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ORIGINAL ARTICLE

$CD34^+$ cell dose and establishment of full donor chimerism at day +100 are important factors for survival with reduced-intensity conditioning with fludarabine and melphalan before allogeneic hematopoietic SCT for hematologic malignancies

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The combination of fludarabine and melphalan as a reduced-intensity conditioning (RIC) regimen extends allogeneic hematopoietic SCT (HSCT) as a therapeutic option for elderly or frail patients with relapsed, refractory or other high-risk hematologic malignancies. Whether any modifiable factors exist that could improve survival before or immediately after HSCT is unknown. We reviewed the medical records of the first 50 patients at our institution to undergo fludarabine/melphalan RIC from September 2000 to September 2007 to determine factors associated with survival. A total of 25 (50%) patients had undergone prior HSCT and as such was a high-risk group of patients. On multivariate analysis, $CD34^+$ cell dose greater than 5.5×10^6 per kg (risk ratio (RR) 0.44, 95% CI 0.19–0.98, $P = 0.02$) and full donor chimerism at day +100 (RR 0.17, 95% CI 0.06–0.64, $P = 0.002$) remained independent prognostic factors. In our series, achievement of full donor chimerism at day +100 was associated with an approximately 70% 2-year survival, a favorable outcome in this high-risk group of patients. Although the infused $CD34^+$ cell dose is a modifiable variable, whether donor lymphocyte infusions or other immunologic interventions should be performed to promote the establishment of full chimerism early post transplant remains unknown.
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Introduction

Allogeneic hematopoietic SCT (HSCT) is a life-saving treatment for patients with high-risk or refractory hematologic malignancies. However, the potential severe toxicities of myeloablative conditioning preclude many patients from candidacy for this therapy. Reduced-intensity conditioning (RIC) regimens extend this modality to patients who would otherwise be deemed medically unsuitable to receive myeloablative conditioning. The combination of fludarabine and melphalan is a commonly used moderately myelosuppressive RIC regimen that has antineoplastic activity in both myeloid malignancies including AML and myelodysplastic syndrome (MDS)[1] and lymphoid malignancies including ALL,[2] Hodgkin's and non-Hodgkin's lymphomas[3,4] and multiple myeloma.[5] Its use results in the rapid establishment of donor chimerism, excellent rates of engraftment and a reduction in relapse risk compared with other RIC regimens.[6–8] Fludarabine/melphalan RIC also has proven tolerable in patients who have failed a previous autologous or allogeneic HSCT, although a high rate of relapse as well as late complications of HSCT may adversely affect outcome in this high-risk group of patients.[9] Because those who undergo RIC allogeneic HSCT have either a guarded prognosis from a disease or comorbidity standpoint or both, we sought to determine modifiable factors associated with survival to identify potential therapeutic targets to improve outcome in this group of patients.

Materials and methods

We reviewed the medical records of the first 50 consecutive patients who underwent fludarabine/melphalan RIC at our institution from September 2000 to September 2007 to determine factors associated with survival. The Mayo Clinic Institutional Review Board approved the study. Overall survival was defined as the time from day 0 to date of death due to any cause. Patients transplanted during 2000–2002 were given fludarabine 25 mg/m^2 on days −6 to

