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Multicenter Analyses Demonstrate Significant Clinical Effects of Minor Histocompatibility Antigens on GvHD and GvL after HLA-Matched Related and Unrelated Hematopoietic Stem Cell Transplantation

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A B S T R A C T

The effect of minor H antigen mismatching on the occurrence of graft-versus-host disease (GvHD) and graft-versus-leukemia (GvL) after HLA-matched hematopoietic stem cell transplantation (HSCT) has mainly been demonstrated in single-center studies. Yet, the International Histocompatibility and Immunogenetics Workshops (IHIW) provide a collaborative platform to execute crucial large studies. In collaboration with 20 laboratories of the IHIW, the roles of 10 autosomal and 10 Y chromosome–encoded minor H antigens were investigated on GvHD and relapse incidence in 639 HLA-identical related donor (IRD) and 210 HLA-matched unrelated donor (MUD) HSCT recipients. Donor and recipient DNA samples were genotyped for the minor H antigens HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, SP110, PANE1, UGT2B17, and HY. The correlations with the primary outcomes GvHD (acute or chronic GvHD), survival, and relapse were statistically analyzed. The results of these multicenter analyses show that none of the HLA class I–restricted HY antigens were found to be associated with any of the primary outcomes. Interestingly, of the HLA class II–restricted HY antigens analyzed, HLA-DQ5 positive recipients showed a significantly increased GvHD-free survival in female-to-male HSCT compared with male-to-female HSCT ($P = .013$). Yet, analysis of the overall gender effect, thus independent of the known HY antigens, between the gender groups demonstrated an increased GvHD incidence in the female-to-male transplantations ($P < .005$) and a decreased GvHD-free survival in the female-to-male

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transplantations ($P < .001$). Of all autosomally encoded minor H antigens, only mismatching for the broadly expressed minor H antigen HA-8 increased the GvHD incidence in IRD HSCT (Hazard ratio [HR] = 5.28, $P < .005$), but not in MUD HSCT. Most striking was the influence of hematopoietic restricted minor H antigens on GvL as mismatching for hematopoietic minor H antigens correlated with lower relapse rates ($P = .078$), higher relapse-free survival ($P = .029$), and higher overall survival ($P = .032$) in recipients with GvHD, but not in those without GvHD. In conclusion, the significant GvHD effect of the broadly expressed minor H antigen HA-8 favors matching for HA-8 in IRD, but not in MUD, patient/donor pairs. The GvHD-GvL association demonstrating a significant lower relapse in hematopoietic minor H antigen mismatched patient/donor pairs underlines their clinical applicability for adoptive immunotherapy, enhancing the GvL effect in a GvHD controllable manner.

INTRODUCTION

Minor histocompatibility (H) antigens are considered to play a key role in the allo-immune responses after HLA-matched stem cell transplantation (HSCT), evoking graft-versus-host disease (GvHD) and the curative reaction designated as graft-versus-leukemia (GvL) [1,2]. The cell and tissue expression of the minor H antigens determine their participation in GvHD and GvL reactions. Whereas the hematopoietic-restricted minor H antigens (hematopoietic minor H antigens) induce important allo-immune responses in GvL, the broadly expressed minor H antigens (broad minor H antigens) participate in both GvHD and GvL [3].

Regarding the latter group of broad minor H antigens, clinical results show that HLA-matched minor H antigen-mismatched transplantation recipients have an increased risk for developing GvHD and a poorer survival. In general, gender mismatching significantly affects HSCT outcome; the highest risk for GvHD has been observed in male recipients of female stem cells [4-7]. Mismatching for the broad autosomally encoded minor H antigen HA-8 or UGT2B17 increases the risk for GvHD [8,9]. Experimental evidence for the involvement of broad minor H antigens, such as HY, in the GvHD arm of HSCT was shown by functional in vitro assays [3] and by an in situ ex vivo skin explant assay [10]. Importantly, HY-specific T cells are detectable during clinical GvHD in blood [11] and skin samples of male patients after gender-mismatched HSCT [12].

The effect of mismatching for the hematopoietic minor H antigen HA-1 on GvHD has been studied by several investigators reporting different outcomes. Whereas some studies observed an association between HA-1 mismatching and the development of GvHD, others did not [13-16]. A plausible explanation for the reported association of HA-1 with GvHD, is the putative presence of recipient's residual dermal antigen-presenting cells after HSCT [10,17]. These antigen-presenting cells reside for various time spans in recipients' skin [10] and are able to stimulate HA-1-specific T cells in ex vivo in situ models [17]. Although not specifically investigated for ACC-1, it may also explain the observed correlation between ACC-1 mismatching and GvHD [17].

The main and crucial activity of the hematopoietic minor H antigens resides in the GvL arm of HSCT. Their hematopoietic-restricted expression also includes leukemic cells and leukemic progenitor cells [18]. In vitro experiments demonstrated lysis of leukemic cells when exposed to cytotoxic T lymphocytes (CTLs) specific for HA-1 and HA-2 [19], ACC-1 and ACC-2 [20], HB-1 [21], PANE1 [22] and SP110 [23]. Clinically, CTLs specific for the hematopoietic minor H antigens HA-1, HA-2, and LRH-1 coincide with remission of hematological malignancies after donor lymphocyte infusion [19]. The therapeutic potency of the latter minor H antigens has been demonstrated in animal models [24].

Clinical evidence for GvL effects of hematopoietic minor H-antigen mismatches is sparse. Two studies reported on the absence of correlation between HA-1 mismatching and relapse [25,26]. To the contrary, HA-1 disparity is correlated with lower leukemia relapse rates in HLA-A2-positive chronic myeloid leukemia (CML) recipients who received myeloablative allo-SCT from HLA-identical related donors (IRD) [15,27]. Moreover, the emergence of HA-1-specific cytotoxic T cells parallels the therapeutic effect of donor lymphocyte infusion [25,28]. Interestingly, a significantly reduced relapse incidence was observed in CML recipients of HA-1 mismatched HSCT grafts but was restricted to patients suffering from GvHD [29].

In the present multicenter study, we investigated the effect of mismatching of 10 autosomally encoded and 10 Y chromosome–encoded minor H antigens on the clinical outcome of 639 HLA-identical related and 210 HLA-matched unrelated HSCT.

Table 1
Patient and Donor Demographics

Characteristic	GvHD	No GvHD
Age, mean (standard error), yr		
Recipient	36.6 (.84)	35.6 (.79)
Donor	37.2 (.88)	38.9 (.96)
Donor type		
Identical related	225 (35%)	414 (65%)
Matched unrelated	86 (41%)	124 (59%)
Underlying disease		
Acute leukemia	131 (39%)	201 (61%)
Chronic leukemia	114 (53%)	102 (47%)
Lymphoma	17 (25%)	51 (75%)
Plasma cell disorders	7 (16%)	36 (84%)
Solid tumors	0	16 (100%)
MDS/MPS	36 (49%)	38 (51%)
Bone marrow aplasia	6 (27%)	16 (73%)
Inherited disorders	0	56 (100%)
Hemoglobinopathies	0	11 (100%)
Other/not reported	0	11 (100%)
Stem cell source		
Bone marrow	225 (42%)	308 (58%)
Peripheral blood	86 (32%)	184 (68%)
Cord blood	0	1 (100%)
Other/not reported	0	45 (100%)

MDS indicates myelodysplastic syndrome; MPS, myeloproliferative syndrome.
Data presented as n (%) unless otherwise indicated.

MATERIALS AND METHODS

Study Population

A total of 849 HLA-A, -B, -C, -DRB1, and -DQB1 allele-matched transplantations, facilitated by the participating centers, were studied. All materials were obtained after informed consent according to the local guidelines of the participating centers. Table 1 summarizes all relevant patient and donor characteristics.

