

Intentional Induction of Mixed Chimerism and Achievement of Antitumor Responses After Nonmyeloablative Conditioning Therapy and HLA-Matched Donor Bone Marrow Transplantation for Refractory Hematologic Malignancies

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ABSTRACT

Mixed lymphohematopoietic chimerism can be induced in mice with bone marrow transplantation (BMT) after a nonmyeloablative preparative regimen that includes cyclophosphamide, anti-T-cell antibody therapy, and thymic irradiation. These mixed chimeras are resistant to the induction of graft-versus-host disease (GVHD) after delayed donor leukocyte infusions (DLIs), despite a potent lymphohematopoietic graft-versus-host reaction that converts the mixed chimeric state to a full donor one. Based on this animal model, we initiated a trial of nonmyeloablative therapy with HLA-matched or -mismatched donor BMT and DLI for refractory hematologic malignancies. Twenty-one of 36 patients enrolled in this trial received a genotypically (n = 20) or phenotypically (n = 1) HLA-matched donor transplant; results reported here are for those patients only. Preparative therapy consisted of cyclophosphamide in doses of 150 to 200 mg/kg; peritransplant antithymocyte globulin; thymic irradiation (in patients who had not received previous mediastinal radiation therapy); and cyclosporine. Eighteen of 20 evaluable patients developed persistent mixed lymphohematopoietic chimerism as defined by >1% donor peripheral white blood cells until at least day 35 posttransplantation. Ten patients received prophylactic DLI beginning 5 to 6 weeks after BMT for conversion of mixed chimerism to full donor hematopoiesis and to optimize a graft-versus-leukemia effect. Fourteen of 20 evaluable patients (70%) achieved an antitumor response; 8 of these responses were complete, and 6 were partial. Of the 8 evaluable patients who received prophylactic DLI, 6 showed conversion to full donor chimerism. Five of the 9 evaluable patients (56%) who received prophylactic DLI achieved a complete response, compared with 3 of 11 patients (27%) who did not receive prophylactic DLI. Currently 11 patients are alive, and 7 of these are free of disease progression at a median follow-up time of 445 days (range, 105–548 days) posttransplantation. Transplantation-related complications included cyclophosphamide-induced cardiac toxicity in 3 of 21 patients (14%) and grade II or greater GVHD in 6 patients (29%). One patient (5%) died from a complication of BMT, and 1 patient (5%) died from GVHD after 2 prophylactic DLIs were given for conversion of chimerism. In summary, mixed lymphohematopoietic chimerism was reproducibly induced after a novel nonmyeloablative preparative regimen incorporating chemotherapy, peritransplant antithymocyte globulin, and thymic irradiation, allowing for early administration of DLI in 10 of 21 patients. After treatment, striking antitumor responses were observed in the majority of patients with chemotherapy-refractory hematologic malignancies.

KEY WORDS

Mixed chimerism • Allogeneic • Bone marrow transplantation • Nonmyeloablative • Hematologic malignancies

INTRODUCTION

Mixed lymphohematopoietic chimerism can be induced in several animal models using myeloablative preparative regimens and mixed autologous (or syngeneic) and allogeneic marrow transplantation [1,2] or, after nonablative preparative regimens, using peritransplant anti-T-cell therapy [3-6]. In a murine model established by Pelot et al. [7], mixed chimerism was induced across full major histocompatibility complex barriers after a preparative regimen that included cyclophosphamide, monoclonal anti-T-cell antibody therapy, and thymic irradiation. These mixed chimeras, as well as mixed chimeras produced with earlier regimens [8], were resistant to the induction of graft-versus-host disease (GVHD) after delayed donor leukocyte infusions (DLIs), despite a potent lymphohematopoietic graft-versus-host reaction that converted the mixed chimeric state to a state of full donor hematopoiesis. These results suggest that graft-versus-leukemia (GVL) effects might be achieved without development of GVHD via the initial induction of mixed chimerism followed by delayed DLI.

In clinical bone marrow transplantation (BMT), transient mixed lymphohematopoietic chimerism occurs commonly after allogeneic transplantation. The reported incidence appears to be dependent on the sensitivity of the chimerism assay used, with a higher likelihood of host cell detection by polymerase chain reaction-based analyses than by conventional cytogenetic assays [9-14]. Mixed chimerism has been shown to be associated with less intensive (particularly non-total body irradiation [TBI]-containing) preparative regimens and likely reflects increased host stem cell survival [15,16]. Moreover, in some clinical series, an association has been seen between mixed chimerism and a reduced incidence of acute GVHD [9,11,17-19].

In an effort to reduce morbidity and mortality after conventional allogeneic BMT, several investigators recently have used nonmyeloablative preparative regimens to achieve alloengraftment. Although data regarding chimerism in these studies are incomplete, it appears that in the majority of cases, full or nearly full allogeneic chimerism was achieved [20-22]. Based on the principle of lessened morbidity accompanying nonmyeloablative conditioning therapy, the potential GVHD modulatory effect of mixed lymphohematopoietic chimerism, and the attempt to capture the potent cell-mediated antitumor effects of delayed donor leukocyte infusions [23-25], we conducted a pilot trial of nonmyeloablative therapy with BMT and DLI for patients with refractory hematologic malignancies.

PATIENTS AND METHODS

Patients

Thirty-six patients with chemotherapy-refractory hematologic malignancies were enrolled in a clinical trial conducted at Massachusetts General Hospital (MGH) in Boston. The trial involved nonmyeloablative conditioning therapy followed by HLA-matched or -mismatched allogeneic BMT for the induction of mixed chimerism. The preliminary results for the recipients of a 1- or 2-antigen-mismatched donor transplant have been published [26]. Only the results for the 21 patients who received a genotypically (n = 20) or phenotypically (n = 1) HLA-identical donor

Table 1. *Patient Characteristics**

No. of patients	21
Median age (range), y	44 (22-62)
Sex (M:F)	13:8
Diagnosis	
NHL	11
Intermediate grade	8
Low grade	3
Hodgkin's disease	4
AML	3
ALL	1
CLL	2
Donor/recipient HLA status	
Genotypically HLA identical	20
Phenotypically HLA identical	1
No. of patients with chemotherapy-refractory disease	20/21†
No. of patients who had received a previous autologous stem cell transplant	3

*ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma.

†One patient was in untreated relapse after autologous peripheral blood stem cell transplantation.

transplant are reported here. All patients were treated under the auspices of a protocol approved by the MGH Subcommittee for Human Studies. Demographic characteristics for the 21 HLA-matched recipients are described in Table 1.