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−2 and melphalan 140 mg/m^2 on day −2. Those transplanted during 2003 and later received split dosing of the melphalan at 70 mg/m^2 on days −3 and −2. Factors analyzed for association with survival included demographic and disease factors (age at transplant, sex, lymphoid vs myeloid disease, karyotype and blast percentage in those with MDS and leukemia, disease status at transplant, previous HSCT, and previous chemotherapy for solid tumors), donor/graft factors (CD34$^+$ cell dose, graft source, related vs unrelated graft, degree of HLA match, CMV serostatus and ABO match), and post transplant factors (time to neutrophil, lymphocyte and platelet engraftment, day +100 chimerism status, development of and severity of acute GVHD, development of and severity of chronic GVHD). Full donor chimerism was defined as $\geqslant$95% as previously described[10,11] and was monitored by a peripheral blood (or less commonly BM) method involving genomic DNA extraction from unsorted mononuclear cells and subsequent PCR amplification of highly polymorphic short tandem repeats. Occasionally, a FISH method of labeling X and Y chromosomes in opposite sex transplants was used. Univariate survival analyses for nominal variables were performed according to the Kaplan–Meier method.[12] The two-tailed log-rank test was applied to determine statistically significant differences.[13] Cox proportional hazards model was applied both for the univariate survival analysis with continuous variables and for the multivariate survival analysis.[14] The following continuous data were also analyzed as binary variables after dichotomization: CD34$^+$ cell dose (above vs equal to or below the median of 5.5×10^6 cells per kg), BM aspirate blast percentage ($\leqslant$5% or >5%) and time to absolute lymphocyte count greater than 500×10^6 cells per liter (15 days or less or greater than 15 days) based on our institution's previous experience with evaluating post transplant immune reconstitution.[15] Univariate variables with $P \leqslant 0.10$ were included in the multivariate model, with the least significant one being eliminated in serial iterations until only statistically significant variables with a $P < 0.05$ remained (backward selection).

Results

Table 1 lists baseline demographic data and univariate analysis. The median age at transplant was 53.5 years (range 20–67 years). Most patients received HSCT for myeloid malignancies (predominantly AML or MDS, 29 patients, 58%). Approximately one-third patients were in CR at the time of transplant (32%). The majority also received stem cells from a sibling donor (74%). Half (25 patients) of the patients in this cohort had received previous HSCT. Seven patients had a prior allogeneic HSCT for AML, and one had a prior allogeneic HSCT for CLL. One patient each had undergone prior autologous HSCT for AML and breast cancer. The remaining 15 patients who had prior transplants had undergone autologous HSCT for lymphoid malignancies. The indication for RIC allogeneic HSCT for most (19 patients) was a relapse of their primary malignancy. Six patients were undergoing transplantation for a new primary malignancy (therapy-related MDS with

Table 1 Patient demographics and univariate analysis

Patient characteristics N = *50*	N *(%)*	P-*value*
Sex		
Male	34 (68)	0.69
Female	16 (32)	
Age, median years (range)	53.5 (20–67)	0.32
Disease[a]		
AML	18 (36)	0.57
Myelodysplastic syndrome	11 (22)	
Non-Hodgkin's lymphoma	8 (16)	
Multiple myeloma	7 (14)	
CLL	2 (4)	
CML	2 (4)	
Hodgkin's lymphoma	1 (2)	
Primary myelofibrosis	1 (2)	
Karyotype in myeloid diseases		
Favorable/standard risk	16 (32)	0.007
Poor risk	15 (30)	
Blast percentage in myeloid diseases		
>5%	7 (14)	0.59
$\leqslant$5%	23 (46)	
Remission status		
CR	16 (32)	0.19
PR	9 (18)	
Relapsed/refractory	13 (26)	
Untreated	12 (24)	
Donor		
Related	37 (74)	0.6
Unrelated	13 (26)	
Graft source		
BM	2 (4)	0.96
PBSC	48 (96)	
Degree of match		
6/6 or 10/10 antigen match	46 (92)	0.33
Single antigen mismatch	4 (8)	
Acute GVHD[b]		
Grade 1–2	9 (18)	0.45
Grade 3–4	14 (28)	
Chronic GVHD[b]		
Limited chronic	2 (4)	0.0003
Extensive chronic	19 (38)	
Prior therapy		
HSCT	25 (50)	0.83
Chemotherapy for solid tumors	3 (6)	
CMV serostatus (donor/recipient)		
Positive/Positive	14 (28)	0.44
Negative/Positive	12 (24)	
Positive/Negative	6 (12)	
Negative/Negative	18 (36)	
ABO mismatch		
None	33 (6)	0.76
Minor	10 (20)	
Major	6 (12)	
Rh incompatible	1 (2)	
CD34$^+$ cell dose[c]		
$>5.5 \times 10(6)$	23 (46)	0.05
$\leqslant 5.5 \times 10(6)$	26 (52)	
CD34$^+$ cell dose (as continuous variable)		0.61

