



Histocompatibility

Donor HLA-E Status Associates with Disease-Free Survival and Transplant-Related Mortality after Non In Vivo T Cell-Depleted HSCT for Acute Leukemia

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Previous studies have suggested that HLA-E may have a significant role in the outcome of matched unrelated hematopoietic stem cell transplantation (HSCT), especially for patients with acute leukemia. We used Center for International Blood and Marrow Transplant Research data and samples of 1840 adult patients with acute leukemia and their 10/10 HLA-matched unrelated donors to investigate the impact of HLA-E matching status as well as of donor/recipient (D/R) HLA-E genotype on post-HSCT outcome. Both patients and donors were HLA-E genotyped by next-generation sequencing. All patients received their first transplant in complete remission between 2000 and 2015. Median follow-up time was 90 months. Overall survival, disease-free survival (DFS), transplant-related mortality (TRM), and relapse incidence were primary endpoints with statistical significance set at .01. D/R HLA-E genotype analysis revealed a significant association of donor HLA-E*01:03/01:03 genotype with DFS (hazard ratio [HR] = 1.35, $P = .0006$) and TRM (HR = 1.41, $P = .0058$) in patients who received T cell replete (ie, without in vivo T cell depletion) transplants ($n = 1297$). As for D/R HLA-E matching, we did not identify any significant effect on any of the clinical outcome endpoints. In conclusion, this is the largest study to date reporting an improvement of DFS and TRM after matched unrelated HSCT by avoidance of HLA-E*01:03 homozygous donors in patients transplanted with T cell replete grafts for acute leukemia.

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INTRODUCTION

HLA-E is a nonclassical HLA class Ib antigen-presenting molecule with a multifaceted albeit not fully explored immunomodulatory role. HLA-E is practically identical to its HLA class Ia counterparts consisting of a 3-domain α heavy chain and an invariant β 2-microglobulin light chain [1,2] It is

constitutively expressed on the surface of immune and endothelial cells [3], but its expression can be ubiquitously induced under inflammatory conditions [4,5]. HLA-E is also considered a surrogate marker of HLA class I expression, as under normal conditions, it mainly presents peptides from the leader sequences of classical HLA class I molecules [2]. Despite the structural similarities, HLA-E exhibits minimal polymorphism and significantly lower expression levels compared with its classical HLA class Ia paralogues, with 43 alleles and 11 distinct proteins identified until now (IMGT/HLA Database). Only 2 isoforms predominate worldwide, HLA-E*01:01 and HLA-E*01:03, which differ in 1 amino acid position in the α 2 heavy

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chain domain. This minimal polymorphism appears to affect the functional and expression features of the 2 alleles through unclear mechanisms [6]. The surface expression of HLA-E*01:01 is significantly lower compared with that of HLA-E*01:03, whereas the 2 alleles have been found to exhibit distinct peptide binding affinity profiles, which in turn could affect their interaction with their corresponding receptors [7–11]. HLA-E has a multifaceted immunomodulatory role. On one hand, it is the only known ligand to the potent inhibitory heterodimeric receptor CD94/NKG2A. On the other hand, HLA-E has been found to participate in immune activation through its interaction with the activating CD94/NKG2C receptor as well as with HLA-E restricted T cell receptors [12]. It is also of note that newly generated donor natural killer (NK) cells first express the NKG2A receptor on their surface with all other NK receptors appearing gradually at a later time point [13–16]. Despite its evident importance from an immunologic standpoint, HLA-E has been investigated only sporadically with regard to its role in hematopoietic stem cell transplantation (HSCT) outcome in a rather small number of heterogeneous studies with often discordant results [17–25]. In our recently published work [25] on the effect of HLA-E polymorphism after a 10/10 HLA-matched unrelated HSCT in a German cohort of 509 patients with acute leukemia, we identified a potentially beneficial effect of HLA-E incompatibility between recipient and donor. Significantly lower nonrelapse mortality rates accounted for the better overall survival (OS) in the HLA-E mismatched cases. The HLA-E mismatch effect was primarily significant in the advanced disease group, whereas analysis of the impact of patient/donor HLA-E genotype on HSCT outcome revealed an association between patient HLA-E*01:03/01:03 genotype and lower survival probability. Herein we conducted a multicenter retrospective analysis of the outcome of 1840 patients with acute leukemia in complete remission undergoing 10/10 HLA-matched unrelated HSCT, using clinical data and samples from the Center for International Blood and Marrow Transplant Research (CIBMTR), with the aim to address the following questions: (1) Does HLA-E match status between recipient and donor affect HSCT outcome? (2) Does a specific HLA-E genotype in patients and/or donors correlate with outcome?

