—Report on Experiments and Clinical Cases—

Stem Cell Transplantation Using Non-myeloablative Conditioning Regimen with Fludarabine for Hematological Malignancies

Kenji Tajika, Hiroki Yamaguchi, Taro Mizuki,
Hiroyuki Nakamura, Kazutaka Nakayama and Kazuo Dan

Department of Pathophysiological Management/Medical Oncology, Graduate School of Medicine, Nippon Medical School

Abstract

Stem cell transplantation (SCT) is a useful treatment for hematological malignancies, but it is limited to younger patients because of its high treatment-related mortality. Fludarabine (Flu), a novel anticancer agent with potent immunosuppressive activity, used as a conditioning regimen (reduced intensity transplantation; RIST), can decrease treatment-related mortality, as recently reported. However, the best drug combination and the best timing for RIST remain unknown. We herein report the SCT outcomes of 36 patients undergoing Flu treatment at our institution since December 2002 and retrospectively analyze the results. RIST conditioning with Flu was well-tolerated. No severe toxicity related conditioning regimens was observed in our patients, even though there were 10 patients with a history of autologous (n=5) or allogeneic stem cell transplantation (n=5). Hematological engraftment was found in 33 patients. The median times for reconstitution of WBCs, RBCs, and platelets were 16 days, 27.5 days and 34 days, respectively. Stable complete donor chimerism after SCT was present in all patients with WBC engraftment, and no patients experienced late rejection. Thirty-two patients were evaluated for acute graft versus host disease (aGVHD). Nine patients had no aGVHD. The incidence of grade I/II and III/IV aGVHD was 78% and 22%, respectively. Skin lesions were the major sites of involvement. Gut involvement was present in 9 patients. All 4 patients with grade IV GVHD had stage four hepatic GVHD. Twenty-two patients were analyzed for chronic GVHD (cGVHD). Twelve patients had no cGVHD, 6 had limited type and 4 had extended type. The overall survival (OS), relapse rate (RR), and non-relapse mortality (NRM) in all patients over 7 years were found to be 41.7%, 20.1%, and 34.6%, respectively. Induction failures were present in 5 cases of AML and 1 case of NHL. Disease progression was the primary cause of death, which occurred in 12 of 21 patients. Six patients died of grade IV GVHD (n=2) or complicated fungal infection contracted during the GVHD treatment (n=4). One patient died of secondary MSD, which originated from donor hematopoietic cells. Two patients died of cerebral bleeding and cardiac rapture, respectively. We found that the patients' state on SCT was the most important factor in long-term survival. The OS of standard risk and high risk patients with hematological malignancies were 75% and 30.3%. We concluded that stem cell transplantation using a non-myeloablative conditioning regimen with Flu was a useful therapeutic approach for patients with hematological malignancies.
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Key words: stem cell transplantation, non-myeloablative conditioning, fludarabine

Correspondence to Kenji Tajika, MD, PhD, Division of Hematology, Department of Internal Medicine, Nippon Medical School, 1–1–5 Sendagi, Bunkyo-ku, Tokyo 113–8603, Japan
E-mail: tajika@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

Introduction

Stem cell transplantation (SCT) is a useful treatment for hematological malignancies, such as acute leukemia and malignant lymphoma. However, it is limited to younger patients because of its high treatment-related mortality (TRM). Fludarabine (Flu), a novel anticancer agent with potent immunosuppressive activity, is a new drug that may overcome this problem[1,2]. Using this new agent as a conditioning regimen (reduced intensity transplantation, or RIST) can reduce TRM, and many patients who would have been excluded from transplantation because of their age or impaired organ function can also obtain the benefits of SCT[1,3–5]. However, the best drug combination and the best timing for RIST remain to be characterized. We herein report our SCT results using Flu at our institution since December 2002 to evaluate the feasibility and efficacy of this type of SCT.

Patients and Methods

Patients

Between December 1, 2002, and December 31, 2008, 36 consecutive patients with hematological malignancies received RIST with Flu at our hospital. Results were analyzed in January 2010. All patients were ineligible for myeloablative SCT because of their age, a history of previous SCT, or impaired vital organ-functions. The patient characteristics are summarized in **Table 1**. There were 23 men and 13 women, and the median age was 57.0 years (range, 19–67).

