grafts. Prophylaxis against graft-versus-host disease (GVHD) mainly consisted of cyclosporine with or without methotrexate ($n=273$), whereas nine patients received tacrolimus-based prophylaxis. One patient received no prophylaxis. Acute and chronic GVHD were scored according to the classic Seattle criteria.[9,10]

Statistical considerations

The primary end point of this study was overall survival (OS) after allo-SCT. Disease-free survival (DFS) and cumulative incidences of relapse, nonrelapse mortality, and acute and chronic GVHD were evaluated as secondary end points. The probabilities of OS and DFS and the cumulative incidence of acute GVHD were calculated using the Kaplan–Meier method. Cumulative incidences of relapse and nonrelapse mortality were calculated using Gray's method, considering each other risk as a competing risk.[11] Univariate comparisons for dichotomous variables between groups were performed with Fisher's exact test or the χ^2-test and multivariate analyses were performed using logistic regression analysis. Proportional hazards modeling was used to assess the influence of confounding factors on time-to-event variables. Potential confounding factors considered in the analysis were recipient age, sex, FAB classification, karyotype, preparative regimen, stem cell source, and the history of chemotherapy before transplantation. The effect of the development of acute and chronic GVHD on the incidence of relapse was analyzed among patients who survived without relapse at 60 and 150 days after transplantation, respectively. This landmark analysis was used to exclude bias that may arise from including patients who died too early to develop GVHD in the group without GVHD.

Results

Patient characteristics

The patient characteristics are summarized in Table 1. There were 171 males and 112 females. The median age at allo-SCT was 41 years (range 16–65 years). The median duration from diagnosis to allo-SCT was 8 months (range 1–204 months). A total of 188 patients had received chemotherapy before allo-SCT (Chemo group), whereas 95 had not (NoChemo group). Among the Chemo group, 81 underwent allo-SCT in complete remission (CR), while 107 were not in remission (NR). The Chemo group included a significantly higher proportion of patients with advanced disease than the NoChemo group ($P<0.0001$). In addition, the proportion of patients with a poor karyotype was significantly higher in the Chemo group ($P=0.004$).

Survival

Of the 283 patients, 159 were alive with a median follow-up of 36.5 months (range 1–139 months). The Kaplan–Meier probability of OS was 67.9% (95% confidence interval (CI) 63.4–72.4%) at 1 year, 48.8% (95% CI 44.7–52.9%) at 5 years, and 42.5% (95% CI 38.8–46.3%) at 10 years (Figure 1). The OS and DFS curves are superimposed, since only 13 patients were alive after relapse at this analysis. In a univariate analysis, age younger than 40 years, disease duration of 1 year or longer, good karyotype, diagnosis of RA, and the NoChemo group were associated with longer OS (Table 2). Among these factors, FAB classification, karyotype, and the history of chemotherapy were identified as independent significant prognostic factors for OS (Table 2, Figure 2).

Table 1 Patient characteristics

		History of previous chemotherapy		
	Total	*Presence*	*Absence*	*P-value*
Age (years)				
≤40	136	94	42	0.38
>40	147	94	53	
Sex				
Male	171	116	55	0.61
Female	112	72	40	
FAB				
RA	61	29	32	<0.0001
RAEB	58	29	29	
RAEBt	70	55	15	
CMML	25	19	6	
LT	69	56	13	
Karyotype				
Good	131	84	47	0.004
Intermediate	60	31	29	
Poor	41	32	9	
Unknown	51	41	10	
TBI				
Presence	173	115	58	>0.99
Absence	110	73	37	
Stem cell				
BM	218	144	74	0.88
PB	65	44	21	

LT = leukemic transformation.