Table 1 Continued

Patient characteristics N = *50*	N *(%)*	P-*value*
Day +100 chimerism		
Full donor	32 (64)	0.001
< full donor	5 (10)	
Days to neutrophil engraftment		
15 days (13–17 days IQR)		0.03
Days to platelet engraftment		
19 days (16–24 days IQR)		0.25
Days to lymphocyte engraftment		
20 days (15–30.5 days IQR)		0.78

Abbreviation: IQR = interquartile range.
[a]Kaplan–Meier analysis on lymphoid vs myeloid disease.
[b]Kaplan–Meier analysis on whether any acute or chronic GVHD was present.
[c]Not available on one patient (only total nucleated cell dose recorded).

high-risk cytogenetics in five patients, and AML and peripheral T-cell lymphoma in one patient each). Most patients received CYA-based GVHD prophylaxis (5 with CYA alone, 30 received CYA in combination with MTX, and 8 received CYA together with mycophenolate mofetil). Six patients received tacrolimus and MTX as GVHD prophylaxis, and the remaining one patient received no GVHD prophylaxis (second allogeneic transplant for relapsed leukemia <100 days before the first transplant from the same sibling donor).

Median time to neutrophil engraftment was 15 days (13–17 days interquartile range), platelet engraftment was 19 days (16–24 days interquartile range) and lymphocyte engraftment $>500\times10^6$ per liter was 20 days (15–30.5 interquartile range). 28% of patients experienced grade 3–4 acute GVHD, and 46% experienced chronic GVHD (38% extensive). Of 50, 27 patients have died, 5 from relapsed disease (Table 2). Only one of the deaths before day +100 was due to relapsed disease, and mortality (primarily due to infections) at day +100 was 26% (13 patients). Three deaths between day +100 and 1 year were due to relapse, and six were due to complications of the transplant. Of those patients surviving more than 1 year, one death was due to relapse, two deaths were due to GVHD, and one death was unexpected and of unknown cause. Twenty-three patients (46%) are alive after a median of 27 months of follow-up.

We performed a multivariate analysis to determine factors independently associated with survival using those factors significant on univariate analysis (karyotype in myeloid diseases, presence of chronic GVHD, $CD34^+$ cell dose, day +100 chimerism and time to neutrophil engraftment). $CD34^+$ cell dose greater than 5.5×10^6 per kg (risk ratio (RR) 0.44, 95% CI 0.19–0.98, $P=0.02$) and full donor chimerism at day +100 (RR 0.17, 95% CI 0.06–0.64, $P=0.002$) remained independent prognostic factors. Figure 1 shows Kaplan–Meier estimates for survival associated with cell dose and chimerism. Achievement of full donor chimerism was not related to $CD34^+$ cell dose ($P=0.46$).

Discussion

Our retrospective study has identified two potentially modifiable factors associated with improved survival in patients receiving fludarabine/melphalan RIC before allogeneic HSCT: cell dose and chimerism. Cell dose has previously been associated with a reduced relapse risk, potentially because of improved post transplant lymphocyte recovery.[16] $CD34^+$ cell dose is also associated with the development of chronic GVHD in the reduced-intensity setting,[17] a factor strongly associated with survival with a similar RIC regimen consisting of fludarabine and BU followed by HSCT for high-risk AML and MDS.[18] An optimal cell dose for RIC has been proposed at 6 to 8×10^6 per kg $CD34^+$.[19]

