Negative effect of KIR alloreactivity in recipients of umbilical cord blood transplant depends on transplantation conditioning intensity

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We examined the clinical impact of killer-immunoglobulin receptor-ligand (KIR-L) mismatch in 257 recipients of single (n = 91) or double (n = 166) unit umbilical cord blood (UCB) grafts after myeloablative (n = 155) or reduced intensity (n = 102) conditioning regimens. Analyses of double unit grafts considered the KIR-L match status of the dominant engrafting unit. After myeloablative conditioning, KIR-L mismatch had no effect on grade III-IV acute graft-versus-host disease (GVHD), transplantation-related mortality (TRM), relapse, and survival. In contrast, after reduced intensity conditioning, KIR-L mismatch between the engrafted unit and the recipient resulted in significantly higher rates of grade III-IV acute GVHD (42% [CI, 27-59] vs 13% [CI, 5-21], P < .01) and TRM (27% [CI, 12%-42%] vs 12% [CI, 5%-19%], P = .03) with inferior survival (32% [CI, 15%-59%] vs 52% [CI, 47%-67%], P = .03). Multivariate analysis identified KIR-L mismatch as the only predictive factor associated with the development of grade III-IV acute GVHD (RR, 1.8 [CI, 1.1-2.9]; P = .02) and demonstrated a significant association between KIR-L mismatch and increased risk of death (RR, 1.8; 95% CI, 1.0-3.1; P = .05). Our results do not support the selection of UCB units based on KIR-L status and suggest that KIR-L mismatching should be avoided in reduced intensity UCB transplantation. (Blood. 2009;113:5628-5634)

Introduction

Natural killer (NK) cells are part of the innate immune system and are involved in viral immunity and cancer surveillance. The physiology of NK cells is tightly regulated to control proliferation, cytotoxicity, and cytokine production.[1] NK-cell alloreactivity in the setting of allogeneic transplantation is determined by the specificity of the killer-immunoglobulin receptors (KIRs) on donor NK cells for recipient MHC class I.[2] Some donors have a subset of NK cells that do not express inhibitory KIRs that recognize their cognate MHC class I ligand on recipient cells. If this potential exists, a donor is said to be KIR-ligand (KIR-L) mismatched. Using retrospective analysis, this type of mismatch between donor and recipient has been associated with decreased rates of relapse and prolonged survival for myeloid leukemia patients after myeloablative, haploidentical transplantation using stringent T-cell depletion (TCD).[3,4] Similar effects were not seen with adult unrelated donors[5,6] unless in vivo TCD was performed using anti–thymocyte globulin.[7] The importance of TCD is supported by studies showing that graft TCD affects NK-cell reconstitution.[8,9]

The use of umbilical cord blood (UCB) as a source of hematopoietic stem cells is increasing.[10-15] The use of 2 UCB units to compose the graft has made this cell source an attractive alternative to treat adult patients with hematologic malignancies using myeloablative (MA) and reduced-intensity (RI) conditioning.[10,12] Compared with adult stem cell grafts, UCB grafts contain approximately 1-log fewer T cells, all of which are naive. Based on the premise that NK-cell alloreactivity dominates in the setting of low graft T-cell numbers, we hypothesized that transplantation with UCB units might favor NK-cell alloreactivity and that the use of KIR-L–mismatched units might result in better clinical outcomes.

Methods

Patients

Two hundred fifty-seven patients who underwent transplantation with 1 (n = 91) or 2 (n = 166) partially HLA-matched UCB units at the University of Minnesota between 1998 and 2006 were included in this analysis if allele-level molecular typing for HLA-C was available for all patients and their UCB donor unit(s). HLA typing for HLA-A, -B, and -DRB1 was also available on all patients. Patients received either an MA (n = 155) or an RI (n = 102) conditioning. Patients who lacked HLA-C information (n = 37) or who had received prior allogeneic transplantation (n = 5) were excluded. The small (n = 19) and clinically heterogeneous group of patients receiving single UCB unit grafts after a RI conditioning were also excluded. In double unit UCB transplantation there is usually a dominant engrafting unit. Because our analysis of the impact of KIR alloreactivity on outcomes assumes that the effect is mediated by the engrafted donor NK cells, we excluded an additional 24 patients who had graft failure (n = 21) or insufficient information on which unit was the long-term, dominant engrafting unit (n = 3). An initial analysis demonstrated that the patients receiving 1 or 2 UCB units after MA conditioning had similar outcomes. Therefore, we divided the patients into 2 cohorts based on the intensity of the conditioning regimen. Patients with acute leukemia in first or second complete remission (CR), chronic myelogenous leukemia in first chronic phase, and chemotherapy-sensitive lymphoma in CR or partial remission were considered standard risk for posttransplantation disease recurrence; the remaining patients were considered high risk for recurrence. For recipients of 2 UCB units, we considered the combined dose of the 2 units as the total cell dose, and used the HLA matching of the less well-matched of the 2 units to assign the degree of HLA matching to the recipient. The supportive care provided to this cohort has been previously reported, along with their clinical outcomes, in an analysis that did not