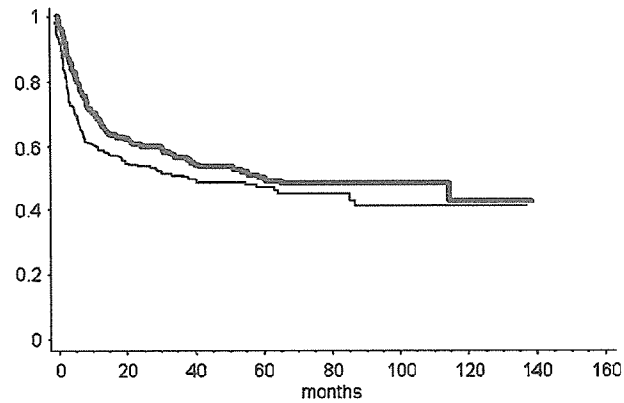

Figure 1 Overall survival (thick line) and disease-free survival (thin line) after transplantation.

Value of chemotherapy before transplantation

To determine the value of chemotherapy before transplantation in further detail, we only analyzed 139 patients with RAEB-t or LT to exclude biases, since the Chemo group included a significantly higher proportion of patients with RAEB-t and LT. Twenty-eight patients belonged to the NoChemo group and 111 patients belonged to the Chemo group. The proportion of

Table 2 Results of proportional hazard modeling for overall survival

Univariate	*Relative risk*	*P-value*
Sex		
Female	1.00	
Male	0.97 (0.68–1.39)	0.88
Age (years)		
<40	1.00	
⩾40	1.46 (1.02–2.09)	0.040
Duration from diagnosis to transplantation		
<1 year	1.00	
⩾1 year	0.64 (0.41–0.99)	0.045
Karyotype		
Good	1.00	
Intermediate	1.36 (0.83–2.21)	0.22
Poor	2.69 (1.65–4.37)	<0.0001
Unknown	1.47 (0.92–2.38)	0.11
FAB		
RA	1.00	
RAEB	1.84 (0.96–3.51)	0.065
RAEBt	2.42 (1.31–4.44)	0.0045
CMML	1.57 (0.71–3.50)	0.27
LT	3.16 (1.91–6.25)	<0.0001
Chemotherapy		
Absence	1.00	
Presence	1.92 (1.26–2.92)	0.0025
Unknown	1.70 (1.08–2.68)	0.023
Stem cell		
BMT	1.00	
PBSCT	1.33 (0.85–2.07)	0.21
Regimen		
TBI (–)	1.00	
TBI (+)	0.99 (0.69–1.42)	0.97
Multivariate		
FAB		
RA	1.00	
RAEB	1.82 (0.96–3.48)	0.069
RAEBt	2.00 (1.08–3.70)	0.029
CMML	1.31 (0.58–2.94)	0.51
LT	2.58 (1.40–4.76)	0.0023
Karyotype		
Good	1.00	
Intermediate	1.41 (0.86–2.30)	0.18
Poor	2.15 (1.30–3.55)	0.0028
Unknown	1.32 (0.82–2.14)	0.25
Chemotherapy		
Absence	1.00	
Presence	1.89 (1.19–2.99)	0.0065

patients with a poor karyotype was equivalent between the 2 groups (39 *vs* 45%, $P=0.44$). The duration from diagnosis to allo-SCT was shorter in the NoChemo group, with a marginal significance (161 *vs* 248 days in median, $P=0.07$). Among the Chemo group, 43 were in CR and 68 were in NR at allo-SCT. The survival curves of the NoChemo group and CR patients in the Chemo group were superimposed (57 *vs* 54%, $P=0.81$), whereas patients who underwent allo-SCT in NR after chemotherapy showed significantly shorter survival (20%, Figure 3a). The cumulative incidence of relapse was 26.2% in the NoChemo group and 27.6% in CR patients in the Chemo group ($P=0.83$, Figure 3b). Nonrelapse mortality was also similar between the two groups (18.8 *vs* 17.8%, $P=0.86$, Figure 3c). In contrast, the cumulative incidence of nonrelapse mortality was significantly higher in NR patients in the Chemo group than the NoChemo group (48.9 *vs* 18.8%, $P=0.014$), whereas difference in the incidence of relapse was not statistically significant (35.0 *vs* 26.2%, $P=0.44$).

The role of chemotherapy was also examined in patients with less-advanced MDS (RA or RAEB). Survival in the NoChemo group, CR patients in the Chemo group, and NR patients in the Chemo group was 73.4, 58.2, and 50.0% at 5 years, respectively. This difference was not statistically significant ($P=0.11$).

