Bone Marrow Transplantation (2005) 35, 549–556

www.nature.com/bmt

Conditioning regimens

Reduced-intensity stem-cell transplantation for adult acute lymphoblastic leukemia: a retrospective study of 33 patients

T Hamaki[1,2], M Kami[2], Y Kanda[3], K Yuji[4], Y Inamoto[5], Y Kishi[2], K Nakai[6], I Nakayama[7], N Murashige[2], Y Abe[8], Y Ueda[9], M Hino[10], T Inoue[11], H Ago[12], M Hidaka[13], T Hayashi[14], T Yamane[10], N Uoshima[7], S Miyakoshi[4] and S Taniguchi[4]

[1]*Department of Transfusion Medicine, Metropolitan Fuchu Hospital, Tokyo, Japan;* [2]*Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo, Japan;* [3]*Department of Cell Therapy & Transplantation Medicine, University of Tokyo, Tokyo, Japan;* [4]*Department of Hematology, Toranomon Hospital, Tokyo, Japan;* [5]*Department of Internal Medicine, Japanese Red Cross Nagoya First Hospital, Japan;* [6]*First Department of Internal Medicine, Kansai Medical University, Osaka, Japan;* [7]*Department of Hematology, Matsushita Memorial Hospital, Osaka, Japan;* [8]*Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan;* [9]*Department of Internal Medicine, Kurashiki Central Hospital, Okayama, Japan;* [10]*Department of Clinical Hematology and Clinical Diagnostics, Graduate School of Medicine, Osaka City University, Osaka, Japan;* [11]*Department of Internal Medicine, Shiga University of Medical Science, Shiga, Japan;* [12]*Department of Hematology and Oncology, Shimane Prefectural Central Hospital, Shimane, Japan;* [13]*Department of Internal Medicine, Kumamoto National Hospital, Kumamoto, Japan; and* [14]*Department of Hematology, Tenri Hospital, Osaka, Japan*

Summary:

Efficacy of reduced-intensity stem-cell transplantation (RIST) for acute lymphoblastic leukemia (ALL) was investigated in 33 patients (median age, 55 years). RIST sources comprised 20 HLA-identical related donors, five HLA-mismatched related, and eight unrelated donors. Six patients had undergone previous transplantation. Disease status at RIST was first remission ($n=13$), second remission ($n=6$), and induction failure or relapse ($n=14$). All patients tolerated preparatory regimens and achieved neutrophil engraftment (median, day 12.5). Acute and chronic graft-versus-host disease (GVHD) developed in 45 and 64%, respectively. Six patients received donor lymphocyte infusion (DLI), for prophylaxis ($n=1$) or treatment of recurrent ALL ($n=5$). Nine patients died of transplant-related mortality, with six deaths due to GVHD. The median follow-up of surviving patients was 11.6 months (range, 3.5–37.3 months). The 1-year relapse-free and overall survival rates were 29.8 and 39.6%, respectively. Of the 14 patients transplanted in relapse, five remained relapse free for longer than 6 months. Cumulative rates of progression and progression-free mortality at 3 years were 50.9 and 30.4%, respectively. These findings suggest the presence of a graft-versus-leukemia effect for ALL. RIST for ALL is worth considering for further evaluation.
Bone Marrow Transplantation (2005) **35**, 549–556.
doi:10.1038/sj.bmt.1704776
Published online 31 January 2005

Correspondence: Dr M Kami, Hematopoietic Stem Cell Transplant Unit, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; E-mail: mkami@ncc.go.jp
Received 7 June 2004; accepted 5 October 2004
Published online 31 January 2005

Keywords: reduced-intensity hematopoietic stem cell transplantation; acute lymphoblastic leukemia; graft-versus-host disease; regimen-related toxicity; graft-versus-leukemia effect

Allogeneic stem-cell transplantation (allo-SCT) represents a curative option for acute lymphoblastic leukemia (ALL). Myeloablative therapy with high-dose radiochemotherapy or chemotherapy alone followed by allo-SCT is the most powerful method for eradicating leukemic cells. Patients who experience relapse are candidates for allo-SCT, since few patients who have relapsed are cured using conventional chemotherapies.[1] In adult patients with ALL in second complete remission (CR2), most studies have indicated a disease-free survival (DFS) rate of approximately 30%.[2,3] In contrast, allo-SCT for adult patients with ALL in first complete remission (CR1) is controversial, since allo-SCT is associated with significant transplant-related mortality (TRM). Whether allo-SCT is beneficial for ALL in CR1 remains unclear.[4,5] Some patients with certain high-risk factors, including specific cytogenetic abnormalities, should be offered allo-SCT in CR1.[6]

Elimination of leukemic cells following allo-SCT is attributable to two processes: the direct effect of chemo-radiotherapy; and graft-versus-leukemia (GVL) effects. The role of GVL effects is less defined in ALL. Some case reports have demonstrated durable remission following donor lymphocyte infusion (DLI),[7,8] and several studies have shown that a GVL effect associated with graft-versus-host disease (GVHD) contributes to a reduction in ALL relapse after allo-SCT.[9–11] However, these case reports and small studies might have suffered from patient selection bias. Two retrospective studies have suggested that GVL effects for ALL are weaker than those for myeloid

malignancies.[9,12] In a study of 44 ALL patients treated using DLI, only three achieved remission ⩾1 year, and 3-year overall survival (OS) was 13%.[13] DLI showed only limited benefit in the treatment of recurrent ALL after allogeneic CST.

A new strategy for transplantation using reduced-intensity conditioning for stem-cell transplantation (RIST) has been developed to reduce regimen-related toxicities while preserving antileukemic effects.[14] This strategy decreases the risk of nonrelapse mortality and allows transplantation in elderly patients or those with organ dysfunction. Since RIST is based on a GVL effect, usefulness for ALL has not yet been clarified. Most prospective studies on RIST have included small numbers of patients with ALL, ranging from 1 to 9.[14 19] Five studies have reported using ⩾10 patients with ALL.[20 24] Two retrospective multicenter studies focused on RIST for ALL.[21,22] Arnold *et al*[21] reported that four of 22 patients were alive and disease-free at 5–30 months after RIST, and that seven died of TRM. In all four patients who survived following RIST, underlying diseases were in CR at the time of transplant. Martino *et al*[22] summarized the features of patients from four prospective studies, including patients with advanced ALL. OS and TRM were 31 and 23%, respectively. Relapse rate was 33% in patients transplanted in CR, and 60% in those with overt disease.

The present study describes our experience of 31 patients with ALL who were treated using RIST.

Patients and methods

Diagnosis and classification of clinical subtypes of ALL

ALL was diagnosed on the basis of cytology, karyotype, and immunophenotyping of marrow cells. Patients had received induction/consolidation chemotherapy in accordance with local protocols.

Patient characteristics

A total of 33 patients with ALL underwent RIST at 18 transplant centers between October 2000 and November 2003. Patients who were not candidates for conventional myeloablative transplantation were considered for RIST. All patients satisfied one or more of the following inclusion criteria: age greater than 50 years, prior autologous transplantation with high-dose chemotherapy, and non-hematologic organ dysfunction. They all gave informed consent. Patient characteristics are shown in Table 1.

Table 1 Patient characteristics

Variables	*n*
Median age; range (years)	55; 17–68
Sex; Male/female	11/22
Performance status[a]; 0–2/3–4	29/3
Lineage; T/B	1/32
Cytogenetics	
Normal	10
t(9:22)(q34;q11)	14
t(1;19)(q23;p13.3)	1
Hypodiploid	1
Others	5
Not available	2
Disease status	
Complete remission first/second	13/6
Refractory primary	5
First/second/third relapse	4/4/1
Indication for RIST[b]	
Age	19
Organ dysfunction	9
Previous transplantation	6
Poor performance status	3
Heavily treated	1
Infection	4
Donor	
HLA-identical related	20
HLA-mismatch related	5
Unrelated	8
Stem cells; Marrow/blood	9/24
Preparative regimens	
Fludarabine/busulfan and related	15
Fludarabine/melphalan	8
Other fludarabine-based	4
Cladribine-based	2
Others	4
GVHD prophylaxis	
Cyclosporin alone	8
Cyclosporin/methotrexate	17
Tacrolimus/methotrexate	3
Others	5

[a]Performance status defined according to ECOG criteria. Information on PS was unavailable for one patient.
[b]Nine patients displayed two indications for RIST.

Preparatory regimen and transfused stem cells

Transplantation procedures are shown in Table 1. Preparatory regimens comprised fludarabine(Flu)/busulfan with ($n=3$) or without rabbit anti-thymocyte globulin (ATG) ($n=9$), or with total body irradiation (TBI) at 4–8 Gy ($n=2$), or with both ATG and TBI ($n=1$),[14,17] Flu/melphalan ($n=8$),[15] another fludarabine-based regimen ($n=4$),[25] cladribine-based regimen ($n=2$), TBI at 2 Gy ($n=2$),[16] and others ($n=2$). Either T-cell depletion or CD34-positive cell selection was performed in all patients. The median number of transfused cells was 3.9×10^6 $CD34^+$ blood stem cells/kg (range, 0.98–7.5 $\times 10^6$ cells/kg) or 3.2×10^8 marrow-nucleated cells/kg (range, 0.43–5.3 cells/kg).

Definition

Reduced-intensity regimens were defined as reported previously.[26,27]

The day of neutrophil engraftment was defined as the first of three consecutive days on which absolute neutrophil count was >500 cells/μl. The day of platelet engraftment was defined as the first of seven consecutive days on which the platelet count was >20 000/μl without platelet transfusion.

The diagnosis of GVHD was based on clinical evidence with histologic confirmation whenever possible. Acute GVHD within the first 100 days after transplantation was graded according to standard criteria.[28,29] Patients who survived at least 100 days were evaluated for chronic GVHD. Chronic GVHD was graded as limited (localized skin or single organ involvement) or clinically extensive.

TRM was defined as death without progression of underlying disease. OS was defined as duration of survival between transplant and either death or last follow-up. Progression-free survival (PFS) was defined as duration of survival after transplant without disease progression, relapse, or death. When ALL recurred before engraftment, the underlying disease was considered to have progressed on the day of transplant.

Study endpoints

The major end point was 1-year PFS following RIST. Secondary end points included incidence of relapse, nonrelapse mortality, incidence and severity of GVHD, engraftment, complications, frequency of DLI, and OS. Patients were considered to have died of nonrelapse cause if no evidence of disease relapse or progression was apparent. Data were analyzed as of March 1, 2004.

Statistical analysis

Cumulative incidences of progression and progression-free mortality were evaluated using Gray's method, considering each other risk as a competing risk.[30] OS and PFS were estimated using the Kaplan–Meier methods. Potential confounding factors considered in the analysis were age, sex, donor type (HLA-matched related donor *vs* alternative donor), stem cell source (bone marrow (BM) *vs* peripheral blood stem cells (PBSC)), HLA-mismatch, disease status, conditioning regimen, and development of grades II–IV acute GVHD. To evaluate the influence of these factors on PFS, proportional hazard modeling was used, treating the development of acute GVHD as a time-dependent covariate. Factors associated with at least borderline significance ($P<0.10$) on univariate analyses were subjected to multivariate analysis using backward stepwise proportional-hazard modeling. Values of $P<0.05$ were considered statistically significant.

Results

Engraftment

Neutrophil counts did not decrease below 500/μl in one patient, while autologous blasts recovered in two patients. The remaining 30 patients achieved neutrophil engraftment within a median interval of 12.5 days (range, 8–26 days). Among 31 patients with neutrophil recovery, two patients never experienced thrombocytopenia and three patients died without platelet engraftment. The other 26 patients achieved platelet engraftment within a median of 13 days (range, 8–50 days).

