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The Role of Missing Killer Cell Immunoglobulin-Like Receptor Ligands in T Cell Replete Peripheral Blood Stem Cell Transplantation from HLA-Identical Siblings

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The contribution of natural killer (NK) cells to graft-versus-malignancy (GVM) effects following hematopoietic stem cell transplantation (HSCT) remains uncertain, particularly in the HLA-identical setting. A model considering missing HLA ligands to the donor's inhibitory killer cell immunoglobulin-like receptor (KIR), termed the missing KIR ligand model, has been established in T cell depleted bone marrow transplantation (BMT), but lacks validity in other cohorts with different treatment characteristics. We hypothesized that the impact of missing KIR ligands on relapse-free survival (RFS) and overall survival (OS) in T cell replete peripheral blood SCT (PBSCT) differs from that in the T cell depleted BMT setting, and retrospectively evaluated 100 consecutive, HLA-identical sibling transplantations for hematologic malignancies. In addition to KIR ligand status, we considered the donors' activating KIRs and grafted NK, T, and $CD34^+$ cell doses. Our findings demonstrate noninferiority for OS ($P = .005$) and RFS ($P = .002$) for the heterozygous HLA-C group KIR ligand status (C1/2; n = 47) compared with patients missing either C1 or C2 (n = 53). Similarly, OS ($P = .031$) and RFS ($P = .034$) of Bw4-positive patients was noninferior to that of patients missing a Bw4 ligand to KIR3DL1. By multivariate analysis, C1/2 heterozygous patients had a favorable risk ratio (RR) for relapse (RR = 0.28; $P = .003$), RFS (RR = 0.56; $P = .046$), and acute graft-versus-host disease grade II-IV (RR = 0.36; $P = .05$). Following reduced-intensity conditioning (RIC), but not standard-intensity conditioning, myeloablative (MA) transplantation, a grafted NK cell dose above the median (3.4×10^7/kg) was associated with a lower risk of relapse (RR = 0.57; $P = .003$) and improved survival (RR = 0.78; $P = .03$). Overall, our findings support a role for NK alloreactivity in HLA-identical HSCT, but argue against a favorable impact of missing KIR ligands in the given setting. We conclude that the mechanism favoring the missing KIR ligand constellation in T cell depleted BMT may not operate in T cell replete PBSCT. The reasons for this differential effect remain unresolved.

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INTRODUCTION

Despite the established role of graft-versus-malignancy (GVM) mechanisms in hematopoietic stem cell transplantation (HSCT), relapsing malignancy remains a major obstacle to its success. The extent to which natural killer (NK) cells contribute to GVM remains a matter of debate, as does the underlying immunologic model. A contribution of NK cells to GVM was first demonstrated in T cell depleted haploidentical HSCT [1,2], where it was shown to depend on killer cell immunoglobulin-like receptor (KIR) ligand mismatch in graft-versus-host (GVH) direction, for example, in the case of an HLA-C group 2 (C2)-negative recipient with a C2-positive, KIR2DL1-positive donor. Subsequent studies confirmed the contribution of GVH-directed KIR ligand mismatch to NK cell alloreactivity in unrelated HSCT [3-7], whereas other studies did not [8-12]. The causes for these divergent results are most likely differences in patient cohorts and transplantation protocols, with particular regard to T cell depletion [9,13].

In HLA-identical as opposed to HLA-mismatched HSCT, NK alloreactivity must, by definition, rely on

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other mechanisms than KIR ligand mismatch. One possible mechanism, the so-called "missing KIR ligand" model, is defined by the patient's lack of one or more HLA class I ligands to the donor's inhibitory KIRs. Because of independent inheritance of KIR and HLA genes, this constellation occurs intraindividually (ie, it does not require an HLA mismatch), and thus it occurs frequently in the HLA-matched setting. Studies addressing the impact of the missing KIR ligand constellation on GVM, graft-versus-host disease (GVHD), and graft rejection have yielded divergent results [7,14-23]. Thus, in contrast to the KIR ligand mismatch model for HLA-mismatched HSCT and the conditions established to favor its clinical emergence (ie, haplotype mismatch, T cell depletion, high $CD34^+$ cell dose, and myeloid malignancy [1,9,13]), no model has yet obtained consensual acceptance to reliably predict NK alloreactivity in HLA-identical HSCT. Of note, those studies showing the missing KIR ligand model to be favorable in terms of transplantation outcome were based largely on bone marrow (BM) and/or T cell depleted grafts [14,17,18]. Importantly, a recent study elucidating the mechanisms behind the missing KIR ligand model was based on $CD34^+$ selected HSCT [23]. In view of the foregoing reports and our seemingly divergent results in T cell replete peripheral blood stem cell (PBSC) transplantation (PBSCT) [20], we speculate that graft source (BM vs PBSCs) and graft manipulation (T cell depleted vs unmanipulated grafts) are major factors determining whether the missing KIR ligand constellation may promote or interfere with GVM effects following HSCT. To address this question, we restricted the present analysis exclusively to unmanipulated PBSC grafts.