Eligibility criteria for transplantation included a diagnosis of chemotherapy-refractory hematologic malignancy, defined as achievement of less than a partial response or disease progression during salvage chemotherapy or relapse after autologous stem cell transplantation; Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2; age ≤ 65 years; and adequate organ function (as specified by the protocol). Patients and their donors were typed for HLA-A and HLA-B using standard serologic techniques and for HLA-DR using sequence specific primer (SSP)- or sequence specific oligonucleotide probe (SSOP)-based analyses. By virtue of their disease status and previous therapy (eg, an autologous stem cell transplant), patients were believed to have a prohibitively high risk of mortality after conventional allogeneic stem cell transplantation or a very high risk of posttransplantation relapse.

Conditioning Therapy

Conditioning therapy consisted of cyclophosphamide 50 mg/kg per day (with dosing based on actual or ideal body weight, whichever was less), administered intravenously for 3 (n = 14) or 4 (n = 7) consecutive days on transplant days -6 or -5 through -3. Thymic irradiation (700 cGy) was given on day -1 in patients who had not received previous mediastinal radiation therapy (n = 13) and antithymocyte globulin (ATG) 30 mg/kg per day (n = 4) on days -2, -1, and 1 or 15 mg/kg per day (n = 17) on days -1, 1, 3, and 5. Intravenous cyclosporine was begun on day -1 at a dose of 5 mg/kg using continuous infusion over 20 hours daily. On transplant day 4, the daily cyclosporine dose was reduced to 3 mg/kg. When patients were

able to tolerate oral medication, cyclosporine was changed to oral administration at a dose of 6 mg/kg every 12 hours.

Bone Marrow Collection and Infusion

Bone marrow was procured in the operating room from donors under an epidural or general anesthetic. A target number of 3×10^8 nucleated cells per kg of recipient body weight was sought. Five patients were recipients of marrow with a minor or major ABO incompatibility. In the case of minor ABO incompatibility ($n = 1$), plasma was removed from donor marrow before transplantation. In the case of major ABO incompatibility ($n = 4$), red blood cells were depleted from the donor bone marrow using a CS-3000 cell separator (Baxter-Fenwal, Round Lake, IL). Bone marrow was infused at a rate of 300 to 500 mL/h.

Donor Leukocyte Collection and Infusion

In the absence of acute GVHD (or suspected GVHD), patients were eligible to receive "prophylactic" DLI beginning on day 35 posttransplantation in an effort to convert their state of mixed chimerism to a state of full donor hematopoiesis.

Unmobilized donor leukocytes were obtained by leukapheresis using a Cobe Spectra cell (Cobe Laboratories, Lakewood, CO) separator according to the manufacturer's instructions. All products were procured by peripheral venous access. Pheresis durations were 120 to 240 minutes and yielded a median of 1.4×10^8 mononuclear cells per kg recipient body weight (range, 0.7 to 2.0×10^8) and 0.8×10^8 CD3⁺ T cells per kg recipient body weight (range, 0.3 to 1.1×10^8). Donor leukaphereses were initially performed on days 35 and 56; fresh leukocytes were either immediately infused into recipients or collected and infused on day 35, with the remainder cryopreserved for later infusion. In some cases (ie, for donors who lived a long distance from the transplant center), donor leukocytes were collected and cryopreserved shortly after bone marrow harvest. The target number of CD3⁺ T cells per infusion for prophylactic DLI was 1×10^7 /kg. Higher numbers of CD3⁺ T cells were given in some cases for treatment of relapse, and in 1 instance, 5×10^7 CD3⁺ T cells/kg were given in a prophylactic DLI on day 56.

Supportive Care

Patients were cared for in either high-efficiency particulate air (HEPA)-filtered or laminar air flow rooms. All patients had triple-lumen Silastic central venous catheters. Anti-infective measures included co-trimoxazole for *Pneumocystis carinii* prophylaxis from admission until day -1, then 3 times per week after resolution of neutropenia; ofloxacin 400 mg twice per day or levofloxacin 500 mg daily until resolution of neutropenia; fluconazole beginning on day -1 at a dose of 400 mg, which was reduced to 200 mg daily on day 0; and intravenous (IV) acyclovir at a dose of 250 mg/m² every 8 hours beginning on day -1, which was changed to a dose of 400 mg twice per day after resolution of neutropenia. Febrile neutropenia was treated with broad-spectrum antibacterial agents, usually vancomycin and ceftazidime. All blood products were irradiated with 2500 cGy using a cesium irradiator and were transfused through a third-generation leukocyte depletion filter.

Antiemetic therapy consisted of dexamethasone 20 mg IV, granisetron 1 mg IV twice per day or ondansetron 24 mg

IV daily, diphenhydramine 25 to 50 mg IV, and lorazepam 1 mg IV before each dose of cyclophosphamide. Diphenhydramine and lorazepam were given as needed for nausea or vomiting.

Treatment of acute GVHD consisted of corticosteroids (IV methylprednisolone, which was changed to oral methylprednisolone when patients could tolerate oral medications), initially at a dose of 1 to 2 mg/kg and then tapered as tolerated. Anti-interleukin-2 receptor (Zenapax; Hoffmann-LaRoche, Nutley, NJ) or anti-CD2 (Biotransplant, Boston, MA) monoclonal antibody therapy was given to 5 patients for steroid-resistant disease.

Analyses of Chimerism

Analyses of microsatellite variable-number-of-tandem-repeat (VNTR) or short-tandem-repeat markers were performed on peripheral blood leukocytes from patients and their donors before BMT. Weekly microsatellite analyses were then performed on peripheral blood leukocytes beginning on day 7 through day 100 posttransplantation and on days 28 and 100 on bone marrow aspirate samples. Peripheral blood analyses were then performed every 6 months beginning on day 180 posttransplantation. Microsatellite analyses were performed using a standard technique [27,28].

Statistical Analysis

The Kaplan-Meier method was used to estimate disease-free and overall survival probability for patients who underwent transplantation [29].

RESULTS

Patient Enrollment

All 21 enrolled patients who had a genotypically or phenotypically HLA-identical donor received their intended transplant (see Table 1). Twenty of the 21 patients had experienced a chemotherapy-refractory relapse of their malignancy. The remaining patient experienced an untreated relapse 11 months after an autologous stem cell transplant.

Engraftment

Median time to absolute neutrophil count greater than 0.5×10^9 /L was 12 days (range, 10-20 days). Median time to a platelet count of greater than 20×10^9 /L in 17 of 21 patients was 16 days (range, 8-30 days). In 2 patients with progressive acute leukemia, platelet counts had not recovered at 76 and 121 days posttransplantation. In 2 patients with Hodgkin's disease, platelet counts never fell below 20×10^9 /L, and platelet transfusions were not required (Table 2).