MATERIALS AND METHODS

Study Population and Clinical Data

Adult patients (N = 1840) diagnosed with acute myeloid leukemia (AML) (n = 1379) and acute lymphoblastic leukemia (ALL) (n = 461) in complete remission undergoing their first HSCT from a 10/10 HLA-matched unrelated donor between 2000 and 2015 were eligible for inclusion in the study cohort. All patients received unmanipulated (ie, without ex vivo T cell depletion) bone marrow or peripheral blood stem cell grafts. All clinical data were obtained from the CIBMTR and initially acquired after signed consent of both recipients and donors in accordance with the Declaration of Helsinki. The study was approved by the National Marrow Donor Program Institutional Review Board in conformity with the federal regulation regarding the protection of human research participants as well as the ethical review board of the University of Ulm (project number 227/16).

Definitions

Early disease stage was defined as AML or ALL transplanted in first complete remission, and intermediate disease stage was defined as AML or ALL transplanted in second complete remission or more. Patients with advanced disease stage, defined as in relapse or primary induction failure, were excluded. Myeloablative conditioning was defined as treatment with total body irradiation ≥ 500 cGy in a single fraction or ≥ 800 cGy fractionated, and/or busulfan (Bu) ≥ 9 mg/kg orally or i.v. equivalent, and/or melphalan > 140 mg/m², or combinations of Bu/cyclophosphamide and cyclophosphamide/total body irradiation. Less intense regimen treatments were classified as reduced-intensity conditioning [26].

HLA Typing and DPB1 Matching

Patients and donors were HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1 genotyped at second field resolution level. Only transplant pairs compatible for the loci HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 at the allelic level (10/10 HLA-matched) were included in the study. HLA-DPB1 mismatches were checked for permissiveness by applying the T cell epitope as previously described [27], using the online DPB1 T cell epitope web tool from the IMGT/HLA database (<https://www.ebi.ac.uk/ipd/imgt/hla/dpb.html>).

HLA-E Typing

Both patients and their unrelated donors were HLA-E genotyped by next-generation sequencing (NGS) on an Illumina (San Diego, CA, USA) Miseq platform using the protocol of a CE-certified H-Seq NGS in-house kit for HLA high-resolution typing at the Department of Transplantation Immunology of the Institute for Transfusion Medicine and Immunogenetics in Ulm, Germany. HLA-E-specific primers were designed for complete exon 2 and 3 sequencing analysis that enabled precise assignment of all known alleles. The oligonucleotide sequences of the 2 sets of forward and reverse HLA-E-specific NGS primers are as follows: exon 2, forward 5'-3': GGAGGAGGGTCCGGCCGATCTC; exon 2, reverse 5'-3': ACCCGAAGATTCGAGGGGACCCGC; exon 3, forward 5'-3': GGCTTGGTGGCGGGACTGACTAAG; and exon 3, reverse 5'-3': GAGAGTAGCCCTGTGGACCTCTTAC (Metabion International AG, Martinsried, Germany). Sequencing data analysis was carried out by SeqPilot-NGS-Software (JSI Medical Systems, Ettenheim, Germany). IPD-IMGT/HLA databank version 3.28 (European Bioinformatics Institute (EMBL-EBI), Cambridgeshire, UK) was used for HLA-E allele assignment.

Outcome Endpoints

OS, disease-free survival (DFS), transplant-related mortality (TRM), relapse, acute graft-versus-host disease (aGVHD) grades II to IV, and chronic GVHD (cGVHD) were set as clinical outcome endpoints. OS was defined as time to death from any cause. DFS was defined as time to treatment failure with death or relapse counting as events. TRM was defined as the time to death without evidence of disease relapse, with the latter constituting a competing risk. Disease recurrence or persistence was defined as relapse. This event was summarized by cumulative incidence estimate with TRM as the competing risk. The cumulative incidence of aGVHD grades II to IV, according to the Glucksberg grading criteria, and cGVHD were calculated with death as the competing risk. Patients were censored at date of last follow-up.

Statistical Analysis

The probabilities of OS and DFS were estimated using the Kaplan-Meier estimator, with the variance estimated by Greenwood's formula. Cumulative incidences of TRM, relapse, aGVHD, and cGVHD were estimated to account for competing risks. Nonrelapse death was a competing risk in the estimation of malignancy relapse, death was a competing risk for graft-versus-host disease (GVHD), and relapse was a competing risk for estimation of TRM. Comparison of survival curves was done using the log-rank test. Comparison of cumulative incidence curves was done based on Fine-Gray's model. Chi-square tests were used to compare the distributions of discrete factors between the HLA-E matched versus HLA-E mismatched groups. For continuous factors, the median and ranges were also calculated. The Kruskal-Wallis test was used to compare the continuous factors between the HLA-E matched versus HLA-E mismatched groups. HLA-E genotype effects in donors and recipients were tested separately and in a joint analysis. Allele-specific mismatches in the GVHD direction were also examined. Transplant pairs with HLA-E genotypes, including other HLA-E alleles (ie, other than HLA-E*01:01 or *01:03), were excluded from this analysis.