PATIENTS AND METHODS

We retrospectively evaluated 100 consecutive granulocyte-colony stimulating factor (G-CSF)–mobilized PBSCTs from HLA-identical siblings performed for hematologic malignancies at our institution between October 2000 and December 2008. According to the rules of the local ethics committee and the Declaration of Helsinki, all patients provided written informed consent before any diagnostic or treatment procedures and agreed to anonymized handling of patient data for research purposes. Treatment characteristics, described in more detail previously [20], are summarized in Table 1. In brief, 55 patients received standard myeloablative conditioning (MAC), and 45 patients received reduced-intensity conditioning (RIC) because of age, comorbidity, or previous high-dose therapy (2 allogeneic and 2 autologous HSCTs). GVHD prophylaxis consisted of a trough level adjusted calcineurin inhibitor (mainly cyclosporine-A [CsA]), and either short-course methotrexate (MTX; predominantly following MAC HSCT) or mycophenolate mofetil (MMF; exclusively following RIC HSCT). All grafts were T cell replete; that is, neither in vivo T cell depletion (by, eg, antithymocyte globulin (ATG) or alemtuzumab) nor in vitro T cell depletion was applied.

Flow cytometric analysis of graft composition (n = 100) and KIR genotyping (n = 86), applying the polymerase chain reaction (PCR) single specific primer (SSP)based KIR-Ready Gene Kit (INNO-TRAIN, Kronberg/Taunus, Germany), have been described previously [20]. KIR gene frequencies in the donor cohort (Table 2) are representative for a Caucasian population (www.allelefrequencies.net).

The missing KIR ligand constellation was evaluated for the inhibitory KIRs 2DL1 (ligand to HLA-C group 2/C2), 2DL2/3 (ligand to HLA-C group 1/C1), 3DL1 (ligand to HLA-Bw4 epitopes including A23, A24, and A32 [24]) and 3DL2 (ligand to HLA-A3 and A11). Because most and virtually all Caucasians are positive for 2DL1 and 3DL2, respectively, and at least one of the C1-specific inhibitory KIRs 2DL2 or 2DL3, several authors have suggested evaluating for the presence or absence of HLA-C KIR ligands [15,16,21] or HLA-A3 and HLA-A11 KIR ligands [20], simply assuming positivity of the cognate inhibitory KIR. In the present cohort, stratification by missing C2, either considering actual donor positivity for 2DL1 (n = 98) or assuming its positivity (n = 100), resulted in 95% identical strata. In case of missing C1, both methods resulted in a 100% identical stratification. In contrast, because a significant proportion of donors (9%) did not express 3DL1, "missing Bw4" was adopted only if 3DL1 was actually present in the donor. Nevertheless, because of the frequent expression of a Bw4 epitope, 98 of 100 transplants were evaluable for the "missing Bw4 ligand to KIR3DL1" condition.

The following activating KIRs were analyzed: 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, and 3DS1. The PCR kit used allows the discrimination of the 2DS4 full-length variants, *001 and *002, from the deletion variants, *003, *004, and *006. The KIR AA genotype was defined by the absence of any B haplotypedefining KIR (2DL2, 2DL5, 3DS1, 2DS1, 2DS2, 2DS3, or 2DS5; www.allelefrequencies.net). The BB genotype was assumed if the KIR A haplotypeassociated 3DL1 and 2DS4 were absent. All others were assigned to the AB genotype.

Data were analyzed as of January 31, 2009. Statistical analyses were performed using NCSS 2001 software (NCSS, Kaysville, UT). For univariate analyses, either Kaplan-Meier survival or cumulative incidence analysis (considering competing risks) was applied. Curves were compared using the log-rank test. To address the problem of multiple comparisons, a significant difference was assumed in cases of P values $\leq .01$.

