In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation

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A novel nonmyeloablative conditioning regimen was investigated in 44 patients with hematologic malignancies. The median patient age was 41 years. Many of the patients had high-risk features, including 19 patients with a previous failed transplant. Recipient conditioning consisted of CAMPATH-1H, 20 mg/day on days −8 to −4; fludarabine, 30 mg/m^2 on days −7 to −3; and melphalan, 140 mg/m^2 on day −2. Thirty-six recipients received unmanipulated granulocyte colony-stimulating factor–mobilized peripheral blood stem cells from HLA-identical siblings, and 8 received unmanipulated marrow from matched unrelated donors. GVHD prophylaxis was with cyclosporine A alone for 38 patients and cyclosporine A plus methotrexate for 6 sibling recipients. Forty-two of the 43 evaluable patients had sustained engraftment. Results of chimerism analysis using microsatellite polymerase chain reaction indicate that 18 of 31 patients studied were full-donor chimeras while the other patients were mixed chimeras in one or more lineages. At a median follow-up of 9 months (range 3 to 29 months), 33 patients remain alive in complete remission or with no evidence of disease progression. Seven patients relapsed or progressed post-transplantation, and 4 of them subsequently died. Four patients died of regimen-related complications. There were no cases of grades III-IV acute GVHD. Only 2 patients developed grade II acute GVHD, and only 1 had chronic GVHD. The estimated probability of nonrelapse mortality was 11%. Although longer follow-up is needed to establish the long-term remission rates, this study demonstrates that this nonmyeloablative preparative regimen is associated with durable engraftment, minimal toxicity, and low incidence of GVHD. (Blood. 2000;96: 2419-2425)

Introduction

High-dose chemoradiotherapy followed by allogeneic stem cell transplantation (SCT) has been extensively used to treat patients with hematologic malignancies. This procedure is often limited to patients in good medical condition because of the increased risk of regimen-related toxicity and graft-versus-host disease (GVHD) that occurs with increasing age and poor performance status.[1,2] The curative potential of transplantation is not solely due to the conditioning regimen but also to the well-documented graft-versus-leukemia (GVL) effect.[3] The most convincing evidence for this GVL effect is that donor leukocyte infusions (DLIs) can reinduce remissions in patients who have relapsed following allogeneic SCT.[4,5] Patients with chronic myeloid leukemia are most likely to respond, but responses have also been documented in patients with acute leukemia, chronic lymphocytic leukemia, myeloma, and lymphoma.[6,7]

In an effort to reduce the transplant-related mortality (TRM) associated with allogeneic SCT, low-intensity fludarabine-based regimens have been developed.[8-11] These have been designed to be immunosuppressive rather than myeloablative to facilitate donor engraftment and thereby limit systemic toxicity. There appears to be a spectrum of hematopoietic toxicity associated with these nonmyeloablative regimens—from minimally cytopenic regimens that use low-dose total body irradiation alone[12] to regimens that combine fludarabine with melphalan or busulfan.[10,13] While these studies have demonstrated impressive allogeneic engraftment with minimal nonhematologic toxicity, there is still a significant morbidity and mortality from acute and chronic GVHD.[8-11]

We have therefore developed a novel nonmyeloablative regimen for allogeneic SCT. Our regimen was designed to suppress the recipient immune system enough to allow allogeneic engraftment without excessive regimen toxicity or GVHD. The use of fludarabine as an immunosuppressant as part of the conditioning regimen was similar to previously published studies of nonmyeloablative SCT.[8-11] The combination of fludarabine with 180 mg/m^2 of melphalan was originally described by the M. D. Anderson group.[14] Our regimen used a different dose of melphalan, 140 mg/m^2; however, the addition of in vivo CAMPATH-1H to the conditioning regimen was new and appears to have been crucial in limiting graft-versus-host reactions.

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Patients and methods

Eligibility criteria

Patients with hematologic malignancies were enrolled at 6 hospitals in England. The study design was approved by the ethics committees at each participating site. All patients gave written informed consent to participate. Patients with lymphoma, acute leukemia, myelodysplasia, multiple myeloma, chronic lymphocytic leukemia, and chronic myeloid leukemia between ages 18 and 60 years were eligible to participate. Patients required an HLA-identical sibling or unrelated donor as determined by serologic typing for HLA A/B and molecular typing for HLA DR/DQ. Data were analyzed as of November 30, 1999.

