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Unrelated-Donor Bone Marrow Transplantation with a Conditioning Regimen Including Fludarabine, Busulfan, and 4 Gy Total Body Irradiation

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Abstract

We investigated the feasibility of reduced-intensity conditioning with 4 Gy total body irradiation, fludarabine (30 mg/m^2 for 6 days), and busulfan (4 mg/kg for 2 days) for bone marrow transplantation from a serologically HLA-matched unrelated donor. Seventeen adult patients (median age, 55 years; range, 27-67 years) with various hematologic malignancies (6 in remission, 11 not in remission) were treated. Successful engraftment was achieved in all patients at a median of day 18 (range, day 14-35) after transplantation, although subsequent secondary graft failure was observed in 2 patients. The cumulative incidence of acute graft-versus-host disease (GVHD) of grades II to IV at day 100 was 48%. With a median follow-up of 286 days (range, 56-687 days), the rates of 1-year overall survival, 100-day nonrelapse mortality, and 1-year nonrelapse mortality were 41%, 14%, and 46%, respectively. Eleven patients died, and the causes of death were relapse (n = 4), pulmonary complications (n = 4), acute GVHD (n = 2), and sepsis (n = 1). The remaining 6 patients (at transplantation, 2 were in remission, and 4 were not in remission) are currently still in remission. These results suggest that this regimen reduces the risk of graft failure, but further studies are needed to ameliorate transplantation-related toxicities, primarily GVHD and/or pulmonary complications.

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Key words: Unrelated donor bone marrow transplantation; Fludarabine; Busulfan, TBI

1. Introduction

Although allogeneic hematopoietic stem cell transplantation (HSCT) is a possible curative approach for patients with various hematologic malignancies, only 30% to 40% of patients in Japan have an appropriate family donor available [1]. Hence, the application of unrelated-donor transplantation using bone marrow or cord blood cells has been expanding. Another area of current interest is the application of reduced-intensity conditioning regimens, mostly incorporating fludarabine as a primary agent, because conventional allogeneic HSCT using a conditioning regimen with high doses of systemic chemotherapy/radiation is associated with significant toxicities. In contrast, HSCT with a reduced-intensity conditioning regimen allows older patients and those who have contraindicating comorbidities to undergo HSCT [2-7].

Nevertheless, special consideration should be paid to developing reduced-intensity conditioning protocols for the unrelated-donor HSCT setting, because the incidences of both graft rejection and graft-versus-host disease (GVHD) are greater than in related-donor transplantation. In addition, the intensity of the reduced-intensity conditioning regimen influences transplantation-related toxicities and the relapse rate, and the stem cell source (ie, peripheral blood stem cells or bone marrow cells) influences engraftment [8]. Accordingly, several reduced-intensity conditioning protocols have been tested to address a variety of problems [8-17]. In this study, we investigated the feasibility of bone marrow transplantation (BMT) from a serologically HLA-matched unrelated donor with a regimen containing

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4 Gy of total body irradiation (TBI), fludarabine (Flu), and busulfan (BU).

2. Patients and Methods

2.1. Patients and Donors

The data for adult patients with hematologic malignancies who underwent unrelated-donor BMT through the Japan Marrow Donor Program between June 2002 and December 2003 at the National Cancer Center Hospital were analyzed retrospectively. This protocol was approved by the Ethics Committee, and written informed consent was obtained from each patient. The patients who were enrolled in this study were ineligible for conventional allogeneic HSCT because of age (older than 50 years) and/or concomitant diseases or preceding intensive therapies, such as autologous HSCT or multiple chemotherapies. Donor-recipient pairs were selected on the basis of serologic matching for HLA-A and HLA-B and molecular matching for HLA-DRB1. HLA allele typing was performed by intermediate-resolution polymerase chain reaction (PCR) analysis. The stem cell source, which was determined by the Japan Marrow Donor Program donor center, was bone marrow in all cases.

2.2. Treatment Plan and Evaluations

The conditioning regimen consisted of 30 mg/m^2 Flu intravenously daily for 6 days (day −8 to day −3), 4 mg/kg BU orally daily for 2 days (days −6 and −5, without BU dose adjustment), and 4 Gy TBI without lung shielding (day −9 or day −1, single dose or 2 divided doses). Non–T-cell–depleted bone marrow was infused on day 0. The time of neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9$/L, and the time of platelet engraftment was defined as the first of 7 consecutive days with a platelet count $\geq 20 \times 10^9$/L without transfusion support. Granulocyte colony-stimulating factor (G-CSF) was administered at 300 μg/m^2 from day 6 and continued until neutrophil engraftment. The degree of donor chimerism among peripheral blood mononucleated cells was evaluated by PCR analysis of short tandem repeat polymorphisms with fluorescently labeled primers. Secondary graft failure was defined as cytopenia with an absolute neutrophil count $< 0.1 \times 10^9$/L or decreasing chimerism not associated with relapsing disease in patients who had recovered in the early posttransplantation period.

GVHD prophylaxis consisted of cyclosporin A (CsA) from day −1 (daily administration of 3 mg/kg by continuous intravenous infusion or 6 mg/kg orally in 2 divided doses) and methotrexate (10 mg/m^2 intravenously on day 1 and 7 mg/m^2 on days 3, 6, and 11). The CsA dosage was adjusted according to the patient's renal function and to maintain therapeutic levels (250-350 ng/mL) with continuous infusion or trough levels (150-250 ng/mL) with oral administration. In patients without GVHD, CsA was tapered from day 100 over a 3- to 6-month period. Standard criteria were used to grade acute and chronic GVHD [18,19]. Chronic GVHD was evaluated in patients who survived at least 100 days and was classified as limited or extensive. Patients who developed acute GVHD ≥grade II were treated with methylprednisolone at 1 to 2 mg/kg per day.

2.3. Supportive Care

Antimicrobial prophylaxis consisted of ciprofloxacin, fluconazole, acyclovir, and trimethoprim/sulfamethoxazole according to our institutional protocol. All patients were nursed in a room equipped with high-efficiency air filtration of particulates. Monitoring for cytomegalovirus (CMV) antigenemia was performed once a week after neutrophil engraftment by means of the horseradish peroxidase–C7 method. Patients positive for CMV antigenemia were started preemptively on ganciclovir therapy.

2.4. Statistical Analysis

Overall survival was calculated from the time of transplantation until death from any cause. Progression-free survival was measured from transplantation until disease progression or death from any cause. Nonrelapse death was defined as death due to any cause other than relapse. Survival curves for overall survival and progression-free survival were estimated by the Kaplan-Meier method.

3. Results

3.1. Patients

The median age of the 17 patients was 55 years (range, 27-67 years; Table 1). The diagnoses were acute myeloid leukemia (AML) (n = 7), myelodysplastic syndrome (MDS) (n = 4), chronic myelogenous leukemia (n = 1), non-Hodgkin's lymphoma (n = 4), and multiple myeloma (n = 1). Six patients were in remission at transplantation, and the remaining 11 were not in remission. Three patients with MDS or AML following MDS underwent unrelated-donor BMT as a primary treatment. Seven donor-recipient pairs were fully matched for HLA-A, HLA-B, and HLA-DRB1 at the allele level, 4 donor-recipient pairs had an allele-level mismatch at the HLA-A locus, and 5 pairs had an allele-level mismatch at the HLA-DRB1 locus. One patient was mismatched with the donor at 3 HLA alleles.

