Ⅳ. 研究成果の刊行物・別刷

Int J Hematol (2008) 87:310–318
DOI 10.1007/s12185-008-0051-1

ORIGINAL ARTICLE

Clinical features of late cytomegalovirus infection after hematopoietic stem cell transplantation

Yuki Asano-Mori · Yoshinobu Kanda · Kumi Oshima · Shinichi Kako · Akihito Shinohara · Hideki Nakasone · Hiroyuki Sato · Takuro Watanabe · Noriko Hosoya · Koji Izutsu · Takashi Asai · Akira Hangaishi · Toru Motokura · Shigeru Chiba · Mineo Kurokawa

Received: 12 October 2007 / Revised: 18 December 2007 / Accepted: 26 December 2007 / Published online: 5 March 2008

Abstract Late cytomegalovirus (CMV) disease beyond day 100 after hematopoietic stem cell transplantation (HSCT) has become an increasing problem after the introduction of preemptive ganciclovir (GCV) administration. To clarify the risk factors and outcome for late CMV reactivation and disease, we retrospectively analyzed the records of 101 Japanese adult patients who underwent allogeneic HSCT between 1998 and 2005 at our hospital. Fifty-one developed late positive CMV antigenemia, with a cumulative incidence of 53%. Recipient CMV seropositivity, the use of alemtuzumab, chronic GVHD, and high-dose steroids were significantly associated with late positive antigenemia. Eight patients developed late CMV disease, with a cumulative incidence of 8%, including retinitis and gastrointestinal disease. None progressed to a fatal disease. The use of alemtuzumab was identified as an independent significant risk factor for late CMV disease, although it was not associated with increased non-relapse mortality. Among the 51 patients with late positive antigenemia, 28 had consistently less than three positive cells, 25 of whom showed negative conversion without antiviral agents. In conclusion, late CMV antigenemia appeared to develop frequently, especially in patients with profound immune suppression; however, a fatal outcome could be prevented by optimal preemptive therapy. Low-level antigenemia may not require antiviral treatments.

Keywords Cytomegalovirus · Antigenemia · Ganciclovir · Preemptive therapy · Hematopoietic stem cell transplantation

Y. Asano-Mori · Y. Kanda · K. Oshima · S. Kako · A. Shinohara · H. Nakasone · H. Sato · T. Watanabe · K. Izutsu · T. Asai · A. Hangaishi · T. Motokura · M. Kurokawa (✉)
Department of Hematology & Oncology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: kurokawa-tky@umin.ac.jp

Y. Asano-Mori
Department of Hematology, Japanese Red Cross Medical Center, Tokyo, Japan

N. Hosoya · S. Chiba
Department of Cell Therapy & Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan

1 Introduction

Despite the widespread use of prophylactic and preemptive ganciclovir (GCV) therapy, cytomegalovirus (CMV) disease remains one of the major causes of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Late occurrence of CMV disease beyond day 100 after HSCT is now increasingly observed, although early CMV disease within the first 100 days has been significantly decreased by the introduction of universal prophylaxis from engraftment or preemptive therapy with monitoring the CMV viral load [1–4]. The delayed CMV-specific immune reconstitution and antiviral drug resistance might have led to an increased incidence of late CMV disease [5, 6]. The main clinical manifestations are pneumonia and gastrointestinal disease [1, 2, 4], whereas retinitis and central nervous system disease are occasionally observed [6, 7]. Late CMV disease has frequently progressed to a fatal outcome with a mortality rate up to 50% [1, 2, 4], probably because most of the recipients are outpatients with less intensive monitoring and therefore, the antiviral agents tend to be administered after CMV-related symptoms are detected.

Although preemptive therapy with CMV monitoring has been successful in preventing early CMV disease [8], the efficacy of such an approach beyond day 100 remains to be evaluated. We have been routinely continuing CMV monitoring beyond day 100 and administered GCV preemptively. In this study, we retrospectively analyzed the incidence, risk factors, and outcome of late CMV reactivation and disease in allogeneic HSCT patients.

2 Patients and methods

2.1 Study population

During a 7-year period (from January, 1998 to September, 2005), 205 adult patients (≥16 years old) underwent allogeneic HSCT for the first time at the University of Tokyo Hospital. Twenty-nine patients died within the first 100 days after HSCT, two of whom developed CMV disease. Among the remaining 176 patients who survived more than day 100 after HSCT, the day of the first negative antigenemia test without any antiviral agents beyond day 100 was defined as the starting point of late CMV antigenemia monitoring. The median period from HSCT to the starting point was 100 days (100–207 days). Eleven patients who developed CMV disease before the starting point and 64 who did not undergo CMV antigenemia assay at least five times after the starting point were excluded. Finally, 101 patients were included in the study.

The median follow-up was 12.6 months (range 4.8–74 months) after HSCT. The patient characteristics are shown in Table 1. Thirty-five, twenty, and forty-six patients received grafts from a HLA-matched related donor, a mismatched related donor, and a matched unrelated donor, respectively. Unrelated HSCT was performed exclusively using bone marrow, whereas 40 out of 55 related donors provided a peripheral blood stem cell graft. Acute leukemia in first remission, chronic myelogenous leukemia in the first chronic phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, and aplastic anemia were defined as low-risk diseases, while others were considered high-risk diseases. Donors other than HLA-matched related donors were defined as alternative donors.

Table 1 Patients' characteristics

Characteristic	Total patients
Sex (male/female)	65/36
Age, median (range)	41.0 (16–66)
Serostatus before transplant	
Recipient CMV-positive/negative	90/11
Donor CMV-positive/negative	80/21
Underlying disease	
Acute leukemia	46
CML	21
MDS	12
NHL/ATL	11
SAA	5
Other	6
Graft source	
PBSC	40
BM	61
Donor type	
Matched related	35
Mismatched related	20
Unrelated	46
Preparative regimen	
Cy/TBI-based regimen	66
Bu/Cy-based regimen	11
ATG-including regimen	3
Flu-based reduced-intensity regimen	21
GVHD prophylaxis	
CsA + MTX	78
Tacrolimus + MTX	10
Alemtuzumab + CsA + MTX	13
Chronic GVHD	
Extensive	53
Limited	21
None	27

CMV cytomegalovirus, *CML* chronic myelogenous leukemia, *MDS* myelodysplastic syndrome, *NHL* non-Hodgkin lymphoma, *ATL* adult T-cell leukemia/lymphoma, *SAA* severe aplastic anemia, *PBSC* peripheral blood stem cell, *BM* bone marrow, *Cy* cyclophosphamide, *TBI* total body irradiation, *Bu* busulfan, *ATG* anti-thymocyte globulin, *Flu* fludarabine, *GVHD* graft-versus-host disease, *CsA* cyclosporine, *MTX* methotrexate

2.2 Transplantation procedure

The conventional preparative regimen for leukemia/lymphoma was mainly performed with either total body irradiation (TBI) regimen [cyclophosphamide (Cy) at 60 mg/kg/day for 2 days and TBI at 2 Gy twice daily for 3 days] or non-TBI regimen [Cy at the same dose combined with busulfan (Bu) at 4 mg/kg/day for 4 days]. In the TBI regimen, the dose of Cy was decreased to 40 mg/kg for 1 day and etoposide at 20 mg/kg for 2 days was added instead, in patients with impaired cardiac function. Fludarabine (Flu)-based regimens, including FB regimen (Flu at 30 mg/m^2/day for 6 days and Bu at 4 mg/kg/day for 2 days) with or without TBI at 4 Gy, FB16 regimen (Flu at the same dose with Bu at 4 mg/kg/day for 4 days), FM regimen (Flu 30 mg/m^2/day for 5 days and melphalan at 140 mg/m^2/day for 1 day), and FC regimen (Flu at

25 mg/m^2/day for 5 days and Cy at 60 mg/kg/day for 2 days), were used as reduced-intensity regimens for elderly or clinically infirm patients [9]. Gemcitabine at 1,000 mg/m^2/day for 3 days was added to the FB regimen for patients with pancreatic cancer [10]. The conditioning regimen for aplastic anemia was either a rabbit antithymocyte globulin (ATG) regimen (Cy at 50 mg/kg/day for 4 days and ATG at 5 mg/kg/day for 5 days with or without TBI at 4 Gy) or an alemtuzumab regimen (Cy at 25 mg/kg/day for 4 days and Flu at 30 mg/kg/day for 4 days combined with alemtuzumab at 0.2 mg/kg/day for 6 days, with or without TBI at 2 Gy).

For prophylaxis against GVHD, cyclosporine A (CsA) at 3 mg/kg/day or tacrolimus at 0.03 mg/kg/day was administered combined with short-term methotrexate (10–15 mg/m^2 on day 1, 7–10 mg/m^2 on days 3 and 6, and optionally on day 11). For patients who received a graft from a haploidentical HLA-mismatched donor, alemtuzumab was added to the TBI regimen or the FB regimen at 0.2 mg/kg for 6 days [11]. Methylprednisolone (mPSL) or prednisolone (PSL) at 1 or 2 mg/kg was added for patients who developed grade 2–4 acute GVHD, whereas PSL at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, fungal, herpes simplex virus, and pneumocystis jirovecii infections consisted of fluconazole, tosufloxacin, acyclovir, and sulfamethoxazole/trimethoprim.