Multivariate analyses were performed using Cox's proportional hazards model. All variables were tested for the affirmation of the proportional hazards assumption (PHA). Factors violating the PHA were adjusted via stratification. A stepwise model-building approach was subsequently used to select variables for the primary and secondary outcomes with a threshold of 0.05 for both entry and retention in the model. All predictors considered for model integration are presented in detail in the supplementary material. No significant interactions between the tested variables (ie, HLA-E match and HLA-E genotype) and the adjusted covariates were detected in any of the models. To adjust for multiple testing, a significance level of .01 was used for association of a main variable. The statistical software SAS version 9.4 (SAS Institute, Cary, NC) was used for all the analyses.

RESULTS

Cohort Characteristics

The demographic, clinical, and immunogenetic features of the study cohort are summarized in Table 1. Median age was 46 years (range, 18 to 77), whereas median follow-up time was 90 months (range, 9 to 185 months). Less than 30% of patients received in vivo T cell depletion (TCD) treatment with

Table 1
Cohort Characteristics

Variable	Value
Number of patients	1840
Number of centers	122
Patient related	
Recipient age at transplant, yr	
Median (range)	46 (18-77)
Recipient sex	
Male	975 (53.0)
Female	865 (47.0)
Recipient race	
White	1732 (94.1)
African American	31 (1.7)
Asian/Pacific Islander	29 (1.6)
Native American	5 (0.3)
Other	4 (0.2)
Missing	39 (2.1)
Karnofsky score prior to transplant	
<90	540 (29.3)
≥90	1209 (65.7)
Missing	91 (5.0)
Disease related	
Disease	
AML	1379 (75.0)
ALL	461 (25.0)
Disease status	
Early	1265 (68.8)
Intermediate	575 (31.2)
Transplant related	
Graft type	
Bone marrow	469 (25.5)
Peripheral blood	1371 (74.5)
Donor age at transplant, yr	
Median (range)	31 (18-61)
Donor-recipient sex match	
M-M	724 (39.4)
M-F	552 (30.0)
F-M	251 (13.6)
F-F	313 (17.0)
T cell epitope matching [27]	
Fully matched	292 (15.9)
Permissive	807 (43.9)
GvH nonpermissive	315 (17.1)
HvG nonpermissive	329 (17.9)
Missing	97 (5.2)
Donor-recipient CMV match	
-/-	508 (27.6)
-/+	679 (36.9)
+/-	204 (11.1)
+/+	414 (22.5)
Missing	35 (1.9)
GVHD prophylaxis	
Tacrolimus based	1502 (81.6)
Cyclosporine based	305 (16.6)
Other GVHD prophylaxis	11 (0.6)
Missing	22 (1.2)
ATG/Campath	
ATG + Campath	1 (<0.1)
ATG alone	490 (26.6)

(continued)

Table 1 (Continued)

Variable	Value
Campath alone	50 (2.7)
No ATG or Campath	1297 (70.5)
Missing	2 (0.1)
Conditioning intensity	
MAC	1414 (76.9)
RIC	426 (23.1)
Year of transplant	
2000-2007	974 (52.9)
2008-2015	866 (47.1)
Time from diagnosis to transplant	6 (<1-252)
Median follow-up of survivors (range), mo	90 (9-185)

Values are presented as number (%) unless otherwise indicated.

M indicates male; F, female; GvH, graft versus host; HvG, host versus graft; CMV, cytomegalovirus; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

antithymocyte globulin (ATG) or Campath (Sanofi Genzyme, Cambridge, MA, USA), whereas about 77% were treated with a myeloablative conditioning regimen. Peripheral blood was the graft source in about two thirds of the cases. The characteristics of the HLA-E matched versus HLA-E mismatched groups were balanced for all of the clinically relevant parameters considered (Supplementary Table S1).

HLA-E Genotyping Results

The HLA-E genotyping results for recipients and donors are summarized in Table 2. The 2 predominant isoforms, HLA-E*01:01 and HLA-E*01:03, comprised >99% of cases in both patients and donors. Three new alleles were identified, whereas no genotyping result was possible for 2 transplant pairs due to poor sample quality and unsuccessful DNA isolation. The frequencies found in recipients and donors were similar and in accordance with those previously reported [28,29]. The loose linkage disequilibrium between HLA-E and HLA class I was confirmed once more [25], with 32.5% (n = 598) of transplant pairs being HLA-E discordant.