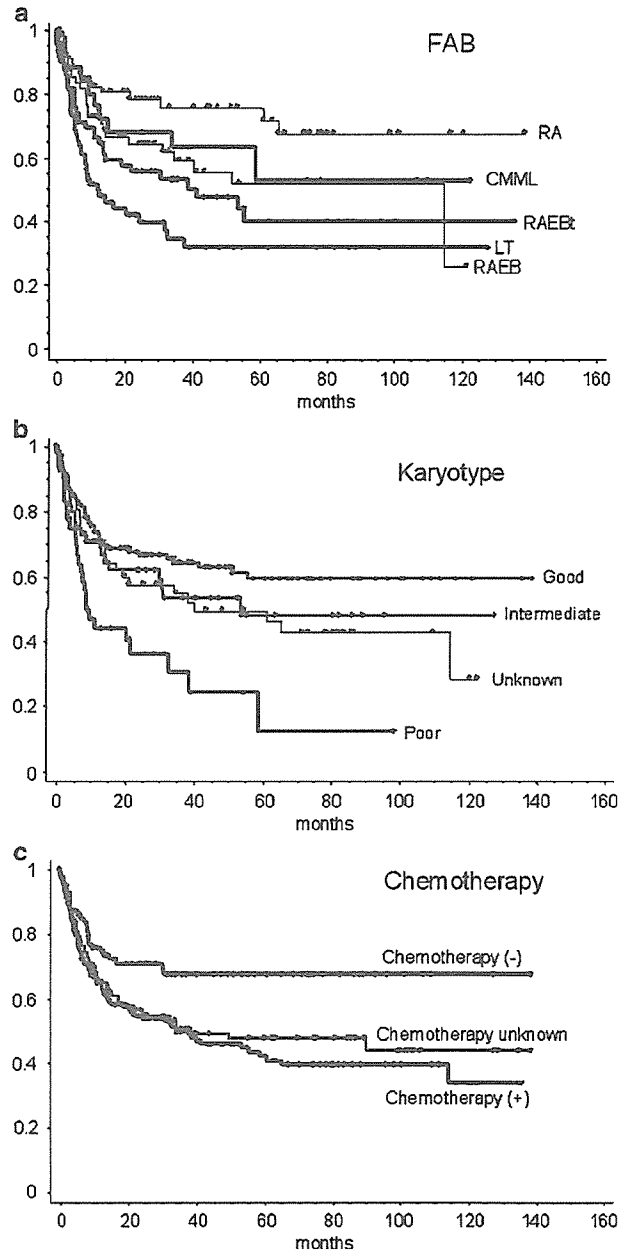

Figure 2 Overall survival grouped according to significant independent prognostic factors. Patients who lacked information regarding the history of chemotherapy before transplantation were included in (c).