2.3 CMV antigenemia assay

CMV antigenemia assay was performed as described previously [12]. In brief, 1.5×10^5 peripheral blood leukocytes were attached to a slide, using a cytocentrifuge and fixed with formaldehyde. The cells were sequentially immunostained with monoclonal antibody C10/11 (Clonab CMV; Biotest, Dreieich, Germany), which targets CMV pp65 antigen, and reacted with goat alkaline phosphatase-labeled anti-mouse immunoglobulin (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan). Under light microscopy, CMV-positive cells were counted and the results are presented as the sum of the number of positive cells per two slides.

2.4 Preemptive therapy for CMV disease

Preemptive therapy against CMV disease was performed by weekly monitoring of CMV antigenemia after engraftment, as described previously [13]. Until June 2001, intravenous GCV was started at an induction dose of 10 mg/kg/day when ten or more CMV-positive cells were detected in patients who underwent HSCT from a HLA-matched related donor and when positive cells were detected at any level in patients who underwent HSCT from an alternative donor. From July 2001, the induction dose was decreased to 5 mg/kg/day and the threshold of antigenemia to start GCV was changed to twenty and three positive cells for patients who underwent HSCT from a HLA-matched related donor and an alternative donor, respectively [14]. The dose of GCV was increased to 10 mg/kg/day when rising antigenemia was observed. The dose of GCV was adjusted according to renal function [15]. GCV was continued until negative antigenemia was observed. Beyond day 100 after HSCT, CMV monitoring was continued at least every other week in 90 patients and at longer intervals in the remaining 11 patients. GCV was administered in a similar manner as before day 100, at the discretion of attending physicians.

2.5 Definition of late positive CMV antigenemia and CMV disease

Late positive CMV antigenemia was defined as the detection of CMV-positive cells at any level after the starting point. Recurrent CMV antigenemia was defined as the detection of CMV-positive cells after the negative conversion of late positive CMV antigenemia. All patients with symptoms compatible with CMV disease, such as interstitial pneumonia, colitis, or gastritis underwent extensive pathological and microbiological examination of biopsy specimens. Biopsy was performed in all cases of interstitial pneumonia, colitis, and gastritis. The diagnosis of these CMV diseases was made by histopathological examination and immunochemical staining of biopsy specimens, which demonstrated typical CMV inclusion bodies. To establish a definite diagnosis of CMV retinitis, patients received PCR to detect CMV-DNA using aqueous humor and/or ophthalmoscopy, which demonstrated typical findings of CMV retinitis, including a white fluffy retinal infiltrate with several areas of hemorrhage or a granular white area without hemorrhage.

2.6 Statistical analysis

The cumulative incidences of late positive CMV antigenemia and CMV disease, and the impact of possible confounding factors on these events were evaluated using Gray's method, considering death without each event as a competing risk [16]. The cumulative incidence of nonrelapse mortality (NRM), and the impact of possible confounding factors on NRM were evaluated, considering relapse as a competing risk. The development of chronic GVHD and the use of systemic corticosteroids at $\geq$0.5 mg/kg/day were treated as time-dependent covariates. Factors associated with at least borderline significance ($P < 0.10$) in univariate analyses were subjected to multivariate

analysis using backward stepwise proportional-hazard modeling. *P*-values <0.05 were considered significant.

3 Results

3.1 Incidence and risk factors for late positive CMV antigenemia

Overall, 51 of 101 patients developed late positive antigenemia at a median of 29 days (range 1–483 days) after the starting point, with a cumulative incidence of 53% (Fig. 1a). In univariate analyses, recipient CMV seropositivity, the use of alemtuzumab in a conditioning regimen, the preceding detection of CMV antigenemia, prior use of GCV, the development of chronic GVHD, and the use of systemic corticosteroids at ≥0.5 mg/kg/day were significantly associated with the development of late positive antigenemia (Table 2). In multivariate analysis, recipient CMV seropositivity, the use of alemtuzumab in a conditioning regimen, the development of chronic GVHD, and the use of systemic corticosteroids at ≥0.5 mg/kg/day were identified as independent risk factors for late positive antigenemia.

Fifty (98%) of the 51 patients with late positive antigenemia showed negative conversion of antigenemia after a median of 21 days (range 2–430 days). The median peak antigenemia level was only two positive cells per two slides (range 1–268). Twenty-eight patients developed late CMV antigenemia with consistently less than three positive cells (low-level antigenemia), 25 of whom showed negative conversion without GCV administration (Table 3A). Of the remaining 23 patients who developed high-level antigenemia with three or more positive cells, all but one who died of invasive aspergillosis (IA) achieved negative conversion, with ($n = 17$) or without ($n = 5$) GCV administration. Twenty-nine of the 50 patients (58%) had recurrent antigenemia at a median of 14 days (range 3–714 days) after the first negative conversion (Table 3B). The second recurrence was observed in 17 of 26 patients, after a median of 21 days (range 4–323 days) after the second negative conversion.

3.2 Incidence and risk factors for late CMV disease

Eight patients developed late CMV disease at a median of 54 days (range 14–248 days) after the starting point, with a cumulative incidence of 8%, (Fig. 1b). Female sex, the use of alemtuzumab in the conditioning regimen, the preceding detection of CMV antigenemia, prior use of GCV, and the development of chronic GVHD were associated with a higher incidence of late CMV disease with at least borderline significance ($P < 0.10$) (Table 4). Among these, the use of alemtuzumab in the conditioning regimen was the only independent risk factor for late CMV disease.

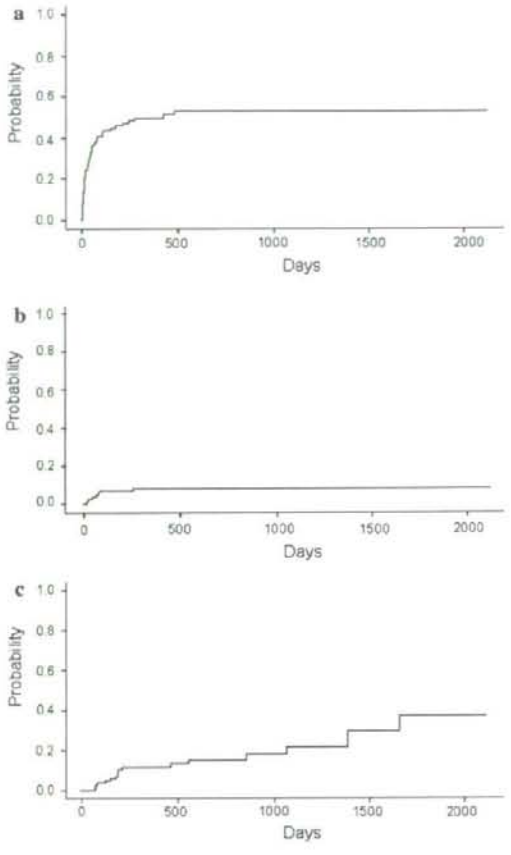

Fig. 1 Cumulative incidences of **a** late positive CMV antigenemia, **b** late CMV disease, and **c** non-relapse mortality. The definition of the starting point is described in the text

Late CMV disease involved retinitis in four, colitis in three, and gastritis in one (Table 5). Three of the four patients with retinitis were asymptomatic. Although all of these CMV diseases were successfully treated with GCV, two patients died of bronchiolitis obliterans and IA, after the resolution of CMV disease. Among the eight patients with late CMV disease, seven developed CMV disease after the development of late positive antigenemia. Five developed CMV disease after the first episode, and the other two after recurrent antigenemia. The median peak antigenemia level was ten positive cells per two slides (range 4–186). The remaining patient developed late CMV disease before the development of late positive antigenemia. All patients achieved negative conversion by the administration of intravenous GCV.

Table 2 Risk factors for late positive CMV antigenemia

Univariate analysis			
Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	63	0.30
≤40 years old	45	44	
Sex			
Male	65	52	0.40
Female	36	54	
Disease risk			
Standard risk	40	51	0.80
High risk	61	54	
Graft source			
Bone marrow	61	46	0.18
Peripheral blood	40	64	
Donor type			
Matched related donor	35	47	0.51
Alternative donor	66	56	
Regimen			
TBI regimen	72	54	0.81
Non-TBI regimen	29	50	
Regimen			
With alemtuzumab	13	69	0.041
Without alemtuzumab	88	47	
Donor CMV			
Seropositive	80	57	0.18
Seronegative	21	33	
Recipient CMV			
Seropositive	90	58	0.0069
Seronegative	11	9	
Prior CMV antigenemia			
Yes	74	61	0.0053
No	27	32	
Prior use of GCV			
Yes	59	62	0.012
No	42	41	
Year of transplant			
Before June 2001	30	50	0.77
After July 2001	71	54	

Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	3.29 (1.81–5.97)	<0.0001
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	2.77 (1.16–4.45)	0.017
No	45		

Table 2 continued

Multivariate analysis			
Factors	Relative risk	95% CI	*P*-value
Steroid at ≥0.5 mg/kg	2.13	1.04–4.38	0.040
Chronic GVHD	2.87	1.54–5.35	0.00095
With alemtuzumab	2.54	1.20–5.37	0.015
Recipient CMV seropositive	13.0	1.77–95.6	0.012

3.3 Incidence and risk factors for non-relapse mortality

Seventeen patients died of non-relapse causes more than 100 days after HSCT, with a 3-year cumulative incidence of 22% (Fig. 1c). Male sex and the use of systemic corticosteroids at ≥0.5 mg/kg were associated with a higher NRM with at least borderline significance (Table 6). The use of systemic corticosteroids at ≥0.5 mg/kg was identified as independently significant for NRM in multivariate analysis. The direct causes of death included non-infectious pulmonary complications (NIPC) in seven patients, infections other than CMV in five, gastrointestinal bleeding in two, multiple organ failure in two, and acute myocardial infarction in one. Fifteen of the seventeen patients received systemic corticosteroids at ≥0.5 mg/kg after the starting point, for severe chronic GVHD in eight, NIPC in four, respiratory failure caused by infections in two, and hemophagocytic syndrome in one.