GVHD and other complications

Two patients died within 100 days of transplant. Causes of death were diffuse alveolar damage and hemophagocytic syndrome.

A total of 15 patients (45%) developed grades II–IV acute GVHD. Maximal ratings were grade II ($n=9$), III ($n=3$), or IV ($n=3$). Acute GVHD was fatal in five patients. Of the 25 evaluable patients (64%) who survived longer than 100 days, 16 developed chronic GVHD.

Infections were documented in seven patients. Causative organisms included methicillin-resistant *Staphylococcus aureus* ($n=3$), *Escherichia coli* ($n=1$), *Pseudomonas aeruginosa* ($n=1$), *Candida tropicalis* ($n=1$), and *Aspergillus* species ($n=1$). Infections were fatal in four patients.

Noninfectious complications other than GVHD occurred in eight patients, and comprised: hepatic veno-occlusive disease ($n=2$); chronic subdural hematoma ($n=1$); hemophagocytic syndrome ($n=1$); interstitial pneumonitis ($n=1$); pleural effusion ($n=1$); idiopathic pulmonary syndrome ($n=1$); and engraftment syndrome ($n=1$).

A total of 21 patients died, and disease progression was absent in nine of these cases. Causes of death included GVHD ($n=6$), idiopathic pulmonary syndrome ($n=1$), hemophagocytic syndrome ($n=1$), and sepsis ($n=1$). The remaining 12 patients died after leukemic progression, with eight deaths due to progressive disease, and other four attributable to complications associated with DLI (acute GVHD $n=2$) or second RIST (thrombotic microangiopathy, $n=2$; hepatic veno-occlusive disease, $n=2$).

Response to RIST

In all, 12 of 14 patients transplanted during relapse achieved durable remission (Table 2). In the other two patients, ALL recurred immediately after neutrophil engraftment. In seven patients with t(9:22)(q34;q11), minimal residual disease was monitored with reverse transcriptase-polymer chain reaction assay (RT-PCR) using bcr-abl-specific primers. Molecular remission was confirmed in four of the seven patients (Patients 4, 13, 26, and 27), with durations of 4.2, 4.6, 11.1, and 27.6 months, respectively.

OS and PFS

As of March 2004, 12 patients were alive in CR ($n=9$) and relapse ($n=3$) with a median follow-up of 11.6 months (range, 3.5–37.3 months). Probability of 2-year PFS and OS was 18.6% (95% confidence interval (CI), 2.4–34.9%) and 29.7% (95% CI, 11.7–47.7%), respectively (Figure 1). The median duration of overall survival was 177 days (range, 40–1119 days). Cumulative rates of progression and progression-free mortality at 3 years were 50.9 and 30.4%, respectively (Figure 2).

Actuarial 1-year PFS rates were 30.6% (95% CI, 7.7–53.5%) for the 19 patients transplanted in CR1/CR2 and 28.6% (95% CI, 4.9-52.2%) for the 14 patients transplanted in relapse or induction failure ($P=0.26$) (Figure 3).

Table 2 Outcomes of patients who received RIST for ALL

Patient	*Age/ sex*	*Karyotype*	*Status before RIST*	*Performance status*	*Donor*	*Relapse*	*Reason for DLI*	*Acute GVHD*	*Chronic GVHD*	*Present status*	*Follow-up, days*	*Cause of death*
1	55/F	Unavailable	CR1	1	Identical related	Day + 28		II	NE	Dead	85	Leukemia
2	59/F	Others	CR1	1	Identical related	Day + 197	Relapse	II	Limited	Dead	299	Severe GVHD following DLI
3	68/F	Normal	CR2	2	Identical related	Day + 315	CNS relapse	0	No	Dead	870	Progression after second RIST
4	43/F	t(9:22)(q34;q11)	Primary refractory	3	Mismatched related	No	Prophylaxis	I	Extensive	Death in CR1	406	Bronchitis obliterans
5	56/F	Normal	CR1	0	Identical related	No		0	Extensive	Alive in CR1	1119	
6	50/M	t(9:22)(q34;q11)	Second relapse	0	Identical related	No		II	NE	Death	71	Hemophagocytic syndrome, VOD, GVHD
7	58/M	t(9:22)(q34;q11)	CR2	0	Mismatched related	Day + 181		II	Not available	Dead	237	Leukemia
8	31/F	t(9:22)(q34;q11)	Second relapse	0	Identical related	Day + 36	Relapse	0	NE	Death	134	Second RIST on day 67, died from TMA/VOD
9	45/F	Normal	CR1	0	Identical related	No		II	Extensive	alIve in CR1	966	
10	50/F	t(9:22)(q34;q11)	CR1	0	Identical related	Day + 92		0	No	Dead	421	Leukemia
11	23/F	Others	First relapse	1	Matched unrelated	No		0	Limited	Alive in CR2	828	
12	35/M	t(9:22)(q34;q11)	First relapse	4	Matched unrelated	No		II	No	Death in CR1	130	Pulmonary hemorrhage after *Pseudomonas* pneumonia
13	56/M	t(9:22)(q34;q11)	Primary refractory	1	Identical related	Day + 712		III	Extensive	Alive with disease	734	
14	64/F	Normal	CR1	0	Identical related	No		0	Extensive	Alive in CR1	516	
15	46/M	t(9:22)(q34;q11)	CR1	0	Matched unrelated	No		I	Extensive	Died in CR1	162	MRSA pneumonia, sepsis
16	53/F	Hypodiploid	First relapse	0	Identical related	No		III	Unavailable	Death in CR2	167	Endotoxic shock
17	55/M	Normal	CR2	0	Identical related	Day + 84	Relapse	0	Extensive	died in CR3	136	Severe GVHD following DLI
18	67/M	t(9:22)(q34;q11)	CR1	1	Identical related	No		I	Extensive	Died in CR1	176	Respiratory failure due to idiopathic pulmonary syndrome
19	55/F	Normal	Primary refractory	1	Identical related	No		0	Extensive	Alive in CR1	333	
20	59/M	t(9:22)(q34;q11)	CR1	1	Identical related	No		0	Extensive	Alive in CR1	347	
21	52/F	Others	CR1	NE	Matched unrelated	No		I	NE	Died in CR1	68	Respiratory failure due to diffuse alveolar damage
22	60/F	t(1;19)(q23;p13.3)	First relapse	2	Mismatched related			IV	NE	Death	54	Severe GVHD after FK tapering for refractory disease
23	60/F	t(9:22)(q34;q11)	CR2	0	Identical related	Day + 99	Relapse	II	Extensive	Dead	148	Leukemia
24	33/F	Others	Second relapse	1	Mismatched unrelated	No		IV	Limited	Death in CR3	186	Fungal pneumonia

Table 2 Continued

Patient	*Age/ sex*	*Karyotype*	*Status before RIST*	*Performance status*	*Donor*	*Relapse*	*Reason for DLI*	*Acute GVHD*	*Chronic GVHD*	*Present status*	*Follow-up, days*	*Cause of death*
25	27/M	Normal	CR2	1	Matched unrelated	No		II	Limited	Alive in CR2	257	
26	59/F	t(9:22)(q34;q11) + complex	Primary refractory	0	Matched unrelated	No		IV	No	Death in CR1	125	Liver GVHD followed by multiple organ failure
27	65/F	t(9:22)(q34;q11)	Second relapse	2	Mismatched related	Day+138		0	No	Death	182	Leukemia
28	55/F	t(9:22)(q34;q11)	CR1	1	Identical related	No		0	No	Alive in CR1	166	
29	17/M	Others	Primary refractory	3	Identical related			0	NE	Death	40	Leukemia in CNS
30	29/F	Normal	Third relapse	1	Identical related	Day+113		II	No	Alive with disease	148	
31	37/F	Complex	CR2	0	Matched unrelated	Day+86		I	No	Alive with disease	131	
32	56/F	Normal	CR1	1	Identical related	Day+99		0	No	Dead	178	Leukemia
33	59/F	Normal	CR1	0	Mismatched related	No		III	No	Alive in CR1	104	

M = male; F = female; CR = complete remission; NE = not evaluated; DLI = donor lymphocyte infusion; GVHD = graft-versus-host disease; CNS = central nervous system, TMA = thrombotic microangiopathy; VOD = veno-occlusive disease.

Association between GVHD and OS

Among the 21 patients who survived without disease progression longer than 100 days, presence of grades II–IV acute GVHD tended to show better PFS compared with those without it; however, the difference was marginal (relative risk 0.45,. 95% CI 0.18–1.16, $P=0.10$) (Figure 4).

DLI

DLI was undertaken in six patients. Patient 4 received prophylactic DLI and achieved durable molecular remission until she died of bronchitis obliterans at 13.5 months after RIST. The remaining five patients underwent DLI following recurrence of ALL. Two patients (Patients 3 and 8) received DLI followed by second RIST. Patient 8 underwent second RIST 38 days after DLI, and died from progressive disease 29 days after second RIST. Patient 3 received DLI for central nervous system (CNS) relapse, and underwent second RIST. She relapsed again in the CNS and bone marrow, and died of disease progression 8.7 months after second RIST.

Response to DLI was evaluated in the remaining three patients (Patients 2, 17, and 23). One patient responded to DLI, and two died of acute GVHD. Patient 2 received DLI for emerging extramedullary disease. The lesion was controlled using local irradiation and DLI. However, ALL recurred in the BM and the patient experienced grade IV acute GVHD that eventually proved fatal. In Patient 17, peripheral blasts disappeared after DLI, but the patient died of gastrointestinal GVHD. Patient 23 died of disease progression without any response to DLI.

Prognostic factors for PFS

In univariate analyses, no variables were identified as significant prognostic factors for PFS (Table 3). Multivariate analysis was discontinued due to the lack of associated factors from univariate analyses.

Discussion

GVL effects against ALL after allo-SCT have been discussed for almost 15 years.[31,32] A small but significant proportion of patients with advanced ALL achieve durable remission following RIST. Of the 14 patients transplanted in relapse, 12 achieved durable remission, and molecular remission was confirmed in four of the seven patients with t(9;22)(q34;q11) in this study. Considering that combination chemotherapy is usually ineffective at producing prolonged survival in patients with advanced ALL, the long-term PFS after allo-SCT suggests that durable allogeneic immune reactions continue to suppress leukemic progression.

Optimal reduced-intensity regimens remain unclear for RIST for ALL. Even after achievement of CR following allo-SCT, patients with ALL display high levels of minimal residual disease associated with increased relapse rates.[33] Most physicians believe that GVL effects are insufficient or may not have enough time to arrest advanced ALL, and

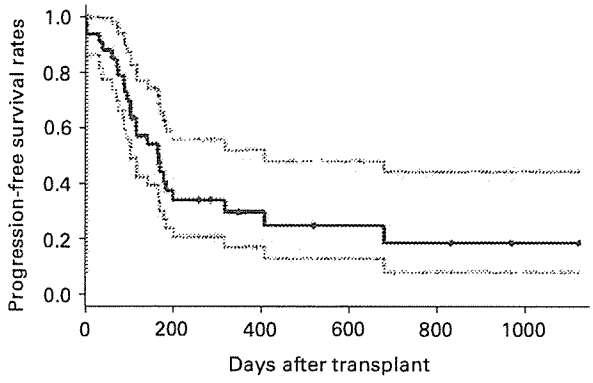

Figure 1 Progression-free survival. Probability of 2-year PFS and OS was 18.6% (95% confidence interval (CI), 2.4–34.9%) and 29.7% (95% CI, 11.7–47.7%), respectively. Broken lines show 95% CI. PFS at 3 years was 18.6% (95% confidence interval, 2.4–34.9%).