Patient characteristics

Detailed characteristics are shown in Table 1. Forty-four patients were enrolled in the study from June 1997 to September 1999. Twenty-eight were male, and 16 were female. Age range at the time of transplantation was 18 to 56 years (median, 41 years). Fourteen patients had non-Hodgkin lymphoma (NHL), 10 Hodgkin disease, 6 acute myeloid leukemia (AML), 7 multiple myeloma, 3 hypoplastic myelodysplastic syndrome, 1 acute lymphoblastic leukemia, 1 chronic lymphocytic leukemia, 1 chronic myeloid leukemia, and 1 plasma cell leukemia. This was a cohort of patients with high-risk features, including 19 patients with a previous failed autologous (18 patients) or allogeneic (1 patient) transplant, renal failure (2 patients), poor left ventricular function (2 patients), or refractory disease (9 patients). The median time interval from first to second transplant was 24 months (range, 8-79 months).

Table 1. Patient characteristics, source of progenitor cells, and GVHD prophylaxis (n = 44)

Patient no.	Age	Sex	Disease	Previous failed transplant/prior lines of therapy	Previous radiotherapy	Status at transplantation	Source of donor cells	$CD34^+ \times 10^6$/kg (PBSC) MNC $\times 10^8$/kg (marrow)	GVHD prophylaxis
1	53	M	MCL	No/3	No	CR-1	PBSC/sibling	6.1	CsA + MTX
2	27	M	HD	No/3	Local	CR-2	PBSC/sibling	3.2	CsA + MTX
3	32	M	AML	No/3	Local	CR-3	PBSC/sibling	6.6	CsA + MTX
4	54	M	LG-NHL-T	Auto/5	Local + TBI	PR	PBSC/sibling	3.1	CsA + MTX
5	25	F	HD	Auto/5	Local	PR	PBSC/sibling	4.6	CsA
6	41	M	HD	Auto/6	Local × 2	PR	PBSC/sibling	4.1	CsA
7	41	M	LG-NHL	No/3	No	CR-1	PBSC/sibling	4.6	CsA
8	45	M	MDS	Auto/4	Local	PR	Marrow/MUD	2.64	CsA
9	38	M	HG-NHL	Auto/3	Local	PR	Marrow/MUD	3.52	CsA
10	48	F	MM	Auto/2	No	PR	PBSC/sibling	4.22	CsA + MTX
11	44	M	MM	Allo/2	No	PR	PBSC/sibling	3.87	CsA + MTX
12	42	F	AML	No/2	No	Refractory	PBSC/sibling	5.9	CsA
13	51	F	AML	No/2	No	Refractory	PBSC/sibling	1 + 6	CsA
14	25	M	HD	Auto/4	Local	CR-4	PBSC/sibling	6.4	CsA
15	35	M	LG-NHL	Auto/4	No	PR	PBSC/sibling	5.6	CsA
16	18	M	ALL	Auto/3	TBI	CR-2	Marrow/MUD	4.3	CsA
17	32	F	HD	Auto/4	No	Refractory	PBSC/sibling	5.7	CsA
18	39	M	HD	Auto/4	Local	PR	PBSC/sibling	12.2	CsA
19	30	M	AML	Auto/3	TBI	CR-2	Marrow/MUD	6.9	CsA
20	35	F	HD	No/4	Local	Refractory	PBSC/sibling	6.9	CsA
21	50	M	LG-NHL	No/2	Local	CR-2	PBSC/sibling	3.19	CsA
22	48	M	LG-NHL-T	No/4	No	Refractory	PBSC/sibling	6.73	CsA
23	40	F	MM	No/2	No	PR	PBSC/sibling	3.9	CsA
24	49	F	MDS	No/0	No	PR	PBSC/sibling	9.3	CsA
25	38	F	LG-NHL-T	No/5	No	Refractory	PBSC/sibling	7.5	CsA
26	44	F	AML	Auto/3	TBI	CR-2	Marrow/MUD	2.9	CsA
27	33	F	HD	No/3	Local	CR-3	PBSC/sibling	4.17	CsA
28	43	F	MM	No/1	No	PR	PBSC/sibling	21.1	CsA
29	50	M	MCL	No/1	No	CR-1	PBSC/sibling	9.25	CsA
30	50	M	AML	No/2	No	CR-2	PBSC/sibling	2.28	CsA
31	20	M	HG-NHL	No/4	Local	Refractory	PBSC/sibling	2.98	CsA
32	49	M	MM	Auto/3	Local	PR	PBSC/sibling	5.1	CsA
33	49	M	MDS	No/0	No	PR	PBSC/sibling	0.55 + 0.65	CsA
34	43	M	LG-NHL	No/4	No	CR-2	PBSC/sibling	8.9	CsA
35	56	M	LG-NHL-T	No/4	Local	Refractory	PBSC/sibling	2.1	CsA
36	31	M	HD	Auto/4	No	CR-3	PBSC/sibling	2.95	CsA
37	44	M	MCL	Auto/3	No	CR-3	PBSC/sibling	5.29	CsA
38	37	M	CLL	No/2	No	PR	PBSC/sibling	4.4	CsA
39	37	M	LG-NHL	No/5	No	PR	PBSC/sibling	3.5	CsA
40	22	F	HD	Auto/4	No	PR	Marrow/MUD	4.79	CsA
41	35	M	PCL	No/1	No	CR-1	Marrow/MUD	2.29	CsA
42	46	F	MM	No/1	No	PR	PBSC/sibling	6.8	CsA
43	48	F	MM	Auto/5	Local	Refractory	PBSC/sibling	5.94	CsA
44	53	F	CML	No/1	No	CP-1	Marrow/MUD	2.6	CsA