evaluate the role of NK-cell alloreactivity.[10-12,16] Written informed consent was obtained from all patients who underwent transplantation and donors in accordance with the Declaration of Helsinki, and approval was received from the University of Minnesota Institutional Review Board (IRB).

UCB graft selection and processing

Selection and processing of UCB units has been previously reported.[10-12,16] In summary, UCB units were required to be matched at 4 (or more) of the 6 HLA antigens based on antigen-level HLA-A and -B and allele-level HLA-DRB1 typing. For recipients of 2 UCB units, they were matched to the recipient and to each other at 4 (or more) of the 6 HLA antigens, not necessarily at the same locus. Matching at HLA-C, -DQ, and -DP or ABO blood-type matching was not considered. Cryopreserved units of UCB were thawed using the method described by Rubinstein et al.[17]

HLA typing

Allele-level molecular typing was performed for class I HLA-A, -B, and -C by sequence-based typing (SBT) using Visible Genetics (Suwanee, GA) reagents and sequencer. Class II HLA DRB1 molecular typing was performed by sequence-specific primers (SSPs) using One Lambda (Canoga Park, CA) reagents. Ambiguities were resolved whenever possible.

KIR-ligand assignment

The presence of KIR-L mismatching in the graft-versus-host (GVH) direction was assigned as initially described by Ruggeri et al based on HLA-B (Bw4) and HLA-C (C1 and C2 ligand groups) mismatches for all clinical end points.[3] A separate analysis also determined KIR-L mismatch by the additional inclusion of HLA-A3 and -A11 where indicated. For the analysis of acute GVH disease (GVHD), transplantation-related mortality (TRM), relapse, and survival only the KIR-L assignment of the dominant engrafting unit was considered.

Preparative regimens and GVHD prophylaxis

Myeloablative conditioning. Between 1995 and 2000, the MA conditioning consisted of cyclophosphamide (Cy) 60 mg/kg intravenously daily for 2 days, total body irradiation (TBI) 1320 cGy, delivered in 8 fractions over 4 days, and anti–thymocyte globulin (ATG, ATGAM; Pharmacia, Kalamazoo, MI) 15/kg intravenously, twice daily for 3 days.[11] Between 2000 and 2006, the MA regimen consisted of same doses of cyclophosphamide and TBI, and fludarabine (Flu) 40 mg/m^2 intravenously daily for 3 days.[12]

Reduced-intensity conditioning. The RI conditioning consisted of Flu 40 mg/m^2 per day intravenously for 5 consecutive days, a single fraction of TBI 200 cGy, and either a single dose of Cy 50 mg/kg intravenously or busulfan (Bu) 2 mg/kg by mouth every 12 hours for 2 consecutive days.[10,16] A subgroup of patients treated with Cy/Flu/TBI200 (n = 30) also received ATG (ATGAM; Pharmacia) at 15 mg/kg every 12 hours intravenously for 3 days.[10]

Posttransplantation immunosuppression. For patients receiving MA conditioning, posttransplantation immune suppression included cyclosporine-A (CSA) from day −3 for at least 3 months (aiming for a trough blood level of > 200 μg/L) with either short course of methylprednisolone 1 mg/kg intravenously on days 5 to 19 (1998-2000)[11] or mycophenolate mofetil (MMF) 2 to 3 grams daily either orally or intravenously from days −3 to 30 (2000-2006).[12] For all recipients of RI conditioning, the posttransplantation immune suppression consisted of CSA and MMF.[10,16]

Statistical considerations

Patient and transplant characteristics were analyzed using the χ^2 test for categoric data, with the Fisher exact test or the Freeman-Halton test used when applicable, and the Wilcoxon rank-sum test for continuous data.[18] Study end points included overall survival, relapse, TRM, grades II-IV and III-IV acute GVHD, and chronic GVHD.