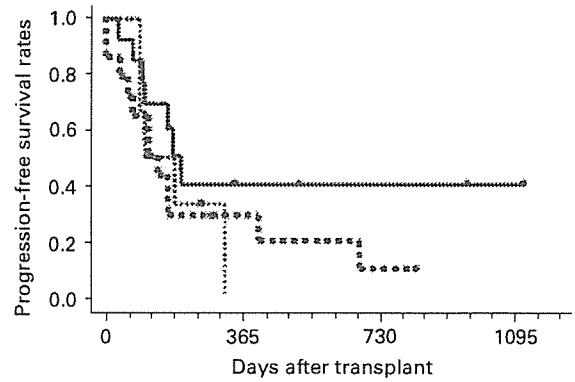

Figure 3 Association between progression-free survival (PFS) rates and disease status at RIST. Actuarial 1-year PFS rates were 30.6% (95% CI, 7.7–53.5%) for the 19 patients transplanted in CR1/CR2 and 28.6% (95% CI, 4.9–52.2%) for the 14 patients transplanted in relapse or induction failure ($P = 0.26$). Solid line indicates patients in CR1 and large broken line indicates patients in CR2. Small broken line indicates the others.

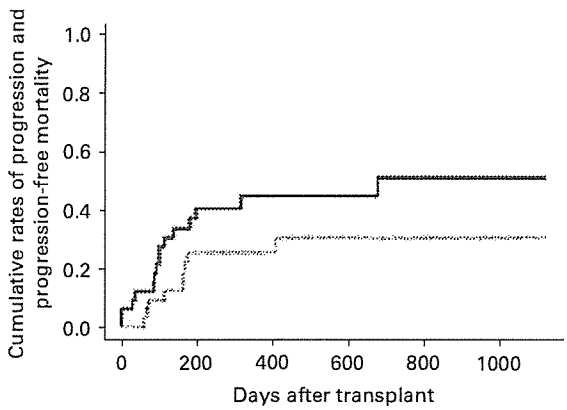

Figure 2 Cumulative rates of progression and progression-free mortality. Cumulative incidence of progression at 3 years was 50.9%. Cumulative incidence of nonprogression mortality at 3 years was 30.4%. Solid line indicates progression and broken line indicates progression-free mortality.

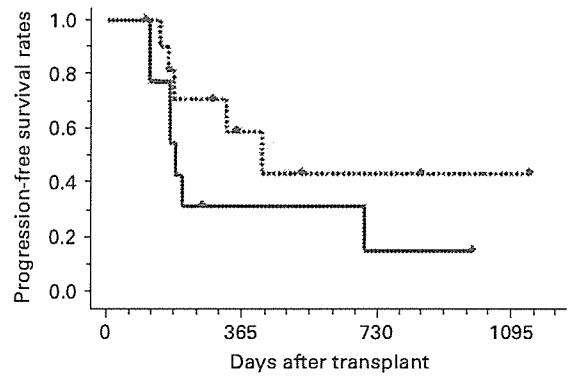

Figure 4 Influence of acute GVHD on progression-free survival (PFS) rates. Among the 21 patients who survived without disease progression longer than 100 days, presence of grades II–IV acute GVHD tended to show better PFS compared with those without it; however, the difference was marginal (relative risk 0.45, 95% CI 0.18–1.16, $P = 0.10$). Solid line indicates patients with grades II–IV acute GVHD ($n = 10$). Broken line indicates patients with acute GVHD less than grade II ($n = 11$).

that preparative regimens should be more intense in RIST for ALL compared with chronic-phase chronic myeloid leukemia or low-grade malignant lymphoma. Purine analog-based regimens were mostly used in this study, and chemotherapeutic agents such as melphalan, busulfan and 4–8 Gy TBI were added in 28 patients. These regimens might have been beneficial for establishing durable engraftment in RIST for advanced ALL, and might have contributed to temporary control of disease. The role of additional chemoradiotherapy needs to be further investigated in RIST for ALL.

Optimal timing of RIST for ALL remains unknown. Estimated 2-year PFS rates after RIST for high- and low-risk ALL were 8 and 42%, respectively. Outcomes for RIST are dismal unless therapy is given in CR1 (Figure 3). These findings are comparable to previous reports on conventional allo-SCT for ALL.[34,35] Disease status remains an important prognostic factor in RIST and decision-making as to when to proceed with allo-SCT in patients with ALL is difficult.

Response rates to DLI in ALL patients were low,[12,13] suggesting a limited GVL effect for ALL, especially after hematologic relapse. Only early intervention before clinical relapse can improve prognosis for these patients, by inducing GVL effects.[13,36] In our study, DLI induced remission in one of five patients who relapsed following RIST, accompanied by severe GVHD. Another two patients died of leukemic progression, and the other two patients died of GVHD following DLI. However, some patients dramatically benefit from DLI such as Patients 2 and 17 in our study, and the one reported by Slavin *et al.*[7] Many publications suggest that the efficacy of DLI may be improved by activation of donor lymphocytes using *in vitro* or *in vivo* interleukin-2 with no prohibitive GVHD.[37] Activation of donor lymphocytes is a possible future approach in an attempt to amplify the already well-documented GVL effect in ALL.

Table 3 Risk factors for overall survival following allogeneic hematopoietic stem-cell transplantation

Factors	*Hazard ratio*	*95% Confidence interval*	*P*
Univariate analysis			
Pre-transplant factors			
Age ⩾50 *vs* <50 years	1.1	0.47–2.57	0.83
Ph1 *vs* others	1.48	0.66–3.31	0.34
Previous SCT (+) *vs* (−)	0.51	0.15–1.73	0.28
CR1 *vs* others	0.53	0.22–1.28	0.16
CR12 *vs* others	0.64	0.28–1.42	0.27
PS34 *vs* PS012	1.7	0.50–5.78	0.39
PB *vs* BM	0.82	0.34–2.00	0.66
Matched related *vs* others	0.78	0.34–1.76	0.54
GVHD prophylaxis single *vs* combined	0.58	0.22–1.57	0.29
Post transplant factor			
GVHD II–IV present *vs* absent	2.2	0.86–5.62	0.10

Ph1 = Philadelphia chromosome 1; SCT = stem cell transplantation; CR1 = first complete remission; CR12 = first or second complete remission; PS = ECOG performance status; PB = peripheral blood stem cells, BM = bone marrow stem cells; GVHD = graft-versus-host disease.

Two basic types of complications are associated with allo-SCT: regimen-related toxicity and GVHD. Nonrelapse mortality rate within 100 days of transplantation was 6.4% in this study. Reduced-intensity regimens cause less organ damage, contributing to lower rates of TRM. These findings are comparable to previous reports.[14,15,38] GVHD represents another significant concern after RIST,[39] which is frequently complicated by infections.[40] Rates of acute and chronic GVHD were 45 and 64%, respectively. The balance between GVHD and GVL is delicate in allo-SCT. Augmentation of GVHD prophylaxis may hamper GVL effects, and leukemic cells cannot be eradicated by reduced-intensity conditioning alone. This study failed to show meaningful advantages of GVHD in preventing relapse of ALL following RIST, while GVHD remains the leading cause of TRM. At present, allo-SCT recipients receive uniform GVHD prophylaxis irrespective of the risk of underlying disease or patient condition. Intensification of GVHD prophylaxis is at least beneficial in RIST for low-risk ALL. Management of GVHD prophylaxis should be optimized considering risk of underlying disease and patient condition.

Our study indicates that RIST is worth considering for further intense evaluation. The technique may offer the best chance for hematologic remission and prolonged survival, given the poor prognosis after conventional chemoradiotherapy. However, this retrospective study was too small to provide definitive conclusions regarding RIST for ALL. Since our study included heterogeneous patient group, centers, and conditioning approaches, interpretation of the results requires caution. Further investigation and data are awaited to determine the indications and optimal transplantation procedures for ALL.

Acknowledgements

We are grateful to Drs Takanori Teshima, Naoki Kobayashi, Takashi Ashida, Atsushi Woke, Issei Hatanaka and Shinji Nakao for their cooperation and detailed descriptions of their cases.

References

1 Giona F, Testi AM, Annino L *et al.* Treatment of primary refractory and relapsed acute lymphoblastic leukaemia in children and adults: the GIMEMA/AIEOP experience. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto. Associazione Italiana Ematologia ed Ocologia Pediatrica. *Br J Haematol* 1994; **86**: 55–61.
2 Herzig RH, Bortin MM, Barrett AJ *et al.* Bone-marrow transplantation in high-risk acute lymphoblastic leukaemia in first and second remission. *Lancet* 1987; **1**: 786–789.
3 Butturini A, Gale RP. Chemotherapy *versus* transplantation in acute leukaemia. *Br J Haematol* 1989; **72**: 1–8.
4 Horowitz MM, Messerer D, Hoelzer D *et al.* Chemotherapy compared with bone marrow transplantation for adults with acute lymphoblastic leukemia in first remission. *Ann Intern Med* 1991; **115**: 13–18.
5 Fiere D, Lepage E, Sebban C *et al.* Adult acute lymphoblastic leukemia: a multicentric randomized trial testing bone marrow transplantation as postremission therapy. The French Group on Therapy for Adult Acute Lymphoblastic Leukemia. *J Clin Oncol* 1993; **11**: 1990–2001.
6 Avivi I, Goldstone AH. Bone marrow transplant in Ph+ ALL patients. *Bone Marrow Transplant* 2003; **31**: 623–632.
7 Slavin S, Naparstek E, Nagler A *et al.* Allogeneic cell therapy with donor peripheral blood cells and recombinant human interleukin-2 to treat leukemia relapse after allogeneic bone marrow transplantation. *Blood* 1996; **87**: 2195–2204.
8 Ferster A, Bujan W, Mouraux T *et al.* Complete remission following donor leukocyte infusion in ALL relapsing after haploidentical bone marrow transplantation. *Bone Marrow Transplant* 1994; **14**: 331–332.
9 Horowitz MM, Gale RP, Sondel PM *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; **75**: 555–562.
10 Appelbaum FR. Graft versus leukemia (GVL) in the therapy of acute lymphoblastic leukemia (ALL). *Leukemia* 1997; **11** (Suppl. 4): S15–S17.
11 Cornelissen JJ, Carston M, Kollman C *et al.* Unrelated marrow transplantation for adult patients with poor-risk acute lymphoblastic leukemia: strong graft-versus-leukemia effect and risk factors determining outcome. *Blood* 2001; **97**: 1572–1577.
12 Kolb HJ, Schattenberg A, Goldman JM *et al.* Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. *Blood* 1995; **86**: 2041–2050.
13 Collins Jr RH, Goldstein S, Giralt S *et al.* Donor leukocyte infusions in acute lymphocytic leukemia. *Bone Marrow Transplant* 2000; **26**: 511–516.
14 Slavin S, Nagler A, Naparstek E *et al.* Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; **91**: 756–763.
15 Giralt S, Thall PF, Khouri I *et al.* Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood* 2001; **97**: 631–637.
16 McSweeney PA, Niederwieser D, Shizuru JA *et al.* Hematopoietic cell transplantation in older patients with hematologic

malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; **97**: 3390–3400.
17 Bornhauser M, Thiede C, Platzbecker U *et al.* Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. *Clin Cancer Res* 2001; **7**: 2254–2262.
18 Niederwieser D, Maris M, Shizuru JA *et al.* Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. *Blood* 2003; **101**: 1620–1629.
19 Maris MB, Niederwieser D, Sandmaier BM *et al.* HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood* 2003; **102**: 2021–2030.
20 Rezvani K, Lalancette M, Szydlo R *et al.* Non-myeloablative stem cell transplant (NMSCT) in AML, ALL and MDS: disappointing outcome for patients with advanced phase disease. *Blood* 2000; **96**: 479a.
21 Arnold R, Massenkeil G, Bornhauser M *et al.* Nonmyeloablative stem cell transplantation in adults with high-risk ALL may be effective in early but not in advanced disease. *Leukemia* 2002; **16**: 2423–2428.
22 Martino R, Giralt S, Caballero MD *et al.* Allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning in acute lymphoblastic leukemia: a feasibility study. *Haematologica* 2003; **88**: 555–560.
23 Michallet M, Bilger K, Garban F *et al.* Allogeneic hematopoietic stem-cell transplantation after nonmyeloablative preparative regimens: impact of pretransplantation and post-transplantation factors on outcome. *J Clin Oncol* 2001; **19**: 3340–3349.
24 Ruiz-Arguelles GJ, Gomez-Almaguer D, Ruiz-Arguelles A *et al.* Results of an outpatient-based stem cell allotransplant program using nonmyeloablative conditioning regimens. *Am J Hematol* 2001; **66**: 241–244.
25 Childs R, Chernoff A, Contentin N *et al.* Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 2000; **343**: 750–758.
26 Bacigalupo A. Second EBMT Workshop on reduced intensity allogeneic hemopoietic stem cell transplants (RI-HSCT). *Bone Marrow Transplant* 2002; **29**: 191–195.
27 Bacigalupo A. Third EBMT/AMGEN Workshop on reduced-intensity conditioning allogeneic haemopoietic stem cell transplants (RIC-HSCT), and panel consensus. *Bone Marrow Transplant* 2004; **33**: 691–696.
28 Glucksberg H, Storb R, Fefer A *et al.* Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* 1974; **18**: 295–304.
29 Przepiorka D, Weisdorf D, Martin P *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
30 Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med* 1999; **18**: 695–706.
31 Weisdorf DJ, Nesbit ME, Ramsay NK *et al.* Allogeneic bone marrow transplantation for acute lymphoblastic leukemia in remission: prolonged survival associated with acute graft-versus-host disease. *J Clin Oncol* 1987; **5**: 1348–1355.
32 Kersey JH, Weisdorf D, Nesbit ME *et al.* Comparison of autologous and allogeneic bone marrow transplantation for treatment of high-risk refractory acute lymphoblastic leukemia. *N Engl J Med* 1987; **317**: 461–467.
33 Bader P, Hancock J, Kreyenberg H *et al.* Minimal residual disease (MRD) status prior to allogeneic stem cell transplantation is a powerful predictor for post-transplant outcome in children with ALL. *Leukemia* 2002; **16**: 1668–1672.
34 Doney K, Fisher LD, Appelbaum FR *et al.* Treatment of adult acute lymphoblastic leukemia with allogeneic bone marrow transplantation. Multivariate analysis of factors affecting acute graft-versus-host disease, relapse, and relapse-free survival. *Bone Marrow Transplant* 1991; **7**: 453–459.
35 Forman SJ, Schmidt GM, Nademanee AP *et al.* Allogeneic bone marrow transplantation as therapy for primary induction failure for patients with acute leukemia. *J Clin Oncol* 1991; **9**: 1570–1574.
36 Bader P, Klingebiel T, Schaudt A *et al.* Prevention of relapse in pediatric patients with acute leukemias and MDS after allogeneic SCT by early immunotherapy initiated on the basis of increasing mixed chimerism: a single center experience of 12 children. *Leukemia* 1999; **13**: 2079–2086.
37 Lonnqvist B, Brune M, Ljungman P. Lymphoblastoid human interferon and low dose IL-2 combined with donor lymphocyte infusion as therapy of a third relapse of CML – a case report. *Bone Marrow Transplant* 1996; **18**: 241–242.
38 Fukuda T, Hackman RC, Guthrie KA *et al.* Risks and outcomes of idiopathic pneumonia syndrome after nonmyeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. *Blood* 2003; **102**: 2777–2785.
39 Mielcarek M, Martin PJ, Leisenring W *et al.* Graft-versus-host disease after nonmyeloablative *versus* conventional hematopoietic stem cell transplantation. *Blood* 2003; **102**: 756–762.
40 Fukuda T, Boeckh M, Carter RA *et al.* Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. *Blood* 2003; **102**: 827–833.

Biology of Blood and Marrow Transplantation 11:429-436 (2005)

1083-8791/05/1106-0003$30.00/0
doi:10.1016/j.bbmt.2005.01.010

Bloodstream Infection after Umbilical Cord Blood Transplantation Using Reduced-Intensity Stem Cell Transplantation for Adult Patients

Hiroto Narimatsu,[1] Tomoko Matsumura,[1] Masahiro Kami,[2] Shigesaburo Miyakoshi,[1] Eiji Kusumi,[1] Shinsuke Takagi,[1] Yuji Miura,[1] Daisuke Kato,[1] Chiho Inokuchi,[2] Tomohiro Myojo,[1] Yukiko Kishi,[2] Naoko Murashige,[2] Koichiro Yuji,[1] Kazuhiro Masuoka,[1] Akiko Yoneyama,[1] Atsushi Wake,[1] Shinichi Morinaga,[1] Yoshinobu Kanda,[3] Shuichi Taniguchi,[1] for the Tokyo Stem Cell Transplantation Consortium

[1]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [2]Hematopoietic Stem Cell Transplant Unit, The National Cancer Center Hospital, Tokyo, Japan; [3]The Department of Cell Therapy and Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan

Correspondence and reprint requests: Masahiro Kami, MD, Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan (e-mail: mkami@ncc.go.jp).

Received December 24, 2004; accepted January 27, 2005

ABSTRACT

Bloodstream infection (BSI) is a significant problem after cord blood transplantation (CBT). However, little information has been reported on BSI after reduced-intensity CBT (RI-CBT). We retrospectively reviewed the medical records of 102 patients. The median age of the patients was 55 years (range, 17-79 years). Preparative regimens comprised fludarabine 125 to 150 mg/m^2, melphalan 80 to 140 mg/m^2, or busulfan 8 mg/kg and total body irradiation 2 to 8 Gy. Prophylaxis against graft-versus-host disease comprised cyclosporin or tacrolimus. BSI developed within 100 days of RI-CBT in 32 patients. The cumulative incidence of BSI was 25% at day 30 and 32% at day 100. The median onset was day 15 (range, 1-98 days). Causative organisms included *Pseudomonas aeruginosa* (n = 12), *Staphylococcus epidermidis* (n = 11), *Staphylococcus aureus* (n = 6), *Enterococcus faecium* (n = 4), *Enterococcus faecalis* (n = 4), *Stenotrophomonas maltophilia* (n = 4), and others (n = 7). Of the 32 patients with BSI, 25 (84%) died within 100 days after RI-CBT. BSI was the direct cause of death in 8 patients (25%). Univariate analysis failed to identify any significant risk factors. BSI clearly represents a significant and fatal complication after RI-CBT. Further studies are warranted to determine clinical characteristics, identify patients at high risk of BSI, and establish therapeutic strategies.

KEY WORDS

Bacterial infection • Bacteremia • Allogeneic hematopoietic stem cell transplantation • *Pseudomonas aeruginosa* • Coagulase-negative *Staphylococcus*

INTRODUCTION

Bacterial infection is a major cause of morbidity and mortality in neutropenic patients after cancer chemotherapy. [1] Approximately half of febrile neutropenic patients have established or occult undiagnosed infection, and approximately 20% of patients with neutrophil counts of $<0.1 \times 10^9$/L acquire bacteremia. [1] The most common portal of bacterial infection is the alimentary tract, where cancer chemotherapy–induced mucosal damage allows invasion by opportunistic organisms. Similarly, damage to the skin integument by invasive procedures, such as placement of vascular access devices, provides portals of entry for infectious organisms.

The frequency of bacterial infection after allogeneic hematopoietic stem cell transplantation (allo-SCT) has decreased with the widespread use of antimicrobiotic prophylaxis and empirical administration of broad-spectrum antimicrobiotics. However, better control of gram-negative infections in neutropenic patients has fostered the increase of infections caused by gram-positive organisms and also gram-negative organisms resistant to β-lactams or fluoroquinolones.

These organisms now account for most bacterial infections. [2-4] Despite improvements in supportive measures, early bacterial infection remains a significant problem in both myeloablative and reduced-intensity stem cell transplantation. [5,6]

Cord blood transplantation (CBT) is an attractive alternative for patients with hematologic diseases in the absence of matched related or unrelated donors. The value of CBT with myeloablative preparative regimens has been confirmed for pediatric patients. [7,8] Myeloablative CBT for adult patients offers a 90% chance of engraftment with a 15% rate of transplant-related mortality, mostly attributable to infection. [9-12] We and other groups recently reported the feasibility of reduced-intensity CBT (RI-CBT) regimens for adult patients with advanced hematologic diseases. [10,13-17]

Delayed immune recovery and graft-versus-host disease (GVHD) contribute to infection being the leading cause of transplant-related mortality after CBT with myeloablative preparative regimens. [8,11,12,17] However, studies on immune recovery after RI-CBT have produced optimism that RI-CBT recipients may experience less GVHD and fewer infectious complications. Although RI-CBT is a promising treatment for patients with hematologic diseases, little information has been reported on infectious complications after RI-CBT. We therefore retrospectively investigated the frequency and clinical features of bacteremia in patients who received RI-CBT for advanced hematologic disease.

PATIENTS AND METHODS

Study Patients and Donors

Between January 2002 and March 2004, 102 patients with hematologic diseases or solid tumors underwent RI-CBT at Toranomon Hospital, Japan. All patients had diseases that were incurable with conventional treatments and were considered inappropriate for conventional allo-SCT because of the lack of an HLA-identical sibling or a suitable unrelated donor, age >50 years, and/or organ dysfunction (generally attributable to previous intense chemotherapy, radiotherapy, or both). All patients provided written informed consent in accordance with the requirements of the Institutional Review Board.

HLA Typing and Donor Matching

A search for unrelated donors was performed by using the Japan Marrow Donation Program [18] for patients without HLA-identical sibling donors. If no appropriate donor was identified, then the Japan Cord Blood Bank Network [19] was searched. A total of 54 patient/cord blood pairs were sex mismatched. Cord blood units were not depleted of T lymphocytes.

Preparative Regimen

All 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 (filgrastim or lenograstim) was administered intravenously from day 1 until neutrophil engraftment.

Supportive Care and Management of GVHD

All patients were managed in reverse isolation in laminar airflow–equipped rooms and received trimethoprim/sulfamethoxazole for prophylaxis against *Pneumocystis carinii*. Fluoroquinolone and either fluconazole or itraconazole were administered for prophylaxis against bacterial and fungal infections, respectively. Prophylaxis against herpesvirus infection was also administered with acyclovir. [20] Neutropenic fever was managed according to the guidelines for the use of antimicrobial agents in neutropenic patients. [1,21] Cytomegalovirus (CMV) pp65 antigenemia was monitored on a weekly basis. If positive results were identified, preemptive therapy was initiated with ganciclovir or foscarnet. Hemoglobin and platelet counts were maintained at >7 g/dL and $>10 \times 10^9$/L, respectively, by using in-line filtered and irradiated blood transfusions.

GVHD was clinically diagnosed in combination with skin or gut biopsies after engraftment or attainment of 100% donor chimerism. Acute and chronic GVHD were graded according to established criteria. [22,23] GVHD prophylaxis involved continuous infusion of cyclosporin 3 mg/kg or tacrolimus 0.03 mg/kg from day −1 until the patient tolerated oral administration (Table 1). The dose was tapered from day 100 to day 150 or according to GVHD status. If grade II to IV acute GVHD developed, prednisolone 1 mg/kg/d was added to cyclosporin or tacrolimus and tapered from the beginning of the clinical response.

