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Reduced-Intensity Conditioning Followed by Related and Unrelated Allografts for Hematologic Malignancies: Expanded Analysis and Long-Term Follow-Up

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A B S T R A C T

Reduced-intensity conditioning (RIC) extends the curative potential of allogeneic hematopoietic cell transplantation (HCT) to patients with hematologic malignancies unable to withstand myeloablative conditioning. We prospectively analyzed the outcomes of 292 consecutive patients, median age 58 years (range, 19 to 75) with hematologic malignancies treated with a uniform RIC regimen of cyclophosphamide, fludarabine, and total body irradiation (200 cGy) with or without antithymocyte globulin and cyclosporine and mycophenolate mofetil graft-versus-host disease (GVHD) prophylaxis followed by allogeneic HCT at the University of Minnesota from 2002 to 6. Probability of 5-year overall survival was 78% for patients with indolent non-Hodgkin lymphoma, 53% for chronic myelogenous leukemia, 55% for Hodgkin lymphoma, 40% for acute myelogenous leukemia, 37% for myelodysplastic syndrome, 29% for myeloma, and 14% for myeloproliferative neoplasms. Corresponding outcomes for relapse were 0%, 13%, 53%, 37%, 39%, 75%, and 29%, respectively. Disease risk index (DRI) predicted both survival and relapse with superior survival (64%) and lowest relapse (16%) in those with low risk score compared with 24% survival and 57% relapse in those with high/very-high risk scores. Recipient cytomegalovirus (CMV)-positive serostatus was protective from relapse with the lowest rates in those also receiving a CMV-positive donor graft (29%). The cumulative incidence of 2-year nonrelapse mortality was 26% and was lowest in those receiving a matched sibling graft at 21%, with low (21%) or intermediate (18%) HCT-specific comorbidity index, and was similar across age groups. The incidence of grades II to IV acute GVHD was 43% and grades III to IV 27%; the highest rates were found in those receiving an unrelated donor (URD) peripheral blood stem cell (PBSC) graft, at 50%. Chronic GVHD at 1 year was 36%.

Future approaches incorporating alternative GVHD prophylaxis, particularly for URD PBSC grafts, and targeted post-transplant antineoplastic therapies for those with high DRI are indicated to improve these outcomes.

INTRODUCTION

Allogeneic hematopoietic cell transplants (HCTs) are potentially curative therapy for a wide range of hematologic malignancies. Advanced age, medical comorbidities, and prior treatment history can preclude the use of the more toxic myeloablative conditioning and limit the applicability of this valuable therapy. Consequently, the development of reduced-intensity conditioning (RIC) regimens expands the use of HCT and potentially limits nonrelapse mortality (NRM). Prior publications have shown that underlying disease type, disease stage at transplantation, comorbidity, and the degree of conditioning intensity reduction impact transplant outcomes [1-3].

We previously reported outcomes on the first 123 patients with hematologic malignancies treated at our institution with a consistent RIC platform of cyclophosphamide, fludarabine, and low-dose total body irradiation (TBI) using sibling donors [3]. We described a well-tolerated platform in an older patient

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population and noted superior outcomes in indolent lymphoma. We have completed protocol enrollment and now report outcomes on 292 patients with advanced hematologic malignancies treated in a prospective trial using both sibling and unrelated donors (URDs).

METHODS

Patients were treated at the University of Minnesota on this protocol between 2002 and 2017 according to protocol NCT00303719. Those transplanted through 2016 were included in the analysis to allow for sufficient follow-up. Criteria for undergoing RIC allogeneic HCT from both related donors and URDs and eligibility criteria were previously reported [3]. In summary, eligible patients were ≤75 years old and received a transplant from a 5-6/6 HLA-matched related donor or an 8/8 allele level HLA-matched URD; mismatched unrelated donors were excluded from the analysis because of low numbers (n = 3). Patients < 55 years old were eligible if they had evidence of organ dysfunction, were heavily pretreated, or had a recent fungal infection precluding myeloablative conditioning. Eligible diseases included acute leukemia in complete remission, chronic myelogenous leukemia (CML; nonblast crisis), chemotherapy-sensitive lymphoma, chronic lymphocytic leukemia or myeloma, myelodysplastic syndrome (MDS) with <5% blasts, and myeloproliferative neoplasms.

