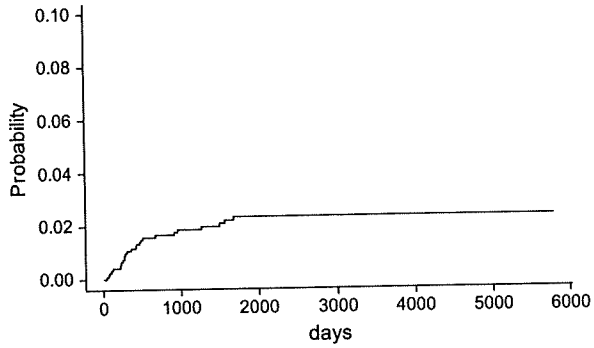

Figure 1. Cumulative incidence of CNS relapse treating death without CNS relapse as competing risk.

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

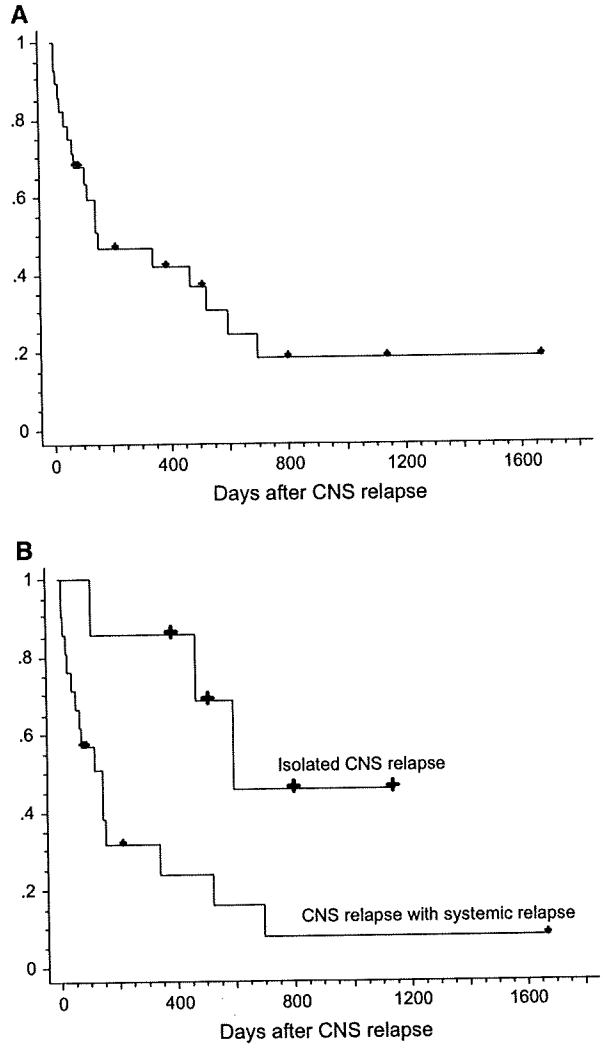

Figure 2. OS after CNS relapse (A) and that grouped according to isolated CNS relapse or CNS relapse associated with systemic relapse (B).

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

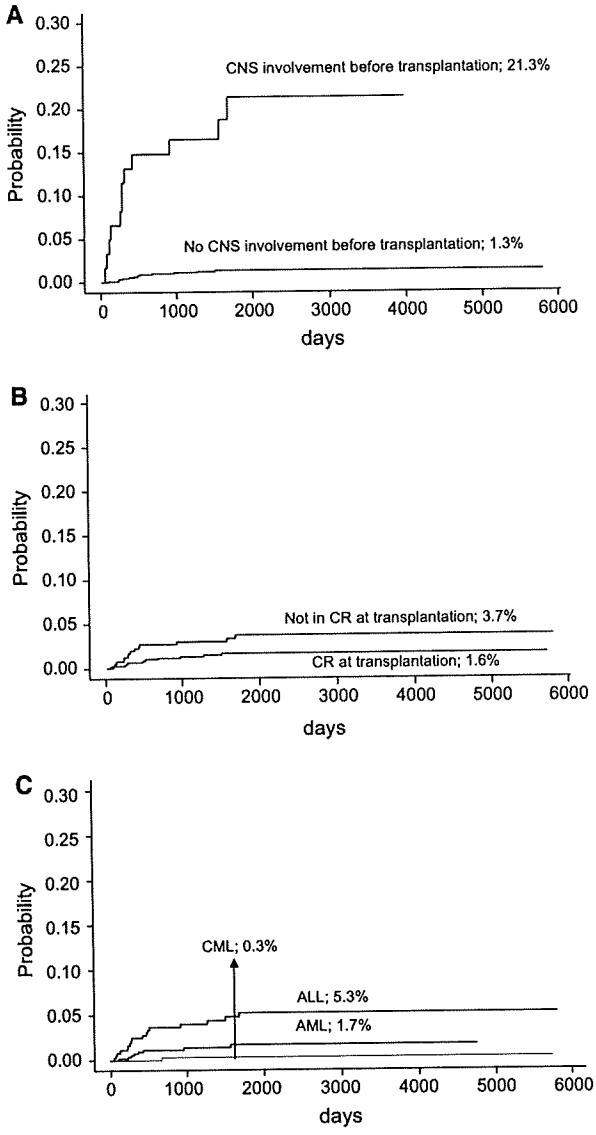

Figure 3. Cumulative incidence of CNS relapse grouped according to the history of CNS involvement before transplantation (A), disease status at transplantation (B), and underlying disease (C).