MCL indicates mantle cell lymphoma; HD, Hodgkin disease; AML, acute myeloid leukemia; LG-NHL, low-grade non-Hodgkin lymphoma; T, transformed low-grade to high-grade lymphoma; MDS, myelodysplastic syndrome; HG-NHL, high-grade non-Hodgkin lymphoma; MM, multiple myeloma; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CR, complete remission; PR, partial remission; CP, chronic phase; PBSC, peripheral blood stem cells; MUD, matched unrelated donor; CsA, cyclosporine A; MTX, methotrexate; TBI, total body irradiation.

Monoclonal antibody

CAMPATH-1H is a humanized immunoglobulin (Ig) G1 monoclonal antibody against the CD52 antigen.[15] It was prepared from the culture supernatant of Chinese hamster ovary cell transfectants cultured in a hollow fiber fermentor. It was purified by affinity chromatography on protein A-sepharose (Amersham Pharmacia Biotech, Little Chalfont, England) and size exclusion chromatography on Superdex 200 (Amersham Pharmacia Biotech) and formulated in phosphate-buffered saline. The half-life of CAMPATH-1H in humans is dependent on the amount of target CD52 antigen in the patient. Based on work in progress, there is persistence on CAMPATH-1H in vivo past day 0 sufficient to cause T-cell lysis by antibody-dependent cell-mediated cytotoxicity.

Conditioning regimen

Treatment consisted of the humanized monoclonal antibody CAMPATH-1H, 20 mg/day intravenous infusion over 8 hours on days −8 to −4; fludarabine, 30 mg/m^2 intravenous infusion over 30 minutes on days −7 to −3; and melphalan, 140 mg/m^2 intravenous infusion over 30 minutes on day −2. Thirty-six recipients received unmanipulated peripheral blood stem cells (PBSCs) from their siblings, and 8 received unmanipulated marrow from matched unrelated donors.

Stem cell and bone marrow collection

Sibling donors received granulocyte colony-stimulating factor (G-CSF) at 10 μg/kg subcutaneously once daily on days −4 to 0. Leukapheresis were performed on days 0 and +1 using conventional techniques for PBSC collection. Unrelated donors had bone marrow collected on day 0 under general anesthesia using conventional techniques. In 2 sibling donors, we failed to collect more than 2×10^6/kg CD34$^+$ cells from G-CSF–mobilized peripheral blood, and therefore bone marrow was also harvested. The total number of CD34$^+$ cells collected from peripheral blood and the number of mononuclear cells collected from the bone marrow are shown in Table 1. Unmanipulated mobilized peripheral blood or bone marrow was infused through central venous catheters on days 0 and +1 and on day 0, respectively.