The conditioning regimen consisted of fludarabine, cyclophosphamide, TBI ± antithymocyte globulin (ATG). Fludarabine was dosed at 40 mg/m^2 i.v. from days −6 to −2 until October 2009. A change to fixed-dose fludarabine 30 mg/m^2 daily on days −6 to −2 thereafter was based on prior institutional pharmacokinetic analyses linking increased fludarabine metabolite levels with higher NRM and diminished survival [4]. Cyclophosphamide 50 mg/kg was given on day −6 and a single dose of 200 cGy TBI on day −1. Equine ATG (dosed at 15 mg/kg i.v. every 12 hours for 6 doses on days −6, −5, and −4 with methylprednisolone 1 mg/kg as a premedication) was administered to those not exposed to combination chemotherapy within the preceding 6 months for related donors or 3 months for URDs.

The primary graft source for related donors was mobilized peripheral blood stem cells (PBSCs), whereas bone marrow was the preferred source for matched URDs. Graft-versus-host disease (GVHD) prophylaxis included cyclosporine (CSA), targeting a trough level of 200 to 400 ng/mL, and mycophenolate mofetil (MMF), beginning day −3 until day +30 [3]. MMF dosing transitioned from 2 g/day to 3 g/day in 2011 based on institutional data highlighting a need for higher MMF dosing to achieve an adequate area under the curve. The higher area under the curve translated into less acute GVHD (aGVHD) in cord blood transplants [5-7]. CSA continued through day +100 and tapered at a rate of 10% per week if no evidence of GVHD was found. Granulocyte colony-stimulating factor 5 μg/kg was administered beginning day +1 and continued until the absolute neutrophil count was >2.5 × 10^9/L for 2 consecutive days. Infectious prophylaxis included antibacterial, antifungal, and antiviral therapies per institutional guidelines.

Disease risk index (DRI) scores were calculated and assigned retrospectively [8] using the Center for International Blood and Marrow Transplant Research (CIBMTR) online calculator. Two independent reviewers (E.D.W. and N.B.) scored all patients, and any discrepancies were resolved. HCT-specific comorbidity index (HCT-CI) [9] scores were calculated and assigned prospectively for more recent transplants and were calculated retrospectively for earlier transplants.

This trial was a prospective clinical study reviewed and approved by the Masonic Cancer Center Protocol Review Committee and Human Subjects Institutional Review Board at the University of Minnesota. All patients signed Institutional Review Board–approved informed consent in accordance with the Declaration of Helsinki.

Study Endpoints and Statistical Analysis

We followed all patients longitudinally until death or last follow-up at the University of Minnesota using standardized collection procedures. The endpoints included neutrophil and platelet recovery defined the first day of an absolute neutrophil count > .5 × 10^9/L for at least 3 consecutive days and a platelet count > 20 × 10^9/L with no transfusion for at least 7 days, overall survival (OS), relapse, NRM, grades II to IV and III to IV aGVHD, and chronic GVHD. Stopping rules were in place and defined as a serious adverse event > 30% NRM at day +100. Continuous monitoring for this endpoint ensued through the trial.

Unadjusted estimates and 95% confidence intervals (CIs) were calculated using Kaplan-Meier curves for OS [10]. Statistical comparisons were completed by the log-rank test. The cumulative incidence function was used to estimate the probability for all other outcomes, considering nonevent death as a competing risk for relapse, GVHD and engraftment, and relapse as a competing risk for NRM [11]. Statistical comparisons were completed by Gray's test.

Cox regression was used to examine the independent effect of factors for OS [12]. Fine and Gray regression was used to examine the independent effect of factors on relapse, NRM, GVHD, and engraftment [13]. Factors considered in regression analyses were fludarabine and MMF dosing combinations (fludarabine 40 mg/m^2 + MMF 2 g versus 30 mg/m^2 + 2 g versus 30 mg/m^2 + 3 gm), age (<50 versus 50 to 60 versus >60), donor type (matched sibling versus mismatched sibling versus matched URD [marrow] versus matched URD [PBSC]), use of ATG (yes versus no), HCT-CI scores (low risk versus intermediate risk versus high risk), gender (male versus female), prior autologous transplant (yes versus no), diagnosis, DRI (low versus intermediate versus high/very high), year of transplant, and cytomegalovirus (CMV) serostatus (donor+/recipient+ versus donor+/recipient− versus donor−/recipient+ versus donor−/recipient−).

All reported P values were 2-sided. All analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC) and R version 3.3.1(R Foundation for Statistical Computing, Vienna, Austria). Outcomes and covariates were all prespecified. All statistical tests were reported, and there was no adjustment for multiple testing.