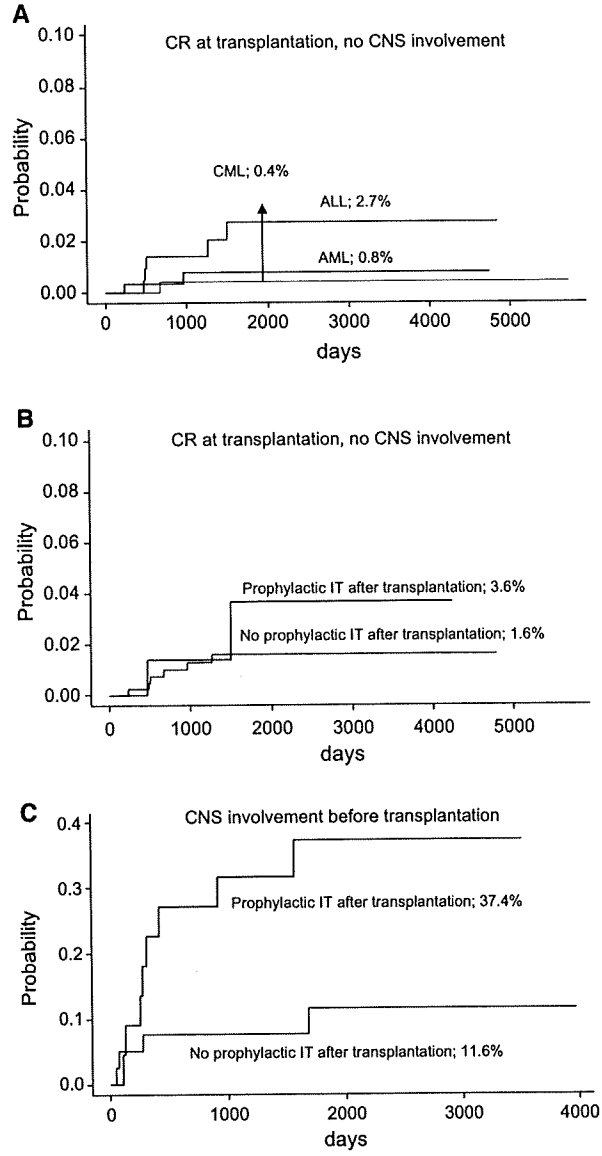

Figure 4. Cumulative incidence of CNS relapse in patients in remission at transplantation without a history of CNS involvement before transplantation grouped according to the underlying disease (A) and the use of prophylactic intrathecal chemotherapy (IT) after transplantation (B). Cumulative incidence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

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ORIGINAL ARTICLE

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

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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.
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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.

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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	
$\leqslant 4.0 \times 10^6$/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.

rate (CR + PR + MR) was 23%. A univariate analysis to identify possible relationships between clinical parameters and overall response failed to show any statistically significant factors. The conditioning regimen did not significantly affect the response rate, although the statistical power was not enough due to the small number of patients in each group. Response was observed in two of the seven patients who received the most intensive regimen including fludarabine, BU and gemcitabine, while it was seen in one of the six patients who received the least intensive regimen with fludarabine and low-dose TBI. None of the patients with mixed chimerism showed a response, but this difference was not statistically significant. DLI (donor lymphocyte infusion) was performed in four patients who had progressive disease after RICT, and the number of infused CD3-positive cells was between 2.7×10^7 and 1.8×10^8 cells/kg. One patient showed tumor shrinkage after DLI, but the response was transient.

Figure 1a shows overall survival after RICT. Median survival was only 139 days and the major cause of death was tumor progression. Other causes of death included infection in one and chronic GVHD in two. In a univariate analysis, ECOG-PS below 2 and infused CD34-positive cell dose greater than 4.0×10^6 cells/kg were associated with significantly longer survival after RICT (Table 2; Figures 1b and c). A multivariate analysis revealed that these two factors were almost independently significant (Table 2). With regard to post transplantation factors, while the development of grade II–IV acute GVHD did not significantly affect survival ($P = 0.76$), the eight patients who developed chronic GVHD tended to survive longer than those who survived longer than 100 days after RICT but did not develop chronic GVHD ($P = 0.092$; Figure 2). This analysis was unlikely to be biased by the fact that patients who survived longer had more chance to develop chronic GVHD, as most of the patients developed chronic GVHD as a progressive type from acute GVHD.

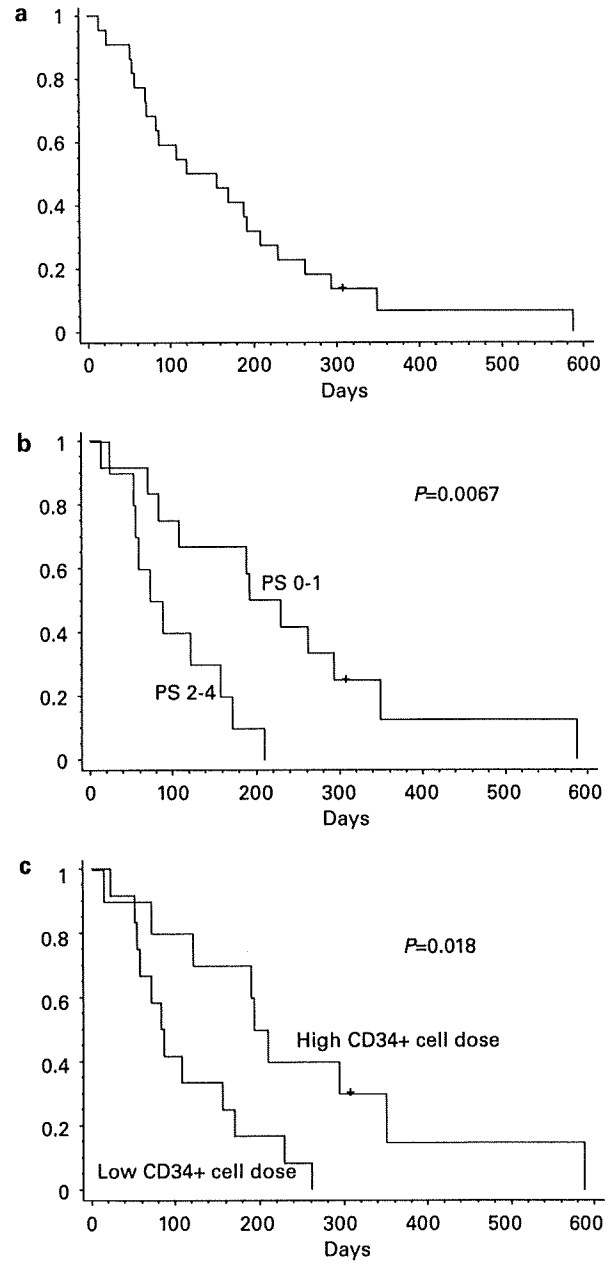

Figure 1 Patient survival, overall (a) and grouped according to risk factors (b and c).

Discussion

To summarize these findings, 23% of the 22 patients in this series showed a response to RICT. However, the duration of the response was generally short and most of the patients eventually died with progressive disease. The median survival after RICT was only 139 days and only one survived longer than 1 year after transplantation. Good ECOG-PS and higher number of CD34-positive cells in the graft were independently associated with longer survival.

The relationship between the number of infused CD34-positive cells and transplant outcome has been studied in PBSC transplantation for hematological malignancies.[17] The infusion of a higher number of CD34-positive cells has been associated with faster recovery of neutrophils and plts, but chronic GVHD was more frequently observed in patients who received a very high dose of CD34-positive cells (that is, $>8.0 \times 10^6$ cells/kg). In this study, two patients failed to achieve engraftment, and both had received less than 4.0×10^6 cells/kg of CD34-positive cells. However, a statistically significant survival advantage was confirmed even after these two patients were excluded from the analysis. If we consider that the major cause of death in this study was progressive disease, the infusion of a higher number of CD34-positive cells might have protected patients from disease progression by a graft-versus-host reaction, although we failed to show a significant difference between the number of infused CD34-positive cells and tumor response or the incidence of chronic GVHD, probably due to the small number of patients. Patients who developed chronic GVHD showed better survival than those who did not, with a borderline significance, suggesting that they had some immunological protection against the progression of pancreatic cancer.