Overall survival was estimated by the Kaplan-Meier method.[19] Comparisons of time-to-event curves were completed by the log-rank test. Cox regression was used to assess the independent effect of KIR alloreactivity

on overall survival.[20] Cumulative incidence was used to estimate the end points of relapse, TRM, and acute and chronic GVHD. Nonevent deaths were treated as competing risks.[21] The proportional hazards model of Fine and Gray was used to assess the independent effect of KIR alloreactivity.[22] All factors were tested for proportional hazards before inclusion in the regression models.[18] Factors considered in the regression models for the MA cohort were as follows: the ligand mismatch in GVH direction for the dominant engrafting unit, age by decade, sex, conditioning regimen, HLA disparity, cytomegalovirus (CMV) serostatus, diagnosis (standard risk vs high risk), number of donor units, infused CD34$^+$ and CD3$^+$ cell doses (in quartiles), use of ATG, and acute GVHD as a time-dependent variable where appropriate. Factors considered in the regression models for the RI cohort were as follows: the ligand mismatch in GVH direction for the dominant engrafting unit, age (< 50 vs ≥ 50 years), sex, HLA disparity, CMV serostatus, diagnosis (standard risk vs high risk), infused CD34$^+$ and CD3$^+$ cell doses (in quartiles), use of ATG, and acute GVHD as a time-dependent variable where appropriate.

Results

Patient, transplantation, and graft characteristics

Patient, transplantation, and graft characteristics stratified by the treatment cohort are summarized in Table 1. The median age (15 vs 51 years, $P < .01$) and weight (82 vs 72 kg, $P < .01$) of the recipients, year of transplantation (2004-2006; MA 48% vs RI 76%, $P < .01$) and posttransplantation immunosuppression (CSA/MMF; MA 34% vs RI 100%, $P < .01$) were significantly different between the 2 cohorts. The proportion of males (60% vs 66%, $P = .36$) and CMV-seropositive recipients (56% vs 57%, $P = .91$) was similar for the MA and RI cohorts. Patients who received a MA conditioning were more likely to have acute leukemia (74% vs 36%, $P < .01$) and less likely to have lymphomas (8% vs 40%, $P < .01$). There were a higher proportion of patients with high relapse risk in the RI conditioning cohort (29% vs 50%, $P < .01$). Total nucleated (3.6 vs 3.5×10^7/kg, $P = .72$), CD34$^+$ (4.3 vs 4.5×10^5/kg, $P = .40$), and CD3$^+$ (1.3 vs 1.3×10^7/kg, $P = .62$) cell doses and HLA matching (0-1 HLA mismatch; 49% vs 45%, $P = .68$) were not significantly different between the MA conditioning and RI conditioning cohorts.

KIR-ligand mismatch

Patients, conditioning regimen, and graft characteristics for the MA and RI conditioning cohorts stratified by the presence of KIR-L mismatch are summarized in Table 1. The prevalence of KIR-L mismatching based on HLA-B and HLA-C ligands was 26% and 32% for recipients of MA and RI conditioning ($P = .31$), respectively. In both the MA and RI conditioning cohorts, there were no significant demographic differences, apart from a higher rate of CMV seropositivity among KIR-L–matched patients (Table 1).

Impact of KIR-ligand mismatch on outcomes after myeloablative conditioning

As summarized in Table 2, after MA conditioning (n = 155) there was no significant difference on the incidence of grades II-IV and III-IV acute GVHD (Figure 1A), chronic GVHD, TRM (Figure 1B), relapse, and overall survival (Figure 1C) when comparing recipients of KIR-L–matched or –mismatched grafts. These outcomes were not significantly different in subset with acute myeloid leukemia (AML; n = 60, Table 3) or when considering HLA-A3/A11 in the KIR-L mismatch analysis, as only 1 transplant had a KIR-L mismatch solely based on HLA-A3 in the donor but not the