Table 2
Minor H Antigens Included in This Study, Encoded by Genes on the Y Chromosome (A) and on the Autosomal Chromosomes (B)

A

Minor H Antigen	HLA Restriction	HUGO Gene Name		Tissue Distribution
A1/HY	A1	*USP9Y*	Ubiquitin specific peptidase 9, Y-linked	Broad
A2/HY	A2	*KDM5D*	Lysine (K)-specific demethylase 5D	Broad
A33/HY	A33	*TMSB4Y*	Thymosin, beta 4, Y-linked	Unknown
B27/HY	B27	*DDX3Y*	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	Restricted
B52/HY	B52	*RPS4Y1*	Ribosomal protein S4, Y-linked 1	Restricted
B60/HY	B60	*UTY*	Ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	Broad
B7/HY	B7	*KDM5D*	Lysine (K)-specific demethylase 5D	Broad
B8/HY	B8	*UTY*	Ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	Restricted
DQ5/HY	DQ5	*DDX3Y*	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	Broad
DR15/HY	DR15	*DDX3Y*	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	Broad
DRB3*0301/HY	DRB3*0301	*RPS4Y1*	Ribosomal protein S4, Y-linked 1	Broad

B

Chrom.	Minor H Antigen	HLA Restriction	Hugo Gene Name		Tissue Distribution
19	HA-1/A2	HLA-A2	*HMHA1*	histocompatibility (minor) HA-1	Restricted
19	HA-1/B60	HLA-B60	*HMHA1*	histocompatibility (minor) HA-1	Restricted
7	HA-2	HLA-A2	*MYO1G*	myosin IG	Restricted
15	HA-3	HLA-A1	*AKAP13*	A kinase (PRKA) anchor protein 13	Broad
9	HA-8	HLA-A2	*KIAA0020*	KIAA0020	Broad
5	HB-1	HLA-B44	*HMHB1*	histocompatibility (minor) HB-1	Restricted
15	ACC-1	HLA-A24	*BCL2A1*	B-cell leukemia/lymphoma 2 related protein A1	Restricted
15	ACC-2	HLA-B44	*BCL2A1*	B-cell leukemia/lymphoma 2 related protein A1	Restricted
22	PANE1	HLA-A3	*CENPM*	centromere protein M	Restricted
2	SP110	HLA-A3	*SP110*	SP110 nuclear body protein	Restricted
4	UGT2B17	HLA-A29	*UGT2B17*	UDP glucuronosyltransferase 2 family, polypeptide B17	Broad
4	UGT2B17	HLA-B44	*UGT2B17*	UDP glucuronosyltransferase 2 family, polypeptide B17	Broad

Minor H Antigen Genotyping

Recipient and donor DNA were genotyped for 10 autosomal minor H antigens, ie, HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, SP110, PANE1, and UGT2B1, using a PCR-SSP-based assay developed at the Leiden University Medical Center, as described previously [30]. The minor H antigen characteristics, their HLA restriction molecules, and the immunogenic alleles have been described before [30]. Primers used for minor H antigen-specific amplification were synthesized and provided by Invitrogen (Carlsbad, CA).

Definitions of Outcomes

The primary outcomes of the analysis were overall survival, defined as time from graft infusion (day 0) to death from any cause, time to acute GvHD (absence or occurence of acute GvHD; information on GvHD grading was not provided by the participating centers), defined by the Glucksberg scale [31], time to chronic GvHD, as defined according to the Seattle criteria [32], time to death after GvHD, and time to non-GvHD–related mortality. Relapse was defined as the time from graft infusion to recurrence of the original disease. Additionally, death after relapse, and nonrelapse-related mortality were analyzed.

Statistical Analysis

Minor H antigen mismatches of the HSCT pairs were classified as GvH mismatched or GvH matched using the dbMinor algorithm (www.lumc.nl/dbminor) [32]. To investigate the effect of minor H antigen mismatches, the association between transplantation outcomes and minor H antigen matched versus minor H antigen mismatched was quantified by using a survival framework on the donor/recipient pairs. Cox proportional hazard analyses were used taking GvHD and death as competing risks. The occurrence of death is censored by the occurrence of GvHD (and vice versa) in complete analogy to the usual analyses of relapse and mortality. The associations of HA-3, HA-8, donor type, and hematopoietic minor H antigen mismatching with transplantation outcome were quantified by the hazard ratio in these models. Outcomes were overall survival, GvHD incidence, GvHD-free survival, and non-GvHD mortality.

Furthermore, GvHD was also treated as a time-dependent risk factor itself, when analyzing its effect on overall survival, relapse incidence, relapse-free survival, and nonrelapse mortality. The hazard ratios, therefore, are always consistent with a competing risk framework and can be used to compute cumulative incidence curves (except when GvHD is used as a time-depending covariate).

P values less than or equal to .05 were considered to be statistically significant. In view of the diminished power of statistical test for interactions, we consider the data to indicate interaction (effect modification) when the *P* value is less than or equal to .10, effectively taking 10% as a significance level.

All line graphs in Figures 1, 2, 3, and 4 are cumulative incidence estimates stemming from the above-mentioned Cox models in the usual framework of competing risks. They are univariate or bivariate estimates (stratified) and not based on modeling the curves themselves. Figure 5 integrates the various estimated curves into 1 stacked graph depicting the estimated proportion of patients in each of the 4 states (equivalent to a multistate model that corresponds to the competing risk framework).

RESULTS

Overall Gender Effect but Little Influence of Single HY Antigens on GvHD

Analyses of 7 HLA class I-restricted HY antigens were carried out on the 7 HY antigens as a group and on each of them separately. Comparing HSCT pairs with at least 1 or more HY antigen mismatch versus gender matched pairs showed no significant influence on GvHD incidence, relapse, or survival, regardless of the donor type (GvHD in Figure 1A, Supplementary Table 1).

For separate statistical analysis of HLA-A33/HY, HLA-B52/HY, and HLA-B60/HY, the number of HY-mismatched HSCT pairs was too low (Supplementary Table 2). For HLA-B7/HY and HLA-B8/HY, no differences between the gender-matched and the gender-mismatched groups were observed (data not shown). For the HLA-A1/HY, a strong, but statistically not significant tendency towards an increased GvHD incidence was found (Figure 1B) (HR = 3.0, $P = .06$), independent of the donor type ($P = .92$). For HLA-A2/HY, no correlation with GvHD incidence was observed, neither in IRD (HR = 1.5, $P = .35$), nor in MUD HSCT (HR = .81, $P = .64$) (Figure 1C). Cox regression analyses showed a weak interaction between HY mismatching and donor type in relation to developing GvHD for HLA-A2/HY (HR = 2.0, $P = .25$).