4 Discussion

This study demonstrated that the cumulative incidence of late CMV disease was successfully decreased and CMV-related mortality was completely avoided by preemptive therapy with extended CMV antigenemia monitoring beyond day 100, in spite of a high frequency of late CMV reactivation. The use of alemtuzumab was the only significant independent risk factor for late CMV disease, while recipient CMV seropositivity, the use of alemtuzumab, chronic GVHD and high-dose steroids were important determinants for late positive antigenemia. A significant correlation between the development of late positive antigenemia and these risk factors is consistent with the clinical observation that the development of chronic GVHD and the use of alemtuzumab or high-dose steroid resulted in delayed recovery of CMV-specific immune response, leading to an increased incidence of late CMV reactivation [17, 18]. Extended CMV-antigenemia monitoring is strongly recommended in such patients with profound immunosuppression.

Table 3 Time course of late positive CMV antigenemia

A. Time course of CMV-Ag in 51 patients who developed late positive antigenemia

Peak level after late positive Ag	Number of patients (*n*)	Use of GCV	Number of patients (*n*)	Peak value of CMV-Ag	Patients with negative conversion
CMV-Ag < 3	28	(−)	25	1 (1–2)	25
		(+)	3	2 (1–2)	3
CMV-Ag ≥ 3	23	(−)	5	4 (3–8)	5
		(+)	18	12 (3–268)	17

B. Recurrence of late positive CMV antigenemia

Late positive CMV antigenemia	Number of patients (*n*)	Patients with late positive Ag (*n*)	Value of CMV-Ag at late positive Ag	Peak value of CMV-Ag	Use of GCV	Patients with negative conversion
First late positive Ag	101	51	1 (1–268)	2 (1–268)	21	50
Second late positive Ag	50	29	1 (1–18)	1 (1–86)	14	26
Third late positive Ag	26	17	2 (1–126)	3 (1–126)	8	16

Table 4 Risk factors for late CMV disease

Univariate analysis

Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	9	0.66
≤40 years old	45	7	
Sex			
Male	65	5	0.094
Female	36	15	
Disease risk			
Standard risk	40	8	0.90
High risk	61	9	
Graft source			
Bone marrow	61	5	0.18
Peripheral blood	40	13	
Donor type			
Matched related donor	35	3	0.17
Alternative donor	66	11	
Regimen			
TBI regimen	72	10	0.29
Non-TBI regimen	29	3	
Regimen			
With alemtuzumab	13	31	0.00097
Without alemtuzumab	88	5	
Donor CMV			
Seropositive	80	9	0.58
Seronegative	21	5	
Recipient CMV			
Seropositive	90	9	0.31
Seronegative	11	0	

Table 4 continued

Univariate analysis

Factors	*n*	Incidence (%)	*P*-value
Prior CMV antigenemia			
Yes	74	11	0.075
No	27	0	
Prior use of GCV			
Yes	59	12	0.080
No	42	2	
Year of transplant			
Before June 2001	30	3	0.26
After July 2001	71	10	

Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	9.27 (1.11–77.5)	0.040
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	1.12 (0.21–5.90)	0.90
No	45		

Multivariate analysis

Factors	Relative risk	95% CI	*P*-value
With alemtuzumab	8.20	2.02–33.3	0.0032

The incidence of late CMV disease in our series was lower than that in previous studies, where antiviral agents were not used preemptively beyond day 100 [1–3]. In a recent study by Boeckh et al. [2], 17.8% of the patients

Table 5 Characteristics of patients who developed late CMV disease

UPN	Sex/age	Diagnosis	Preparative regimen	cGVHD grade	Steroid at CMV disease	Starting point to late positive Ag / disease (days)	Treatment	CMV disease	Lymphocyte counts at CMV disease	Peak value of late CMV antigenemia	Negative conversion	Survival months (months)	Cause of death
1	M/38	ALL	Cam/Cy/TBI	Limited	0	18/60	Intravenous GCV	Retinitis (asymptomatic)	1,505	10	+	29.8+	Alive
2	F/43	NHL	Cy/TBI	Extensive	0	1/14	Intravenous GCV / vitrectomy	Retinitis (asymptomatic)	840	6	+	21.4+	Alive
3	M/42	AA	Cam/Flu/Cy/TBI	Extensive	PSL 30 mg	2/48	Intravenous GCV	Colitis	1,125	12	+	12.1+	Alive
4	F/57	AML	Cam/Flu/Bu/TBI	None	0	4/27	Intravenous/ intreavetreal GCV	Retinitis (asymptomatic)	<400	28	+	6.2	IA
5	F/41	ALL	Cam/Cy/TBI	Limited	0	4/77	Intravenous GCV	Retinitis(symptomatic)	1,890	4	+	9.6+	Alive
6	F/25	CML	Cy/TBI	Extensive	mPSL 20 mg	42/17	Intravenous GCV /foscarnet	Gastritis	748	0	+	33.5	BO
7	F/38	AML	Cy/TBI	Extensive	0	7/248	Intravenous GCV	Colitis	704	6	+	48.6+	Alive
8	M/56	NHL	Flu/BU	Extensive	PSL 10 mg	36/69	Intravenous GCV	Colitis	2,465	186	+	38.0+	Alive

UPN unique patient number, *cGVHD* chronic graft-versus-host-disease, *CMV* cytomegalovirus, *GCV* ganiclovir, *AA* aplastic anemia, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *CML* chronic myelogenous leukemia, *NHL* non-Hodgkin lymphoma, *TBI* total body irradiation, *Cam* alemtuzumab, *Cy* cyclophsphamide, *Flu* fludarabine, *Bu* Busulfan, *PSL* predonisolone, *mPSL* methylpredomisolone, *IA* invasive aspergillosis, *BO* bronchiolitis obliterans

Table 6 Risk factors for 3-year non-relapse mortality

Univariate analysis			
Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	21	0.55
≤40 years old	45	9	
Sex			
Male	65	22	0.092
Female	36	3	
Disease risk			
Standard risk	40	14	0.65
High risk	61	16	
Graft source			
Bone marrow	61	16	0.60
Peripheral blood	40	15	
Donor type			
Matched related donor	35	21	0.10
Alternative donor	66	12	
Regimen			
TBI regimen	72	14	0.26
Non-TBI regimen	29	18	
Regimen			
With alemtuzumab	13	8	0.56
Without alemtuzumab	88	16	
Donor CMV			
Seropositive	80	15	0.60
Seronegative	21	18	
Recipient CMV			
Seropositive	90	15	0.14
Seronegative	11	22	
Prior CMV antigenemia			
Yes	74	15	0.30
No	27	18	
Prior use of GCV			
Yes	59	18	0.65
No	42	12	
Year of transplant			
Before June 2001	30	14	0.78
After July 2001	71	16	

Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	1.35 (0.35–5.21)	0.67
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	10.5 (2.34–47.0)	0.0021
No	45		

Multivariate analysis			
Factors	Relative risk	95% CI	*P*-value
Steroid at ≥0.5 mg/kg	1.05	2.34–47.0	0.0021

who did not receive antiviral agents preemptively more than 3 months after HSCT, developed late CMV disease, including pneumonia and gastrointestinal disease predominantly, with a mortality rate of 46%. Other studies have also confirmed that CMV pneumonia was the leading manifestation of late CMV disease with an associated mortality rate ranging from 60 to 80%, in the absence of preemptive therapy [1, 4]. In contrast, we exclusively observed late CMV retinitis and/or gastrointestinal disease, none of which was directly related to death. These findings suggest that preemptive therapy with extended CMV antigenemia monitoring, reduced the incidence of late CMV disease and eradicated fatal CMV disease.

The use of alemtuzumab was the only independent significant risk factor for late CMV disease, although it was not associated with increased non-relapse mortality. This finding is inconsistent with the conclusion of previous studies that the use of alemtuzumab did not result in an increased incidence of CMV disease in two previous studies despite a strong association with the high frequency of CMV reactivation [11, 17]. In this study, four of the 13 patients who received alemtuzumab developed late CMV disease, three of whom had retinitis. Two cases of retinitis were asymptomatic and diagnosed by ophthalmologic screening. We performed routine ophthalmologic screening as a standard practice only in patients who received alemtuzumab, based on the association between a high incidence of CMV reactivation and delayed posttransplant immune reconstitution by alemtuzumab [11, 17]; therefore, asymptomatic retinitis might have been overlooked in patients who did not receive alemtuzumab in this study or in patients who received alemtuzumab in other studies.