Supportive care

Patients were managed in reverse isolation in conventional or laminar airflow rooms. All patients received prophylaxis with cotrimoxazole or pentamidine against *Pneumocystis carinii* infection. Acyclovir and fluconazole or itraconazole prophylaxis were routinely used. Blood products were irradiated to 25 Gy. Red cell and platelet transfusions were given to maintain hemoglobin levels above 9 g/dL and platelet count above 10 to 15×10^9/L. The cytomegalovirus (CMV)-seronegative patients received only CMV-negative blood products; seropositive patients received CMV-unscreened blood products. Febrile neutropenic patients received broad-spectrum intravenous antibiotics according to each hospital's policy for the management of neutropenic sepsis. G-CSF at 5 μg/kg per day was administered subcutaneously at the discretion of the transplant physician to speed hematologic recovery in 38 patients until the patient's absolute neutrophil count was at least 1000/μL for 3 consecutive days (Table 2).

GVHD prophylaxis and grading

GVHD prophylaxis consisted of cyclosporine A (CsA), 3 mg/kg starting on day −1, and methotrexate (MTX) at a dose of 10 mg/m^2 on days +1, +3, and +6 for 6 sibling recipients, and it consisted of CsA alone for the other 38 patients. Intravenous CsA was switched to an oral dose as soon as the patients would tolerate medications by mouth and was continued for a median of 4 months (range, 1 to 8 months). Patients who survived 100 days or longer were evaluable for chronic GVHD. Acute and chronic GVHD were graded according to the consensus criteria.[16]

Follow-up

Patients had regular follow-up at 3 monthly intervals post-transplantation to assess disease response and remission status. These evaluations varied depending on the underlying diagnosis but included bone marrow aspirates or biopsies, cytogenetics, computed tomography scans, paraprotein levels, and skeletal surveys.

Chimerism analysis

DNA was prepared from pretransplantation recipient blood and donor blood. Following transplantation, either buffy coat or granulocyte T-cell and B-cell preparations were obtained from peripheral blood as previously described.[17] We used 4 different primer sets each flanking highly polymorphic short tandem repeat units on different human chromosomes. With primers VWA31 (Perkin-Elmer) and THO1 (Perkin-Elmer), polymerase chain reaction (PCR) volumes were 50 μL containing Genamp PCR buffer II (Perkin-Elmer), 1.5-mmol/L MgCl, 0.2 mmol/L of each deoxyribonucleoside triphosphate (dNTP), 1 mmol/L of each primer, 0.5 U of AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, CA), and 5 μL of DNA. Cycling conditions were 95°C for 45 seconds, 54°C for 30 seconds, and 72°C for 1 minute for 30 cycles. With primers ACPP (forward: ACTGTGCCTAGCCTATACTT, backward: AGTGAGCCAAGAGTGCACTA) and HUMSTRX1 (forward: CTCCTTGTGGCCTTCCTTAAATGG, backward: CTTCTCCAGCACCCAAGGAAGTCA), PCR volumes were 50 μL containing VNTR buffer (45-mmol/L Tris-HCl, 11-mmol/L NH_4SO_4, 6.7-mmol/L 2-mercaptoethanol, 4.5-μmol/L ethylenediaminetetraacetic acid, 110-μg/mL bovine serum albumin), 5 mmol/L of each dNTP, 1 mmol/L of each primer, 0.5 U of AmpliTaq polymerase (Perkin-Elmer), and 5 μL of DNA. Cycling conditions were 95°C for 30 seconds, 58°C for 30 seconds, and 95°C for 30 seconds for 30 cycles. The forward primer of each pair was labeled with either JOE or FAM fluorescent dyes. One microliter of PCR product was denatured in 12 μL of formamide and electrophoresed through Performance Optimized Polymer 4 (Perkin-Elmer) on an ABI 110 automated sequencer (Perkin-Elmer) in the presence of Rox 500 size standard (Perkin-Elmer). Genescan software 2.1 (Perkin-Elmer) was used to analyze the data. Primers that gave rise to recipient/donor–specific peaks were identified and used for post-transplantation determination of chimeric status in the various cell populations.

Study endpoints

The primary study endpoint was the successful durable hemopoietic engraftment and TRM. There were secondary endpoints, including regimen-related toxicity, incidence and severity of GVHD, and progression-free survival.