Analyses of the 3 HLA class II–restricted HY antigens as a group yielded no significant differences (data not shown). Analyses of the 3 HLA class II–restricted HY antigens separately demonstrated a nonsignificant increased GvHD

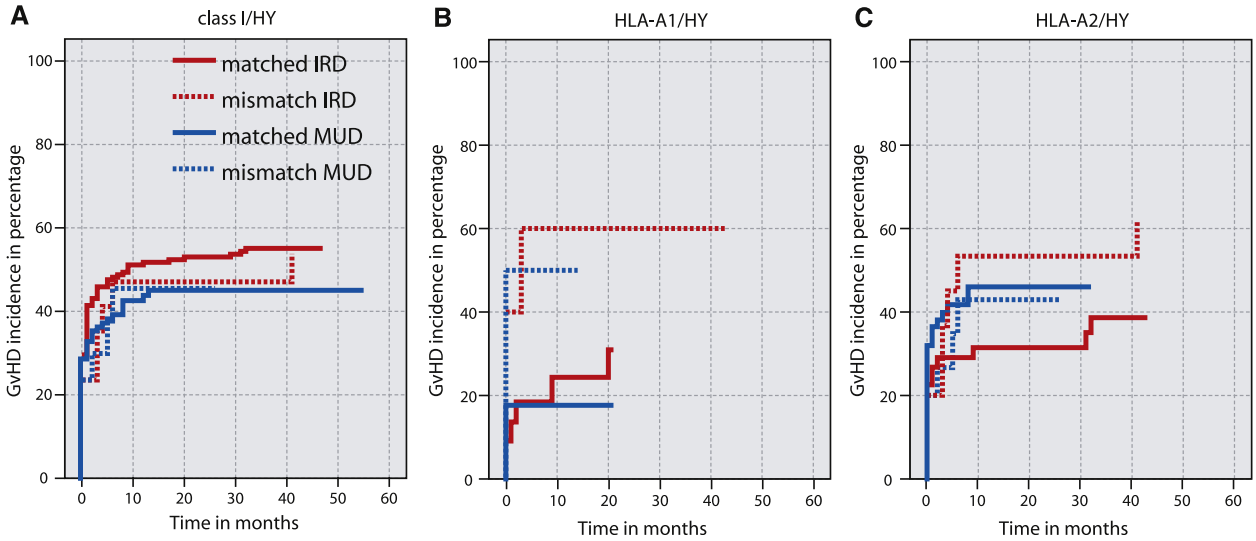

Figure 1. The effect of mismatching for HLA class-I restricted HY minor H antigens on grat-versus-host disease (GvHD) incidence. Blue lines represent the matched unrelated donor (MUD) recipients, and red lines represent the identical related donor (IRD) recipients. Solid lines are minor H antigen matched and the dotted lines are minor H antigen mismatched. (A) IRD recipients with 1 or more HY mismatches (dotted red line) show comparable GvHD incidence when compared to IRD recipients who are fully matched for all HY minor H antigens tested and to MUD recipients (blue line and blue dotted line, respectively). (B) HLA-A1/HY mismatching increases the GvHD incidence in both IRD and in MUD recipients. (C) Mismatching for HLA-A2/HY affects the GvHD incidence in IRD recipients but not in MUD recipients. None of these trends were statistically significant. See Supplementary Table 1 for detailed statistics.

incidence in HLA-DR15 IRD HY-mismatched recipients when compared with HY-matched HLA-DR15 IRD recipients (HR = 1.8, $P = .19$) (Figure 2A). The number of HLA-DR15 MUD recipients was too low for adequate analyses. The role of HLA-DRB3*03:01/HY could not be evaluated, as none of the centers reported allelic typing for the HLA-DRB3 locus.

When analyzing the effect of HLA-DQ5/HY on GvHD incidence, a nonsignificant trend toward a lower incidence of GvHD was observed in the female-to-male HSCT pairs as opposed to the combined HLA-DQ5 gender-matched (ie, male-to-male and female-to-female) and male-to-female HSCT pairs (HR = .45, $P = .07$) (Figure 2B). This trend was present both in IRD (HR = .41, $P = .15$) and in MUD HSCT (HR = .50, $P = .27$). Thus, there was no interaction between donor type and HY mismatching (data not shown). When comparing HLA-DQ5 female-to-male pairs with the HLA-DQ5 male-to-female pairs (Figure 2C), HLA-DQ5 female recipients (IRD and MUD combined) displayed a statistically significant lower GvHD rate (HR = .30, $P < .05$). Moreover, HLA-DQ5 male-to-female HSCT demonstrated a significantly increased GvHD-free survival when compared with HLA-DQ5 female-to-male HSCT (HR = .037, $P < .05$). None of the HLA class II–restricted HY mismatches correlated with GvL (data not shown).

The overall gender effect between the gender groups, thus independent of HY presenting HLA class I or class II molecules,

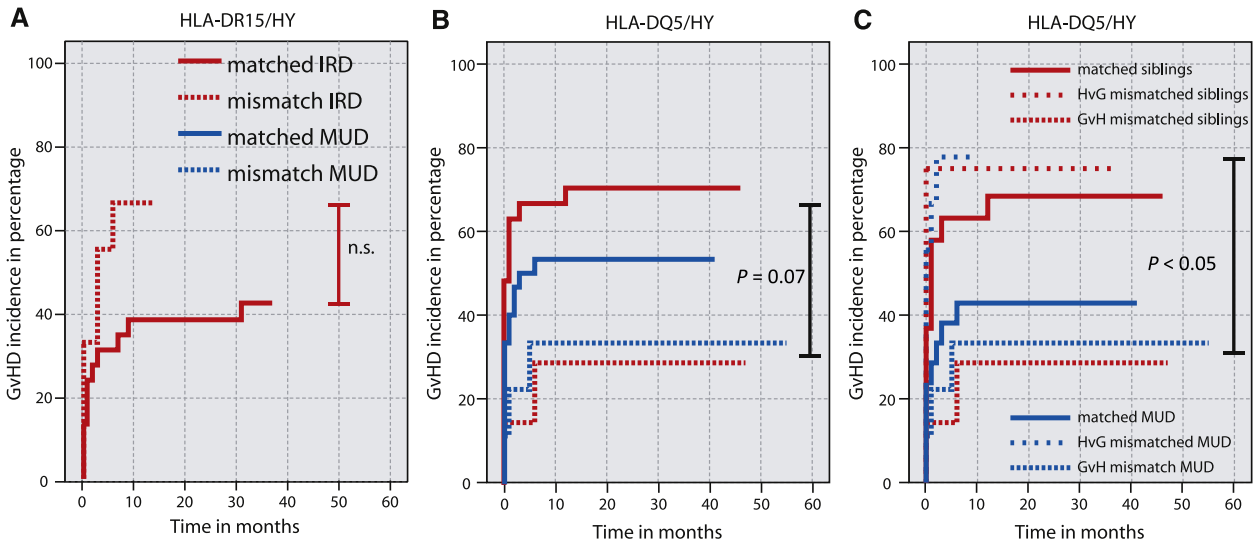

Figure 2. The effect of mismatching for HLA class II–restricted HY minor H antigens on GvHD incidence. Blue lines represent the matched unrelated (MUD) recipients, and red lines represent the identical related donor (IRD) recipients. Solid lines are minor H antigen matched and the dotted lines are minor H antigen mismatched. (A) IRD recipients with an HLA-DR15/HY mismatch (dotted red line) show a trend to more GvHD incidence when compared to IRD recipients who are matched. MUD recipients with an HLA-DR15/HY mismatch were not observed in this study. (B) HLA-DQ5/HY mismatching significantly decreases the GvHD incidence in both IRD and in MUD recipients. (C) Female recipients of a male HLA-DQ5/HY-mismatched graft display significantly more GvHD when compared with male recipients of a female graft.