Table 1. Patients, conditioning regimen, and graft characteristics

Factor	MA conditioning			RI conditioning			MA vs RI, P
	KIR-L mismatched, n=41	KIR-L matched, n=114	P	KIR-L mismatched, n=33	KIR-L matched, n=69	P	
Median age at transplantation, y (range)	15 (0.6-53)	15.9 (1.0-59)	.84	48 (22-69)	52 (6-68)	.10	< .01
Median weight, kg (range)	75.8 (57.2-130.3)	69.8 (33.4-148.6)	.30	86.9 (55.9-125.7)	78.5 (22.1-121.8)	.31	< .01
Male, no. (%)	27 (66)	66 (58)	.37	22 (67)	45 (65)	.89	.36
Recipient CMV positive, no. (%)	14 (34)	73 (64)	< .01	14 (42)	44 (64)	**.04**	.91
Diagnosis, no. (%)							
AML	18 (44)	42 (37)		8 (24)	20 (29)		
ALL	13 (32)	42 (37)		1 (3)	8 (11)		
CML	3 (7)	11 (9)	.86	2 (6)	3 (4)	.47	< .01
MDS	2 (5)	3 (3)		2 (6)	8 (12)		
NHL/Hodgkin	4 (10)	8 (7)		17 (52)	24 (35)		
Other	1 (2)	8 (7)		3 (9)	6 (9)		
Year of transplantation, no. (%)							
1998-2000	9 (22)	23 (20)	.12	0	0	.89	< .01
2001-2006	54 (78)	91 (80)		33 (100)	69 (100)		
High-risk disease,* no. (%)	13 (32)	32 (28)	.66	21 (64)	30 (43)	.06	< .01
ATG with conditioning, no. (%)	17 (41)	43 (38)	.67	8 (24)	22 (32)	.43	.13
GVHD prophylaxis, no. (%)							
CSA/MPD	16 (39)	44 (39)	.99	0	0	na	< .01
CSA/MMF±MPD	25 (61)	70 (61)		36 (100)	69 (100)		
HLA matching,† no. (%)							
4/6	25 (61)	59 (52)		28 (85)	45 (65)		
5/6	14 (34)	42 (37)	.42	5 (15)	18 (26)	.08	.68
6/6	2 (5)	13 (11)		0	6 (9)		
No. of donors, no. (%)			.28			na	na
1	27 (66)	64 (56)		033 (100)	069 (100)		
2	14 (34)	50 (44)					
Conditioning, no. (%)							
Bu containing	1 (2)	8 (7)		1 (3)	1 (1)	.54	< .01
Cy/Flu/TBI	24 (59)	70 (61)	.75	32 (97)	68 (99)		
Cy/TBI	16 (39)	36 (32)					
Median infused TNC, ×10⁷ (range)	3.4 (1.0-10.3)	3.8 (1.2-10.8)	.51	3.3 (2.0-6.8)	3.6 (1.5-5.9)	.26	.72
Median infused CD34⁺, ×10⁵ (range)	4.6 (0.9-21.6)	4.0 (0.6-34.8)	.49	4.0 (1.1-16.6)	4.6 (1.1-13.7)	.84	.40
Median infused CD3⁺, ×10⁷ (range)	1.2 (0.1-2.6)	1.3 (0.2-3.2)	.32	1.1 (0.1-2.7)	1.4 (0.2-3.1)	.07	.62
Median follow-up among survivors, y (range)	2.2 (1.0-6.8)	2.1 (0.9-7.8)	.99	2.0 (1.0-3.5)	1.8 (0.9-5.3)	.94	**.02**

P values less than .05 are shown in bold for easy identification.

ALL indicates acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; and NHL, non-Hodgkin lymphoma.

*Disease was defined as standard risk if patients underwent transplantation with AML and ALL in first or second complete remission, CML in first chronic phase, and chemotherapy-sensitive lymphoma in partial or complete remission. Other patients were considered to have high-risk disease.

†For recipients of 2 UCB units the HLA matching reflects the worst matched of the 2 units.

recipient (data not shown). Multivariate models were then performed using demographic factors and transplantation variables in univariate analysis with a P value of .10 or less and those with biologic significance (Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). In multivariate analysis, there was a higher risk of grade II-IV acute GVHD among recipients of 2 UCB units (RR, 2.3; 95% confidence

interval [CI], 1.5-37; P < .01) with no increased risk for patients with a dominant engrafting KIR-L–mismatched unit (RR, 1.0; 95% CI, 0.6-1.6; P = .91). The only independent predictor of a higher risk of TRM after MA conditioning was age of 18 years or older (RR, 3.4; 95% CI, 1.3-9.2; P = .02) as adjusted for the number of donor units (2 UCB units: RR, 0.7; 95% CI, 0.3-1.8; P = .42) and a dominant engrafting KIR-L–mismatched unit (RR, 1.7; 95% CI,

Table 2. Outcomes of all patients after UCB transplantation based on the presence or absence of NK-cell alloreactivity in the GVHD direction

Outcome	Myeloablative conditioning			Reduced-intensity conditioning		
	KIR-L mismatched, n=41	KIR-L matched, n=114	P	KIR-L mismatched, n=33	KIR-L matched, n=69	P
Grade II-IV GVHD, % (95% CI)	46 (30-64)	46 (36-56)	.82	79 (59-99)	57 (44-70)	**.01**
Grade III-IV GVHD, % (95% CI)	17 (6-28)	17 (10-24)	.97	42 (27-59)	13 (5-21)	< .01
Chronic GVHD at 1 year, % (95% CI)	10 (1-19)	21 (13-29)	.16	12 (1-23)	14 (6-22)	.91
TRM at 2 years, % (95%CI)	27 (14-40)	18 (11-25)	.19	27 (12-42)	12 (5-19)	**.03**
Relapse at 2 years, % (95% CI)	18 (6-30)	28 (19-37)	.37	39 (21-57)	47 (34-60)	.72
Survival at 3 years, % (95% CI)	50 (32-68)	57 (47-67)	.46	32 (15-59)	52 (47-67)	**.03**

P values less than .05 are shown in bold for easy identification.