RESULTS

Patients

Of 349 patients transplanted on our RIC protocol using related donors and URDs from 2002 to 2016, 292 were included in this analysis. Fifty-seven patients were excluded from the analysis for prior allogeneic transplant (n = 8), transplant during postchemotherapy marrow aplasia or postradiotherapy-labeled antibody (n = 13), prior natural killer cell therapeutic infusion (n = 12), mismatched URDs (n = 3), nonmalignancy (n = 4), renal cell cancer (n = 4), fraternal twin or cousin donor (n = 4), pediatric age (n = 2), and transplants in 2017 with insufficient follow-up (n = 7).

Table 1 summarizes the patient and donor characteristics, diseases, and transplant characteristics. The median age was 58 years (range, 19 to 75) with a median follow-up of 8 years (interquartile range [IQR], 4 to 12). Median time from diagnosis to transplant was 13 months (IQR, 2 to 198), 61% were men, and 61% had an HCT-CI score of 0 to 2. Eighteen percent had a prior autologous transplant, and 30% received ATG with their conditioning. Sibling donors accounted for most of the transplants (HLA matched bone marrow, 1%; matched PBSCs, 76%; mismatched PBSCs, 4%) with HLA matched unrelated marrow (13%) and matched unrelated PBSCs (6%) accounting for the remainder. DRI was intermediate risk (65%) for most patients followed by low risk (20%) and high/very high risk (14%).

Engraftment

Two hundred eighty-nine of 292 patients achieved neutrophil engraftment > 500/μL by day +42 for an incidence of 99% (95% CI, 98% to 100%). The median time to engraftment was 9 days (IQR, 7 to 11). Platelet engraftment of 20 × 10^9/L by 6 months was 91% (95% CI, 84% to 98%) for the entire cohort. The median time to platelet recovery was 16 days (IQR, 0 to 19). We observed a slightly lower incidence of platelet engraftment by 6 months in those with CML (67%; 95% CI, 40% to 94%) and myeloproliferative neoplasm (79%; 95% CI, 52% to 100%) compared with over 87% in all other disease groups. At day +28 the median donor chimerism/engraftment was 98% (IQR, 91% to 100%) and by day +100, 100% (IQR, 96% to 100%). There was no difference in chimerism based on DRI or donor source.

Survival

After a median follow-up of 8 years (range, .8 to 15), 130 patients survived for a 5-year OS of 42% (95% CI, 36% to 48%). Five-year survival was superior in those receiving matched sibling donor transplants (46%; 95% CI, 39% to 52%) and was inferior in those using matched URD PBSCs (15%; 95% CI, 3% to 36%) or mismatched sibling PBSCs (25%; 95% CI, 6% to 50%; P < .01). Most deaths occurred within the first 2 years.

Table 1
Demographics and Characteristics (N = 292)

Characteristic	Value
Gender, male	178 (61)
Age at transplant, yr	
Median (range) [IQR]	58 (19-75) [51-63]
<50	61
50-60	123
61-70	99
70+	9
Fludarabine and MMF dosing groups	
Fludarabine 40 mg/m^2 + MMF 2 g/day	136 (47)
Fludarabine 30 mg/m^2 + MMF 2 g/day	24 (8)
Fludarabine 30 mg/m^2 + MMF 3 g/day	132 (45)
Year of transplant	
2002-2005	70 (24)
2006-2010	88 (30)
2011-2016	134 (46)
Donor type	
Sibling match (marrow)	2 (1)
Sibling match (PBSCs)	223 (76)
Sibling mismatch (PBSCs)	12 (4)
URD match (marrow)	37 (13)
URD match (PBSCs)	18 (6)
Prior autologous	54 (18)
ATG with prep	89 (30)
Diagnosis group	
AML	81 (28)
MDS	47 (16)
Aggressive NHL	29 (10)
Indolent NHL	23 (8)
Hodgkin lymphoma	21 (7)
CML	15 (5)
CLL	22 (8)
Multiple myeloma	24 (8)
MPN	14 (5)
ALL	16 (5)
Time from diagnosis to treatment, mo	
Median (range) [IQR]	13 (2-198) [5-39]
DRI	
Unknown	3 (1)
Low	57 (20)
Intermediate	190 (65)
High	39 (13)
Very high	3 (1)
Comorbidity (HCT-CI)	
Low risk	82 (28)
Intermediate risk	95 (33)
High risk	112 (38)
Missing Data	3 (1)
Karnofsky performance status	
≤80	54 (18)
90	159 (54)
100	79 (27)
CMV serostatus	
D+R+	86 (29)
D+R−	35 (12)
D−R+	83 (28)
D−R−	88 (30)
Follow-up (reverse Kaplan-Meier), yr	
Median (IQR)	8 (4-12)