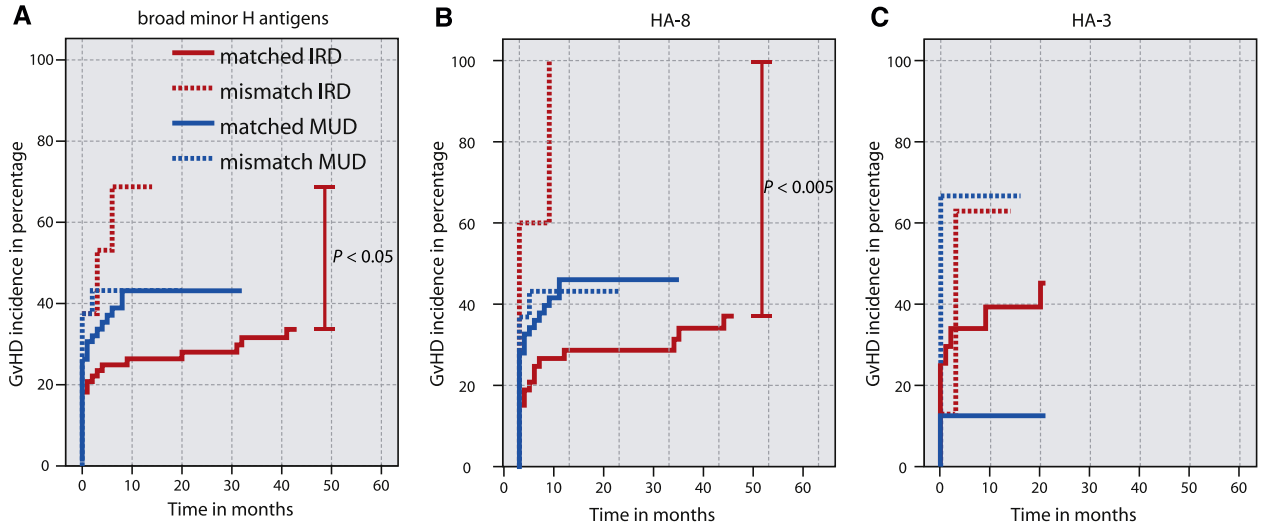

Figure 3. The effect of mismatching for broad autosomal minor H antigens on graft-versus-host disease (GvHD) incidence. Blue lines represent the matched unrelated donor (MUD) recipients, and red lines represent the identical related donor (IRD) recipients. Solid lines are minor H antigen matched and the dotted lines are minor H antigen mismatched. All statistically significant differences are visualized in the graphs. (A) IRD recipients with 1 or more mismatches for the broad autosomal minor H antigens (dotted red line) show an increased GvHD incidence when compared with IRD recipients who are fully matched for all broad autosomal minor H antigens tested. These differences were not observed for minor H antigen matched and mismatched MUD recipients (blue line and blue dotted line, respectively). (B) HA-8 mismatching increases the GvHD incidence in IRD recipients, but not in MUD recipients. (C) Mismatching for HA-3 affects the GvHD incidence in both the IRD and the MUD groups. See Table 3 for statistics.

demonstrated a significant increase GvHD incidence in the female-to-male transplantations compared with the other groups combined (HR = 2.45, $P < .005$) and a decrease of GvHD-free survival in the female-to-male transplantations (HR = 2.19, $P < .001$) (Supplementary Figure 1).

In conclusion, of all single HLA class I– and II–restricted HY antigens analyzed, only the HLA-DQ5 female recipients of male grafts reached a statistically significant interaction with higher GvHD-free survival. Overall gender analysis on the whole study population, regardless of the known HY antigens, showed significant higher GvHD incidence and decreased GvHD-free survival in male recipients of female grafts.

Mismatches for Broad Autosomal Minor H Antigens Correlate with GvHD

Disparities between HLA-matched recipients and their donors for broad autosomal minor H antigens, ie, HA-3, HA-8, and UGT2B17, were analyzed for their impact on GvHD development, relapse, and recipient survival. In IRD pairs, mismatching for at least 1 broad autosomal minor H antigen resulted in a 4-fold increased GvHD risk (HR = 3.93, $P < .05$) (Figure 3A). This increased risk was not observed in the MUD group (HR = 1.18, $P = .64$).

The increased GvHD risk in the study population was mainly due to HA-8 mismatching (Table 3; Figure 3B) (HR = 3.9, $P < .05$). Statistical analyses on the interaction between HA-8 mismatching and donor type (ie, IRD or MUD) in Cox regression analyses, showed a significant effect of HA-8 mismatching in IRD but not in the MUD pairs; Cox regression analysis resulted in a HR of 5.28 ($P < .005$) in the IRD group. Notably, in the IRD group, all recipients of an HA-8-mismatched graft (n = 5) developed GvHD.

HA-3 mismatching displayed a trend to an increased risk for GvHD (HR = 3.31, $P = .078$) (Figure 3C). Moreover, HA-3-mismatched recipients showed a statistically significance of 3-fold decreased GvHD-free survival (HR = .32, $P < .05$). Interaction analyses ($P = .37$) did not justify separation of the IRD group from the MUD group.

The role of mismatches for the minor H antigens UGT2B17/A29 and UGT2B17/B44 could not be addressed because of the low numbers of relevant mismatched pairs (Supplementary Table 3). In summary, of all broad autosomal minor H antigens analyzed in this study, only the minor H antigen HA-8 demonstrated a significant influence on the development of GvHD in IRD but not in MUD transplantations. Analysis of the impact of mismatching on relapse and recipient survival yielded no significant results.

Mismatches for Hematopoietic Autosomal Minor H Antigens do Not Correlate with GvHD or with GvL

The influence of mismatching for hematopoietic autosomal minor H antigens on GvHD, relapse, and survival was analyzed. One or more hematopoietic minor H antigen mismatches within a single HSCT pair did not lead to a significant increase in GvHD incidence neither in the MUD nor in the IRD pairs (overall HR = 1.075, $P = .71$) (Figure 2A). Analysis of each minor H antigen separately showed a significant increase in GvHD in ACC-1-mismatched recipients of an IRD HSCT (HR = 3.7, $P = .05$) only, confirming earlier reports. Individual analyses of all other hematopoietic minor H antigens yielded no significant differences in any of the outcome parameters between matched and mismatched groups (data not shown).

The restricted expression of hematopoietic minor H antigens assumes their effect on the GvL activity. However, in the present study, the relapse rates in recipients with 1 or more hematopoietic minor H antigen mismatches were equal to the relapse rate in recipients matched for all hematopoietic minor H antigens. These results were similar both for the IRD and the MUD transplantations (HR = 1.051, $P = .88$) (Figure 3B). Equal relapse rates were observed for HA-1-mismatched and HA-1-matched recipients, as depicted in Figure 3C (IRD: HR = .653, $P = .59$; MUD: HR = 1.011, $P = .98$). Consequently, no correlation was observed between