In 18 of 20 evaluable patients, donor hematopoiesis (as defined by >1% donor cells in the peripheral blood) was observed until at least day 35 posttransplantation. Three of the 18 patients, however, lost evidence of donor hematopoiesis, 1 on day 42, 1 on day 43, and 1 on day 45 posttransplantation.

Engraftment Syndrome

Fourteen of 21 patients developed signs and symptoms consistent with engraftment syndrome [30]. This syndrome occurred shortly before or coincident with engraftment and was characterized by fever, weight gain, and a predominantly

Table 2. Posttransplantation Patient Outcomes*

UPN	Disease	Age, y	Remission Status	Previous Autologous SCT	Cy Dose, mg/kg	TI	Median Days to		Maximum Pre-DLI GVHD Grade	Organ(s) Affected	Response	Outcome
							ANC >0.5	PC >20,000				
181	T-cell rich B-cell lymphoma	41	RR	N	200	Y	15	23	0		CR	Died day 180, GVHD (post-DLI)
194	Lymphomatoid granulomatosis	44	RR	N	200	Y	16	8	I	Skin	PR	Died day 193, PD
201	B-cell DLCL	45	RR	N	200	N	17	15	II	Skin	PR	Died day 518, PD
218	AML	44	RR	N	200	Y	18	>76	II	Skin	PR	Died day 76, PD
234	B-cell DLCL	22	RR	N	200	N	18	17	0		CR	Alive day 560 in CCR
21	Follicular mixed-cell lymphoma	35	RR	Y	150†	N	12	30	0		CR	Alive day 548 in CCR
238	Hodgkin's disease	27	UR	Y	200	N	10	11	II	Skin	CR	Alive day 520 with PD
247	AML	27	RR	N	200	Y	18	22	IV	Skin, Gut	CR	Died day 77, PTLD
254	Hodgkin's disease	27	RR	N	150‡	N	20	14	0		PR	Died day 167, PD
259	T-cell ALL	40	RR	N	150‡	Y	15	10	0		PD	Died day 36, PD
268	CLL	55	RR	N	150‡	Y	13	15	II	Skin	PR	Alive day 343 in PR
275	Follicular small cleaved-cell lymphoma	43	RR	N	150‡	Y	12	12	0		CR	Alive day 329 with PD
279	B-cell DLCL	62	RR	N	150‡	Y	12	12	II	Skin	PD	Died day 51, PD
282	Diffuse mixed-cell lymphoma	57	RR	N	150‡	Y	11	14	0		PD	Died day 43, PD
283	AML	44	RR	N	150‡	N	12	NE	0		PD	Alive day 273 with PD
285	CLL	50	RR	N	150‡	N	14	24	0		CR	Alive day 267 in CCR
317	B-cell DLCL	39	RR	N	150‡	Y	14	15	0		PD	Died day 90 with PD
322	Follicular mixed-cell lymphoma	50	RR	N	150‡	Y	14	14	0		NR	Alive day 127
328	T-cell rich B-cell lymphoma	61	RR	N	150‡	Y	9	9	0		PR	Alive day 110
329	Hodgkin's disease	55	RR	N	150‡	N	10	—§	0		CR	Alive day 105 in CCR
334	Hodgkin's disease	24	RR	Y	150‡	N	14	—§	0		NE	Alive day 85

*ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; ANC, absolute neutrophil count; CCR, continuous complete response; CLL, chronic lymphocytic leukemia; CR, complete response; Cy, cyclophosphamide; DLCL, diffuse large-cell lymphoma; DLI, donor leukocyte infusion; GVHD, graft-versus-host disease; NE, not evaluable; NR, no response; PC, platelet count; PD, progressive disease; PR, partial response; PTLD, posttransplantation lymphoproliferative disease; RR, refractory relapse; SCT, stem cell transplantation; TI, thymic irradiation; UPN, unique patient number; UR, untreated relapse.

†Cyclophosphamide dose reduced to 150 mg/kg because of cardiac toxicity.

‡Cyclophosphamide dose reduced to 150 mg/kg because of change in protocol.

§Platelets never fell below $20 \times 10^9/L$, and platelet transfusions were not required.

truncal and facial erythematous rash. In each case histopathologic analysis of skin biopsy samples was not diagnostic of acute GVHD, and the syndrome responded promptly to corticosteroids.

Six of the 14 patients who developed engraftment syndrome were ultimately believed to have grade II or greater GVHD. Four of 8 patients without GVHD showed no sign of GVHD after a rapid taper of their immunosuppressive medication (ie, cyclosporine and corticosteroids), and they were able to receive a DLI beginning at 5 weeks posttransplantation.

Graft-V ersus-Host Disease

In the absence of grade II or \geq grade II GVHD (or the absence of initial suspicion of GVHD), cyclosporine was tapered and discontinued at a median of 37 days (range, 19-154 days) posttransplantation. The median duration of cyclosporine therapy for all 21 patients was 67 days (range, 19-301 days) posttransplantation.

Six of 21 patients (29%) developed biopsy-confirmed grade II or greater GVHD after BMT and before DLI, often during the rapid corticosteroid taper that followed treatment of the initial signs and symptoms of engraftment syndrome. In 5 of the 6 patients, the grade II GVHD was limited to the skin. One patient (5%) developed grade IV GVHD involving both the skin and gastrointestinal (GI) tract. An additional 5 patients developed grade II or greater acute GVHD after prophylactic DLI, and 1 patient developed de novo grade IV GVHD after DLI for treatment of posttransplantation relapse.

Donor Leukocyte Infusions

Prophylactic DLIs, beginning 5 to 6 weeks post BMT, were given to 10 patients (Table 3). Six patients received a single DLI consisting of $1 \times 10^7/kg$ CD3⁺ T cells on day 35, and 4 patients received a DLI ($1 \times 10^7/kg$ CD3⁺ T cells) on day 35 and a second DLI ($1 \times 10^7/kg$ CD3⁺ T cells [n = 3] and

Table 3. Prophylactic DLI and Patient Outcome*

UPN	Day(s) of DLI	% Donor Chimerism		Time, wk†	GVHD		Response
		Before DLI	After DLI		Pre-DLI	Post-DLI	
181	35, 56	30-70	99	5	0	IV	CR
234	35, 62	50-70	>99	8	0	—‡	CR
254	36, 57	1-10	>99	7	0	IV	PR
322	36, 64	10-30	<1	8	0	0	PR
21	35	50-70	>99	4	0	0	CR
285	37	30-70	>99	2	0	II	CR
329	41	30-50	>99	5	0	II	CR
317§	35	50-70	70-90	2	0	NE	PD
328	40	30-70	30-50	8	0	0	PR
334	44	10-30	30-50	2	I	III	NE

*CR indicates complete response; DLI, donor leukocyte infusion; GVHD, graft-versus-host disease; NE, not evaluable; PD, progressive disease; PR, partial response.