HLA-E*01:03/01:03 Genotype Is Associated with Lower DFS

To assess the effect of donor and patient HLA-E genotype on HSCT outcome, we excluded from the analysis transplant pairs with rare or unidentified HLA-E genotypes (n = 13), leaving 1827 patients and their unrelated donors in the analysis. The respective effect of donor and recipient HLA-E genotype on outcome was analyzed in separate multivariate models. HLA-E genotype was overall significantly associated with DFS probability, whereas the impact on other clinical outcome endpoints did not reach statistical significance (data not shown), with the exception of relapse incidence, where a trend was detected. Specifically, the HLA-E*01:03 homozygous genotype in both donors and recipients was found to have an unfavorable association with DFS (hazard ratio [HR] = 1.28, P = .0027 and HR = 1.31, P = .0017, respectively) in the separate analyses of donor and recipient HLA-E genotype effect, where the HLA-E*01:01/01:01 genotype was set as baseline. Pairwise comparisons of the heterozygous (HLA-E*01:01/01:03) donors showed more favorable DFS compared with HLA-E*01:03/01:03 donors in a statistically significant manner (HR = 0.79, P = .0022), whereas no such effect was seen in patients (HR = 0.90, P = .1678). This finding is suggestive of a detrimental impact of donor HLA-E*01:03/01:03 genotype along with a putatively protective effect of patient HLA*01:01/01:01 genotype. Although overall impact of HLA-E genotype on relapse did not reach formal

Table 2
HLA-E Genotyping Results

HLA-E Genotypes, n (%)								
HLA-E*	01:01/01:01	01:01/01:03	01:03/01:03	01:01/01:05	01:03/01:05	01:01/01new	01:03/01new	Missing
Patients	592 (32.2)	915 (49.7)	324 (17.6)	3 (0.16)	0 (0.00)	1 (0.05)	2 (0.11)	3 (0.16)
Donors	583 (31.7)	916 (49.8)	337 (18.3)	1 (0.05)	1 (0.05)	0 (0.00)	0 (0.00)	2 (0.11)

statistical significance in either donor or recipient multivariate models (overall level), HLA-E*01:03/01:03 genotype in both donors and recipients associated with statistically significant higher risk of relapse when individually evaluated. After stepwise backward exclusion, the covariates maintained in the multivariate models for DFS were recipient age, conditioning intensity, disease, and Karnofsky score, whereas the analysis was stratified on donor/recipient sex match and graft type as they violated the PHA. For relapse incidence analysis, significant covariates included donor/recipient cytomegalovirus match, conditioning intensity, disease status, time from diagnosis to HSCT, and Karnofsky score, whereas the analysis was stratified for disease type due to violation of the PHA. The multivariate results of donor/recipient (D/R) HLA-E genotype associations for DFS are presented in Table 3. The results for relapse are presented in Supplementary Table S3 along with a table summarizing the effect of D/R HLA-E genotype on all clinical outcome endpoints assessed (Supplementary Table S4).

Donor HLA-E Genotype Is More Important

Due to the high correlation between recipient and donor HLA-E genotype ($P < .0001$), we also explored the joint effect of recipient and donor HLA-E genotype through a 4-level joint D/R analysis: (a) donor = 01:01+, patient = 01:01+ (set as baseline); (b) donor = 01:01+, patient = 01:03/01:03; (c) donor = 01:03/01:03, patient = 01:01+; and (d) D/R = 01:03/01:03, where 01:01+ = HLA-E*01:01/01:01 or *01:01/01:03. This joint D/R HLA-E genotype analysis revealed that it is the donor HLA-E genotype mostly driving the results, as patient HLA-E*01:03/01:03

genotype was not associated with worse DFS when combined with donor HLA-E* genotype other than 01:03/01:03 (ie, donor HLA-E*01:01+, patient HLA-E*01:03/01:03, HR = 1.08, $P = .4906$). The results of relapse incidence analysis did not meet the stringent criteria for statistical significance but were in line with those of DFS, with donor HLA-E*01:03/01:03 cases exhibiting higher risk of relapse regardless of patient HLA-E genotype. The multivariate results of joint D/R HLA-E genotype analysis for DFS are presented in Table 3. Those for relapse are presented in Supplementary Table S3. No interactions between donor and recipient HLA-E genotype were identified in the joint analysis. A visual presentation of the joint D/R HLA-E genotype analysis for DFS is depicted in Figure 1. The corresponding data regarding the DFS rates for years 1, 2, 3, 4, and 5 after transplantation are summarized in Table 4. The distribution of most clinically relevant parameters in patients transplanted with HLA-E*01:03/01:03 versus those receiving HLA-E*01:01/01:01 and/or *01:01/01:03 grafts was balanced. These data are summarized in Supplementary Table S5.

In Vivo T Cell Depletion Abrogates the Role of Donor HLA-E Genotype

To further explore the possible mechanism implicated in the observed association between donor HLA-E genotype and DFS, we analyzed separately the patients who received T cell-depleted grafts with either ATG or Campath ($n = 540$) from those who received no in vivo TCD treatment ($n = 1297$). These analyses revealed that the donor HLA-E genotype was only relevant in T cell replete transplantations, whereas donor HLA-E