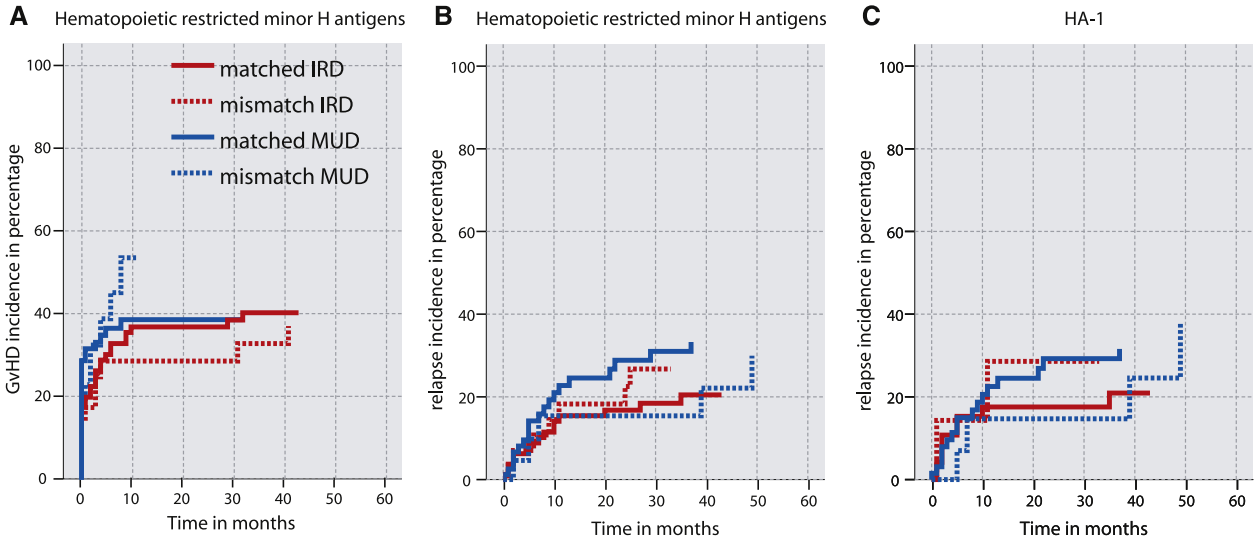

Figure 4. The effect of mismatching for hematopoietic minor H antigens on graft-versus-host disease (GvHD) and graft-versus-leukemia (GvL) incidence. Blue lines represent the matched unrelated donor (MUD) recipients, and red lines represent the identical related donor (IRD) recipients. Solid lines are minor H antigen matched and the dotted lines are minor H antigen mismatched. None of the differences are statistically significant. (A) Recipients with 1 or more mismatches in the hematopoietic minor H antigen (dotted lines) show no increased GvHD incidence when compared with recipients who are fully matched for all hematopoietic minor H antigens tested, neither in the IRD group (red), not in the MUD group (blue). (B) Recipients with at least 1 mismatch for a hematopoietic minor H antigen (dotted lines) show no decreased relapse incidence when compared to recipients who are fully matched for all hematopoietic minor H antigens tested, neither in the IRD group (red), nor in the MUD group (blue). (C) HA-1 mismatching had no effect on relapse incidence.

mismatching for hematopoietic minor H antigens and relapse in either donor type.

Mismatches for Hematopoietic-Restricted Autosomal Minor H Antigens Correlate with GvL in Recipients with GvHD

We earlier demonstrated the GvHD-dependent effect of HA-1 mismatching on relapse [29]. In the latter study, GvHD was analyzed as a static parameter and comprised only CML patients. In the present study, the role of GvHD on the relapse incidence was analyzed as a time-dependent risk factor, comparing all recipients with 1 or more hematopoietic minor H antigen mismatches with the pairs matched for all studied hematopoietic minor H antigens. Moreover, we here analyzed all patients, regardless of the underlying disease.

Table 4 lists the effect of hematopoietic minor H antigen mismatching on relapse incidence (HR = .30, $P = .078$), relapse-free survival (HR = .347, $P < .05$), and overall survival (HR = .315, $P < .05$); all significantly dependent on the presence of GvHD. As time-dependent Cox regression analyses cannot be depicted graphically, we illustrated these effects via incidence curves with GvHD as a static parameter (Figure 5). Note that this depiction cannot be envisaged as a predictive model, but only describes our data set. The incidence curves support the above-observed lower relapse, improved relapse-free survival, and better overall survival in hematopoietic minor H antigen-mismatched recipients with GvHD when compared with those without GvHD for IRD HSCT recipients (Figure 5E versus Figure 5G). The majority (82%) of the patients with GvHD and a hematopoietic minor H antigen mismatch (Figure 5G) survive free of relapse; the first relapse in this group was observed only after 23 months in 1 patient and 25 months after HSCT in the second. One of the latter patients died 28 months after HSCT.

No correlations with reduced relapse rates were observed in the hematopoietic minor H antigen-matched groups (Figure 5A versus Figure 5C). The occurrence of GvHD did not influence the effect of matching/mismatching on relapse, on relapse-free survival, on nonrelapse mortality, and on overall survival in the MUD recipients (Figure 5B versus Figure 5D, Figure 5F versus Figure 5H). Our data set did not allow statistical analysis of a triple interaction analysis including donor type, minor H antigen mismatching, and GvHD status. In summary, hematopoietic minor H antigens significantly influence the GvL effect in patients suffering from GvHD. Data sets were too small to reliably analyze this effect for each hematopoietic minor H antigen separately (Supplementary Table 3).

DISCUSSION

This multicenter study, comprising 639 IRD and 210 MUD HSCT pairs, investigated the effect of minor H antigen mismatching on HSCT outcome. This comprehensive study yields thorough insights in the role of 10 autosomally and 10 Y chromosome–encoded minor H antigens in GvHD, GvL effect, and overall survival.

Most HY antigens included in our study showed a broad tissue distribution (Table 2A). Consequently, an effect of HY mismatching on the outcome of HSCT can be expected. A previous report suggested that the effects of gender mismatching on GvHD are mainly confined to patients surviving more than 6 months after transplantation, leading to an increased relative risk for chronic but not acute GvHD [33]. Although the numbers in the current study are too low to analyze these late effects, this phenomenon seems to be absent when analyzing the HLA class I-restricted HY antigens as a group, as displayed by the virtually overlapping curves in Figure 1A. Whether or not the HLA-A2/HY shows an effect in IRD recipients, as suggested by the divergence of the GvHD incidence curves in Figure 1C, remains to be elucidated in larger cohorts. Likewise the HLA class I-restricted HY antigens, our data of the HLA class II–restricted HY antigens revealed, with 1 exception, no significant correlations with the measured clinical parameters. The 1 correlation observed was found for HLA class II DQ5/HY. Namely, HLA-DQ5/HY mismatching in the female-to-male HSCT resulted in

