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Allogeneic Hematopoietic Cell Transplantation after Fludarabine and 2 Gy Total Body Irradiation for Relapsed and Refractory Mantle Cell Lymphoma

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ABSTRACT

We carried out HLA-matched related (n=16) and unrelated (n=17) hematopoietic cell transplantation (HCT) in 33 patients with relapsed and refractory mantle cell lymphoma after nonmyeloablative conditioning with fludarabine and 2 Gy total body irradiation. Postgrafting immunosuppression consisted of cyclosporine and mycophenolate mofetil. Fourteen patients had failed high-dose autologous HCT. Thirty-one of the 33 patients had stable engraftment, while 2 patients experienced non-fatal graft rejections. The incidences of acute grades II, III and IV, and chronic GVHD were 27%, 17%, 13%, and 64%, respectively. The overall response rate in the 20 patients with measurable disease at the time of HCT was 85% [n=17; 75% complete remissions (CR) and 10% partial remissions (PR)], while 3 patients had progressive disease. Only 1 of the 17 patients who responded and none of the 13 transplanted in CR had disease relapse with a median follow-up of 24.6 months. Relapse and non-relapse mortalities were 9% and 24%, respectively, at 2 years. The Kaplan-Meier probabilities of overall and disease-free survivals at 2 years were 65% and 60%, respectively. Allogeneic HCT after nonmyeloablative conditioning is a promising salvage strategy for patients with relapsed and refractory mantle cell lymphoma. The high response and low relapse rates with this approach suggest that mantle cell lymphoma is susceptible to graft-versus-tumor responses.

INTRODUCTION

The median life expectancy after the diagnosis of mantle cell lymphoma has been 36-52 months[1] and recurrent disease has been considered incurable with conventional chemotherapy.[1-6] Most patients present with advanced stage (IV) disease, and while the disease is often sensitive to chemotherapy, relapse and resistance to chemotherapy continue to be significant problems.[7] The use of rituximab in front-line therapy might improve disease-free survival; however, treatment with R-CHOP (Rituximab-Cyclophosphamide, Vincristine, Adriamycin, Prednisone) has led to median progression-free survival of only 16 months despite the achievement of molecular remissions.[6] The use of R-Hyper-CVAD (Rituximab-Cyclophosphamide, Vincristine, Adriamycin, Prednisone, Methotrexate, Cytarabine) also has been associated with high complete remission (CR) rates; however, late relapses continue to be observed. Incorporation of high-dose therapy and autologous hematopoietic cell transplantation (HCT) after intensive induction therapy has been associated with progression-free survivals in the 60-80% range at 3-5 years.[8-11]

For patients with relapsed and refractory disease, standard high-dose therapy and autologous HCT has been ineffective.[9,12,13] The use of radiolabeled antibodies in the conditioning regimen might provide added tumor-specific therapy and improve outcome.[14] However, patients who relapsed after high-dose therapy and autologous HCT have had few options for long-term disease control. Most salvage attempts with additional intensive chemotherapy with or without autologous HCT have not been effective due to disease recurrence or toxicities.[12,15,16] As a consequence, there are no accepted salvage treatment options for patients with relapsed or refractory mantle cell lymphoma.

Long-term remissions and possibly cures have been described for patients with mantle cell lymphoma with allogeneic HCT after conventional high-dose conditioning regimens.[9,17,18] However, high-dose allogeneic HCT was associated with a 30-40% risk of early non-relapse mortality, which has tempered enthusiasm for exploring this approach in patients with mantle cell lymphoma, especially in those who previously failed autologous HCT.[19-22] More recently, promising results have been seen with reduced-intensity conditioning and allogeneic HCT in chemotherapy responsive[23] compared to more advanced-stage patients.[15,16] Some of these trials used in vivo T-cell depletion with alemtuzamab to reduce GVHD risk.[15,16]

We hypothesized that a nonmyeloablative conditioning regimen consisting of fludarabine, 30 mg/m^2/day given on three consecutive days, and 2 Gy total body irradiation (TBI) in concert with post-grafting immunosuppression by cyclosporine (CSP) and mycophenolate mofetil (MMF)[24,25] would allow stable engraftment of allogeneic hematopoietic cells. The low dose regimen was expected to reduce toxicities and mortality in heavily pretreated patients. We speculated that donor immune cells might eradicate malignant cells through graft-versus-tumor (GVT) reactions. In this report, we describe the clinical outcomes of 33 patients with mantle cell lymphoma, 28 of whom had advanced disease, who were given HCT grafts from related or unrelated donors after nonmyeloablative conditioning.

PATIENTS, MATERIALS AND METHODS

Eligibility criteria

Thirty-three patients with mantle cell lymphoma received nonmyeloablative conditioning and allogeneic HCT between February 5, 1997 and August 29, 2003 on seven separate phase I/II multi-institutional Fred Hutchinson Cancer Research Center (FHCRC) Protocols (#1225, n=2; #1409, n=3; #1463, n=7; #1533, n=1; #1591, n=2; #1596, n=10; and #1641, n=8). The primary differences among the protocols were related to the use of HLA-identical siblings, 10 of 10 HLA antigen-matched unrelated individuals, and one HLA antigen-mismatched related and unrelated individuals as HCT donors, and variations in the duration and intensity of postgrafting immune suppression with CSP and MMF. The latter changes were aimed at reducing the risks of both graft-versus-host disease (GVHD) and graft rejection. Three patients received planned autologous HCT as cytoreduction 40-80 days before allogeneic HCT.

The studies were performed at seven academic centers including the FHCRC, University of Washington, Seattle Veterans Affairs Medical Center, Oregon Health and Science University, Stanford University, Baylor University, Emory University, and University of Utah. The FHCRC served as the coordinating center for all protocols. Protocols were approved by the Institutional Review Boards (IRB) at the FHCRC and each of the collaborating sites. Patients signed consent forms approved by the local IRB. Patients were eligible for the protocols if they were more than 50 years old. Younger patients (n=10) were included if they were at prohibitive risk for non-relapse mortality with conventional conditioning due to comorbid conditions or because of failed autologous HCT.[15,19,26]

Patients were not eligible for protocols if they were pregnant, had decompensated liver disease, corrected pulmonary diffusion capacity <35%, cardiac ejection fraction of <30%, Karnofsky performance status <50, or serologic evidence of infection with the human immunodeficiency virus. No exclusions were made for disease status or chemotherapy sensitivity, though most patients received salvage chemotherapy in attempts to reduce disease bulk. No exclusions were made for renal insufficiency or active bacterial or fungal infections. However, most recent protocols required that fungal pneumonitis be stable on therapeutic azole therapy for at least 1 month before HCT.