Table 1. Patient and Treatment Characteristics

	Total	C1/2 Heterozygous	C1 or C2 Homozygous	*P*
n	100	47	53	
Follow-up of survivors, months, median (range)	38.2 (1.4–100.7)	39 (1.7–100.7)	36 (1.4–91.3)	.38
Median recipient age, years (range)	49 (17–74)	47	51	.36
Median donor age, years (range)	46 (13–79)	41	49	.07
Donor/recipient sex				.90
Female donor; male recipient	24	11 (23%)	13 (25%)	
Other	76	36 (77%)	40 (75%)	
Diagnosis				.13
Acute myelogenous leukemia	43	21 (45%)	22 (42%)	
Acute lymphoblastic leukemia	16	8 (17%)	8 (15%)	
CML	10	5 (11%)	5 (9%)	
Myelodysplastic syndrome	9	3 (6%)	6 (11%)	
Lymphoma*	10	2 (4%)	8 (15%)	
Myeloma†	8	7 (15%)	1 (2%)	
Other	4	1 (2%)	3 (6%)	
Risk category*				.14
Standard risk	35	20 (43%)	15 (28%)	
High risk	65	27 (57%)	38 (72%)	.20
Conditioning regimen				
Standard MAC	55	29 (62%)	26 (49%)	
fTBI 12 Gy/Cy	24	14	10	
Bu/Cy ± VP-16	25	12	13	
Other	6	3	3	
RIC	45	18 (38%)	27 (51%)	
Bu/Flu ± thiotepa	39	16	23	
Flu/TBI (2 Gy)	3	1	2	
Other	3	1	2	

MAC indicates myeloablative conditioning; RIC, reduced-intensity conditioning; CML, chronic myelogenous leukemia; Flu, fludarabline; Cy, cyclophosphamide 2 × 60 mg/kg i.v.; Bu, busulfan (12.8 mg/kg i.v. for MAC; 6.4 mg/kg in the majority of RIC regimens; (f)TBI, (fractionated) total body irradiation. Standard-risk disease was defined as acute leukemia in first remission or CML in first chronic phase; all other indications were classified as high-risk disease (see below).

*Remission state of patients with non-Hodgkin lymphoma: progressive/refractory, n = 6; relapse after HSCT, n = 3; second complete remission, n = 1.

†Remission state of patients with myeloma: progressive disease, n = 2; relapse following tandem HSCT, n = 2; relapse following allo-BMT, n = 1; partial remission following induction therapy, n = 3.

Noninferiority was assessed by the log-rank test using EquivTest software (Statistical Solutions, Saugus, MA) for equivalence analyses, with equivalence margins for the risk ratio (RR) set at 0.8-1.25. For univariate analyses, graft cell counts were dichotomized as "high" versus "low" according to the respective median values (Table 2). Cox regression multivariate analysis was calculated for overall survival (OS; death), relapse-free survival (RFS; death or relapse), relapse, acute GVHD (aGVHD), and nonrelapse mortality (NRM), considering the continuous variables (patient and donor age and graft cell counts [NK cells, T cells, $CD34^+$ cells, total mononuclear cells (MNCs)]); the categorical variables

Table 2. Graft Composition and KIR Genotyping

Graft composition, median cell dose (range)	MNC	11.8×10^8 ($5.2–25.3 \times 10^8$)
	$CD34^+$ cells	6.0×10^6 ($1.1–18.2 \times 10^8$)
	T cells	3.1×10^8 ($1.3–7.9 \times 10^8$)
	NK cells	3.4×10^7 ($0.6–14.8 \times 10^8$)
KIR gene expression (donor)	2DL1	97%
	2DL2	55%
	2DL3	91%
	2DL2 or 2DL3	100%
	3DL1	91%
	3DL2	100%
	2DS1	35%
	2DS2	56%
	2DS3	30%
	2DS4 (full-length variant)	40%
	2DS4 (deletion variant)	84%
	2DS5	30%
	3DS1	38%
KIR group haplotype frequencies (donor)	AA	31%
	AB	61%
	BB	8%

KIR indicates killer cell immunoglobulin-like receptor.

(cytomegalovirus [CMV] serostatus of patient and donor, disease risk category, and sex match [female donor to male recipient vs other]), the missing KIR ligand status for HLA-A, -B, and -C; and the KIR group haplotype (AA vs other).

