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Association of Disparities in Known Minor Histocompatibility Antigens with Relapse-Free Survival and Graft-versus-Host Disease after Allogeneic Stem Cell Transplantation

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Allogeneic stem cell transplantation (allo-SCT) can induce remission in patients with hematologic malignancies due to graft-versus-tumor (GVT) responses. This immune-mediated antitumor effect is often accompanied by detrimental graft-versus-host disease (GVHD), however. Both GVT and GVHD are mediated by minor histocompatibility antigen (MiHA)-specific T cells recognizing peptide products from polymorphic genes that differ between recipient and donor. In this study, we evaluated whether mismatches in a panel of 17 MiHAs are associated with clinical outcome after partially T cell–depleted allo-SCT. Comprehensive statistical analysis revealed that DNA mismatches for one or more autosomal-encoded MiHAs was associated with increased relapse-free survival in recipients of sibling transplants ($P = .04$), particularly in those with multiple myeloma ($P = .02$). Moreover, mismatches for the ubiquitous Y chromosome–derived MiHAs resulted in a higher incidence of acute GVHD grade III-IV ($P = .004$), whereas autosomal MiHA mismatches, ubiquitous or restricted to hematopoietic cells, were not associated with severe GVHD. Finally, we found considerable differences among MiHAs in their capability of inducing in vivo T cell responses using dual-color tetramer analysis of peripheral blood samples collected after allo-SCT. Importantly, detection of MiHA-specific T cell responses was associated with improved relapse-free survival in recipients of sibling transplants ($P = .01$). Our findings provide a rationale for further boosting GVT immunity toward autosomal MiHAs with a hematopoietic restriction to improve outcomes after HLA-matched allo-SCT.

INTRODUCTION

Allogeneic stem cell transplantation (allo-SCT) combined with donor lymphocyte infusion (DLI) is a potent treatment for patients with hematologic malignancies [1,2]. Numerous clinical and experimental studies in HLA-identical allo-SCT provide evidence that both the potentially curative graft-versus-tumor (GVT) effect and graft-versus-host disease (GVHD) develop as a result of donor T cell responses directed against disparate minor histocompatibility antigens (MiHAs) [3-5]. These MiHAs are polymorphic HLA-bound peptides derived from cellular proteins that can induce powerful alloreactive T cell responses. It has been demonstrated that emergence of MiHA-specific T cells precedes clinical remission in patients treated with DLI [4,6,7]. Although various MiHAs, including Y chromosome–encoded MiHAs, are expressed ubiquitously, increasing numbers of autosomal-encoded MiHAs expressed exclusively by hematopoietic cells and their malignant counterparts are being identified [8-10]. The molecular identification of these GVHD- and GVT-associated MiHAs has made it possible to study the clinical impact of MiHA mismatches and their specific T cell responses after allo-SCT.

Several studies of HLA-matched allo-SCT have reported an association between MiHA mismatches and clinical outcome. Mismatches in individual MiHAs, including HA1, HA2, and HA8, have been associated with increased rates of GVHD and lower rates of relapse in some studies [11-13], although other studies did not confirm these results [14-16]. Furthermore, previous studies have mainly investigated cohorts of HLA-matched non–T cell–depleted transplants and found an increased rate of chronic GVHD (cGVHD) and a reduced relapse rate only with HY MiHA disparity [17,18]. Moreover, investigations of the role of MiHA incompatibility in transplantation outcome have been hampered by the requirement

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to restrict studies to specific HLA types and the low frequencies of particular MiHA alleles. Recently, however, it was reported that patients with HLA-A2$^+$ chronic myelogenous leukemia who developed acute GVHD (aGVHD) showed improved overall survival (OS) and relapse-free survival (RFS) when receiving a transplant from an HA1-mismatched donor [19].

In this study, we performed a retrospective analysis on the impact of a panel of 17 immunogenic MiHA mismatches in a relatively large cohort of patients who underwent partially T cell–depleted allo-SCT. In recipients of sibling transplants, mismatches in one or more of the studied autosomal-encoded MiHAs resulted in improved RFS ($P = .04$), especially in patients with multiple myeloma (MM) ($P = .02$). In contrast, no significant association between autosomal MiHA mismatches and aGVHD or cGVHD was observed, whereas an HY disparity was associated with an increased rate of grade III-IV aGVHD ($P = .004$). Finally, here we describe for the first time the potential of disparate MiHAs to induce productive T cell responses posttransplantation. Tetramer analysis revealed that a strong variation in the ability of different MiHAs to mount specific CD8$^+$ T cell responses post-transplantation (0%-60%). More important, the presence of MiHA-specific T cell immunity was associated with improved RFS, without inducing severe aGVHD or cGVHD. Taken together, these data provide a rationale for further boosting GVT immunity toward autosomal hematopoietic-restricted MiHAs to improve RFS in recipients of HLA-matched allo-SCT.

PATIENTS AND METHODS

Patients and Donors

This study included a total of 327 adult allo-SCT recipients and their donors. They were selected from the total transplantation cohort of our center, who underwent HLA-matched, partially T cell–depleted allo-SCT for a hematologic malignancy between 1995 and 2010. HLA was typed using sequence-specific PCR. Recipients of sibling transplants underwent transplantation with an HLA-identical sibling donor graft, and recipients of matched unrelated donor (MUD) grafts underwent transplantation with an 8-10 of 10 HLA-matched voluntary donor, not considering HLA-DP. Only donor–recipient couples with HLA types HLA-A1, -A2, -A3, -A24, -B7, -B8, or -B44 were included, because the selected set of MiHAs was restricted to these HLA types. Furthermore, couples were selected based on the availability of both patient and donor material. Table 1 reports characteristics of patients, donors, and allo-SCT procedures. All patients and donors had provided informed consent for the prospective collection of data and samples for investigational use. The study was approved by the Radboud University Nijmegen Medical Centre's Institutional Review Board.