HLA typing and matching

Patients and donors were typed for HLA-A, -B, and -C by intermediate resolution DNA typing to a level at least as sensitive as serology, and -DRB1 and -DQB1 by high-resolution techniques.[27] All sixteen related donors were matched with their donors for 10 of 10 HLA alleles. Of the 17 unrelated recipients, 13 were matched with their donors at the allele level for 10 HLA loci (HLA-A, B, C, DR-B1 and DQ-B1), two had single HLA allele level mismatches, one had a single HLA antigen mismatch, and one had a combined single HLA antigen and allele mismatch. All allele and antigen mismatches were for HLA class I antigens (Table 1).

Patients

Patient characteristics are shown in Table 1. The median patient age was 53.5 (range 32.6 to 69.6) years. Twenty-seven were males and 6 females. Comorbidities scores were assigned to patients using criteria modified from Charlson et. al [28-30].Using this methodology, 20 patients had

score “0”, 7 had score “1”, 2 had score “2”, 1 patient each had scores “3”, “4” and “5”, and 1 patient could not be scored. The patients’ diagnoses were confirmed before HCT with review of pathology specimens, flow cytometry reports, and, if available, cytogenetic analyses and molecular studies. The median time from disease diagnosis to HCT was 2.8 (range 0.4 to 9.5) years. The median number of treatment regimens was 4 (range 1 to 10). Recipients of unrelated grafts were more heavily pretreated than those of related grafts (median treatment regimens 5 versus 3, respectively). Forty-two percent (10/17 unrelated recipients versus 4/16 sibling recipients) of patients had failed autologous HCT. Three additional patients had planned autologous HCT 1–3 months before allogeneic HCT in an attempt to reduce disease burden. Before nonmyeloablative HCT, patients were defined as having “responsive disease” if patients had at least a partial (50% or greater reduction in tumor masses) remission (PR) or CR (no detectable disease by radiologic, endoscopic, marrow morphology or marrow phenotyping) to the last chemotherapy administered. Patients were considered “refractory” to salvage therapy if they had less than a PR (i.e. stable or progressive tumor growth) to the last therapy administered. Patients with recurrent disease after having previously documented responsive disease were considered to have “untested relapse”. Thirty-nine percent of patients were refractory to salvage chemotherapy or planned autologous HCT (Table 1). Similar proportions of unrelated recipients (41%) and related recipients (38%) had refractory disease. Of 20 patients with measurable disease, 9 had minimal (largest lymph node <1.5 cm), 5 had moderate (largest lymph node ≥ 1.5 cm and <5cm), and 6 had large disease burdens (largest lymph node ≥5cm). Forty-one percent of the unrelated recipients had moderate to large disease burdens compared to 25% of the related recipients. The median follow-up after HCT for surviving patients was 24.6 (range 2.7 to 41.7) months. Early results in 7 of the 33 patients have been reported previously.[25]

Table 1. Patient characteristics

Donor	Pt No.	Pt Age (yrs)	HCT date	Allele mismatch	Antigen mismatch	CCI score	No. prior regimens	Auto. HCT Failed (planned)	Months from Diagnosis to HCT	Disease Status at HCT*	Disease burden**	Marrow	FLOW cytometry	Beta-2 microglob-ulin μg	LDH
MRD	1	39	3/7/00			0	2	0	7.4	CR1	minimal	negative	negative	1.5	187
	2	52	4/29/00			0	2	0	12.1	refractory	moderate	positive	negative	ND	ND
	3	52	5/2/00			0	2	(1)	4.6	CR1	minimal	negative	negative	1.6	215
	4	59	6/28/00			4	5	1	20.7	PR	minimal	negative	negative	5.6	255
	5	39	11/17/00			1	9	0	35.0	refractory	minimal	negative	negative	1.6	314
	6	68	5/3/01			1	10	(1)	57.9	refractory	moderate	positive	positive	ND	256
	7	61	6/29/01			0	3	0	115.6	PR	moderate	negative	negative	ND	ND
	8	55	7/25/01			3	3	0	5.1	CR1	minimal	ND	ND	ND	ND
	9	49	8/14/01			1	1	0	12.6	refractory	minimal	negative	positive	ND	224
	10	44	9/19/01			0	6	1	31.1	refractory	large	positive	NA	ND	ND
	11	53	3/6/02			0	7	1	91.3	CR5	minimal	ND	ND	ND	ND
	12	67	4/10/02			5	4	0	56.1	refractory	large	negative	negative	3.1	176
	13	49	6/21/02			2	2	0	10.8	untested relapse	minimal	positive	positive	ND	219
	14	63	9/6/02			1	2	0	36.9	CR2: MRD	minimal	negative	positive	1.9	137
	15	53	1/30/03			0	1	(1)	8.0	CR1	minimal	negative	negative	1.8	223
	16	58	2/11/03			0	4	1	63.8	CR2	minimal	negative	negative	1.4	172
URD	17	43	2/5/00			0	4	1	64.1	refractory	minimal	positive	negative	ND	ND
	18	47	3/3/00			0	3	1	45.7	untested relapse	minimal	negative	negative	1.3	123
	19	62	6/7/00			0	8	0	41.8	PR	moderate	negative	positve	ND	
	20	70	10/6/00			1	8	1	34.2	refractory	large	negative	negative	ND	357
	21	64	11/15/00			0	3	0	17.5	refractory	large	positive	ND	ND	125
	22	58	12/8/00	B: 3508 vs. 3501		1	8	0	34.7	CR2	minimal	negative	negative	ND	150
	23	54	6/7/01	A*02 mm		0	7	1	76.3	CR3	minimal	negative	negative	2.3	158
	24	61	5/18/02			0	7	1	60.8	refractory	large	negative	negative	1.1	189
	25	51	6/20/02			1	5	1	58.6	CR2	minimal	negative	negative	3.7	140
	26	66	1/28/03			0	5	1	34.7	CR2	minimal	negative	negative	2.1	153
	27	48	1/30/03			2	3	1	28.0	CR2	minimal	negative	negative	2.2	165
	28	48	5/17/03			0	3	0	32.1	refractory	moderate	negative	ND	4.1	167

29	57	5/20/03			0	2	0	10.5	CR1†	minimal	negative	negative	2.4	225
30	50	5/29/03			0		1	91.3	refractory	moderate	positive	positive	1.4	169
31	56	7/8/03	A: 0301 vs. 0302	C: 0602 vs. 0401/09N	ND	5	0	10.0	refractory	moderate	negative	positive	ND	770
32	33	7/31/03		C: 0303 vs. 0401	0	6	1	87.6	PR	minimal	negative	negative	1.4	234
33	52	8/29/03			0	6	0	12.1	PR	large	positive	positive	3.0	162

Abbreviations: Pt=patient; HCT=hematopoietic cell transplant; No.=number; CCI Dn=donor; Chemo=chemotherapy; BM=bone marrow; Flow=flow cytometry; LDH=lactate dehydrogenase; F=female; M=male; CR=complete remission; ND=not done; NA=not available.

