Non-myeloablative conditioning regimen of fludarabine, busulfan, anti-thymocyte globulin, and methylprednisolone for allogeneic peripheral blood hematopoietic cell transplantation

Eighteen adult patients were transplanted with hematopoietic cells from HLA-identical siblings after a fludarabine-based non-myeloablative conditioning. Seventeen achieved granulocyte engraftment on a median of day 11. The proportion of patients having complete donor chimerism increased from 40% at 1 month to 82% at 2 and 100% at 4 months.

We initiated a study to investigate whether a fludarabine-based non-myeloablative conditioning is sufficiently immunosuppressive[1-6] to allow engraftment of allogeneic hematopoietic cells in a majority of the patients.

Eligible patients belonged to one of the following categories; patients with leukemia not eligible for conventional bone marrow transplantation (BMT) because of age (>50 years) or comorbidities, patients with indolent hematologic disorders such as myelodysplastic syndrome (MDS) or paroxysmal nocturnal hemoglobinuria (PNH), and patients with various treatment-refractory malignancies. Patients were nursed in regular hospital beds and given fludarabine (Berlex Laboratories) 30 mg/m^2 iv days –7 to –2, busulfan 4 mg/kg po days –7 and –6, antithymocyte globulin (ATG) (Upjohn) 20 mg/kg iv days –5 to –2, and methylprednisolone 2 mg/kg iv days –5 to –2. Peripheral blood mononuclear cells were collected from the donors on the fourth and fifth days of granulocyte colony-stimulating factor (G-CSF) administration (10 µg/kg 4 days) and infused into the patients on the same days (days 0 and 1). Cyclosporine 1.5 mg/kg iv was given every 12 hours starting day –1, then switched to an appropriate oral dose when feasible. Cyclosporine dose was tapered starting day 30 (first 9 patients) or 60 by 10% each month. G-CSF 450 µg iv was administered daily starting day 5 until absolute neutrophil count (ANC) was over 3,000/µL. Acute and chronic graft-versus-host disease (GVHD) and veno-occlusive disease of the liver (VOD) were classified according to appropriate criteria.[7-9] Regimen-related toxicities were classified according to WHO criteria. Hematopoietic chimerism was evaluated using peripheral blood and polymerase chain reaction (PCR) amplification of short tandem repeats or amelogenin loci[10] monthly for 6 months, then once every 3 months for 2 years.

Between July 1999 and December 2000, 18 patients were enrolled into the study and all completed conditioning and received donor cell infusions (Table 1). The median age of the patients was 39.5 years (range, 21-59). Seventeen of the 18 patients achieved an ANC over 500/µL on a median of day 11 (range, 9-16). Thirteen of the 18 patients achieved a platelet count over 20,000/µL on a median of day 12 (range, -7-53). One each among 18 patients did not require red cell or platelet transfusion. Two patients (both with MDS) experienced graft failure (Table 2). Eight (44%) developed acute GVHD (grade II, 3; grade III, 2). Eight of 11 evaluable patients (72%) developed chronic GVHD (extensive, 6). All patients developed fever associated with ATG. Six (33%) suffered grade III/VI stomatitis. Three had mild VOD with maximum bilirubin levels of 5.3-7.0 mg/dL. Five (UPNs 122, 132, 138, 146, and 169) died of progressive disease of underlying malignancies. Two (UPNs 137 and 168) died of GVHD. One each died of graft failure (UPN 114), CNS bleeding (UPN 161), and sepsis (UPN 162).

Two patients died before the 1-month hematopoietic chimerism assay was performed (Table 2). The proportion of patients having complete donor chimerism (CC) increased from 40% at 1 month to 82% at 2 months and 100% at 4 months after transplantation. Two of 8 patients with normal or remission bone marrow showed recipient DNA at 1 month while all of 7 patients with abnormal marrow showed recipient DNA at 1 month (p=0.004 by chi-squared test).

Table 1. Patient and donor characteristics.

Characteristics	N=18
Median age, yr (range)	39.5 (21-59)
Sex	
Male	11
Female	7
Diagnosis and disease status at HCT	
Leukemia with old age or cormorbid condition	3
AML, 1st CR	2
Age over 55	1
Candida abscess in the liver	1
ALL, 1st CR	1
Age over 55	1
Low risk hematologic disorders	4
MDS	2
RA	1
RARS	1
PNH	2
Refractory malignancies	
NHL	5
PTL, chemo-refractory	2
Mantle cell, chemo-refractory	1
Angiocentric, chemo-refractory	1
Lymphoblastic, sensitive relapse	1
HD, chemo-refractory	1
MM, in partial remission	1
RCC, IFN-refractory	2
Ovarian cancer, chemo-refractory	1
MFH, chemo-refractory	1
Donor median age, yr (range)	40.5 (16-55)
Donor sex	
Male	12
Female	6
HLA matched sibling	18
Number of cells infused, median (range)	
Mononuclear cells (×10^8/kg)	6.70 (2.98-12.87)
CD34$^+$ cells (×10^6/kg)	5.34 (0.11-17.45)
CD3$^+$ cells (×10^8/kg)	4.33 (1.70-8.75)

HCT, hematopoietic cell transplantation; MDS, myelodysplastic syndrome; RA, refractory anemia; RARS, refractory anemia with ringed sideroblast; NHL, non-Hodgkin's lymphoma; PTL, peripheral T-cell lymphoma; HD, Hodgkin's disease; MM, multiple myeloma; RCC, renal cell carcinoma; IFN, interferon; MFH, malignant fibrous histiocytoma; PNH, paroxysmal nocturnal hemoglobinuria.