Statistical methods

Actuarial curves were estimated according to the Kaplan-Meier method. Surviving patients were censored on the last day of follow-up. The significance of differences between the curves was estimated by the log-rank test. Cox multivariate regression analysis was performed to calculate the independent effects of various risk factors influencing nonrelapse mortality, overall survival, and disease-free survival. The proportional hazards assumption was tested using a time-dependent covariant approach.

Results

Toxicities

All patients were assessable for toxicity. The conditioning regimen was generally well tolerated in patients who only received CsA as GVHD prophylaxis. The use of MTX in addition to CsA was associated with severe mucositis and delayed engraftment (Table 2). The original intention was to give MTX to all patients, but its use was abandoned because of toxicity. There were no cases of veno-occlusive disease. Four patients died of regimen-related toxicity. One patient died on day +21 of gram-negative septicemia while still aplastic. The second patient died on day +24 of idiopathic pneumonitis after engrafting on day +14. The third

Table 2. Transplant outcome of 44 patients receiving a nonmyeloablative regimen

Patient no.	Toxic death	Neutrophils > 0.5×10^9/L, days	Platelets > 20×10^9/L, days	Acute GVHD	Chronic GVHD	Current status, months	Cause of death
1		20*	24	—	No	Dead	GVHD post-DLI
2		23*	96	—	No	Alive in CR 26+	—
							Methicillin-resistant *Staphylococcus aureus*
3	Yes; day +153	23*	24	—	No	Dead	Pneumonia
4	Yes; day +21	NE*	NE	—	NE	Dead	Sepsis
5		11	11	Grade I skin	No	Alive in CR 22+	
6		12	11	—	No	Alive in CR 18+	
7		11	11	—	No	Alive in CR 16+	
8	Yes; day +24	13	N/A	—	NE	Dead	Pneumonitis
9		10	15	—	No	Alive in CR 12+	
10	Yes; day +148	21*	23	—	No	Dead	Renal failure
11		18*	13	—	No	Alive in CR 13+	
12		15†	13	—	No	Dead	Disease
13		12†	22	Grade II GI	No	Dead	Disease
14		13†	15	Grade I skin	No	Alive-progressive disease 15+	
15		12	13	—	No	Alive in CR 14+	
16		18	30	—	No	Alive in CR 11+	
17		14	11	—	No	Alive-progression-free 10+	
18		11	9	—	No	Alive-progression-free 9+	
19		11	10	—	No	Alive in CR 9+	
20		8	3	—	No	Alive-progression-free 8+	
21		16	18	—	No	Alive in CR 10+	
22		13	16	Grade I skin	Skin, liver	Alive-progression-free 10+	
23		16	11		No	Alive in PR 12+	
24		11†	11	Grade II skin & GI	No	Alive in CR 11+	
25		12	14	—	No	Dead	Disease
26		14	18	—	No	Alive in CR 8+	
27		15	12	—	No	Alive in CR 7+	
28		12	10	—	No	Alive in PR 7+	
29		13	9	—	No	Alive in CR 6+	
30		13	18	—	No	Alive in CR 6+	
31		10	9	—	No	Alive in CR 6+	
32		10	8	—	No	Alive in PR 5+	
33		22†	18	—	No	Alive in CR 8+	
34		12	13	—	No	Alive in CR 7+	
35		13	24	—	No	Alive-progressive disease 5+	
36		12	12	—	No	Alive in CR 4+	
37		13†	15	—	No	Alive in CR 5+	
38		17	18	—	No	Alive-progression-free 3+	
39		14	14	—	No	Alive-progression-free 3.5+	
40		12	12	—	No	Alive-progression-free 3+	
41		14	18	—	No	Alive in CR 3+	
42		23	30	—	No	Alive in PR 3+	
43		13	27	—	No	Alive-progressive disease 3+	
44		15	11	—	No	Alive-cytogenetic CR 3+	

NE indicates nonevaluable; CR, complete remission.
*Patient received MTX as GVHD prophylaxis.
†Patient did not receive post-transplantation G-CSF.

patient died of MRSA pneumonia on day +153. The fourth patient with myeloma, whose creatinine clearance prior to transplantation was 23 mL/min, died of renal failure on day +148.