Table 2 Univariate and multivariate analyses for overall survival

Factor	*Median survival (days)*	P-*value*
A. Univariate		
Age (years)		
<55	115	0.63
⩾55	180	
Sex		
Male	87	0.69
Female	170	
ECOG-PS		
0–1	211	0.0067
2–4	80	
Stage		
Locally advanced	192	0.21
Metastatic	121	
Serum CEA		
Negative	122	0.70
Positive	192	
Serum CA19-9		
Negative	157	0.84
Positive	132	
Regimen		
Flu+BU based	191	0.25
Flu+CY	156	
Flu+TBI	71	
CD34+ cell dose		
$\leqslant 4.0 \times 10^6$/kg	85	0.018
$>4.0 \times 10^6$/kg	201	
GVHD prophylaxis		
CsA alone	132	0.55
CsA+MTX	191	
CsA+MMF	96	
B. Multivariate	*Relative risk (95% CI)*	P-*value*
ECOG-PS		
0–1	1.00	0.032
2–4	3.39 (1.11–10.3)	
CD34+ cell dose		
$\leqslant 4.0 \times 10^6$/kg	1.00	0.068
$>4.0 \times 10^6$/kg	0.37 (0.13–1.07)	

Abbreviations: CI = confidence interval; ECOG-PS = Eastern Cooperative Oncology Group performance status; Flu = fludarabine; MMF = mycophenolate mofetil.

This study was limited by the heterogeneity of transplantation procedures among centers. However, considering the difficulty of performing a large-scale prospective study on RICT against pancreatic cancer, this small survey may currently represent the best evidence of the efficacy of this novel treatment strategy against advanced pancreatic cancer and may suggest a future direction for improving the treatment outcome. We showed that pancreatic cancer can be a possible target for allogeneic immunotherapy. However, the immunological effect was not strong or durable enough to prevent tumor progression. A possible strategy for enhancing a graft-versus-tumor effect against pancreatic cancer without enhancing GVHD is a combination with specific immunotherapy using antigens including CA19-9,

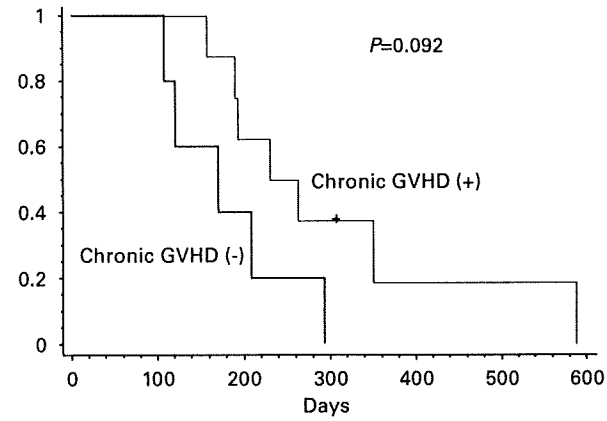

Figure 2 Overall survival of patients who survived at least 100 days after transplantation grouped according to the presence or absence of chronic GVHD.

CA242, CEA, Her-2, mutated K-ras and MUC-1.[12] Among these, CEA is attractive, since it is expressed in 85–90% of pancreatic cancer, and a specific immunotherapy against CEA could also be applied to other gastrointestinal cancers. An increase in the serum anti-CEA antibody level associated with a tumor response was observed in the University of Tokyo Study.[15] In addition, Kim *et al.*[18] showed that a peptide CEA652, TYACFVSNL, binds to HLA-A24 and induces CEA-specific cytotoxic T cells. Therefore, vaccination with such a peptide may be promising as a post transplantation immunotherapy against pancreatic cancer. Another approach is to add molecular targeting agents such as erlotinib after RICT. This may induce tumor cell death, leading to the enhanced presentation of tumor antigens to donor T cells. In addition, RICT can be combined with surgical resection, since the prognosis of pancreatic cancer is very poor even after complete resection.[19,20] Maximum graft-versus-tumor effect can be expected when the tumor load is at its lowest level.

In conclusion, a tumor response was observed in approximately one-fourth of the patients who underwent RICT against advanced pancreatic cancer. Although the response was not durable, our findings, such as the relationship between longer survival and the infusion of a higher number of CD34-positive cells or the development of chronic GVHD, should support a future study to enhance the specific immunological effect against pancreatic cancer.

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Decreased incidence of acute graft-versus-host disease by continuous infusion of cyclosporine with a higher target blood level

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Cyclosporine A (CsA) is the mainstay of pharmacologic prevention of acute graft-versus-host disease (GVHD). We previously reported that continuous infusion of CsA with a target blood level between 250 and 400 ng/ml significantly increased the incidence of acute GVHD compared to twice-daily infusion with a target trough level between 150 and 300 ng/ml. Thus, we raised the target level of CsA continuous infusion to 450–550 ng/ml. We treated 33 patients with the higher target level (CsA500) and compared the efficacy and toxicity with those in the 33 historical control patients (CsA300 group). Other transplantation procedures were not changed. The patients' characteristics were equivalent. The average CsA concentration was adjusted around 500 ng/ml and the actual daily dose was maintained at the initial dose (CsA 3mg/kg/day). Toxicities were equivalently observed among the two groups. The incidence of grades II–IV acute GVHD was significantly lower in the CsA500 group (27 vs. 52%, $P = 0.033$). The target level of CsA was identified as an independent significant risk factor for grades II–IV acute GVHD ($P = 0.039$), adjusted for the presence of HLA mismatch. The incidence of chronic GVHD was also decreased in the CsA500 group (47 vs. 73%, $P = 0.016$). We conclude that the toxicity of the continuous CsA infusion with a target level of 450–550 ng/ml is acceptable and the efficacy to prevent acute GVHD is significant. A larger comparative study is warranted to confirm these findings. Am. J. Hematol. 83:226–232, 2008. © 2007 Wiley-Liss, Inc.

Introduction

Cyclosporine A (CsA) is one of the most commonly used immunosuppressive agents for the prevention of acute graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). However, the dose, target blood level, and schedule of infusion vary among protocols and have not been optimized [1]. On the other hand, the importance of blood CsA concentration as well as administered dose has been shown in several reports [2–5]. We previously compared continuous infusion of CsA with a target blood level between 250 and 400 ng/ml and twice-daily infusion targeted to a trough level between 150 and 300 ng/ml in the early period after transplantation in a retrospective study [6]. The incidence of grades II–IV acute GVHD was significantly higher in patients who received the continuous CsA infusion, adjusted for the other significant factors. The actual daily dose of CsA in the continuous infusion group was decreased from the starting dose of 3–1.9 mg/kg/day on average at 4 weeks after transplantation, which might have adversely affected the incidence of acute GVHD. However, the incidences of renal dysfunction and relapse were significantly lower in these patients. The lower incidence of relapse in the continuous infusion group resulted in better disease-free survival in patients with high-risk diseases (43 vs. 16% at 2 years, $P = 0.039$), but not in standard-risk patients (72 vs. 80%, $P = 0.45$). We thus considered that the target CsA level of 250–400 ng/ml in the continuous infusion group was appropriate in high-risk patients, but too low in standard-risk patients. Therefore, we raised the target level of CsA to 450–550 ng/ml when we continuously infuse CsA in standard-risk patients [7]. In this report, we evaluated the safety and efficacy of the continuous infusion of CsA with this high target blood concentration at 500 ng/ml.