The duration of administration of conditioning was 6 days in our study as opposed to 10 days in the study by Slavin *et al.*,[6] although the total doses of fludarabine and busulfan were same. Our study showed that allogeneic hematopoietic cell transplantation utilizing fludarabine-based non-myeloablative conditioning achieved reliable engraftment in a majority of the patients. After BMT with BuCy2 conditioning in our hospital, 53% and 67% of 76 patients showed CC at 1 and 2 months, respectively (unpublished data). Our experience in a limited number of patients showed that the kinetics of donor cell chimerism is comparable between the two groups of patients. The conditioning regimen utilized in our study was tolerated well even in the patients who were older than 50 years. The degree and dura-

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Table 2. Results of assays of hematopoietic chimerism and clinical outcome.

UPN	Age/ sex	Dx	Pro- cedure	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	9 mo	12 mo	15 mo	18 mo	21 mo	24 mo	Status (post-HCT months)
107	31/F	PNH	HCT	**10***	CC	CC	CC	CC	ND	CC	CC	CC	CC	CC	CC	Alive and NED (24.0)
111	57/F	ALL	HCT	**15**	CC	CC	CC	CC	ND	CC	CC	CC	CC	CC		Alive and NED (22.6)
114	50/M	MDS	HCT DLI	**100** CC	CC	death										Primary engraftment failure Died with aGVHD and CMV pneumonia after cytoxan + DLI (3.5)
115°	41/F	AML	HCT	donor	donor	donor	donor	ND	donor	ND	ND	ND	ND	ND		Alive and NED (21.9)
122	29/F	OC	HCT	CC	death											Died with progressive disease (1.6)
128	21/F	PNH	HCT	**<5**	CC	**10**	CC	CC	ND	CC	CC	CC	CC			Alive and NED (17.3)
132	38/M	MFH	HCT	death												Died with progressive disease (0.9)
137	59/F	RCC	HCT	CC	death											Died in PR with aGVHD (1.9)
138	25/M	NHL	HCT DLI	**20** **31**	**40** **13**	**85** CC	CC	CC	death							Progressive disease Died with progressive disease (9.2) after salvage chemotherapy+ DLI
143	35/M	NHL	HCT	**19**	CC	CC	CC	ND	CC	ND						Alive in PR (16.0)
146	27/M	NHL	HCT	CC	CC	death										Died with progressive disease (2.3)
148	29/M	MM	HCT DLI	**10** CC	CC	CC	CC	ND	ND	CC	CC					PD after PR Alive in PR (15.6)
156	57/M	AML	HCT	CC	CC	CC				death						Died in relapse (9.7)
161	48/M	NHL	HCT	death												Died with CNS bleeding (0.5)
162	56/M	NHL	HCT	CC	death											Died with sepsis (1.6)
168	53/M	HD	HCT	CC	CC	CC										Died in PR with chronic GVHD (4.0)
169	46/M	RCC	HCT	**25**	CC	CC	CC	CC		death						Died with progressive disease (7.7)
175	32/F	MDS	HCT DLI	**42** **88**	**73** **83**											Secondary graft failure Alive in secondary graft failure (7.1)

The numbers in **bold are the proportions of recipient DNA. °The patient-donor pair of UPN 115 did not have a suitable marker gene for the recipient DNA. However, post-HCT PCR analysis showed that the patient's blood cell DNA was donor-origin. UPN, unique patient number; Dx, diagnosis; mo, month; HCT, hematopoietic cell transplantation; DLI, donor leukocyte infusion; CC, complete donor chimerism; ND, not done; PNH, paroxysmal nocturnal hemoglobinuria; MDS, myelodysplastic syndrome; OC, ovarian cancer; MFH, malignant fibrous histiocytoma; RCC, renal cell carcinoma; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; NED, no evidence of disease; PR, partial remission.*

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tion of myelosuppression was less with the non-myeloablative conditioning regimen. Our data on hematopoietic chimerism showed that the likelihood of achieving CC at 1 month after HCT was related to bone marrow status at the time of transplantation. Both of the two patients with MDS experienced graft failure. Further studies are warranted to investigate the effect of bone marrow status at the time of transplantation on donor hematopoietic cell chimerism and engraftment.

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