More than half of the patients with late positive antigenemia developed low-level antigenemia with consistently less than three positive cells, and 90% showed negative conversion without GCV administration. In all high-level antigenemia patients, except for one who died of IA while on preemptive GCV, negative conversion without progression to fatal disease was obtained by preemptive therapy; therefore, three positive cells per two slides might be an appropriate threshold to start GCV beyond day 100 after transplantation.

In conclusion, late positive antigenemia was frequently observed beyond day 100 after transplantation, especially in profoundly immunosuppressed patients who received alemtuzumab, high-dose steroids, or who developed chronic GVHD. Preemptive therapy with extended CMV antigenemia monitoring beyond day 100, not only reduced the incidence of late CMV disease, but also completely prevented fatal CMV disease; therefore, extended CMV monitoring is recommended at least for patients with such risk factors.

Acknowledgments This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare.

References

1. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood. 1996;88:4063–71.
2. Boeckh M, Leisenring W, Riddell SR, et al. Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. Blood. 2003;101:407–14.
3. Einsele H, Hebart H, Kauffmann-Schneider C, et al. Risk factors for treatment failures in patients receiving PCR-based preemptive therapy for CMV infection. Bone Marrow Transplant. 2000;25:757–63.
4. Nguyen Q, Champlin R, Giralt S, et al. Late cytomegalovirus pneumonia in adult allogeneic blood and marrow transplant recipients. Clin Infect Dis. 1999;8:618–23.
5. Krause H, Hebart H, Jahn G, Müller CA, Einsele H. Screening for CMV-specific T cell proliferation to identify patients at risk of developing late onset CMV disease. Bone Marrow Transplant. 1997;19:1111–6.
6. Wolf DG, Lurain NS, Zuckerman T, et al. Emergence of late cytomegalovirus central nervous system disease in hematopoietic stem cell transplant recipients. Blood. 2003;101:463–5.
7. Crippa F, Corey L, Chuang EL, Sale G, Boeckh M. Virological, clinical, and ophthalmologic features of cytomegalovirus retinitis after hematopoietic stem cell transplantation. Clin Infect Dis. 2001;32:214–9.
8. Kanda Y, Mineishi S, Saito T, et al. Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic hematopoietic stem cell transplantation: a single-center experience in Japan. Bone Marrow Transplant 2001;27:437–44.
9. Niiya H, Kanda Y, Saito T, et al. Early full donor myeloid chimerism after reduced-intensity stem cell transplantation using a combination of fludarabine and busulfan. Hematologica 2001;86:1071–4.
10. Kanda Y, Komatsu Y, Akahane M, et al. Graft-versus-tumor effect against advanced pancreatic cancer after allogeneic reduced-intensity stem cell transplantation. Transplantation. 2005;79:821–7.
11. Kanda Y, Oshima K, Asano-Mori Y, et al. In vivo alemtuzumab enables haploidentical HLA-mismatched hematopoietic stem cell transplantation without ex vivo graft manipulation. Transplantation. 2005;79:1351–7.
12. Kurihara T, Hayashi J, Matsuoka T, Ito A. HCMV pp65 antigenemia assay using indirect alkaline phosphatase staining method. Bio Med Res. 1995;16:125–9.
13. Asano-Mori Y, Oshima K, Sakata-Yanagimoto M, et al. High-grade cytomegalovirus antigenemia after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2005;36:813–9.
14. Kanda Y, Mineishi S, Saito T, et al. Response-oriented preemptive therapy against cytomegalovirus disease with low-dose ganciclovir: a prospective evaluation. Transplantation. 2002;73:568–72.
15. Asano-Mori Y, Kanda Y, Oshima K, et al. Pharmacokinetics of ganciclovir in haematopoietic stem cell transplantation recipients with or without renal impairment. J Antimicrob Chemother. 2006;57:1004–7.

16. 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.
17. Chakrabarti S, Mackinnon S, Chopra R, et al. High incidence of cytomegalovirus infection after nonmyeloablative stem cell transplantation: potential role of alemtuzumab in delaying immune reconstitution. Blood. 2002;99:4357–63.
18. Hakki M, Riddell SR, Storek J, et al. Immune reconstitution to cytomegalovirus after allogeneic hematopoietic stem cell transplantation: impact of host factors, drug therapy, and subclinical reactivation. Blood. 2003;102:3060–7.

CLINICAL RESEARCH

Central Nervous System Relapse of Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation

Kumi Oshima,[1] Yoshinobu Kanda,[1] Takuya Yamashita,[2] Satoshi Takahashi,[3] Takehiko Mori,[4] Chiaki Nakaseko,[5] Katsumichi Fujimaki,[6] Akira Yokota,[7] Shin Fujisawa,[8] Takafumi Matsushima,[9] Hiroyuki Fujita,[10] Tohru Sakura,[11] Shinichiro Okamoto,[4] Atsuo Maruta,[12] Hisashi Sakamaki,[2] for the Kanto Study Group for Cell Therapy

Little information is available regarding central nervous system (CNS) relapse of adult leukemia after allogeneic hematopoietic stem cell transplantation (HSCT). Therefore, we reviewed the data of 1226 patients with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia (CML) who received first allogeneic HSCT between 1994 and 2004, using the database of the Kanto Study Group for Cell Therapy (KSGCT), and analyzed the incidence, risk factors, and outcome of patients with CNS relapse. Twenty-nine patients developed CNS relapse at a median of 296 (9-1677) days after HSCT with a cumulative incidence of 2.3%. Independent significant factors associated with CNS relapse included ALL as the underlying diagnosis (relative risk [RR] = 9.55, 95% confidence interval [CI] = 1.26-72.2, P = .029), nonremission at HSCT (RR = 2.30, 95% CI = 1.03-5.15, P = .042), the history of CNS invasion before HSCT (RR = 5.62, 95% CI = 2.62-12.0, $P = 9.2 \times 10^{-6}$), and the prophylactic intrathecal chemotherapy after HSCT (RR = 2.57, 95% CI = 1.21-5.46, P = .014). The 3-year overall survival (OS) after CNS relapse was 18%. In 7 of 29 patients with CNS relapse, leukemia was observed only in CNS. Three of 7 patients were alive without systemic relapse, resulting in 3-year survival after CNS relapse of 46%. Although the outcome of patients with CNS relapse was generally poor, long-term disease-free survival could be achieved in some patients.

Biol Blood Marrow Transplant 14: 1100-1107 (2008)

KEY WORDS: Leukemia, Central nervous system, Relapse, Allogeneic hematopoietic stem cell transplantation

INTRODUCTION

Relapse of the original disease remains 1 of the most important causes of failure after allogeneic hematopoietic stem cell transplantation (HSCT) for leukemia. Although majority of the patients develop systemic relapse, extramedullary relapse has been also observed after HSCT. The incidence of central nervous system (CNS) relapse after allogeneic HSCT ranged from 2.9% to 11% [1-3]. Risk factors for CNS relapse identified in previous studies included CNS involvement before HSCT [2] and nonremission at HSCT [1]. Prophylactic intrathecal administration of methotrexate (MTX) was shown to decrease the incidence of CNS relapse of acute lymphoblastic leukemia (ALL) in the Seattle study [1], whereas the other 2

From the [1]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [2]Division of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [3]Division of Molecular Therapy, The Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [4]Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan; [5]Department of Hematology, Chiba University School of Medicine, Chiba, Japan; [6]Department of Internal Medicine and Clinical Immunology, Yokohama City University, Graduate School of Medicine, Kanagawa, Japan; [7]Department of Internal Medicine, Chiba Aoba Municipal Hospital, Chiba, Japan; [8]Department of Hematology, Yokohama City University Medical Center, Kanagawa, Japan; [9]Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Gunma, Japan; [10]Division of Hematology, Shizuoka Red Cross Hospital, Shizuoka, Japan; [11]Division of Hematology, Saiseikai Maebashi Hospital, Gunma, Japan; and [12]Department of Hematology, Kanagawa Cancer Center, Kanagawa, Japan

Correspondence and reprint requests to: Yoshinobu Kanda, MD, PhD, Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847, Amanuma-cho, Omiya-ku, Saitama-shi, Saitama 330-8503, Japan (e-mail: ycanda-tky@umin.ac.jp).

Received March 23, 2008; accepted July 2, 2008
1083-8791/08/1410-0001$34.00/0
doi:10.1016/j.bbmt.2008.07.002

studies failed to find the benefit of prophylactic intrathecal administration of MTX on CNS relapse in patients with acute leukemia [2,3]. There has been no generalized consensus on intrathecal administration of MTX, and in fact, a survey of the European Group for Blood and Marrow Transplantation (EBMT) had reported that the practice varied widely among centers [4].

We examined the incidence, risk factors, and outcome of CNS relapse after allogeneic HSCT in adult patients with acute myelogenous leukemia (AML), ALL, and chronic myelogenous leukemia (CML), and also evaluated the prophylactic effect of intrathecal administration of MTX on CNS relapse.

MATERIALS AND METHODS

Study Population

The study population consisted of 1226 patients, who underwent allogeneic HSCT for AML, ALL, and CML for the first time between January 1994 and December 2004 at 10 hospitals participating in the Kanto Study Group for Cell Therapy (KSGCT).