Results

Patient characteristics

We performed allogeneic HSCT for 33 standard-risk patients with the higher target CsA level at 450–550 ng/ml (CsA500 group). The historical control group treated with the original target CsA level at 250–400 ng/ml (CsA300 group) also included 33 patients [6]. The characteristics of the patients were equivalent between the two groups, except for the underlying disease (Table I). The number of patients with chronic myelogenous leukemia (CML) was only 2 in the CsA500 group, including one with chronic neutrophilic leukemia in uncontrollable leukocytosis, due to the introduction of imatinib in the treatment of such patients.

Blood concentration and actual daily dose of CsA

The dose of CsA was adjusted to maintain the blood CsA concentration between 450 and 550 ng/ml in the CsA500 group. All patients required repeated dose adjustments of CsA to maintain the targeted blood level. This adjustment was successful and the mean CsA concentration was 488 ±

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TABLE I. Characteristics of the Patients

	CsA500 group (n = 33)	CsA300 group (n = 33)	P-value
Sex			
Male	20	26	0.18
Female	13	7	
Age			
<40	16	17	>0.99
≥40	17	16	
Underlying disease			
AL	24	13	0.017
CML	2	12	
MDS	2	1	
NHL	3	6	
Others	2	1	
Donor			
Related	12	16	0.46
Unrelated	21	17	
HLA			
Match	28	25	0.54
Mismatch	5	8	
Stem cell source			
BM	25	26	>0.99
PB	8	7	
Regimen			
Non-TBI	4	9	0.21
TBI	29	24	
MTX dose			
<31mg/m^2	16	11	0.32
≥31mg/m^2	17	22	

BM, bone marrow; PB, peripheral blood; TBI, total body irradiation; AL, acute leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma.

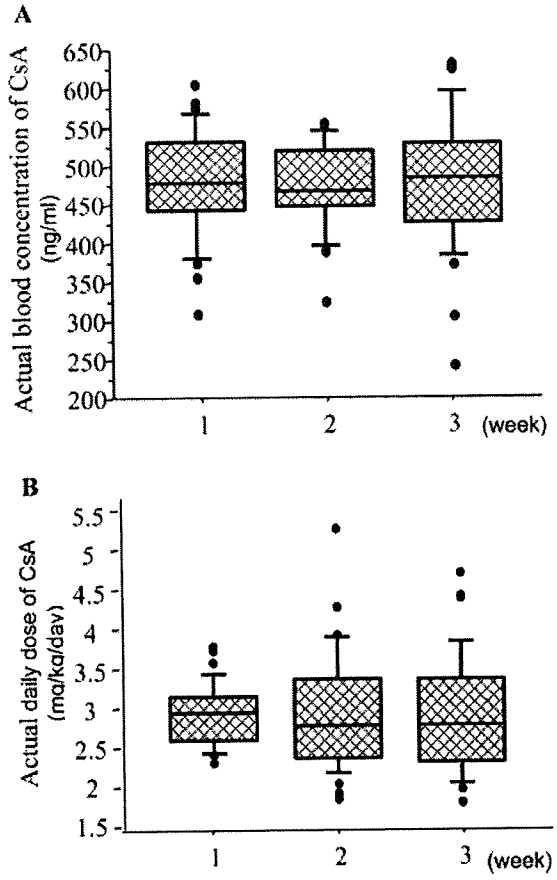

Figure 1. Actual blood concentration (A) and daily dose (B) of cyclosporine. The mean CsA concentration was 488 ± 89, 475 ± 41, and 482 ± 69 ng/ml and the actual dose was 2.9 ± 0.4, 2.8 ± 0.8, and 2.7 ± 0.7 mg/kg at the 1st, 2nd, and 3rd week after HSCT, respectively.

89, 475 ± 41, and 482 ± 69 ng/ml at the 1st, 2nd, and 3rd week after HSCT, respectively (Fig. 1A). The actual dose was 2.9 ± 0.4, 2.8 ± 0.8, and 2.7 ± 0.7 mg/kg at the 1st, 2nd, and 3rd week after HSCT, respectively (Fig. 1B). The median duration of intravenous cyclosporine was 41 days (range 16–74 days) after transplantation.

Toxicity

The incidence of renal dysfunction defined as elevation of the serum creatinine level above ×1.5 and ×2.0 the baseline value was equivalent between the CsA500 group and the CsA300 group (Table II, 24 vs. 24%, P = 0.96 and 15 vs. 13%, P = 0.71, respectively). Liver dysfunction defined as elevation of the total bilirubin level above 2 mg/dl was also similar (30 vs. 24%, P = 0.78). Thrombotic microangiopathy was not observed in any patients. No central nerves system toxicities were observed. In the CsA500 group, we decreased the target level of CsA to 300 ng/ml due to hyperbilirubinemia 9 days after HSCT in one patient and substituted prednisolone for CsA in another patient due to hyperbilirubinemia and renal dysfunction at day 21 after HSCT. The latter patient had already had liver cirrhosis classified to Child-Pugh A due to hepatitis C virus infection before HSCT.

TABLE II. Incidences of Adverse Events Due to Cyclosporine

	(−)	(+)	P-value
Incidence of serum creatinine > 1.5 × baseline value			
CsA500	25	8 (24%)	>0.99
CsA300	25	8 (24%)	
Incidence of serum creatinine > 2.0 × baseline value			
CsA500	28	5 (15%)	0.71
CsA300	30	3 (13%)	
Incidence of bilirubin > 2.0 mg/dl			
CsA500	23	10 (30%)	0.78
CsA300	25	8 (24%)	
Incidence of TMA			
CsA500	33	0 (0%)	>0.99
CsA300	33	0 (0%)	

TMA: thromobtic microangiopathy.