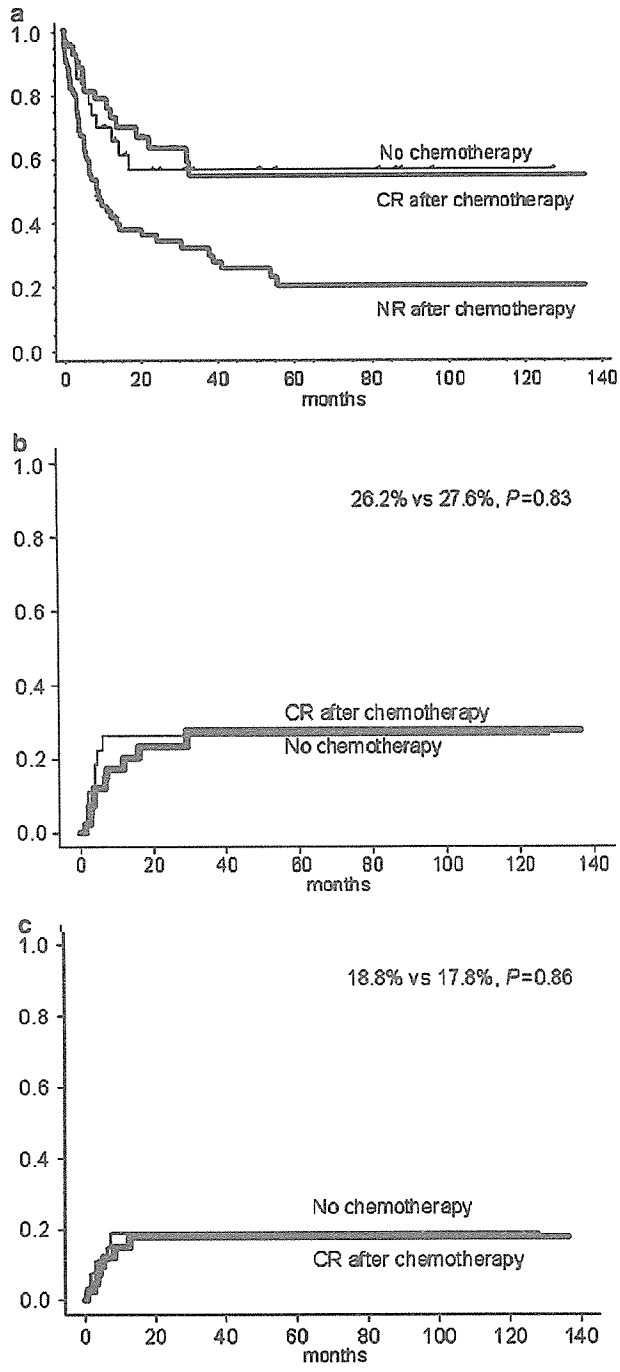

Figure 3 Overall survival (a) and cumulative incidences of relapse (b) and nonrelapse mortality (c) grouped according to the presence or absence of previous history of chemotherapy. These are compared between patients who achieved remission after chemotherapy and those who did not undergo chemotherapy. Only patients with RAEB-t or LT were included in the analyses.

Influence of acute and chronic GVHD on relapse

The cumulative incidence of grade II–IV and III–IV acute GVHD among those who achieved engraftment was 33 and 10%, respectively. Independent significant risk factors for grade II–IV acute GVHD were the karyotype (relative risk (RR) 1.98, 3.02, and 1.87, $P=0.016$, 0.0005, and 0.047 for intermediate, poor, and unknown groups, respectively) and the absence of a previous history of chemotherapy (RR 1.64, $P=0.033$). Chronic GVHD developed in 110 (53%) of 209 patients who survived more than 100 days. Logistic regression analysis identified higher age and the use of peripheral blood stem cell graft as independent significant risk factors for the development of chronic GVHD (RR 1.75 and 2.97, $P=0.049$ and 0.019, respectively). The cumulative incidence of relapse in those who developed grade II–IV acute GVHD and those who did not was 39.6 and 19.5%, respectively ($P=0.086$). The cumulative incidence of relapse in those who developed chronic GVHD and those who did not was 22.0 and 20.8%, respectively ($P=0.82$).

Discussion

Although intensive chemotherapy may cure rare young patients with advanced MDS,[10,12] allo-SCT is the only curative treatment for most MDS patients. The recent application of nonmyeloablative or reduced-intensity conditioning extended the indication of allo-SCT to older patients with MDS.[13,14] Factors known to be associated with the outcome of allo-SCT include patient age, disease duration, bone marrow blast counts, FAB classification, karyotype, and IPSS.[1–4,7,15,16] The fact that a lower bone marrow blast count before allo-SCT was associated with a better outcome after allo-SCT has encouraged physicians to administer intensive chemotherapy before allo-SCT. In fact, two registry reports from the European Group for Blood and Marrow Transplantation (EBMT) and National Marrow Donation Program showed that DFS for patients who underwent allo-SCT in first complete remission was better than that for patients who underwent allo-SCT as primary treatment for RAEB-t or LT.[1,2] However, this might only reflect the fact that patients with disease that originally responded well to chemotherapy were selected in the low blast count group. Also, these studies did not take into account patients who received induction chemotherapy but died or developed toxicity that precluded allo-SCT. Thus, these data do not justify the administration of induction chemotherapy before allo-SCT.