* At the last staging prior to HCT relative to the last pre-HCT therapeutic intervention made. CR-no detectable disease. PR- 50% reduction relative to the last staging. Patients were considered "refractory" to salvage therapy if they did not have a PR or CR to the last therapy given. Patients not administered therapy after having previously documented responsive disease were considered to have "untested relapse".

**Disease burden: minimal-largest lymph node <1.5 cm, moderate-largest lymph node ≥1.5 cm and <5cm and large-lymph node ≥5cm.

†Failed induction

Conditioning regimen and postgrafting immunosuppression

Patients received fludarabine, 30 mg/m^2/day, on days -4, -3, and -2 before, and 2 Gy of TBI at a rate of 0.07 Gy/min from linear accelerators on the day of HCT (day 0). Immunosuppressive therapy with CSP, 5.0-6.25 mg/kg given orally twice a day, was started on day –3, and with MMF, 15 mg/kg orally twice or three times a day, started 4-6 hours after HCT on day 0. Intravenous formulations of CSP and MMF were administered if patients were not able to tolerate oral medications. Discontinuation of immunosuppression for patients without GVHD was mandated by individual protocols. All recipients of HLA-identical related grafts had MMF stopped at day 28, while CSP was tapered beginning on day 56 over 21 days for eight patients with advanced stage disease and two in first CR, or over 124 days for three patients with cytoreductive autologous HCT and two in first CR. For patients with HLA matched or single allele mismatched unrelated donors, MMF was tapered at day 40 over 56 days and CSP was tapered at day 100 over 80 days. In eight of the unrelated recipients, MMF was given 15 mg/kg, 3 times a day, with the same tapering schedule. For recipients of single HLA antigen and combined HLA antigen and allele mismatched grafts, MMF was given 3 times a day and was tapered at day 100 over 2 months and CSP was tapered at day 180 over 180 days.

Collection of hematopoietic cells

Sibling donors received granulocyte colony-stimulating factor (G-CSF) at 16 μg/kg on days -5 through day 0. Leukapheresis procedures were performed on days -1 and day 0. G-CSF-mobilized peripheral blood mononuclear cells (G-PBMC) were given with median doses of 9.33 (range 3.83 to 15.21) CD34 x 10^6/kg and 3.26 (range 1.94 to 7.6) x 10^8/kg CD3, respectively. The collections of unrelated marrow (n=2) and G-PBMC (n=15) were coordinated through

unrelated donor centers.[25] The median CD34 and CD3 cell doses were 7.05 (range 1.57 to 25.98) x 10^6/kg and 2.23 (range 0.23 to 6.9) x 10^8/kg, respectively. Portions of the unrelated G-PBMC products, containing 1 x 10^7 CD3+ cells/kg, were cryopreserved for possible later use as donor lymphocyte infusions. Red blood cell (RBC)-depleted HCT was performed in ABO-mismatched patients with high titers of anti-donor isohemagglutinins.

Supportive care

Patients were managed in the ambulatory care clinic except during the scheduled infusions of unrelated hematopoietic cells or whenever complications mandated closer medical supervision and/or therapy. Anti-microbial prophylaxis, cytomegalovirus prophylaxis, and blood product support have been described.[25]

GVHD grading and therapy

The local investigators used standard criteria to grade acute and chronic GVHD.[31] Treatment of acute and chronic GVHD was per each institution's standard practice guidelines. First line of therapy for isolated proximal gut GVHD often consisted of methylprednisolone at 1 mg/kg/day or oral beclomethasone with or without systemic corticosteroids.

Follow-up

Health-care providers examined patients at least 3 times a week for the first month and then weekly or more frequently depending on the patients' clinical status. Disease-dependent restaging evaluations after HCT occurred at 2 months, 3 months, 6 months, 1 year, and then yearly thereafter, or more frequently depending on clinical circumstances. Evaluations consisted

of marrow aspirates for pathology, cytogenetics, flow cytometry, molecular studies, and radiographic examinations. Patients were referred to the care of their local physicians approximately 100 days after HCT.

Treatment of persistent/progressive or relapsed malignancies

Progression or relapse of lymphoma in the absence of severe manifestations of acute or chronic GVHD was treated by rapid discontinuation of systemic immunosuppression in order to initiate GVT effects. Persistent disease not meeting the definition for progression was generally observed without changes in protocol-specified immunosuppressive therapy. Only 1 of the 33 patients received a donor lymphocyte infusion, and this was for falling donor T-cell chimerism to prevent graft rejection.

Chimerism analyses

Confirmation of donor engraftment was made by chimerism analyses of flow cytometrically sorted peripheral blood granulocytes and T cells, and nucleated marrow cells collected on days 28, 56, 84, 180, and 365, and then yearly after HCT. For recipients of sex-mismatched HCT, chimerism analyses were based on fluorescent in situ hybridization (FISH) for X and Y chromosomes[32] and for the remainder on polymerase chain reaction-based amplification of variable number tandem repeat (VNTR) sequences unique to donors and hosts.[33] For all patients transplanted at Stanford University, VNTR analyses were used exclusively.

Study end points

Data were analyzed as of December 1, 2003. The primary study end points were hematopoietic engraftment, survival, progression-free survival, and non-relapse mortality. Secondary outcomes included the incidence and severity of GVHD, and evaluation of GVT responses. Patients were considered to have died of non-relapse mortality if there was no evidence of disease relapse or progression. Patients with persistent disease with no evidence of progression were considered at risk for non-relapse mortality. Disease responses and progression were defined by standard criteria.[34] Neutrophil recovery was defined as the first of three consecutive days with neutrophil counts >0.5 x 10^9/L. Platelet recovery was defined as the first of three consecutive days with platelet counts >20 x 10^9/L without transfusion support.