Table 3
Analysis of D/R HLA-E Genotype Effect on DFS

Factor	N	Event	HR	95% Confidence Interval	P Value
1. Donor HLA-E genotypes					.0039
01:01/01:01	577	368	1.00		
01:01/01:03	905	573	1.01	0.89-1.16	.8348
01:03/01:03	333	240	1.28	1.09-1.51	.0027
01:03/01:03 versus 01:01/01:03			1.27	1.09-1.47	.0022
2. Recipient HLA-E genotypes					.0045
01:01/01:01	584	345	1.00		
01:01/01:03	908	608	1.18	1.03-1.34	.0164
01:03/01:03	318	224	1.31	1.11-1.55	.0017
01:03/01:03 versus 01:01/01:03			1.11	0.96-1.30	.1678
3. Joint D/R HLA-E genotypes					.0055
(a) Dnr = 01:01+	1352	853	1.00		
Pat = 01:01+					
(b) Dnr = 01:01+	123	82	1.08	0.86-1.36	.4906
Pat = 01:03/01:03					
(c) Dnr = 01:03/01:03	136	98	1.29	1.04-1.59	.0184
Pat = 01:01+					
(d) Dnr = 01:03/01:03	195	142	1.31	1.09-1.56	.0035
Pat = 01:03/01:03					

01:01+ = HLA-E*01:01/01:01 and/or *01:01/01:03, 01:03/01:03 = HLA-E*01:03/01:03. Statistical significance is marked with bold (ie, $P < .01$). Adjusted variables: patient age, disease type, conditioning intensity, Karnofsky score, donor/recipient sex match, graft type. Pairwise comparisons: d versus c, HR = 1.01, 95% confidence interval = 0.78 to 1.31, $P = .9170$.

Dnr indicates donor; Pat, patient.

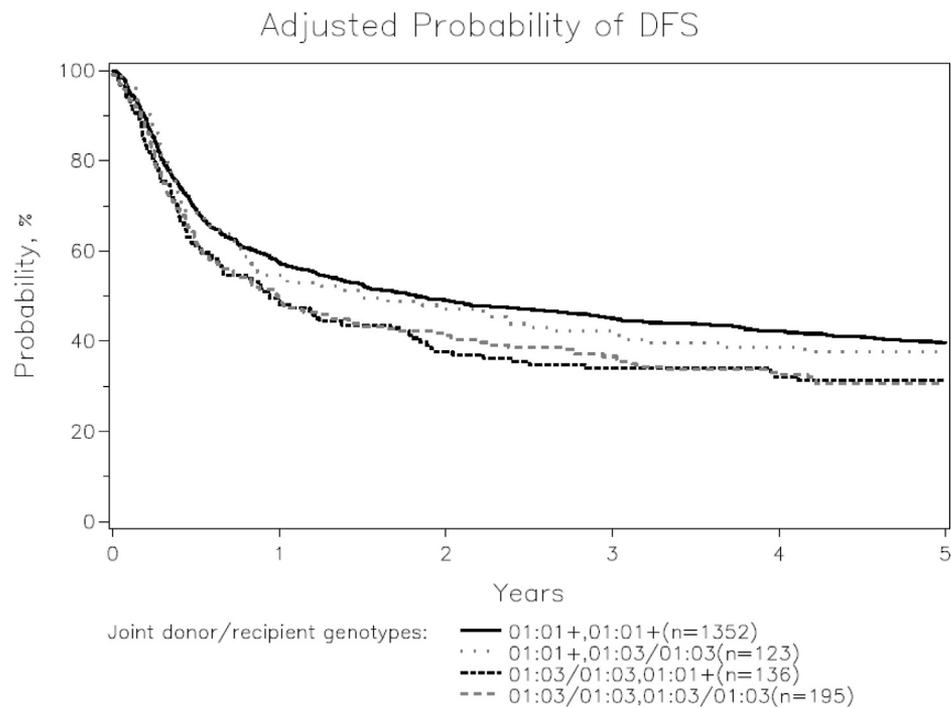


Figure 1. Joint D/R HLA-E genotype analysis and DFS. Adjusted curves of DFS probability with respect to joint D/R HLA-E genotype analysis. 01:01+, 01:01+ = donor and recipient HLA-E*01:01/01:01 and/or *01:01/01:03 (n = 1352); 01:01+, 01:03/01:03 = donor HLA-E*01:01/01:01 and/or *01:01/01:03 and recipient HLA-E*01:03/01:03 (n = 123); 01:03/01:03, 01:01+ = donor HLA-E*01:03/01:03 and recipient HLA-E*01:01/01:01 and/or *01:01/01:03 (n = 136); 01:03/01:03, 01:03/01:03 = donor and recipient HLA-E*01:03/01:03 (n = 195). Data on DFS rates are summarized in Table 4.

genotype appeared to also correlate with TRM in this subset of patients. Furthermore, this interaction between HLA-E genotype and in vivo TCD underscored the predominant role of the donor's HLA-E genotype, as the recipient's HLA-E genotype did not associate with any HSCT outcome in these subset analyses (see Supplementary Table S6). The results of the aforementioned multivariate models of donor HLA-E genotype for DFS and TRM are summarized in Tables 5 and 6, respectively. Given that the previous analyses showed that no significant differences were observed between HLA-E*01:01/01:01 and HLA-E*01:01/01:03 donors, these 2 levels were conjoined in the in vivo TCD analysis. For TRM analysis, significant covariates included donor and recipient age, disease type, disease status, time from diagnosis to HSCT, and GVHD prophylaxis, whereas the analysis was stratified for D/R sex match and graft type due to violation of the PHA.