Stem cell transplantation

Eighteen patients were treated with fludarabine (Flu, 25 mg/m^2/day ×6), oral busulfan (Bu, 1.0 mg/kg every 8 hours ×6), and total body irradiation (TBI). Since July 2007, new forms of Bu for intravenous use have been available in Japan; 8 patients received Flu (25 mg/m^2/day ×6), intravenous Bu (ivBu, 0.8 mg/kg every 8 hours ×6), and TBI. Five patients were conditioned with Flu (30 mg/m^2/day ×3), cyclophosphamide (CY, 750 mg/m^2/day ×3), and TBI. Two patients were conditioned with Flu (25 mg/m^2/day ×6), one dose of melphalan (MEL, 140 mg/m^2) and TBI. The dose of TBI for each case is shown in **Table 1**. Three patients were conditioned without TBI; Flu (25 mg/m^2/day ×5) and Bu (1.0 mg/kg ×6) (n=2), Flu (30 mg/m^2/day ×3) and CY (750 mg/m^2/day ×3) (n=1). Twenty-four patients received cord blood cells (CB) from HLA-mismatched unrelated donors, seven patients received granulocyte colony-stimulating factor (G-CSF)-stimulated peripheral blood cells (G-PB) from HLA identical siblings, 3 patients received bone marrow cells (BM) from HLA-identical unrelated donors, and 2 patients received BM from HLA-identical siblings, respectively.

Cicrosporin (CSA) or tacrorimus (FK) was used for prophylaxis against acute graft-versus-host disease (GVHD), accompanied by or without short-term methotrexate.

Chimerism Analysis

The chimerism of hematopoietic cells after SCT was assessed by typing the informative alleles of short tandem repeat (STR) loci on several chromosomes or by fluorescent *in situ* hybridization (FISH) with X and Y chromosome probes in sex-mismatched SCT.

Statistical Analyses

The overall survival (OS) was estimated by the Kaplan-Meier method. Comparison of the survival between the standard and high-risk groups was analyzed with the generalized Wilcoxon test. A high-risk disease status was defined as acute leukemia beyond a second complete remission (CR) or without hematological remission, chronic myelogenous leukemia (CML) with accelerated phase, myelodysplastic syndrome (MDS) beyond refractory anemia with excess of blast-1 (REAB-1), and non-Hodgkin lymphoma (NHL) beyond CR or a good partial remission (PR).