Statistical methods

Survival and progression-free survival were estimated by the Kaplan-Meier method. Cumulative incidence estimates were calculated for graft rejection, acute GVHD, relapse, and non-relapse mortality. Hazard ratios were estimated from Cox regression models. Deaths were treated as competing events in analyses of graft rejection, GVHD, and disease progression. Rejection was treated as a competing event in analyses of GVHD. Progression and non-relapse mortality were the components of progression-free survival and were treated as competing events. Factors considered in univariate analysis for non-relapse mortality, relapse/progression, overall and progression free survival included detectable lymphoma by flow cytometry or marrow morphology, donor status, Charlson comorbidity score, donor-recipient sex mismatch, HLA antigen or allele mismatch, prior autologous HCT, chemotherapy sensitivity, disease burden, β2 microglobulin above or below the median, lactate dehydrogenase level, number of prior

treatment regimens, patient age, CD34 cell dose, CD3 cell dose. Multivariate models were not constructed because of the small sample size, small number of events, and lack of multiple significant univariate risk factors. All p-values were derived from likelihood ratio statistics and were two-sided.

Putative graft-versus-tumor effects were evaluated using time-dependent Cox regression models. To accommodate changes in GVHD with time after transplant, patients were assigned to two time-dependent acute GVHD comparison groups (no acute GVHD versus grade I-IV), and to two time-dependent chronic GVHD groups (no or limited chronic GVHD versus clinical extensive chronic GVHD). All patients were considered to be in the "no acute GVHD" and "no chronic GVHD" groups on day 0. They were then assigned to their acute GVHD group and/or their chronic GVHD group at the time of onset of each grade of acute or chronic extensive GVHD, respectively, and their subsequent probabilities of achieving complete remission was compared with patients surviving a similar length of time without developing acute or chronic extensive GVHD.

RESULTS

Engraftment

The median neutrophil nadir and median duration of neutropenia were 200 cells/μL and 4 days, respectively. The median platelet nadir was 38,000/μL. The median levels of day 28 donor CD3, CD33 and marrow chimerism were 95%, 98% and 97%, respectively. Thirty of the 33 patients

had sustained donor engraftment and progressive increases of donor chimerism in all lineages to 100%, usually by 6 months after HCT.

Two (6%) patients rejected their HLA allele-matched unrelated donor marrow (n=1) or G-PBMC (n=1) grafts, while a third patient developed marrow aplasia after DLI for low (33%) donor T cell chimerism. One of the three patients had mixed chimerism in peripheral blood CD3 and granulocyte lineages and marrow cells for more than 18 months before eventually rejecting his unrelated marrow graft and experiencing autologous marrow recovery. This patient had developed transient myelodysplastic marrow and clonal abnormalities in host cells for 19 months (chromosome 7 deletion and a translocation between chromosome 7 and short arm of chromosome 18 in 4/16 cells). Recently, both the myelodysplastic features and the cytogenetic abnormalities have resolved, and the patient's lymphoma has remained in remission. A second patient had early rejection of the G-PBMC graft with prolonged neutropenia; a subsequent G-PBMC graft from the same donor after fludarabine, 90 mg/m^2, and 400 cGy of TBI was also rejected; and a third graft from a second unrelated donor after cyclophosphamide, 120 mg/kg, fludarabine, 150 mg/m^2, and thymoglobulin, 7.5 mg/kg over 6 days, was successful.

The final patient with an HLA-identical sibling graft developed aplasia (but maintained donor cell engraftment) after a single dose of pentostatin (2 mg/m^2) followed by donor lymphocyte infusion for low donor T-cell chimerism and suspected impending graft rejection. This patient had successful hematologic recovery after another G-PBMC infusion from the same donor following fludarabine (90 mg/m^2) and 300 cGy of TBI conditioning, but eventually died of pulmonary aspergillosis.

Toxicity

Toxicities other than those due to GVHD included 66 grade III and 22 grade IV organ-specific episodes (Table 2). Eight grade III cardiovascular episodes were from calcineurin-induced hypertension and 5 were from transient episodes of atrial fibrillation. The grade IV episodes included hematological (neutropenia and thrombocytopenia), gastrointestinal (anorexia requiring total parenteral nutrition and colonic perforation from colonoscopy), pulmonary (failure requiring mechanical ventilation) and renal (failure requiring dialysis) events.

Table 2: Toxicity events* after allogeneic transplantation

Organ system	Grade III (No. of events)	Grade IV (No. of events)
Hematologic	8	4
Cardiovascular	17	1
Endocrine	2	0
GI	3	4
Hemorrhage	3	1
Hepatic	6	2
Infection	7	0
Metabolic	3	1
Neurology	6	2
Pain	2	0
Pulmonary	7	4
Renal	2	3
Total	66	22

*Toxicity scoring was performed using the National Cancer Institute's Common Toxicity Criteria Manual (CTC), version 2.0 (http://ctep.cancer.gov/reporting/ctc.html).

GVHD

Forty-five percent of patients had either no or fleeting grade I acute GVHD not requiring therapy. Grades II, III, and IV acute GVHD occurred in 27%, 17% and 13% of patients, respectively (57% overall) (Figure 1A). Chronic extensive GVHD occurred in 18/28 (64%) patients who were evaluable beyond day 100 (Figure 1B). Complications from acute and chronic GVHD were responsible for the deaths of 4 (12%) patients.

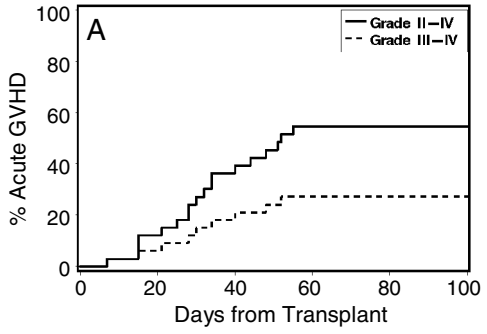

Figure 1. Cumulative probabilities of **A)** acute grades II-IV and III-IV GVHD and **B)** chronic GVHD.

