Biol Blood Marrow Transplant 25 (2019) 949–954

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Donor Killer Cell Immunoglobulin-Like Receptor Genotype Does Not Improve Graft-versus-Leukemia Responses in Chronic Lymphocytic Leukemia after Unrelated Donor Transplant: A Center for International Blood and Marrow Transplant Research Analysis

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Article history:
Received 29 June 2018
Accepted 20 December 2018

Key Words:
Chronic lymphocytic leukemia
KIR
Allogeneic transplantation
Genotype
NK cells

A B S T R A C T

Allogeneic hematopoietic cell transplantation (alloHCT) remains the sole curative therapy for patients with chronic lymphocytic leukemia (CLL), leading to 40% to 45% long-term survival. The impact of donor killer immunoglobulin-like receptor (*KIR*) genotype on outcomes of unrelated donor (URD) alloHCT for CLL is unknown. We examined 573 adult URD CLL recipient pairs. *KIR* genotype (presence/absence) was determined for each donor, and comprehensive modeling of interactions with recipient HLA class I loci (KIR ligands) was used to evaluate their effect on relapse and survival. Recipients had a median age of 56 years, and most were not in remission (65%). Both 8/8 HLA-matched (81%) or 7/8 HLA matched grafts (19%) were studied. Factors associated with improved overall survival (OS) were reduced-intensity conditioning (hazard ratio [HR] of death, .76) and good performance status (HR, .46), whereas alloHCT in nonremission (HR, 1.96) and mismatched donors (HR, 2.01) increased mortality. No models demonstrated a relationship between donor *KIR* genotype and transplant outcomes. Cox regression models comparing donors with *A/A* versus *B/x KIR* haplotypes and those with *KIR* gene content scores of 0 versus 1 versus ≥2 yielded similar rates of nonrelapse mortality, relapse, acute graft-versus-host disease (GVHD), and chronic GVHD and the same progression-free survival and OS. Relapse risk was not different for grafts from donors with *KIR3DL1* transplanted into HLA C1/1 versus C2 recipients. This large analysis failed to demonstrate an association between URD *KIR* genotype and transplant outcome for patients with CLL, and thus *KIR* genotyping should not be used as a donor selection criterion in this setting.

INTRODUCTION

Chronic lymphocytic leukemia (CLL), the most common leukemia in adults, is a biologically heterogenous disease. High-risk CLL harbors adverse genetic aberrations including mutations in chromosomes 17 or 11 or the tumor suppressor gene *TP53*. Clinically, high-risk CLL exhibits a relapsing course with short and incomplete remissions after standard chemotherapy. High-risk CLL can be temporarily controlled with the small molecule inhibitors ibrutinib, idelalisib, or venetoclax; however, donor allogeneic hematopoietic cell transplantation (alloHCT) is the only known curative therapy [1]. AlloHCT can cure about 40% to 50% of patients with CLL, in part by inducing a potent donor-derived graft-versus-leukemia (GVL) response. Donor T cells contribute to GVL by recognition of minor antigen peptides for which the donor and patient are disparate presented by self-HLAs [2,3]. Disease progression in CLL may be influenced by both HLA homozygosity and haplotype bias [4]. However, donor-derived natural killer (NK) cells can also generate potent

Financial disclosure: See Acknowledgments on page 953.
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https://doi.org/10.1016/j.bbmt.2018.12.763
1083-8791/

alloreactivity toward tumor cells and may play a significant role in eradicating residual tumor after alloHCT [5].