UCB indicates umbilical cord blood; NK, natural killer; GVHD, graft-versus-host disease; KIR-L, killer-cell immunoglobulin–like receptor ligand; and TRM, treatment-related mortality.

Figure 1. KIR-L mismatch adversely affects GVHD, TRM, and survival in patients receiving reduced-intensity conditioning. Outcomes of myeloablative (A-C) and reduced-intensity (D-F) umbilical cord blood transplantation for patients engrafted with a KIR-ligand mismatch (····) or match (—).
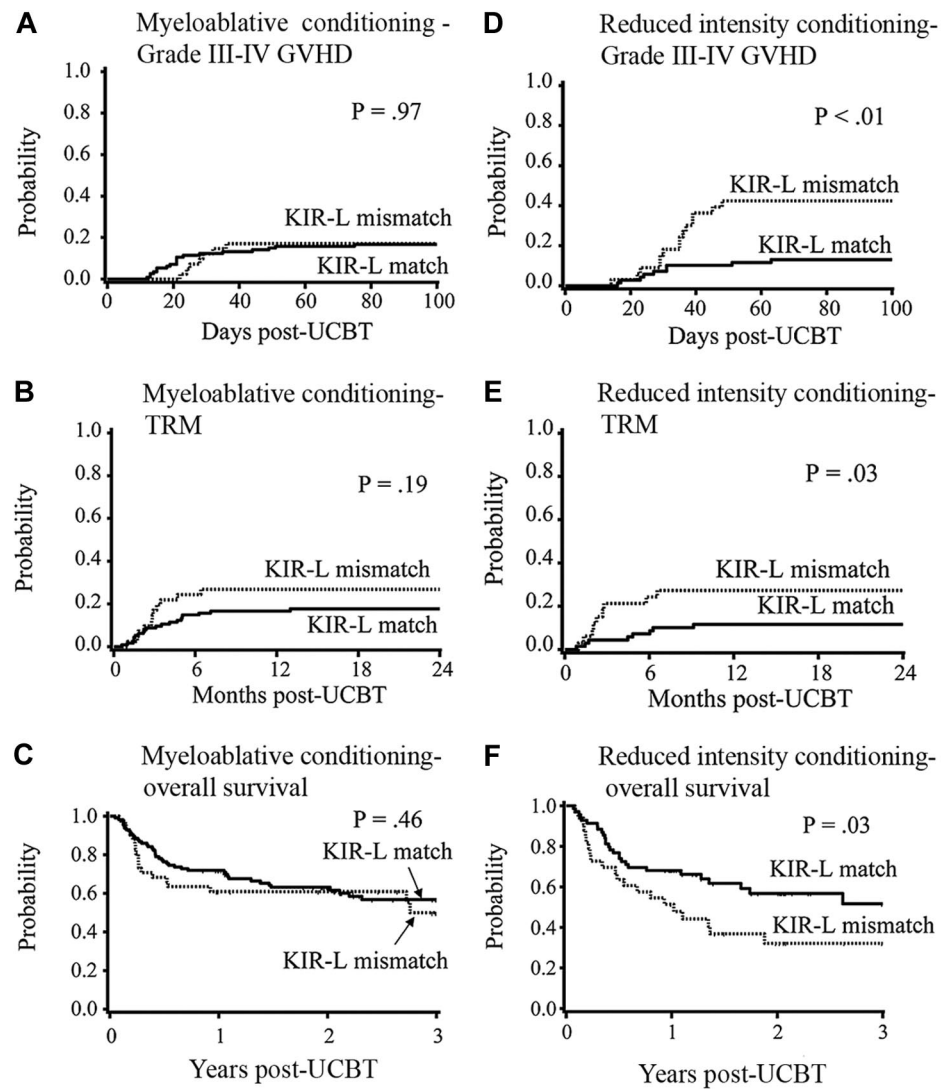
0.8-3.5; $P = .18$). The risk of relapse was significantly lower for patients who were older ($\geq$ 18 years: RR, 0.5; 95% CI, 0.2-1.0; $P = .04$). Relapse risk was higher for patients with high-risk disease (RR, 2.3; 95% CI, 1.1-4.5; $P = .02$), whereas the number of donors (2 UCB: RR, 1.0; 95% CI, 0.5-2.2; $P = .91$) and a dominant engrafting KIR-L–mismatched unit (RR, 0.6; 95% CI, 0.3-1.4; $P = .22$) had no significant impact. Patients who received better HLA-matched grafts (5-6/6 HLA-matched) had better survival (RR, 0.6; 95% CI, 0.3-1.0; $P = .04$) after adjusting for the number of donors (2 UCB units: RR, 0.9; 95% CI, 0.5-1.5;