GVT and Disease-Free Survival

At the time of HCT, 19 patients had measurable disease by CT scan, and one patient had evidence for disease by flow cytometry (Table 3). The disease response rate after transplant was 85%. An example of an anti-tumor response after an unrelated donor nonmyeloablative HCT in a heavily pre-treated patient with bulky disease who had failed autologous HCT is shown in Figure 2. Fifteen patients, including the one with flow cytometry evidence of disease had CR (75%), 2 patients had PR (10%), and 3 experienced disease progression (15%). The median time to CR was 87 (range 21 to 244) days. One of the 15 patients who achieved CR had disease relapse 5 months after HCT, while the others have remained in CR. In the time-dependent analysis of the relationship of disease response with GVHD, there was no association between disease response and acute grades I-IV acute GVHD ($p=0.36$), but a trend was observed with chronic extensive GVHD ($p=0.09$). With an observation time of 24.6 months, all 13 patients transplanted without evidence of disease have remained in CR. In the patients with progressive disease, one refused further direction or follow-up from the transplant center, one could not have immunosuppression discontinued due to GVHD and concurrent aspergillus infection, while the other two had fulminate early lymphoma progression unresponsive to immunosuppression discontinuation. The Kaplan-Meier probability of overall progression-free survival was 60% (95% confidence intervals: 43-78%) at 2 years, and corresponding figures for recipients of related and unrelated grafts were 56% and 66%.

Table 3. Patient outcomes

Donor	Pt No.	Graft Rejection	GVHD		Disease Response	Follow-up Days	Causes of Death
			Acute Grade	Extensive Chronic			
MRD	1	No	0	Yes	CR	>1073	
	2	No	0	No	PR	†156	NRM: Organizing Pneumonia secondary to ARDS
	3	No	0	Yes	CR	>1155	
	4	No	2	No	CR*	†21	NRM: pancreatitis
	5	No	0	Yes	CR	>1105	
	6	No	1	Yes	CR	>901	
	7	No	1	Yes	CR	>732	
	8	No	0	Yes	CR	>749	
	9	No	4	Yes	CR	†108	NRM: MOF from Grade 4 GVHD.
	10	No	3	Yes	CR	†117	NRM: Aspergillus pneumonia, sepsis, MOF
	11	No	4	No	CR	†53	NRM: Grade IV GVHD, aspergillosis
	12	No	2	No	Progression	†146	Progression, Aspergillus pneumonia
	13	No	3	No	CR	>361	
	14	No	3	No	CR	>398	
	15	Yes	0	No	CR	†202	NRM: Aspergillus pneumonia
	16	No	2	Yes	CR	>217	
URD	17	No	0	No	Relapse	>1250	
	18	Yes	2	Yes	CR	>1113	
	19	No	2	Yes	CR	>1136	
	20	No	2	Yes	CR	>915	
	21	No	3	Yes	CR	>742	
	22	No	2	Yes	CR	>894	
	23	No	4	No	CR	†70	NRM: CHF 2° cardiac fibrosis and grade IV GVHD
	24	No	1	Yes	PR	>577	
	25	No	4	No	CR	†143	NRM: Sepsis syndrome secondary to Grade IV GVHD.
	26	No	3	Yes	CR	>231	

27	No	1	No	CR	>210	
28	N	0	N	CR	>87	
29	Y	0	N	CR	>81	
30	N	2	Y	CR	>102	
31	N	NE	NE	Progression	†10	Disease progression
32	N	2	N	CR	>93	
33	N	NE	NE	Progression	†44	Disease progression

Abbreviations: Pt=patient; aGVHD=acute graft versus host disease; HCT=hematopoietic cell transplant; No.=number; MRD=HLA-matched related donor; URD=unrelated donor; Y=yes; N=no; CR=complete remission; PR=partial remission; NRM=non-relapse mortality; ARDS=acute respiratory distress syndrome; MOF=multi-organ failure; CHF=congestive heart failure; NE=not evaluable.
* on autopsy

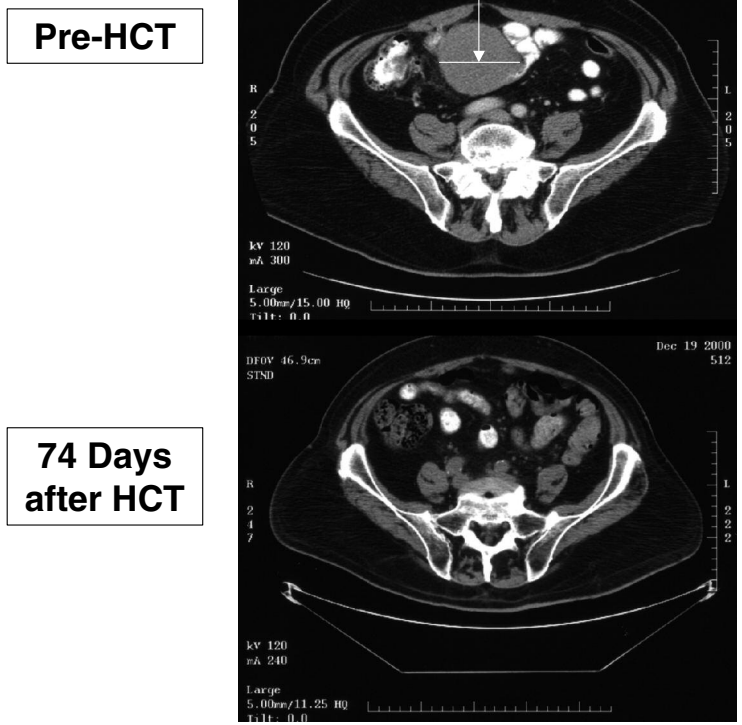

Figure 2. Example of graft-vs-tumor response. Patient #20 (from Table 1), a sixty-nine year-old man with rapidly progressive mantle cell NHL. Prior treatments included 8 lines of chemotherapy with only a 6-month remission to high-dose radiolabeled antibodies with autologous PBSC support. At the time of transplant, he had kinetically failed ESHAP with rapidly progressive disease during the pretransplant workup. **A)** Pretransplant CT scan image (day-27) through the upper pelvis demonstrating an 8 x 7 cm mass that extended through twelve 0.5 cm cuts. **B)** CT scan image through the same region demonstrating complete resolution of the mass on day +74 post nonmyeloablative transplantation from a matched unrelated donor. He remains in remission 30 months posttransplant with no evidence of GVHD.