NK cells kill malignant cells by direct or antibody-dependent cellular cytotoxicity [6]. NK cell recognition and killing of target cells are controlled by the balance of signals from both inhibitory and activating NK cell receptors [6]. These include inhibitory and activating killer cell immunoglobulin-like receptors (KIRs) that interact with "self" class I HLA-C1, HLA-C2, and Bw4 to regulate NK cell education and target killing. Inhibitory KIRs include KIR2DL2/3 (specific for HLA-C1 epitopes), 2DL1 (specific for HLA-C2), and 3DL1 (specific for HLA-Bw4) [5,6]. The ligands for activating KIR (2DS1, 2DS2, 2DS3, 2DS5, and 3DS1) are less well characterized but include HLA-A11 (2DS2), HLA-C2 (2DS1), and HLA-F (3DS1). *KIR* genes are organized into *A* or *B* haplotypes combined to form genotypes *A/A* or *B/x* (*A/B* or *B/B*) [6]. Haplotype *B* contains variable numbers of activating *KIR* genes, whereas group *A* has a fixed number of inhibitory and fewer activating *KIR* genes. About 70% of the white population has at least 1 *KIR B* haplotype. NK cell recognition and killing of target cells is controlled by the balance of signals from both inhibitory and activating NK cell receptors [6]. *KIR* genes and *HLA* genes segregate independently on different chromosomes (19 and 6, respectively), and even fully HLA-matched allogeneic donors may have a high frequency of NK cells mismatched between KIR and their cognate HLA class I ligands.

Unrelated donors (URDs) with *KIR B/x* genotype were demonstrated to protect against relapse and improve survival in a cohort of patients with acute myeloid leukemia, especially when donors had centromeric *KIR B* genes [7–9]. Relapse protection was enhanced in recipients who had 1 or 2 C1-bearing HLA-C allotypes compared with C2 homozygous recipients. Donor *KIR B/x* genotype and higher numbers of *B* genes have also been associated with antitumor responses in cohort of patients with non-Hodgkin lymphoma and pediatric acute lymphoblastic leukemia [10,11]. Here, we studied whether URD *KIR* gene content influences GVL reactions against CLL by evaluating the incidence of relapse and other outcomes after alloHCT in a large cohort of B cell CLL patients reported to the Center for International Blood and Marrow Transplant Research (CIBMTR).

METHODS

We studied 573 HCT recipients with CLL and their corresponding donors transplanted between 1995 and 2014 at 106 different centers. All HCTs used URD grafts identified through the National Marrow Donor Program. Donor DNA samples were available in the National Marrow Donor Program/CIBMTR Biorepository. Stored donor samples were genotyped for the presence or absence of *KIR* genes. Outcome data were collected from the CIBMTR. Patient-related variables in the analysis included age, sex, coexistence of disease or organ impairment, Karnofsky and comorbidity scores, donor–recipient HLA match status, and recipient HLA-based KIR ligands. Disease-related characteristics including presence/absence of chromosome 17p, immunoglobulin heavy-chain variable mutation status, Rai stage, lactate dehydrogenase levels, presence of bulky adenopathy at the time of HCT, and fludarabine responsiveness were examined in the CIBMTR database; these variables were missing for many subjects and were not included in the analysis. Disease status at transplantation was recorded as complete response, partial response, partial response nodal, stable disease, and progressive disease. Transplant-related variables included the type of preparative regimen (myeloablative, reduced-intensity conditioning, or nonmyeloablative), graft source (bone marrow versus peripheral blood), use of antithymocyte globulin and/or alemtuzumab, type of graft-versus-host disease (GVHD) prophylaxis, number of lines of therapy, and transplant period. The study protocol was approved by the Institutional Review Board of the National Marrow Donor Program in accordance with the Declaration of Helsinki.