Table 1 Patient characteristics

Age	Sex	Diagnosis	Status	Conditioning	SCT	aGVHD	cGVHD	Results
61	F	AML	>3 CR	Flu/Bu/TBI (4)	CBT	Ⅱ	NE	dead
32	M	Ph-ALL	>CR	Flu/Bu/TBI (4)	CBT-2	Ⅳ	NE	dead
58	M	MDS/AL	failure	Flu/Bu/TBI (4)	CBT	Ⅱ	NE	dead
56	M	MDS	RA	Flu/Bu/TBI (4)	PB	Ⅱ	extent	dead
50	F	NHL	>3 relpase	Flu/Bu/TBI (4)	CBT-2	Ⅱ	limit	dead
61	M	MM	good PR	Flu/Cy/TBI (4)	CBT-2	Ⅰ	limit	dead
57	F	NHL	>3 relapse	Flu/Bu/TBI (4)	CBT-2	Ⅱ	limit	alive
59	M	Ph-ALL	relpase	Flu/Cy/TBI (8)	CBT	Ⅱ	—	dead
57	M	NHL	>3 CR	Flu/Bu	PB-2	0	—	alive
65	M	NHL	relpase	Flu/Bu/TBI (4)	CBT	NE	NE	dead
30	M	MDS	RAEB-1	Flu/Cy/TBI (4)	CBT	Ⅰ	—	alive
67	M	AML	failure	Flu/Bu/TBI (9)	CBT	Ⅰ	extent	alive
41	M	AML	2 relpase	Flu/Bu/TBI (4)	PB-2	Ⅳ	NE	dead
42	M	MDS/AL	no treat	Flu/Bu/TBI (4)	CBT	Ⅰ	NE	dead
56	M	CML	AP	Flu/Bu	PB	0	extent	dead
64	F	NHL	CR	Flu/Bu/TBI (4)	CBT	0	—	alive
57	M	NHL	2 relpase	Flu/Cy/TBI (4)	CBT	Ⅱ	—	dead
58	F	NHL	good PR	Fly/Cy	PB	0	—	alive
64	F	MDS/AL	no treat	Flu/Bu/TBI (4)	BM-2	Ⅳ	NE	dead
63	F	CML	AP	Flu/Bu/TBI (4)	CBT	Ⅰ	—	alive
45	M	NHL	failure	Flu/Bu/TBI (9)	PB	Ⅳ	NE	dead
47	M	MDS	RAEB-2	Flu/Cy/TBI (4)	CBT	Ⅱ	extent	alive
19	M	AML	relpase	Flu/Bu/TBI (9)	BM-2	Ⅰ	—	alive
50	F	AML	relpase	Flu/MEL/TBI (9)	PB-2	Ⅲ	NE	dead
48	F	NHL	failure	Flu/Bu/TBI (4)	CBT	0	—	alive
58	M	hypo-AML	no treat	Flu/Bu/TBI (9)	CBT	0	NE	dead
49	F	MF	—	Flu/Bu/TBI (4)	CBT	0	NE	dead
55	M	NHL	CR	Flu/ivBu/TBI (4)	u	0	—	alive
58	F	Ph-ALL	CR	Flu/ivBu/TBI (4)	u	Ⅰ	—	alive
63	M	MDS/AL	no treat	Flu/ivBu/TBI (4)	CBT	NE	NE	dead
41	F	AML	relpase	Flu/ivBu/TBI (4)	CBT-2	Ⅱ	limit	dead
54	M	NHL	CR	Flu/ivBu/TBI (4)	CBT	Ⅱ	limit	alive
59	M	Ph-AML	failure	Flu/ivBu/TBI (4)	CBT	NE	NE	alive
60	M	AML	failure	Flu/ivBu/TBI (4)	CBT	Ⅱ	limit	dead
44	F	AML	failure	Flu/MEL/TBI (6)	CBT	NE	NE	dead
60	M	Ph-ALL	CR	Flu/ivBu/TBI (4)	u	0	—	alive

AML; acute myelogenous leukemia, Ph-ALL; Ph-positive acute lymphoblastic leukemia, Ph-AML; Ph-positive AML, MDS/AL; myelodysplastic syndrome/acute leukemia, NHL; non-Hodgkin lymphoma, MM; multiple myeloma, CML; chronic myelogenous leukemia, Hypo-AML; hypoplastic AML, MF; myelofibrosis
Status; status on SCT, >3 CR; beyond third complete remission, failure; induction failure, RA; refractory anemia, 3> relpase; beyond third relpase, PR; partial remission, relapse; first relapse, RAEB; refractory anemia with excess of blast, 2 relapse; second relapse, no treat; no treatment, AP; accelerated phase,
TBI (4); total body irradiation (4 Gy), CBT; cord blood transplantation, CBT-2; second CBT, PB; allogeneic peripheral blood transplantation, PB-2; second allogeneic peripheral blood transplantation, BM-2; second bone marrow transplantation,
u; unrelated bone marrow transplantation
NE; not evaluated

Results

Engraftment

Hematological engraftment was found in 33 patients. The median time for reconstitution of myelopoiesis (WBCs more than 1,000/μL) was 16 days (range; 8–36 days). Autologous hematological recovery was found in a patient with Ph-positive AML. Two other patients could not be evaluated

because of the early appearance of leukemic cells. RBCs (reticulocytes greater than 1%) and platelet engraftments (platelets more than 20,000/μL) were found at a median time of 27.5 days (n= 28, range; 13–98 days) and 34 days (n= 26, range; 12–121 days), respectively.

Complete donor chimerism was found 30 days after SCT in all patients with WBC engraftment. Complete donor chimerism was also stable, and no patients experienced late rejection.