†Time in weeks after initial DLI or until conversion to >99% donor hematopoiesis.

‡Limited chronic GVHD.

§Patient subsequently received a second DLI (6×10^7 CD3⁺ cells/kg) for progressive disease on day 49 and could not be evaluated for chimerism conversion or postprophylactic DLI GVHD.

5×10^7 /kg CD3⁺ T cells [n = 1]) between days 56 and 64 posttransplantation. In 6 patients, the mixed chimeric state converted to a state of full donor hematopoiesis at a median of 5 weeks (range, 2-8 weeks) after 1 (n = 3) or 2 (n = 3) DLIs.

In 3 of the 4 evaluable recipients of a single prophylactic DLI, conversion to full donor chimerism occurred within 2 to 5 weeks. In the remaining patient, stable mixed chimerism was maintained 8 weeks after DLI. Two patients who received a single DLI were not evaluable. One (unique patient number [UPN] 317) showed increasing donor hematopoiesis after a single prophylactic DLI and received a second DLI on day 49 for disease progression; the other (UPN 334) had not yet been evaluated 5 weeks post-DLI. Grade II or greater GVHD developed in 3 patients who received a single DLI; 2 patients developed grade II skin GVHD that improved after an increase in cyclosporine dose or initiation of low-dose corticosteroids, and the third patient experienced grade III GVHD of the skin and GI tract.

In 3 of the 4 patients who received 2 DLIs, conversion to full donor chimerism occurred between 5 and 8 weeks post-DLI. Severe (grade IV) acute GVHD led to the death of 1 patient (UPN 181) after 2 DLIs (1×10^7 CD3⁺ T cells/kg at 5 weeks and 5×10^7 CD3⁺ T cells/kg at 8 weeks); one patient (UPN 254) developed grade IV GVHD that improved with corticosteroids and anti-IL-2 receptor monoclonal antibody therapy. One patient (UPN 234) did not develop acute GVHD but had biopsy evidence of chronic GVHD of the liver on day 421 posttransplantation. The fourth patient (UPN 322) had no detectable donor cells 1 week after receiving the first prophylactic DLI and received a second DLI on day 64; donor cells remained undetectable 8 weeks after the second DLI.

An additional 4 patients received either 2 or 3 DLIs for treatment of posttransplantation relapse. One of these patients (UPN 194) had an undetectable level of donor chimerism at the time of his relapse on day 44 posttransplantation. One DLI of approximately 5×10^7 CD3⁺ T cells/kg was given on day 49, resulting in prompt regression of his lymphoma and the de novo appearance of a donor-derived

band on VNTR analysis of his blood, indicating the presence of approximately 1% donor chimerism. A second DLI of 5×10^7 CD3⁺ T cells/kg on day 121 was followed by conversion to >99% donor chimerism and the development of grade IV GVHD. Although the clinical manifestations of the patient's GVHD resolved after treatment with corticosteroids, cyclosporine, and ATG, he developed a fulminant recurrence of his lymphoma and died on day 193 posttransplantation. In another patient (UPN 201), non-Hodgkin's lymphoma showed a mixed response after 3 DLIs—given on days 142, 187, and 230—and had symptoms associated with chronic GVHD-like clinical manifestations (eg, rash and keratoconjunctivitis sicca). The patient died from progressive pulmonary lymphoma on day 518 posttransplantation. One patient (UPN 238) received 3 DLIs for relapsed Hodgkin's disease. After the third DLI, given in conjunction with recombinant interferon alfa, this patient developed grade II cutaneous GVHD and demonstrated conversion from 60% to >99% donor cells. No antitumor response was observed in 1 patient with acute myeloid leukemia (AML) (UPN 283) after 2 DLIs for progressive disease.

Chimerism Studies

Eighteen of 20 evaluable patients developed persistent mixed lymphohematopoietic chimerism after BMT, as defined by >1% donor peripheral blood white blood cells until at least day 35 posttransplantation (the day of the initial prophylactic DLI). One patient (UPN 259) experienced early progression of his acute lymphoblastic leukemia, and chimerism studies were not obtained after day 27 posttransplantation.

The pattern of chimerism as determined by microsatellite analysis of peripheral blood over time in 10 recipients of either 1 or 2 prophylactic DLIs is illustrated in Figure 1. A fall in the percentage of donor cells shortly after engraftment can be seen in the majority of cases. Six of 8 evaluable patients (followed for at least 8 weeks after initial DLI) demonstrated conversion to full donor hematopoiesis.

Seven patients with a diagnosis other than acute leukemia did not receive DLI for chimerism conversion.

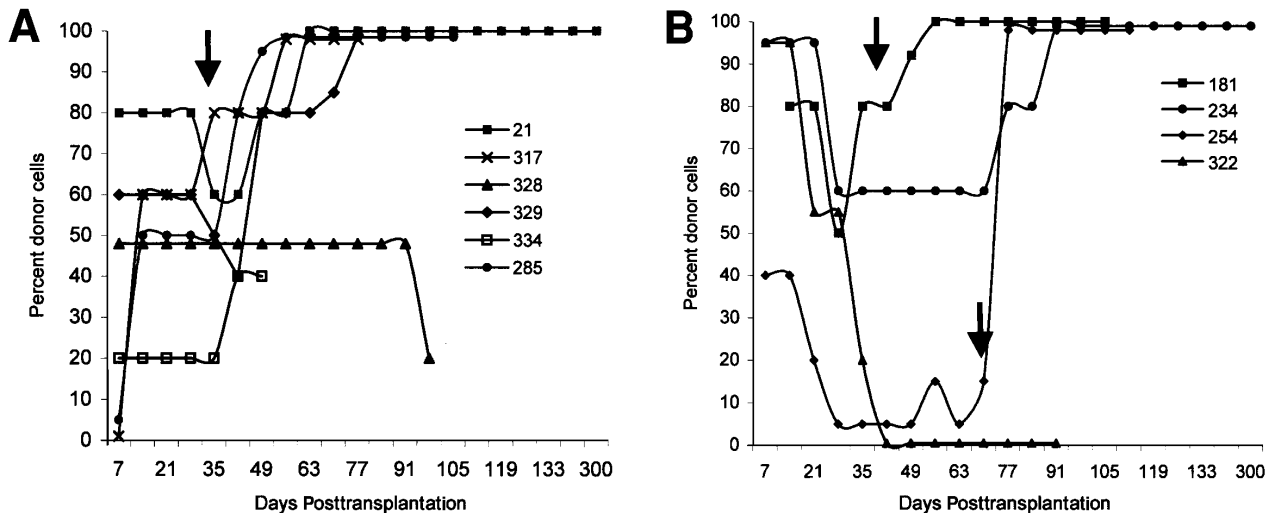


Figure 1. The pattern of chimerism as determined by microsatellite analysis of peripheral blood over time in 6 patients who received a single prophylactic DLI beginning on day 35 (one patient [UPN 317] was included until day 49, when DLI was given for relapse) (A) and in 4 patients who received 2 prophylactic DLIs (B). Black arrows indicate time of DLI administration.