KIR Genotyping

Donor KIR genotyping was performed via gene-specific or group-specific multiplex PCR and next-generation sequencing (Illumina, San Diego, CA) for 16 individual *KIR* genes: *KIR2DL1-5, KIR2DS1-5, KIR3DL1-3, KIR3DS1, KIR2DP1,* and *KIR3DP1*. Sequences were analyzed using an American Society of Histocompatibility and Immunogenetics approved multiplex KIR typing algorithm developed by Histogenetics (using IMGT KIR database v2.6.0, Anthony Nolan Research Institute, Royal Free Hospital, Pond Street, Hampstead, London), and each KIR gene was defined as present or absent. We identified *A* and *B KIR* haplotypes and assigned donor genotypes (*A/A* or *B/x*). For *B/x* donors we further determined whether their *B*-defining genes were in the centromeric or telomeric part of the *KIR* locus or in both (*Cen AA* versus *B/x, Tel AA* versus *B/x*) [10]. The *KIR B*–gene motifs content score (*KIR B*-score) for each donor was calculated as the sum of centromeric and telomeric gene-content motifs containing *B* haplotype–defining genes (values ranged from 0 to 4) [8].

Outcome Definitions

Overall survival (OS) was defined as time from HCT to death from any cause. Relapse and progression-free survival (PFS) were defined per CIBMTR criteria [12]. Nonrelapse mortality (NRM) was defined as death in absence of relapse. Grades II to IV and III to IV acute GVHD (aGVHD) were defined by the Glucksberg scale [13]. Chronic GVHD (cGVHD) was defined according to the Seattle criteria [14]. There was incomplete reporting in a small subset of the population (1%) that affected the cohort assessable for aGVHD, relapse, PFS, and NRM. Six cases from the *B/x* group had a missing relapse status, and 1 case from the AA group had missing aGVHD status; therefore, the total sample size for OS differs from aGVHD, relapse, and NRM.

Statistical Analysis

In univariate analysis we evaluated PFS and OS using Kaplan-Meier estimates. Relapse, NRM, and aGVHD were evaluated using the cumulative incidence function. In multivariable analysis Cox proportional hazard models were used for the following endpoints: OS, PFS, relapse, NRM, aGVHD grades II to IV, aGVHD grades III to IV, and cGVHD. All clinical variables including donor source, GVHD prophylaxis, conditioning regimen, HLA match, time from diagnosis to transplant, disease status, in vivo T cell depletion, recipient age, and Karnofsky performance score were tested for the proportional hazard assumption and adjusted as needed through stratification. Stepwise forward–backward selection was performed to build Cox regression models with a threshold of .05 for model entry.

The main objective was to test for association of each donor *KIR* genotype with relapse and PFS. Each *KIR* variable was forced into each outcome model and tested separately with the adjustment for the selected clinical risk factors. Interactions between each *KIR* variable and the adjusted clinical variables were tested, and none was statistically significant at the .05 nominal level. To adjust for multiple testing, a significance level of .01 was used for declaring a significant association of donor *KIR* genotypes. To test for donor–recipient *KIR–KIR* ligand genetic interactions, we conducted a comprehensive analysis using previously established models: donor *KIR A/A* versus *B/x* genotype [15]; donor *KIR Cen AA* versus *AB* versus *BB* [8]; *KIR B-gene* score 0 versus 1 to 4 [8]; missing ligand in recipients (HLA-C1/x versus C2) [16,17]; HLA C1/C1 versus C2/2 versus C2/C1 in the presence of activating *KIR2DS1* [18]; and missing Bw4 ligand for inhibitory *KIR3DL1* [16,17]. All p values were 2-sided. SAS version 9.4 (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Patients, Disease, and Transplant Characteristics

Median age at alloHCT of the 573 CLL patients was 56 years (range, 21 to 74), and 31% were older than 60 years of age; 78% were men, and 64% had a good performance status (Karnofsky performance score > 90%) (Table 1). Median time from diagnosis to HCT was 4.9 years (range, 1 month to 26 years). Most patients were not in remission (65%). Data on 17p chromosome abnormalities were available for 27% of patients; 52 patients (9%) harbored chromosome 17p deletion. Most recipients received reduced-intensity/nonmyeloablative conditioning (77%), and about one-third had in vivo T cell depletion. About one-third were cytomegalovirus seropositive.