3.2. Engraftment and Chimerism

The median number of infused nucleated cells was 2.7×10^8/kg (range, $0.65\text{-}5.5 \times 10^8$/kg). All patients achieved neutrophil recovery, but 5 patients did not become independent of platelet transfusion during their follow-up period (Table 2). The median times until neutrophil and platelet recoveries were 18 days (range, 14-35 days) and 26 days (range, 15-112 days), respectively (Figure 1). Late graft failure was observed in 2 patients, one of whom had secondary graft failure due to myelosuppression caused by ganciclovir treatment for CMV colitis. In this patient, donor chimerism was not assessed after day 30 when complete donor chimerism was confirmed. In the other case, donor chimerism decreased from 89% on day 30 to 33% on day 60, despite the tapering of CsA from day 30. Chimerism was

Table 1.
Patient and Disease Characteristics*

Patient No.	Age, y/Sex	Disease	Status	Time from Dx to HSCT, mo	HLA Allelic Mismatch	GVH Vector	HVG Vector	Contraindications to Conventional HSCT	Pretransplantation Comorbidities
1	55/F	AML	CR3	117				Age	No
2	52/F	AML	Primary Ref	13	DRB1	1	1	Age + comorbidity	Pneumonia
3	57/F	AML	Rel2	28				Age	Atrial fibrillation
4	55/M	MDS	Primary Ref	3				Age	Atrial fibrillation
5	57/M	MDS	CR1	8				Age	No
6	59/M	CML	CP2	8				Age	No
7	55/M	PTCL	PR	16	DRB1	1	1	Age	Gastric ulcer
8	58/M	AML	Untreated	10	DRB1	1	1	Age	Bronchial asthma, FEV_1 75%
9	59/M	AML	Untreated	33	DRB1	1	1	Age	Bilirubin 1.5 mg/dL
10	52/M	AML	CR1	11	A	1	1	Age	FEV_1 67%
11	57/M	MDS	CR1	13				Age	Prior gastric cancer
12	61/M	AML	CR2	58	A, both DRB1	3	3	Age	No
13	67/F	FL	Primary Ref	58	A	1	1	Age + comorbidity	Dyspnea requiring oxygen
14	27/M	DLBCL	Rel3	38	A	1	0	Prior autologous HSCT	No
15	48/F	MM	Primary Ref	80				Comorbidity	Ventricular septal defect
16	52/F	MDS	Untreated	130	A	1	1	Age	No
17	49/M	FL	Rel1	28	DRB1	1	1	Prior multiple chemotherapies	No

*Dx indicates diagnosis; HSCT, hematopoietic stem cell transplantation; GVH, graft-versus-host; HVG, host-versus-graft; AML, acute myeloid leukemia; CR3, third complete remission; Ref, refractory; Rel2, second relapse; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CP2, second chronic phase; PTCL, peripheral T-cell lymphoma; PR, partial remission; FEV_1, forced expiratory volume in 1 second; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; MM, multiple myeloma.

evaluated by analysis of short tandem repeats in 14 patients, and complete donor chimerism was confirmed in 12 of these patients. One patient who relapsed on day 32 had exhibited 54% donor chimerism on day 30. In the remaining 3 patients who relapsed after transplantation, complete donor chimerism had been achieved by day 30. In the patient who relapsed on day 78, donor chimerism decreased from 100% on day 30 to 64% on day 60. Mixed chimerism was not confirmed in the other 2 patients before disease progression or relapse. The patients without graft failure or relapse did not have mixed chimerism during their follow-up periods.

3.3. Regimen-Related Toxicities and Infections

Regimen-related toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0, and maximum toxicities are shown in Table 3. Fifteen of the 17 patients had grade III oral/pharyngeal mucositis that required morphine as an analgesic. Reversible elevation (grades III-IV) in transaminase and bilirubin levels occurred in 35% and 12% of the cases, respectively. No veno-occlusive disease was observed. Four patients developed transient grade III hyponatremia within 28 days after transplantation. Four patients developed transient pulmonary infiltration or congestive heart failure due to hypercytokinemia at engraftment, and 2 of these patients developed grade II acute GVHD after engraftment. No histologic findings of acute GVHD were seen in the other 2 patients. One patient developed reversible paroxysmal supraventricular tachycardia. One patient developed bloody diarrhea and abdominal pain even after improvement of acute GVHD of the skin, and we diagnosed intestinal thrombotic microangiopathy from the results of a gut biopsy. This patient was successfully managed by diminishing immunosuppressive treatment. Four patients who had blood cultures positive for bacterial infection (*Pseudomonas aeruginosa*, *Acinetobacter lwoffii*, *Corynebacterium* sp, and *Staphylococcus* sp) within 28 days after transplantation were successfully treated with antibiotics. Invasive aspergillosis was encountered in 2 patients (1 proven and 1 possible case). In the proven case, the patient had bronchiolitis obliterans, which was the ultimate cause of death. Of the 17 patients, CMV antigenemia was detected in 12 patients, 2 of whom had CMV colitis.

3.4. Graft-versus-Host Disease

Acute GVHD of grades II to IV was diagnosed in 8 patients (48%; 95% confidence interval [CI], 36%-59%); the GVHD was grade II in 3 patients and grade IV in 5. The median time to the onset of acute GVHD was 32 days (range, 20-81 days) after transplantation (Figure 2A). Two of 4 patients who skipped methotrexate treatment on day 11 because of severe mucositis developed grade IV acute GVHD. Two of the 5 patients with grade IV acute GVHD subsequently died. One of these patients had acute GVHD after the withdrawal of CsA treatment at the time of leukemia relapse, and the other patient had received bone

Table 2.
Transplantation Outcomes*

Patient No.	Time to ANC >0.5 × 10^9/L, d	Time to Platelets >20 × 10^9/L, d	Acute GVHD						Chronic GVHD (Involved Organs)	Follow-up, d	Current Disease Status	Cause of Death
			Grade	Skin	Liver	Gut	mPSL, mg/kg	Response				
1	16	26	IV	3	4	4	2	PG	NE	121	Dead	Acute GVHD
2	35	30	0	0	0	0	—	—	NE	133	Dead	Relapse
3	17	—	0	0	0	0	—	—	NE	56	Dead	Relapse
4	14	15	IV	4	0	0	2	CR	Ext (skin, mouth, eyes, liver, lung)	439	Dead	BO
5	15	22	I	1	0	0	—	—	Ext (skin, mouth, liver)	286	Dead	IP
6	21	38	II	3	0	0	—	—	NE	260	Dead	Relapse
7	14	25	I	2	0	0	—	—	Ext (mouth, liver)	687+	CR, alive	
8	20	30	II	3	1	0	1	PR	Ext (skin)	667+	CR, alive	
9	22	—	II	3	0	0	—	—	Ext (skin, mouth, eyes)	336	Dead	Organizing pneumonia
10	18†	—	0	0	0	0	—	—	NE	94	Dead	Secondary graft failure
11	16	23	0	0	0	0	—	—	Ext (skin, mouth)	564+	CR, alive	
12	16†	—	IV	2	4	3	2	PG	NE	69	Dead	Acute GVHD
13	18	23	I	1	0	0	1	CR	Ext (mouth, eyes, liver)	525+	CR, alive	
14	18	—	IV	3	4	2	1	UE	NE	64	Dead	Relapse
15	14	16	0	0	0	0	—	—	Ext (mouth, eyes)	511+	CR, alive	
16	26	112	0	0	0	0	—	—	Lim (mouth)	463+	CR, alive	
17	22	38	IV	4	0	0	2	CR	Ext (skin, mouth, eyes, liver, lung)	276	Dead	BO + aspergillosis

*ANC indicates absolute neutrophil count; GVHD, graft-versus-host disease; mPSL, methylprednisolone; PG, progressive response; NE, not evaluable; CR, complete response; Ext, extensive disease; BO, bronchiolitis obliterans; IP, interstitial pneumonitis; PR, partial response; UE, unevaluated; Lim, limited disease.

†Secondary graft failure occurred after neutrophil recovery.

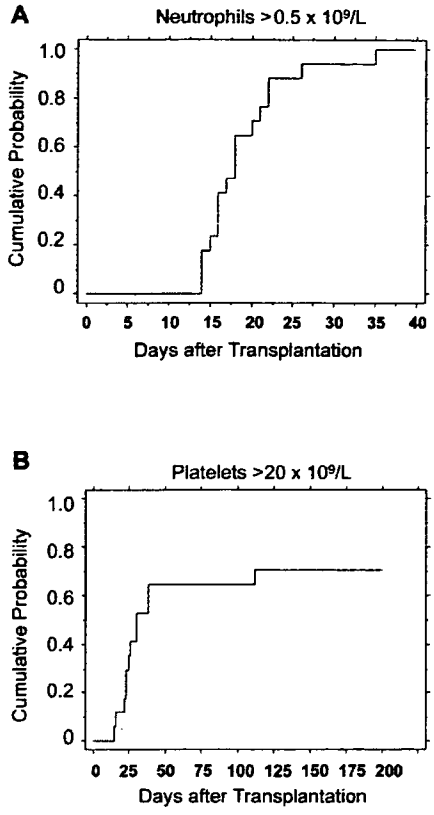

Figure 1. Engraftment after unrelated-donor bone marrow transplantation following reduced-intensity conditioning expressed as the cumulative probability of a neutrophil count >0.5 × 10^9/L (A) and a platelet count >20 × 10^9/L (B). All patients achieved neutrophil recovery, but 5 patients did not achieve platelet recovery. The median times until neutrophil and platelet recoveries were 18 days (range, 14-35 days) and 26 days (15-112 days), respectively. Late graft failure was observed in 2 patients.

marrow from a donor with allele-level mismatches at 3 HLA loci. Two patients with grade IV acute GVHD involving only the skin were successfully treated with methylprednisolone. Grade II acute GVHD involving only the skin was treated solely with CsA in 2 patients (Table 2). In 7 patients without relapse or secondary graft failure, CsA was tapered from a median of day 120 (range, day 96-169). Only 2 of the 7 patients were able to discontinue CsA (at days 203 and 288). Chronic GVHD was documented in all patients who survived beyond day 100 (1 with limited GVHD, 9 with extensive disease). There was no significant correlation between HLA disparity at the allele level and the incidence of GVHD, although it was difficult to analyze the data statistically because of the small number of patients in this study.