Three patients (UPNs 282, 194, and 275) had nondetectable (<1%) donor chimerism by days 28, 34, and 43, respectively, posttransplantation. Three patients (UPNs 238, 268, and 279) maintained a state of stable mixed lymphohematopoietic chimerism. Two patients (UPNs 194 and 238) demonstrated conversion to full donor chimerism after DLI for disease relapse. Spontaneous conversion to full donor chimerism occurred by day 91 posttransplantation in 1 patient (UPN 201).

The median percentage of donor cells present over time in 10 patients who received prophylactic DLI and in 8 of the 11 patients who did not receive DLI because of the presence or initial suspicion of acute GVHD is illustrated in Figure 2. Three patients with acute leukemia were excluded from the data used for this graph because they either did not achieve remission or had early disease progression; thus, the contribution from host leukemic cells to the cell populations analyzed complicated the interpretation of their chimerism data.

Transplant-Related Toxicities

Three patients (UPNs 21, 181, and 285) developed cyclophosphamide-induced cardiac toxicity, which included a significant decrease in left ventricular ejection fraction (LVEF) and the development of atrial fibrillation in 2 patients (UPNs 181 and 285) with symptoms and signs of congestive heart failure. One of the latter 2 patients (UPN 285) still had persistent left ventricular dysfunction and was receiving digoxin, metoprolol, and milrinone 9 months posttransplantation. A third patient (UPN 21) had chest tightness with ischemic electrocardiographic changes (ie, T-wave inversions in lateral leads) and a fall in LVEF from 46% to 39%.

Because of severe ATG-related toxicities, including hypotension, renal insufficiency, and shock liver in 2 patients, ATG dose was reduced to 15 mg/kg (based on ideal or actual body weight, whichever was less). The timing of ATG was also changed in an attempt to reduce the incidence of acute GVHD (from days -2, -1, and 1 to days -1,

1, 3, and 5). No serious toxicities occurred after the reduction of ATG dose from 30 to 15 mg/kg.

One patient (5%) died from a transplantation-related complication. After treatment with high-dose corticosteroids, cyclosporine, and anti-interleukin-2 receptor monoclonal antibody therapy for grade IV GVHD, this patient (UPN 247) with AML died on day 77 posttransplantation because of a widely disseminated B-cell non-Hodgkin's lymphoma. A second patient (UPN 181) developed grade IV GVHD after a second prophylactic DLI (5.0×10^7 CD3⁺ cells/kg) and died on day 180 from progressive hepatic failure and polymicrobial sepsis.

Response

Fourteen of 20 evaluable patients (70%) achieved a response; 8 responses were complete, and 6 were partial (see Table 2). Of 13 evaluable patients with chemotherapy-refractory non-Hodgkin's or Hodgkin's lymphoma who underwent BMT, 6 achieved a complete response and 4 a partial response. Of 9 evaluable patients who received prophylactic DLI, 5 (56%) achieved a complete response, compared with 3 of 11 patients (27%) who did not receive prophylactic DLI.

The computed tomography scans and bone marrow biopsy specimens shown in Figures 3 and 4 illustrate the striking antitumor responses in patients with advanced chronic lymphocytic leukemia (CLL) and refractory non-Hodgkin's lymphoma. One patient (UPN 268) with chemotherapy-refractory CLL had a peripheral white blood cell count of 29,800/ μ L (80% lymphocytes), massive mediastinal and intra-abdominal lymphadenopathy, and splenomegaly (Figure 3A). He received an HLA-matched donor marrow transplant and achieved near-complete remission of his disease. Figure 3B shows resolution of his intra-abdominal lymphadenopathy and splenomegaly, and B cells were still declining at follow-up, as shown in Figure 3C. He has no evidence of disease progression on day 343 posttransplantation but has chronic cutaneous GVHD.

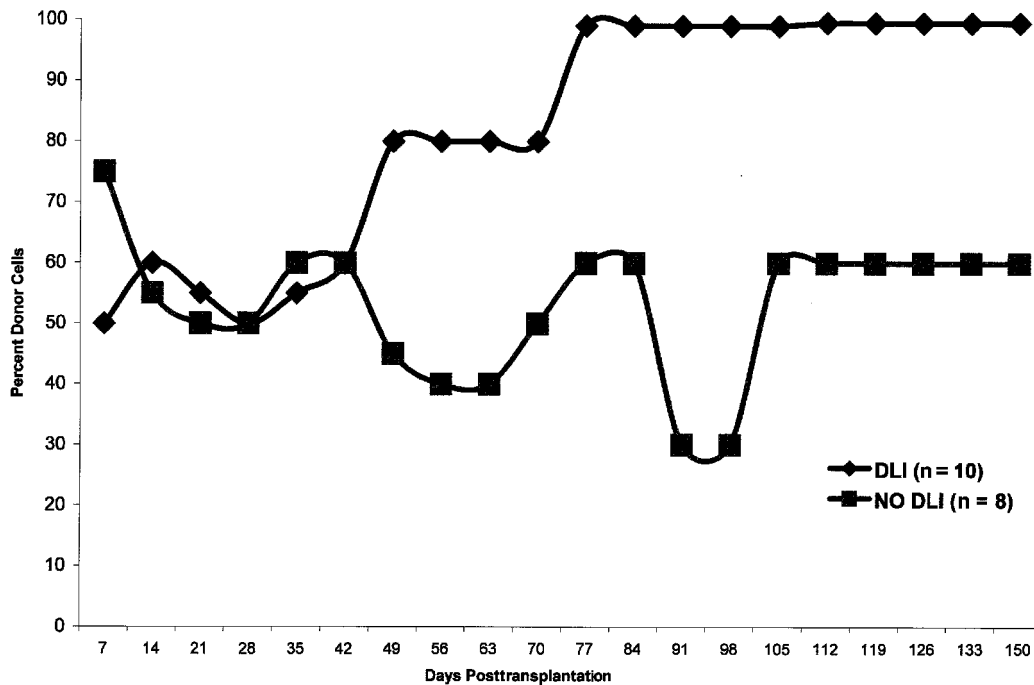


Figure 2. The median percentage of donor cells found in peripheral white blood cells over time in 10 recipients of an HLA-matched donor BMT who received DLI beginning on day 35 and between days 56 and 64 posttransplantation, and 8 patients who did not receive DLI posttransplantation because of the presence or suspicion of GVHD. Patterns of chimerism were determined by microsatellite analysis and are shown as estimates of the percentage of donor DNA present.