Tacrolimus-based GVHD prophylaxis was the most frequently used (60%). The frequency of KIR ligands in recipients was as follows: HLA-C1, 82%; HLA-C2, 57%; and HLA-Bw4, 58%. Donor *KIR* genotype reflected the general pattern in the white population: 67% (n = 386) were *B/x* genotype (only 4% had *B/B*), and 23% were *A/A* genotype (n = 183). Almost all donors carried *KIR3DL1* (96%), and 36% carried the activating *KIR2DS1*. Most donor–recipient pairs were 8/8 high-resolution, allele-level matched at *HLA-A, -B, -C, -DRB1*, and *-DQB1* (n = 379, 66%), and the remainder were mismatched at 1 HLA allele. Donor *KIR*

Table 1
Patient, Donor, Disease, and Transplant Characteristics (N = 573)

Characteristics	Value
Age, median (range), yr	56 (21-74)
≥60	184 (32)
Sex, male	428 (75)
Race/ethnicity	
White	530 (92)
African-American	25 (4)
Asian	1 (<1)
Multiple	2 (<1)
Unknown	15 (3)
Karnofsky performance score	
<80%	44 (8)
80-100%	492 (86)
Missing	37 (6)
HCT-CI	
0-2	273 (47)
3+	63 (11)
Not available	237 (41)
Donor age, median (range), yr	33 (19-60)
Lines of prior chemotherapy	
≤3	223 (39)
>3	35 (6)
Unknown/missing	315 (55)
Disease status	
CR/PR/nPR	193 (34)
Stable/progressive/untreated	372 (65)
Missing	8 (1)
Time from diagnosis to transplant, median (range), mo	59 (1-323)
URD HLA match	
8/8	466 (81)
7/8	107 (19)
Graft source	
Bone marrow	118 (21)
Peripheral blood	455 (79)
Donor–recipient cytomegalovirus status	
Recipient positive	166 (29)
Recipient negative–donor positive	214 (37)
Recipient negative–donor negative	161 (28)
Conditioning regimen intensity	
Myeloablative	131 (23)
RIC/NMA	438 (77)
Unknown	4 (<1)
TBI in conditioning	220 (38)
In vivo T cell depletion	
None	356 (62)
Antithymocyte globulin	150 (26)
Alemtuzumab	67 (12)
GVHD prophylaxis	
Tac+ MMF ± other(s)	141 (25)
Tac MTX ± other(s) and Tac ± other(s)	251 (43)
CSA + MTX or MMF ± other(s)	111 (19)
CSA ± other(s)	64 (12)
Other(s)	6 (1)
Year of transplant	
1995-1999	29 (5)
2000-2004	146 (25)
2005-2009	299 (52)
2010-2014	99 (17)
Recipient expressing HLA C1	467 (82)
Recipient expressing HLA C2	325 (57)
Recipient expressing HLABw4	331 (58)
Donor *KIR* genotype	
KIR AA	187 (33)
KIR Bx	386 (67)
Donor with *KIR2DS1*	205 (36)
Donor with *KIR3DL1*	549 (96)

Values are n (%) unless otherwise defined. HCT-CI indicates hematopoietic cell transplantation–specific comorbidity index; CR, complete response; nPR, nodal partial response; PR, partial response; RIC, reduced-intensity conditioning; NMA, nonmyeloablative conditioning; TBI, total body irradiation; MMF, mycophenolate mofetil; MTX, methotrexate; Tac, tacrolimus; CSA, cyclosporine A.

genotypes were similar in the HLA matched and single allele mismatched HCTs.