RESULTS

Impact of HLA-Inhibitory KIR Constellations

Of the 100 transplantations evaluated, 47 pairs were C1/2 heterozygous (both HLA-C ligand groups present), 13 pairs were C2/2 homozygous (missing C1), and 40 pairs were C1/1 homozygous (missing C2). The probability of OS at 3 years for the C1/2 heterozygous patients (0.58; 95% confidence interval [CI] = 0.42-0.74) was not inferior to that of the C1 or C2 homozygous patients (0.42; 95% CI = 0.27-0.57; P = .085 for difference; P = .0049 for noninferiority of C1/2 heterozygosity) (Figure 1A). The probability for RFS also was not inferior in the C1/2 heterozygous patients (0.56; 95% CI = 0.41-0.71) compared with the C1 and C2 homozygous patients (0.35; 95% CI = 0.21-0.49; P = .035 for difference; P = .002 for noninferiority of heterozygosity) (Figure 1B). The cumulative relapse incidence at 3 years was significantly lower in the C1/2 heterozygous patients (0.23; 95% CI = 0.14-0.40) compared with the C1 or C2 homozygous recipients (0.51; 95% CI = 0.38-0.67; P = .006 for difference) (Figure 2A). By multivariate analysis, C1/2 heterozygosity was significantly associated with a lower relapse incidence (RR = 0.28; P = .003), had a marginal association with superior RFS (RR = 0.56; P = .046), and had an OS at least equivalent to that of C1 or C2 heterozygosity (RR = 0.59; P = .095) (Table 3).

Furthermore, the C1/2 heterozygous recipients tended to experience less aGVHD grade II-IV compared with the C1 or C2 homozygous recipients (cumulative incidence, 0.11, [95% CI = 0.05-0.25] vs 0.29 [95% CI = 0.19-0.44]; P = .011) (Figure 2B). This trend was confirmed by multivariate analysis (RR = 0.36; P = .05) (Table 3).

Similarly, with regard to KIR3DL1, the presence of a cognate ligand (ie, Bw4 epitope) had no adverse effect on any clinical endpoint (regardless of whether all Bw4 ligands or only those with isoleucine at residue 80 were considered; data not shown). Bw4 positivity was not inferior to the missing Bw4 ligand constellation in terms of OS (Bw4 present [n= 74]: 0.53, 95% CI = 0.39-0.66; Bw4 missing [n =24]: 0.44, 95% CI = 0.22-0.65; P = .031 for noninferiority) and RFS (Bw4 present [n = 74]: 0.49, 95% CI = 0.36-0.61; Bw4 missing [n = 24]: 0.37, 95% CI = 0.17-0.58; P = .034 for noninferiority). Likewise, by multivariate analysis, the presence or absence of a Bw4 ligand did not influence any clinical endpoint (data not shown).

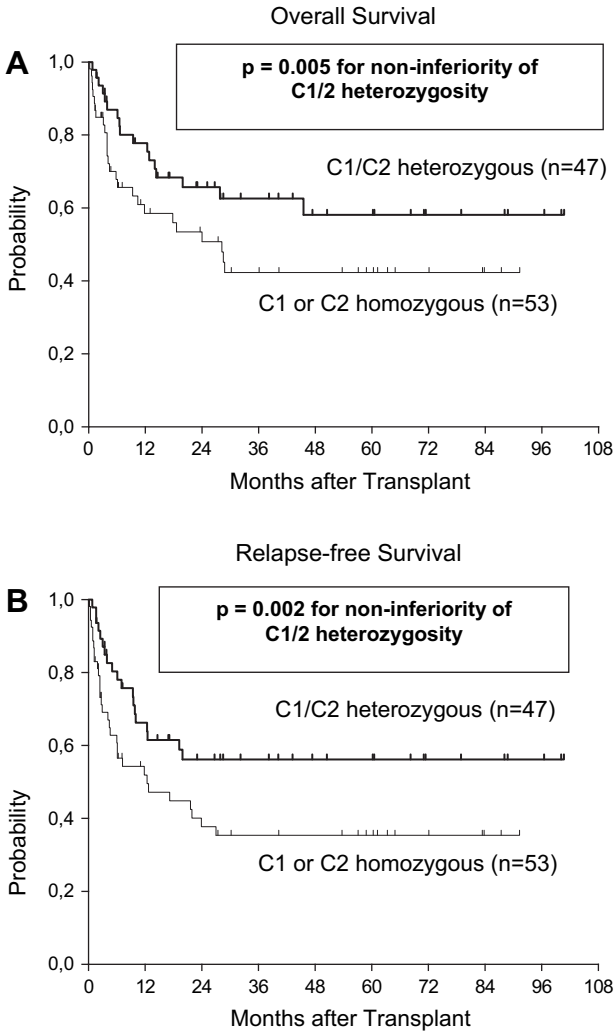

Figure 1. OS (A) and RFS (B) stratified by HLA-C KIR ligand group (n = 100).