Transplantation Procedure

Of the 1226 patients, the sources of stem cell was bone marrow (BM) in 903, peripheral blood stem cells (PBSC) in 178, BM plus PBSC in 10, and cord blood (CB) in 134. Conventional myeloablative conditioning regimens such as total body irradiation (TBI) and cyclophosphamide (Cy), busulfan (Bu), and Cy, and their modified regimens were performed in 1168 patients. Among them, TBI of at least 10 Gy was performed in 815 patients. Reduced-intensity conditioning (RIC) regimens were conducted in 53 patients. Prophylaxis of graft-versus-host disease (GVHD) was attempted with calcineurin inhibitors (cyclosporine [CsA] or tacrolimus) with or without short-term MTX in the majority of patients.

Definition of CNS Relapse

CNS relapse was diagnosed as the presence of leukemic cells in the cerebrospinal fluid (CSF). Isolated CNS relapse was defined as CNS relapse without any other sites of relapse of leukemia.

Statistical Considerations

Overall survival (OS) was calculated using the Kaplan-Meier method. Cumulative incidence of CNS relapse was calculated using Gray's method, considering death without CNS relapse as a competing risk [5]. Cumulative incidence of isolated CNS relapse was calculated using Gray's method, treating systemic relapse and death without relapse as a competing risk [5]. The protective effect of chronic GVHD (cGVHD) on CNS relapse was evaluated among patients who developed bone marrow relapse within 100 days after HSCT. Factors associated with at least borderline significance ($P < .10$) in the univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. Finally, P values of $<.05$ were considered statistically significant.

RESULTS

Characteristics of the Patients

Characteristics of patients included in the study were listed in Table 1. The median age was 36 years, ranging from 15 to 69 years. The underlying diseases were AML (n = 533), ALL (n = 352), and CML (n = 341). Eighty-one patients had the history of CNS involvement before HSCT. Eight hundred and nine patients were in complete remission of acute leukemia or in chronic phase of CML at HSCT, and the remaining patients had active disease. In the following analyses, CML in the chronic phase was included in leukemia in complete remission.

CNS Relapse

Twenty-nine patients developed CNS relapse at a median of 296 days (9-1677 days) after HSCT, giving the cumulative incidence of 2.3% (Figure 1). The median age was 31 years (range: 17-47). The underlying disease was ALL in 18, AML in 9, and CML in 2. Sixteen patients had CNS involvement before HSCT and

Table 1. Characteristics of Patients

Median age (range) at transplantation	36 (15-69)
Sex	
Male	762
Female	464
Underlying disease	
AML	533
ALL	352
CML	341
Disease status	
CR	809
non-CR	416
History of CNS disease	
Yes	81
No	802
Type of conditioning	
Conventional	1168
Reduced intensity	53
TBI ≥10 Gy in conditioning	
Yes	815
No	404
Donor type	
Related	478
Unrelated	548
Stem cell source	
BM	902
PBSC	178
BM + PBSC	10
CB	134

CB indicates cord blood.

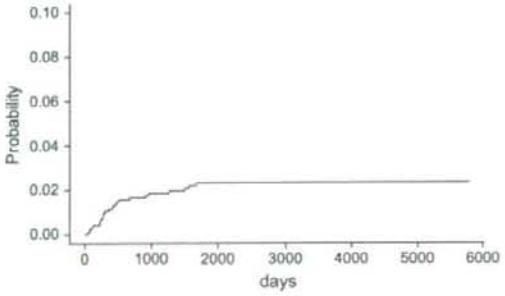

Figure 1. Cumulative incidence of CNS relapse treating death without CNS relapse as competing risk.

6 of them had active CNS disease at HSCT. OS after CNS relapse was 42% at 1 year and 18% at 3 years (Figure 2A). OS of the whole patient cohort, patients with CNS relapse, and those without CNS relapse was 59.8%, 33.2%, and 60.6%, respectively, at 3 years after transplantation.

Pretransplant factors that affected the incidence of CNS relapse after HSCT with at least borderline significance were ALL as the underlying disease, active disease at HSCT, a history of CNS leukemia, the use of TBI regimens, HSCT from an unrelated donor, and the use of prophylactic intrathecal chemotherapy after HSCT (Table 2). Among them, multivariate analysis showed that ALL as the underlying disease, active disease at HSCT, the history of CNS involvement, and the use of intrathecal chemotherapy after HSCT were independently significant (Table 2 and Figure 3). The cumulative incidences of CNS relapse in patients with and without a history of CNS involvement before HSCT were 21.3% and 1.3%, respectively (Figure 3A). Patients with ALL were at higher risk for CNS relapse even in patients in remission at HSCT without a history of CNS involvement before HSCT (ALL 2.7%, AML 0.8%, and CML 0.4%, $P = .088$, Figure 4A). Twenty-three patients who had active leukemia at HSCT had persistent disease after HSCT. Among these, only 2 patients developed CNS relapse after HSCT. However, median survival of this cohort was only 90 days after HSCT producing a 1-year survival of 14%, and thus, majority of the patients died very early, before developing CNS relapse.

Effect of Intrathecal Chemotherapy on the Incidence of CNS Relapse

The practice of intrathecal chemotherapy in allogeneic HSCT recipients varied among the 10 institutions of the KSGCT. Half of them never used prophylactic intrathecal chemotherapy before and after HSCT. The remaining half administered intrathecal prophylaxis routinely before HSCT, of which 2 institutions added intrathecal chemotherapy after

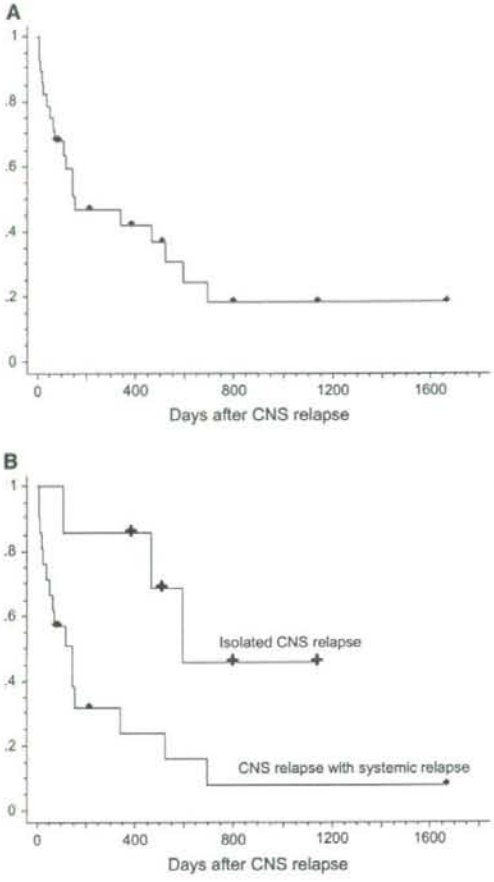

Figure 2. OS after CNS relapse (A) and that grouped according to isolated CNS relapse or CNS relapse associated with systemic relapse (B).

HSCT for high-risk patients such as those with ALL or the history of CNS involvement. In this cohort, intrathecal prophylaxis before HSCT was conducted in 701 of 887 patients and intrathecal chemotherapy after HSCT was done in 141 of 807 patients whose information about intrathecal chemotherapy was available. Antineoplastic agents used for intrathecal chemotherapy mainly consisted of MTX. The median numbers of intrathecal chemotherapy before and after HSCT were 1 (range: 1-4) and 2 (range: 1-4), respectively.

We failed to find a significant prophylactic effect of intrathecal chemotherapy for CNS relapse. The relative risk for CNS relapse was 1.52 (95% CI 0.61-3.79, $P = .37$) for intrathecal chemotherapy before HSCT and 3.92 (95% CI 1.80-8.51, $P = .00057$) for intrathecal chemotherapy after HSCT (Table 2). This adverse influence of intrathecal chemotherapy after HSCT was significant even after adjusted for the

Table 2. Impact of Pretransplant Factors on the Incidence of CNS Relapse after Transplantation

Factor		Univariate RR (95% CI)	P value	Multivariate RR (95% CI)	P value
Age		1.00 (1.00-1.00)	.15		
Sex		1.01 (0.65-1.59)	.95		
Disease	CML	1.00		1.00	
	AML	5.58 (0.70-44.5)	.10	3.60 (0.46-28.4)	.22
	ALL	17.7 (2.36-132.8)	.0052	9.55 (1.26-72.2)	.029
CR/non-CR		2.33 (1.08-5.04)	.031	2.30 (1.03-5.15)	.042
History of CNS disease		17.9 (8.30-38.6)	2.0×10^{-13}	5.62 (2.62-12.0)	9.2×10^{-6}
TBI		2.91 (1.00-8.44)	.050		
Conventional/reduced intensity		0.99 (0.47-2.07)	.97		
Related/unrelated		1.85 (1.06-3.23)	.030		
Source	BM	1.00			
	PBSC	0.24 (0.03-1.77)	.16		
	CB	0.70 (0.17-2.96)	.63		
Sex mismatch		1.06 (0.42-2.66)	.90		
HLA mismatch		0.46 (0.06-3.46)	.45		
Prophylactic IT before HSCT		1.52 (0.61-3.79)	.37		
Prophylactic IT after HSCT		3.92 (1.80-8.51)	.00057	2.57 (1.21-5.46)	.014

IT indicates intrathecal chemotherapy; CNS, central nervous system; RR, relative risk.

underlying disease, disease status at HSCT, and the history of CNS involvement before HSCT (relative risk 2.57, 95% CI 1.21-5.46, $P = .014$). Among patients without a history of CNS involvement before HSCT who were in remission at HSCT, the incidences of CNS relapse after HSCT were 3.6% and 1.6% who received and did not receive intrathecal chemotherapy after HSCT, respectively ($P = .057$, Figure 4B). In patients with a history of CNS involvement before HSCT, the incidences of CNS relapse after HSCT were 37.4% and 11.6%, respectively, who received and did not receive intrathecal chemotherapy after HSCT ($P = .018$; Figure 4C). When we limited the analysis in patients with ALL, the incidences of CNS relapse after HSCT were 6.2% and 3.7% who received and did not receive intrathecal chemotherapy after HSCT ($P = .17$), respectively, in patients without a history of CNS involvement before HSCT who were in remission at HSCT and they were 55.6% and 15.5%, respectively, in patients with a history of CNS involvement before HSCT ($P = .0081$).