HLA-E Matching Status between Recipient and Donor Is Not Associated with HSCT Outcome

No particular effect of D/R HLA-E incompatibility was identified on any clinical endpoint and in any of the analysis models

(Supplementary Table S2). No interaction was observed between HLA-E matching status and the covariates analyzed in the multivariable models. These findings were also repeated in the in vivo TCD subanalysis (see Supplementary Table S6).

DISCUSSION

The impact of HLA-E polymorphism on unrelated HSCT outcome has been investigated only sporadically by a rather small number of studies and therefore is yet to be clearly defined. Our study is, to our knowledge, the largest to address this question in an acute leukemia population receiving transplants from 10/10 HLA-matched unrelated donors. Although we did not detect any effect of D/R HLA-E matching status, donor and recipient HLA-E*01:03 homozygous genotype was associated with significantly lower DFS and higher risk of relapse compared with the other two genotypes (ie, HLA-E*01:01 and HLA-E*01:01/01:03). Joint D/R HLA-E genotype analysis disclosed that it is the donor HLA-E genotype mainly driving the results. Further subanalysis on account of in vivo TCD revealed that this association was detectable only in T cell replete transplantations. Interestingly, donor HLA-E genotype also associated with TRM in this subset of

Table 4
DFS Rates after Transplantation by Joint D/R HLA-E Genotype Groups

Group	1 Year (%)	2 Years (%)	3 Years (%)	4 Years (%)	5 Years (%)
Dnr = 01:01+	57.5 (54.9, 60.1)	49.1 (46.6, 51.8)	45.1 (42.5, 47.7)	42.2 (39.6, 44.9)	39.7 (37.1, 42.3)
Pat = 01:01+	n = 767	n = 641	n = 561	n = 489	n = 418
Dnr = 01:01+	54.7 (46.0, 63.4)	47.2 (38.5, 55.9)	42.2 (33.6, 50.8)	38.7 (30.2, 47.1)	37.7 (29.3, 46.2)
Pat = 01:03/01:03	n = 68	n = 59	n = 48	n = 41	n = 38
Dnr = 01:03/01:03	48.8 (40.7, 56.9)	37.8 (29.9, 45.7)	34.1 (26.4, 41.8)	32.3 (24.6, 39.9)	31.3 (23.7, 38.9)
Pat = 01:01+	n = 65	n = 49	n = 41	n = 34	n = 32
Dnr = 01:03/01:03	49.0 (42.2, 55.8)	41.3 (34.6, 48.0)	36.6 (30.0, 43.1)	33.2 (26.8, 39.7)	30.8 (24.4, 37.2)
Pat = 01:03/01:03	n = 95	n = 80	n = 67	n = 58	n = 47

01:01+ = HLA-E*01:01/01:01 and/or *01:01/01:03, 01:03/01:03 = HLA-E*01:03/01:03.

Table 5
Analysis of Donor HLA-E Effect on DFS in T Cell Replete Transplants

Factor	Level	Count	Event	HR	HR_Low	HR_Up	P Value
Donor HLA-E genotypes	Overall*0006
01:01+	1+3	1044	649	1.00	.	.	.
01:03/01:03	2	232	169	1.35	1.14	1.60	.0006
Recipient age at transplant	Overall*0001
18-29	1	275	165	1.00	.	.	.
30-39	2	206	108	0.82	0.64	1.04	.1044
40-49	3	320	200	1.11	0.90	1.38	.3134
50-59	4	320	230	1.35	1.09	1.66	.0051
60+	5	155	115	1.50	1.14	1.98	.0042
Conditioning intensity	Overall*0204
MA	1	1077	667	1.00	.	.	.
RIC	2	199	151	1.28	1.04	1.58	.0204
Disease	Overall*0421
AML	10	934	588	1.00	.	.	.
ALL	20	342	230	1.18	1.01	1.38	.0421
Karnofsky score	Overall*0179
<90%	1	370	250	1.00	.	.	.
≥90%	2	838	515	0.87	0.75	1.01	.0756
Missing	99	68	53	1.25	0.93	1.69	.1423

01:01+ = HLA-E*01:01/01:01 and/or *01:01/01:03, 01:03/01:03 = HLA-E*01:03/01:03. For this analysis, levels 1 and 3 (ie, HLA-E*01:01/01:01 and *01:01/01:03, respectively) of the donor HLA-E genotype were collapsed in 1 level as pairwise comparison revealed no differences between the 2 groups. Statistical significance is marked with bold (ie, $P < .01$). Covariable's cutoff for inclusion in model is $P < .05$.

MA indicates myeloablative.

* The analysis was stratified on donor/recipient sex match and graft type due to PHA violation.