Graft Versus Host Disease (GVHD)

Thirty-two patients were evaluated for acute graft versus host disease (aGVHD). Nine patients had no aGVHD. The incidence of grade I/II and III/IV aGVHD was 78% and 22%, respectively. Skin lesions were the major sites of involvement (19 of 32 patients). Gut involvement was found in 9 patients. All 4 patients with grade IV GVHD had stage four hepatic GVHD. Twenty-two patients could be analyzed for chronic GVHD (cGVHD). Twelve patients had no cGVHD, 6 had limited type and 4 had extended type.

Early and Late Complications

RIST conditioned with Flu was well-tolerated. No severe toxicity related conditioning regimens was observed in our patients, even though there were 10 patients with a history of autologous (n=5) or allogeneic stem cell transplantation (n=5) (**Table 1**).

The major late complications included bacterial or fungal infection during the treatment of GVHD. Five patients suffered from pneumonia (2 were bacterial, and 3 were fungal), and 1 patient suffered from pneumococcal meningitis.

Three patients developed malignancy after SCT; one had secondary myelodysplastic syndrome (MDS) 537 days after SCT, one had uterine cancer 2,516 days after SCT, and one had post-transplantation lymphoproliferative disorder related with Epstein-Barr virus (EBV) 126 days after SCT; this patient was successfully treated with rituximab and involved field-radiation.

Survival and Causes of Death

Figure 1 shows OS, relapse rate (RR) and non-relapse mortality (NRM) of all patients. OS, RR and NRM 7 years after SCT were 41.7%, 20.1% and 34.6%, respectively. Five patients with AML and 1 patient with NHL could not achieve remission after SCT. Relapse was documented in patients with AML (n=2), ALL (n=2), MM (n=1), and NHL (n=1). Disease progression was the major cause of death, which occurred in 12 (55%) of 21 patients. Six patients died because of grade IV GVHD (n=2) or complicated fungal infection during the treatment of GVHD (n=4). One patient died because of secondary MDS which originated from donor hematopoietic cells. Two other patients died because of cerebral bleeding and cardiac rapture, respectively.

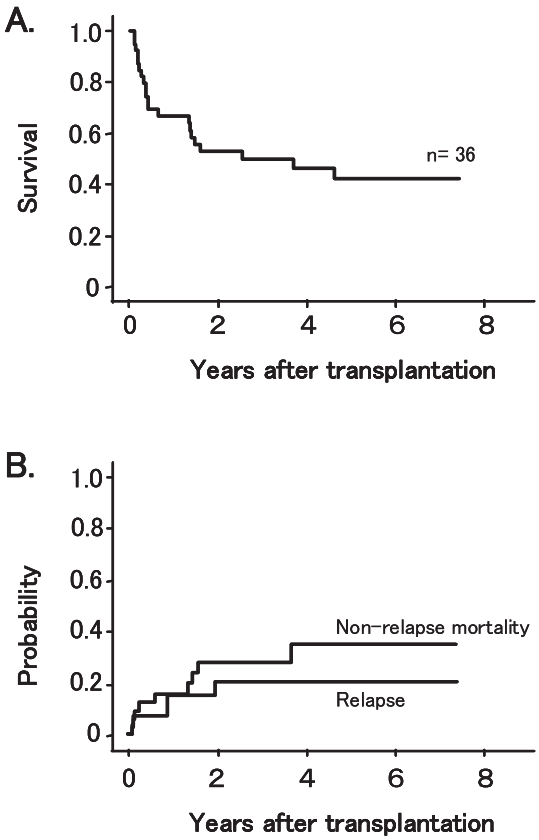

Fig. 1 Overall survival (OS) (A) and cumulative incidence of relapse and non-relapse mortality (B) are shown. The OS of 7 years was found to be 41.7%. The cumulative incidence of relapse and non-relapse mortality of 7 years were 20.1% and 34.6%, respectively.

Factors in Survival

Figure 2 shows the difference of the overall survival of patients with leukemia and lymphoma

according to the disease status on SCT. The OS of standard risk and high-risk patients were 75% (n=9) and 30.3% (n=26) (P<0.01). Patients with aggressive lymphoma such as NK blastic cell lymphoma (n=1), AITL (n=1), and ATLL (n=1) or with Ph-positive ALL (n=1) who were considered to be incurable using conventional chemotherapy, received SCT on complete remission (CR) and all could obtain a long-term complete remission after SCT.