Incidences of acute and chronic GVHD

We performed a univariate analysis to evaluate the impact of potential confounding factors on the incidence of grades II–IV acute GVHD and identified two significant factors; the presence of HLA-mismatch including allele-mismatch and the target level of CsA (Table IIIA). As shown in Fig. 2A, the incidence of grades II–IV acute GVHD in the CsA300 group was significantly higher than that in the CsA500 group (52 vs. 27%, P = 0.033). Corticosteroids therapy for acute GVHD was more frequently required in the CsA300 group (39 vs. 15%, P = 0.051). The percentage of patients who received corticosteroids to treat GVHD was lower than the incidence of grades II–IV acute GVHD, because we did not use systemic corticosteroids for grades II acute GVHD with skin involvement only. The difference in the incidence of

TABLE III. Factors Associated the Incidences of Grades II–IV Acute GVHD and Nonrelapse Mortality

A. Univariate analyses

Factor	Acute GVHD	*P*-value	Nonrelapse mortality	*P*-value
Sex				
Male	20 (44%)	0.31	12 (30%)	0.020
Female	6 (30%)		0 (0%)	
Age				
<40 years	15 (46%)	0.30	4 (14%)	0.21
≥40 years	11 (33%)		8 (30%)	
Underlying disease				
CML	7 (50%)	0.25	2 (14%)	0.49
Non-CML	19 (37%)		10 (25%)	
Donor				
Related	11 (39%)	0.97	8 (36%)	0.052
Unrelated	15 (40%)		4 (13%)	
HLA				
Match	17 (32%)	0.0037	10 (23%)	0.78
Mismatch	9 (69%)		2 (18%)	
Stem cell source				
BM	19 (37%)	0.46	9 (21%)	0.68
PBSC	7 (47%)		3 (24%)	
Regimen				
Non-TBI	4 (31%)	0.56	5 (49%)	0.035
TBI	22 (42%)		7 (15%)	
MTX dose				
<31mg/m^2	12 (44%)	0.32	7 (19%)	0.87
≥31mg/m^2	14 (36%)		5 (24%)	
Target levels of CsA				
CsA500	9 (27%)	0.033	2 (8%)	0.051
CsA300	17 (52%)		10 (27%)	

B. Multivariate analyses

Factor	RR of acute GVHD	*P*-value	RR of nonrelapse mortality	*P*-value
Target levels of CsA				
CsA300	1.00	0.039	1.00	0.064
CsA500	0.43 (0.19–0.96)		0.24 (0.053–1.09)	
HLA				
Match	1.00	0.0062		
Mismatch	3.14 (1.39–7.14)			

grades II–IV acute GVHD between the two groups was more prominent in unrelated HSCT (Fig. 2B, 44 vs. 33% in related HSCT and 59 vs. 24% in unrelated HSCT).

Next, we performed a multivariate analysis to identify independent risk factors for the development of Grades II–IV acute GVHD. Two factors were independently significant with a relative risk (RR) of 3.14 (95% confidence interval [CI] 1.39–7.14, $P = 0.0062$) for the presence of HLA-mismatch and RR of 0.43 (95% CI 0.19–0.96, $P = 0.039$) for the CsA500 group, respectively (Table IIIB). The cumulative incidence of Grades III, IV acute GVHD was only 11%. The target level of cyclosporine (CsA500 vs. CsA300: 3 vs. 18%, $P = 0.045$) was identified as the only significant risk factor for the development of Grades III, IV acute GVHD.

The number of patients who developed limited and extensive chronic GVHD was 5 and 18, respectively, in the CsA300 group and 4 and 11, respectively, in the CsA500 group. The incidence of chronic GVHD was also significantly decreased in the CsA500 group (Table IV and Fig. 3, 47 vs. 73%, $P = 0.016$).

Transplantation outcome

The lower incidence of acute GVHD in the CsA500 group translated into the lower incidence nonrelapse mortality (Table III, 8 vs. 27%, $P = 0.051$). On the other hand, the incidence of relapse tended to be higher in the CsA500 group (Table V, 20 vs. 6%, $P = 0.065$), although this difference became smaller when we excluded patients with CML (19 vs. 10%, $P = 0.29$). Finally, there was no significant difference in disease-free survival between the CsA500 group and the CsA300 group (Fig. 4, 72 vs. 63%, $P = 0.68$).

Discussion

We successfully maintained the blood CsA concentration at around 500 ng/ml and the actual dose at around 3 mg/kg/day by twice a week monitoring for the first 3 weeks after transplantation. The preliminary data in these 33 patients suggested the feasibility and efficacy of the continuous infusion of CsA at this higher target level.

Several studies have reported the relationship between the blood concentration of CsA and the efficacy to prevent GVHD after allogeneic HSCT [2–5]. Especially, the area under the concentration–time curve (AUC) has been believed to be the most important pharmacokinetic parameter for the efficacy of calcineurin inhibitors [8,9]. The monitoring of AUC, however, requires frequent blood sampling and is not suitable for daily practice. Therefore, the trough concentration (C_{TL}) has been measured as a surrogate for AUC in twice-daily infusion of CsA, although recent reports

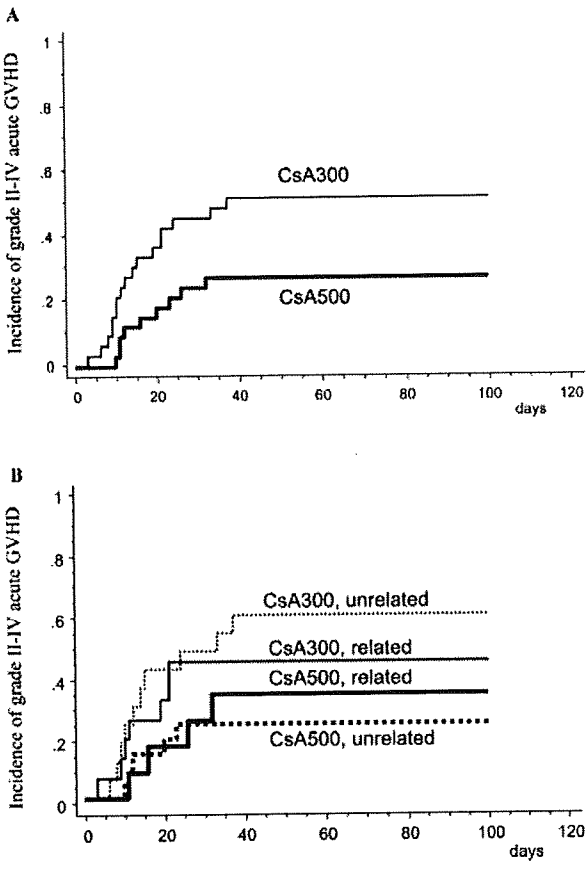

Figure 2. Incidence of Grades II–IV acute GVHD grouped according to the target level of cyclosporine. (A) all patients, (B) stratified by the donor type.