Nine patients developed leukoencephalopathy with a median onset of 288 days after HSCT. The incidence of leukoencephalopathy was significantly higher in patients who underwent intrathecal chemotherapy after HSCT (3.5% versus 0.5%, $P = .0076$).

Isolated CNS Relapse

Seven patients developed isolated CNS relapse at a median of 671 days (125-1677 days) after HSCT, presenting the cumulative incidence of 0.70%. Characteristics of these 7 patients were listed in Table 3. All received bone marrow as stem cell source. Prognostic factors associated with isolated CNS relapse with at least borderline significance were age, active disease at HSCT, CNS involvement before HSCT, stem cell source, the use of intrathecal chemotherapy after HSCT, and the absence of HLA mismatch. Among these, independent significant factors for isolated CNS relapse included the history of CNS involvement before HSCT, the use of PBSC or CB as stem cell source, and the absence of HLA mismatch (Table 4). The treatment of isolated CNS relapse consisted of intrathecal chemotherapy and/or cranial irradiation and CNS disease was successfully controlled in 5 of the 7 patients. Four patients developed bone marrow relapse within 1 year. However, the remaining 3 patients were alive without systemic relapse at 518, 807, and 1149 days after CNS relapse and 1283, 1478, and 2195 days after HSCT, respectively. Survival after CNS relapse was significantly better in patients who developed isolated CNS relapse than those who developed CNS relapse with systemic relapse (46% versus 8% at 3 years, $P = .023$, Figure 2B).

Effect of cGVHD on CNS Relapse

Among the 378 patients who experienced bone marrow relapse within 100 days after HSCT but were free from CNS relapse at day 100, 21 (6.1%) showed CNS relapse later on. The incidence of CNS relapse after bone marrow relapse was 7.1% in patients with cGVHD and 2.0% in those without cGVHD ($P = .14$).

Analysis Excluding CML Patients

We repeated these analyses excluding patients with CML, because the incidence of CNS relapse was extremely low, as shown in Figure 3C. The cumulative incidence of CNS relapse was 3.2%. Independently significant pretransplant factors for CNS relapse

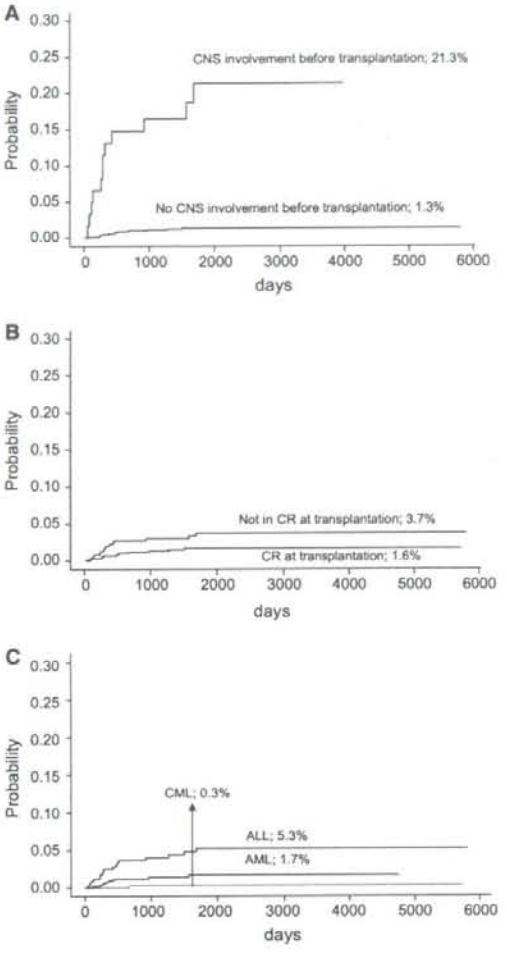

Figure 3. Cumulative incidence of CNS relapse grouped according to the history of CNS involvement before transplantation (A), disease status at transplantation (B), and underlying disease (C).

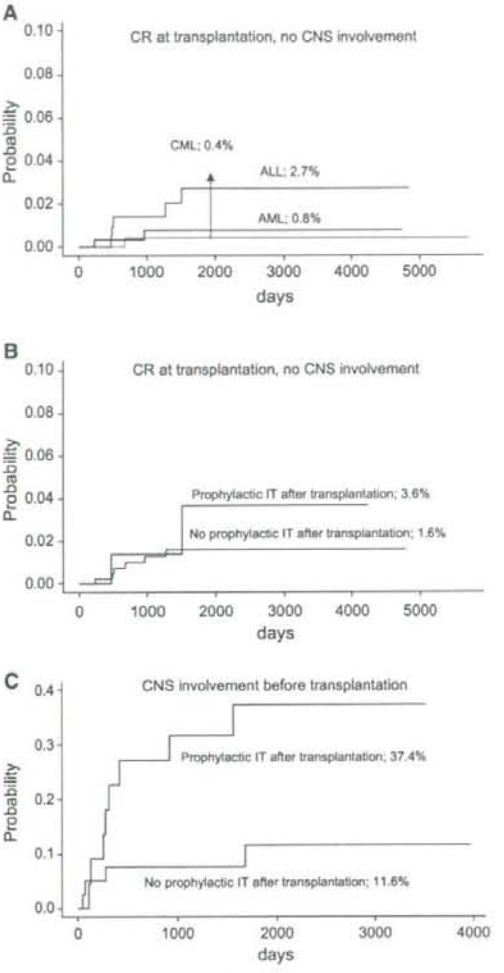

Figure 4. Cumulative incidence of CNS relapse in patients in remission at transplantation without a history of CNS involvement before transplantation grouped according to the underlying disease (A) and the use of prophylactic intrathecal chemotherapy (IT) after transplantation (B). Cumulative incidence of CNS relapse in patient with CNS involvement before transplantation grouped according to the use of prophylactic IT after transplantation (C).

were the same as the analyses including CML patients; ALL compared to AML as the underlying disease (RR 2.68, 95% CI 1.18-6.11, $P = .019$), active disease at HSCT (RR 2.49, 95% CI 1.08-5.73, $P = .032$), the history of CNS involvement (RR 5.64, 95% CI 2.60-12.3, $P = .000012$), and the use of intrathecal chemotherapy after HSCT (RR 2.69, 95% CI 1.25-5.81, $P = .012$). The cumulative incidence of isolated CNS relapse was 0.9%. Independently significant pretransplant factors for CNS relapse included ALL compared to AML as the underlying disease, the history of CNS involvement, the use of PBSC as stem cell source, the absence of HLA mismatch, and the use of intrathecal chemotherapy after HSCT.

DISCUSSION

The cumulative incidences of CNS relapse and isolated CNS relapse were 2.3% and 0.70% in this cohort, respectively, which were almost comparable with those in previous studies (Table 5) [1-3]. The history of CNS leukemia before HSCT was identified as the strongest predictive factor for CNS relapse after HSCT in our study as previously reported [1,2].

We could not show a beneficial effect of prophylactic intrathecal chemotherapy on the incidence of

Table 3. Characteristics of Patients Who Developed Isolated CNS Relapse after Transplantation

Patient No.	1	2	3	4	5	6	7
Age	23	31	24	35	26	41	18
Sex	M	M	M	M	F	M	M
Disease	CML	CML	ALL	AML	ALL	ALL	ALL
Disease status	CP2	BC	RL2	RL1	RL2	CR1	RL2
History of CNS disease	Yes	Yes	Yes	Yes	Yes	No	No
Stem cell source	BM	BM	BM	BM	BM	BM	BM
Donor type	R	R	U	R	R	R	U
HLA mismatch	No	Yes	No	No	No	No	Yes
Conditioning regimem	Bu+Cy	CA+Cy+TBI	ETP+Cy+TBI	CA+Cy+TBI	ETP+Cy+TBI	Cy+TBI	CA+Cy+TBI
Days to an isolated CNS relapse	671	134	125	1565	276	1265	1677
CNS treatment	IT+RT	IT+RT	IT	IT+DLI	RT	IT+RT	IT
Systemic relapse	No	No	Yes	Yes	Yes	Yes	No
Days from HSCT to systemic relapse	164	1680	444	1572			
Day from CNS relapse to systemic relapse	39	115	168	307			
Outcome	Alive	Alive	Dead	Dead	Dead	Alive	Alive
Follow-up duration (days)	1478	1283	236	2031	870	1661	2195

IT indicates intrathecal chemotherapy; RT, radiation; DLI, donor lymphocyte infusion; BU, busulfan; CY, cyclophosphamide; CA, cytarabine; ETP, etoposide; CNS, central nervous system; HSCT, hematopoietic stem cell transplantation.