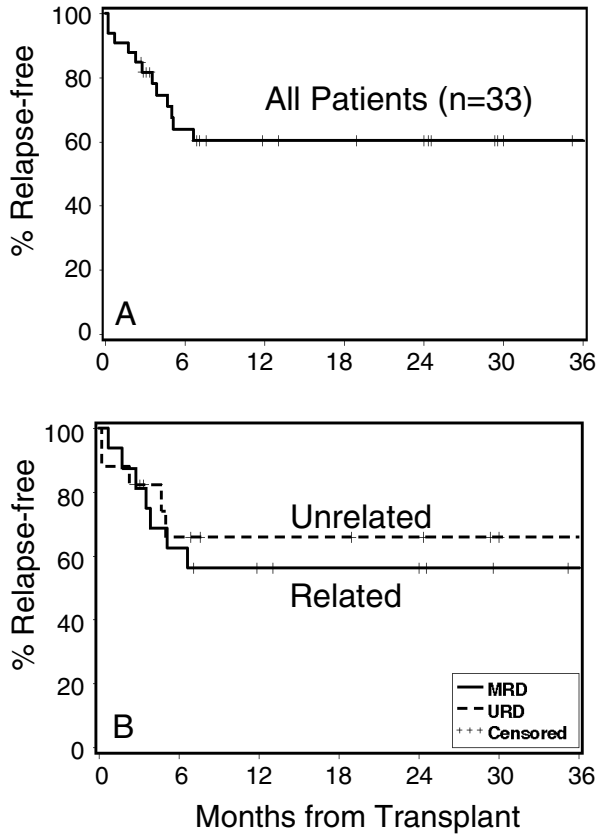

Figure 3. Kaplan Meier estimates of progression-free survival for **A)** all 33 patients and **B)** recipients of unrelated (n=17) and related (n=16) HCT.

respectively (Figure 3). The cumulative probability of relapse at 2 years was 16%. In univariate analysis, the only pretransplant factor predictive of relapse was the number of preceding treatment regimens. None of 14 patients with <4 treatment regimens experienced relapse compared to 4 of 19 patients with ≥4 regimens (p=0.01).

Overall survival and non-relapse mortality

Eleven patients have died. Three (9%) died of disease progression and eight (24%) of non-relapse causes. The cumulative probability of non-relapse mortality at 2 years was 24% (Figure 4). The Kaplan-Meier probability of overall survival was 64% (95% confidence intervals: 46-81%) at 2 years, and corresponding figures for recipients of related and unrelated donor grafts were 56% and 74%, respectively (Figure 5). On univariate analysis, the only

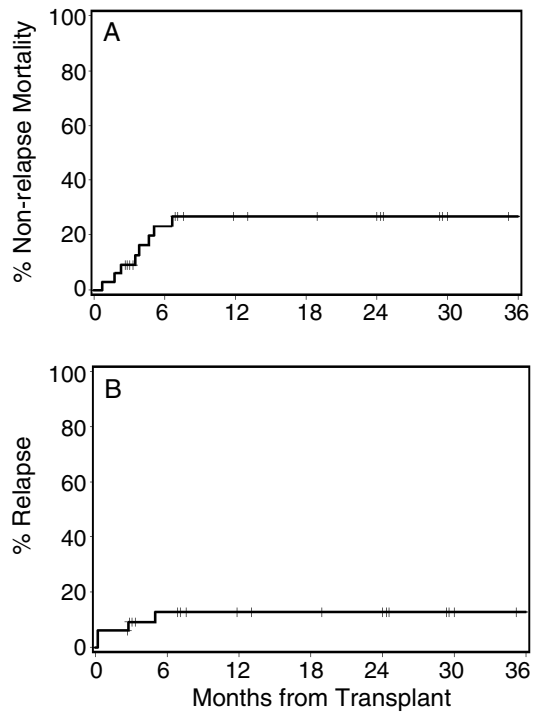

Figure 4. Cumulative probabilities of **A)** non-relapse mortality and **B)** disease relapse

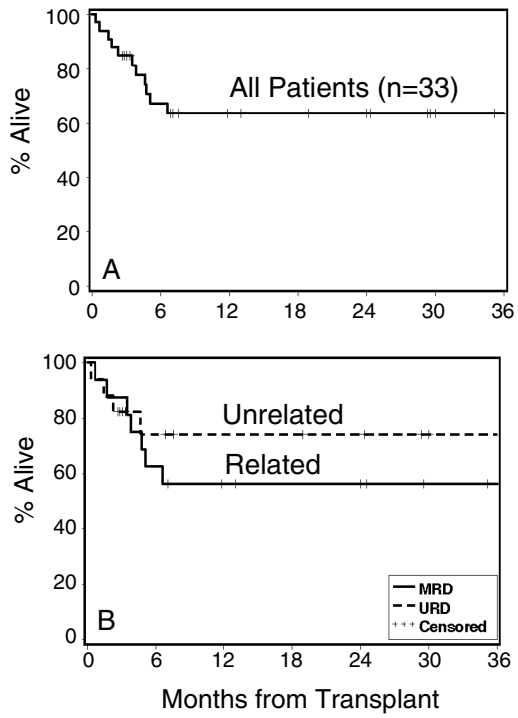

Figure 5. Kaplan Meier estimates of survival for **A)** all 33 patients and **B)** recipients of unrelated (n=17) and related (n=16) HCT.

significant factor that predicted worse survival was β2 microglobulin level greater than the median of 1.85 (p=0.03). However, this is based on only 6 events in the 19 patients who had β2 microglobulin levels available at the time of HCT (Table 1).

Non-relapse causes of death included four episodes of grade IV GVHD associated with multi-organ failure (n=2) or infections (n=2), infections with aspergillus (n=2), acute respiratory distress syndrome (n=1), and pancreatitis with multi-organ failure (n=1). On univariate analysis, patients less than 55 years old had worse non-relapse mortality compared to patients who were older than 55 years (p=0.003), presumably because a higher proportion of patients ≤55 years old had failed previous autologous HCT (11 versus 6 patients, respectively) and had chemotherapy refractory disease or untested relapse (10 versus 6 patients, respectively). There were no differences in the distribution of co-morbidity scores between the two patient groups.