Runde *et al*[16] analyzed the outcome of patients who underwent BMT from HLA-identical siblings as first-line treatment for MDS and were reported to the EBMT. OS at 5 years was 42, 24, and 28% for patients with RAEB, RAEB-t, and LT, respectively. They concluded that allo-SCT should be performed as primary treatment for patients with a low blast cell count. Anderson *et al*[17] retrospectively compared the outcome of allo-SCT as an initial treatment for secondary AML and that of allo-SCT for chemotherapy-sensitive secondary AML. DFS at 5 years was not significantly different (24 *vs* 15%, $P=0.33$). The findings of the present analysis of JSHCT registry data were similar. OS at 5 years was 57% for patients who underwent allo-SCT for RAEB-t or LT without induction chemotherapy and 54% for those who underwent allo-SCT in remission ($P=0.81$). The difference was not significant even after adjusting for age and karyotype (RR 0.90, $P=0.80$). Although there might be a bias that patients with an indolent clinical outcome tended to undergo allo-SCT without induction chemotherapy, it was unlikely, because the duration from diagnosis to transplantation was rather shorter in the NoChemo group. Thus, the administration of remission induction chemotherapy before allo-SCT was not recommended from these data.

Information regarding the type of chemotherapy was not collected in this study and thus, it was supposed that low-dose

chemotherapy was also included, especially in patients with RA. However, most CR patients in the Chemo group must have received AML-type intensive chemotherapy, because CR can be rarely achieved with low-dose chemotherapy. A major shortcoming of this analysis was the lack of information regarding blast cell count at the time of transplantation. However, in the comparison between NoChemo group and CR patients in the Chemo group, blast count at transplantation was greater than 20% in the former group, whereas it was less than 5% in the latter group, because only patients with RAEB-t or LT were included in this analysis. Nevertheless, survival after transplantation was similar between the two groups.

We did not collect data of patients who underwent induction chemotherapy but did not proceed to allo-SCT. It was supposed that there were many patients who gave up transplantation due to lack of response to chemotherapy or toxicity of chemotherapy. Therefore, the CR patients in the Chemo group included only patients who had good response to chemotherapy and maintained good physical condition enough to undergo allo-SCT. Nevertheless, survival after transplantation was similar between the NoChemo group and the CR patients in the Chemo group.

OS at 5 years among patients with RAEB-t or LT in this study was better than those in previous reports. Considering the relapse rate of 26–27% and nonrelapse mortality rate of 17–18%, this good outcome resulted from the low nonrelapse mortality rate, probably due to the low incidence of acute GVHD. Not only the homogeneity of HLA and minor histocompatibility antigens but also the high frequency of the IL10-592A allele among the Japanese population may account for the low incidence of severe acute GVHD.[18–20]

The study from IBMTR did not demonstrate that the development of acute or chronic GVHD affected the incidence of relapse after allo-SCT from an HLA-identical sibling donor,[4] while the NMDP study showed that patients who developed grade II–IV acute GVHD had a lower incidence of relapse after allo-SCT from an unrelated donor than those who did not develop acute GVHD.[1] In this study, the development of GVHD did not appear to have a positive impact on the incidence of relapse. This discrepancy might be explained by a hypothesis that an anti-MDS effect associated with GVHD may be stronger in unrelated allo-SCT than in that from an HLA-identical sibling donor. Also, the anti-MDS effect associated with GVHD in this study might have been masked by the bias that the incidence of GVHD was significantly higher in patients with a poor karyotype.

In conclusion, this study confirmed that allo-SCT from an HLA-identical sibling donor offers the potential for long-term survival in patients with MDS. Induction chemotherapy before allo-SCT did not appear to offer any benefit. Although only a randomized controlled trial will be able to establish a definite conclusion, it seems that allo-SCT may be beneficial as primary treatment for patients with a low blast cell count or those with an advanced disease but with an indolent clinical course. Relapse is the major cause of failure after allo-SCT for advanced MDS, but we should not intend to induce GVHD, since GVHD did not appear to have a clear beneficial effect on the incidence of relapse.