CNS relapse after HSCT. The incidence of CNS relapse was rather higher in patients who received intrathecal chemotherapy after HSCT. This was probably biased by the fact that significantly higher proportion of patients received intrathecal chemotherapy after HSCT among patients with CNS involvement before HSCT than those without CNS leukemia (47.4% versys 13.4%, $P < .0001$). However, intrathecal chemotherapy after HSCT significantly adversely affected the incidence of CNS relapse even after adjusted for the underlying disease, disease status at HSCT, and the history of CNS involvement before HSCT. Also, a benefit of intrathecal chemotherapy after HSCT was not shown in patients with ALL, in contrast with the previous reports [1,6]. This discrepancy might have resulted from the difference in the intensity of the intrathecal chemotherapy. Intrathecal chemotherapies were administered 6 times after HSCT in the Seattle group, whereas the medium number of intrathecal chemotherapy in the current study was only 2 (range: 1-4). Therefore, the intensity of intrathecal chemotherapy might be important to sufficiently prevent CNS relapse after HSCT. However, they observed the development of leukoencephalopathy in 7 of the 415 patients and we also observed leukoencephalopathy significantly more frequently in patients who received intrathecal chemotherapy after HSCT than those who did not. Therefore, such an intensive intrathecal chemotherapy should be avoided for patients at low risk for CNS relapse. We had a concern that the use of intrathecal chemotherapy after HSCT might delay immune recovery and thereby

Table 4. Impact of Pretransplant Factors on the Incidence of Isolated CNS Relapse after Transplantation

Factor		Univariate RR (95% CI)	P-Value	Multivariate RR (95% CI)	P-Value
Age		0.99 (0.98-1.00)	.055		
Sex		1.05 (0.47-2.34)	.90		
Disease	CML	1.00			
	AML	0.73 (0.04-11.9)	.82		
	ALL	5.31 (0.61-45.9)	.13		
CR/non-CR		4.98 (0.97-25.7)	.055		
History of CNS disease		48.3 (9.37-249.4)	3.6×10^{-6}	48.1 (9.40-245.9)	3.3×10^{-6}
TBI		3.21 (0.38-26.8)	.28		
Conventional/reduced intensity		1.08 (0.26-4.49)	.92		
Related/unrelated		1.45 (0.65-3.24)	.37		
Source	BM	1.00		1.00	
	PBSC	N.A.	<.0001	N.A.	<.0001
	CB	N.A.	<.0001	N.A.	<.0001
Sex mismatch		1.58 (0.26-9.41)	.62		
HLA mismatch		N.A.	<.0001	N.A.	<.0001
Prophylactic IT before HSCT		1.09 (0.21-5.61) 0.92			
Prophylactic IT after HSCT		7.11 (1.62-31.2) 0.0094			

N.A. indicates not assessable because no events were observed in the group; IT, intrathecal chemotherapy; RR, relative risk.

Table 5. Cumulative Incidence of CNS Relapse after HSCT in Prior Studies and Our Study

	n	Underlying Disease (AML/ALL/CML)	History of CNS Leukemia (%)	CR at Transplant (%)	Allogeneic Transplant (%)	Incidence of CNS Relapse (%)	Reference
1	415	217/198/0	23.4	47.7	100	2 in AML, 13 in ALL	1
2	92	0/92/0	22.8	100	71.7	11	2
3	487	366/121*/0	3.5	100	67.6	2.9	3
4	1226	533/352/341	9.2	65.8	100	2.3	Present report

*Including 5 patients with acute unclassified leukemia.

increase the risk of systemic relapse, but the incidence of systemic relapse was not significantly different between those who received intrathecal chemotherapy and those who did not (relative risk 1.11, 95% CI 0.79-1.55, $P = .56$). The use of total body irradiation (TBI) in the conditioning regimen has been considered to prevent CNS relapse, because irradiation is effective for so called sanctuary sites of chemotherapy. However, the incidence of CNS relapse was also rather higher in patients who received the TBI regimen. This may be again because of the fact that significantly higher proportion of patients received the TBI regimen among patients with CNS involvement before HSCT than those without CNS leukemia (81.5% versus 57.9%, $P < .0001$).

As for stem cell source, isolated CNS relapse was observed exclusively after BMT. A possible explanation for this may be the year effect, because allogeneic PBSCT and CBT started after 2000 in Japan. However, the year of HSCT of patients who developed isolated CNS relapse evenly ranged between 1997 and 2002. Another possible explanation is the presence of graft-versus-CNS relapse effect enhanced by increased incidence of cGVHD after allogeneic PBSCT and the presence of HLA-mismatch in CBT. The significantly higher incidence of CNS relapse after autologous HSCT than that after allogeneic HSCT suggested the existence of such an immunologicprotection against CNS relapse [2]. Isolated extramedullary relapse was also reported to be observed earlier in autologous HSCT than in allogeneic HSCT [7]. Furthermore, successful treatment of CNS relapse with reduced-intensity transplantation may suggest the presence of graft-versus-leukemia CNS leukemia effect [8], although the other reports doubted such effect against for CNS lesions [9-12]. The observed tendency toward a lower CNS relapse incidence after bone marrow relapse in patients with cGVHD than those without cGVHD in the current study might support this speculation, although we have no immunologic evidence.

The prognosis of patients who developed relapse after allogeneic HSCT has been reported to be extremely poor [13,14]. Also, survival after isolated CNS relapse was reported to be no better than that after bone marrow relapse in pediatric patients with AML and adult patients with ALL [15,16]. However, in the current study, 3 of the 7 patients who developed isolated CNS relapse were alive for more than a year without leukemia, resulting in the significantly better survival than those who developed CNS relapse after or simultaneously with systemic relapse. We could not identify the reason for this discrepancy, but the age and underlying disease of the study population differed between our study and the previous report. We consider that an intensive treatment against CNS leukemia is warranted for adult patients with isolated CNS relapse.

In conclusion, we confirmed that ALL as the underlying disease, active disease at HSCT, and the history of CNS involvement before HSCT were significant predictors for CNS relapse after HSCT. We failed to show a significant prophylactic effect of intrathecal chemotherapy to prevent CNS relapse and such a prophylactic treatment should be avoided for patients at low risk for CNS relapse. The prognosis for isolated CNS relapse was surprisingly good.

ACKNOWLEDGMENTS

The authors wish to thank the all clinicians who have assisted with the provision of data for this project.

REFERENCES

1. Thompson CB, Sanders JE, Flournoy N, et al. The risks of central nervous system relapse and leukoencephalopathy in patients receiving marrow transplants for acute leukemia. *Blood*. 1986;67:195-199.
2. Ganem G, Kuentz M, Bernaudin F, et al. Central nervous system relapses after bone marrow transplantation for acute lymphoblastic leukemia in remission. *Cancer*. 1989;64:1796-1804.
3. Singhal S, Powles R, Treleaven J, et al. Central nervous system relapse after bone marrow transplantation for acute leukemia in first remission. *Bone Marrow Transplant*. 1996;17:637-641.
4. Ruutu T, Corradini P, Gratwohl A, et al. Use of intrathecal prophylaxis in allogeneic haematopoietic stem cell transplantation for malignant blood diseases: a survey of the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant*. 2005;35:121-124.
5. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
6. Nagatoshi Y, Kawano Y, Nagayama J, et al. Treatment of isolated central nervous system relapse in high-risk lymphoid malignancy with allogeneic bone marrow transplantation and extended intrathecal therapy. *Br J Haematol*. 2004;125:766-768.

7. Potenza L, Luppi M, Riva G, et al. Isolated extramedullary relapse after autologous bone marrow transplantation for acute myeloid leukemia: case report and review of the literature. *Am J Hematol.* 2006;81:45-50.
8. Ostronoff M, Domingues MC, Ostronoff F, et al. Reduced intensity conditioning allogeneic bone marrow transplantation following central nervous system (CNS) relapse of acute promyelocytic leukemia: evidence for a graft-versus-leukemia effect in the CNS. *Am J Hematol.* 2006;81:387-388.
9. Au WY, Lie AK, Liang R, et al. Isolated extramedullary relapse of acute lymphoblastic leukaemia after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 1999;24:1137-1140.
10. Seo S, Kami M, Honda H, et al. Extramedullary relapse in the so-called "sanctuary" sites for chemotherapy after donor lymphocyte infusion. *Bone Marrow Transplant.* 2000;25:226-227.
11. Salutari P, Sica S, Micciulli G, et al. Extramedullary relapse after allogeneic bone marrow transplantation plus buffy-coat in two high risk patients. *Haematologica.* 1996;81:182-185.
12. Wilson DB, Michalski JM, Grossman WJ, et al. Isolated CNS relapse following stem cell transplantation for juvenile myelomonocytic leukemia. *J Pediatr Hematol Oncol.* 2003;25:910-913.
13. Bosi A, Laszlo D, Labopin M, et al. Second allogeneic bone marrow transplantation in acute leukemia: results of a survey by the European Cooperative Group for Blood and Marrow Transplantation. *J Clin Oncol.* 2001;19:3675-3684.
14. Breems DA, Van Putten WL, Huijgens PC, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. *J Clin Oncol.* 2005;23:1969-1978.
15. Johnston DL, Alonzo TA, Gerbing RB, et al. Risk factors and therapy for isolated central nervous system relapse of pediatric acute myeloid leukemia. *J Clin Oncol.* 2005;23:9172-9178.
16. Sancho JM, Ribera JM, Oriol A, et al. Central nervous system recurrence in adult patients with acute lymphoblastic leukemia: frequency and prognosis in 467 patients without cranial irradiation for prophylaxis. *Cancer.* 2006;106:2540-2546.