IQR-Interquartile range; MM-HLA mismatch; pbsc-peripheral blood stem cell; URD-unrelated donor; RIC-reduced intensity conditioning; Cy-Cytoxan; Flu-Fludarabine; TBI-Total body irradiation; ATG-antithymocyte globulin; CSA-Cyclosporine; MMF-Mycophenolate mofetil; AML-acute myeloid leukemia; MDS-Myelodysplasia; NHL indicates non-Hodgkin lymphoma; CML-Chronic Myeloid Leukemia; CLL, chronic lymphoid leukemia; eoplasms – MmnMPN, Myelproliferative Neoplasms (3 CMML = Chronic Myelomonocytic Leukemia, 3 MDS/MPN, 5 myelofibrosis, 3 myeloproliferative disease not otherwise defined)HCT-CI-Hematopoietic Cell Transplant Comorbidity Index; CMV-Cytomegalovirus; D-Donor; R-Recipient; K-M-Kaplan-Meier. Aggressive Lymphoma (Diffuse Large B Cell, T cell lymphoma, Mantle Cell Lymphoma, Burkitt Lymphoma, Lymphoblastic Lymphoma). Indolent Lymphoma (Follicular lymphoma, Waldenstroms).

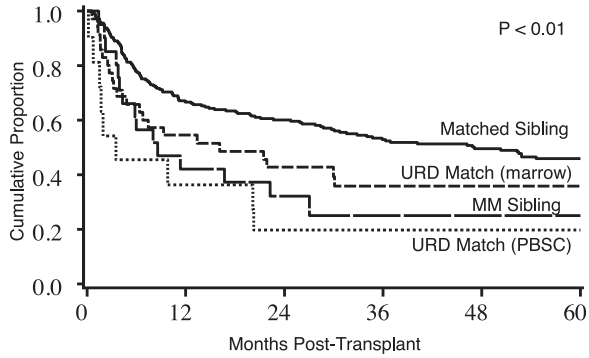

Figure 1. Survival by donor type adjusted for DRI and age.

Underlying diagnosis and DRI significantly influenced OS with superior survival in those with indolent non-Hodgkin lymphoma (78%; 95% CI, 55% to 90%), Hodgkin lymphoma (55%; 95% CI, 31% to 74%), or CML (53%; 95% CI, 25% to 74%) and those with low-risk DRI (64%; 95% CI, 50% to 75%). Younger age was associated with superior survival with the best 5-year survival in those younger than 50 (56%; 95% CI, 43% to 68%) with similar survival in those aged 50 to 60 (41%; 95% CI, 32% to 50%) and aged > 60 (34%; 95% CI, 25% to 44; $P = .04$). Figure 1 details OS stratified by donor, DRI, and age.

Cox regression analysis confirmed the independent predictive value of donor type, DRI, and age on survival outcomes. Conditioning/GVHD prophylaxis combinations (fludarabine/MMF dosing) had no effect on survival. In comparison with those transplanted with a matched sibling, those with a mismatched sibling PBSC donor (n = 12; hazard ratio [HR], 3.1; 95% CI, 1.5 to 6.2) or a matched PBSC URD (n = 18; HR, 2.3; 95% CI, 1.3 to 4.0) or matched marrow URD (n = 37; HR, 1.7; 95% CI, 1.0 to 2.6) had inferior outcomes, as did those with an intermediate-risk (n = 190; HR, 1.9; 95% CI, 1.2 to 3.1) and high/very-high-risk DRI (n = 42; HR, 2.9; 95% CI, 1.6 to 5.1 when compared with low-risk patients (Tables 2 and 3).

Relapse

The cumulative incidence of relapse at 5 years for the entire cohort was 38% (95% CI, 32% to 44%). Donor type, DRI, disease subtype, and recipient CMV serostatus best predicted relapse. Risk of relapse was lowest in those with indolent non-Hodgkin lymphoma (0%) and CML (13%; 95% CI, 0% to 29%) and highest in patients with multiple myeloma at 75% (95% CI, 52% to 98%; $P < .01$). Likewise, relapse was lowest in those with low-risk DRI (16%; 95% CI, 6% to 25%) compared with 57% (95% CI, 40% to 74%) in those with high/very-high-risk DRI ($P < .01$) (Figure 2). Recipient CMV status correlated with relapse risk, with the lowest risk in patients who were CMV seropositive (Figure 3).