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High-grade cytomegalovirus antigenemia after hematopoietic stem cell transplantation

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Summary:

Clinical impact of high-grade (HG) cytomegalovirus (CMV) antigenemia after hematopoietic stem cell transplantation has not been clarified. Therefore, in order to investigate the risk factors and outcome for HG-CMV antigenemia, we retrospectively analyzed the records of 154 Japanese adult patients who underwent allogeneic hematopoietic stem cell transplantation for the first time from 1995 to 2002 at the University of Tokyo Hospital. Among 107 patients who developed positive CMV antigenemia at any level, 74 received risk-adapted preemptive therapy with ganciclovir (GCV), and 17 of these developed HG-antigenemia defined as $\geqslant$50 positive cells per two slides. The use of systemic corticosteroids at $\geqslant$0.5 mg/kg/day at the initiation of GCV was identified as an independent significant risk factor for HG-antigenemia. Seven of the 17 HG-antigenemia patients developed CMV disease, with a cumulative incidence of 49.5%, which was significantly higher than that in the low-grade antigenemia patients (4%, $P<0.001$). However, overall survival was almost equivalent in the two groups. In conclusion, the development of HG-antigenemia appeared to depend on the profound immune suppression of the recipient. Although CMV disease frequently developed in HG-antigenemia patients, antiviral therapy could prevent a fatal outcome.

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Cytomegalovirus (CMV) disease is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Two strategies have been tested for the prevention of CMV disease after HSCT. First, universal prophylaxis with ganciclovir (GCV) after engraftment has led to a decrease in early CMV disease.[1,2] However, it also increased invasive fungal infections due to neutropenia and did not improve survival. Alternatively, preemptive therapy with GCV only in patients who are at high risk for CMV disease was investigated. Preemptive therapy with monitoring of the CMV viral load by antigenemia assay or polymerase chain reaction (PCR) resulted in a marked reduction of CMV disease without a significant increase in the incidence of bacterial or fungal infections.[3,4]

Although the level of CMV was expected to predict the development of CMV disease,[5] there has been some discrepancy regarding the correlation between the level of CMV antigenemia and the clinical outcome. Nichols *et al*[6] reported that rising levels of CMV antigenemia during preemptive therapy did not correlate with CMV disease among allogeneic HSCT recipients. On the other hand, other studies have shown a significant correlation between high viral load and CMV disease.[7,8] Therefore, we retrospectively analyzed the clinical impact of high-grade (HG) CMV antigenemia in allogeneic HSCT patients.

Patients and methods

Study population

We analyzed the records of 154 consecutive adult patients ($\geqslant$16 years old) who underwent allogeneic HSCT from an HLA-matched or a one-locus-mismatched donor for the first time at the University of Tokyo Hospital between June 1995 and December 2002. Nine patients who received reduced-intensity conditioning were included. The patient characteristics are shown in Table 1. In total, 66, 23, and 65 patients received graft from an HLA-matched related donor, a one-locus-mismatched related donor, and a matched unrelated donor, respectively. Unrelated HSCT was performed exclusively using bone marrow, whereas 33 related donors chose to donate peripheral blood stem cell graft. Acute leukemia in first remission, chronic myelogenous leukemia in 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 as high-risk diseases. Donors other than HLA-matched related donors were defined as alternative donors.

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Table 1 Patients' characteristics

Characteristic	*Total patients*
Sex (male/female)	106/48
Age, median (range)	35.0 (16–60)
Serostatus before transplant	
Recipient CMV-positive/negative	137/17
Donor CMV-positive/negative	129/25
Underlying disease	
Acute leukemia	73
CML	40
MDS	17
NHL/ATL	11
SAA	8
Other	5
Graft source	
PBSC	33
BM	121
Donor type	
Matched related	66
Mismatched related	23
Unrelated	65
Preparative regimen	
CY/TBI – based regimen	104
BU/CY – based regimen	34
ATG – including regimen	7
Nonmyeloablative regimen	9
GVHD prophylaxis	
CyA + MTX	137
Tacrolimus + MTX	17
Acute GVHD	
Grades 0–I	85
Grades II–IV	69

CMV = cytomegalovirus; PBSC = peripheral blood stem cell; BM = bone marrow; CY = cyclophosphamide; TBI = total body irradiation; BU = busulfan; ATG = antithymocyte globulin; GVHD = graft-versus-host disease; CyA = cyclosporine; MTX = methotrexate.