Cell dose did not influence rates of full donor chimerism, the other independent prognostic factor we identified in this series. Although mixed chimerism may not be detrimental to survival in allogeneic transplants for nonmalignant diseases,[20] whether any degree of mixed chimerism is acceptable in allogeneic transplantations for hematologic malignancies is subject to debate. In our series, lineage-specific (T-cell vs myeloid) chimerism was not available on many patients and thus not formally evaluated for association with survival. In another series of fludarabine-based RIC, delayed T-cell chimerism was clearly associated with poorer PFS in those with myeloid malignancies (40% relapse rate compared to 0% in those with mixed vs complete T-cell chimerism, respectively, $P=0.002$).[11] Whether lineage-specific chimerism should be routinely monitored or whether any immunologic therapies such as prophylactic donor lymphocyte infusions[21] or cytokine therapies such as IL-2[22] should be undertaken in an attempt to convert mixed chimerism to full donor chimerism in the early post transplant period is worthy of prospective study.

Our study has important limitations due to our limited number of patients from a single institution and retrospective analysis. Chimerism from earlier time points (days +30 and +60) was performed on an insufficient number of patients to be included in analysis. In addition, lineage-specific chimerism and lymphocyte subset analysis was not performed on most patients. Two other important and potentially modifiable factors that we did not have the statistical power to analyze in this cohort are the impact of donor source and various GVHD prophylaxis regimens on lymphocyte recovery and survival. These shortcomings could be addressed with a prospective study. Finally, fludarabine and melphalan RIC may not be suitable conditioning before unrelated cord blood transplants without additional immunomodulation owing to high rates of graft failure (4 of 10 reported patients),[23] and consequently our study results may not be generalizable to those undergoing cord blood transplants.

GVHD remains a significant cause of morbidity and mortality in RIC allogeneic HSCT.[8] The addition of alemtuzumab to fludarabine and melphalan RIC before allogeneic HSCT for MDS and AML has recently been associated with a sixfold reduction in rates of GVHD, although this was associated with a nonsignificant increase in disease recurrence rates compared with a historical cohort.[24] GVHD was the cause of death in four patients

Table 2 Patient deaths

Patient number	*Sex*	*Age*	*Disease*	*Cause of death*	*Day post HSCT at death*	*Disease status at HSCT*	*Day +100 disease response*	*Grade II–IV acute GVHD*
1	M	51	Multiple myeloma	Infection	7	Relapse 1	Dead	—
2	M	62	AML, transformed from MDS	MOF	15	Refractory	Dead	—
3	M	63	AML, transformed from MDS	ARDS	19	Untreated	Dead	—
4	M	54	MDS	Infection	22	Untreated	Dead	—
5	F	50	Follicular non–Hodgkin's lymphoma	ICH	31	Relapse 2+	Dead	—
6	M	65	AML, transformed from MDS	MOF	31	Refractory	Dead	—
7	M	45	CLL	Infection	34	Relapse 1	Dead	—
8	F	26	AML, M5, monocytic	MOF	44	Relapse 2+	Dead	Liver
9	M	65	AML, transformed from MDS	Infection	53	Refractory	Dead	—
10	F	49	Hodgkin's lymphoma	Cardiac arrest	59	CR 2+	Dead	—
11	M	61	MDS-treatment related	Infection	73	Untreated	Dead	—
12	M	25	AML, M1, myeloblastic	MOF	77	CR 2+	Dead	Liver
13	M	59	AML, NOS	Relapse	78	CR 1	Dead	—
14	M	67	AML, M7, megakaryoblastic	GVHD	98	CR 1	Dead	Gut
15	M	61	Multiple myeloma	Infection	110	PR 2+	PR	Gut
16	F	51	AML, transformed from MDS	Relapse	124	Relapse 1	Relapse	—
17	F	64	CML	GVHD	135	Untreated	Persistent disease	Skin, gut, liver
18	F	56	AML, M2, myelocytic	Infection	137	CR 1	CR	Gut, liver
19	M	59	Mantle cell lymphoma	Infection	199	PR 2+	CR	Gut
20	F	46	MDS	Relapse	215	Untreated	Persistent disease	Liver
21	M	46	AML, M2, myelocytic	ICH	249	CR 1	CR	Gut, liver
22	M	51	Diffuse large B-cell lymphoma	ARDS	260	PR 2+	CR	—
23	F	59	AML, M4, myelomonocytic	Relapse	278	CR 2+	CR	Gut
24	M	53	Multiple myeloma	Relapse	389	PR 1	CR	—
25	F	59	CML	GVHD	542	Untreated	CR	Gut
26	M	29	γ-δ T-cell lymphoma	Unknown	655	CR 2+	CR	—
27	F	61	MDS	GVHD	659	Refractory	CR	Gut

