared with ATG platforms, showing significantly better LFS, lower incidence of GVHD, and nonrelapse mortality of the former regimen compared with the latter in patients with AML in complete remission. In the study by Ruggeri et al,²⁷ the incidence of aGVHD was not influenced by the stem cell source or intensity of conditioning regimens in multivariate analysis

adjusting for the adopted GVHD prophylaxis. We hypothesize that the inclusion of advanced status diseases and ALL patients in our cohort could partly explain these results. In addition, different statistical approaches were used in the 2 studies as we separately analyzed the outcomes according to the 2 GVHD platforms and focused on match status at each specific HLA locus as a main effect term in our multivariate model.

Our study has several limitations. In particular, although this is the largest number of haploidentical transplants in which the HLA effect and the GVHD prophylaxis effect has been studied so far, the study might still have limited power to detect subtle associations, in particular for the transplants with high-resolution allele typing which represented an 85% subgroup of the entire cohort.

For the same reasons, we were unable to analyze whether transplantation outcomes in our cohort could be influenced by the permissibility of HLA disparities depending on their immunogenicity. Larger cohorts and implementation with functional biological assays are needed to examine whether prioritization of haploidentical donors should be based on specific mismatches predicted to elicit differential T-cell allorecognition.

Moreover, we were unable to assess the impact of HLA-DQ and -DP because the relevant typing data were not available. Even if HLA-DQ is in strong linkage disequilibrium with other HLA class II loci, we cannot exclude that we missed some information potentially derived from this locus. For HLA-DP, where the existence of permissive mismatches based on functional data has been reported to be associated with clinical outcome of unrelated HSCT,⁴²⁻⁴⁴ we

could not analyze whether this applied also to our haploidentical cohort. The role of noninherited maternal antigens⁴⁵ could also not be investigated here because only a small minority of patients had a complete HLA familiar typing that could allow assignment of mismatched haplotypes, given that parents are not usually typed, particularly for adult patients.

The clinical success obtained with haploidentical transplantation both with the ATG and the PTCy conditioning regimen platform has allowed this potentially curative treatment modality to be offered over recent years to many patients who before were precluded from it. This has led to a dramatic increase in the clinical application and relevance of this therapeutic approach not only in Europe, with over 1500 such transplants performed in 2014 alone,⁴⁶ but also worldwide, in particular in the United States and China. The finding from this study that the role of HLA mismatching on the unshared is not sufficiently prominent to justify its consideration in haploidentical donor selection, and that the role of HLA and other variables appears to be modulated by the adopted GVHD prophylaxis, will be of clinical relevance in this setting. Retrospective and prospective confirmation of these data in further homogeneous and well-sized cohorts are clearly warranted.

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Authorship

Contribution: F.L. designed, performed, and coordinated the research, and collected, analyzed, and interpreted the data; F.L. and K.F. wrote the manuscript; M.L. designed the research and performed the statistical analyses; F.C., K.F., A.N., and M.M. designed the research, contributed to data, and commented on the manuscript; C.R.M. performed the high-resolution HLA imputation; A.R., A.B., and M.B. commented on the manuscript; and Z.G., Y.K., W.A., B.B., J.T., A.B., D.B., G.M., A.S., M.B., and D.W.B. contributed to data.

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