Transplantation procedure

The preparative regimen for leukemia/lymphoma was mainly performed with either a total body irradiation (TBI) regimen (cyclophosphamide (Cy) at 60 mg/kg for 2 days and TBI at 200 cGy twice daily for 3 days) or a non-TBI regimen (Cy at the same dose combined with busulfan (Bu) at 4 mg/kg for 4 days). Reduced-intensity regimens included the FB regimen (fludarabine (Flu) at 30 mg/m^2 for 6 days and Bu at 4 mg/kg for 2 days) and the FB16 regimen (Flu at the same dose with Bu 4 mg/kg for 4 days), and were used for elderly or clinically infirm patients.[9] Gemcitabine at 1000 mg/m^2 for 3 days was added to the FB regimen for patients with pancreatic cancer.[10] The anti-thymocyte globulin (ATG) regimen for aplastic anemia consisted of Cy at 50 mg/kg for 4 days and rabbit ATG at 5 mg/kg for 5 days with or without TBI at 200 cGy.

For prophylaxis against graft-versus-host disease (GVHD), cyclosporine A 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). Methylprednisolone or prednisolone at 1 or 2 mg/kg was added for patients who developed grade II–IV acute GVHD. Prophylaxis against bacterial, fungal, herpes virus, and *Pneumocystis carinii* infections consisted of fluconazole, tosufloxacin, acyclovir, and sulfamethoxazole/trimethoprim.

CMV antigenemia assay

CMV antigenemia assay was performed at least once a week after engraftment as described previously.[11] 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 number of positive cells per two slides.

Preemptive therapy for CMV disease

Preemptive therapy against CMV disease was performed by monitoring CMV antigenemia weekly after engraftment. Until June 2001, intravenous GCV was started at an induction dose of 10 mg/kg/day when 10 or more CMV-positive cells were detected in patients who underwent HSCT from an 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 20 and three positive cells for patients who underwent HSCT from an HLA-matched related donor and an alternative donor, respectively.[12] The dose of GCV was increased to 10 mg/kg/day when rising antigenemia was observed. The dose of GCV was adjusted according to the weekly monitoring of the creatinine clearance. GCV was continued until negative antigenemia was observed. Foscarnet at an induction dose of 120 mg/kg/day was substituted for GCV for patients with severe neutropenia or progressive CMV infection during GCV administration.

Definition of HG-CMV antigenemia and CMV disease

Positive antigenemia was defined as a detection of CMV-positive cells at any level. HG-antigenemia was defined as a positive result with 50 or more positive cells per two slides and low-grade (LG) antigenemia as the presence of less than 50 positive cells, because the 75 percentile value of maximal antigenemia in each patient was 48 positive cells per two slides. All patients with symptoms compatible with CMV disease such as interstitial pneumonia, colitis, and gastritis underwent extensive pathological examination of biopsy specimens. Patients with symptoms compatible with CMV retinitis received opthalmoscopy and/or PCR to detect CMV-DNA using aqueous humor, to establish a definite diagnosis of CMV disease.

Statistical analysis

Univariate and multivariate analyses for time-to-event covariates were performed using the log-rank test and proportional-hazard modeling, respectively. Factors associated with at least borderline significance ($P<0.10$) in univariate analyses were subjected to a multivariate analysis and stepwisely deleted from the model. The cumulative incidence of CMV disease was evaluated using Gray's method, considering death without CMV disease as a competing risk.[13]

Results

Incidence and risk factors for positive CMV antigenemia

Of the 154 patients, 107 (69.5%) developed positive antigenemia at a median of 42 days (range 12–637 days) after transplantation. In univariate analyses, higher age, recipient CMV seropositivity, HLA disparity, HSCT from an alternative donor, and grades II–IV acute GVHD were associated with the development of positive antigenemia (Table 2). In a multivariate analysis, recipient CMV seropositivity, HLA disparity, and grades II–IV acute GVHD were identified as independent risk factors for positive antigenemia.