Engraftment

One patient was not evaluable for engraftment because of early death on day +21. Of the 43 patients eligible for assessment of engraftment, 42 had sustained engraftment as defined by neutrophil counts above 0.5×10^9/L and an untransfused platelet count of above 20×10^9/L for at least 3 consecutive days. Details of the neutrophil and platelet reconstitution are shown in Table 2. The median time to recover an absolute neutrophil count of 0.5×10^9/L was 13 days (range, 8-23 days) and of 1.0×10^9/L was 17 days (range, 8-47 days). The median time to achieve platelets above 20×10^9/L was 13 days (range, 3-96 days) and above 50×10^9/L was 17 days (range, 8-118 days). Patient No. 7 developed graft rejection. After initial engraftment on day +11, the patient became cytopenic on day +20 and reconstituted recipient hemopoiesis on day +31 without autologous stem cell support.

Chimerism

Thirty-one patients had chimerism studies performed using microsatellite PCR or fluorescent in situ hybridization for X and Y chromosomes on peripheral blood. Detailed results are shown in

Table 3. Patient No. 7, who rejected his graft, had only recipient myeloid and lymphoid cells present. Of the other 30 patients, 18 had only donor cells present. Of the 12 patients with mixed chimerism, 5 had detailed lineage-specific studies performed using microsatellite PCR. Three of these patients were full-donor chimeras in the myeloid and B-cell lineages but were mixed T-cell chimeras. Two patients were mixed chimeras in all lineages tested (Figure 1). Six of the 8 unrelated recipients had chimerism studies performed, and all 6 were found to have only donor cells present following transplantation (Table 3). Two patients (Nos. 1 and 11) who were mixed chimeras post-transplantation became full-donor chimeras following DLI therapy.

Graft-versus-host disease

No grade III-IV acute GVHD was observed post-transplantation. Three patients developed grade I skin, 1 patient grade II gastrointestinal, and 1 patient grade II skin and gastrointestinal (Table 2) acute GVHD. Only 1 patient has developed chronic GVHD, limited to skin and liver involvement. Two patients developed GVHD following DLI to treat relapse of disease. One of these patients had steroid-resistant grade IV acute GVHD, and the other had limited chronic GVHD.

Disease response and relapses

Current disease status is shown in detail in Table 2. The conditioning regimen induced remissions in 2 of 2 patients with refractory AML and 2 of 2 evaluable patients with myelodysplasia. Of the 6 patients with Hodgkin disease in partial remission or with refractory disease at transplantation, 2 achieved a complete remission (CR) following transplantation, and 4 are progression-free. Seven patients with NHL in partial remission or with refractory disease prior to transplantation were evaluable for disease response (Table

Table 3. Chimerism analysis

Patient no.	Months post-transplantation	Unseparated peripheral blood	Granulocytes	B cells	T cells
1	6		Donor	Donor	Mixed
	10 (post-DLI)		Donor	Donor	Donor
2	4		Donor	Donor	Donor
	14		Donor	Donor	Donor
	25		Donor	Donor	Donor
5	1		Donor	Donor	Donor
	3		Donor	Donor	Donor
	21		Donor	Donor	Donor
6	5		Donor	Donor	Mixed
7	1	Recipient			
9	1		Donor	Donor	Donor
10	1	Mixed			
11	4	Mixed			
12	1		Donor	Donor	Donor
13	1		Donor	Donor	Donor
15	1	Donor			
	5	Donor			
16	1	Donor			
	9	Donor			
19	9		Donor	Donor	Donor
20	8		Mixed	Mixed	Mixed
21	1	Mixed			
	8	Mixed			
	10	Mixed			
22	1	Mixed			
23	3	Donor			
26	1	Donor			
	3	Donor			
27	1	Donor			
	5	Donor			
29	1	Donor			
	5	Donor			
30	1	Donor			
	5	Donor			
31	1	Donor			
32	1	Donor			
34	6		Donor	Donor	Mixed
36	1	Donor			
	5	Donor			
37	3		Mixed	Mixed	Mixed
38	1	Mixed			
	4	Mixed			
39	1	Mixed			
40	3		Donor	Donor	Donor
43	3		Donor	Donor	Mixed
44	3	Donor			