The pre- and posttransplantation bone marrow biopsy specimens pictured in Figure 4 show resolution of diffuse marrow involvement by a follicular mixed large- and small-cell lymphoma in 1 patient (UPN 21). This patient had previously received an autologous stem cell transplant for relapse of stage IV disease. He then experienced a second relapse and received an HLA-matched donor transplant under our protocol for his progressive, chemotherapy-refractory disease. On day 35 posttransplantation, he received prophylactic DLI, which led to conversion to full donor chimerism within 4 weeks, without the development of GVHD. He is presently disease free and was without evidence of GVHD on day 548 posttransplantation.

Survival

Eleven of the 21 patients (52%) were alive at a median of 445 days (range, 105-548 days) posttransplantation; 7 of these were free of disease progression. Two patients, UPNs 247 and 18, died from treatment-related complications on days 77 and 180, respectively. Eight patients (5 patients with non-Hodgkin's lymphoma, 2 with acute leukemia, and 1 with Hodgkin's disease) died from progressive malignancy posttransplantation. Kaplan-Meier graphs of progression-free and overall survival probability for the 21 patients who underwent transplantation are shown in Figures 5 and 6, respectively.

DISCUSSION

Treatment options for patients with chemotherapy- and radiotherapy-refractory hematologic malignancies are very limited. Early transplantation series using conventional myeloablative preparative regimens demonstrated long-

term disease-free survival rates of $\leq 15\%$ for this group of patients [31-33]. Although more recent series have shown an apparent improvement in the outcome of patients with advanced hematologic malignancies [34,35], the survival probabilities for patients with chemotherapy-refractory disease remain poor.

This trial has demonstrated for the first time the ability to intentionally achieve stable mixed lymphohematopoietic chimerism after conditioning with a nonmyeloablative chemotherapy regimen that includes pretransplantation thymic irradiation and peritransplantation anti-T-cell antibody therapy. Although cyclophosphamide conditioning (with or without ATG) has been used in patients undergoing transplantation for severe aplastic anemia and hematologic malignancies and appears to be sufficient to achieve donor engraftment in the majority of cases [9,36], documentation of sustained mixed chimerism is lacking. Our regimen differs significantly from previous regimens in both the treatment scheme (ie, posttransplantation ATG and thymic irradiation) and intent (ie, intentional induction of mixed chimerism to diminish the toxicity of host conditioning, to ameliorate GVHD, and to enable subsequent administration of DLI).

Based on a murine model that showed that anti-T-cell antibodies are still circulating at the time of transplantation, and therefore deplete donor T cells in vivo, posttransplantation ATG was used in an attempt to deplete donor T cells in vivo. With the establishment of a mixed chimeric state in the mouse, DLI in doses that would cause severe GVHD in freshly myeloablated recipients can be given later, without causing significant GVHD [7,8]. Elimination of recipient-type hematopoietic cells after DLI, without development of GVHD, is evidence that a graft-versus-host reaction can be confined to the lymph-

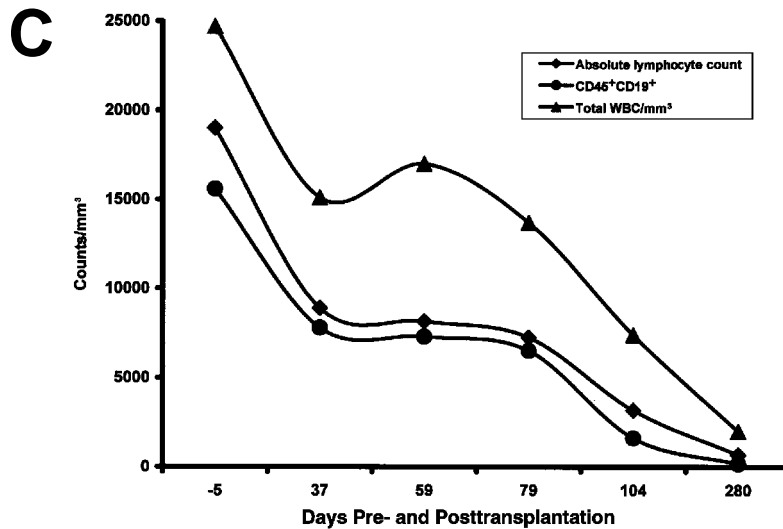
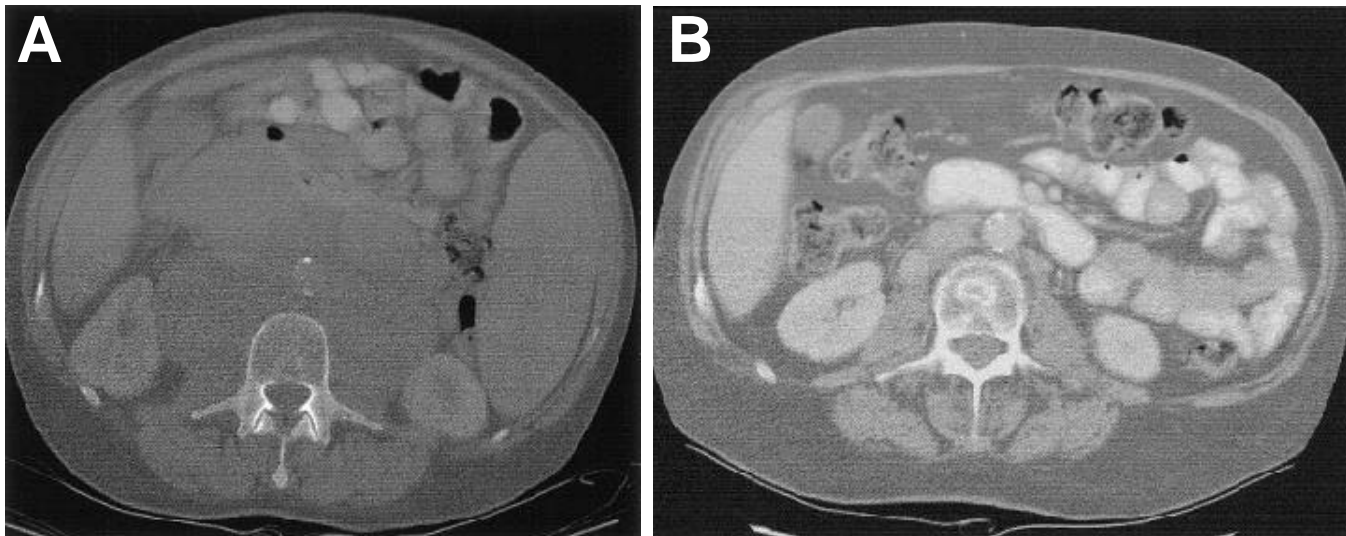