Effect of KIR Genotype on Transplant Outcomes

In this CLL cohort transplants from donors with *KIR B/x* and *A/A* genotypes yielded similar rates of relapse, PFS (Figure 1), and OS after alloHCT. After adjusting for important clinical variables, multivariate analysis confirmed that donor *KIR* genotype (*AA* versus *B/x*), *KIR B*-score, and individual *Cen KIR B*-gene localization also conferred similar rates of NRM, aGVHD grades II to IV, aGVHD grades III to IV, cGVHD, relapse, PFS, and OS (Table 2, Figure 1). Comprehensive modeling evaluating missing ligand, missing C1 ligand (HLA-C1 versus -C2), missing Bw4 ligand for inhibitory *KIR3DL1* versus ligand present, and HLA C1/C1 versus C2/2 versus C2/C1 in the presence of activating *KIR2DS1* did not reveal significant effects on the main transplant outcomes (Table 2). In addition, no differences were seen on outcome based on *KIR A* versus *KIR B/x* donors when the HLA-matched (n = 367) or HLA-mismatched transplants (n = 169) were analyzed separately (data not shown). In aggregate, none of the models tested showed any statistically significant association between donor *KIR* genotype and transplant outcomes.

Clinical Factors Affecting Transplant Outcomes

At median follow-up of 84 months (range, 6 to 218), the probability of OS and PFS at 5 years for the entire cohort were 34% (95% confidence interval [CI], 30% to 38%) and 22% (95% CI, 19% to 36%), respectively. Cumulative incidence of relapse and NRM were 40% (95% CI, 36% to 45%) and 37% (95% CI, 33% to 42%), respectively. The main cause of death was disease recurrence (n = 190; 49%) (see Supplemental Table 1). In multivariate analysis factors associated with improved OS included reduced-intensity conditioning regimen (hazard ratio [HR] of death, .76; P = .05), Karnofsky score ≥ 80% (HR, .46; P = .0006), 8/8 HLA-matched URD (HR, .5; P = .004), and GVHD prophylaxis using tacrolimus with methotrexate as compared with tacrolimus with mycophenolate (HR, .64; P = .0016). Shorter OS was associated with disease not in remission at the time of transplant as compared with complete response/partial response (HR, 1.49; P = .003), >3 lines of prior therapy (HR, 2.54; P < .001), and use of alemtuzumab (HR, 1.46; P = .049) (Table 3).

Cumulative incidence of aGVHD grades II to IV at 100 days was 47% (95% CI, 43% to 51%), aGVHD grades III to IV at 100 days was 19% (95% CI, 16% to 23%), and cGVHD occurred at 1 year in 50% (95% CI, 45% to 54%). GVHD contributed to death in 45 patients (12%).

DISCUSSION

The importance of donor *KIR* genotype in affecting outcome after URD alloHCT has been clearly demonstrated in acute myeloid leukemia and non-Hodgkin lymphoma, where URD *KIR B/x* genotype confers an independent reduction in relapse and improved PFS [6,7]. In the non-Hodgkin lymphoma cohort, relapse protection was strongest in HLA-matched transplants. Here we studied the potential contribution of donor NK cells to GVL responses in a large cohort of CLL patients. We tested all published models, none of which demonstrated any significant association between donor *KIR* genotype or *KIR*/KIR-ligand genetic polymorphisms on alloHCT outcomes for CLL, in contrast to reported associations in acute myeloid leukemia, non-Hodgkin lymphoma, and pediatric acute lymphoblastic leukemia. We evaluated HLA-matched and HLA-mismatched subsets in our CLL cohort but found no significant effect of *KIR*