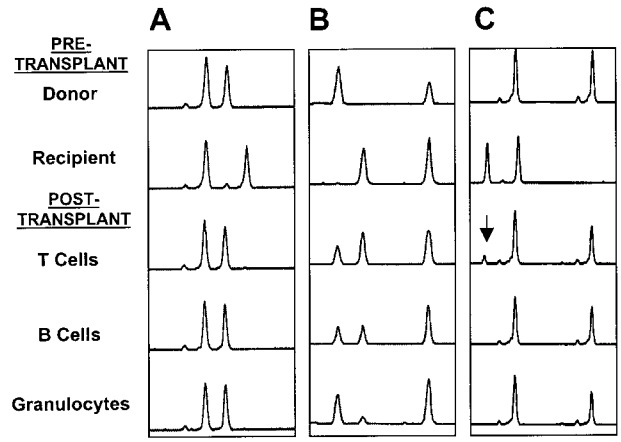

Figure 1. Microsatellite analyses. (A) Full-donor chimerism in all 3 lineages; (B) mixed chimerism in all 3 lineages; and (C) mixed T-cell chimerism (arrow shows recipient T-cell marker) with full-donor chimerism and B cells and granulocytes.

2). Two of these patients achieved a CR, and 3 remain progression-free; however, 2 patients with transformed low-grade NHL had early post-transplantation disease progression (Table 2). None of the 6 patients with myeloma achieved a CR following the transplantation procedure alone, although patient No. 11 achieved a CR following post-transplantation DLI.

Seven patients relapsed or progressed following transplantation (Table 2). Patient No. 1, with mantle cell lymphoma, relapsed 9 months post-transplantation and was treated with DLI. He died 11 months post-transplantation with steroid-resistant grade IV GVHD. Patient No. 12, with relapsed AML, progressed 3 months post-transplantation and was treated with DLI but died 3 months later of disease progression. Patient No. 13, with refractory AML, relapsed 6 months post-transplantation, was treated with DLI, and achieved transient remission but relapsed and died 14 months post-transplantation. Patient No. 14, with Hodgkin disease, relapsed 15 months post-transplantation and was treated with DLI and has not yet responded to this therapy. Patient No. 25, with refractory transformed low-grade NHL, progressed shortly after engraftment and, despite DLI, died a month later. Another patient with transformed low-grade NHL, patient No. 35, progressed 3 months post-transplantation. Patient No. 43 had progression of myeloma following transplantation.

Survival analyses

The median follow-up of the patients is only 9 months. The Kaplan-Meier estimated probabilities of nonrelapse mortality, progression-free survival, and overall survival for all 44 patients are shown in Figure 2. The estimated probability of nonrelapse mortality at 12 months was 11% (95% confidence interval [CI], 4%-26%). The estimated probability of progression-free survival at 12 months was 71% (95% CI, 53.6%-84.2%). The estimated probability of overall survival at 12 months was 73.2% (95% CI, 44.3%-88.7%).

Discussion

TRM remains a major obstacle to successful allogeneic SCT. The introduction of nonmyeloablative purine analogue conditioning regimens has facilitated allogeneic engraftment while limiting regimen-related mortality.[8-11,13] Despite this, GVHD remains a significant cause of mortality and morbidity following nonmyeloablative conditioning. Previously published results reported using other nonmyeloablative conditioning regimens have shown a 38% to 60% incidence of grade II-IV acute GVHD.[8-11,13] This was the primary cause of death in some patients.

In our study, the incidence of GVHD was exceptionally low. No patients had grade III-IV acute GVHD, and only 2 patients (5%) developed grade II acute GVHD. The incidence of chronic GVHD was also low, with only 1 patient developing limited skin GVHD. While the incidence of chronic GVHD cannot yet be fully assessed in some of the patients because of relatively short follow-up, given the fact that only 1 patient has experienced this complication and that all but 3 of the patients are off all immunosuppression, we anticipate a very low rate of chronic GVHD. Because the use of post-transplantation CsA was similar to other nonmyeloablative regimens, the differences in the incidence and severity of GVHD may in part reflect the in vivo use of the humanized monoclonal antibody CAMPATH-1H as part of the conditioning regimen.[18] This was administered to the patients on days −8 to −4 prior to transplantation. Because CAMPATH-1H has a prolonged half-life in humans, there was significant circulating antibody when the unmanipulated donor PBSCs or bone marrow was infused into the recipient, resulting in a degree of in vivo T-cell depletion. This combination of in vivo CAMPATH-1H together with CsA appears to have been very effective in preventing GVHD in both sibling and unrelated donor allograft recipients. A number of these patients have had their CsA discontinued between 1 and 3 months post-transplantation without the development of GVHD.