Figure 3. Pretransplantation (A) and day 100 posttransplantation (B) computed tomography (CT) scans of the abdomen in a patient (UPN 268) with CLL and lymphadenopathy and splenomegaly. Pretransplantation CT scan shows massive intra-abdominal lymphadenopathy and splenomegaly. Day 100 posttransplantation CT scan demonstrates resolution of the intra-abdominal lymphadenopathy and splenomegaly. C, Declining B-cell population over time in the same patient. Total white blood cell (WBC) count, absolute lymphocyte count, and CD45⁺CD19⁺ cell populations, as determined by flow cytometry, continued to decline at more than 280 days posttransplantation.

phohematopoietic system, a situation that is desirable to achieve GVL effects without the development of GVHD. Although the outcome of our trial reflects some of the same results obtained in the murine model, the initial GVHD observed in some patients indicates that posttransplantation ATG does not deplete donor T cells in humans to the same extent as in the mouse. Nevertheless, the conversion to full donor chimerism after 1 DLI in 3 of the 4 patients evaluated, without the development of acute GVHD in 1 patient and with treatment-responsive GVHD limited to the skin in 2 patients, is evidence that a similar phenomenon can be seen in humans. In addition, the achievement of complete remission in these 3 patients is evidence that GVL effects can be achieved in this manner without the development of severe GVHD.

After a series of protocol revisions intended to reduce transplantation-related morbidity, including a reduction in cyclophosphamide dose and a change in the dosing and timing of ATG, the present regimen has been very well tolerated. These protocol changes were necessitated by cardiac toxicity in 2 patients who received a cyclophosphamide dose of 200 mg/kg, unexpectedly severe ATG-related toxicities that occurred in 2 patients who received ATG in a dose of 30 mg/kg, and the desire to reduce toxicity in this group of heavily pretreated patients, many of whom had received previous mediastinal radiation therapy. Furthermore, we sought to further reduce the risk of GVHD by administering an increased proportion of the ATG posttransplantation in recipients of the modified regimen.

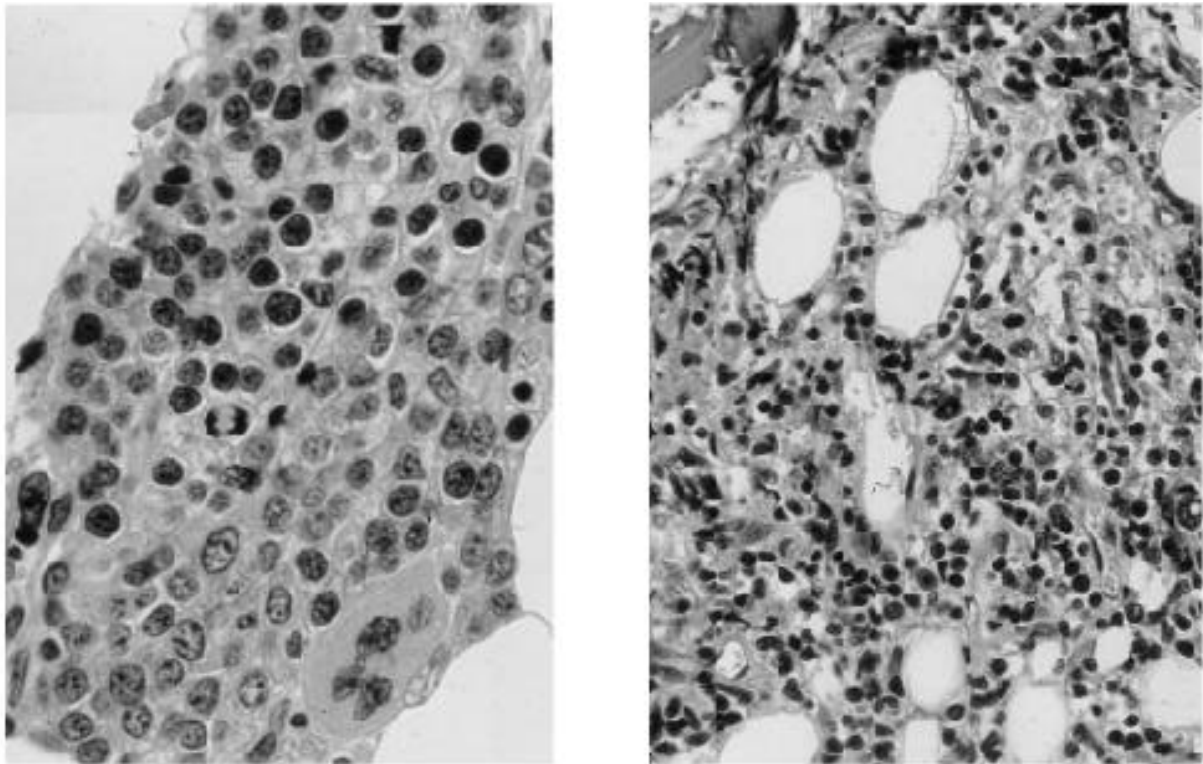


Figure 4. *Pretransplantation (A) and posttransplantation (B) bone marrow biopsy specimens in 1 patient (UPN 21). The pretransplantation specimen demonstrates hypercellularity with diffuse involvement by predominantly small to medium-sized cleaved lymphoma cells. The posttransplantation (day 106) specimen shows trilineage hematopoiesis with no evidence of lymphoma.*

Presently the most prominent and common toxicity after transplantation is engraftment syndrome, which occurred in 14 of 21 patients, often early posttransplantation (as early as day 7). Cytokine-mediated engraftment syndromes have been described previously, including a “hyper-acute GVHD syndrome” that occurred after HLA-mismatched donor transplantation [30,37]. Manifestations of this syndrome, however, were exquisitely sensitive to corticosteroids in most of our patients, thereby allowing an early and rapid taper of immunosuppressive therapy. GVHD could not be confirmed in biopsy specimens obtained while patients had this syndrome, and in more than half of patients, no definitive evidence of GVHD developed after discontinuation of the steroids.

Weekly determinations of lymphohematopoietic chimerism in our trial allowed evaluation of the relationship of host and donor cell populations to the engraftment process. The curious fall in the percentage of donor cells shortly after engraftment in most cases could signify a transient host-versus-graft reaction, which might provide cytokines that contribute to the clinical syndrome observed or may reflect the recovery of host hematopoietic cells after conditioning. Thus, donor hematopoietic cells may become “diluted” by recovering host elements.