suggested that the measurement of blood concentration at 2–4 hr after infusion may be more appropriate [10]. In continuous infusion, the intradaily variation of the blood concentration of CsA should be minimal and we can evaluate the blood concentration regardless of the timing (steady-state concentration; C_{SS}). However, the relationship between Css in continuous infusion and C_{TL} in twice-daily infusion has not been clarified. Recently, Nakamura et al. reported that the target Css in the continuous infusion of CsA should be 2.55 times the C_{TL} to provide an equal AUC during the twice-daily infusion with a target C_{TL} [11]. Therefore, for example, the target C_{SS} in the continuous infusion of CsA should be 383–638 ng/ml to obtain a similar AUC during the twice-daily infusion with a target C_{TL} at 150–250 ng/ml, that is generally used in daily practice. However, the target blood concentration between 250 and 350 ng/ml is widely used in the continuous infusion of CsA [4]. The expected AUC will be far lower than that during the twice-daily infusion of CsA at the generally used target level. The target C_{SS} in this study at 500 ng/ml (450–550 ng/ml) would be appropriate according to the calculation model. In fact, the actual dose of CsA was maintained at 2.7 and 3.0 mg/kg on average. We had a concern that the incidence of renal dysfunction would be increased, since the relationship between the blood CsA level and drug-induced nephrotoxicity has been shown [12]. The incidence of renal dysfunction, however, was not increased by the dose adjustment and appropriate hydration when CsA levels above the target range were observed.

TABLE IV. Factors Associated the Incidence of Chronic GVHD

A. Univariate analyses

Factor	Chronic GVHD	*P*-value
Sex		
Male	24 (67%)	0.63
Female	10 (56%)	
Age		
<40 years	16 (60%)	0.31
≥40 years	18 (70%)	
Underlying disease		
CML	10 (77%)	0.12
Non-CML	24 (60%)	
Donor		
Related	14 (63%)	0.75
Unrelated	20 (66%)	
HLA		
Match	28 (64%)	0.74
Mismatch	6 (58%)	
Stem cell source		
BM	25 (60%)	0.20
PBSC	9 (81%)	
Regimen		
Non-TBI	27 (64%)	0.75
TBI	7 (65%)	
MTX dose		
<31mg/m^2	15 (64%)	0.81
≥31mg/m^2	19 (68%)	
Target levels of CsA		
CsA500	11 (47%)	0.016
CsA300	23 (73%)	

B. Multivariate analyses

Factor	RR	*P*-value
Target levels of CsA		
CsA300	1.00	0.014
CsA500	0.44 (0.23–0.85)	

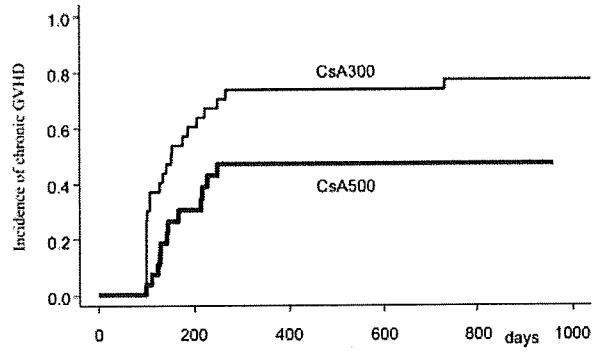

Figure 3. Incidence of chronic GVHD grouped according to the target level of cyclosporine.

Previous randomized control studies that compared continuous infusion of CsA and tacrolimus as GVHD prophylaxis had showed the superiority of tacrolimus to prevent acute GVHD [13–16]. However, these studies employed the lower target level of CsA between 150 and 400 ng/ml. Yanada et al. have also reported that tacrolimus-based regimen was better than cyclosporine-based regimen to prevent GVHD in unrelated bone marrow (BM) transplantation in Japan [17]. However, it was a retrospective analysis

TABLE V. Factors Associated the Incidence of Relapse and Disease-Free Survival

A. Univariate analyses

Factor	Relapse	*P*-value	Disease-free survival	*P*-value
Sex				
Male	5 (12%)	0.90	29 (58%)	0.054
Female	2 (15%)		18 (85%)	
Age				
<40 years	4 (16%)	0.72	25 (71%)	0.35
≥40 years	3 (10%)		22 (60%)	
Underlying disease				
CML	1 (7%)	0.51	11 (79%)	0.34
Non-CML	6 (15%)		36 (60%)	
Donor				
Related	1 (4%)	0.10	19 (60%)	0.53
Unrelated	6 (20%)		28 (67%)	
HLA				
Match	6 (13%)	0.74	37 (63%)	0.64
Mismatch	1 (10%)		10 (72%)	
Stem cell source				
BM	7 (16%)	0.15	35 (63%)	0.55
PBSC	0 (0%)		12 (76%)	
Regimen				
Non-TBI	0 (0%)	0.14	8 (51%)	0.41
TBI	7 (16%)		39 (69%)	
MTX dose				
<31 g/m^2	1 (4%)	0.071	21 (77%)	0.19
≥31 g/m^2	6 (21%)		26 (55%)	
Target levels of CsA				
CsA500	5 (20%)	0.069	26 (72%)	0.68
CsA300	2 (6%)		21 (63%)	

B. Multivariate analyses

Factor	RR of relapse	*P*-value	RR of disease-free survival	*P*-value
Target levels of CsA				
CsA300	1.00	0.065	1.00	0.68
CsA500	4.08 (0.92–18.1)		0.82 (0.32–2.12)	

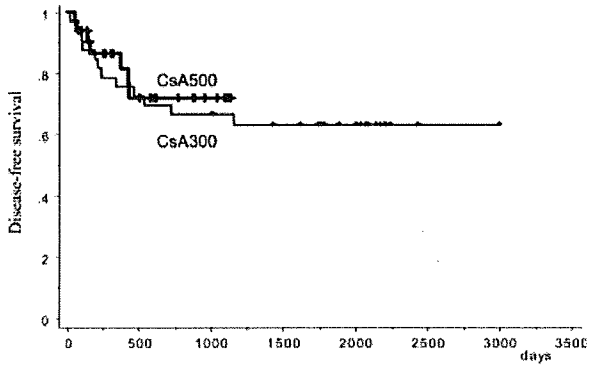

Figure 4. Disease-free survival grouped according to the target level of cyclosporine.

using the database of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and therefore the dose, target blood level, and infusion schedule of both cyclosporine and tacrolimus were various. Especially, the target level of CsA is generally low in the daily practice in Japan. Therefore, the results of these previous studies that compared CsA and tacrolimus as GVHD prophylaxis might have been affected by the target blood concentration [13–17].