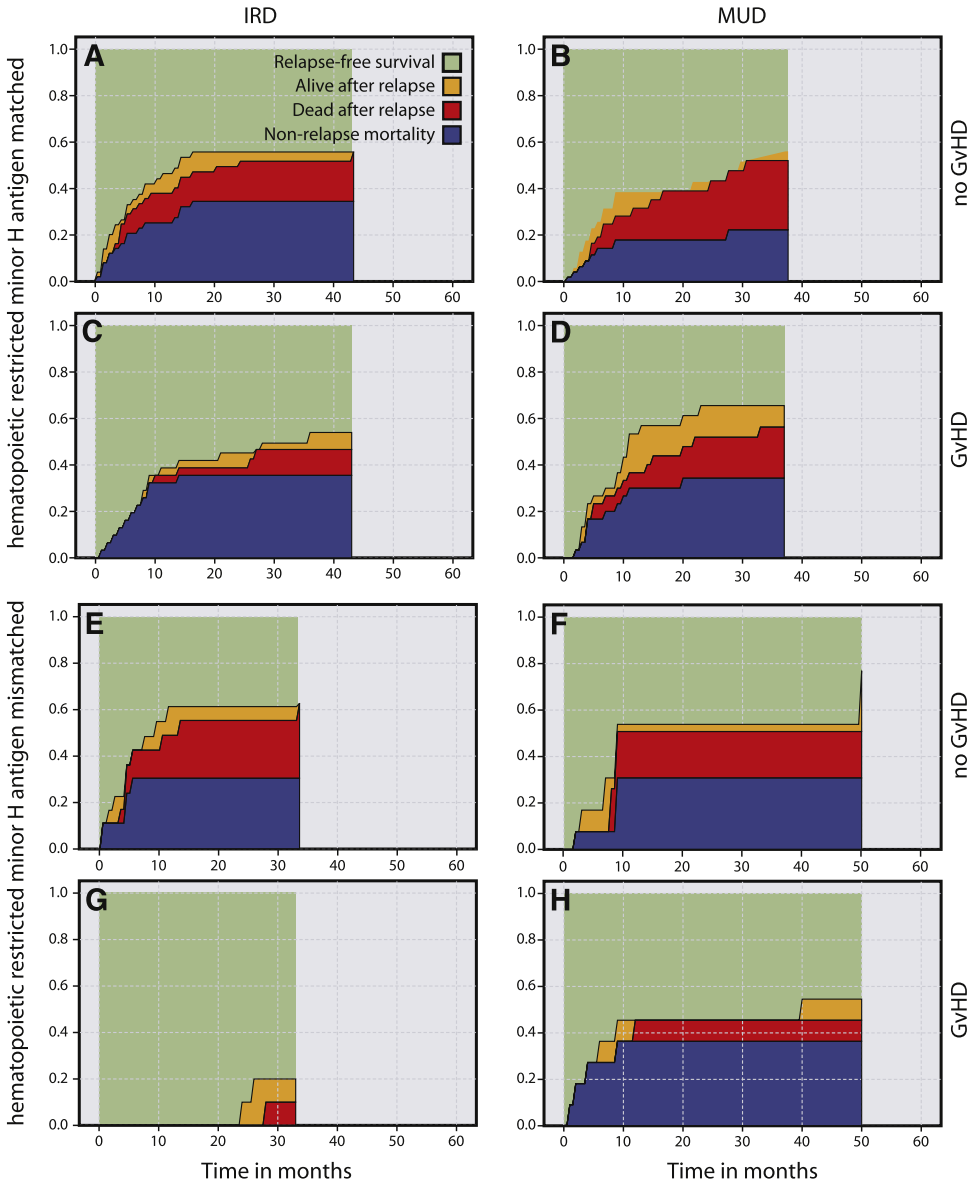

Figure 5. Effect of hematopoietic minor H antigen disparity on all outcome parameters, depending on the graft-versus-host disease (GvHD) status, assuming GvHD as a time-independent risk factor. All curves estimated in a competing risk framework; the 4 panels arise from fitting a competing risk model on each of the 4 subgroups separately (ie, 4 univariate analyses without further model assumptions apart from the competing risks framework). Note that these figures do not correctly predict the outcome correctly, as GvHD must be regarded as a time-dependent risk factor (see Table 4 for the correct statistics). Blue area: non-relapse-related mortality. Red area: dead after relapse; Yellow area: alive after relapse. The remaining green area represents the relapse-free survival. Left row: identical related donor (IRD); Right row: matched unrelated donor (MUD). A) hematopoietic minor H antigen-matched IRD in recipients without GvHD; B) hematopoietic minor H antigen-matched MUD in recipients without GvHD; C) hematopoietic minor H antigen-matched IRD in recipients with GvHD; D) hematopoietic minor H antigen-matched MUD in recipients with GvHD; E) hematopoietic minor H antigen-mismatched IRD in recipients without GvHD; F) hematopoietic minor H antigen-mismatched MUD in recipients without GvHD; G) hematopoietic minor H antigen-mismatched IRD in recipients with GvHD; H) hematopoietic minor H antigen-mismatched MUD in recipients with GvHD.

a significantly ($P < .05$) lower GvHD incidence when compared with male-to-female HSCT. Although intriguing, we currently have no explanation for the latter correlation. Our analyses on all study objects of an overall gender effect, thus independent of the known HY antigens, confirmed the known detrimental influences of female grafts to male recipients on the GvHD incidence ($P < .005$) and on GvHD-free survival ($P < .001$). Whether or not parity of the female donors influenced these results is unknown; our data set lacks that specific information.

Like most of the molecular identified HY antigens, the autosomally encoded minor H antigens HA-3, HA-8, and UGT2B17, show broad tissue distribution. Therefore, their possible impact on GvHD was investigated in the underlying study as well. Moreover, the effect of minor H antigen mismatching is assumed to be most pronounced in the HLA identical HSCT setting as opposed to the (partially) HLA-matched unrelated donor setting [13]. This assumption was recently demonstrated in separate studies on the effect of HA-8 mismatching on GvHD in either IRD [8,34] or in MUD transplantations [16]. Anticipating the latter, we executed all analyses for both of these donor types. Studying our large cohort, we demonstrated that the effect of HA-8 mismatching on GvHD is indeed restricted to the IRD HSCT ($P < .05$ for interaction between HA-8 mismatching and donor type). Because MUD pairs are expected to differ

Table 3
Cox Proportional Hazards Regression Analysis of Autosomally-Encoded Broad Minor H Antigen Mismatches as Risk Factors for Graft-Versus-Host Disease, Survival, and Relapse

Outcome	*P* Value	Hazard Ratio (95% Confidence Interval)
GvHD incidence		
Broad	.10	1.61 (.9 to 2.8)
HA-3	.08	3.31 (.9 to 12.5)
HA-3* donor type	.37	.27 (0 to 4.8)
HA-3 (IRD)	.59	1.87 (.2 to 4.8)
HA-3 (MUD)	.09	5.43 (.8 to 38.8)
HA-8	.13	1.63 (.9 to 3.1)
HA-8* donor type	<.05	3.93 (1.0 to 15.1)
HA-8 (IRD)	<.005	5.28 (1.7 to 16.3)
HA-8 (MUD)	.82	1.09 (.5 to 2.4)
GvHD-free survival		
Broad	.30	1.30 (.8 to 2.1)
HA-3	<.05	3.14 (1.0 to 9.8)
HA-3* donor type	.86	.81 (.1 to 8.0)
HA-3 (IRD)	.23	2.56 (.6 to 11.9)
HA-3 (MUD)	.18	3.39 (.6 to 20.5)
HA-8	.35	1.30 (.8 to 2.3)
HA to 8* donor type	.19	2.30 (.7 to 7.9)
HA-8 (IRD)	.05	2.82 (1.0 to 8.1)
HA-8 (MUD)	.80	1.09 (.6 to 2.1)
Overall survival		
Broad	.55	.82 (.4 to 1.6)
HA-3	.50	1.74 (.3 to 8.7)
HA-3* donor type	.94	81866 (0 to 10^{134})
HA-3 (IRD)	.11	3.81 (.7 to19.8)
HA-3 (MUD)	.68	.03 (.1 to 10^{6})
HA-8	.50	.77 (.3 to 1.6)
HA-8* donor type	.39	.39 (.1 to 1.9)
HA-8 (IRD)	.34	.38 (.1 to 2.8)
HA-8 (MUD)	.96	.98 (.4 to 2.3)
Relapse incidence		
Broad	.33	.04 (0 to 24)
HA-3	.60	.58 (.1 to 4.6)
HA-3* donor type	.99	.00 (0 to ∞)
HA-3 (IRD)	.63	.04 (0 to 14,331)
HA-3 (MUD)	.59	.54 (.1 to 5.2)
HA-8	.97	1.02 (.4 to 2.9)
HA-8* donor type	.97	.00 (0 to 10^{293})
HA-8 (IRD)	.44	.04 (0 to 138)
HA-8 (MUD)	.54	1.37 (.5 to 3.8)
Relapse-free survival		
Broad	.45	.63 (.2 to 2.1)
HA-3	.71	1.28 (.4 to 4.6)
HA-3* donor type	.34	3.67 (.3 to 53.7)
HA-3 (IRD)	.33	2.171 (.5 to 10.3)
HA-3 (MUD)	.59	.54 (.1 to 5.2)
HA-8	.74	.89 (.5 to 1.7)
HA-8* donor type	.23	.274 (0 to 2.3)
HA-8 (IRD)	.25	.31 (0 to 2.3)
HA-8 (MUD)	.69	1.16 (.6 to 2.4)

IRD indicates HLA-identical related donor; MUD, HLA-matched unrelated donor.
<.05 and <.005 indicate a *P* value from .01 to .05 and from .001 to .005 respectively.
* Interaction analyses have been performed for these 2 factors. HA-3 and HA-8 were tested in a univariate model.

in more minor H antigens than the IRD pairs, we reasoned that in the MUD recipients, a single HA-8 effect may not be measurable. Alternatively, this effect may be explained by the current different treatment of most MUD HSCT with T cell depleted grafts. Our data set did not allow including T cell depletion in the statistical analyses. It is important to note that the group size for MUD was significantly smaller than the size of the IRD group. However, given the complete overlap of the survival curves in Figure 3B, it is highly unlikely that HA-8 matching in MUD leads to a significant increase in GvHD.