The lack of both HLA-A3 and HLA-A11 (the cognate ligands to KIR3DL2) had no significant effect on RFS when the entire cohort was analyzed (n = 62: 0.48, 95% CI = 0.34-0.61 vs n = 38: 0.41, 95% CI = 0.24-0.58; P = .44). On the other hand, noninferiority of RFS and OS in the presence of HLA-A3 and/or A11 cannot be assumed with certainty (P = .47 for RFS; P =.46 for OS). However, the effects of diverse activating KIRs on survival were dependent on HLA-A3/A11 status. In patients expressing HLA-A3 and/or -A11, donor positivity for KIR2DS5 was associated with improved RFS (0.90, 95% CI = 0.71-1.00 vs 0.21, 95% CI = 0.03-0.39; P = .006) (Figure 3A) and improved OS (0.90, 95% CI = 0.71-1.00 vs 0.23, 95% CI = 0.04-0.42; P = .014). This was because of the absence of relapse in all of 10 HLA-A3 or A11positive patients with a 2DS5-positive donor, as opposed to a relapse incidence of 0.51 (95% CI = 0.34-0.78) in those with 2DS5-negative donors (P = .01) (Figure 3B). Multivariate analysis confirmed the impact of a 2DS5-positive donor on RFS in the

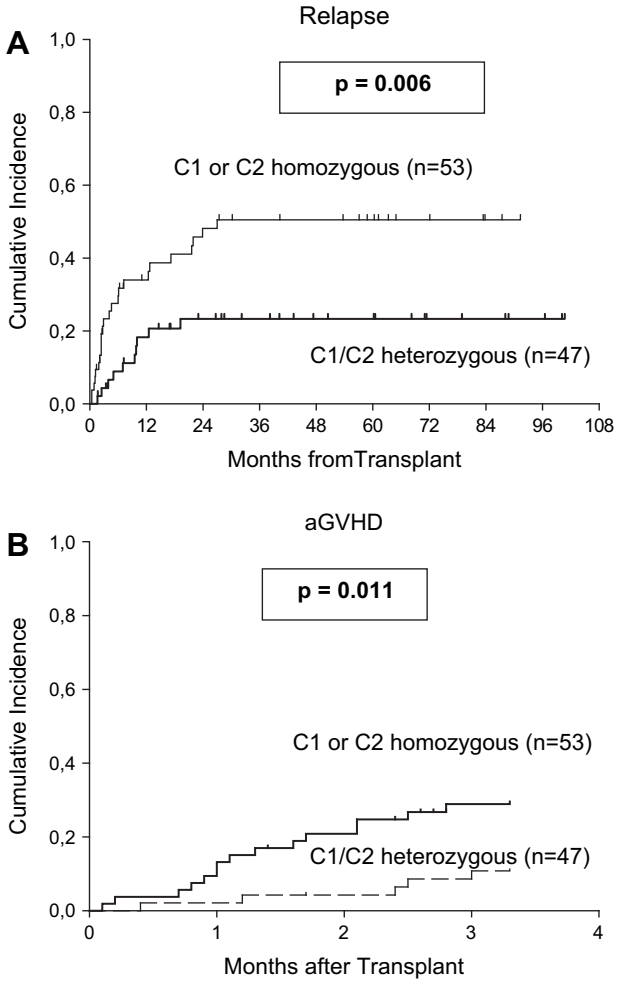

Figure 2. Cumulative incidence of relapse (A) and aGVHD grade II-IV (B), stratified by HLA-C KIR ligand group (n = 100).

HLA-A3/11positive population (P = .026). Similar to KIR2DS5, KIR2DS1 had, by multivariate analysis, a beneficial effect on OS (P = .05) and RFS (P = .026) among HLA-A3 or -A11positive patients. On the other hand, in HLA-A3/A11negative patients, a 2DS4 full-length variant positive donor was associated with a trend toward improved RFS (0.58, 95% CI = 0.33-0.82 vs 0.31, 95% CI = 0.14-0.47; P = .043).