3.5. Survival and Causes of Death

The median follow-up period was 286 days (range, 56-687 days). Overall, 11 patients died, but 6 patients are currently in remission (2 in remission and 4 not in remission at the time of transplantation). The estimated 100-day and 1-year nonrelapse mortality rates were 14% (95% CI, 12%-17%) and 46% (95% CI, 33%-57%), respectively (Figure 2B). Estimated 1-year overall survival and progression-free survival rates were both 41% (95% CI, 32%-51%; Figure 3). There were 4 deaths due to recurrent or progressive disease at a median time of 55 days (range, 32-93 days). The causes of the 7 treatment-related deaths included acute GVHD (n = 2), secondary graft failure with sepsis (n = 1), interstitial pneumonitis (n = 1), organizing pneumonia (n = 1), bronchiolitis obliterans (n = 1), and bronchiolitis obliterans with invasive aspergillosis (n = 1).

4. Discussion

In our previous study in an unrelated-donor BMT setting, 5 patients underwent conditioning with a combination of Flu (30 mg/m^2 for 6 days) or cladribine (0.11 mg/kg for 6 days), BU (4 mg/kg for 2 days), and antithymocyte globulin (2.5 mg/kg for 4 days) without TBI, but secondary graft failure in 2 of these patients alerted us to a possible higher risk of graft rejection when we used bone marrow instead of peripheral blood cells as the stem cell source. In this study, we demonstrated that the addition of 4 Gy of TBI to the widely applied combination of Flu (30 mg/m^2 for 6 days) and BU (4 mg/kg for 2 days) reduces the risk of graft failure and enables the rapid achievement of full donor chimerism without donor lymphocyte infusion (DLI) and that the regimen-related toxicity was acceptable. Nevertheless, a relatively high incidence of nonrelapse mortality was observed. We lost 4 patients who developed extensive chronic GVHD and subsequent pulmonary complications in the later phase, more than 6 months after transplantation. Because many patients develop extensive GVHD, we assume that the pulmonary complications were primarily due to GVHD and not the consequence of our reduced-intensity stem cell transplantation (RIST) regimen incorporating 4 Gy of TBI. However, Deeg et al reported that more pulmonary compli-

Table 3.
Maximum Toxicities (N = 17)*

Grade	Cardiac, n	Mucositis, n	GI, n	Hepatic, n	CNS, n	Hyponatremia, n	Pulmonary, n	Renal, n
0	12	0	9	1	16	6	11	15
I	4	0	3	2	0	7	2	0
II	0	2	4	7	0	0	0	2
III	1	15	1	5	1	4	4	0
IV	0	0	0	2	0	0	0	0

*GI indicates gastrointestinal tract; CNS, central nervous system.

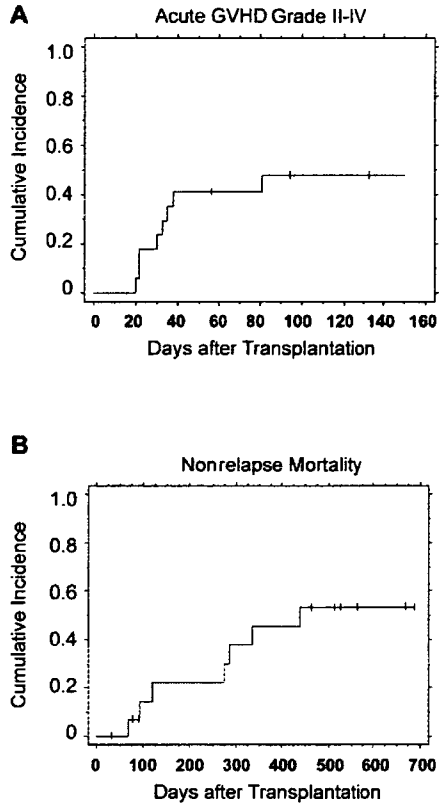

Figure 2. Cumulative incidence of acute GVHD (grades II-IV) (A) and nonrelapse mortality (B) after unrelated bone marrow transplantation following reduced-intensity conditioning. Acute GVHD (grades II-IV) was diagnosed in 8 patients (48%) (grade II in 3 patients and grade IV in 5) at a median of day 32 (range, day 20-81). The estimated 100-day and 1-year nonrelapse mortality rates were 14% and 46%, respectively.

cations developed in patients with aplastic anemia who received 4 to 6 Gy of TBI in combination with cyclophosphamide/antithymocyte globulin for unrelated-donor BMT than in patients who received 2 Gy TBI [20]. These investigators recommended that a 2-Gy TBI dose is sufficient to allow stable engraftment without increased toxicities, and this proposal should be evaluated in future studies. On the other hand, Maris et al described a nonmyeloablative conditioning regimen consisting of 2 Gy TBI and Flu (90 mg/m^2) for unrelated-donor HSCT [8]. In their study, the use of bone marrow rather than G-CSF–mobilized peripheral blood cells as the source of hematopoietic stem cells led to a lower engraftment rate (56% versus 85%), as well as lower rates of overall survival (33% versus 57%) and progression-free survival (17% versus 44%). Because bone marrow is currently the only stem cell source available from volunteer donors in Japan, we may need a more intensified regimen than the combination of 2 Gy TBI and 90 mg/m^2 Flu.

In this study, the rates of acute GVHD of grades II to IV and extensive chronic GVHD in patients who survived for more than 100 days were 48% and 90%, respectively. Grade IV acute GVHD was the primary cause of death in 2 patients. Moreover, the quality of life of patients who develop extensive chronic GVHD rapidly deteriorates, particularly in elderly patients. Although CsA was tapered from a median of day 120 in this series, it might be better to delay the start of CsA tapering in elderly patients, who are associated with higher GVHD rates. Studies have incorporated in vivo T-cell depletion through the addition of antithymocyte globulin or alemtuzumab in order to reduce the risk of GVHD [21-26]. In the study reported by Chakraverty et al, severe GVHD following RIST from an unrelated donor was decreased with in vivo use of alemtuzumab in the preparative regimen [23]. In their study, the rates of acute GVHD (grades II to IV) and chronic GVHD were 21% and 8%, respectively. The long half-life of alemtuzumab (15-21 days) may disturb the induction of full donor chimerism, however. If patients cannot achieve full donor chimerism, the usual option is DLI, which carries a risk of GVHD [26]. Moreover, lymphocytes for DLI are not always available for every patient, particularly in unrelated-donor transplantation settings. In this regard, we think that a regimen that routinely involves DLI after transplantation cannot be considered a universal strategy. In the present study, 2 patients who had

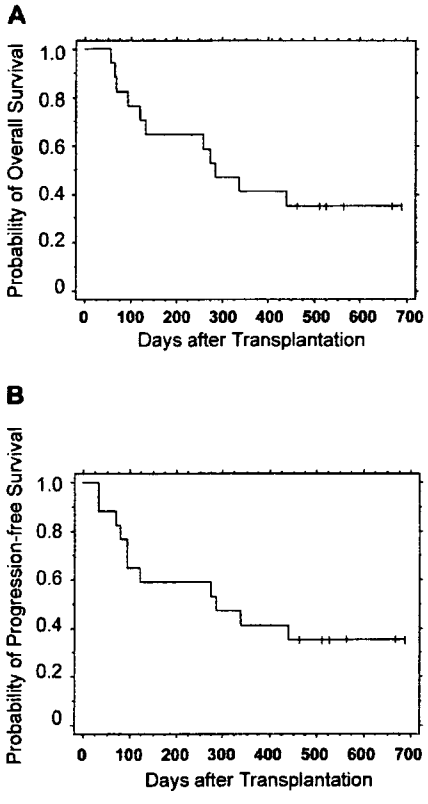

Figure 3. Kaplan-Meier actuarial probability of overall survival (OS) (A) and progression-free survival (PFS) (B) after unrelated-donor bone marrow transplantation following reduced-intensity conditioning. The median follow-up was 286 days (range, 56-687 days). The 1-year OS and PFS rates were both 41%. All 6 of the surviving patients (2 in remission and 4 not in remission at transplantation) remain in remission.

secondary graft failure did not receive DLI, because of grade IV acute GVHD in 1 patient and a reduced performance status in the other. Another approach to preventing severe GVHD is the use of novel immunosuppressive regimens. Several combinations of agents for GVHD prophylaxis, including CsA/mycophenolate mofetil [8,14,16] and tacrolimus/methotrexate [10,15,27], have been reported previously, and their value should be tested in prospective trials.