Treatment Protocol

All patients were treated in accordance with previously reported protocols [20-22]. Myeloablative conditioning regimens consisted of cyclophosphamide 60 mg/kg for 2 days in combination with either total body irradiation (TBI; 4.5 Gy for 2 days) or busulfan (4 mg/kg for 4 days). Idarubicin (42 mg/m^2 over 48 hours i.v.) was often added to reduce the risk of relapse in the setting of partially T cell–depleted SCT [23]. Nonmyeloablative conditioning regimens consisted mainly of cyclophosphamide (1200 mg/m^2 for 4 days) in combination with fludarabine (30 mg/m^2 for 4 days), and sometimes only TBI (2 Gy). Patients receiving a MUD graft received antithymocyte globulin (ATG) (2 mg/kg for 4 days). After conditioning, patients received a partially T cell–depleted graft derived either from bone marrow (BM) or mobilized peripheral blood stem cells (PBSCs). The median number of CD34$^+$ stem cells in the graft was 1.9×10^6 cells/kg (range, $0.5\text{-}6.5 \times 10^6$ cells/kg) for BM and 5.6×10^6 cells/kg (range, $1.3\text{-}13.8 \times 10^6$ cells/kg) for PBSCs, and the median number of CD3$^+$ T cells was 0.7×10^6 T cells/kg in BM grafts and 0.5×10^6 T cells in PBSC grafts (Table 1). GVHD prophylaxis consisted of cyclosporine A (CsA) only in the majority of patients, at a dosage of 1.5 mg/kg i.v. twice daily for the first 2 weeks and 1 mg/kg i.v. twice daily or 2.5-3 mg/kg orally twice daily thereafter. CsA was tapered in the absence of GVHD starting at 2 months posttransplantation and stopped at 3 months. Several patients received prophylactic or therapeutic DLI after allo-SCT. Prophylactic DLI was restricted to patients who had been off CsA therapy for at least 3 months and had not developed aGVHD grade II-IV or cGVHD.

Table 1
Recipient, Donor, and SCT Characteristics (n = 327)

Characteristic	Value
Recipient age, years, mean (range)	46 (18-67)
Donor age, years, mean (range)	46 (11-71)
Recipient male sex, n (%)	195 (60)
Donor male sex, n (%)	191 (58)
Donor–recipient sex match, n (%)	
Male patient–female donor	76 (23)
Other	251 (77)
Donor relation, n (%)	
Matched sibling donor	264 (81)
Matched unrelated donor	63 (19)
Disease category, n (%)	
Acute myelogenous leukemia/myelodysplastic syndrome	118 (36)
Acute lymphoblastic leukemia	34 (10)
Chronic myelogenous leukemia	53 (16)
Non-Hodgkin lymphoma/chronic lymphocytic leukemia	67 (20)
MM	55 (17)
Stem cell source	
Mobilized peripheral blood, n (%)	175 (54)
CD34 $\times 10^6$/kg, median (range)	5.6 (1.3-13.8)
CD3 $\times 10^6$/kg, median (range)	0.5 (0.04-1.7)
Bone marrow, n (%)	152 (46)
CD34 $\times 10^6$/kg, median (range)	1.9 (0.5-6.5)
CD3 $\times 10^6$/kg, median (range)	0.7 (0.5-1.1)
Date of SCT, n (%)	
1995-1999	98 (30)
2000-2004	83 (25)
2005-2010	146 (45)
Conditioning regimens, n (%)	
Myeloablative	
(Idarubicin)-cyclophosphamide-busulfan	18 (5.5)
(Idarubicin)-cyclophosphamide-TBI	182 (55.5)
Cyclophosphamide-ATG-busulfan	8 (2.5)
Cyclophosphmycophenolate mofetilamide-ATG-TBI	39 (12)
Nonmyeloablative	
Fludarabine-cyclophosphamide	50 (15.5)
Fludarabine-cyclophosphamide-ATG	16 (5)
TBI alone	14 (4)
GVHD prophylaxis, n (%)	
Cyclosporine alone	305 (93)
Cyclosporine/mycophenolate mofetil	13 (4)
None	9 (3)
Disease status, n (%)	
Early	193 (59)
Intermediate	81 (25)
Advanced	53 (16)
Interval between diagnosis and SCT, n (%)	
≤1 year	205 (62.5)
>1 year	122 (37.5)
Cytomegalovirus status, n (%)	
Negative/negative	76 (23)
Other combination	229 (70)
Missing	22 (7)
Gratwohl score, n (%)	
0	30 (9)
1	183 (56)
2	93 (28.5)
3	21 (6.5)

Definition of Outcome Variables

Acute GVHD was graded according to the criteria of Przepiorka et al. [24], and cGVHD was classified according to the revised Seattle criteria of Lee et al. [25] Patient risk scores for outcome were determined based on the Gratwohl score [26]. OS, RFS, and nonrelapse mortality (NRM) were defined according to the standard criteria proposed by the European Group for Blood and Marrow Transplantation.

MiHA Genotyping Using the KASPar System

HLA-matched SCT donor–recipient pairs were genotyped for a panel of 17 MiHAs with the KASPar assay system (KBioscience, Hoddesdon, UK), a fluorescence-based competitive allele-specific PCR using nonlabeled primers. Details of this method are available at http://www.kbioscience.co.uk.

Tetramer Staining and Validation by T Cell Culture

PE- and allophycocyanin (APC)-labeled MiHA tetramers were produced as described previously [27]. Tetramer staining was performed directly on cryopreserved peripheral blood mononuclear cells (PBMCs) after thawing and at 7 days after ex vivo restimulation with the appropriate MiHA peptide [28]. For this, PBMCs were stimulated once with MiHA peptide–pulsed (10 μM) Epstein-Barr virus lymphoblastoid cell lines (EBV-LCLs) on day 0. MiHA peptides were loaded on a corresponding EBV-LCL stably transduced with either HLA-A2, -A3, or -B7. For additional HLA types (eg, HLA-A24, HLA-B8), matching healthy donor EBV-LCL were used. After initial ex vivo restimulation, 100 IU/mL of IL-2 (Chiron, Emeryville, CA) and 10 ng/mL of IL-15 (Immunotools, Friesoythe, Germany) were added at days 2 and 5. For tetramer staining, $\sim 1 \times 10^6$ cells were stained with 0.2 μg of tetramer for 15 minutes at room temperature. The stability of all tetramers was verified by HPLC analysis in combination with an HLA-binding assay (MHC-ELISA or MHC-bead assay) [29,30]. In addition, the functional reactivity of all tetramers, except HEATR, was confirmed by staining of cytotoxic T lymphocyte clones specific for the corresponding epitope and subsequent flow cytometry analysis. After tetramer staining, cells were washed with PBS/0.5% BSA (Sigma-Aldrich, St. Louis, MO) and labeled with Alexa Fluor 700–conjugated CD8 (Life Technologies, Grand Island, NY) in combination with FITC-conjugated CD4, CD14, CD16, and CD19 (Beckman Coulter, Brea, CA) for 30 minutes at 4°C. Finally, cells were washed and resuspended in PBS/0.5% BSA containing 0.2 μM Sytox blue (Life Technologies), to allow exclusion of dead cells. Data acquisition was performed on a Cyan-ADP analyzer (Beckman Coulter) and analyzed with Kaluza 1.1 software (Beckman Coulter). $CD8^+$ T cells were defined as viable Sytox blue–negative, CD8-positive, and CD4-, CD14-, CD16-, and CD19-negative (on FITC) single-cell lymphocytes. Within the $CD8^+$ T cell population, cells positive for both tetramers (APC and PE) were quantified (Supplemental Figure 1). Patients were classified as having a positive tetramer response when MiHA-specific $CD8^+$ T cells (≥0.01% tetramer-positive cells within the $CD8^+$ T cell population) were found either directly after thawing and/or at 7 days after peptide ex vivo restimulation using peripheral blood samples obtained during the effector or memory phase of the immune response.