The incidence of grades II–IV acute GVHD in the CsA500 group in this study was suppressed to 24% in unrelated HSCT including three HLA allele-mismatched transplants. This incidence was similar to that in the tacrolimus group of patients who underwent HSCT from an alternative donor (30 from an HLA-matched unrelated donor and 4 from the other alternative donor) in a Japanese randomized controlled trial (21%) [13]. Adverse drug reactions were more frequently observed in the tacrolimus group than in the CsA group in this Japanese randomized trial [13], whereas the toxicities in the CsA500 group were equivalent to those in the CsA300 group in the current study. Therefore, the continuous infusion of CsA with a target concentration at 500 ng/ml may provide similar efficacy of GVHD prophylaxis with less frequent toxicities compared to tacrolimus. Wingard et al. have reported that an important relationship between blood concentration of these agents and their efficacy and toxicity using data of a randomized controlled trial [16]. They showed that the efficacy of CsA to prevent GVHD could be improved by elevating the target blood concentration of CsA, whereas the toxicity of tacrolimus could be reduced by lowering the target blood concentration of tacrolimus. Therefore, a randomized controlled trial to compare CsA and tacrolimus with their appropriate target blood concentration is required to draw a definite conclusion.

Another concern about the elevation of the target concentration of CsA was the possible increase in the inci-

dence of relapse [18,19]. We previously showed that the incidence of relapse was significantly lower after the continuous infusion of CsA with the low target CsA concentration at 300 ng/ml compared to twice-daily infusions targeted to 150–300 ng/ml, because the actual dose of CsA was obviously decreased in the continuous infusion group [6]. In this study, the incidence of relapse tended to be higher in the CsA500 group (20 vs. 6%, $P = 0.065$), although there was no significant difference in disease-free survival. A possible explanation of the tendency toward higher relapse rate in the CsA500 group was the impaired graft-versus-leukemia effect due to the higher CsA concentration. Another explanation was the fact that the CsA300 group included significantly more patients with CML in the first chronic phase, the relapse rate of which is expected to be very low. Actually, the difference in the incidence of relapse became smaller when we excluded patients with CML. In addition, relapse in the CsA500 group mainly occurred in patients with relatively poor underlying diseases, including one with chronic neutrophilic leukemia in uncontrollable leukocytosis, one with acute myeloblastic leukemia with monosomy 7, and one with acute lymphoblastic leukemia with minimal residual disease detected by flow cytometry. Therefore, it might be important to make an appropriate definition of standard-risk disease. Currently, we are excluding acute leukemia in first remission with poor cytogenetic abnormalities, such as the presence of Philadelphia chromosome or monosomy 7, from standard-risk disease.

In conclusion, the continuous infusion of CsA with a target level of 450–550 ng/ml appeared to be safe and effective to prevent acute and chronic GVHD. A randomized controlled trial is being planned to confirm the appropriateness of this higher target level of CsA.

Patients and Methods

Patients

A continuous infusion of CsA with the target blood level between 450 and 550 ng/ml was started as GVHD prophylaxis for standard-risk patients at our institute in March 2003. We compared the safety and efficacy of this GVHD prophylaxis with those in the historical standard-risk patients in whom the blood CsA level was targeted to 250–350 ng/ml [6]. Standard-risk disease included acute leukemia in complete remission, CML in chronic phase, myelodysplastic syndrome without leukemic transformation, chemosensitive lymphoma, and nonmalignant disorders such as chronic active Epstein-Barr virus infection, while the others were considered high-risk diseases.

Transplantation procedure

Conditioning regimen was mainly a combination of cyclophosphamide (60 mg/kg for 2 days) with either busulfan (4 mg/kg/day for 4 days) or total body irradiation (TBI; 2 Gy twice daily for 3 days). BM was exclusively used as stem cell source in unrelated HSCT, whereas peripheral blood (PB) or BM was chosen in HSCT from a relative. GVHD prophylaxis consisted of CsA and short term methotrexate (MTX). The dose of MTX was 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6 in HLA-matched related HSCT. MTX at 7 mg/m^2 was added on day 11 in HLA-mismatched related HSCT and HLA-matched unrelated HSCT. In HLA allele-mismatched unrelated HSCT, the doses of MTX were increased to 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11.

CsA was administered as a 24-hr continuous infusion. The concentration of CsA was measured twice a week by fluorescence polarization immunoassay with a specific monoclonal antibody, using whole blood samples [20]. The dose of CsA was adjusted based on the ratio of the measured blood concentration and the target blood concentration of cyclosporine at 500 ng/ml to maintain the blood CsA concentration between 450 and 550 ng/ml. For example, when the measured blood concentration was 400 ng/ml using a daily cyclosporine dose of 200 mg, we multiplied the dose of cyclosporine by the ratio and determined the next cyclosporine dose at 200 mg × 500/400 = 250 mg. The route of CsA administration was converted to oral at a ratio of 1:2 when patients were able to tolerate oral intake after engraftment. Acute GVHD was graded as previously described [21]. Prophylaxis against bacterial, fungal, and *Pneumocystis carinii* infection consisted of fluconazole, tosufloxacin, and sulfamethoxazole/trimethoprim or inhalation of pentamidine. As prophylaxis against herpes simplex virus infection, acyclovir was given from days 7–35. Pre-emptive therapy with ganciclovir for cytomegalovirus infection was performed by monitoring cytomegalovirus antigenemia. The initial dose of ganciclovir was 5 mg/kg once daily and the dose was elevated to 5 mg/kg twice daily, when an increasing antigenemia was observed [22]. Other supportive procedures were not changed.

Statistical considerations

Toxicities were evaluated until the route of CsA was changed to oral. Renal dysfunction was defined as elevation of serum creatinine level above ×1.5 or ×2.0 the baseline value. Liver dysfunction was defined as elevation of the total bilirubin level above 2 mg/dl. Dichotomous variables of the patients' characteristics in the two groups were compared using Fisher's exact test. Overall survival, disease-free survival, and the cumulative incidence of acute GVHD were calculated using the Kaplan–Meier method, whereas the cumulative incidences of relapse and nonrelapse mortality were calculated using Gray's method considering each other event as a competing risk [23]. Potential confounding factors for the analyses included age, sex, donor types (related or unrelated), stem cell sources (BM or PB), conditioning regimens (TBI or non-TBI), HLA-mismatch, total doses of MTX, and the target levels of CsA. To evaluate the influence of the confounding factors on these events, the log-rank test and proportional hazards modeling were used for univariate and multivariate analyses, respectively. Factors that showed at least borderline significance ($P < 0.10$) in univariate analyses were included in the multivariate analyses and stepwisely deleted from the model, although the target level of CsA was persistently stayed in the model. All P-values were two-sided and P-values of 0.05 or less were considered statistically significant.