Table 6
Analysis of Donor HLA-E Effect on TRM in T Cell Replete Transplants

Factor	Level	Count	Event	HR	HR_Low	HR_Up	P Value
Donor HLA-E genotypes	Overall*0058
01:01+	1+3	1038	306	1.00	.	.	.
01:03/01:03	2	232	84	1.41	1.11	1.81	.0058
Recipient age at transplant	Overall*	<.0001
18-29	1	274	71	1.00	.	.	.
30-39	2	204	48	0.83	0.57	1.20	.3240
40-49	3	320	95	1.20	0.87	1.64	.2654
50-59	4	320	114	1.65	1.21	2.25	.0014
60+	5	152	62	2.36	1.64	3.39	<.0001
Donor age	Overall*0117
18-32	1	719	190	1.00	.	.	.
33-49	2	470	171	1.35	1.10	1.67	.0051
≥50	3	81	29	1.42	0.95	2.11	.0874
Disease	Overall*0748
AML	10	930	276	1.00	.	.	.
ALL	20	340	114	1.23	0.98	1.55	.0748
Disease status	Overall*0151
Early	1	843	275	1.00	.	.	.
Intermediate	2	427	115	0.71	0.54	0.94	.0151
GVHD prophylaxis	Overall*2054
TAC + other(s)	47	1018	300	1.00	.	.	.
CSA + other(s)	81	241	83	1.14	0.89	1.46	.2921
Missing	99	11	7	1.80	0.83	3.89	.1354
Time from diagnosis to transplant, mo	Overall*0203
≤6.25	1	633	186	1.00	.	.	.
>6.25	2	637	204	1.34	1.05	1.71	.0203

01:01+ = HLA-E*01:01/01:01 and/or *01:01/01:03, 01:03/01:03 = HLA-E*01:03/01:03. For this analysis, levels 1 and 3 (ie, HLA-E*01:01/01:01 and *01:01/01:03, respectively) of the donor HLA-E genotype were collapsed in 1 level as pairwise comparison revealed no differences between the 2 groups. Statistical significance is marked with bold (ie, $P < .01$). Covariable's cutoff for inclusion in model is $P < .05$.

TAC indicates tacrolimus; CSA, cyclosporine.

* The analysis was stratified on donor/recipient sex match and graft type due to PHA violation.

patients. Despite a noticeable trend, neither recipient nor donor HLA-E genotype appeared to influence OS substantially. No statistically significant effect was identified with respect to aGVHD and cGVHD. In line with previous reports [30,31], nonpermissive HLA-DPB1 mismatches were correlated with higher risk of aGVHD grades II to IV. It is of note that no potential confounding between HLA-E and HLA-DPB1 matching was identified.

These findings contrast with our recent study on a similar German cohort [25], which also consisted of patients with acute leukemia receiving 10/10 HLA-matched unrelated HSCT transplant grafts, in whom we detected an unexpected beneficial effect of HLA-E mismatch between recipient and donor on OS, which correlated primarily with significantly reduced non-relapse mortality and was mainly observed in the subset of advanced disease patients. On the other hand, a detrimental effect of patient HLA-E*01:03/01:03 genotype on HSCT outcome was detected in the multivariate models of both studies. The additional joint D/R HLA-E genotype analysis in the CIBMTR study made clear that the patient HLA-E genotype contribution to the overall effect is mainly driven by the highly associated donor HLA-E genotype. The latter had no significant effect in the German cohort. The use of ATG in the majority of the German cohort patients possibly accounts for this discordance. The 2 cohorts, apart from ATG use, also differed in disease status. Due to differences in the definition of advanced disease between the German (ie, transplant in more than second complete remission, more than first relapse) and the American cohorts (ie, AML or ALL transplanted in relapse or primary induction failure), no patients with advanced disease stage were included in the CIBMTR cohort. Even though no interaction was found between HLA-E matching status and these parameters in any of the multivariate models, one cannot exclude the possibility that an ATG-related effect may only be detected in an advanced disease setting. Validation studies are needed to clarify if HLA-E mismatch could be beneficial in a combined advanced disease stage and ATG treatment context. Meanwhile, both studies agree that additional matching for HLA-E does not confer any advantage to the outcome of patients with acute leukemia receiving HSC grafts from unrelated donors.