The induction of adequate antileukemic activity is another primary concern with a RIST procedure, particularly for patients with refractory diseases. de Lima et al reported a promising regimen that consisted of once-daily intravenous BU (130 mg/m^2 for 4 days) and Flu (40 mg/m^2 for 4 days) for patients with AML or MDS [27]. Replacement of oral BU with an intravenous preparation may result in an improved toxicity/survival profile. In our series, 4 patients achieved remission after RIST, although they were not in remission at the time of transplantation. Hence, it is likely that the antileukemic effect exerted by 4 Gy TBI in combination with Flu and BU is valuable even for the immediate control of leukemic blasts, although this possibility needs to be confirmed in further studies. The use of DLI has allowed the rescue of relapsed patients after allogeneic HSCT. In this study, however, we did not give DLI to 4 patients with progressive or relapsed diseases after transplantation because the relevance of the graft-versus-leukemia effect in rapidly proliferating diseases was not fully established and 2 of the patients had developed acute GVHD.

In conclusion, our regimen of 4 Gy TBI, Flu (180 mg/m^2), and BU (8 mg/kg) was effective in reducing the risk of graft failure following unrelated-donor transplantation. We confirmed, however, that a high incidence of nonrelapse mortality, primarily due to GVHD and/or pulmonary complications, still remains a major obstacle for the wider application of this procedure to elderly or medically infirm patients. Further studies to identify ways to ameliorate transplantation-related toxicities are urgently required.

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ORIGINAL ARTICLE

Rituximab does not compromise the mobilization and engraftment of autologous peripheral blood stem cells in diffuse-large B-cell lymphoma

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To investigate effects of the preautografting administration of rituximab on the mobilization and engraftment of peripheral blood stem cells (PBSC), we retrospectively analyzed the outcomes of 43 newly diagnosed diffuse-large B-cell lymphoma patients who received CHOP chemotherapy with or without rituximab as a first-line treatment before autologous PBSC transplantation (PBSCT). There was no difference in the number of $CD34^+$ cells among PBSC between the non-rituximab and the rituximab groups. Although B-cells were completely depleted from PBSC in the rituximab group, we found no difference in the expression of CXCR-4, VLA-4 and c-Kit on PBSC, indicating that rituximab did not affect the expression of these adhesion molecules, which might be involved in the mechanism of mobilization. There was no significant difference in the recovery of neutrophils and platelets, transplant-related toxicity and post-transplant complications between the two groups. Despite the short follow-up, there was no significant difference in progression-free survival between the two groups. These results indicated no adverse effect of rituximab on the mobilization and engraftment of PBSC. Larger studies are required to determine the impact of rituximab on the mobilization and function of PBSC as well as whether a survival advantage exists in patients who undergo auto-PBSCT with rituximab.

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Introduction

The emergence of new, more effective therapies has benefited patients with aggressive non-Hodgkin's lymphoma (NHL). As high-dose chemotherapy with autologous stem cell rescue has been shown to provide a survival advantage over salvage chemotherapy in relapsed patients with NHL,[1,2] many investigators have attempted to extend the use of auto-SCT approaches for the treatment of aggressive NHL. Recent reports have revealed that high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (auto-PBSCT) is superior to standard chemotherapy as a primary treatment for newly diagnosed patients with aggressive NHL.[3,4] Moreover, rituximab has changed the treatment paradigm of CD20-positive lymphoma, and has improved response and survival rates in combination with chemotherapy,[5–7] and rituximab-containing chemotherapy is increasingly becoming the primary standard for patients with diffuse-large B-cell lymphoma (DLBCL). Recent trials have focused on how to incorporate rituximab into high-dose chemotherapy followed by auto-PBSCT as a first-line treatment,[8,9] including the concept of *in vivo* purging of lymphoma cells from the circulation before the collection of auto-PBSC.[10,11] However, there is little evidence to support an effect of rituximab on the mobilization and engraftment of auto-PBSC,[12,13] though rituximab might be associated with a poor mobilization and impaired engraftment of PBSC. In an attempt to clarify this issue, we retrospectively compared characteristics of collection and transplantation of auto-PBSC in DLBCL patients treated with a protocol consisting of six courses of the CHOP regimen and high-dose conditioning chemotherapy followed by auto-PBSCT with or without rituximab as a primary treatment. In addition, we also tested the expression of adhesion molecules such as VLA-4, CXCR-4, and c-Kit on mobilized PBSC in the two groups to elucidate whether rituximab affects the expression of these molecules, as degradation of adhesion molecules could lead to the release of stem/progenitor cells from bone marrow into peripheral blood.[14,15]

Patients and methods

Patient

Patients aged 15–65 years with newly and histologically diagnosed DLBCL, were enrolled in this study. CD20 expression was determined at each participating institution, and further immunostaining was performed on central review if the lineage assignment was ambiguous. Eligible patients had an ECOG performance status of 0–3, high-intermediate to high risk according to the International Prognostic Index,[16] and at least one objective, measurable disease parameter. Exclusion criteria included transformed follicular lymphoma, central nervous system involvement, inadequate organ function, concomitant malignancy and an active viral infection such as hepatitis B, hepatitis C and human immunodeficiency virus. This study was conducted in accordance with the ethical guidelines mandated by the Declaration of Helsinki. All patients signed informed consent forms approved by the institutional review board at each participating hospital.

Between May 2001 and January 2006, a total of 43 patients were enrolled into this retrospective analysis: 20 patients were treated with CHOP chemotherapy and autologous PBSCT from May 2001 to May 2003 before the era of rituximab treatment (non-rituximab group). After September 2003 when rituximab was approved for the treatment of DLBCL in Japan, 23 patients enrolled in our study were treated with exactly the same regimen mentioned above plus rituximab (rituximab group) as a first option. Patient characteristics were well balanced and not statistically different between the two groups with regard to sex, age and clinical conditions (Table 1).

Treatment

All patients were treated with a standard CHOP regimen consisting of cyclophosphamide 750 mg/m^2 on day 1, doxorubicin 50 mg/m^2 on day 1, vincristine 1.4 mg/m^2 to a maximum of 2 mg on day 1 and prednisone 100 mg/m^2 on days 1 through 5, every 21 days for six courses. Patients in complete remission (CR), CR of undetermined significance (CRu) or partial remission (PR) after three cycles of CHOP received high dose of etoposide 500 mg/m^2 for 3 days followed by granulocyte colony-stimulating factor (G-CSF) to mobilize PBSC as described previously.[17] PBSC were collected using a COBE Spectra (Gambro JAPAN Inc., Tokyo, Japan) blood cell separator.[17] The target cell dose was 2×10^6 CD34$^+$ cells/kg. Harvested PBSC were cryopreserved until used as described previously.[18]

After the collection of PBSC, all patients received additional three courses of CHOP. Thereafter, the patients in CR, CRu or PR underwent autologous PBSCT: the conditioning regimen consisted of ranimustine 200 mg/m^2 on day −8 and day −3, carboplatin 300 mg/m^2 on day −7 through −4, etoposide 500 mg/m^2 on day −6 through −4 and cyclophosphamide 50 mg/kg on day −3 and day −2. On day 0, unpurged PBSC were reinfused followed by administration of G-CSF 5 μg/kg. Engraftment was confirmed by a granulocyte count $>0.5 \times 10^9$/l and platelet counts $>20 \times 10^9$/l or independence of platelet transfusion.

On the other hand, 23 patients in the rituximab group received rituximab (Chugai Pharmaceutical Co., Tokyo, Japan) 375 mg/m^2 one day before the 2nd, 3rd, 5th and 6th CHOP regimen, on day −9 and day + 1 after autologous PBSCT. Rituximab was also administered one day before the high-dose etoposide regimen, and one day before PBSC collection again for *in vivo* purging of circulating lymphoma cells to avoid contamination from lymphoma cells in the PBSC harvest. In total, patients in the rituximab group received eight courses of rituximab 3000 mg/m^2 in this protocol.