Furthermore, expression of HLA-A3 or -A11 was associated with a significantly increased incidence of aGVHD grade II-IV (0.35, 95% CI = 0.22-0.54 vs 0.12, 95% CI = 0.06-0.23; P = .003) (Figure 3C), as confirmed by multivariate analysis (P = .021) (Table 3). The effect of HLA-A3/11 on aGVHD was particularly pronounced in the MAC subgroup (0.41, 95% CI = 0.23-0.73 vs 0.11, 95% CI = 0.04-0.27; P = .005). There was no association between aGVHD II-IV and relapse incidence (RR = 1.22; P = .63) or RFS (RR = 1.03; P = .93). Finally, the KIR group haplotype (A/A vs B/x) had no significant impact on any clinical endpoint (data not shown).

Impact of the Grafted NK Cell Dose

The grafted NK cell dose (above vs below the median [ie, 3.4 × 10^7/kg]) did not influence OS in the entire cohort. However, after stratification for conditioning type, differential effects were observed. In the RIC subgroup, a high NK cell dose was associated with a trend toward lower relapse incidence (0.26, 95% CI = 0.12-0.55 vs 0.59, 95% CI = 0.41-0.85; P = .028), superior RFS (0.50, 95% CI = 0.27-0.72 vs 0.21, 95% CI = 0.03-0.40; P = .062), and superior OS (0.60, 95% CI = 0.37-0.82 vs 0.22; 95% CI = 0.03-0.41; P = .12). By multivariate analysis of the RIC subgroup, a high NK cell dose improved all 3 endpoints (Table 3). Conversely, a high T cell dose was associated with an increased relapse incidence in the RIC subgroup (Table 3). An opposite effect of NK cell dose was found in the MAC subgroup, with a trend toward inferior OS after transplantations with a high NK cell dose (0.49, 95% CI = 0.28-0.70 vs 0.72, 95% CI = 0.55- 0.90; P = .09). This trend was confirmed by multivariate analysis (P = .019; Table 3).

DISCUSSION

The present study in unmanipulated, T cell replete PBSCT failed to reproduce the GVM-promoting effect of missing KIR ligands shown previously for T cell depleted BMT and PBSCT [14,23]. Likewise, other studies providing evidence supporting the benefit of missing KIR ligands have largely referred to transplantations with BM as the graft source and/or including T cell depleted grafts [17,18]. On the other hand, 2 other studies demonstrated an adverse outcome of C2 homozygous patients (missing C1) following HLA-matched related HSCT [15] or HLA-C matched unrelated HSCT [16]. Furthermore, the effect of KIR ligands was analyzed in a cohort of patients with chronic myelogenous leukemia (CML) undergoing BMT or PBSCT [21]. Although not explicitly addressed by the authors, the data imply that only those C1/2 heterozygous patients who underwent BMT fared worse than the C1/1 and C2/2 homozygous patients; those who underwent PBSCT experienced a comparatively favorable course. Our data, although limited by the heterogeneity of the diagnoses, are strictly homogenous with respect to the graft source (ie, T cell replete PBSC), and show that in this setting, C1/2 heterozygous patients have a lower relapse risk and are not inferior to HLA-C homozygous patients in terms of OS and RFS. This finding, along with results from the aforementioned studies [15,16,21], suggest that the benefit of missing KIR ligands may be restricted to transplants using BM grafts and/or applying T cell depletion. The explanation for the opposing effects of KIR ligands in T cell

Table 3. Multivariate Analyses

Cohort/Endpoint	Variable	RR	P
Entire cohort			
Death	C1/C2 heterozygosity	0.59	.095
	Standard risk indication	0.46	.029
	Donor CMV seronegativity	2.03	.025
Death or relapse	C1/C2 heterozygosity	0.56	.046
	Standard risk indication	0.49	.031
Relapse	C1/C2 heterozygosity	0.28	.003
	Standard risk indication	0.41	.053
	Donor age (per year)	0.94	.023
	Recipient age (per year)	1.05	.044
NRM	Donor age (per year)	1.04	.04
aGVHD II-IV	HLA-A3 or -A11-positive	3.00	.021
	C1/C2 heterozygosity	0.36	.05
MAC subgroup			
Death	C1/C2 heterozygosity	0.33	.035
	NK cell dose (per 10^7 cells)	1.53	.019
Death or relapse	C1/C2 heterozygosity	0.27	.025
	Recipient age (per year)	1.12	.006
	Donor age (per year)	0.89	.008
Relapse	C1/C2 heterozygosity	0.09	.0006
	Donor age (per year)	0.84	.0003
	Recipient age (per year)	1.16	.0007
RIC subgroup			
Death	NK cell dose (per 10^7 cells)	0.78	.03
Death or relapse	NK cell dose (per 10^7 cells)	0.79	.02
Relapse	NK cell dose (per 10^7 cells)	0.57	.003
	T cell dose (per 10^8 cells)	1.44	.002