Statistical Analysis

The outcome variables aGVHD and cGVHD, relapse, RFS, OS, and NRM after allo-SCT were analyzed in relation to MiHA disparity. Associations with RFS and OS were analyzed using Kaplan-Meier curves and log-rank tests. Statistical differences in the RFS rates at specific time points were analyzed using Kaplan-Meier point estimates and their associated errors. Associations with the cumulative incidence of aGVHD, cGVHD, relapse, and NRM were estimated respecting the presence of competing risks using the Gray test Competing risks were death within 100 days from other toxicities or relapse for aGVHD, death after 100 days from other toxicities or relapse for cGVHD, NRM for relapse, and death from relapse for NRM.

In cases with a P value ≤.20 in univariate analysis, Cox regression analysis (for the endpoints RFS and OS) and Fine-Gray regression analysis (for the endpoints aGVHD, cGVHD, relapse, and NRM) were used to adjust for the following confounding risk factors: patient age, stem cell source, year of transplantation, conditioning regimen, diagnosis subgroup, cytomegalovirus seropositivity of either recipient and/or donor, and aGVHD grade II-IV. Analyses were performed using SAS 8.2 software (SAS Institute, Cary, NC) and the cmprsk package of open source language R version 2.6.2. (www.r-project.org). P values <.05 were considered statistically significant. MiHA mismatch parameters were defined as disparate HY (HY MiHA restricted to HLA-A2, -B7, or -B8), disparate HA1, and disparate autosomal MiHA (mismatched for one or more of the MiHAs listed in Table 2, excluding HY).

RESULTS

Clinical Outcome Parameters after HLA-Matched Allo-SCT

We analyzed clinical outcomes after partially T cell–depleted allo-SCT in 327 patients with a hematologic malignancy. The median follow-up was 7.1 years (range, 0.5-17 years) in patients alive at last follow-up and 1.1 years (range, 0.05-12.6 years) in those who died. Figure 1A and B show the survival curves for OS (5 year: 68%) and RFS (5 year: 44%) for the complete cohort. NRM after 5 years was 15.6% (ie, 43.4% of all deaths; Figure 1C), and the relapse rate was 40.4%. Acute GVHD occurred in 19.6% of patients but was severe (grade III-IV) in only 6.5%. Of the evaluable patients, 22.7% developed limited cGVHD, and 14.2% had extensive cGVHD.

Table 2
MiHA Disparity Rate

MiHA	HLA Restriction	Peptide Sequence	Reference	Number*	Disparate Pairs, n (%)†
HA3	A1	VTEPGTAQY	[40]	82/87	3 (3.7)
HA1	A2	VLHDDLLEA	[41]	195/197	32 (16.4)
HA2	A2	YIGEVLVSV	[42,43]	195/197	6 (3.1)
HA8	A2	RTLDKVLEV	[44]	194/197	22 (11.3)
HY	A2	FIDSYICQV	[9]	197/197	42 (21.3)
ADIR	A2	SVAPALALAFPA	[45]	195/197	30 (15.4)
HwA11	A2	CIPPDSLLFPA	[46]	195/197	6 (3.1)
SP110	A3	SLPRGTSTPK	[47]	92/93	7 (7.6)
PANE1	A3	RVWDLPGVLK	[48]	92/93	5 (5.4)
ACC1	A24	DYLQYVLQI	[49]	54/54	6 (11.1)
ACC2	B44	KEFEDDIINW	[49]	64/65	16 (25.0)
LRH1	B7	TPNQRQNVC	[35]	97/98	19 (19.6)
HY	B7	SPSVDKARAEL	[8]	98/98	26 (26.5)
ECGF	B7	RPHAIRRPLAL	[50]	97/98	5 (5.2)
ZAPHIR	B7	IPRDSWWVEL	[51]	97/98	12 (12.4)
HY	B8	LPHNMTDL	[52]	67/67	14 (20.9)
HEATR	B8	ISKERAEAL	[53]	64/67	8 (12.5)

* Number of patient donor couples typed among the total number of couples presenting with the correct HLA molecule.

† Number of disparate pairs and disparity rate of a particular MiHA were determined within all typed couples presenting with the correct HLA molecule.

MiHA Allele Frequency and Phenotype Disparity Rate

Recipient and donor DNA were typed to determine MiHA allele frequency and disparity rates. Paired couples were genotyped only when the appropriate HLA molecule to which the MiHA is restricted was expressed, leading to actual immunogenic disparity rates. HY.A2, HY.B7, or HY.B8 disparity was recorded when a female donor to male recipient transplantation was performed. The highest disparity rates within the restricting HLA type were observed for all Y chromosome–derived MiHAs (20.9%-26.5%), as well as for ACC2 (25.0%), LRH1 (19.6%), and HA1 (16.4%). Overall, 60 (18.2%) and 137 (41.6%) of the 327 pairs were disparate for Y-chromosome–encoded and autosomal chromosome–encoded MiHA, respectively (Table 2).

HY Disparity Associated with Increased aGVHD

The incidence of grade III-IV aGVHD at day 100 after allo-SCT was significantly higher in HY-mismatched patients than in HY-matched patients (19% versus 4%; $P < .001$, univariate analysis) when respecting the presence of competing risks. Multivariate analysis confirmed this association for HY incompatibility with the increased risk of aGVHD grade III-IV (hazard ratio [HR], 4.1; 95% confidence interval [CI], 1.6-10.3; $P < .001$) (Figure 2A). Notably, none of the non–sex-linked MiHA mismatched categories (HA1 or autosomal MiHA mismatched) showed an association with aGVHD or cGVHD (Figure 2A and B), possibly owing to the hematopoietic-restricted tissue expression pattern of the majority of the autosomal MiHAs tested.