Table 1 Patient and disease characteristics

Characteristics	*R-patients (n = 23)*	*Non-R patients (n = 20)*	P
Age, median (range)	58 (21–65)	52 (15–65)	0.61
Male/female	10/13	12/8	0.87
IPI at diagnosis			
Intermediate-high	17	16	0.39
High	6	4	
Disease status at PBSCT			
CR	13	9	0.37
Cru	7	8	
PR	3	3	
Time in months to auto-PBSCT, median (range)	5.8 (4.4–8.5)	5.6 (4.6–9.8)	0.43

Abbreviations: IPI = international prognostic index; NS = not significant ($P > 0.05$); R = rituximab.

Cell staining and fluorescence activated cell sorter (FACS) analysis

Peripheral blood mononuclear cells (PBMNC) were prepared by thawing the frozen PBSC harvest samples, which were stored at −80°C.[18] PBMNC were stained with a Cy5-PE-conjugated lineage (Lin) cocktail (anti-CD3, CD4, CD7, CD8, CD11b, CD16, CD56 and glycophorin A; Caltag, Burlingame, CA, USA), fluorescein isothiocyanate-conjugated anti-CD19 (Becton Dickinson (BD) Pharmingen, San Jose, CA, USA), PE-conjugated anti-CXCR-4, VLA-4 and c-Kit (BD Pharmingen), allophycocyanin-conjugated anti-CD34 (BD Pharmingen) and PE-Cy7-conjugated anti-CD38 (Caltag) antibodies. Nonviable cells were excluded by propidium iodide staining. Expression of adhesion molecules was detected on progenitors using a highly modified triple laser (488 nm argon laser, 633 nm helium-neon laser and 407 nm crypton laser) FACS (FACS Aria; BD) as described previously.[15]

Statistical analysis

The test of independence between the rituximab and non-rituximab groups was made with the χ^2 test, Fisher's exact test, or the Kruskal–Wallis test, where appropriate. Distribution of time to progression-free survival (PFS) was summarized with Kaplan–Meier product limit estimators and compared by log-rank test.

Table 2 Peripheral blood stem cell mobilization characteristics

Characteristics	*R-patients (n = 23)*	*Non-R patients (n = 20)*	P
Duration from HD-VP to collection of PBSC, median days (range)	20 (16–22)	20 (15–21)	0.36
WBC count at PBSC collection, mean (range) ($\times 10^9$/l)	9.49 ± 4.17 (4.30–18.26)	8.72 ± 4.51 (3.70–19.30)	0.55
Neutrophil count at PBSC collection, mean (range) ($\times 10^9$/l)	7.07 ± 4.14 (1.70–16.25)	6.33 ± 4.29 (1.67–16.87)	0.44
Platelet count at PBSC collection, mean (range) ($\times 10^9$/l)	168.1 ± 85.2 (49.3–373.0)	188.7 ± 97.4 (37.3–282.7)	0.29
$CD34^+$ cell dose collected in PBSC ($\times 10^6$/kg), mean (range)	11.10 ± 8.04 (2.23–27.9)	13.00 ± 10.30 (6.35–51.00)	0.64
Percent of $CD34^+$ cells per PBSC MNC, mean (range)	3.66 ± 3.27 (0.52–16.1)	3.98 ± 2.09 (1.52–10.71)	0.85
Percent of $CD19^+$ B-cells per PBSC MNC, mean (range)[a]	0.06 ± 0.05 (0.013–0.150)	0.89 ± 0.39 (0.50–1.46)	<0.005

Abbreviations: R = rituximab; HD-VP = high-dose etoposide for PBSC mobilization; NS = not significant ($P > 0.05$).
[a]Content of $CD19^+$ B cells was analyzed in seven R-patients and seven non-R patients, respectively.

Table 3 Autologous PBSCT outcomes

Characteristics	*R-patients (n = 23)*	*Non-R patients (n = 20)*	P
$CD34^+$ cell dose reinfused in PBSC ($\times 10^6$/kg), median (range)	9.82 ± 6.08 (2.28–25.85)	10.99 ± 5.62 (3.41–25.50)	0.46
Time in days to neutrophils ($> 0.5 \times 10^9$/l), median (range)	9 (8–12)	9 (8–11)	0.39
Time in days to platelets ($> 20 \times 10^9$/l), median (range)	10 (8–15)	10 (8–14)	0.57
Time in days to platelets ($> 50 \times 10^9$/l), median (range)	12 (10–17)	13 (11–16)	0.8
Time in days to dependency of platelet transfusion, median (range)	8 (6–13)	9 (7–11)	0.15
Rate of > grade III of transplant-related toxicity[a]	17%	15%	0.45
Rate of any documented infection[b]	26%	30%	0.42
1-year PFS	80.20%	78.30%	0.59

Abbreviations: R = rituximab; NS = not significant ($P > 0.05$); PFS = progression-free survival.
[a]Transplant-related toxicities were scored using National Cancer Institute Common Toxicity Criteria.
[b]Any documented infection = any positive culture.

Results

Stem cell mobilization

One course of apheresis for the collection of PBSC was sufficient to obtain the number of $CD34^+$ cells needed for transplantation in all patients of both groups. There was no significant difference in white blood cell count (WBC) at collection (R vs NR group, mean WBC 9.49×10^9/l vs 8.72×10^9/l, $P = 0.55$), platelet counts at collection (mean, 168.1 vs 188.7×10^9/l, $P = 0.29$), duration from administration of etoposide to collection of PBSC (median day, 20 vs 20, $P = 0.36$), percentage of $CD34^+$ cells at harvest (mean, 3.66 vs 3.98%, $P = 0.85$), or $CD34^+$ cell dose collected (mean, 11.10 vs 13.00 $\times 10^6$/kg, $P = 0.64$) between the two groups (Table 2).

Engraftment of PBSC

The characteristics of auto-PBSCT are shown in Table 3. Engraftment was rapid and documented in all patients. There was no significant difference in the $CD34^+$ cell dose reinfused (R vs NR group, mean $CD34^+$ cell dose 9.82 vs 10.99×10^6/kg, $P = 0.46$), days taken to achieve a granulocyte count of 0.5×10^9/l (median day, 9 vs 9, $P = 0.39$), and a platelet count of 20×10^9/l in platelet counts (median day, 10 vs 10, $P = 0.57$), or independence of platelet transfusion (median day, 8 vs 9, $P = 0.15$) (Table 3). There were no treatment-related deaths, and no significant difference in the frequency of more than grade 3 adverse events scored using the National Cancer Institute Common Toxicity Criteria between two groups (17 vs 15%, $P = 0.45$). Neutropenic fever occurred in the majority of patients in both groups; however, we found no difference in the prevalence of documented infections (26 vs 30%, $P = 0.42$).

Survival

The median follow-up period was 51 months in the non-rituximab group and 31 months in the rituximab group. At the time of reporting, there was no significant difference in 1-year PFS (R vs NR groups, 80.2 vs 78.3%, $P = 0.59$) (Table 3).

Expression of adhesion molecules on PBSC

Four courses of rituximab were administered before the collection of PBSC to eliminate DLBCL cells. Therefore, we compared the contents of B-cells in the harvest products to evaluate the efficacy of B-cell purging by rituximab. PBSC harvests contained a small fraction of $CD19^+$ mature-B cells (0.89 ± 0.39% of MNC, $n = 7$) in the non-rituximab group, whereas only a few B cells were circulating (0.06 ± 0.05% of MNC, $n = 7$) in the rituximab group ($P < 0.001$) (Table 2).

We next tested for the expression of c-Kit and adhesion molecules such as VLA-4 and CXCR-4 on immature $CD34^+$ stem/progenitor cells in the PBSC harvest, as downregulation of these molecules resulted in release of stem/progenitor cells from marrow and mobilization into the circulation.[15] Figure 1 shows the mean fluorescence intensity (MFI) for these molecules in seven patients in the non-rituxiamb group and seven in the rituximab group. There was no significant difference in MFI of c-Kit (NR vs R groups, 206.4 ± 35.9 vs 182.0 ± 22.0, $P = 0.15$), CXCR-4

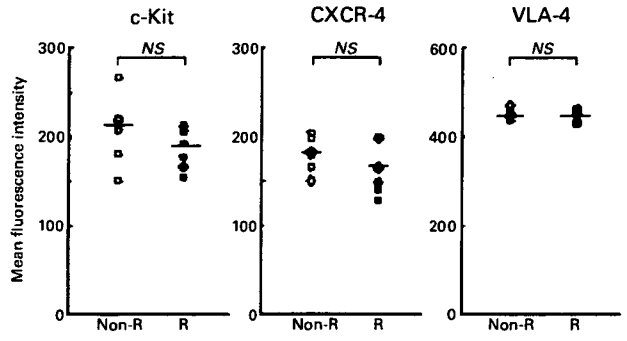

Figure 1 Comparative expression of c-Kit, CXCR-4 and VLA-4 on $CD34^+$ immature stem/progenitors in auto-PBSC harvests among the non-rituximab group (Non-R, open circle) and rituximab group (R, closed circle). Circles indicate median MFI for these molecules. NS, not significant.