$P = .62$) and a dominant engrafting KIR-L–mismatched unit (RR, 1.2; 95% CI, 0.7-2.1; $P = .50$). Causes of death were similar in patients with a KIR-L–matched or –mismatched dominant engrafting UCB unit (Table 4).

Impact of KIR-L mismatch after reduced-intensity conditioning

Univariate analysis demonstrated significantly higher incidences of grades II-IV and III-IV acute GVHD (Figure 1D), TRM (Figure 1E), and poorer survival (Figure 1F) for RI transplantation patients

Table 3. Outcomes of patients with AML after UCB transplantation based on the presence or absence of NK-cell alloreactivity in the GVHD direction

Outcome	Myeloablative conditioning			Reduced-intensity conditioning		
	KIR-L mismatched, n=18	KIR-L matched, n=42	P	KIR-L mismatched, n=8	KIR-L matched, n=20	P
Grade II-IV GVHD, % (95% CI)	50 (26-74)	38 (23-53)	.59	75 (45-100)	50 (27-73)	.07
Grade III-IV GVHD, % (95% CI)	28 (8-48)	17 (6-28)	.36	38 (8-68)	5 (0-14)	**.03**
Chronic GVHD at 1 year, % (95% CI)	6 (0-16)	26 (12-40)	.13	13 (0-33)	24 (4-44)	.76
TRM at 2 years, % (95% CI)	22 (2-42)	18 (6-28)	.49	25 (0-53)	5 (0-14)	.08
Relapse at 2 years, % (95% CI)	30 (8-52)	21 (8-34)	.29	50 (16-84)	53 (29-77)	.49
Survival at 3 years, % (95% CI)	44 (18-68)	66 (48-79)	.15	21 (1-59)	54 (27-75)	.26

A *P* value less than .05 is shown in bold for easy identification.

AML indicates acute myeloid leukemia; UCB, umbilical cord blood; NK, natural killer; GVHD, graft-versus-host disease; KIR-L, killer-cell immunoglobulin–like receptor ligand; and TRM, treatment-related mortality.

Table 4. Causes of death after UCB transplantation based on the presence or absence of NK-cell alloreactivity in the GVHD direction

Cause of death	Myeloablative conditioning		Reduced-intensity conditioning	
	KIR-L mismatched (%)	KIR-L matched (%)	KIR-L mismatched (%)	KIR-L matched (%)
Disease recurrence	6 (33)	23 (49)	13 (62)	17 (61)
Infection	7 (39)	5 (11)	2 (9)	1 (4)
GVHD	2 (11)	4 (8)	3 (14)	4 (14)
ARDS	0	1 (2)	0	0
Graft failure	2 (11)	2 (4)	0	0
Organ failure	1 (6)	8 (17)	1 (5)	4 (14)
Secondary malignancy	0	2 (4)	0	2 (7)
Hemorrhage	0	2 (4)	2 (8)	0

P values were calculated using a Freeman-Halton test. For myeloablative conditioning, all causes of death had P values of .16. For reduced-intensity conditioning, all causes of death had P values of .41.

UCB indicates umbilical cord blood; NK, natural killer; GVHD, graft-versus-host disease; KIR, killer-cell immunoglobulin–like receptor; and ARDS, adult respiratory distress syndrome.

with a dominant engrafting KIR-L–mismatched UCB unit (n = 102, Table 2). In the subset with AML only, the incidence of grade III-IV was significantly higher in those receiving a dominant engrafting KIR-L–mismatched unit. There was no impact on TRM, relapse, or survival (Table 3). When KIR-L matching through HLA-A3/A11 was included, 7 additional transplants were classified as KIR-L mismatch. Of those, 2 were based on HLA-A11 and 5 on HLA-A3 in donor where neither HLA-A3 nor -A11 was present in the recipient. Significantly higher incidences of grades II-IV (76% vs 57%, $P = .02$) and III-IV acute GVHD (38% vs 14%, $P < .01$) were apparent with inclusion of KIR-L–mismatched donors by HLA-A. However, univariate rates for KIR-L–mismatched versus –matched transplantation outcomes for TRM (24% vs 12%, $P = .09$) and survival (36% vs 50%, $P = .10$) were no longer significant. There was no impact on relapse rate (38% vs 49%, $P = .95$).