DISCUSSION

There is no accepted treatment for patients with advanced mantle cell lymphoma.[2,3,35] Survival has been poor for heavily treated patients[12,36] or those with advanced disease features.[8,9] Conventional high-dose allogeneic HCT is rarely used because of a 30-40% risk of early non-relapse mortality even in younger patients with lymphoma[19-21]. Recently, reduced intensity regimens have been developed in attempts to reduce non-relapse mortality while exploiting GVT effects.[15,16,23-25]

The current study used HCT from related or unrelated donors after nonmyeloablative conditioning to treat patients with advanced mantle cell lymphoma. Despite the low intensity conditioning regimen, a high rate of clinical remissions were observed. It is unlikely that the mild regimen of fludarabine, 90 mg/m^2, and 2 Gy TBI produced the sustained responses and more likely that remissions were due to graft-versus-lymphoma effects. Overall, 75% of patients had CR and 10% PR, and only 12% have relapsed/progressed (6% related and 17% unrelated). The high response rate and the low incidence of relapse/progression were remarkable as 85% of patients had failed frontline or salvage treatments, 40% had failed preceding high dose therapy and autologous HCT, and 36% were refractory to the last treatment regimen. At 2 years the estimated overall survival was 65% and progression-free survival was 60%. This compares favorably to other published studies of autologous HCT for relapsed and refractory patients.[12,13] The only factor identified that adversely affected survival was the pre-HCT β_2 microglobulin level. This finding should be interpreted with caution since more than a third of patients did not have this parameter measured at the time of HCT. The only factor predicting a high risk of relapse/progression was exposure to more than three lines of preceding therapy. This was also noted previously in patients undergoing autologous HCT.[12] It is remarkable that chemotherapy refractoriness was not identified as a risk factor for disease progression/relapse. The reason for the high response rate and low relapse rate in these patients is unclear, but it may be due to differences in cell killing mechanisms between donor immune cell-mediated graft-versus-tumor responses to those of chemotherapy.

The non-relapse mortality at 2 years was 24% with most events occurring by 6 months. Given the small numbers of patients and clinical events, multivariable analysis of this end-point was not

possible. However, univariate analysis showed that patients older than 54 years had a lower probability of non-relapse mortality. Since there was no difference in CCI scores between older and younger patients, this might be explained by a lower proportion of older patients with relapsed/refractory disease at HCT and previous high-dose autologous HCT compared to younger patients. The major causes of non-relapse death were GVHD (n=2), GVHD and infections (n=2), and infections (n=2), emphasizing the need for better prophylaxis and treatment for acute GVHD and infections. Comorbidities have been shown to play major roles in patients' abilities to tolerate allogeneic HCT,[37,38] but in this small series of patients, they did not impact outcomes.

The current study compares favorably to two other reported series, using reduced-intensity conditioning for allogeneic transplantation for comparable patients with mantle cell lymphoma. Robinson et al.[16] reported the experience of the European Bone Marrow Transplant Lymphoma Working Group's experience of various reduced intensity conditioning regimens for non-Hodgkin lymphoma (NHL), 22 of whom had mantle cell lymphoma. The 1-year overall and progression-free survival, incidence of progression, and treatment-related mortality were 38%, 31%, 48%, and 46%, respectively. Faulkner et al.[15] reported the experience using BCNU, etoposide, Ara-C, melphalan, and alemtuzamab for various lymphoproliferative disorders, including 5 patients with mantle cell lymphoma analyzed with 8 patients with high-grade NHL. At 2 years, the rates of relapse, overall, and event-free survivals were 67.9%, 45.4%, and 16.8%. In a study by Khouri et. al.[23]of 18 mantle cell patients (17 of whom were in complete remission at the time of HCT), non-relapse mortality occurred in 2 of 18 patients and 3 of 18 patients had disease progression. The improved outcomes observed in the current study compared with the

studies by Robinson and Faulkner might have been due to the less intensive conditioning, resulting in less toxicity to heavily pretreated patients as well as the lack of T-cell depletion from the use of alemtuzamab for GVHD prophylaxis. T-cell depletion after reduced-intensity conditioning would be expected to attenuate or even abrogate allogeneic GVT responses. Differences in the patient groups prevent ready comparison of the current study to the better-risk patients included in the Khouri study. However, T cell depletion was not used in the Khouri study, which may explain the similar low relapse rates in that and the current study.

In summary, nonmyeloablative conditioning allowed for successful allogeneic HCT in patients with advanced mantle cell lymphoma and was associated with a high response rate and low risk of relapse at 2 years. We advocate the early identification of appropriately HLA-matched related or unrelated donors so that salvage allogeneic nonmyeloablative HCT can be performed if patients relapse after aggressive chemotherapy induction and high-dose consolidation strategies. Alternatively, the current results also support consideration of earlier integration of allogeneic HCT after nonmyeloablative conditioning as a consolidation strategy for newly diagnosed patients. The treatment of patients earlier in their disease course could result in higher response and lower progression rates, and less risk of non-relapse mortality. Finally, improvements in supportive care, in particular the prophylaxis and treatment of GVHD and infections, might result in improvements of allogeneic HCT outcomes using nonmyeloablative conditioning for patients with mantle cell lymphoma.

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REFERENCES

1. Malignant Lymphomas. Hamilton, Ontario: American Cancer Society; 2002.

2. Densmore JJ, Williams ME. Mantle cell lymphoma. Current Treatment Options in Oncology. 2003;4:281-287.

3. Fisher RI, Dahlberg S, Nathwani BN, Banks PM, Miller TP, Grogan TM. A clinical analysis of two indolent lymphoma entities: mantle cell lymphoma and marginal zone lymphoma (including the mucosa-associated lymphoid tissue and monocytoid B-cell subcategories): a Southwest Oncology Group study. Blood. 1995;85:1075-1082.

4. Bosch F, Lopez-Guillermo A, Campo E, et al. Mantle cell lymphoma: presenting features, response to therapy, and prognostic factors. Cancer. 1998;82:567-575.

5. Romaguera JE, Khouri IF, Kantarjian HM, et al. Untreated aggressive mantle cell lymphoma: results with intensive chemotherapy without stem cell transplant in elderly patients. Leukemia & Lymphoma. 2000;39:77-85.

6. Howard OM, Gribben JG, Neuberg DS, et al. Rituximab and CHOP induction therapy for newly diagnosed mantle-cell lymphoma: molecular complete responses are not predictive of progression-free survival. J Clin Oncol. 2002;20:1288-1294.

7. Velders GA, Kluin-Nelemans JC, De Boer CJ, et al. Mantle-cell lymphoma: a population-based clinical study. J Clin Oncol. 1996;14:1269-1274.