Autosomal MiHA Disparity Is Associated with Improved RFS in Sibling Transplants

Statistical analysis revealed that mismatches in the studied MiHAs had no impact on RFS in the complete cohort (Figure 3A). Because recipients of MUD grafts might have an HLA-DP mismatch, as well as a higher rate of unknown MiHA mismatches, we analyzed the effect of known MiHA disparities on clinical outcome separately in the sibling cohort (n = 264). Interestingly, recipients of an autosomal MiHA-mismatched sibling graft had significantly better RFS in

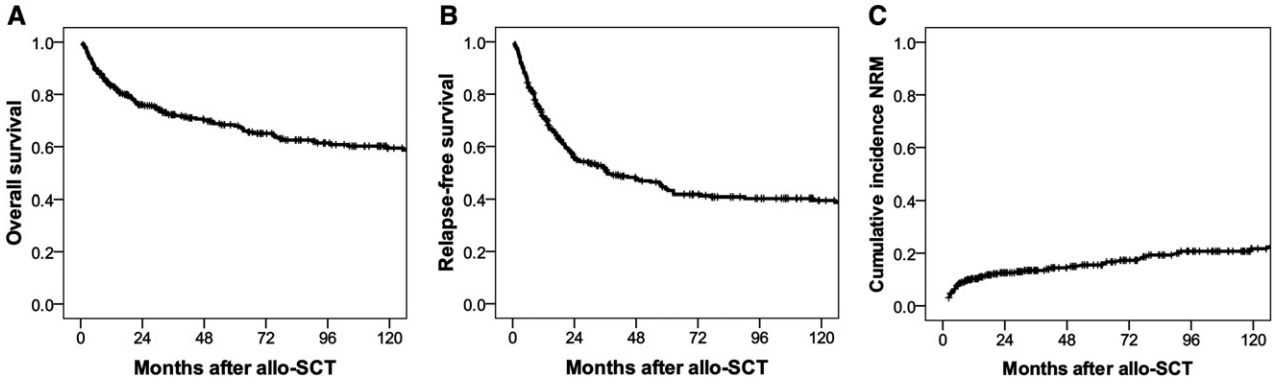

Figure 1. Clinical outcome parameters after partially T cell–depleted allo-SCT in the complete cohort of allo-SCT recipients. OS (A), RFS (B), and cumulative incidence of NRM (C) were determined by Kaplan-Meier analysis.

multivariate analysis (HR, 0.68; 95% CI, 0.48-0.98; $P = .04$) (Figure 3B and Table 3). This beneficial effect of autosomal MiHA disparity on RFS could be attributed to trends toward improved NRM (HR, 0.59; 95% CI, 0.29-1.25; $P = .19$) and decreased relapse (HR, 0.77; 95% CI, 0.50-1.11; $P = .14$), as assessed in multivariate Fine-Gray competing-risk analyses. Furthermore, in sibling allo-SCT, HY disparity also was associated with a higher incidence of grade III-IV aGVHD (HR, 4.2; 95% CI, 1.6-10.9; $P = .004$) (Table 3).

Analysis of clinical outcomes in recipients of an MUD graft ($n = 61$) showed no significant associations between MiHA disparity and either RFS (Figure 3C) or aGVHD (data not shown). Taken together, these data indicate that recipients of an HLA-identical sibling donor stem cell graft mismatched for the studied autosomal MiHA may induce beneficial GVT immunity post-SCT.

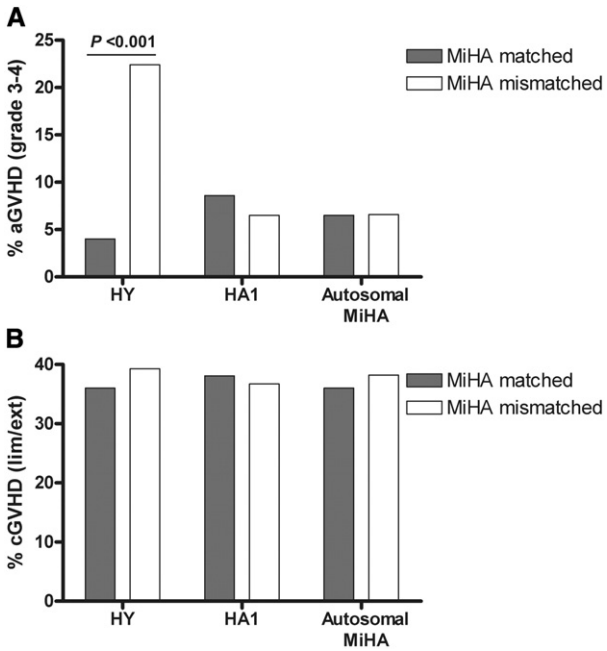

Figure 2. Occurrence of GVHD after MiHA-mismatched allo-SCT. In the complete cohort, the incidence rates of aGVHD grade III-IV (A) and limited/extensive cGVHD (B) were determined using Fine-Gray competing-risk regression models. Groups were categorized based on MiHA disparities in mismatching and matching at the DNA level.

Patients with MM Show Improved RFS after Transplantation with an MiHA-Mismatched Sibling Graft

Interestingly, our multivariate analysis including correction for diagnosis subgroups revealed an association between autosomal MiHA disparity and improved RFS in HLA-matched sibling allo-SCT, indicating a diagnosis-independent effect. To identify which patients would benefit the most from a disparity in the studied MiHAs, we analyzed the influence of MiHA mismatches on clinical outcome in different diagnosis subgroups separately. Notably, we were able to confirm a significant correlation between the occurrence of disparate autosomal MiHA and clinical outcome only in the MM subgroup. Of the 22 mismatched patients with MM, 15 were mismatched for 1 MiHA, 6 were mismatched for 2 MiHAs, and 1 was mismatched for 4 MiHAs. Importantly, patients with MM who underwent allo-SCT had improved RFS when at least one autosomal MiHA mismatch was present (HR, 0.41; 95% CI, 0.19-0.89; $P = .02$) (Figure 4A). In addition, patients with an autosomal MiHA mismatch developed significantly less relapse, as detected by univariate Fine-Gray competing-risk analysis (HR, 0.46; 95% CI, 0.21-1.00; $P = .049$) (Figure 4B). MiHA disparity was also correlated with an increased rate of limited cGVHD ($P = .03$, univariate analysis) (Figure 4C). Notably, the effects on clinical outcome parameters were not related to differences in disease status between MiHA-matched and MiHA-mismatched patients (Figure 4D). Interestingly, 22.6% of the MiHA-mismatched patients with MM had an HA1 disparity (Figure 4E). HA1 disparity has been reported to be associated with improved RFS in patients with chronic myelogenous leukemia who developed aGVHD [19]. Taken together, these data suggest that mismatches in the studied MiHAs induce improved graft-versus-myeloma immunity after partially T cell–depleted sibling allo-SCT.