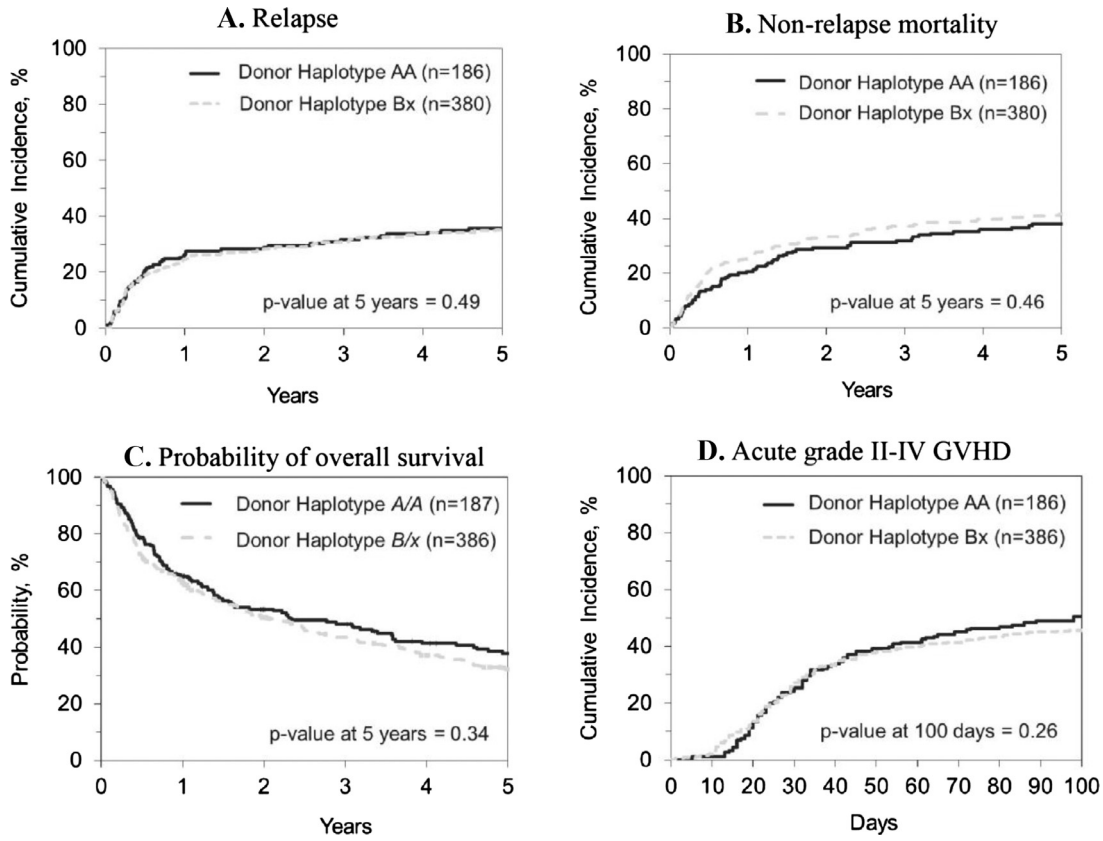

Figure 1. Unadjusted cumulative incidence of relapse (A), NRM (B), probability of OS (C), and acute grades II to IV GVHD (D) by KIR genotype.

genotype associated with HLA matching. A similar lack of significant association was observed in adult acute lymphoblastic leukemia but not in pediatric acute lymphoblastic leukemia [8,11]. These results support the conclusion that donor NK cell–host interactions are specific to disease biology and the lineage of the underlying leukemia. We speculate that in CLL, T cell–mediated GVL may be more potent than NK cell–mediated alloreactivity and thus play more of a role on clinical outcome.

The role of NK cell interactions with HLA KIR ligands in CLL is supported by studies in the nontransplant setting. Karabon et al. [19] examined autologous *KIR/HLA* gene combinations in almost 200 CLL patients and reported that PFS differed by HLA C allele. The 10-year PFS was 75% for C1/C1 patients, 58% for C2/C2 patients, and 43% for C1/C2 patients. Among HLA-C2/C2 patients, PFS was significantly higher in the patients carrying the activating *KIR2DS1* (77% versus 34%). However in our study, although 205 (36%) allogeneic donors had *KIR2DS1* we found no significant differences in relapse or PFS based on recipient HLA class C1 versus C2. Another nontransplant study in CLL showed a striking reduction in the frequency and viability of autologous NK cells expressing inhibitory *KIR2DL1* (for which the ligand is HLA-C2) and/or *KIR3DL1* (for which the ligand is HLA-Bw4), an effect that progressed over time [20]. The NK cells in untreated CLL patients exhibited enhanced susceptibility to activation-induced cell death and poor degranulation when exposed to targets and rituximab. URDs carrying *KIR2DL1* and/or *KIR3DL1*, contained in *KIR A* haplotypes, did not confer improved protection from relapse or better survival. In our analysis genotype *A/A* versus *B/x* donors conferred the same survival regardless of C2 or B4w ligand expression in recipients. These findings suggest that the contribution of NK cell *KIR* polymorphisms to GVL alloreactivity is weak and likely surpassed by other immunologic variables or stronger clinical factors such as HLA matching, disease status, and in vivo T cell depletion. For example, CLL cells have been shown to