In regression analysis DRI and recipient CMV serostatus were independently significant predictors of relapse. Fludarabine/MMF dosing did not impact relapse. Compared with low-risk DRI, the risk of relapse for those with an intermediate-risk DRI was 2.9 (95% CI, 1.5 to 5.8) and 4.9 (95% CI, 2.3 to 10.8) in the high/very-high DRI group. Positive recipient CMV serostatus was associated with significantly less risk of relapse (HR, .6; 95% CI, .4 to .8; $P < .01$) (Table 3). Rates of CMV reactivation at day +100 highly correlated with recipient CMV serostatus (D+/R+, 40% [95% CI, 30% to 40%]; D+/R−, 9% [95% CI, 1% to 18%]; D−/R+, 49% [95% CI, 38% to 60%]; D−/R−, 3% [95% CI, 1% to 6%]). Interestingly, CMV reactivation itself did not significantly impact relapse (HR, 1.2; 95% CI, .8 to 1.8) for those with reactivation.

Table 2
Univariate Analysis

			5-Year Relapse			5-Year Survival			2-Year NRM			aGVHD Grades II-IV		
Factor	Strata	N	Estimate (%)	95% CI (%)	*P*	Estimate (%)	95% CI (%)	*P*	Estimate (%)	95% CI (%)	*P*	Estimate (%)	95% CI (%)	*P*
All patients		292	38	32- 44		42	36- 48		26	20-31		43	37-49	
Donor type	Sibling match	225	39	32- 46	.75	46	39- 52	**<.01**	21	16-27	**<.01**	39	32-46	**<.01**
	Sibling mismatch	12	33	8- 59		25	6- 50		50	27-78		45	17-74	
	URD match (marrow)	37	38	22- 55		38	23- 54		30	15-46		54	36-72	
	URD match (PBSCs)	18	22	3- 41		15	3- 36		56	30-81		67	42-92	
Diagnosis	AML	81	37	25- 48	**<.01**	40	28- 51	**.02**	25	15-35	.09	36	25-46	.12
	MDS	47	39	24- 54		37	22- 51		28	15-42		40	26-55	
	Aggressive NHL	29	35	17- 53		41	22- 59		18	4-32		48	29-67	
	Indolent NHL	23	0			78	55- 90		17	2-33		32	13-50	
	Hodgkin	21	53	30- 76		55	31- 74		14	0-29		33	13-53	
	CML	15	13	0- 29		53	25- 74		41	16-66		47	21-73	
	CLL	22	42	20- 64		44	23- 64		27	9-46		68	46-91	
	Multiple myeloma	24	75	52- 98		29	13- 48		17	2-31		54	33-75	
	MPN	14	29	6- 51		14	2- 37		57	29-85		29	5-52	
	ALL	16	46	20- 73		33	11- 57		31	9-53		63	36-89	
HCT-CI	0 (low)	82	38	27- 49	.68	46	33- 57	.46	21	12-30	**<.01**	44	33-55	.08
	1-2 (intermediate)	95	44	33- 55		45	34- 55		18	10-25		36	26-46	
	3+ (high)	112	34	24- 43		36	27- 45		37	27-46		49	39-59	
DRI	Low	57	16	6- 25	**<.01**	64	50- 75	**<.01**	23	12-34	.26	36	23-49	.70
	Intermediate	190	41	33- 49		38	31- 46		27	21-34		45	37-52	
	High/very high	42	57	40- 74		24	12- 38		24	11-37		43	27-58	
Age	<50	61	43	30- 57	.56	56	43-68	**.04**	18	8-28	**.23**	52	39-66	.20
	50-60	123	40	31-50		41	32-50		25	17-33		41	31-51	
	>60	108	31	22-40		34	25-44		30	21-40		40	31-49	
Fludarabine/ MMF dosing	40 mg/m^2/2 g/day	136	32	21-41	.13	45	46-53	.5	26	19-34	.86	42	34-51	.79
	30 mg/m^2/2 g/day	24	50	28-72		46	26-64		21	5-37		50	29-71	
	30 mg/m^2/3 g/day	132	41	32-50		38	28-47		26	18-34		42	34-51	