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False-positive *Aspergillus* galactomannan antigenaemia after haematopoietic stem cell transplantation

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***Objectives*: Although *Aspergillus* galactomannan (GM) antigen detection is widely applied in the diagnosis of invasive aspergillosis (IA), false-positive reactions with fungus-derived antibiotics, other fungal genera or the passage of dietary GM through injured mucosa are a matter of concern. The aim of this study was to investigate the cumulative incidence and risk factors for false-positive GM antigenaemia.**

***Patients and methods*: The records of 157 adult allogeneic haematopoietic stem cell transplantation (HSCT) recipients were retrospectively analysed. Episodes of positive GM antigenaemia, defined as two consecutive GM results with an optical density index above 0.6, were classified into true, false and inconclusive GM antigenaemia by reviewing the clinical course.**

***Results*: Twenty-five patients developed proven or probable IA with a 1 year cumulative incidence of 12.9%, whereas 50 experienced positive GM antigenaemia with an incidence of 32.2%. Among the total 58 positive episodes of the 50 patients, 29 were considered false-positive. The positive predictive value (PPV) was lower during the first 100 days than beyond 100 days after HSCT (37.5% versus 58.8%). Gastrointestinal chronic graft-versus-host disease (GVHD) was identified as the only independent significant factor for the increased incidence of false-positive GM antigenaemia (PPV 0% versus 66.7%, $P = 0.02$).**

***Conclusions*: GM antigen results must be considered cautiously in conjunction with other diagnostic procedures including computed tomography scans, especially during the first 100 days after HSCT and in patients with gastrointestinal chronic GVHD.**

Keywords: fungal infections, invasive aspergillosis, chronic GVHD, gastrointestinal tract, mucosal damage

Introduction

Invasive aspergillosis (IA) remains one of the leading infectious causes of death after allogeneic haematopoietic stem cell transplantation (HSCT), despite new antifungal agents that have become available in recent years.[1] The high mortality rate of IA was mainly attributed to the difficulty of diagnosis at the early stage of the disease, because histopathological examinations require invasive procedures and fungal cultures have low specificity and sensitivity in detecting IA.

Monitoring of the circulating *Aspergillus* galactomannan (GM) antigen by the sandwich enzyme-linked immunosorbent assay (ELISA) is a feasible non-invasive biological method for early diagnosis of IA.[2] The GM ELISA test has sensitivity of 67% to 100% and specificity of 81% to 99% in neutropenic patients and allogeneic transplant recipients,[3–6] and was introduced as microbiological evidence in the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) criteria for opportunistic invasive fungal infection.[7] However, a concern is the false-positive reactions,

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which may lead to inappropriate invasive investigation or over-treatment with antifungal agents. Previous studies have reported various risk factors for the false-positive results, including early childhood,[3] the development of chronic graft-versus-host disease (GVHD),[8] the passage of GM of food origin[9,10] and certain exoantigens from other fungal genera[11] or fungus-derived antibiotics.[12,13] However, little is known about the exact mechanism of false-positive reactions with these factors.

To clarify the cause of false-positive results, we retrospectively analysed the incidence and risk factors for false-positive GM antigenaemia in allogeneic HSCT recipients.

Patients and methods

Study population

GM ELISA became available at the University of Tokyo Hospital as a routine diagnostic test in February 2000. During a 5 year period (February 2000 to May 2005), 163 consecutive adult patients (>16 years old) underwent allogeneic HSCT at the University of Tokyo Hospital. The medical records of 157 patients who had at least two GM ELISA tests after HSCT were available for a retrospective analysis of positive GM antigenaemia. The median follow-up was 519 days (range, 15–2090 days) after HSCT. The patient characteristics are shown in Table 1. Acute leukaemia in first remission, chronic myelogenous leukaemia in first chronic phase, myelodysplastic syndrome with refractory anaemia or refractory anaemia with ringed sideroblasts, and aplastic anaemia were defined as low-risk diseases, whereas others were considered high-risk diseases. Donors other than human leucocyte antigen (HLA)-matched sibling donors were defined as alternative donors.

Transplantation procedure

The conventional preparative regimen for leukaemia/lymphoma was mainly performed with either cyclophosphamide/total body irradiation (TBI)-based regimens or busulfan/cyclophosphamide-based regimens. In cyclophosphamide/TBI-based regimens, the dose of cyclophosphamide was decreased and etoposide was added instead in patients with impaired cardiac function. Fludarabine-based regimens were used as reduced-intensity regimens for elderly or clinically infirm patients.[14] Cyclosporin A or tacrolimus was administered combined with short-term methotrexate for prophylaxis against GVHD. Alemtuzumab was added for patients who received a graft from an HLA-mismatched donor.[15] Methyl-prednisolone or prednisolone at 1 or 2 mg/kg was added for patients who developed grade II–IV acute GVHD, whereas prednisolone at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, herpes simplex virus and *Pneumocystis jirovecii* infections consisted of tosufloxacin, aciclovir and sulfamethoxazole/trimethoprim.

Antigen detection

GM assay was performed at least every other week after HSCT until discharge from the hospital in the majority of patients. In the outpatient setting, the monitoring of GM was continued at each visit in patients who were receiving immunosuppressive therapy, at the discretion of attending physicians. Circulating *Aspergillus* GM was detected using a sandwich immunocapture ELISA (Platelia Aspergillus, Bio-Rad, Marnes-la-Coquette, France) using a rat anti-GM monoclonal antibody.[2] The technique was performed as recommended by the manufacturer. The optical absorbance of specimens and controls was determined with a spectrophotometer set at 450 and 620 nm wavelengths. The optical density (OD) index for each sample was calculated by dividing the optical absorbance of the clinical sample by that of the threshold control. Two consecutive serum samples with an OD index of 0.6 or more were considered positive.[16]

Table 1. Patients' characteristics

Characteristic	Total patients
Sex (male/female)	105/52
Age, median (range)	41 (16–66)
Underlying disease	
acute leukaemia	70
CML	26
MDS	22
SAA	8
other	31
Graft source	
PBSC	69
BM	88
Donor type	
matched sibling	58
mismatched related	15
unrelated	84
Preparative regimen	
Cy (Etp)/TBI-based regimens	105
Bu/Cy-based regimens	15
ATG-based regimens for SAA	5
Flu-based RIC	32
GVHD prophylaxis	
CsA+MTX	115
tacrolimus+MTX	18
alemtuzumab+CsA+MTX	24
Acute GVHD	
grade 0–I	87
grade II–IV	69
Chronic GVHD	
extensive	57
limited	30
none	47

CML, chronic myelogenous leukaemia; MDS, myelodysplastic syndrome; SAA, severe aplastic anaemia; PBSC, peripheral blood stem cell; BM, bone marrow; Cy, cyclophosphamide; Etp, etoposide; TBI, total body irradiation; Bu, busulfan; ATG, antithymocyte globulin; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporin A; MTX, methotrexate.

Antifungal prophylaxis and treatment for IA

As antifungal prophylaxis, fluconazole at 200 mg was principally given daily from day −14 until the end of immunosuppressive therapy. For patients with a history of IA, intravenous micafungin at 150–300 mg or oral itraconazole at 200 mg was administered instead. All patients were isolated in high-efficiency particulate air (HEPA)-filtered rooms from the start of the conditioning regimen to engraftment. Febrile neutropenia was treated with broad-spectrum antibiotics in accordance with