NRM indicates nonrelapse mortality; aGVHD, acute graft-versus-host disease; RIC, reduced-intensity conditioning. The continuous variables included in the multivariate analysis were recipient age, donor age, and the graft cell counts (NK cells, T cells, $CD34^+$ cells, total MNC). The categorical variables were CMV serostatus of patient and donor, disease risk category, sex match (female donor to male recipient vs other), the missing KIR ligand status for HLA-A, -B, and -C, and the KIR group haplotype. Only variables with a *P* value < .10 are listed.

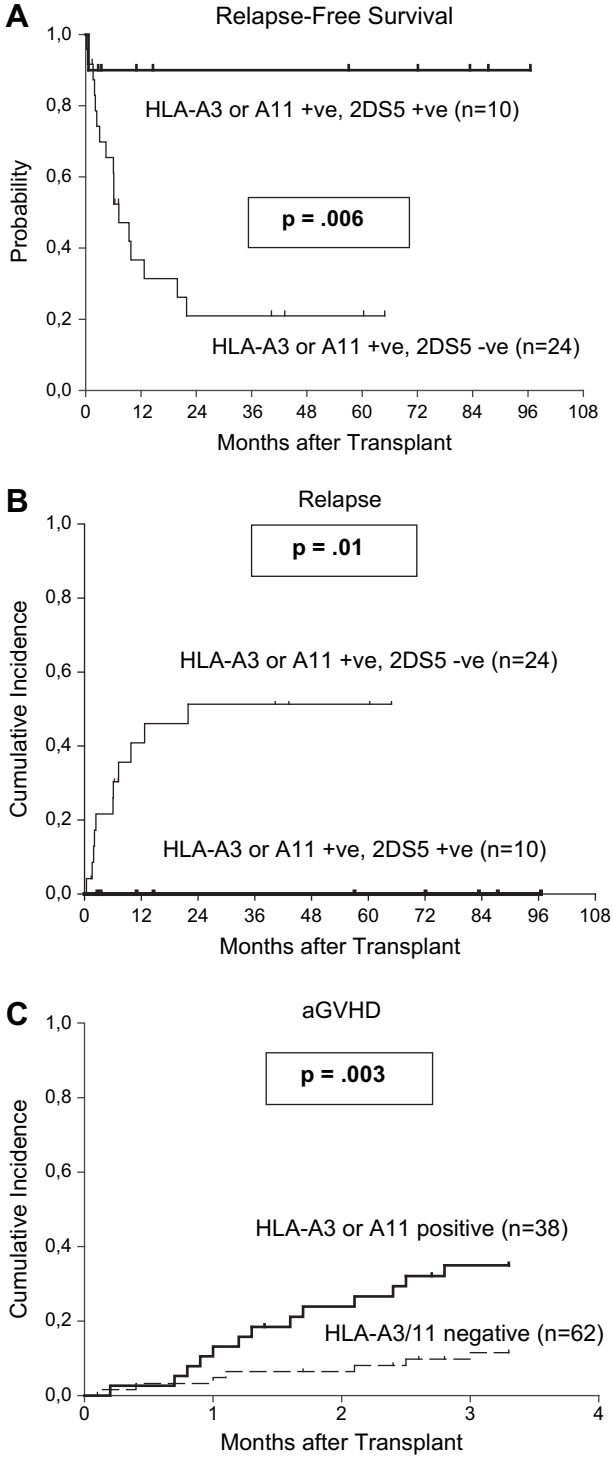

Figure 3. (A) RFS in HLA-A3 or -A11positive patients, stratified by donor KIR2DS5 expression (n = 34). (B) Cumulative incidence of relapse in HLA-A3 or -A11positive patients, stratified by donor KIR2DS5 expression (n = 34). (C) Cumulative incidence of aGVHD grade II-IV, stratified by HLA-A3 or HLA-A11 positivity (n = 100).

depleted BMT compared with unmanipulated PBSCT may lie in the differing reconstitution of the NK cell compartment. In T cell depleted or $CD34^+$-selected HSCT, reconstitution of NK cells relies largely on the development from stem/progenitor cells. As shown previously [23], these immature NK cells are responsible for alloreactivity, because they may respond to missing KIR ligands before the acquisition of self-tolerance. This may explain why Ruggeri et al. [1] observed alloreactive NK cell clones only during the early period after transplantation. Nonetheless, it is theoretically possible that these "not-yet self-tolerant" NK cells may target physiological tissues, resulting in GVHD to a similar extent as they mediate GVM. In contrast, in unmanipulated PBSCT, the large number of mature NK cells infused with the graft may dominate the early posttransplantation period with a self-tolerant profile.