Statistically significant results in bold

Nonrelapse Mortality

NRM at 2 years was 26% (95% CI, 20% to 31%) for the entire cohort. Univariate analysis revealed that donor type and HCT-CI influenced outcomes. Two-year NRM was lowest in those with matched sibling donors (21%; 95% CI, 16% to 27%) and low (21%; 95% CI, 12% to 30%) and intermediate HCT-CI scores (18%; 95% CI, 10% to 25%) and similar across age groups (Table 2). We observed no impact of fludarabine and MMF dosing on NRM. Regression analysis confirmed the significance of donor type and HCT-CI on NRM. The HR was 3.9 (95% CI, 1.6 to 9.6) for mismatched siblings, 3 (95% CI, 1.5 to 6.2) for URD matched PBSCs, and 2.2 (95% CI, 1 to 4.7) for URD matched marrow ($P < .01$). High-risk HCT-CI had an HR of 2.4 (95% CI, 1.5 to 3.9) when compared with low/intermediate-risk HCT-CI ($P < .01$) (Table 3).

Cause of death was most often disease relapse (48%), with complications of aGVHD (18%), infection (8%), chronic GVHD (6%), organ failure (6%), with graft failure, acute respiratory distress syndrome, new malignancy, hemorrhage, accidental death, and encephalitis representing the others (14%). Cause of death differed by donor type. For matched PBSC URD transplants most deaths were aGVHD related (40%), infectious (20%), and disease relapse (20%). Comparatively, with matched sibling donor transplants most deaths were disease relapse (52%) and aGVHD related (18%).

Graft-versus-Host Disease

At 100 days the cumulative incidence of aGVHD grades II to IV was 43% (95% CI, 37% to 49%) and 27% (95% CI, 22% to 32%) for grades III to IV. The incidence of day +100 aGVHD was primarily influenced by donor source, with highest rates of grade II to IV and grades III to IV in PBSC URD at 67% (95% CI, 42% to 92%) and 50% (95% CI, 26% to 74%), respectively, versus 37% (95% CI, 32% to 46%) and 24% (95% CI, 19% to 30%) in matched sibling transplants. In regression analysis the risk of severe aGVHD grades III to IV was highest in URD PBSCs (HR, 2.9; 95% CI, 1.3 to 6.4; $P = .01$) compared with matched sibling donor transplants. We found no other patient, disease, or treatment variables (including fludarabine and MMF dosing) that significantly altered overall or severe aGVHD rates.

The cumulative incidence of chronic GVHD at 2 years was 36% (95% CI, 30% to 42%) for the entire cohort. Only a few URD PBSC recipients survived to 2 years, and thus this subset was not recognized to have a higher incidence of chronic GVHD.

DISCUSSION

This prospective trial using a uniform RIC and GVHD prophylaxis platform for patients with hematologic malignancies highlights superior outcomes in patients with indolent lymphomas and CML, younger patients, those with sibling donors, and those with lower DRI and HCT-CI and highlights need for improvement for those with high-risk DRI and those using unrelated PBSC donors.

Relapse remains a substantial problem, compromising long-term success of HCT for hematologic malignancies. Our cohort of patients revealed high rates of relapse in patients with high-risk DRI, with multiple myeloma, and with Hodgkin lymphoma and modest rates for those with acute myelogenous leukemia (AML) and MDS. Requiring stringent remission status before transplant is a crucial initial step toward diminishing relapse risk post-transplant, especially those with AML/MDS [14-16], and may be equally important for those with high-risk DRI. Augmenting conditioning intensity using myeloablative approaches when possible [2] or considering an intermediate conditioning approach based on disease subtype/DRI are potential approaches for upfront mitigation of relapse risk. Implementing post-HCT antineoplastic treatments with maintenance therapy may further reduce risk of relapse after transplant [16]. Numerous approaches for post-transplant