Ability to Induce Tetramer-Positive MiHA-Specific T Cell Responses Varies among MiHAs

Although genetic MiHA incompatibility has a significant effect on clinical outcome post-SCT, this does not necessarily mean that corresponding T cell responses actually occur. Thus, we investigated the potential of 15 of the 17 studied MiHAs to induce productive MiHA-specific T cell responses in vivo. For this, we analyzed recipient PBMC material obtained at the median of 9 months (range, 2-70 months) after allo-SCT using a dual-color MiHA-multimer approach. Patients were classified as having a positive tetramer response when MiHA-specific $CD8^+$ T cells were detected directly after thawing and/or after a single ex vivo peptide

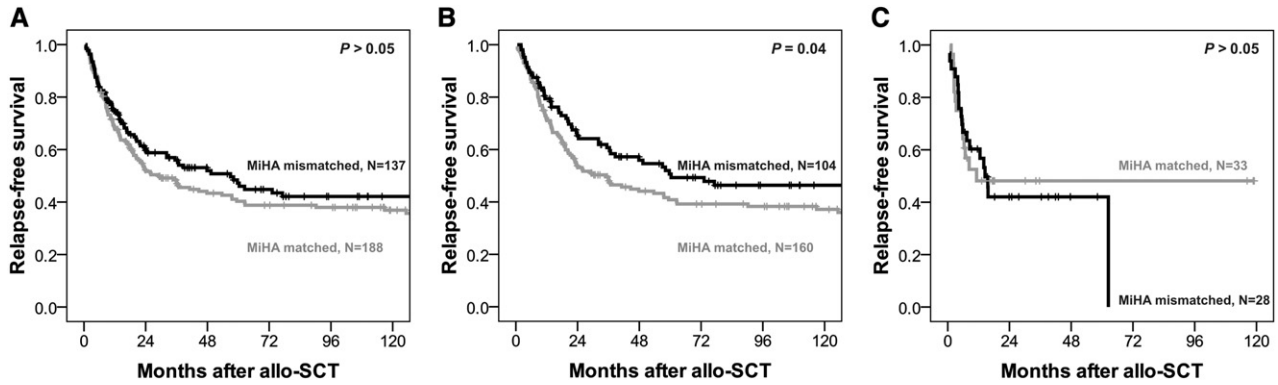

Figure 3. Autosomal MiHA disparity is associated with increased RFS after allo-SCT with a sibling graft. RFS was analyzed in the complete cohort (A) versus recipients of a sibling graft (B) or an MUD graft (C) using the log-rank test. Groups were categorized based on autosomal MiHA disparity in mismatching (black line) and matching (gray line) at the DNA level. Significant *P* values of multivariate analyses are shown.

restimulation. Although we were unable to analyze all patients at the same time interval, we believe that detection of MiHA tetramer–positive $CD8^+$ T cells at variable time points reflects either an ongoing effector immune response or a sustained effector-memory response after immune contraction. Figure 5A shows representative tetramer screenings of 3 different allo-SCT recipients. In freshly thawed samples, low numbers of MiHA-specific $CD8^+$ T cells were detected in 27 of 40 (67.5%) MiHA T cell–responsive patients. In addition, in 13 of 40 (32.5%) responsive patients, tetramer-positive T cells were detectable after 1 week of stimulation with MiHA peptide–pulsed EBV-LCL. Notably, in the whole cohort of MiHA-mismatched recipients, certain MiHA-specific T cell responses occurred more frequently than others (Figure 5B). In particular, disparity for HA1, HA2, PANE1, LRH1, ACC1, and the HY chromosome–encoded antigens HY.A2 and HY.B7 resulted in MiHA-specific $CD8^+$ T cell responses in 25%-60% of the MiHA-mismatched patients. HA8-, SP110-, and ZAPHIR-specific $CD8^+$ T cells were found in 10%-20% of these patients. In contrast, no productive $CD8^+$ T cell responses against ADIR, HwA11, ECGF, HEATR, and HY.B8 were observed, despite genetic disparities. These results indicate that certain MiHAs appear to be relatively more productive than others in inducing MiHA-specific $CD8^+$ T cell responses after partially T cell–depleted allo-SCT.

Finally, we examined whether the presence of an MiHA-specific T cell response, including those targeting autosomal MiHAs (n = 25 in the complete cohort; n = 20 in the sibling graft recipients) or HY (n = 15 in both the complete cohort and the sibling graft recipients) was associated with improved outcome posttransplantation. There were no significant differences in RFS curves between these groups (complete cohort: HR, 0.82; 95% CI, 0.52-1.30; $P = .39$; sibling graft recipients: HR, 0.73; 95% CI, 0.44-1.22; $P = .23$). Nevertheless, in the first years after allo-SCT, RFS clearly differed in patients with an MiHA-specific T cell response and those without such an response. Thus, we compared RFS at 3 years posttransplantation in the 2 groups, and found that detection of MiHA-specific T cell responses was associated with improved RFS in both the complete cohort (RFS of 69% versus 51%; $P = .03$, univariate analysis) (Figure 5C), as well as in the sibling graft recipients (RFS of 73% versus 52%; $P = .01$, univariate analysis) (Figure 5D). Notably, this association with improved RFS at 3 years after allo-SCT can be attributed

Table 3
Univariate Analysis of Patient Characteristics and Clinical Outcomes in the SIB Cohort