The most important finding of this study is the observation that donors with homozygous HLA-E*01:03 are associated with lower DFS and higher TRM but only in a T cell replete transplant setting. A rather limited number of studies have addressed so far the importance of patient and/or donor HLA-E genotype, but most found that the HLA-E*01:03 homozygous genotype in donor and/or patient correlated with lower risk of aGVHD and cGVHD [17,20,21] or lower relapse and hence higher DFS [22,23,32]. Ludajic et al. [21], on the other hand, reported a similar result regarding the adverse effect of donor HLA-E*01:03 homozygosity on relapse and early TRM. Interestingly, the same group reported higher risk of aGVHD and cGVHD for patients being transplanted with HLA-E*01:03 homozygous grafts [21], something we did not observe. Moreover, Tamouza et al. [18] detected an association between higher severe bacterial infection incidence as well as early transplantation-related mortality and HLA-E*01:01 homozygous donors. Last, Fürst et al. [24], in their analysis of 116 unrelated HSCT transplant pairs, observed no effect of HLA-E genotype in either patient or donor significantly affecting outcome. These studies were highly variable in terms of design (ie, related versus unrelated allogeneic HSCT, HLA-E matched or not, only malignant versus also nonmalignant disease entities, only adult versus not only adult patients, in vivo TCD in some patients versus no in vivo TCD, etc) and were also small in size as the largest among them consisted of 187 patients.

Our current findings, contrary to many of the aforementioned studies, suggest that donor HLA-E*01:03 homozygous genotype in a T cell replete HSCT setting may adversely affect DFS due to less efficient leukemia control and higher TRM risk.

It is known that peptide/HLA-E complexes serve as ligands to a restricted albeit functionally variable repertoire of receptors found on NK-, NKT-, and CD8⁺ T cells. Through their multifaceted interactions with those cells, they accordingly tune innate as well as adaptive immune responses in a series of distinct conditions such as infection, pregnancy, cancer, transplantation, and autoimmune disease [5,12,33–37]. It has also been repeatedly demonstrated that the 2 basic HLA-E allelic variants (namely, 01:01 and 01:03) exhibit remarkably different surface expression levels, which are most likely attributed to post-transcriptional regulation factors such as peptide binding affinity and thermal stability [6,7,9,38,39]. The differential cell-surface expression levels and the distinct peptide-binding profiles of the 2 HLA-E protein isoforms, in conjunction with their codominant prevalence worldwide, imply that this seemingly minor genetic variation leads to considerable functional diversity [28]. Our findings suggest that a compromised activation/priming T cell-dependent mechanism probably accounts for the hampered leukemia control and the higher TRM risk in a donor HLA-E*01:03 homozygous setting. To date, there is no molecular-based evidence underpinning this hypothesis. However, pairwise comparison of the 3 potential donor HLA-E genotypes showed that 1 copy of HLA-E*01:01 is enough to abrogate the negative effect of the 01:03 copy, as no differences in DFS and TRM were observed between cases with HLA-E*01:01/01:01 and HLA-E*01:01/01:03 donors, respectively. This might imply that HLA-E*01:01 alleles bind disease-related peptides more efficiently. Moreover, the fact that the 2 allelic forms have been found to have distinct peptide repertoires strengthens this notion [6,8–10,40]. If this hypothesis holds, donor APCs carrying the 01:01 HLA-E isoform may prime a more potent T cell-mediated Graft versus Leukemia-specific response through presentation of leukemia-derived peptides to HLA-E restricted cytotoxic T lymphocytes. Although it is unclear how the donor HLA-E*01:03/01:03 genotype may increase the risk of TRM, a combination of more GVHD and inferior infection control is possible. Additionally, it remains to be clarified if NK priming is less efficient in an HLA-E*01:03/01:03 milieu, considering that newly reconstituted NK cells after T cell replete HSCT exhibit delayed Killer-cell Immunoglobulin-like Receptor (KIR) reconstitution and higher prevalence of CD94/NKG2A [14,15]. Furthermore, an accumulation of potentially inhibitory CD94/NKG2A⁺ CD8⁺ cytotoxic T lymphocytes has been also described elsewhere [41].

In conclusion, after HLA-E genotyping of 1840 unrelated 10/10 HLA-matched transplant pairs and analysis of D/R HLA-E polymorphism with relation to HSCT outcome, we identified a clear association of donor HLA-E*01:03 homozygous genotype with DFS and TRM in transplantations without in vivo TCD. It is of note that up to date, we are the second [21] to report an association between donor HLA-E*01:03 homozygous genotype, DFS, and TRM in a T cell replete transplantation setting. Limitations of our study were the missing data on disease cytogenetic risk, severe infection prevalence rates, and donor KIR haplotypes. Nevertheless, the markedly larger size of this cohort, its homogeneity in terms of several clinical parameters, and the long median follow-up time (90 versus 20 months of follow-up reported in certain studies) provide statistical strength and credibility. Undoubtedly, HSCT constitutes a very complex immunologic milieu, where, due to multiplex interactions among various patient and donor-related parameters, it becomes really

challenging to identify and isolate specific factors that can determine outcome in a significant and independent fashion. Regardless of this, however, our results certainly stress the potential future consideration of unrelated donor HLA-E genotype for donor selection in an acute leukemia T cell replete HSCT setting, as avoidance of HLA-E*01:03/01:03 donors may improve DFS and TRM. Our findings also underscore the need for further research, especially on a functional molecular basis.

DECLARATION OF COMPETING INTEREST

There are no conflicts of interest to report.

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SUPPLEMENTARY MATERIALS

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