Diagnosis and Definition of Bloodstream Infection

When febrile episodes occurred, multiple blood cultures were obtained after the skin or catheter hub was swabbed with 10% povidone-iodine. Bloodstream infection (BSI) must have met at least 1 of the following criteria, [24] and bacteremia was defined as the condition described in criterion 2.

Criterion 1: patient had a recognized pathogen cultured from ≥1 blood culture, and the organism cultured from blood was unrelated to any infection at another site.

Table 1. *Patient Characteristics in RI-CBT*

Variable	Data
Age, y, median (range)	55 (17–79)
Sex (men/women)	55/47
Primary diseases	
Acute lymphoblastic leukemia	13
Acute myeloid leukemia	29
Chronic myelogenous leukemia	1
Adult T-cell leukemia	14
Myelodysplastic syndrome	12
Malignant lymphoma	23
Multiple myeloma	4
Solid tumor	2
Aplastic anemia	4
Risk of underlying deseases (high/low)	77/25
Preparative regimens	
Flud 125 mg/m^2 + L-PAM (80-140 mg/m^2) + TBI (2-8 Gy)	92
Flud 150 mg/m^2 + BU 8 mg/kg + TBI (4-8 Gy)	8
Flud 150 mg/m^2 + L-PAM 140 mg/m^2	1
L-PAM 100 mg/m^2	1*
Number of infused nuclear cells, median (range)	2.9 × 10^7/kg (1.7–5.2)
Number of infused CD34$^+$ cells, median (range)	0.77 × 10^5/kg (0.01–3.3)
GVHD prophylaxis	
Cyclosporine	88
Tacrolimus	14

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 chronic phase, malignant lymphoma in complete remission, multiple myeloma in complete remission, myelodysplastic syndrome in refractory anemia, and aplastic anemia were defined as low risk. All others were considered high risk.

*Patient was treated with fludarabine-containing chemotherapy before transplantation.

Criterion 2: patient had ≥1 clinical symptom, such as temperature >38°C, chills, or hypotension (systolic blood pressure <90 mm Hg), and at least 1 of the following:

a. Common skin contaminant such as diphtheroids, *Bacillus* species, *Propionibacterium* species, coagulase-negative staphylococci, and micrococci, cultured from ≥2 blood cultures drawn on separate occasions.

b. Common skin contaminant such as diphtheroids, *Bacillus* species, *Propionibacterium* species, coagulase-negative staphylococci, and micrococci, cultured from ≥1 blood culture from a patient with an intravascular line, for whom appropriate antimicrobial therapy had been instituted.

Fevers associated with GVHD, engraftment syndrome, [25] pre-engraftment noninfectious fever, [16] pharmacotherapies, and primary malignancies were diagnosed on the basis of a clearly documented clinical history and physical examination, without direct confirmation of infection, as described previously.

Mortality was considered directly attributable to a bloodstream pathogen if the patient died within 7 days after the last positive blood culture without any other probable cause of death. Patients were considered to have responded to antibiotics on the basis of decreasing fever (to <38°C) within 96 hours of initiating therapy with the antibiotic.

End Points

The aims of this study were (1) to determine the incidence of BSI within 100 days of RI-CBT, (2) to describe pathogens involved in BSI, (3) to investigate clinical features of BSI, and (4) to identify risk factors of BSI.

Statistical Analysis

Cumulative incidences of BSI were evaluated by using Gray's method, [26] and death without BSI was considered as a competing risk. Potential confounding factors considered in the analysis included age, sex, stem cell dose, HLA disparity, GVHD prophylaxis, conditioning regimen, regimen-related gut toxicity, neutrophil count, CMV antigenemia, acute GVHD, and the presence of pre-engraftment fever. Proportional hazard modeling was used to evaluate the influences of these factors on the incidence of BSI: neutrophil count, corticosteroid dose, CMV antigenemia, and the development of acute GVHD were treated as time-dependent covariates. Factors associated with at least borderline significance ($P < .10$) in univariate analyses were subjected to multivariate analysis with backward stepwise proportional hazard modeling. Values of $P < .05$ were considered statistically significant.

RESULTS

Patient Characteristics and Clinical Outcomes

Patient characteristics are shown in. Table 1 Primary neutrophil engraftment was achieved by 79 patients within 100 days of RI-CBT. The median day of engraftment was day 20 (range, 11-53 days). Transplant-related mortality occurred in 16 patients (16%) within 30 days of RI-CBT and in 44 patients (43%) within 100 days. Progression of underlying diseases was not noted in any patients within 30 days of RI-CBT, but it occurred in 13 patients and was responsible for 4 deaths within 100 days. As of October 2004, the median follow-up of surviving patients was 5.5 months (range, 4.1-31 months). Overall survival rates were 83% at day 30 and 50% at day 100.

Noninfectious pre-engraftment fever [16] developed in 58 patients. Grade II to IV acute GVHD was present in 23 patients, whereas grade III to IV acute GVHD occurred in 18 patients. The median onset of grade II to IV acute GVHD was day 22 (range, 11-44

days). Corticosteroids, comprising prednisolone or methylprednisolone >0.2 mg/kg/d, were used in 60 patients for underlying diseases, pre-engraftment fevers, engraftment syndrome, or acute GVHD.

Incidence of BSI

BSI developed within 100 days of RI-CBT in 32 patients. The median onset of BSI was day 15 (range, 1-96 day). The cumulative incidence of BSI was 25% at day 30 and 32% at day 100 (Figure 1).

Clinical Features of BSI

The clinical features of BSI are shown in Table 2.

Causative Organisms

Gram-positive bacteremia was identified in 23 patients. Causative organisms included *Staphylococcus epidermidis* (n = 11), *Staphylococcus aureus* (n = 6), *Enterococcus faecium* (n = 4), *Enterococcus faecalis* (n = 4), *Streptococcus mitis* (n = 1), *Stomatococcus mucilaginosus* (n = 1), *Streptococcus agalactiae* (n = 1), *Streptococcus oralis* (n = 1), *Streptococcus sanguis* (n = 1), *Streptococcus* species (n = 1), and gram-positive rods (n = 1).

Gram-negative bacteremia was observed in 16 patients. Causative organisms included *Pseudomonas aeruginosa* (n = 12), *Stenotrophomonas maltophilia* (n = 4), *Enterobacter cloacae* (n = 2), *Escherichia coli* (n = 2), and *Alcaligenes xylosoxidans* (n = 1).

Multiple pathogens were cultured in 14 patients. *Staphylococcus aureus* indicative of methicillin resistance was cultured in 6 patients. *Pseudomonas aeruginosa* indicative of multidrug resistance was cultured in 3 patients. Fungemia due to *Trichosporon beigelii* was noted in 1 patient.

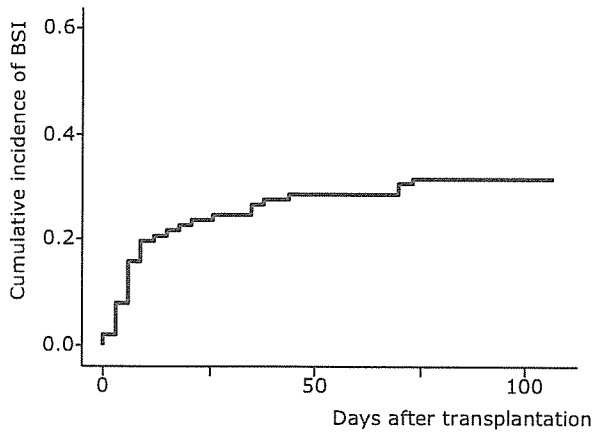

Figure 1. Cumulative incidence of BSI. BSI developed within 100 days in 32 patients. The median onset of BSI was day 15 (range, 1-96 days). The cumulative incidence of BSI was 25% at day 30 and 32% at day 100. BSI indicates bloodstream infection.

Table 2. *Patient Characteristics at Onset of BSI and Treatment Outcomes*

Variable	Data
Onset, day (range)	15 (1-96)
Before engraftment/after engraftment	24/8
Patient status (febrile/afebrile)	31/1*
Inserted catheter at onset day (central/peripheral)	24†/8‡
Median neutrophil count at onset day (/L)	0
Presence of grade II-IV acute GVHD at onset day (yes/no)	0/32
Corticosteroid use*‡ at onset day (yes/no)§	8/24
Antibiotic use for bacterial infection at onset day†	
Fluoroquinolones	12
β-Lactams	8
Carbapenems	7
Vancomycin	5
Aminoglycosides	2
None	2
Response to antibiotics (responded/nonresponded)	7‖/24¶,#,**,††
Reccurence of BSI	3‡‡

GVHD indicates graft-versus-host disease.
*Blood culture was obtained because of persistent low-grade fever.
†Causative organisms included gram-negative (n = 21) and gram-positive (n = 11) bacteria.
‡Causative organisms included gram-negative (n = 5) and gram-positive (n = 3) bacteria and Trichosporon beigelii (n = 1).
§Use of predonisolone or methylpredonisolone >0.2 mg/kg/d.
‖Six patients responded before engraftment.
¶Causative organisms included *Pseudomonas aeruginosa* (n = 9); *Staphylococcus aureus* (n = 5); *Staphylococcus epidermidis* (n = 5); *Stenotrophomonas maltophilia* (n = 4); *Enterococcus faecium* (n = 4); *Streptococcus agalactiae* (n = 1); *Stomatococcus mucilaginosus* (n = 1); *Trichosporon beigelii* (n = 1); *Enterococcus faecalis* (n = 1); *Alcaligenes xylosoxidans* (n = 1); *Enterobacter cloacae* (n = 1); *Escherichic coli* (n = 1); and α-*Streptococcus* (n = 1).Six patients responded before engraftment.
#Four patients did not recoverafter engraftment.
**Fever subsided 16 days after onset in 1 patient. The remaining 3 patients recovered after engraftment.
††Twenty-one patients died.
‡‡Causative organisms were not identical in those patients.

Other Infectious Complications

CMV antigenemia developed in 54 patients (53%) within 100 days, and 53 were successfully treated with preemptive ganciclovir or foscarnet. The remaining patient died of duodenal perforation due to duodenum CMV enterocolitis ulceration, despite prior preemptive therapy. Another 2 patients died within 100 days of RI-CBT because of other fatal infectious complications, namely, miliary tuberculosis (n = 1) and invasive aspergillosis (n = 1).

Mortality of BSI

Within 100 days after RI-CBT, 25 patients (84%) with definitive BSI died because of sepsis (n = 12),

pneumonia (n = 5), disease progression (n = 2), intestinal hemorrhage (n = 2), acute GVHD (n = 2), thrombocytic microangiopathy (n = 1), and invasive aspergillosis (n = 1). BSI was directly associated with death in 8 patients (mortality rate, 25%).

Mortality rates were 13% for gram-positive bacteria and 38% for gram-negative bacteria. Organisms causing fatal BSI included *Pseudomonas aeruginosa* (n = 5), *Stenotrophomonas maltophilia* (n = 2), *Staphylococcus aureus* (n = 2), *Alcaligenes xylosoxidans* (n = 1), and gram-positive rods (n = 1).

Risk Factors of BSI

Univariate analysis failed to identify any significant risk factors (Table 3), and multivariate analysis was therefore not conducted.