Outcome Parameter	MiHA Disparity	Univariate Analysis		Multivariate Analysis		
		HR (95% CI)	*P* Value	Confounding Risk Factors	HR (95% CI)	*P* Value
aGVHD grade III-IV	HY	4.80 (2.00-11.20)	<.001	Patient age, diagnosis, year of transplantation, stem cell source, conditioning regimen	4.20 (1.60-10.90)	.004
	MiHA	1.10 (0.50-2.50)	.74	NA	NA	NA
cGVHD limited/extensive	HY	1.30 (0.70-2.20)	.44	NA	NA	NA
	MiHA	1.01 (0.63-1.67)	.96	NA	NA	NA
Time to relapse	HY	0.58 (0.35-0.98)	.04	Diagnosis, year of transplantation, stem cell source, conditioning regimen	0.65 (0.40-1.08)	.095
	MiHA	0.77 (0.53-1.11)	.16	Diagnosis, year of transplantation, stem cell source, conditioning regimen	0.77 (0.50-1.11)	.14
RFS	HY	0.75 (0.49-1.16)	.20	Patient age, diagnosis, year of transplantation, stem cell source, conditioning regimen	0.81 (0.52-1.27)	.35
	MiHA	0.73 (0.52-1.03)	.07	Patient age, diagnosis, year of transplantation, stem cell source, conditioning regimen	0.68 (0.48-0.98)	.04
OS	HY	0.90 (0.52-1.55)	.70	NA	NA	NA
	MiHA	0.88 (0.56-1.35)	.56	NA	NA	NA
NRM	HY	1.30 (0.70-2.70)	.43	NA	NA	NA
	MiHA	0.59 (0.29-1.25)	.13	Patient age, year of transplantation, cytomegalovirus seropositive, aGVHD grade II-IV	0.59 (0.29-1.25)	.19

NA indicates not applicable.
Associations with OS and RFS were analyzed using Kaplan-Meier curves and log-rank tests. Associations with the cumulative incidence of aGVHD, cGVHD, relapse, and NRM were estimated respecting the presence of competing risks using the Gray test. Furthermore, in case a *P* value was ≤0.20 in univariate analyses, Cox regression analyses (for the endpoints OS and RFS) and Fine and Gray regression analyses (for the endpoints aGVHD, cGVHD, relapse, and NRM) were used to adjust for known confounding risk factors.

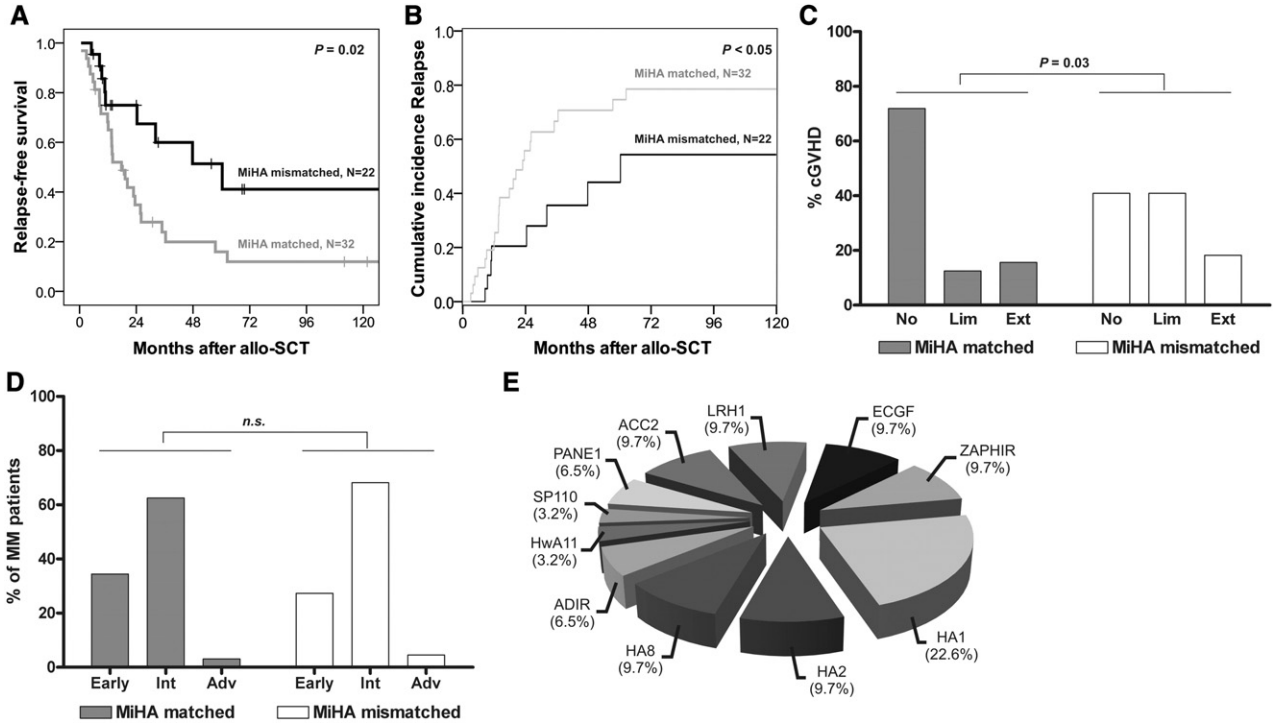

Figure 4. Patients with MM undergoing allo-SCT with a related MiHA-mismatched graft show improved RFS. Recipients of sibling grafts were grouped based on autosomal MiHA disparity in mismatching and matching at the DNA level. (A and B) Differences in RFS (A) and relapse rate (B) between these subgroups were analyzed using the log-rank test and Fine-Gray competing-risk regression model, respectively. *P* values of univariate analyses are given. (C and D) The incidence of cGVHD (C) and disease status (D) were analyzed using the Fisher exact test. (E) Within the total of mismatched MiHA, the relative contribution of each MiHA is depicted. Lim indicates limited; Ext, extensive; Int, intermediate disease; Adv, advanced disease; ns, not significant.

mainly to less relapse in patients with an MiHA-specific T cell response (complete cohort: relapse rate of 26% versus 39%, $P = .09$; sibling graft recipients: relapse rate of 24% versus 40%, $P = .047$), and to a lesser extent to improved NRM (complete cohort: NRM of 5% versus 10%, $P = .26$; sibling graft recipients: NRM of 3% versus 9%, $P = .10$), as assessed in univariate Fine-Gray competing-risk analyses. Despite the inclusion of T cell responses against ubiquitously expressed MiHAs, including HA8 and HY (ie, 17 of 40 in the complete cohort and 17 of 35 in the sibling graft recipients), the rates of grade III-IV aGVHD ($P > .70$) and limited/extensive cGVHD were not affected ($P > .20$; both univariate Fine-Gray competing-risk analysis). To conclude, these results indicate that productive MiHA-specific T cell responses contribute to the beneficial GVT immunity after partially T cell–depleted allo-SCT.