(176.6±17.9 vs 161.5±27.2, $P=0.25$) and VLA-4 (457.7±18.0 vs 454.0±16.9, $P=0.69$) between the two groups.

Discussion

Promising results have been obtained for an initial treatment for NHL patients with high-dose chemotherapy followed by auto-PBSCT[3] as well as the addition of rituximab to standard chemotherapy.[6,7] Emerging new treatment strategies have focused on a combination of rituximab and high-dose chemotherapy with auto-PBSCT. Several trials have been conducted to design the optimal timing of rituximab administration during the treatment course of chemotherapy and auto-PBSCT.[9] A large number of protocols have incorporated rituximab administration preceding chemotherapy and pretransplant conditioning to enhance the chemosensitivity of lymphoma cells by exposure to rituximab.[19] In addition, to reduce or eliminate circulating lymphoma cells contaminating the PBSC harvest, concurrent administration of rituximab with cytotoxic chemotherapy for PBSC mobilization has achieved *in vivo* purging in most patients whose residual lymphoma cells were undetectable in the harvest products by polymerase chain reaction assay.[11,20] However, few studies have examined the effect of rituximab on mobilization and engraftment of auto-PBSC.[12,13] In general, factors associated with poor mobilization include extensive treatment before mobilization,[21] and rituximab administration may impair the efficiency of PBSC mobilization compared to that in the non-rituximab group. Hoerr *et al.*[13] reported that patients in the rituximab group had delays in platelet recovery post-transplant, but rituximab did not affect PBSC mobilization, and post-transplant neutrophil recovery, early complications, and mortality rates. In contrast, Benekli *et al.*[12] have shown the detrimental effect of rituximab on the mobilization and engraftment of PBSC because there was a significantly lower number in collected PBSC and a prolonged neutrophil recovery in the rituximab group. However, both studies were performed in patients with relapsed or refractory NHL who had been heavily treated previously, and the exact effect of rituximab on PBSC mobilization still remains unclear. Therefore, in the newly diagnosed DLBCL patients who were treated with the same treatment protocol consisting of chemotherapy with or without rituximab and auto-PBSCT as a primary treatment, we evaluated the characteristics of mobilization and transplantation of auto-PBSC. In our study, we found no disadvantage in the number of $CD34^+$ cells collected, recovery of neutrophils and platelets or post-transplant complications. As none of our patients were previously treated and this study was conducted as a primary therapy, mobilization potential might not have been impaired, resulting in no adverse effect of rituximab on the mobilization and engraftment of PBSC.

Mechanisms of PBSC mobilization may involve chemotherapy/G-CSF-induced modulation of chemokines, adhesion molecules and proteolytic enzymes.[14] Proteolytic enzymes such as neutrophil elastase, cathepsin G and matrix metalloproteinase-9 released from the activated neutrophils and monocytes can degrade and/or inactivate adhesion molecules such as VCAM-1/VLA-4, chemokines such as stromal-derived factor (SDF)-1/CXCR-4 and soluble Kit ligand, resulting in the disruption of contact between stem/progenitor cells and the bone marrow microenvironment, and then stem/progenitor cells would be released to migrate into peripheral blood.[14,15] However, recently late-onset neutropenia has been reported following rituximab-based chemotherapy,[22,23] and Dunleavy *et al.*[24] have suggested that rituximab may induce perturbations of SDF-1/CXCR-4 interaction, which could retard the egress of neutrophils from bone marrow. Therefore, we investigated expression levels of adhesion molecules on PBSC in the two groups. Low levels were documented in both groups, but we did not find any difference in expression levels of VLA-4, CXCR-4 and c-Kit on PBSC. Moreover, there was no difference in neutrophil counts, which might partially contribute to mobilization, at the time of PBSC collection, and the recovery in neutrophil and platelet counts was equally rapid following auto-PBSCT in the two groups. These results indicated that rituximab might not impair the mobilization as well as homing, engraftment and repopulation of PBSC, without altering the expression of adhesion molecules including at least VLA-4, CXCR-4 and c-Kit.

In summary, our data provide evidence that rituximab has no adverse effect on the mobilization and engraftment of PBSC, when rituximab is employed in the first-line treatment for newly diagnosed DLBCL patients. As rituximab received approval in September 2003 for the treatment of DLBCL in Japan, the median follow-up is too short to evaluate its effect on survival. Moreover, a high incidence of late-onset neutropenia following rituximab-containing chemotherapy and/or autologous stem cell transplantation has been reported, however, its mechanism still remains to be solved.[25,26] Larger studies and longer follow-ups are necessary to confirm these findings and to determine more optimal combinations of rituximab and auto-PBSCT as well as the impact of rituximab on disease-free survival in the treatment of NHL.

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INTERNAL MEDICINE

□ CASE REPORT □

Repeated Relapses of Acute Myelogenous Leukemia in the Isolated Extramedullary Sites Following Allogeneic Bone Marrow Transplantations

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Abstract

Isolated extramedullary (EM) relapses of acute myelogenous leukemia (AML) after allogeneic hematopoietic stem cell transplantation (allo-HSCT) have been reported to be rare, and are usually followed by bone marrow relapses. We report a 49-year-old man with AML with the unfavorable chromosome abnormality 7q-, who was treated by allo-HSCT. Fifteen months after allo-HSCT, the patient initially developed a relapse only in his inguinal lymph nodes, and then bone marrow relapse became evident one month after the EM relapse. Subsequently, the patient received chemotherapy and a second allo-HSCT from another donor, but he suffered another relapse in different EM sites including the skin and central nervous system with a persistently normal marrow. This case is characterized by repeated relapses in isolated EM sites after allo-HSCT and suggests that the anti-leukemic effects of chemotherapy and/or graft-versus-leukemia effects in the EM sites might not be so uniformly effective as that in the marrow. Accordingly, we should be aware that AML relapses can occur repeatedly only in isolated EM sites post allo-HSCT, resulting in treatment failure and a poor prognosis.

Key words: extramedullary, relapse, AML, allo-BMT, GVL

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative therapy for patients with acute myelogenous leukemia (AML) based upon graft-versus-leukemia (GVL) effects in addition to the intensive conditioning chemo-radiotherapy. However, some patients eventually develop a relapse following allo-HSCT, resulting in treatment failure and a poor prognosis. AML relapse usually occurs in the bone marrow, but a small fraction of patients develop extramedullary (EM) relapses either alone or concomitant with bone marrow relapse (1-5). Bekassy et al reported isolated EM relapse occurred after allo-BMT in 20 out of 3,071 AML patients (0.65%) (1). Little is known concerning the mechanism of EM relapse; however, the prognosis of patients with an EM relapse of AML is generally considered to be less favorable than that of AML patients with bone marrow relapse only (1, 6, 7).

We present a case of AML who developed a relapse confined to his inguinal lymph nodes 15 months after allogeneic bone marrow transplantation (allo-BMT). Following a second allo-BMT from another donor, the patient had multiple relapses in different EM sites such as the skin and central nervous system, with a persistently normal marrow. Frequent relapses in EM sites suggest that the GVL effect in the EM sites was not as potent as that in the bone marrow, where it remained effective. Thus, we should note that AML relapses can occur in isolated EM sites because the GVL effect might not be uniformly effective throughout the body following allo-HSCT.

Case Presentation

In June 2002, a 49-year-old Japanese man was referred to us for evaluation of leukocytosis and anemia. At the time of

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hospitalization, hemoglobin was 7.4 g/dl; platelet count, 135×10^9/l; white blood cell count, 32.3×10^9/l with 2% neutrophils, 9% lymphocytes, and 89% myeloblasts that stained positive for myeloperoxidase (MPO). A bone marrow aspirate was hypercellular with 91% myeloblasts, which were positive for CD7, CD13, CD15, CD33, CD34 and HLA-DR. Cytogenetic analysis of the bone marrow cells revealed 46 XY, 7q- in all metaphase cells. A diagnosis of AML-M1 type was made according to the French-American-British classification, and this patient with the 7q- chromosome abnormality was categorized as having a poor prognosis with an approximately 75% chance of relapse (8, 9). Extramedullary leukemia was not documented at diagnosis. The patient was treated with a conventional induction regimen consisting of idarubicin and cytarabine (CA), and he achieved a complete remission (CR). He was treated with two further cycles of consolidation chemotherapy consisting of intermediate-dose CA combined with mitoxantrone in the first course and etoposide in the second course, as described previously (10). During the consolidation chemotherapy, the patient developed invasive pulmonary aspergillosis, which was treated by administration of Amphotericin-B. Finally, local lung resection of left upper lobe was performed prior to allo-BMT.