Table 3
Multivariate Regression Analysis

Outcome	Factor	HR (95% CI)	*P*
5-Year OS	Donor type*		
	Sibling matched	1.0 (ref)	
	Sibling mismatched	3.1 (1.5-6.2)	<**.01**
	URD matched (marrow)	1.7 (1.0-2.6)	**.03**
	URD matched (PBSCs)	2.3 (1.3-4.0)	<**.01**
	DRI		
	Low	1.0 (ref)	
	Intermediate	1.9 (1.2-3.1)	**.01**
	High/very high	2.9 (1.6-5.1)	<**.01**
	Age, yr		
	<50	1.0 (ref)	
	50-60	1.7 (1.1-2.7)	**.02**
	>60	1.8 (1.1-2.9)	**.02**
5-Year relapse	DRI†		
	Low	1.0 (ref)	
	Intermediate	2.9 (1.5-5.8)	<**.01**
	High/very high	4.9 (2.3-10.8)	<**.01**
	Recipient CMV		
	Negative	1.0 (ref)	
	Positive	.6 (.4-.8)	<**.01**
	Fludarabine, MMF		
	40 mg/m^2, 2 g/day	1.0 (ref)	
	30 mg/m^2, 2 g/day	1.7 (1.0-3.1)	.07
	30 mg/m$_2$, 3 g/day	1.5 (1.0-2.2)	.056
2-Year NRM	Donor type‡		
	Sibling matched	1.0 (ref)	
	Sibling mismatched	3.9 (1.6-9.6)	<**.01**
	URD matched (marrow)	2.2 (1.0-4.7)	**.04**
	URD matched (PBSCs)	3.0 (1.5-6.2)	<**.01**
	DRI		NS (.81)
	Low	1.0 (ref)	
	Intermediate	1.3 (.6-2.5)	
	High/very high	1.1 (.5-2.7)	
	Age, yr		
	<50	1.0 (ref)	
	50-60	1.9 (1.0-4.7)	.06
	>60	2.2 (1.0-4.7)	**.04**
	Comorbidity (HCT-CI)		
	Low/intermediate risk	1.0 (ref)	
	High risk	2.4 (1.5-3.9)	<**.01**
	Recipient CMV		
	Negative	1.0 (ref)	
	Positive	1.1 (.7-1.8)	NS (.65)
	Fludarabine, MMF		NS (.45)
	40 mg/m^2, 2 g/day	1.0 (ref)	
	30 mg/m^2, 2 g/day	.8 (.3-2.3)	
	30 mg/m^2, 3 g/day	.7 (.4-1.2)	

Statistically significant results in bold

* Only sibling mismatched and URDs are statistically significant in comparison with sibling matched donors. No other pairwise comparisons are significant.

† High risk is statistically significantly different from intermediate risk at the .05 level.

‡ Only sibling mismatched and URD matched (PBSCs) are statistically significant in comparison with sibling matched donors. No other pairwise comparisons are significant.

maintenance strategies are in development: FLT-3 ITD (fms like tyrosine kinase 3 internal tandem duplication) AML with post-transplant TKI maintenance [17], MDS with post-transplant azacitidine or decitabine maintenance [18-19], myeloma using lenalidomide, proteasome inhibition maintenance (Blood and Marrow Transplant Clinical Trials Network 1302), or lenalidomide + DLI approaches [20]. Finally, immune modulation post-HCT could diminish relapse risk. Based on recently published data highlighting successful IL-15 superagonist (ALT-803) induced natural killer cell and CD8$^+$ T cell expansion in patients relapsing after allogeneic transplant [21], we are now using ALT-803 prophylaxis after RIC allogeneic transplant for

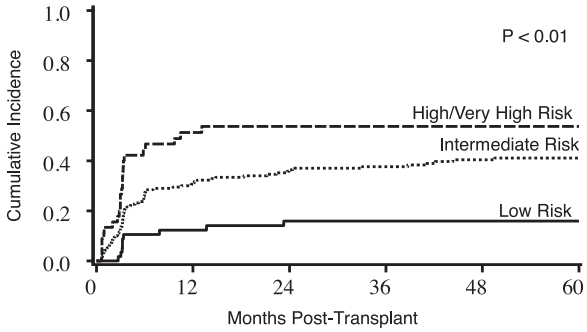

Figure 2. Relapse by DRI.

patients with AML and MDS in attempts to minimize relapse risk. Combining pretransplant disease status–specific requirements, personalized conditioning intensity approaches, and post-HCT maintenance pathways for high relapse risk diseases will improve long-term outcomes.

Our RIC platform was well tolerated in an older patient population with overall low NRM that was not impacted by age as supported by prior publications [22,23]. Unfortunately, despite prior reports from our institution correlating diminished NRM with lower fludarabine metabolite levels, decreasing the fixed dose of fludarabine to 30 mg/m^2 in our study did not translate to diminished NRM. These findings suggest that diminishing NRM with fludarabine dosing would require true pharmacokinetic dosing on an individual patient basis. Those with high-risk HCT-CI and those using a PBSC URD experienced higher NRM and presents a population of patients where adjusted supportive care or adjusted GVHD prophylaxis modifications is needed.