Bone Marrow Transplantation (2008) 42, 99–103

www.nature.com/bmt

ORIGINAL ARTICLE

Allo-SCT using reduced-intensity conditioning against advanced pancreatic cancer: a Japanese survey

Y Kanda[1], Y Omuro[2], E Baba[3], K Oshima[1], K Nagafuji[3], Y Heike[4], Y Takaue[4], T Sasaki[2], H Sakamaki[5] and M Harada[3]

[1]*Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan;* [2]*Department of Chemotherapy, Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, Tokyo, Japan;* [3]*First Department of Internal Medicine, Kyushu University, Fukuoka, Japan;* [4]*Division of Hematology and Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan and* [5]*Hematology Division, Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, Tokyo, Japan*

Pancreatic cancer is a frequent cause of cancer-related mortality and has an extremely poor prognosis. To evaluate the efficacy of allogeneic hematopoietic SCT with reduced-intensity conditioning (RICT) against pancreatic cancer, we analyzed the clinical data of 22 patients. After a fludarabine-based conditioning regimen followed by the infusion of PBSCs, all but two achieved engraftment. Complete, partial and minor response was observed in 1, 2 and 2 patients, respectively, with an overall response rate of 23%. Median survival was only 139 days and the major cause of death was tumor progression. Poor performance status before RICT and a lower number of infused CD34-positive cells were associated with shorter survival after RICT. Patients who developed chronic GVHD tended to survive longer than those who did not. These findings support the investigation of a novel treatment strategy to enhance the immunological effect against pancreatic cancer.
Bone Marrow Transplantation (2008) **42,** 99–103;
doi:10.1038/bmt.2008.94; published online 7 April 2008
Keywords: reduced-intensity conditioning; SCT; mini-transplantation; pancreatic cancer; graft-versus-tumor effect

Introduction

Allogeneic hematopoietic SCT is an established treatment for a variety of hematological disorders. However, its application has been limited to young patients because of various complications including regimen-related toxicities, GVHD, infection and so on. Therefore, SCT with reduced-intensity conditioning (RICT) has been investigated for use in older or clinically infirm patients. The antitumor effect of this therapeutic approach depends not only on the antineoplastic agents and/or irradiation in the conditioning regimen, but also on the immunological graft-versus-tumor effect after RICT.[1] Although RICT has not been clearly shown to have a clinical advantage over conventional chemotherapy, some studies have suggested that RICT may be beneficial in elderly patients with hematological malignancies.[2]

Since the late 1990s, several studies of RICT against advanced solid tumors have been performed to harness the graft-versus-tumor effect.[3] A clinical tumor response after RICT was observed in several solid tumors, especially in renal cell cancer and breast cancer.[4–6] Pancreatic cancer is the fifth most common cause of cancer-related mortality in Japan and the United States, and carries an extremely poor prognosis. The median duration of survival in advanced pancreatic cancer is less than 6 months, even when patients are treated with gemcitabine.[7] The combination of gemcitabine with the other chemotherapeutic agents failed to significantly improve survival.[8–10] Furthermore, although the combination of gemcitabine and erlotinib, a molecular targeting agent against epidermal growth factor receptor, significantly prolonged survival, the difference in median survival was only 2 weeks.[11] Because of this poor prognosis by chemotherapy, treatment strategies to enhance immunological effects against pancreatic cancer have been investigated. One of these is a vaccination targeting tumor-specific antigens such as CA19-9 and CEA.[12] Another strategy is RICT to harness a strong allogeneic immunological antitumor effect. The first successful application of RICT against pancreatic cancer was reported in 2001.[13] Several other reports have suggested the existence of an immunological graft-versus-tumor effect against pancreatic cancer, but the number of patients in each report was too small to draw any meaningful conclusion.[14,15] Therefore, we collected the clinical results of RICT against pancreatic cancer from transplantation centers in Japan, in which a prospective clinical trial of RICT against pancreatic cancer had been performed.

Correspondence: Dr Y Kanda, Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma, Omiya-ku, Saitama City, Saitama 330-8503, Japan.
E-mail: ycanda-tky@umin.ac.jp
Received 28 January 2008; revised 28 February 2008; accepted 5 March 2008; published online 7 April 2008

Patients and methods

We surveyed transplantation centers in Japan and identified three centers (Komagome Hospital, Kyushu University and National Cancer Center Hospital) that were performing a prospective clinical trial against various advanced solid tumors including pancreatic cancer. The University of Tokyo Hospital was performing a trial that exclusively included patients with advanced pancreatic cancer. Two of these trials have already been published.[14,15] We collected the clinical results of all patients with pancreatic cancer who participated in these studies from the published papers or using a questionnaire.

The reduced-intensity conditioning regimens were exclusively fludarabine-based, but varied among centers. The most intensive regimen was the combination of fludarabine (30 mg/m^2/day for 6 days), BU (4 mg/kg/day for 2 days) and gemcitabine (1000 mg/m^2/day for 3 days) at the University of Tokyo Hospital, whereas the combination of fludarabine (30 mg/m^2/day for 3 days) and TBI at 2 Gy (Kyushu University) was the least intensive. CY (60 mg/kg/day for 2 days) was combined with fludarabine (25 mg/m^2/day for 5 days) in the Komagome Hospital. Prophylaxis against GVHD was performed with CYA either alone or in combination with MTX or mycophenolate mofetil. PBSCs were mobilized with G-CSF, cryopreserved using standard techniques without *ex vivo* manipulation, thawed and infused on day 0. Host/donor T-cell chimerism was analyzed by sex-chromosome FISH or the short tandem repeat method after transplantation.[16]

The tumor response to treatment was evaluated as described previously.[15] Briefly, CR (complete response) was defined as disappearance of all clinical evidence of tumor for a minimum of 4 weeks by computed tomography scan. MR (minor response) and PR (partial response) were defined as decreases of 25–50% and greater than 50%, respectively, in the sum of the products of the maximum diameter and its perpendicular diameter of all measurable lesions for a minimum of 4 weeks.[7]

Engraftment was defined as a neutrophil count more than 500/mm^3 for 3 consecutive days after RICT. Engraftment failure was diagnosed as when engraftment was not achieved at any time after transplantation. The probability of survival was calculated using the Kaplan–Meier method. The incidence of chronic GVHD was evaluated in 13 patients who survived longer than 100 days after RICT. Univariate comparisons for dichotomous and time-to-event variables between groups were performed with the Fisher exact test and the log-rank test, respectively, and multivariate analyses were performed using logistic regression analysis and proportional hazards modeling, respectively. Factors associated with at least borderline significance ($P<0.10$) in the univariate analysis were subjected to a multivariate analysis using backward stepwise selection of covariates. All P-values were two sided and values of 0.05 or less were considered statistically significant.

Results

Clinical data of 22 patients with a median age of 57 years (range: 36–68 years) were collected (Table 1). There were 15 male and seven female patients. Fifteen patients had metastatic disease, whereas 7 had locally advanced diseases. All but one patient had received chemotherapy with gemcitabine either alone or in combination with other antineoplastic agents before RICT. In all, 10 had received local irradiation in addition to chemotherapy. Eastern Cooperative Oncology Group performance status (ECOG-PS) was equal to or greater than 2 in 10 patients. The conditioning regimen was fludarabine-BU-based in 10, fludarabine-CY in 7 and fludarabine-TBI in 5. The donors were HLA-matched relatives except in one patient who received graft from an HLA-mismatched family donor. The number of CD34-positive cells infused was greater than 4.0×10^6 cells/recipient body weight (kg) in 10 patients. CYA was used for GVHD prophylaxis: alone in 8, combined with MTX in 10 and combined with mycophenolate mofetil in 4.

Engraftment was observed in all but two patients with a median duration from RICT of 12 days (range: 6–42 days). Complete donor-type T-cell chimerism was confirmed in 18 patients, whereas mixed chimerism persisted in 4 patients. A total of 12 patients developed grade II–IV acute GVHD. Limited and extensive chronic GVHD was observed in three and five patients, respectively, among the 13 patients who survived longer than 100 days after RICT.

The best response after RICT was CR in one, PR in two, MR in two and stable disease in eight. The overall response

Table 1 Characteristics of the patients

Age (years)	
Median	57
Range	36–68
Sex	
Male	15
Female	7
Disease	
Locally advanced	7
Metastatic	15
ECOG-PS	
0–1	12
2–4	10
Regimen	
Flu + BU + Gem	7
Flu + CY	6
Flu + TBI	6
Flu + BU	3
Donor	
HLA-matched sibling	21
Mismatched family donor	1
CD34+ cells in graft	
$\leq 4.0 \times 10^6$/kg	12
$>4.0 \times 10^6$/kg	10
GVHD prophylaxis	
CsA alone	8
CsA + MTX	10
CsA + MMF	4

Abbreviations: ECOG-PS = Eastern Cooperative Oncology Group performance status; Flu = fludarabine; Gem = gemcitabine; MMF = mycophenolate mofetil.