Table 2
Overall *P* Values for Impact of *KIR* Genotype on Main Transplant Outcomes Using Models Shown

KIR Variants	OS	PFS	Relapse	NRM	aGVHD Grades II-IV*	cGVHD
Model A: *Haplotype AA* vs. *Bx*	.54	.68	.91	.44	.71	.24
Model B: *Centromeric AA* vs. *AB* vs. *BB*	.84	.69	.82	.57	.73	.23
Model C: *B-gene* score (0 vs. 1 vs. 2 vs. 3-4)	.70	.87	.72	.61	.92	.39
Model D: Missing recipient HLA C2	.59	.43	.54	.21	.19	.24
Missing recipient HLA C1	.92	.81	.64	.63	.33	.95
Missing recipient HLA Bw4	.50	.53	.02	.22	.82	.86
Model E: Donor *KIR2DS1* present in C1/C1 vs. C2/x recipient	.15	.58	.86	.55	.82	.83
Model F: Donor *KIR3DL1* present in Bw4 recipient vs. missing Bw4 recipient	.95	.91	.76	.61	.82	.90

All $P \geq .02$. Hazard ratios are shown in Supplemental Table 2.
* aGVHD grades III-IV results nonsignificant and similar to aGVHD grades II-IV; data not shown.

Table 3
Multivariate Analysis of Clinical Factors Affecting OS and Relapse

	OS		Relapse	
Variables	HR (range)	*P*	HR (range)	*P*
In vivo T cell depletion				
Antithymocyte globulin*	1.00	.023	1.00	.001
Alemtuzumab	1.46 (1.0-2.13)	.049	1.10 (.71-1.70)	.66
No in vivo depletion	.89 (.7-1.14)	.362	.57 (.4-.81)	.0014
Conditioning regimen				
Myeloablative*	1.00	.049	NS	NS
RIC	.76 (.57-1.0)	.05		
NMA	.70 (.52-.94)	.02		
Disease status				
CR/PR/nPR*	1.0		1.0	
no CR/PR/nPR	1.49.(1.14-7.56)	.0035	1.66 (1.13-2.45)	.010
Donor type				
8/8 HLA matched URD*	1.0		NS	NS
7/8 HLA matched URD	1.56 (1.22-2.01)	.0004		
GVHD prophylaxis				
Tac+/-others*	1.00	.0130	NS	NS
Tac+MTX or MMF+/-other(s)	.64 (.49-.85)	.0016		
CSA+/-others	.80 (.61-1.05)	.1117		
Karnofsky score				
<80%*	1.0		1.0	
80-100%	.46 (.32-.66)	.0001	.42 (.26-.69)	.0005
Number of chemotherapy line				
3*	1.0		1.0	
>3	2.54 (1.64-3.94)	.0001	1.82 (.99-3.34)	.053

NS indicates not significant (not included in multivariate model for outcome).
* Reference category.

overexpress HLA-E, the natural ligand for the inhibitory receptor NKG2A expressed on NK cells [21]. NKG2A/HLA-E interaction has been shown to be a mechanism of tumor evasion in CLL patients and may be a mechanism relevant for immune escape from GVL [22]. Recent studies showed that blocking NKG2A on NK cells from patients with CLL was sufficient to restore their direct cytotoxicity against HLA-E–expressing targets. This mechanism needs to be studied further in CLL where it may outweigh any KIR-mediated effects.