DISCUSSION

As the dominant target antigens in HLA-matched allo-SCT, MiHAs play pivotal roles in both GVT responses and GVHD. A precise understanding of involved MiHA-specific T cell responses not only may lead to better prediction of clinical outcome in allo-SCT recipients, but also may provide a rationale for selection of the most potent MiHAs in post-transplantation immunotherapy. Interestingly, our statistical analysis of immunogenic MiHA disparity rates in sibling graft recipients revealed that DNA mismatches in autosomal-encoded MiHAs are associated with improved clinical outcome. In particular, patients with MM had a lower rate of relapse and increased RFS when undergoing allo-SCT with an MiHA-mismatched sibling transplant. In addition, we found considerable variance in the relative immunogenicity of different MiHAs in inducing productive T cell responses posttransplantation. Most important, the presence of these MiHA-specific T cell responses was associated with improved GVT immunity after partially T cell–depleted allo-SCT.

Characteristics of all recipients and their corresponding donors were analyzed for clinical outcome parameters (Table 3). Importantly, we observed that mismatched autosomal MiHA, including those with a ubiquitous expression pattern, were not correlated with higher incidence rates of severe acute or chronic GVHD after partially T cell-depleted SCT. In accordance with previous reports, only the HY MiHA was associated with increased frequency of grade III-IV aGVHD. Similar findings have been reported by Gratwohl et al. [18] and Stern et al. [31], who used female-to-male alloreactivity as a model for HY MiHA mismatches. However, along with MiHAs, noninherited maternal antigens (NIMAs) and noninherited paternal antigens (NIPAs) are also involved in female donor to male recipient transplants. Reported rates of aGVHD are lower in non–T cell–depleted haploidentical sibling NIMA-mismatched allo-SCT than in NIPA-mismatched allo-SCT [32]. In the present study, we have no information on the NIMA or NIPA status of the transplant couples and cannot exclude a possible influence of these antigens on clinical outcome after partially T cell–depleted allo-SCT. Moreover, unknown MiHAs not included in our panel might be important, as may other general genetic disparities. Thus, we analyzed sibling graft recipients separately, thereby circumventing the greater likelihood of unknown polymorphic differences between recipients and donors. Analysis again revealed a role of HY MiHA disparity, and although more cases of severe aGVHD (grade III-IV) were observed, this sex mismatch showed a trend toward a lower incidence of relapse ($P = .095$) (Table 3), as had been reported by Gratwohl et al. [18].