While our nonmyeloablative conditioning regimen facilitated engraftment in all but one of the evaluable patients, many of the patients were mixed chimeras. Some patients were mixed chimeras in all lineages tested, while others were only mixed chimeras in the T-cell lineage. It has been demonstrated that patients who are mixed chimeras may experience less GVHD than full-donor chimeras.[19,20] On the other hand, mixed chimerism may diminish the potential benefit of the GVL effect seen in the allograft setting.[21,22] While mixed chimeras can be converted to full-donor chimeras following DLI,[5] this was not attempted as part of this pilot study. The primary end points of this study were to explore the incidence of durable engraftment and acute and chronic GVHD. DLIs were only given for overt relapse of disease and were not given prophylactically or pre-emptively because we wished to assess the impact of the conditioning regimen on disease control and relapse. In the present study, we were generally not able to show the benefit of DLI in the setting of post-transplantation

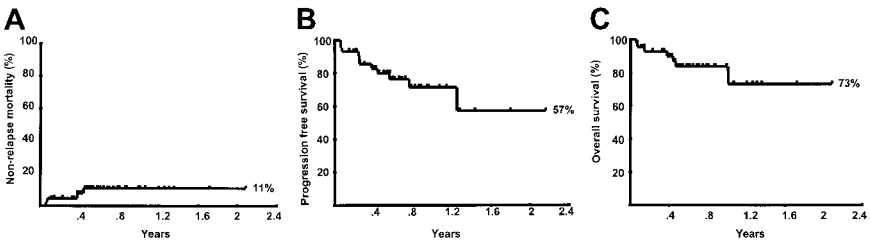

Figure 2. Kaplan-Meier curves. (A) Nonrelapse mortality; (B) progression-free mortality; and (C) overall survival for the entire group of 44 patients.

relapse. DLIs were either ineffective or led to toxicity from GVHD in all but one of the patients treated. This is not surprising, because the response rate in relapsed AML is low and there are few data to show that DLIs are effective in inducing remissions in patients with aggressive lymphomas.[23,24] Only a single patient with multiple myeloma has achieved CR following DLI therapy.

The use of this conditioning regimen has been relatively safe in a group of patients that had many high-risk features: patients who had prior high-dose therapy, patients with renal or cardiac impairment, or patients with high-risk diagnoses for allogeneic SCT such as Hodgkin disease or multiple myeloma. Indeed, allogeneic transplantation using myeloablative conditioning following failed autologous transplantation has been associated with a treatment mortality ranging between 50% and 80%.[25,26] Undoubtedly, such a high mortality rate may offset a potential for cure, and therefore conventional transplants have generally been avoided in such patients. In our study, 19 patients received a second transplant, and only 3 patients (17%) died of transplantation-related complications, demonstrating that this nonmyeloablative approach could be attempted if a second transplant has to be considered.

While a conditioning regimen containing fludarabine and melphalan appears to have been active in tumor control, particularly in patients with Hodgkin disease and NHL, the follow-up period is still very limited and all patients remain at risk of relapse. In such a high-risk group of patients, any conditioning regimen is likely to be associated with a significant relapse risk, and therefore the survival curves shown in Figure 2 should be interpreted with caution. This is particularly so in some hematologic malignancies, such as acute leukemia, where the GVL effect of DLI for the treatment of relapse is of limited efficacy.[23] However, the antitumor responses seen with the conditioning regimen might allow the use of DLI to be delayed until 6 to 12 months post-transplantation, when this intervention might be associated with less GVHD.[5,27,28]

In summary, our results show that our nonmyeloablative regimen facililitates allogeneic engraftment, with a low incidence of GVHD and TRM. The long-term antitumor activity of this regimen remains unknown; however, if used in combination with the prophylactic or pre-emptive use of DLI, prolonged remissions might be obtained in some types of hematologic malignancies.

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