Table 3. *Univariate and Multivariate Analyses for the Risk of BSI Onset*

Univariate Factor	Relative Risk (95% CI)	P Value
Age	1.01 (0.99-1.04)	.42
Sex	1.48 (0.72-3.02)	.29
Infused cell dose	0.88 (0.51-1.52)	.64
Infused cell dose ($CD34^+$ cells)	0.98 (0.54-1.78)	.95
HLA match		
5/6	0.38 (0.039-3.69)	.40
4/6	0.62 (0.084-4.59)	.64
GVHD prophylaxis (cyclosporine versus tacrolimus)	0.90 (0.35-2.34)	.83
Conditioning regimen (melphalan versus busulfan)	0.37 (0.05-2.69)	.32
Pre-engraftment fever	1.91 (0.95-3.84)	.071*
Gut RRT	0.91 (0.32-2.59)	.85
Neutrophil count	1.18 (0.87-1.59)	.30
CMV antigenemia	1.22 (0.38-3.92)	.74
Steroid dose	43.1 (0.084-22300)	.24
Grade II-IV acute GVHD	1.71 (0.44-6.70)	.44

RRT indicates regimen-related toxicity; CMV, cytomegalovirus; GVHD, graft-versus-host disease; CI, confidence interval.

DISCUSSION

This study identified BSI as a significant complication after RI-CBT. The incidence of BSI within 100 days of transplantation was 32% after RI-CBT. Little information is available on BSI after CBT, but Saavedra et al. [17] reported that the incidence of BSI was 55% after CBT with myeloablative regimens. The BSI incidence after RI-CBT is probably lower than that after CBT with myeloablative regimens. The incidence in this study was lower than that after CBT with myeloablative regimens. Although previous studies on CBT have mostly involved pediatric patients, [7-9,19,27,28] subjects in this study were elderly and at high risk of BSI. Reducing the intensity of conditioning regimens in CBT can probably decrease the risk of infection, similar to the situation in bone marrow and peripheral blood stem cell transplantation. [5,6,29,30] The incidences of BSI were 20% to 30% after reduced-intensity stem cell transplantation (RIST) from an HLA-identical related donor, [5,6] which were comparable to our result of BSI incidence after RI-CBT. The incidences after myeloablative allo-SCT from a matched sibling or matched unrelated donor were slightly higher (40%-50%). [6,31] The rate of fatal bacterial infection after RI-CBT is comparable to that of 12% after haploidentical T cell–depleted peripheral blood transplantation with myeloablative preparative regimens. [32]

BSI after RI-CBT tends to develop early after transplantation and to be followed by severe clinical courses compared with that after RIST. The median onset of BSI was day 15, and 84% of patients died within 100 days of RI-CBT in this study, whereas a median onset of day 44 and death in 13% of patients within day 100 were reported in RIST with marrow or peripheral blood stem cells. [6] The early onset and severity of BSI are similar to those seen in tuberculosis after RI-CBT. [33] These trends can be explained by a few basic mechanisms. One is the delayed engraftment leading to prolonged neutropenia after RI-CBT compared with that after RIST of peripheral blood stem cells. [7-9,16,19,27,28,34-36]

Because neutrophils represent the major protective factor against bacterial infections, prolonged neutropenia could be expected to contribute to the severe BSI. Another contributing factor is the fact that immune cells in cord blood are mostly naive and carry low protective effects against infection. [37] Because cord blood does not contain antigen-exposed cells during early-phase immune recovery with peripheral expansion of mature T and B lymphocytes transferred with the graft, immune reconstitution is slow, and this leads to an increased risk of infectious complications. Conditioning regimens containing purine analogs and total body irradiation are so immunosuppressive that patients remain highly immunosuppressed in the early phase after RI-CBT.

In contrast to the high incidence of early infections, the incidence of late infections after engraftment is not high in RI-CBT. In this study, fatal fungal infection developed in only 1 patient. This is in sharp contrast to RIST with peripheral blood. [37,38] The low incidence of late infections may be associated with the low incidence of GVHD after CBT, although further clinical studies are necessary. Advances in prophylaxis against fungal infections have decreased the incidence of these during neutropenia and have resulted in a shift in onset to the time at which GVHD commonly develops. [38-40] Risk factors for fungal infection during this period include GVHD and use of steroids. The low frequency of both factors after RI-CBT is probably associated with the reduced inci-

dence of late infections. The overall incidence of BSI after RI-CBT is comparable to that after RIST of bone marrow and peripheral blood stem cells, where early infections have decreased and late ones have increased.

Identification of high-risk patients and intense infection management are important if treatment outcomes for BSI after RI-CBT are to be improved. Identification of risk factors for BSI is required to determine therapeutic strategies. In this study, none of the conventional risk factors for BSI after allo-SCT [3,5,41,42] was significantly associated with BSI. Our study might not have statistical power because of the small sample size, but the mechanisms of BSI development after RI-CBT may differ from those after conventional allo-SCT. Further studies are warranted.

The organisms causing BSI after RI-CBT are similar to those responsible for infection after conventional allo-SCT and RIST. The frequency of coagulase-negative staphylococci was high, and 58% of causative bacteria were gram-positive cocci. Gram-negative bacteria were resistant to treatments and were associated with high mortality, but they were less common than gram-positive bacteria. Multiple drug–resistant *Pseudomonas aeruginosa* caused most of the fatal gram-negative BSIs. *Pseudomonas* species are known as the cause of severe BSI after allo-SCT. [3] Because most patients in our study had been administered antibiotics during previous chemotherapies, along with prophylactic fluoroquinolones after RI-CBT, multiple drug–resistant *Pseudomonas* species had probably colonized. Evaluation for *Pseudomonas* species colonization before RI-CBT may aid in the identification of patients at high risk of BSI. Prophylactic administration of antibiotics to which the cultured *Pseudomonas* species is sensitive might help to prevent BSI. We are planning a prospective study to investigate the usefulness of surveillance blood culture to identify patients at high risk of BSI.

We used fluoroquinolones as prophylaxis in RI-CBT, because antibacterial prophylaxis with fluoroquinolones has reduced the frequency of gram-negative bacteremia. [43] We are also concerned that routine use of fluoroquinolones may lead to the evolution of antibiotic-resistant bacteria, [44] and there is no evidence about a survival benefit with prophylactic fluoroquinolones. Careful clinical studies are needed on the use of fluoroquinolones.

Although this study provided novel information on BSI after RI-CBT, the limitations of the study must be considered. Unrecognized biases might have affected the results of this small, retrospective study. Because all patients received prophylactic antibiotics, the sensitivity of blood cultures would not have been high. [45] The presence of bacteria that are difficult to isolate in blood cultures can also be underestimated. [46]

In conclusion, this study showed that BSI is a significant complication after RI-CBT. BSI before engraftment is a significant problem of RI-CBT that leads to a high mortality rate within 100 days of RI-CBT. Appropriate management thus needs to be established to improve outcomes after RI-CBT. Identification of high-risk patients is crucial. Because most BSIs develop during neutropenia, modification of conditioning regimens to attenuate mucositis, promotion of engraftment by using multiple cord blood units, and prophylactic granulocyte infusions may prove helpful. Appropriate use of antibiotics before RI-CBT is necessary to prevent the emergence of resistant bacteria. Further prospective clinical studies are awaited to evaluate the efficacy of these proposed strategies.

ACKNOWLEDGMENTS

We are grateful to Dr. Kunio Yano (Hamamatsu Medical Center), Dr. Takahiro Fukuda (School of Medicine, Kyushu University), and Tetsuzo Fukuda (Becton, Dickinson and Company) for their critical reading of the manuscript.

REFERENCES

1. Hughes WT, Armstrong D, Bodey GP, et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis.* 2002;34:730-751.
2. Collin BA, Leather HL, Wingard JR, Ramphal R. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. *Clin Infect Dis.* 2001;33:947-953.
3. Sayer HG, Longton G, Bowden R, Pepe M, Storb R. Increased risk of infection in marrow transplant patients receiving methylprednisolone for graft-versus-host disease prevention. *Blood.* 1994;84:1328-1332.
4. Wisplinghoff H, Seifert H, Wenzel RP, Edmond MB. Current trends in the epidemiology of nosocomial bloodstream infections in patients with hematological malignancies and solid neoplasms in hospitals in the United States. *Clin Infect Dis.* 2003;36:1103-1110.
5. Hori A, Kami M, Kim SW, et al. Development of early neutropenic fever, with or without bacterial infection, is still a significant complication after reduced-intensity stem cell transplantation. *Biol Blood Marrow Transplant.* 2004;10:65-72.
6. Junghanss C, Marr KA, Carter RA, et al. Incidence and outcome of bacterial and fungal infections following nonmyeloablative compared with myeloablative allogeneic hematopoietic stem cell transplantation: a matched control study. *Biol Blood Marrow Transplant.* 2002;8:512-520.
7. Gluckman E. Hematopoietic stem-cell transplants using umbilical-cord blood. *N Engl J Med.* 2001;344:1860-1861.
8. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1998;339:1565-1577.
9. Sanz GF, Saavedra S, Planelles D, et al. Standardized, unrelated donor cord blood transplantation in adults with hematologic malignancies. *Blood.* 2001;98:2332-2338.

10. Goggins TF, Rizzieri DR. Nonmyeloablative allogeneic stem cell transplantation using alternative donors. *Cancer Control.* 2004;11:86-96.
11. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med.* 2004;351:2276-2285.
12. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med.* 2004;351:2265-2275.
13. Rizzieri DA, Long GD, Vredenburgh JJ, et al. Successful allogeneic engraftment of mismatched unrelated cord blood following a nonmyeloablative preparative regimen. *Blood.* 2001;98: 3486-3488.
14. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, Miller JS, Wagner JE. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. *Blood.* 2003;102: 1915-1919.
15. Koh LP, Chao NJ. Umbilical cord blood transplantation in adults using myeloablative and nonmyeloablative preparative regimens. *Biol Blood Marrow Transplant.* 2004;10:1-22.
16. Miyakoshi S, Yuji K, Kami M, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin Cancer Res.* 2004;10:3586-3592.
17. Saavedra S, Sanz GF, Jarque I, et al. Early infections in adult patients undergoing unrelated donor cord blood transplantation. *Bone Marrow Transplant.* 2002;30:937-943.
18. Kodera Y, Morishima Y, Kato S, et al. Analysis of 500 bone marrow transplants from unrelated donors (UR-BMT) facilitated by the Japan Marrow Donor Program: confirmation of UR-BMT as a standard therapy for patients with leukemia and aplastic anemia. *Bone Marrow Transplant.* 1999;24:995-1003.
19. Nishihira H, Kato K, Isoyama K, et al. The Japanese cord blood bank network experience with cord blood transplantation from unrelated donors for haematological malignancies: an evaluation of graft-versus-host disease prophylaxis. *Br J Haematol.* 2003;120:516-522.
20. Kanda Y, Mineishi S, Saito T, et al. Long-term low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2001;28:689-692.
21. Hughes WT, Armstrong D, Bodey GP, et al. 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. *Clin Infect Dis.* 1997;25:551-573.
22. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995;15:825-828.
23. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol.* 1991;28:250-259.
24. O'Grady NP, Alexander M, Dellinger EP, et al. Guidelines for the prevention of intravascular catheter-related infections. *Infect Control Hosp Epidemiol.* 2002;23:759-769.
25. Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2001;27: 893-898.
26. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med.* 1999;18:695-706.
27. Kurtzberg J, Laughlin M, Graham ML, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. *N Engl J Med.* 1996;335:157-166.
28. Wagner JE, Rosenthal J, Sweetman R, et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. *Blood.* 1996;88:795-802.
29. Mossad SB, Avery RK, Longworth DL, et al. Infectious complications within the first year after nonmyeloablative allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transplant.* 2001;28:491-495.
30. Khouri IF, Saliba RM, Giralt SA, et al. Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. *Blood.* 2001;98:3595-3599.
31. Ringden O, Remberger M, Runde V, et al. Peripheral blood stem cell transplantation from unrelated donors: a comparison with marrow transplantation. *Blood.* 1999;94:455-464.
32. Aversa F, Tabilio A, Velardi A, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *N Engl J Med.* 1998;339:1186-1193.
33. Maeda T, Kusumi E, Kami M, et al. Disseminated tuberculosis following reduced-intensity cord blood transplantation for adult patients with hematological diseases. *Bone Marrow Transplant.* 2005;35:91-97.
34. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood.* 2004;104:3813-3820.
35. Ooi J, Iseki T, Takahashi S, et al. Unrelated cord blood transplantation for adult patients with advanced myelodysplastic syndrome. *Blood.* 2003;101:4711-4713.
36. Ooi J, Iseki T, Takahashi S, et al. Unrelated cord blood transplantation for adult patients with de novo acute myeloid leukemia. *Blood.* 2004;103:489-491.
37. Kojima R, Kami M, Nannya Y, et al. Incidence of invasive aspergillosis after allogeneic hematopoietic stem cell transplantation with a reduced-intensity regimen compared with transplantation with a conventional regimen. *Biol Blood Marrow Transplant.* 2004;10:645-652.
38. Fukuda T, Boeckh M, Carter RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. *Blood.* 2003;102:827-833.
39. Marr KA. Antifungal prophylaxis in hematopoietic stem cell transplant recipients. *Oncology (Huntingt).* 2001;15:15-19.
40. Marr KA. New approaches to invasive fungal infections. *Curr Opin Hematol.* 2003;10:445-450.
41. Junghanss C, Boeckh M, Carter RA, et al. Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. *Blood.* 2002;99:1978-1985.
42. Engelhard D, Elishoov H, Strauss N, et al. Nosocomial coagulase-negative staphylococcal infections in bone marrow transplantation recipients with central vein catheter. A 5-year prospective study. *Transplantation.* 1996;61:430-434.
43. Cruciani M, Rampazzo R, Malena M, et al. Prophylaxis with fluoroquinolones for bacterial infections in neutropenic patients: a meta-analysis. *Clin Infect Dis.* 1996;23:795-805.