Our study confirmed previously established disease-specific prognostic factors for CLL, such as lack of remission and >3 lines of therapy pretransplant. Notable host-related factors associated with increased mortality include low Karnofsky performance score but not patient age. We also showed that mortality was increased by myeloablative conditioning, donor–recipient HLA mismatch, in vivo T cell depletion with alemtuzumab, and GVHD prophylaxis using tacrolimus/mycophenolate mofetil compared with tacrolimus/methotrexate. In conclusion, our large retrospective analysis testing all published models of donor *KIR* and KIR/KIR-ligand interactions did not identify any effects on outcome to warrant their use in donor selection from the available HLA-matched URD for patients with CLL.

ACKNOWLEDGMENTS

Funding and Financial disclosures: Research reported in this publication was supported by the National Center for Advancing Translational Sciences and the National Institutes of Health (NIH) Award Number UL1TR000114 (to V.B.) and NCI P01 111412 (to D.J.W., T.W., S.G.E.M., E.T., M.H., S.R.S., L.A.G., M.L., P.P., J.S.M., and S.C.). This work was supported in part by NIH P30 CA77598 using the Masonic Cancer Center, University of Minnesota Oncology Medical Informatics and Services shared resource. The CIBMTR is supported primarily by Public Health Service Grant/Cooperative Agreement 5U24CA076518 from the National Cancer Institute (NCI), the National Heart, Lung and Blood Institute (NHLBI), and the National Institute of Allergy and Infectious Diseases; Grant/Cooperative Agreement 1U24HL138660 from NHLBI and NCI; contract HHSH250201700006C with Health Resources and Services Administration; 3 grants (N00014-17-1-2388, N00014-17-1-2850, and N00014-18-1-2045) from the Office of Naval Research; and grants from Adaptive Biotechnologies; *Amgen, Inc.; anonymous donation to the Medical College of Wisconsin; Astellas Pharma US; Atara Biotherapeutics, Inc.; Be the Match Foundation; *bluebird bio, Inc.; *Bristol Myers Squibb Oncology; *Celgene Corporation; *Chimerix, Inc.; *CytoSen Therapeutics, Inc.; Fred Hutchinson Cancer Research Center; Gamida Cell Ltd.; Gilead Sciences, Inc.; HistoGenetics, Inc.; Immucor; *Incyte Corporation; Janssen Scientific Affairs, LLC; *Jazz Pharmaceuticals, Inc.; Karius, Inc.; Karyopharm Therapeutics, Inc.; *Kite Pharma, Inc.; Medac, GmbH; *Mediware; The Medical College of Wisconsin; *Merck & Co, Inc.; *Mesoblast; MesoScale Diagnostics, Inc.; Millennium, the Takeda Oncology Co.; *Miltenyi Biotec, Inc.; Mundipharma EDO; National Marrow Donor Program; Novartis Pharmaceuticals Corporation; PCORI; *Pfizer, Inc; *Pharmacyclics, LLC; PIRCHE AG; *Sanofi Genzyme; *Seattle Genetics; Shire; Spectrum Pharmaceuticals, Inc.; St. Baldrick's Foundation; Swedish Orphan Biovitrum, Inc.; *Takeda Oncology; and University of Minnesota. (Asterisks denote Corporate Members.) The views expressed in this article do not reflect the official policy or position of the NIH, the Department of the Navy, the Department of Defense, Health Resources and Services Administration, or any other agency of the US Government.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: V.B., D.J.W., J.S.M., and S.C. contributed to the study design, analyzed and interpreted data, and wrote the manuscript. S.R.S., S.J.L., and M.D.H. contributed to the study design, sample procurement, data management, and manuscript editing. T.W. performed the biostatistical analyses for the study. E.T. and N.C. performed KIR genotyping. S.G.E.M., L.A.G., and P.P. reviewed and edited the manuscript.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.12.763.

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