Co-grafted T cells also may impair the recovering NK cells by affecting their acquisition of KIR [13]. Furthermore, application of G-CSF for stem cell mobilization may functionally alter the NK cell compartment by mechanisms involving both $CD34^+$ progenitor cells and T cells [25]. Thus, in unmanipulated PBSCT, mature (and hence self-tolerant) NK cells should not contribute to aGVHD. Nevertheless, they have the possibility of recognizing loss or down-regulation of HLA class I, a phenomenon frequently observed in malignant cells [26-28]. This possibility may be greatest if the transferred NK cells have been

licensed by both C1 group and C2 group HLA, as is the case in C1/2 heterozygous individuals, and/or by Bw4. Accordingly, the "KIR ligand presence" constellation would allow GVM reactivity without the need for concomitant GVHD. Indeed, we found no association between aGVHD and GVM effects. The observed trend toward a reduced incidence of aGVHD grade II-IV in C1/2 heterozygous patients may be explained by the destruction of host antigen-presenting cells (APCs) in the case of alloreactive donor NK cells, as reported by Ruggeri et al. [2]. Furthermore, a reduced incidence of aGVHD in C1/2 heterozygous patients has been observed in matched related and unrelated HSCT [22].

As with KIR3DL1 missing Bw4, we did not find a direct influence of the KIR3DL2 ligands HLA-A3 and HLA-A11 on relapse incidence or survival. This may be due to a hyporesponsive behavior of KIR3DL2-positive NK cells because of a low and peptide-dependent affinity of KIR3DL2 to its HLA-A ligands [29-31]. A novel finding concerning the KIR3DL2 ligands HLA-A3 and HLA-A11 is their modulating influence on the effects of particular activating KIRs. Most striking is the favorable outcome in HLA-A3 or -A11positive patients in case of a KIR2DS5 (or 2DS1)-positive donor, while HLA-A3/11negative recipients profited by a KIR2DS4 full-length variant-positive donor. We believe that these novel findings are worth being reexamined in larger cohorts. Previous studies addressing the role of donor activating KIRs revealed different effects of either individual activating KIR genes or their cumulative number [12,32-37]. Most of these studies did not explicitly investigate the effect of the full-length forms of KIR2DS4 (ie, *001 and *002), however. These variants have been distinguished from the truncated forms (*003, *004, and *006) by the PCR-SSP kit used in this study. They are less frequently expressed than the truncated forms (Table 2), as demonstrated previously [38]. Although the full-length forms of 2DS4 are bound to the cell because of transmembrane and cytoplasmatic domains, the 22-bp truncated forms, lacking these domains, encode a soluble form of the protein. This may explain why the functional characterization of KIR2DS4 requires discrimination between the full-length and the truncated variants.

Finally, our data confirm the benefit of a large number of grafted NK cells in RIC, but not MAC, transplantations, as has been suggested previously [20]. Indirect support for this finding has been provided by others as well. One study revealed an association between quantitative NK cell reconstitution and RFS following RIC, but not MAC, transplantations [39], whereas another study on nonmyeloablative (NMA) transplantation demonstrated associations between early NK cell chimerism and GVM and between early T cell chimerism and aGVHD [40]. The reason for the differential impact of the NK cell dose in the RIC and MAC settings remains to be elucidated.

We conclude that the benefit of the missing KIR ligand constellation, as shown in the T cell depleted setting [14,23], is not reproducible in unmanipulated PBSCT. Thus, separate assessment of T cell depleted versus T cell replete grafts, as well as BMT versus PBSCT cohorts, may be needed to improve the understanding of KIR-related clinical effects. However, a deeper insight into the mechanisms responsible for the differential impact of missing KIR ligands in the aforementioned settings will also require a sophisticated analysis of NK cell function following unmanipulated PBSCT in a way similar to that done previously for T cell depleted HSCT [23].

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