44. Garau J, Xercavins M, Rodriguez-Carballeira M, et al. Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. *Antimicrob Agents Chemother.* 1999;43:2736-2741.
45. Serody JS, Berrey MM, Albritton K, et al. Utility of obtaining blood cultures in febrile neutropenic patients undergoing bone marrow transplantation. *Bone Marrow Transplant.* 2000;26: 533-538.
46. Woo PC, Wong SS, Lum PN, Hui WT, Yuen KY. Cell-wall-deficient bacteria and culture-negative febrile episodes in bone-marrow-transplant recipients. *Lancet.* 2001;357:675-679.

Early Immune Reaction after Reduced-Intensity Cord-Blood Transplantation for Adult Patients

Yukiko Kishi,[1,9] Masahiro Kami,[1] Shigesaburo Miyakoshi,[2] Yoshinobu Kanda,[3] Naoko Murashige,[1] Takanori Teshima,[4] Eiji Kusumi,[2] Shigeo Hara,[5] Tomoko Matsumura,[2] Koichiro Yuji,[2] Kazuhiro Masuoka,[2] Atsushi Wake,[2] Shinichi Morinaga,[2] Mineo Kanemaru,[6] Tatsuyuki Hayashi,[7] Yuji Tanaka,[8] and Shuichi Taniguchi,[2] for the Tokyo Stem Cell Transplant Consortium

Background. To investigate immune reactions after reduced-intensity cord-blood transplantation (RI-CBT).
Materials and Methods. We reviewed medical records of 57 adult RI-CBT recipients. Preparative regimen comprised fludarabine, total-body irradiation, and either melphalan (n=51) or busulfan (n=6). Graft-versus-host disease (GvHD) prophylaxis was cyclosporine. PostRI-CBT immune reactions were classified according to time course: pre-engraftment immune reactions (PIR), engraftment syndrome (ES), and GvHD.
Results. Forty-five patients achieved engraftment at a median of day 19. PIR was characterized by high-grade fever and weight gain and developed on a median of day 9 in 35 of the 45 evaluable patients, including 3 who did not achieve engraftment. PIR subsided spontaneously in 12 patients, whereas corticosteroids were required in the other 23. ES and grade I to IV acute GvHD developed in 36 and 29 patients, respectively. GvHD could not be distinguished from preceding PIR or ES in 10 patients. Causes of the 32 nonrelapse mortalities included GvHD (n=5) and PIR (n=1). There were no significant differences in relapse and nonrelapse deaths between patients with PIR and those without it (18% vs. 5%, and 60% vs. 65%, respectively).
Conclusions. Immune reactions after RI-CBT can be categorized into three distinct subtypes.

Keywords: Graft-versus-host disease, Engraftment syndrome, Preengraftment immune reaction, Allogeneic hematopoietic stem-cell transplantation, Nonmyeloablative stem-cell transplantation.

(*Transplantation* 2005;80: 34–40)

Cord-blood transplantation (CBT) is a promising approach for patients with advanced hematologic malignancies who lack a suitable donor. Cord blood has many theoretic advantages as a stem-cell source. Hematopoietic progenitors from cord blood are enriched in primitive stem cells, producing in vivo long-term repopulating stem cells (*1*). Another advantage of cord blood is immaturity of immune function. Long-lasting unresponsiveness and lack of proliferation of cord-blood lymphocytes on rechallenge with alloantigen might lead to reduced incidence of graft-versus-host disease (GvHD) after CBT (*2*), whereas a graft-versus-leukemia (GvL) effect might be maintained owing to the presence of precursor T and natural killer cells (*3, 4*). The feasibility of related and unrelated CBT has been demonstrated for pediatric patients (*5–8*), and the technique has been successfully applied to adults (*9–12*). Moreover, in adult patients with advanced hematologic malignancies, the feasibility of CBT using reduced-intensity preparative regimens (reduced-intensity CBT [RI-CBT]) has been demonstrated by us and other researchers (*13–16*).

Several types of immune reactions have been reported after allogeneic stem-cell transplantation (allo-SCT). With the exception of acute GvHD (*6, 17–19*), little information is available regarding immune reactions after CBT. The incidence and severity of acute GvHD after unrelated CBT have been low compared with those after allo-SCT from a matched unrelated donor or a mismatched family donor, despite the infusion of human leukocyte antigen (HLA)-mismatched graft (*20*); however, the reported incidence of grade II to IV acute GvHD varies from 25% to 72% (*5, 7–9, 11, 21–24*). Sanz et al. (*10*) reported that 21 of 22 adult CBT recipients developed grade I to IV acute GvHD and that median time to development of GvHD was 9 (range 4–14) days. Considering that median time to neutrophil engraftment was 25.5 (range 14–64) days, the majority of patients developed acute GvHD before engraftment. Similar findings have been reported by other groups (*14*). The circumstances of these immune reactions appear different from those seen in conventional allo-SCT.

Different immune reactions may occur after RI-CBT, and we postulated that characterization of the clinical features of these reactions in relation to engraftment would be useful. We investigated clinical features of immune reactions in 57 patients who underwent RI-CBT at Toranomon Hospital.

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor and Welfare.
[1] Hematopoietic Stem-cell Transplantation Unit, the National Cancer Center Hospital, Tokyo, Japan.
[2] Department of Hematology, Toranomon Hospital, Tokyo, Japan.
[3] Department of Cell Therapy and Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan.
[4] Center for Cellular and Molecular Medicine, Kyushu University, Fukuoka, Japan.
[5] Department of Pathology, Toranomon Hospital, Tokyo, Japan.
[6] Department of Internal Medicine, Higashijyujyo Hospital, Tokyo, Japan.
[7] Department of Internal Medicine, Tokyo Metropolitan Police Hospital, Tokyo, Japan.
[8] Medical Research Information Center, Carson City, NV.
[9] Address correspondence to: Dr. Masahiro Kami, Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, 5–1–1 Tsukiji, Chuo-ku, Tokyo 104–0045, Japan.
E-mail: mkami@ncc.go.jp.
Received 28 November 2004. Revision requested 4 January 2005. Accepted 17 January 2005.

ISSN 0041-1337/05/8001-34
DOI: 10.1097/01.TP.0000163289.20406.86

PATIENTS AND METHODS

Patients

Fifty-seven patients underwent RI-CBT at Toranomon Hospital between January 2002 and August 2003. Patient characteristics and transplantation procedures are shown in Table 1. All patients had hematologic disorders or solid tumors that were incurable with conventional treatments and were considered inappropriate for conventional allo-SCT because of the lack of an HLA-identical sibling or a suitable unrelated donor, age greater than 50 years, or organ dysfunction. All patients provided written informed consent in accordance with the requirements of the institutional review board.

Transplantation Procedures

Cord-blood units that were matched for four or more of six HLA antigens and contained at least 2.0×10^7 nucleated cells/kg of recipient body weight before freezing were used. Cord-blood units were not depleted of T lymphocytes.

The preparative regimen comprised fludarabine 25 mg/m^2 on days −7 to −3, melphalan 80 mg/m^2 on day −2 (n=51) or busulfan 4 mg/kg for 2 days (n=6), and 4 or 8 Gy total-body irradiation (TBI) in 2 fractions on day −1 (Table 1). Granulocyte colony stimulating factor at 300 μg/m^2/day was administered intravenously from day 1 until neutrophil engraftment. Laboratory data including C-reactive protein (CRP) were obtained three times a week.

For GvHD prophylaxis, a continuous infusion of cyclosporine at 3 mg/kg from day −1 until toleration of oral administration was administered. Acute GvHD was graded according to the established criteria (*25*). Patients with grade II to IV acute GvHD were given 0.5 to 2.0 mg/kg per day of methylprednisolone. Treatment of immune reactions other than GvHD was at physicians' discretion. Management of infections was reported previously (*13*).

Chimerism Analysis

Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, polymerase chain reaction for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10% (*26*). Whole-blood and CD3+ cell chimerism was assessed at the time of granulocyte engraftment. When engraftment was delayed, chimerism was assessed on day 30. For those who died before engraftment, chimerism was assessed at least once during life.

Definition of Engraftment and Immune Reactions

Engraftment was defined as white blood cell count greater than 1.0×10^9/L or absolute neutrophil count greater than 0.5×10^9/L for 2 consecutive days. Graft failure was defined as peripheral cytopenia and marrow hypoplasia occurring later than day 60, accompanied by failure to detect donor markers using cytogenetic or molecular techniques.