In February 2003, the patient underwent an allo-BMT from an HLA matched unrelated donor after receiving busulfan (16 mg/kg) and cyclophosphamide (120 mg/kg). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine (CyA) and a short course of methotrexate (MTX). During the neutropenic period, the patient developed pulmonary abscess caused by Streptococcus pneumoniae, however, it gradually stabilized in response to antibiotic therapy. Engraftment was obtained on day 17, and a bone marrow examination demonstrated continuing CR. A chimeric analysis of the minisatellite variable number of tandem repeats disclosed that his bone marrow cells consisted entirely of donor-derived cells. On day 38, the patient developed grade I acute GVHD confined to his skin, which disappeared without any treatment. Four months after allo-BMT, the patient disclosed typical oral manifestations of chronic GVHD, which were resolved after adjusting the dose of CyA.

In May 2004, 15 months after BMT, the patient developed bilateral inguinal lymphadenopathy. He was otherwise asymptomatic with no organomegaly and a normal complete blood count. A biopsy of a left inguinal lymph node revealed the infiltration of medium-sized blastic cells with moderately irregular nuclei (Fig. 1). These cells were positive for CD7, CD13, CD33, CD34 and HLA-DR, the same phenotype as that seen in his bone marrow at presentation. Chimeric analysis demonstrated that the lymph node cells were recipient derived, and a deletion of 7q was documented by cytogenetic analysis. In contrast, a bone marrow aspirate showed no infiltration by AML cells and also a donor-derived pattern by chimeric analysis. Further systemic investigation including computed tomography of the whole body and lumbar puncture of his cerebrospinal fluid showed no leukemic involvement of his other organs except for the inguinal lymph nodes. Based on these observations, isolated EM relapse of AML confined to the lymph nodes was diagnosed. Another donor search was initiated immediately for a second allo-SCT. The immunosuppressant was discontinued to induce a GVL effect; however, no improvement in his lymphadenopathy was seen. One month after the discontinuation of immunosuppression, a bone marrow aspirate showed an increase in the number of AML blasts, which accounted for up to 11% of the bone marrow nucleated cells. Cytogenetic analysis also demonstrated the emergence of cells with a deletion of 7q, and 15% of his bone marrow cells were recipient-derived by chimeric analysis. Thus, the patient suffered a relapse of AML in his bone marrow following an isolated EM relapse in his lymph nodes. Re-induction chemotherapy consisting of daunorubicin and CA was administered. The inguinal lymphadenopathy disappeared soon after chemotherapy; however, the number of AML blasts still increased peripherally and in his marrow. Another two courses of chemotherapy, using the CAG-regimen (11), were ineffective in inducing a CR.

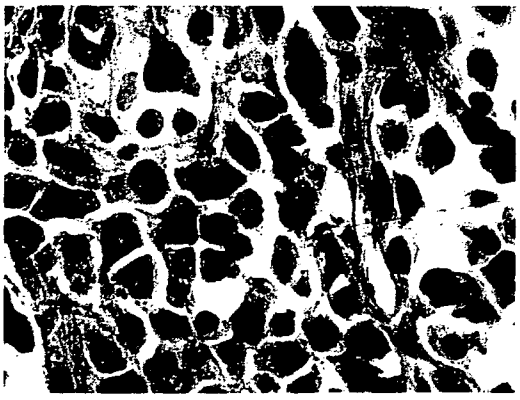

Figure 1. Left inguinal lymph node biopsy specimen showing diffuse infiltration of myeloid leukemic cells. Original magnification was × 1,000 (Hematoxylin & eosin stain).

In November 2004, the patient underwent a second allo-BMT from another HLA matched unrelated donor after receiving a reduced intensified conditioning (RIC) regimen consisting of fludarabine (180 mg/m^2), busulfan (8 mg/kg), and total body irradiation (2 Gy). Acute GVHD prophylaxis consisted of tacrolimus and a short course of MTX. Engraftment was confirmed on day 21, and the patient obtained and continued in CR without GVHD.

On day 150 after the second allo-BMT, the patient presented with multiple raised red-brown nodules, widely scattered over his trunk. A biopsy of a skin lesion showed medium sized blast cells, positive for MPO, CD34 and CD45, which were consistent with AML infiltrates in the skin (Fig. 2). The bone marrow aspirate remained normocellular, with normal maturation of all three lineages and the second donor-derived pattern. CT scans of the whole body showed

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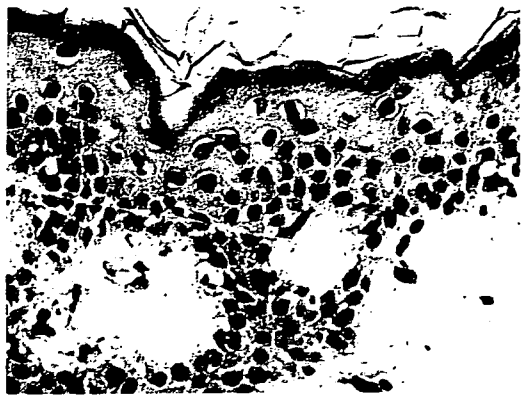

Figure 2. Biopsy specimen of skin lesions on chest demonstrating infiltration of leukemia cells. Original magnification was × 400 (Hematoxylin & eosin stain).

no lymphadenopathy or organomegaly, suggesting no evidence of leukemic infiltration into other sites. Thus, his relapse was documented in a different EM site after the second allo-BMT. Tacrolimus was discontinued to induce a GVL effect; however, his skin lesions expanded rapidly without developing any signs of GVHD. Thereafter, the patient developed a left facial nerve paralysis. His cerebrospinal fluid was infiltrated with AML cells despite repeated intrathecal injections of CA and MTX for prophylaxis of central nervous system (CNS) leukemia prior to allo-BMT. The patient died shortly after the documentation of CNS involvement.

Discussion

AML eventually relapses in 20% to 50% of AML patients after allo-HSCT. AML relapse following allo-HSCT usually occurs in the bone marrow where the leukemic burden is the heaviest, but isolated EM relapse can be detected rarely (1). Isolated EM relapse is usually accompanied by bone marrow relapse within 1 year, and is known to be a very poor prognostic factor, compared to that of medullary relapse after allo-HSCT (1, 6, 12). Thus, isolated EM relapse in AML is an increasingly recognized cause of treatment failure after allo-HSCT.

Predisposing factors that may contribute to the EM relapse of AML after allo-HSCT have been suggested. One factor may be associated with the intrinsic properties of the leukemic cells such as neural adhesion molecule NCAM (CD56) expression, chromosomal aberrations, which include t(8; 21), inv (16), and MLL rearrangement, and M4 and M5 by the FAB classification (1, 7). The present patient with AML-M1 did not have these risk factors for EM disease, and did not exhibit EM involvement at the initial presentation of relapse. The surviving leukemic cells might have transformed after the second round intensive chemotherapy followed by allo-BMTs, and have acquired the ability to adhere to dermal fibroblasts, facilitating their binding to EM tissues other than the bone marrow stroma, leading to the multiple isolated EM relapses (7, 13).

As another possible explanation, the EM sites might serve as sanctuary sites for the dormant leukemic clone after allo-HSCT, since the effect of anti-cancer drugs and/or immune cells and cytokines can be diminished in the EM sites due to the presence of a barrier (2, 7). Thus, following allo-HSCT, when the leukemic cells are still sensitive to the GVL effect, they may be suppressed in the marrow, but may escape from the immunosurveillance in the EM sites where the GVL may be less uniformly effective.