In multivariate analysis, univariate results based on demographics and transplantation variables (Table S2) were considered using a similar strategy to the MA cohort. RI patients who engrafted with a KIR-L–mismatched (HLA-B and HLA-C) unit had significantly higher risks of grades II-IV (RR, 1.8; 95% CI, 1.1-2.9; $P = .02$) and III-IV (RR, 3.4; 95% CI, 1.4-8.1; $P < .01$) acute GVHD. TRM risk was higher for patients with high-risk disease (RR, 3.3; 95% CI, 1.1-9.7; $P = .03$) after adjusting for a dominant engrafting KIR-L–mismatched unit (RR, 2.2; 95% CI, 0.8-5.5; $P = .11$). No factors, including a dominant engrafting KIR-L–mismatched unit (RR, 0.9; 95% CI, 0.5-1.7; $P = .74$), were independent predictors of the risk of relapse after transplantation after RI conditioning. However, the risk of death from any cause was significantly higher for patients with a dominant engrafting KIR-L–mismatched unit (RR, 1.8; 95% CI, 1.0-3.1; $P = .05$), whereas disease risk (high risk: RR, 1.4; 95% CI, 0.8-2.4; $P = .27$) had no significant impact. Causes of death were similar in patients with a KIR-L–matched or –mismatched dominant engrafting UCB unit (Table 4).

Discussion

Based on the premise that NK-cell alloreactivity dominates in the setting of low graft T-cell numbers,[3] we evaluated the impact of NK-cell alloreactivity, as determined by KIR-L mismatch, on outcomes after UCB transplantations. We observed no reduction in the risk of relapse after transplantation with KIR-L–mismatched UCB units after either RI or MA conditioning. Furthermore, after MA conditioning there was no impact of KIR-L mismatching on any of the studied outcomes. In contrast, in the RI conditioning

cohort KIR-L–mismatched UCB grafts were associated with significantly higher risks of acute GVHD, TRM, and death. Most treatment-related deaths occurred early with rare events observed after 1 year. Increased TRM and reduced survival in the setting KIR-L mismatch has been reported by Malmberg et al who studied recipients of adult unrelated donor grafts receiving fully ablative conditioning.[23] These authors found a significantly higher risk of infection-related death in their series. This is in agreement with our MA cohort where infection was the most frequent cause of death with a KIR-L–mismatched donor compared with a KIR-L–matched donor, where relapse was the most frequent cause of death; however these differences were not significant. Relapse accounted for most of the deaths in our RI conditioning cohort irrespective of KIR-L status and infection causes of death were low.

UCB grafts contain fewer T cells than adult donor grafts,[13-15] and reconstitution of NK cells occurs earlier than T cells,[24,25] suggesting that the milieu after UCB transplantation could favor NK-cell alloreactivity. However, our data do not show the same beneficial effect of KIR-L mismatch reported by Ruggeri et al[3,4] and Giebel et al[7] who reported on patients receiving grafts with either ex vivo or in vivo T-cell depletion. Others have found no association between KIR-L mismatch and a reduction in relapse when T cell–replete grafts were used.[5,6] It is hypothesized that in the setting of T-cell depletion more robust NK-cell reconstitution is favored due to less competition for factors (ie, cytokines) required for T-cell reconstitution. This may result in more NK cell–mediated graft-versus-leukemia effects through KIR-L mismatching.[8,9] The concept of T- and NK-cell competition is supported in mouse[26] and human[27-30] studies and the importance of lymphodepletion to expand lymphocytes in vivo is well documented. Lymphodepleting chemotherapy provides both lymphocyte space and a release from a cytokine "sink" resulting in a surge of endogenous IL-15 and IL-7, cytokines that act on both T cells[31] and NK cells.[32,33] However, it is possible that although UCB units are relatively T-cell depleted, the composition of T cells may influence outcomes. Compared with the T cells in adult grafts, which contain a mixture of both naive and memory T cells, UCB grafts contain essentially naive T cells, which may be highly responsive to use endogenous cytokines despite the lower graft T-cell inoculum and, therefore, the competition for factors used in common by NK cells may not be eliminated.