Table 4
Cox Proportional Hazard Analyses for Relapse and Survival with GvHD as a Time-Dependent Risk Factor

Outcome	*P* Value	Hazard Ratio (95% Confidence Interval)
Relapse incidence		
Minor H antigen matching	.13	1.80 (.846 to 3.848)
Donor type	.33	1.32 (.754 to 2.307)
GvHD	.86	.94 (.482 to 1.834)
Minor H antigen matching* GvHD	<.10	.30 (.078 to 1.146)
Relapse-free survival		
Minor H antigen matching	.27	1.37 (.784 to 2.403)
Donor type	.64	1.40 (.747 to 1.604)
GvHD	.77	1.07 (.689 to 1.654)
Minor H antigen matching* GvHD	<.05	.35 (.134 to .898)
Overall survival		
Minor H antigen matching	.32	1.4 (.746 to 2.439)
Donor type	.76	1.10 (.709 to 1.598)
GvHD	.99	1.00 (.628 to 1.582)
Minor H antigen matching* GvHD	<.05	.32 (.110 to .906)
Non-relapse mortality		
Minor H antigen matching	.46	1.23 (.711 to 2.137)
Donor type	.14	1.33 (.912 to 1.933)
GvHD	<.01	.63 (.390 to 1.019)
Minor H antigen matching* GvHD	.46	.72 (.302 to 1.718)

GvHD indicates graft-versus-host disease.
For the analyses on the interaction between hematopoietic minor H antigen mismatching and GvHD, *P* values lower than .10 were considered statistically significant. For all other analyses, *P* values below .05 were used. Hematopoietic minor H antigen mismatching was defined as the presence of at least 1 mismatch for a hematopoietic minor H antigen in GvL direction. The current data set did not allow a triple interaction analysis including donor type, minor H antigen mismatching, and GvHD status.
<.10 and <.05 indicate a *P* value from .05 to .10 and from .05 to .01 respectively.
* Interaction analyses have been performed for these 2 factors.

An effect of the broadly expressed HLA-A1/HA-3 antigen on GvHD was not observed, though in unrelated partially HLA-matched cornea transplantations, mismatching for HLA-A1/HA-3 was associated with rejection [35]. Although trends were seen in both the IRD and the MUD study cohorts (Figure 3B), none of them were statistically significant. There was no interaction with donor type. These analyses may, however, be hampered by the relatively low phenotype frequency of HA-3, as only 10% of the HSCT pairs had an HA-3 mismatch. The observed trends in the HLA-mismatched cornea transplantation study and in the present HSCT study justify a detailed study on larger cohorts of HLA-A1-positive HSCT pairs in order to evaluate the role of HA-3 in GVHD after IRD and/or MUD HSCT.

Finally, in our opinion, the most important observation of this study is the significant enhancing effect of hematopoietic minor H antigens on GvL. Notably, in line with an earlier report on HA-1 [29], mismatching for hematopoietic minor H antigens in patients suffering from GvHD resulted in a reduced relapse incidence ($P = .078$), an increased relapse-free survival ($P < .05$), and a better overall survival ($P < .05$). All outcomes significantly depended on the presence of GvHD. This GvL effect of mismatching was absent in patients who did not develop GvHD. The earlier observation by Mutis et al. [29] was confined to CML patients receiving HLA-matched but HA-1-mismatched HSCT, using GvHD status of the recipient as a binary variable. In the present cohort of patients with various underlying diseases, the initial observation on HA-1 has been confirmed for all hematopoietic minor H antigens analyzed (Figure 5). More importantly, we here included the GvHD status as a time-dependent variable, showing a statistically significant interaction between GvHD and hematopoietic specific minor H antigens on relapse

incidence, relapse-free survival, and overall survival (Table 4). Although our data set did not allow analyzing this triple interaction because of sample size, these effects seem to be stronger in IRD HSCT than in MUD HSCT. Further investigation on the role of donor type is of importance, as it would yield information on the applicability of minor H antigen-based immunotherapy in both IRD and MUD. Controllable GvHD before or during such a therapy may well be essential for the success of this approach. Indications supporting the putative beneficial effect of GvHD on the antitumor reaction have been reported in a mouse study on tumor-specific antigens, showing that tumor-specific T cells develop most efficiently only under GvHD conditions [36]. Similar conclusions were drawn from the clinical observation that WT1-specific CTL only emerged shortly after the occurrence of GvHD, leading to the hypothesis that GvHD stimulates the development and/or expansion of WT1-specific CTL [37]. In line with these observations, development of GvHD is crucial for the success of minor H antigen vaccination in our currently ongoing phase 1 clinical trial with multiple myeloma patients. Herein, recipient DCs, instead of donor dendritic cells, are loaded with hematopoietic minor H peptides (Lokhorst, Mutis, Hambach, Goulmy, unpublished results). In the latter study, we chose for recipient dendritic cells as antigen-presenting cells to induce a clinically relevant GvL effect. Because multiple myeloma patients regularly fail to induce an adequate GvH response, we assumed that recipients' dendritic cells may induce controllable GvHD alongside antiminor H antigen T cell responses.

In conclusion, this study, executed under the auspices of the IHIW with the participation of 20 laboratories, enabled statistical analyses of 10 autosomally and 10 Y-chromosomally encoded minor H antigens on 849 HLA matched patient/donor pairs for their presumed effects on the outcome of HSCT. The present study comprised multiple testing, analyzing the effect of various minor H antigens on GVHD and relapse without formal correction for the number of analyses. As such, the character of this study should be regarded as indicative for the general influence of minor H antigens on stem cell transplantation. The described observations, such as the effect of mismatching for broadly expressed HA-8 on GvHD, thus require confirmation on dedicated cohorts. Notwithstanding the relatively small number of hematopoietic minor H antigens analyzed, their presumed role in the GvL response is endorsed. Our statistical interaction analyses indicate that the latter response is dependent on active anti-host responses after HSCT showing significant effects on relapse ($P = .023$), survival ($P = .069$) and relapse-free survival ($P = .025$). These antileukemic responses may be relevant information for the transplantation centers that intend to apply minor H antigen-based immunotherapy in HLA-matched hematopoietic minor H antigen-mismatched transplantation recipients.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.06.001