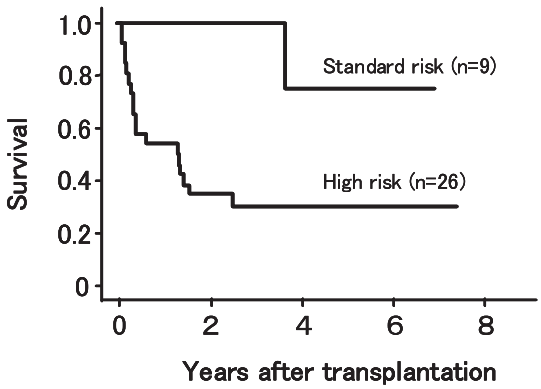

Fig. 2 Differences of OS between standard and high-risk patients are shown. The OS of 7 years was 75% in patients with a standard risk on SCT and 30.3% in those with a high risk. The difference of OS between these two groups was statistically significant (P<0.01).

Discussion

In the present retrospective study, we confirmed the feasibility of RIST conditioned with Flu. Saito et al. demonstrated that RIST from HLA-identical sibling G-PB conditioned with Flu and Bu was associated with acceptable toxicities in older patients for hematological malignancies[3]. Sobecks reported that 400 cGy TBI and Flu are tolerated for RIST with HLA-identical sibling or unrelated G-PB[4]. In the present study, the median subject age was 57 years, and 10 patients had received SCT prior to RIST. We used the BM from HLA-identical sibling donor or unrelated donors, G-PB from sibling donors, or CB as the source of stem cells. Acute toxicities in the present series were also tolerable, even for patients at the second transplantation, as previously mentioned by other authors[2–5].

We also evaluated the efficacy of RIST on hematological malignancies. The 7-year OS among all patients in the patient series was 41.7%, which is similar to the survival rate in other recent reports. Valcárcel et al. demonstrated that they could sustain remissions for patients with high-risk AML and MDS after RIST with 45% of 4-year OS[6]. Armand et al. showed the usefulness of RIST for lymphoma[7]. The 3-year OSs of patients with Hodgkin's lymphoma, indolent lymphoma, and aggressive lymphoma were found to be 56%, 81%, and 42%, respectively. Cho et al. concluded that RIST is a potential therapeutic approach for adults with high-risk ALL in remission with 64% of 3-year OS[8]. There were some differences in the factors influencing OS among the previous reports. This may be due to the patient heterogeneity, sample size, or analysis methods. However, many authors commonly insisted that transplantation at the first CR had favorable effects on the SCT results. Our study confirmed that the most important factor for the success of SCT was the patient's disease status. As shown in **Figure 2**, patients with standard-risk malignancies obtained long-term survival (P<0.01), even though the disease itself was refractory to conventional chemotherapy such as angioimmunoblastic T cell lymphoma, NK/blastic lymphoma and adult T cell leukemia lymphoma.

The impact of the conditioning drugs on survival remains to be characterized. Sobecks et al. demonstrated that 4 Gy TBI with Flu was a better conditioning regimen than the 2 Gy TBI regimen[4]. Shimoni et al. reported that Flu/MEL is associated with a lower incidence of relapse than Flu/Bu[9]. Recently, Simoni et al. also reported that Flu and treosulfan is not only fully intensive but is also a fully acceptable novel conditioning regimen for allogeneic SCT[10]. We were therefore unable to conclude from the present study which was the best conditioning regimen for RIS-SCT.

There were two secondary malignancies after SCT (MDS and uterine carcinoma). Baker et al. reported that the cumulative incidence for the development of any secondary malignancy was 6.9% at 20 years post-SCT. For MDS or AML, the cumulative incidence plateaued at 1.4% by 10 years post-SCT. However, the cumulative incidence of

developing a solid tumor did not plateau and was found to be 3.8% at 20 years post-SCT[11]. Therefore, we must maintain close follow-up for identifying secondary malignancies in patients after RIST.

We conclude that stem cell transplantation using a non-myeloablative conditioning regimen with fludarabine is a useful therapeutic approach for hematological malignancies.

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