8. Khouri IF, Saliba RM, Okoroji GJ, Acholonu SA, Champlin RE. Long-term follow-up of autologous stem cell transplantation in patients with diffuse mantle cell lymphoma in first disease remission: the prognostic value of β_2-microglobulin and the tumor score. Cancer. 2003;98:2630-2635.

9. Milpied N, Gaillard F, Moreau P, et al. High-dose therapy with stem cell transplantation for mantle cell lymphoma: results and prognostic factors, a single center experience. Bone Marrow Transplant. 1998;22:645-650.

10. Mangel J, Leitch HA, Connors JM, et al. Intensive chemotherapy and autologous stem-cell transplantation plus rituximab is superior to conventional chemotherapy for newly diagnosed advanced stage mantle-cell lymphoma: a matched pair analysis. Ann Oncol. 2004;15:283-290.

11. Gianni AM, Magni M, Martelli M, et al. Long-term remission in mantle cell lymphoma following high-dose sequential chemotherapy and in vivo rituximab-purged stem cell autografting (R-HDS regimen). Blood. 2003;102:749-755.

12. Vose JM, Bierman PJ, Weisenburger DD, et al. Autologous hematopoietic stem cell transplantation for mantle cell lymphoma. Biol Blood Marrow Transplant. 2000;6:640-645.

13. Vandenberghe E, Ruiz dE, Loberiza FR, et al. Outcome of autologous transplantation for mantle cell lymphoma: a study by the European Blood and Bone Marrow Transplant and Autologous Blood and Marrow Transplant Registries. Br J Haematol. 2003;120:793-800.

14. Gopal AK, Rajendran JG, Petersdorf SH, et al. High-dose chemo-radioimmunotherapy with autologous stem cell support for relapsed mantle cell lymphoma. Blood. 2002;99:3158-3162.

15. Faulkner RD, Craddock C, Byrne JL, et al. BEAM-alemtuzumab reduced-intensity allogeneic stem cell transplantation for lymphoproliferative diseases: GVHD, toxicity, and survival in 65 patients. Blood. 2004;103:428-434.

16. Robinson SP, Goldstone AH, Mackinnon S, et al. Chemoresistant or aggressive lymphoma predicts for a poor outcome following reduced-intensity allogeneic progenitor cell transplantation: an analysis from the Lymphoma Working Party of the European Group for Blood and Bone Marrow Transplantation. Blood. 2002;100:4310-4316.

17. Kroger N, Hoffknecht M, Kruger W, et al. Allogeneic bone marrow transplantation for refractory mantle cell lymphoma. Ann Hematol. 2000;79:578-580.

18. Kröger N, Hoffknecht M, Dreger P, et al. Long-term disease-free survival of patients with advanced mantle-cell lymphoma following high-dose chemotherapy. Bone Marrow Transplant. 1998;21:55-57.

19. Izutsu K, Kanda Y, Ohno H, et al. Unrelated bone marrow transplantation for non-Hodgkin lymphoma: a study from the Japan Marrow Donor Program. Blood. 2004;103:1955-1960.

20. van Besien K, Loberiza FRJ, Bajorunaite R, et al. Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma. Blood. 2003;102:3521-3529.

21. Peniket AJ, Ruiz DEM, Taghipour G, et al. An EBMT registry matched study of allogeneic stem cell transplants for lymphoma: allogeneic transplantation is associated with a lower relapse rate but a higher procedure-related mortality rate than autologous transplantation. Bone Marrow Transplant. 2003;31:667-678.

22. van Besien K, Sobocinski KA, Rowlings PA, et al. Allogeneic bone marrow transplantation for low-grade lymphoma. Blood. 1998;92:1832-1836.

23. Khouri IF, Lee MS, Saliba RM, et al. Nonablative allogeneic stem-cell transplantation for advanced/recurrent mantle-cell lymphoma. J Clin Oncol. 2003;21:4407-4412.

24. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001;97:3390-3400.

25. Maris MB, Niederwieser D, Sandmaier BM, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood. 2003;102:2021-2030.

26. Radich JP, Gooley T, Sanders JE, Anasetti C, Chauncey T, Appelbaum FR. Second allogeneic transplantation after failure of first autologous transplantation. Biol Blood Marrow Transplant. 2000;6:272-279.

27. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. Blood. 1998;92:3515-3520.

28. Charlson ME, Sax FL, MacKenzie CR, Fields SD, Braham RL, Douglas RG, Jr.. Assessing illness severity: does clinical judgment work? J Chronic Dis. 1986;39:439-452.

29. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373-383.

30. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. Journal of Clinical Epidemiology. 1994;47:1245-1251.

31. Sullivan KM. Graft-versus-host-disease. In: Thomas ED, Blume KG, Forman SJ, eds. Hematopoietic Cell Transplantation, 2nd Edition. Boston: Blackwell Science; 1999:515-536.

32. Durnam DM, Anders KR, Fisher L, O'Quigley JO, Bryant EM, Thomas ED. Analysis of the origin of marrow cells in bone marrow transplant recipients using a Y-chromosome-specific in situ hybridization assay. Blood. 1989;74:2220-2226.

33. Ugozzoli L, Yam P, Petz LD, et al. Amplification by the polymerase chain reaction of hypervariable regions of the human genome for evaluation of chimerism after bone marrow transplantation. Blood. 1991;77:1607-1615.

34. Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood. 1996;87:4990-4997.

35. Hiddemann W, Unterhalt M, Herrmann R, et al. Mantle-cell lymphomas have more widespread disease and a slower response to chemotherapy compared with follicle-center lymphomas: results of a prospective comparative analysis of the German Low-Grade Lymphoma Study Group. J Clin Oncol. 1998;16:1922-1930.

36. Khouri IF, Romaguera J, Kantarjian H, et al. Hyper-CVAD and high-dose methotrexate/cytarabine followed by stem-cell transplantation: an active regimen for aggressive mantle-cell lymphoma. J Clin Oncol. 1998;16:3803-3809.

37. Diaconescu R, Flowers CR, Storer B, et al. Morbidity and mortality with nonmyeloablative compared to myeloablative conditioning before hematopoietic cell transplantation from HLA matched related donors. Blood. In press.

38. Sorror ML, Maris MB, Storer B, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplant comorbidities. Blood; prepublished online April 27, 2004; DOI 10.1182/blood-2004-02-0545.

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Allogeneic Hematopoietic Cell Transplantation after Fludarabine and 2 Gy Total Body Irradiation for Relapsed and Refractory Mantle Cell Lymphoma

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