REFERENCES

1. Hambach L, Goulmy E. Immunotherapy of cancer through targeting of minor histocompatibility antigens. *Curr Opin Immunol*. 2005;17:202-210.
2. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*. 1990;75:555-562.
3. de Bueger MM, Bakker A, van Rood JJ, et al. Tissue distribution of human minor histocompatibility antigens. Ubiquitous versus restricted tissue distribution indicates heterogeneity among human cytotoxic T lymphocyte-defined non-MHC antigens. *J Immunol*. 1992;149:1788-1794.
4. Zwaan FE, Hermans J, Gratwohl A, EBMT Leukaemia Party (EBMT-LWP). The influence of donor-recipient sex mismatching on the outcome of allegeneic BMT in leukemia. *Bone Marrow Transplant*. 1989;4(Suppl 2):8.
5. Gratwohl A, Hermans J, Niederwieser D, et al. Female donors influence transplant-related mortality and relapse incidence in male recipients of sibling blood and marrow transplants. *Hematol J*. 2001;2:363-370.
6. Urbano-Ispizua A, Rozman C, Pimentel P, et al. Risk factors for acute graft-versus-host disease in patients undergoing transplantation with CD34(+) selected blood cells from HLA-identical siblings. *Blood*. 2002;100:724-727.
7. Loren AW, Bunin GR, Boudreau C, et al. Impact of donor and recipient sex and parity on outcomes of HLA-identical sibling allogeneic hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant*. 2006;12:758-769.
8. Akatsuka Y, Warren EH, Gooley TA, et al. Disparity for a newly identified minor histocompatibility antigen, HA-8, correlates with acute graft-versus-host disease after haematopoietic stem cell transplantation from an HLA-identical sibling. *Br J Haematol*. 2003;123:671-675.
9. McCarroll SA, Bradner JE, Turpeinen H, et al. Donor-recipient mismatch for common gene deletion polymorphisms in graft-versus-host disease. *Nat Genet*. 2009;41:1341-1344.
10. Dickinson AM, Wang XN, Sviland L, et al. In situ dissection of the graft-versus-host activities of cytotoxic T cells specific for minor histocompatibility antigens. *Nat Med*. 2002;8:410-414.
11. Mutis T, Gillespie G, Schrama E, et al. Tetrameric HLA class I-minor histocompatibility antigen peptide complexes demonstrate minor histocompatibility antigen-specific cytotoxic T lymphocytes in patients with graft-versus-host disease. *Nat Med*. 1999;5:839-842.
12. Kim YH, Faaij CM, Van Halteren AG, et al. In Situ Detection of HY-Specific T Cells in Acute Graft-versus-Host Disease-Affected Male Skin after Sex-Mismatched Stem Cell Transplantation. *Biol. Blood Marrow Transplant*. 2012;18:381-387.
13. Goulmy E, Schipper R, Pool J, et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med*. 1996;334:281-285.
14. Tseng LH, Lin MT, Hansen JA, et al. Correlation between disparity for the minor histocompatibility antigen HA-1 and the development of acute graft-versus-host disease after allogeneic marrow transplantation. *Blood*. 1999;94:2911-2914.
15. Gallardo D, Arostegui JI, Balas A, et al. Disparity for the minor histocompatibility antigen HA-1 is associated with an increased risk of acute graft-versus-host disease (GvHD) but it does not affect chronic GvHD incidence, disease-free survival or overall survival after allogeneic human leucocyte antigen-identical sibling donor transplantation. *Br J Haematol*. 2001;114:931-936.

16. Spellman S, Warden MB, Haagenson M, et al. Effects of mismatching for minor histocompatibility antigens on clinical outcomes in HLA-matched, unrelated hematopoietic stem cell transplants. *Biol. Blood Marrow Transplant*. 2009;15:856-863.
17. Kim YH, Vyth-Dreese FA, Schrama E, et al. Exogenous addition of minor H antigen HA-1+ dendritic cells to skin tissues ex vivo causes infiltration and activation of HA-1 specific cytotoxic T cells. *Biol Blood Marrow Transplant*. 2011;17:69-77.
18. Hambach L, Spierings E, Goulmy E. Risk assessment in haematopoietic stem cell transplantation: minor histocompatibility antigens. *Best Pract Res Clin Haematol*. 2007;20:171-187.
19. van der Harst D, Goulmy E, Falkenburg J, et al. Recognition of minor histocompatibility antigens on lymphocytic and myeloid leukemic cells by cytotoxic T-cell clones. *Blood*. 1994;83:1060-1066.
20. Akatsuka Y, Nishida T, Kondo E, et al. Identification of a Polymorphic Gene, BCL2A1, Encoding Two Novel Hematopoietic Lineage-specific Minor Histocompatibility Antigens. *J Exp Med*. 2003;197:1489-1500.
21. Dolstra H, Fredrix H, Maas F, et al. A human minor histocompatibility antigen specific for B cell acute lymphoblastic leukemia. *J Exp Med*. 1999;189:301-308.
22. Brickner AG, Evans AM, Mito JK, et al. The PANE1 gene encodes a novel human minor histocompatibility antigen that is selectively expressed in B-lymphoid cells and B-CLL. *Blood*. 2006;107:3779-3786.
23. Warren EH, Gavin MA, Xuereb SM, et al. A single nucleotide polymorphism in the SP110 nuclear phosphoprotein gene creates a minor histocompatibility antigen whose expression is regulated by interferon-gamma. *Blood*. 2002;100:73A.
24. Hambach L, Nijmeijer BA, Aghai Z, et al. Human cytotoxic T lymphocytes specific for a single minor histocompatibility antigen HA-1 are effective against human lymphoblastic leukaemia in NOD/scid mice. *Leukemia*. 2006;20:371-374.
25. Marijt W, Heemskerk M, Kloosterboer F, et al. Hematopoiesis-restricted minor histocompatibilty antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc Natl Acad Sci*. 2003;100:2742-2747.
26. de Rijke B, van Horssens-Zoetbrood A, Beekman JM, et al. A frame-shift polymorphism in P2X5 elicits an allogeneic cytotoxic T lymphocyte response associated with remission of chronic myeloid leukemia. *J Clin Invest*. 2005;115:3506-3516.
27. Katagiri T, Shiobara S, Nakao S, et al. Mismatch of minor histocompatibility antigen contributes to a graft-versus-leukemia effect rather than to acute GVHD, resulting in long-term survival after HLA-identical stem cell transplantation in Japan. *Bone Marrow Transplant*. 2006;38:681-686.
28. Kircher B, Stevanovic S, Urbanek M, et al. Induction of HA-1-specific cytotoxic T-cell clones parallels the therapeutic effect of donor lymphocyte infusion. *Br J Haematol*. 2002;117:935-939.
29. Mutis T, Brand R, Gallardo D, et al. Graft-versus-host driven graft-versus-leukemia effect of minor histocompatibility antigen HA-1 in chronic myeloid leukemia patients. *Leukemia*. 2010;24:1388-1392.
30. Spierings E, Drabbels J, Hendriks M, et al. A uniform genomic minor histocompatibility antigen typing methodology and database designed to facilitate clinical applications. *PLoS ONE*. 2006;1:e42.
31. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295-304.
32. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.
33. Stern M, Brand R, de WT, et al. Female-versus-male alloreactivity as a model for minor histocompatibility antigens in hematopoietic stem cell transplantation. *Am J Transplant*. 2008;8:2149-2157.
34. Perez-Garcia A, de la CR, Torres A, et al. Minor histocompatibility antigen HA-8 mismatch and clinical outcome after HLA-identical sibling donor allogeneic stem cell transplantation. *Haematologica*. 2005;90:1723-1724.
35. Bohringer D, Spierings E, Enczmann J, et al. Matching of the minor histocompatibility antigen HLA-A1/H-Y may improve prognosis in corneal transplantation. *Transplantation*. 2006;82:1037-1041.
36. Stelljes M, Strothotte R, Pauels HG, et al. Graft-versus-host disease after allogeneic hematopoietic stem cell transplantation induces a CD8+ T cell-mediated graft-versus-tumor effect that is independent of the recognition of alloantigenic tumor targets. *Blood*. 2004;104:1210-1216.
37. Morita Y, Heike Y, Kawakami M, et al. Monitoring of WT1-specific cytotoxic T lymphocytes after allogeneic hematopoietic stem cell transplantation. *Int. J. Cancer*. 2006;119:1360-1367.