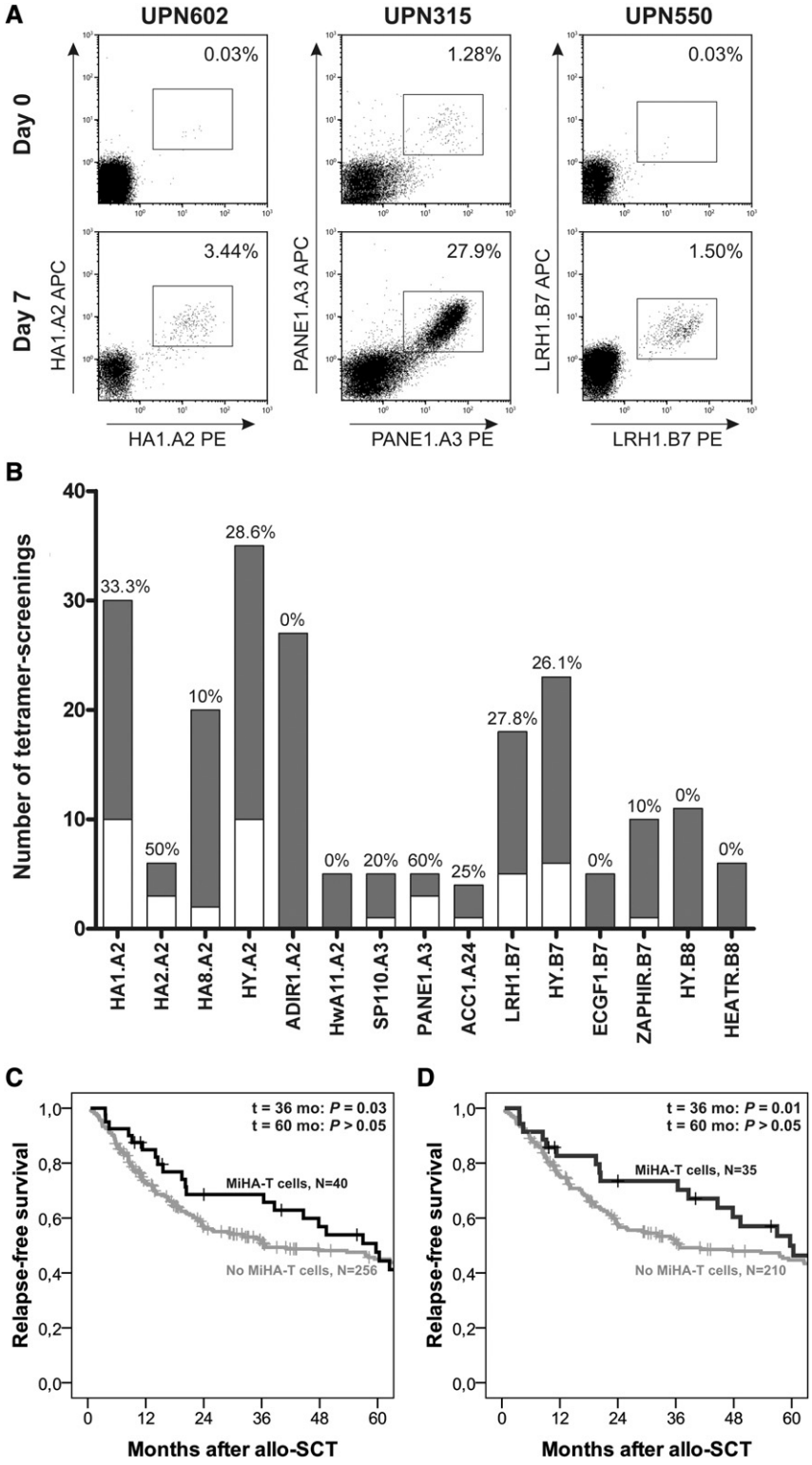

Figure 5. Detection of tetramer-positive MiHA-specific $CD8^+$ T cell responses is associated with improved RFS after allo-SCT. Recipient PBMC samples obtained after allo-SCT were analyzed for the presence of MiHA-specific $CD8^+$ T cells using a dual-color MiHA-multimer flow cytometry assay. Patients were considered to have a positive tetramer response when MiHA-specific $CD8^+$ T cells were found directly after thawing or at 7 days after stimulation with peptide-loaded EBV-LCL. (A) The numbers in the dot plots indicate the percentage of MiHA-specific cells positive for both tetramers (PE and APC) in the $CD8^+$, $CD4^-$, $CD14^-$, $CD16^-$, and $CD19^-$ T cell populations. Three representative examples are shown. (B) For each MiHA disparity, the number of tetramer-positive responses (white bars) within the total number of screenings (gray bars) is depicted for the complete cohort of MiHA-mismatched recipients. Percentages indicate the relative number of productive responses. (C and D) RFS was analyzed for the complete cohort of recipients (C) versus recipients of a sibling graft (D) using the log-rank test. Groups were categorized based on detection of MiHA-specific T cell responses (black line) versus no MiHA-specific T cell responses or no mismatching for any of the studied MiHAs (gray line) after allo-SCT. Statistical differences in RFS incidence were analyzed using Kaplan-Meier point estimates and their associated errors; univariate *P* values are given.

Our findings indicate that a disparity in at least one known autosomal MiHA was associated with higher RFS, with no associated increase in aGVHD incidence or severity after partially T cell–depleted allo-SCT. This contribution of autosomal MiHA mismatches to improved RFS in the sibling transplant setting is even more apparent when compared with recipients of an MUD graft. No effects of mismatched MiHA on RFS were seen in the MUD graft recipients, which might be attributed to the smaller size of this cohort. Furthermore, these MUD recipients received ATG treatment [20], which results in an additional in vivo T cell depletion, thereby reducing the likelihood of inducing tumor-reactive MiHA-specific T cell responses.

Along with the role of alloreactive T cells in the GVT response, the underlying malignancy of the recipients might be important as well. We found that autosomal MiHA disparity in sibling transplants was associated with improved RFS in multivariate analysis, which included correction for diagnosis subgroups, suggesting a diagnosis-independent effect; however, we could confirm a significant correlation between disparate autosomal MiHA and improved RFS only in the MM subgroup. The lack of similar effects in the other diagnosis subgroups could be related to differences in immune susceptibility of the various tumors and the number of patients included in this study. Furthermore, partially T cell–depleted SCT results in low rates of aGVHD and cGVHD, which might have downgraded the clinical impact of MiHA mismatches. When focusing on patients with MM in the sibling graft recipients, we noted an evident association between MiHA disparity and both relapse incidence and RFS independent of disease status. Moreover, MiHA disparity was also associated with a higher incidence of limited cGVHD. These findings indicate that patients with MM can derive particular benefit from an MiHA-driven GVT effect in the presence of an acceptable degree of cGVHD. MM is an immunogenic tumor, and affected patients can respond well to DLI [33,34]. This phenomenon could be linked to multiple factors, likely including high antigen presentation, good susceptibility to killing, and possibly a good window for immune recognition and killing owing to the relatively slow tumor growth. Furthermore, posttransplantation treatment with immunomodulatory drugs, such as lenalidomide, has a promoting effect on GVT immunity.

Overall, we found a low incidence of aGVHD (6.5% grade III-IV) in our partially T cell–depleted setting, even when ubiquitously expressed MiHA mismatches were present. This indicates a potential safe clinical application for transplant mismatching of certain strongly immunogenic MiHAs in allo-SCT. In previous studies, the MiHAs HA1, HA2, LRH1, and ACC1 have been implicated in selective induction of a GVT effect without GVHD [7,35-37]. We have shown that these MiHAs are also potent in inducing MiHA-specific T cell responses in vivo, adding to the promise of these MiHA not only in transplant mismatching, but also in vaccination and adoptive T cell strategies. However, timing of the tetramer-based analysis of posttransplantation PBMC samples is crucial in detecting MiHA-specific T cell responses, and our study had a rather wide time frame of sampling after allo-SCT, which likely resulted in underscoring the frequency of positive responses. In addition, some of the MiHA-specific T cell responses could be masked (subdominant) by other (un) known MiHAs. Moreover, the underlying malignancy might skew the MiHA-specific T cell repertoire toward a particular hematopoietic compartment such as the BM, which may prevent the detection of MiHA-specific T cells in peripheral blood. Thus, the absence of tetramer-positive T cells in our analyses does not necessarily mean a complete lack of MiHA-specific T cells in vivo. Nevertheless, we found that the presence of MiHA-specific T cell responses resulted in improved RFS at 3 years after allo-SCT despite the heterogeneity of our cohort. After 5 years, the RFS curves of patients with and without MiHA-specific immunity no longer differed, however. Because the group size was relatively limited at the start of the study and decreased even further over time owing to positive events or censoring of patients, our data are likely less reliable at later time points. Furthermore, due to the heterogeneity of the cohort, over time the patient group is likely skewed toward late-relapsing malignancies with low immunogenicity. It also could be that the patients who develop late relapse have impaired MiHA-specific T cell immunity due to immune escape mechanisms exploited by surviving tumor cells, such as the PD-1/PD-L1 and BTLA/HVEM coinhibitory pathways, as we reported previously [38,39]. Owing to this negative signaling, T cells can become exhausted over time, and patients may lose the advantage of MiHA-specific immunity posttransplantation.

Importantly, the occurrence of in vivo MiHA-specific T cell responses, including those recognizing ubiquitously expressed MiHAs, amongst which the HY antigens, was not associated with an increased incidence of severe aGVHD or cGVHD. Unfortunately, the group of patients with MiHA-specific T cell immunity was too small to allow us to focus on diagnosis subgroups or perform multivariate analyses. Thus, our findings need to be confirmed in a larger and more homogenous cohort of allo-SCT recipients with longer follow-up.

In conclusion, this study shows that MiHA mismatch is associated with improved clinical outcome after partially T cell–depleted HLA-matched allo-SCT, particularly in patients with MM and likely in patients with other hematologic malignancies as well. The observed positive effects might be attributed to the induction of GVT responses by MiHA-specific $CD8^+$ T cells. Not all MiHAs seem to have the same potential to induce MiHA-specific T cells, however, as demonstrated by our tetramer analysis of PBMC samples collected posttransplantation. Further study of the MiHAs that are most productive in this respect will allow selection of hematopoietic-restricted MiHAs as safe and potent target antigens in allo-SCT to prevent or treat tumor recurrence with posttransplantation immunotherapeutic strategies.

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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.2012.09.008.

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