Abbreviations: ARDS = acute respiratory distress syndrome; F = female; ICH = intracranial hemorrhage; M, male; MDS = myelodysplastic syndrome; MOF = multiorgan failure.

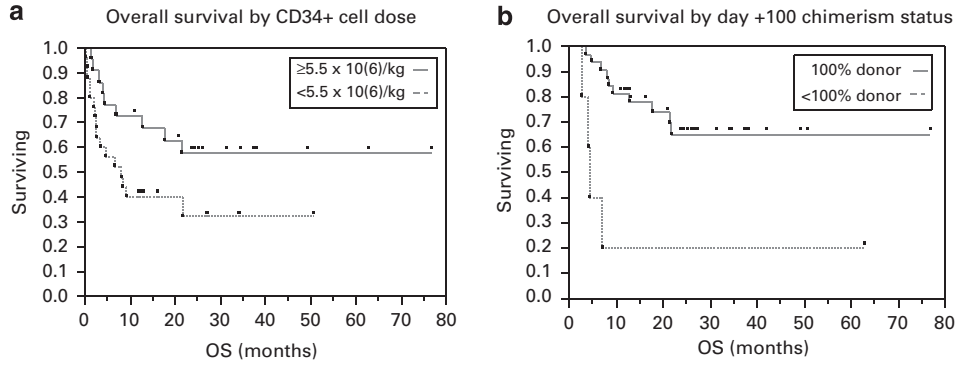

Figure 1 Kaplan–Meier estimates for overall survival based on (**a**) $CD34^+$ cell dose and (**b**) day +100 chimerism status.

(8%) in our series. However, 3 of the 12 patients who died of infections (two with bacterial sepsis and one with disseminated herpes simplex virus) were receiving CYA and tapering doses of steroids for acute GVHD, bringing the total deaths attributable to GVHD or complications of its treatment to 7 patients (14%). Infections/multiorgan failure without GVHD and disease relapse were more frequently the cause of death (nine and five patients, respectively) in our series, arguing for the greater need for improved immunologic recovery rather than augmenting immunosuppression in our group.

Reduced-intensity conditioning regimens such as the combination of fludarabine and melphalan have extended allogeneic transplantation as a potentially life-saving modality for patients with high-risk or refractory hematologic malignancies who would otherwise be medically unsuitable for myeloablative conditioning. Because the toxicities of conditioning were relatively tolerable even in our heavily pretreated high-risk population, developing methods of rapid and effective immune reconstitution to reduce the risk of infectious complications and disease relapse becomes even more important. In this study, we identified day +100 full donor chimerism and $CD34^+$ cell dose greater than 5.5×10^6 per kg as potentially modifiable prognostic factors in RIC allogeneic transplantation. Both frequent monitoring of chimerism status and early

intervention with cytokines, donor lymphocyte infusions or other immunomodulatory treatments when <95% donor chimerism is identified as well as optimizing $CD34^+$ cell dose may improve patient outcomes and could be studied prospectively.

Conflict of interest

The authors declare no conflict of interest.

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