The reported clinical presentation of engraftment or pre-engraftment syndrome (ES) varies, primarily because of the lack of uniform diagnostic criteria (*27*). When patients with no evidence of infection or adverse effects of medication exhibited skin eruption, diarrhea, jaundice (serum levels of

TABLE 1. Characteristics of patients receiving cord-blood transplantation

Characteristics	n=57
Age (yr)	
Median	56
Range	21–72
Sex	
Male	32
Female	25
Diagnosis	no. of patients
Cancer	
Acute lymphoblastic leukemia	
1st complete remission	1
Advanced disease	7
Acute myeloblastic leukemia	
1st complete remission	1
Advanced disease	20
Myelodysplastic syndrome	
Refractory anemia	1
Others	2
Chronic myeloid leukemia	
Advanced disease	1
Malignant lymphoma	
1st complete remission	1
Advanced disease	16
Multiple myeloma	
Advanced disease	1
Solid tumor	2
Bone marrow failure syndrome	
Severe aplastic anemia	4
Conditioning regimen	
Fludarabine/Busulfan/Total body irradiation 4 Gy	5
Fludarabine/Busulfan/Total body irradiation 8 Gy	1
Fludarabine/Melphalan/Total body irradiation 4 Gy	48
Fludarabine/Melphalan/Total body irradiation 8 Gy	3
Graft-versus-host disease prophylaxis	
Cyclosporin	57
Infused CD34+ cells ($\times10^5$/kg)	
Dose	2.9
Range	2.1–4.4
Body weight (kg)	
Median	53.8
Range	37.3–77.4
No. of HLA-A, B, and DRB1 mismatches	
0	1
1	8
2	48

HLA, human leukocyte antigen.

total bilirubin>2.0 mg/dL), or body weight gain greater than 10% of baseline, these parameters were defined as immune reactions. Reactions were classified into the following three subtypes according to timing: pre-engraftment, peri-engraftment, and postengraftment. Immune reactions which developed 6 or more days before engraftment were defined as pre-engraftment immune reactions (PIR). Those within 5 days of engraftment were defined as ES. Others were defined as postES, which generally corresponded to acute GvHD. Acute and chronic GvHD were graded according to the consensus criteria (*25*, *28*). In the treatment of PIR, ES, and GvHD, response to corticosteroid was evaluated as reported previously (*29*).

Primary Endpoints and Statistical Analysis

The primary endpoint of this study was to investigate clinical characteristics of immune reactions after RI-UCBT. Immune reactions were divided into three categories: PIR, ES, and acute GvHD. The following variables were assessed: fever, serum levels of CRP, skin eruption, diarrhea, jaundice, central nervous system complications, weight gain greater than 10% of baseline, documented infections, and response to corticosteroid. The secondary endpoint was to investigate whether these reactions had a prognostic impact.

Overall survival (OS) and relapse-free survival (RFS) were determined using the Kaplan-Meier method. Final follow-up was conducted in July 2004, with a median follow-up of surviving patients being 16.0 (range 13.8–32.4) months. Surviving patients were censored on the last day of follow-up. ES and GvHD were analyzed in patients who achieved initial engraftment. Cumulative incidence of PIR, ES, GvHD, relapse, and nonrelapse-related mortality (NRM) were calculated using Gray's method (*30*), treating death without each type of immune reaction as a competing risk. A multivariate Cox proportional hazards model was used to identify independent and significant prognostic factors for OS and RFS. The variables entered in each analysis were patient age, sex, primary diseases, risks, number of transfused mononuclear cells, HLA-disparity, and dose of TBI. PIR and acute GvHD were included as a time-dependent covariate. A significance level of 5% was set as the limit for inclusion in the model. Prognostic factors that were significant at $P<0.05$ in the stepwise proportional model analysis were considered to be important in influencing survival.

RESULTS

Engraftment and Chimerism Analysis

Forty-five patients achieved engraftment. Median day of engraftment was day 19 (range 11–55). Cumulative incidences of engraftment and death without engraftment at day 100 were 79% and 18%, respectively (Fig. 1). Rescue of primary graft failure occurred in two patients after second RI-UCBT. The remaining 10 patients died before engraftment after a median of 24.5 (range 15–45) days. Causes of death included regimen-related toxicity (n=2), infection (n=6), progression of underlying disease (n=1), and multiple organ failure caused by pre-ES (n=1).

Chimerism data were obtained from 52 patients. Cumulative incidence of complete donor chimerism at day 60 was 97%, and median time to complete donor chimerism was 22 (range 8–54) days. Complete donor chimerism was documented in the seven patients who died of NRM before engraftment.

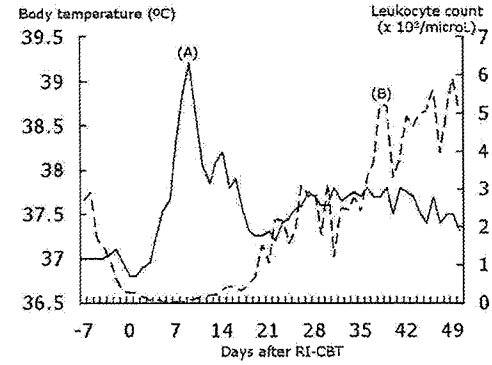

FIGURE 1. Typical clinical course of pre-engraftment immune reaction (PIR). High-grade fever developed on a median of day 9, during severe neutropenia. (A) Body temperature; (B) leukocyte count. RI-CBT, reduced-intensity cord blood transplantation.

Pre-engraftment Immune Reaction

Twelve patients who developed documented infection before engraftment were excluded from the analysis of PIR. Thirty-five of the remaining 45 (78%) patients developed PIR on a median of day 9 (range 6–13). Typical clinical courses of PIR are shown in Figure 2. PIR was observed in three patients who had never engrafted as well as those who had achieved engraftment.

Compared with ES and GvHD, body weight gain, high-grade fever, and elevation of serum levels of CRP were more frequent in PIR. In contrast, jaundice was more common in ES and GvHD than in PIR (Table 2). Histologic examination of the skin was conducted in six patients. Infiltration of mononuclear cells was not prominent in any of the six patients. Common findings were vascular dilatation (n=4) and intercellular edema in the dermis (n=4).

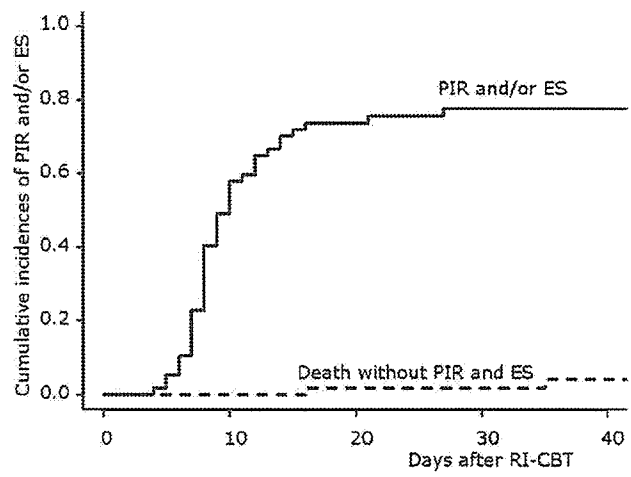

FIGURE 2. Days to PIR or engraftment syndrome (ES), treating death without these complications as a competing risk. Cumulative incidence of PIR or ES was 78%.

TABLE 2. Clinical characteristics of immune responses after reduced-intensity cord-blood transplantation

Type of immune responses	PIR	ES	GvHD
Number of patients with immune responses/number of evaluable patients	35/45	36/44	29/44
Fever (median, range)	39.8 (37.5–41.2)	39.0 (36.8–40.4)	39.2 (37.2–40.7)
Skin rash	16	15	21
Diarrhea	19	20	23
T-Bil >2.0 mg/dL	10	21	18
Body weight gain >10% of baseline	14	3	0
Central nervous system complications	0	5	0
Serum levels of C-reactive protein (mg/dL) (median, range)	14.1 (2.3–25.6)	6.5 (0.2–23)	8.3 (0.9–38.6)
Use of corticosteroid	23	25	25
Response to cortisosteroid (CR/PR/MR/NC)	14/5/3/1	4/4/1/16	15/7/1/2

PIR, preengraftment immune reactions; ES, engraftment syndrome; GvHD, graft-versus-host disease; CR, complete response; PR, partial response; MR, minimal response; NC, no change.

Among the 23 patients given corticosteroid, response was as follows: complete response (CR) (n=14), partial response (PR) (n=5), minimal response (MR) (n=3), and no change (NC) (n=1). PIR subsided spontaneously in the remaining 12 patients in whom corticosteroid had not been administered.

Engraftment Syndrome

Of the 45 patients who achieved engraftment, 44 were included in the analyses of ES, with the remaining patient being excluded because of documented *P. aeruginosa* septicemia. ES developed in 36 patients. Cumulative incidence of PIR or ES was 78% (Fig. 3). Clinical characteristics of ES are shown in Table 2. Five patients with ES developed central nervous system toxicity: cyclosporine neurotoxicity (n=1), limbic encephalopathy (n=2), and metabolic encephalopathy (n=2). No pathogens including bacteria, fungi, or viruses were cultured from cerebrospinal fluid in the five patients. Corticosteroid was given to 25 patients with ES, with the following response: CR (n=4), PR (n=4), MR (n=1), and NC (n=16). Corticosteroid was more frequently required in the patients with preceding PIR and ES than in those with de novo ES (21/27 vs. 4/9), and in the 14 patients with preceding PIR, ES was refractory to corticosteroid.

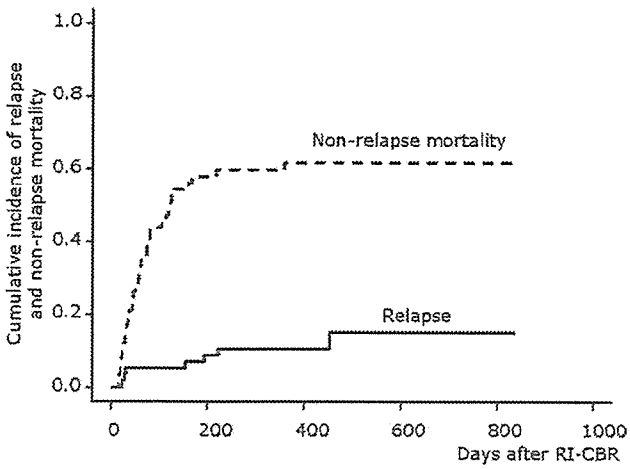

FIGURE 3. Cumulative incidences of relapse and nonrelapse mortality at day 180 were 62% and 15%, respectively.

Postengraftment Immune Reactions (Acute GvHD)

Of the 45 patients who survived longer than 6 days after engraftment, 44 patients were included in the analysis of GvHD. The other patient was excluded because of *E. fecalis* bacteremia. Thirty patients developed acute GvHD: grade I (n=1), II (n=9), III (n=13), and IV (n=7). Skin or gastrointestinal biopsy was conducted in 25 patients. GvHD was histopathologically confirmed in all of these patients. Histopathologic examination was not conducted in the remaining five patients. Cumulative incidence of grade I to IV acute GvHD, treating death without GvHD as a competing risk, was 51% (Fig. 4). It was not possible to differentiate GvHD from

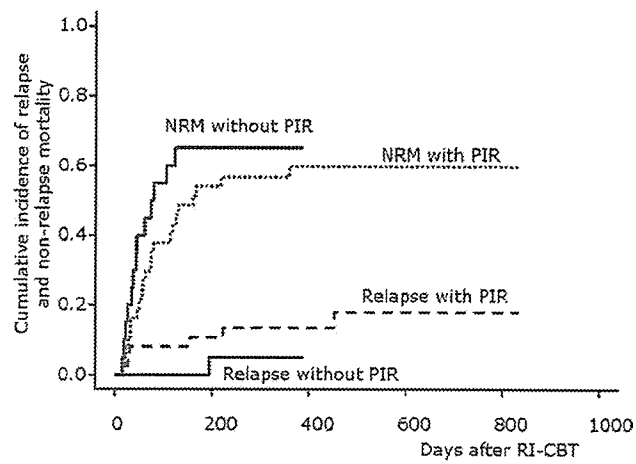

FIGURE 4. Cumulative incidences of relapse and nonrelapse mortality (NRM) for patients grouped by the presence or absence of PIR. Cumulative incidences of relapse were 18% in patients with PIR and 5% in those without it (P=0.32). Cumulative incidences of nonrelapse mortality were 60% in patients with PIR and 65% in those without it (P=0.35).