KIR-L mismatch was not associated with either reduced relapse or better survival in our study. Although our results differ from those recently reported by Willemze et al,[34] interpretation of this contrast requires careful analysis. Willemze et al found a reduction in risk of relapse and improved survival for recipients of KIR-L–

mismatched grafts after MA UCB transplantation for acute leukemia.[34] Patient selection and methodologic differences may explain discrepancies between the 2 studies. Whereas Willemze et al[34] included only patients with acute leukemia, like Ruggeri et al,[3,4] our study included patients with all hematologic malignancies, similar to Giebel et al.[7] Another difference is our use of 2 UCB unit grafts in our cohort of RI and a subgroup of MA conditioning transplantations. In our series, approximately one-third of the patients received ATG as part of the conditioning regimen in both the MA and RI cohorts. In contrast, in the report by Willemze et al[34] approximately 80% of the patients received ATG as part of the conditioning regimen. It is possible that the in vivo T-cell depletion secondary to ATG administration may have contributed to posttransplantation expansion of functional NK cells and favored alloreactivity in the presence of KIR-L mismatch. Ultimately, the impact of ATG on the outcomes of KIR-L–mismatched transplantations needs to be addressed in prospective studies.

We defined KIR-L mismatch based on earlier and clinically validated studies.[3,4,7] Yet consideration of HLA-A may be important as well. HLA-A3 and -A11 may engage KIR3DL2, but the functional result of this interaction is uncertain. Inclusion of HLA-A ligands in our analysis compared with HLA-B and -C only diluted out significant adverse effects on clinical outcomes. This may be unique to KIR3DL2 interactions, which become functional only in the presence of certain peptides,[35] whereas most other inhibitory KIR interactions are thought to be predominantly peptide independent. When HLA-A3/A11 was included, the impact of HLA-A was modest as it resulted in a change of assignment in only 8 (3%) of 257 transplants. We also considered that some HLA-A alleles include the Bw4 epitope. However, the mere presence of Bw4 sequences does not necessarily correlate with function.[2] Foley et al have recently shown that although HLA-A*2402 and HLA*3201 function as bona fide Bw4 epitopes, HLA-A*2501 and HLA-A*2301 were functionally weak.[36] The role of Bw4 HLA-A alleles was also small and rarely changed KIR-L assignment in those patients where allele-level HLA-A typing was available. In this analysis, inclusion of HLA-A did not modify KIR-L NK-cell interactions.

The mechanism by which KIR-L mismatch leads to worse outcomes in the RI setting is not clear. It is possible that the increased incidence of acute GVHD in the RI setting could be, at least in part, due to a contribution from NK-cell damage to GVHD target tissues even though NK cells alone do not cause GVHD. It is also possible that NK cells, know to coactivate dendritic cells, will affect antigen presentation and indirectly affect T-cell activation. Lastly, we acknowledge the some T-cell populations also express KIR, especially those T cells that also express the NK-cell antigen CD56. Therefore, the higher GVHD risk, higher TRM, and poorer survival may not be entirely due to NK cells themselves and may involve T cells as well.

In summary, KIR-L mismatch offers no advantage to UCB graft recipients and may potentially confer higher risk of GVHD and mortality in the setting of RI conditioning. This is the first report suggesting that divergent effects of NK cell alloreactivity may be unmasked when comparing MA and RI conditioning platforms. Growing evidence suggests that in some settings recovering NK cells are not fully mature,[8,9] are affected by immunosuppression,[37] and may not exhibit normal expansion and effector function. Detailed functional studies in NK-cell immune reconstitution may better correlate with clinical outcomes than the simple ligand analysis proposed here. Although this analysis cannot determine whether the detrimental effects of the KIR-L–mismatched UCB units were related to NK-cell alloreactivity itself, indirectly related to T cells, or due to degrees of HLA mismatching, further studies examining the genetic linkage of KIR and HLA loci may unravel the clinical consequences of this complex NK-cell biology. Although additional studies are still needed, these results do not support the selection of KIR-L–mismatched UCB units for allogeneic transplantation.

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Authorship

Contribution: C.G.B. collected and analyzed data, and wrote the paper; J.E.W. designed the study and prepared the paper; D.J.W. designed the study and prepared the paper; S.C. prepared the paper; H.N. collected data; J.N.B. designed the study and prepared the paper; T.D. analyzed data and prepared the paper; M.R.V. prepared the paper; B.R.B. designed the study and prepared the paper; and J.S.M. designed the study, collected and analyzed data, and prepared the paper.

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