Recently the results of pilot clinical trials using nonmyeloablative preparative strategies for HLA-matched donor allogeneic BMT have been published [20-22]. Patients with various hematologic malignancies have received fludarabine or low-dose busulfan-based regimens and allogeneic BMT. The tolerability of these regimens is evidenced by the accept-

able toxicities that have been observed, even in older patients and in patients with comorbid illnesses. Infrequently performed chimerism analyses, however, have shown a predominance of donor chimerism in most cases. A high probability of relapse has been reported in 1 of the series [20], and follow-up durations in these studies have been short.

The role of thymic irradiation in induction of the mixed chimeric state in this clinical trial is unclear. In the murine models of Sharabi and Sachs [3] and Pelot et al. [7], thymic irradiation was a critical component, as it eliminated alloreactive host thymocytes that could mediate intrathymic rejection of donor cells and prevent the establishment of a mixed chimeric state. However, the role of thymocytes in resisting establishment of a mixed chimeric state in adult humans is questionable. Only 13 of the 21 patients received thymic irradiation; the other patients had received previous mediastinal radiation therapy, possibly ablating whatever thymic function may have been present previously. There has been no obvious difference in the establishment of a mixed chimeric state or in the incidence or severity of GVHD among recipients of HLA-matched or -mismatched donor marrow to whom thymic irradiation was or was not administered.

The striking antitumor responses in most patients with chemotherapy-refractory hematologic malignancies appear to be largely a result of a GVL effect. Most patients in this trial previously received and were resistant to cyclophosphamide, the only cytoreductive agent used in our conditioning regimen. In patients with easily measurable disease

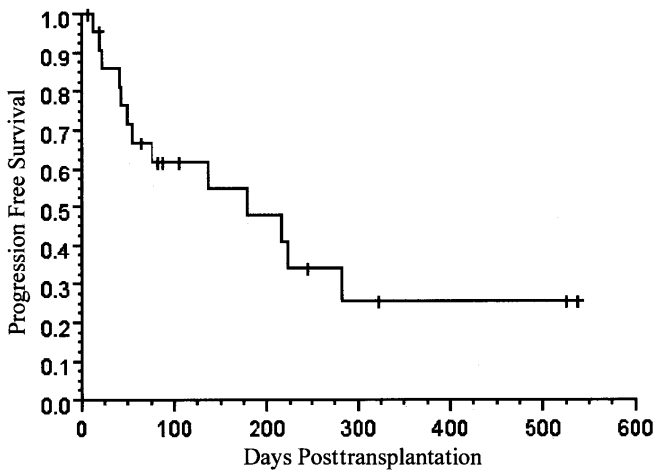


Figure 5. Kaplan-Meier progression-free survival probability for the 21 recipients of an HLA-matched donor bone marrow transplantation.

(eg, palpable lymphadenopathy or skin lesions), antitumor responses were not maximal until after engraftment had been achieved or even after DLI. Although there was a suggestion of an increased complete response rate after prophylactic DLI, the number of patients in each group was too small to reach a meaningful conclusion. Moreover, acute GVHD was a common accompaniment of prophylactic DLI. The impact of DLI on patient response rate and progression-free and overall survival will therefore have to be addressed in future prospective, randomized trials. Because early relapses occurred in 5 of these 21 patients with aggressive hematologic malignancies, further efforts will be required to determine which patients are not likely to benefit from a strategy using a nonmyeloablative chemotherapeutic preparative regimen and immunotherapy with post-transplantation DLI.

Despite this favorable early experience with deliberate induction of mixed chimerism after nonmyeloablative transplant preparative therapy, several problems remain. First, the incidence of GVHD does not appear to be significantly different (and may even be higher when GVHD after DLI

is included) from that after conventional allogeneic stem cell transplantation using myeloablative conditioning therapy. Consideration is therefore being given to the substitution of a monoclonal anti-T-cell antibody for ATG (which may effect a more significant T-cell depletion in vivo and eliminate potential ATG-related toxicities). Second, the timing, and perhaps the dosing, of DLI may be suboptimal. In most situations, cyclosporine had not been completely tapered by day 35, the intended day of the first DLI in our study. In 2 of 3 cases in which a second DLI was given on day 56, severe GVHD developed. In our current protocol the second DLI is omitted, given the observation that the full effects of DLI, in terms of both GVHD and GVL, may not be seen for many weeks after an infusion [38]. Finally, efforts are under way to determine the cellular mechanisms of GVHD and GVL in this mixed chimeric model so that the dissociation of the 2 can be maximized.

In summary, a state of mixed lymphohematopoietic chimerism is reliably induced after a novel nonmyeloablative preparative regimen and BMT from an HLA-matched related donor. DLI can often be given 5 weeks after BMT to promote conversion from the mixed chimeric state to a state of full donor chimerism and thereby more fully capture the important GVL effects of these transplants. This is possible without induction of GVHD. This regimen also appears to be well tolerated, even among heavily pretreated patients with advanced disease. Although this treatment strategy shows considerable promise in the treatment of patients with chemotherapy-refractory hematologic malignancy, its role in specific diseases and the role of prophylactic DLI in augmenting a GVL effect remain to be determined.

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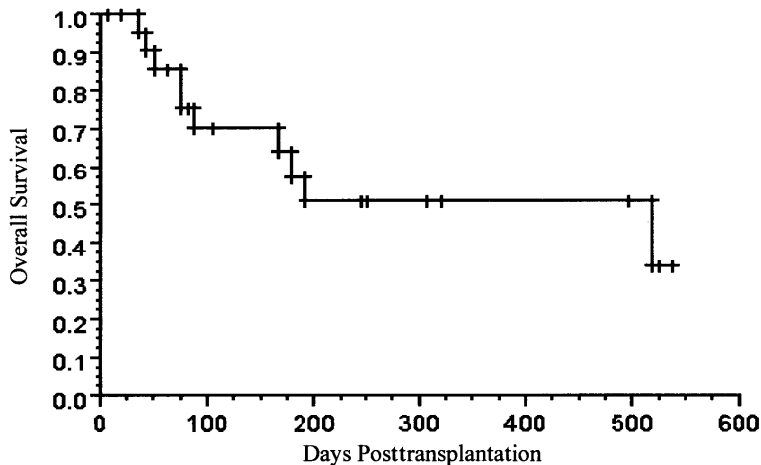


Figure 6. Kaplan-Meier overall survival probability for the 21 recipients of an HLA-matched donor bone marrow transplantation.

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