There is no established treatment strategy for EM relapse post allo-HSCT but experience has shown that the vast majority of patients with isolated EM relapse subsequently develop marrow disease and have a poor prognosis and rapidly succumb to their disease (1, 6, 7). This indicates that a small fraction of leukemic cells might be present in the bone marrow even in those patients in whom extramedullary disease appears to be isolated. To eliminate the residual leukemic cells resistant to prior therapies throughout the entire body, an aggressive treatment with chemotherapy plus a second allo-HSCT from another donor should be considered in some selected cases, especially in younger patients. However, the treatment of EM relapse post allo-HSCT is extremely difficult because of the cumulative toxicities of the previous high-dose chemo-radiotherapy and immunosuppression caused by GVHD and/or immunosuppressants administered. Most patients, including even the younger ones, cannot tolerate aggressive systemic chemotherapy and conventional conditioning regimen followed by a second allo-HSCT (1, 14). Localized therapy such as irradiation to the EM lesions cannot manage leukemic relapse, and these patients usually develop overt leukemia. Patients who have not suffered from GVHD may be offered discontinuation of immunosuppression and donor lymphocyte infusion (DLI) therapy to augment the GVL effects; however, data on the efficacy of DLI in patients with isolated EM relapse post allo-HSCT has not yet shown encouraging results (7, 15). Furthermore, the patient had histories of invasive pulmonary aspergillosis and pulmonary abscess. Based on these observations, in this case we performed a second allo-BMT with the RIC regimen from another donor at the documentation of EM relapse following the first allo-BMT to gain another GVL effect different from that of the first donor. Unfortunately, 5 months later he again developed isolated EM relapse confined to the skin and CNS without marrow disease. In this case, the AML relapse occurred in EM sites that might be inaccessible to chemotherapy and/or the GVL effect, while a full hematopoiesis from the donor was retained in his marrow where the GVL effect could have functioned well after the second allo-BMT. In addition, the prolonged immunosuppression by the double allo-BMT might impair the immunosurveillance against the residual leukemic cells. Thus, physicians should note that AML relapse can occur in isolated EM sites because the GVL effect might not be uniformly effective throughout the body and the surviving leu-

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kemic cells might acquire affinity to the EM sites as a consequence of transformation following allo-HSCT.

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Cytomegalovirus Infections following Umbilical Cord Blood Transplantation Using Reduced Intensity Conditioning Regimens for Adult Patients

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ABSTRACT

Cytomegalovirus (CMV) infection is a major complication after allogeneic hematopoietic stem cell transplantation (Allo-HSCT); however, we have little information on the clinical features of CMV reactivation after cord blood transplantation using reduced-intensity regimens (RI-CBT) for adults. We reviewed medical records of 140 patients who underwent RI-CBT at Toranomon Hospital between January 2002 and March 2005. All the patients were monitored for CMV-antigenemia weekly, and, if turned positive, received preemptive foscarnet or ganciclovir. Seventy-seven patients developed positive antigenemia at a median onset of day 35 (range, 4-92) after transplant. Median of the maximal number of CMV pp65-positive cells per 50,000 cells was 22 (range, 1-1806). CMV disease developed in 22 patients on a median of day 35 (range, 15-106); 21 had enterocolitis and 1 had adrenalitis. CMV antigenemia had not been detected in 2 patients, when CMV disease was diagnosed. CMV disease was successfully treated using ganciclovir or foscarnet in 14 patients. The other 8 patients died without improvement of CMV disease. In multivariate analysis, grade II-IV acute graft-versus-host disease was a risk factor of CMV disease (relative risk 3.48, 95% confidential interval 1.47-8.23). CMV reactivation and disease develop early after RI-CBT. CMV enterocolitis may be a common complication after RI-CBT.

KEY WORDS

Graft-versus-host disease • Ganciclovir • Foscarnet • Cytomegalovirus antigenemia • CD34-positive cells

INTRODUCTION

Cord blood transplantation (CBT) is an attractive alternative for patients with hematologic diseases who lack a matched related or unrelated donor. The value of CBT using myeloablative preparative regimens has been confirmed for pediatric patients [1,2]. Myeloablative CBT for adult patients achieves engraftment in 90% of the patients, but carries a 50% risk of transplant-related mortality (TRM), mostly resulting from infection [3,4]. We and other groups have reported the feasibility of CBT using reduced-intensity regimens (RI-CBT) for adult patients with advanced hematologic diseases [5,6].

Because of delayed immune recovery and graft-versus-host disease (GVHD), infection is the leading cause of TRM after CBT using myeloablative preparative regimens [2-4,7]. However, studies on immune recovery following RI-CBT gave us hope that RI-CBT recipients may less frequently experience GVHD and infectious complications. Cytomegalovirus (CMV) has been 1 of the most feared infectious complications in CBT [8], as well as in conventional allogeneic marrow or peripheral blood stem cell trans-

plantation (PBSCT) [9,10], although we have little information on CMV infection following RI-CBT. We investigated its frequency and clinical features in patients who underwent RI-CBT for advanced hematologic diseases.

PATIENTS AND METHODS

Study Patients and Donors

We reviewed medical records of 140 patients who underwent RI-CBT at Toranomon Hospital between January 2002 and March 2005. All the patients had diseases that were incurable with conventional treatments, and were considered inappropriate for conventional allogeneic stem cell transplantation (allo-SCT) because of the lack of an human leukocyte antigen (HLA)-identical sibling or a suitable related/unrelated donor, aged >50 years old and/or organ dysfunction (generally attributable to previous intense chemotherapy and/or radiotherapy). All the patients provided written informed consent in accordance with the requirements of the institutional review board.

HLA Typing and Donor Matching

An unrelated cord blood donor was searched through the Japan Cord Blood Bank Network [11] for patients without an HLA-identical sibling donor or a suitable related/unrelated donor.

Preparative Regimen

All the patients received purine analog-based preparative regimens (Table 1).

Engraftment

Engraftment was defined as white blood cell counts $>1.0 \times 10^9$/L or absolute neutrophil counts $>0.5 \times 10^9$/L for 2 consecutive days. Granulocyte-colony stimulating factor (G-CSF) was administered i.v. from day 1 until engraftment.

Supportive Care and Management of Preengraftment Fever and GVHD

All the patients were managed in reverse isolation in laminar airflow-equipped rooms, and received trimethoprim/sulfamethoxazole for *Pneumocystis jiroveci* prophylaxis. Fluoroquinolone and fluconazole or itraconazole were administered for prophylaxis of bacterial and fungal infections, respectively. Prophylaxis of herpes virus infection with acyclovir 600 mg/day was also given [12]. Neutropenic fever was managed according to the guidelines [13].

Diagnosis and management of preengraftment immune reaction were reported previously [14]. GVHD was clinically diagnosed in combination with skin or gut biopsies after engraftment or attainment of 100% donor chimerism. Acute (aGVHD) and chronic GVHD (cGVHD) were graded according to the established criteria [15,16]. GVHD prophylaxis was a continuous infusion of cyclosporine 3 mg/kg or tacrolimus 0.03 mg/kg from day −1 until the patients tolerated oral administration. It was tapered off from day 60 until day 150 or depending on the status of GVHD. If grade II-IV aGVHD developed, 0.5-1.0 mg/kg/day of prednisolone was added to cyclosporine or tacrolimus, and tapered from the beginning of clinical response.

Table 1. *Patient Characteristics*

Variable	Number
Age (median [range])	55 (17-79)
Sex (men/women)	81/59
Primary diseases	
Acute lymphoblastic leukemia	19
Acute myeloid leukemia	44
Chronic myelogenous leukemia	5
Adult T cell leukemia	19
Myelodysplastic syndrome	14
Malignant lymphoma	29
Multiple myeloma	4
Aplastic anemia	6
Risk of underlying diseases (high/low)*	99/41
Preparative regimens	
Flud 125 mg/m² + L-PAM (80 mg/m²) + TBI (2-8 Gy)	121
Flud 125 mg/m² + L-PAM (140 mg/m²) + TBI (4-8 Gy)	5
Flud 125 mg/m² + L-PAM (100 mg/m²) + TBI (4-8 Gy)	2
Flud 150 mg/m² + BU 8 mg/kg + TBI (4-8 Gy)	8
Flud 125 mg/m² + L-PAM (80-140 mg/m²)	2
Flud 150 mg/m² + BU 8 mg/kg	1
L-PAM 140 mg/m²	1
Number of infused mononuclear cells ×10E7/kg (median [range])	2.7 (0.4-5.7)
Number of infused CD34⁺ cells ×10E5/kg (median [range])	0.73 (0.01-5.7)
HLA antigen disparity 0/1/2/3	3/21/114/2
GVHD prophylaxis	
Cyclosporine	85
Tacrolimus	55

Flud indicates fludarabine; L-PAM, melphalan; BU, busulfan; TBI, total body irradiation; GVHD, graft-versus-host disease.

*Acute leukemia in complete remission, chronic myelocytic leukemia in the chronic phase, malignant lymphoma in complete remission, multiple myeloma in complete remission, myelodysplastic syndrome in refractory anemia (RA), and aplastic anemia were defined as low risk. All others were considered high risk.

Management of CMV Infection

CMV-specific IgM antibodies in the cord blood units were not examined. Because most patients had been heavily treated and received multiple transfusions, anti-CMV antibodies were not examined before transplantation. Anti-CMV high-titer i.v. immunoglobulin was not regularly administered. All packed