We observed a statistically significant decreased risk of relapse in those recipients who were CMV seropositive. Of the 169 CMV-seropositive recipients in our cohort, 75 (44%) experienced CMV reactivation. In our analysis, interestingly, CMV reactivation as a time-dependent covariate in regression analysis for the endpoint of relapse did not show an association. The data are mixed regarding impact of CMV seropositivity or CMV reactivation on post-transplant relapse and NRM. Our group has previously demonstrated a diminished risk of relapse in those undergoing RIC allogeneic transplant who experienced CMV reactivation citing expansion of a specific population of natural killer cells (CD56^{dim} CD57$^+$NKG2C$^+$) in response to the CMV reactivation. Those data revealed diminished relapse in the setting of CMV reactivation in comparison with CMV-negative patients, suggesting a protective effect of CMV reactivation on relapse after RIC allogeneic HCT [24]. Teira et al. [25] reported a CIBMTR analysis showing no impact of CMV

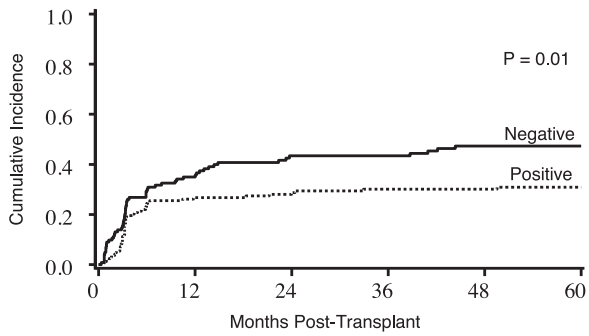

Figure 3. Relapse by CMV serostatus.

reactivation on relapse rates regardless of type of hematologic malignancy but did highlight increased NRM and diminished OS. In an European Society for Blood and Marrow Transplantation analysis Schmidt-Hieber et al. [26] showed similar outcomes with diminished survival and leukemia-free survival, no change in relapse, and increased NRM in CMV-seropositive patients. In contrast, Green et al. [27] reported diminished relapse in the setting of CMV reactivation for those with AML but not acute lymphoblastic leukemia, MDS, or lymphoma with offsetting increased NRM but no difference in survival. Thus, our findings of diminished relapse but similar NRM in the setting of CMV seropositivity only are in partial contrast to the other published data. The differences may be explained by the smaller sample size in our study as compared with the large CIBMTR/ European Society for Blood and Marrow Transplantation analyses.

Finally, we observed rates of aGVHD higher than expected in comparison with contemporary studies. Our overall rates of 43% for grades II to IV and 27% for grade III to IV were modestly higher with the largest contribution from unrelated PBSCs and marrow. Clinical Trials Network 0901 reported 31% for grades II to IV and only 6.8% for grades III to IV using mini-methotrexate + CSA or tacrolimus, tacrolimus + sirolimus, or CSA + MMF [1]. CALGB 100103/Clinical Trials Network 0502 reported even lower rates of 9.6% for gradeg II to IV aGVHD with a mini-methotrexate + tacrolimus approach [2]. Our data suggest that alternative GVHD prophylaxis strategies may be preferred, especially for PBSC URD transplants or mismatched sibling transplant. Post-transplant cyclophosphamide has become an effective GVHD prophylaxis strategy for haploidentical bone marrow transplants yielding very low aGVHD rates [28,29] and is now extending to related donor and URD transplants [30-33]. Numerous other strategies for GVHD prevention and treatment including histone deacetylase inhibitors, IL-6 antibodies, and proteasome inhibitors, are also in development [34]. Given the improvement in rates of severe aGVHD with post-transplant cyclophosphamide in addition to an MMF + tacrolimus backbone, future investigation of this GVHD prophylaxis with our RIC regimen may be valuable, especially for transplants using peripheral blood URD sources or mismatched sibling donors.

In summary, our fludarabine + cyclophosphamide +TBI RIC platform produced highly successful outcomes in indolent lymphoma and CML and those with low-risk DRI. More stringent disease burden criteria at transplant, modifications to conditioning intensity approaches, use of alternative GVHD prophylaxis, and initiation of targeted post-transplant maintenance therapy approaches will strive to improve outcomes for the remaining hematologic malignancy patients treated with our RIC platform.

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