Incidence and risk factors for HG-CMV antigenemia

In total, 74 patients received GCV as preemptive therapy, and 17 of these developed HG-antigenemia at a median of 49 days (range 36–637 days) after HSCT. The use of tacrolimus, grades II–IV acute GVHD, and the use of systemic corticosteroids at any doses, $\geqslant 0.5$ mg/kg/day and $\geqslant 1.0$ mg/kg/day, upon the initiation of GCV were associated with a high incidence of HG-antigenemia with at

Table 2 Risk factors for positive CMV antigenemia

Univariate analysis *Factors*	*Variables*	*n*	*Incidence (%)*	*P-value*
Age	<40 years old	90	68	0.05
	⩾40 years old	64	83	
Donor CMV serostatus	Donor (−)	25	81	0.27
	Donor (+)	129	73	
Recipient CMV serostatus	Recipient (−)	17	33	0.001
	Recipient (+)	137	79	
Donor type	Identical sibling donor	66	69	0.05
	Alternative donor	88	79	
HLA	Match	103	70	0.04
	Mismatch	51	85	
Disease risk	High	81	80	0.69
	Low	73	70	
Graft source	Bone marrow	121	70	0.13
	Peripheral blood	33	100	
Preparative regimen (1)	Non-ATG containing	147	74	0.76
	ATG containing	7	82	
Preparative regimen (2)	Non-TBI regimen	116	66	0.18
	TBI regimen	38	78	
Acute GVHD	Grades 0–I	86	66	0.001
	Grades II–IV	68	85	
GVHD prophylaxis	CyA + MTX	137	74	0.56
	Tacrolimus + MTX	17	76	
Multivariate analysis *Factors*	*Relative risk*	*95% CI*	*P-value*	
Recipient CMV serostatus	5.2 (Positive *vs* negative)	2.1–13.0	<0.001	
Donor	1.5 (Alternative *vs* identical sib.)	1.0–2.3	0.03	
Grade II–IV acute GVHD	2.1 (Grades II–IV *vs* 0–I)	1.4–3.1	<0.001	

least borderline significance (Table 3). Among these, the use of systemic corticosteroids of ⩾0.5 mg/kg/day upon the initiation of GCV was the only independent risk factor for HG-antigenemia.

Clinical outcome of patients who developed HG-antigenemia

The median peak antigenemia level of the 17 patients who developed HG-antigenemia was 95 positive cells per two slides (range 50–821) (Table 4). They received preemptive GCV for a median duration of 22 days (range 9–72), all of whom developed HG-antigenemia after starting GCV. A total of 16 patients had been receiving corticosteroids at the detection of HG-antigenemia, whereas the remaining one who had received ATG as conditioning had not been taking corticosteroid. GCV was replaced with foscarnet in four patients with persistent HG-antigenemia or severe neutropenia during GCV administration. Five patients developed recurrent HG-antigenemia at a median of 70.5 days after the first episode.

Seven of the 17 HG-antigenemia patients developed CMV disease with a cumulative incidence of 49.5%, at a median onset of 50 days (range 0–309 days) from the initiation of GCV. CMV pneumonia developed in three patients, colitis in four, gastritis in one, and retinitis in one (Table 4). The incidence of CMV disease was significantly higher in patients who developed HG-antigenemia than in

Table 3 Risk factors for high-grade CMV antigenemia among 74 patients who received preemptive ganciclovir after transplantation

Univariate analysis *Factors*	*Variables*	*n*	*Incidence (%)*	*P-value*
Age	<40 years old	39	26	0.68
	⩾40 years old	35	20	
Donor CMV serostatus	Donor (−)	15	15	0.33
	Donor (+)	59	23	
Recipient CMV serostatus	Recipient (−)	4	25	0.98
	Recipient (+)	70	23	
Donor type	Identical sibling donor	22	14	0.22
	Alternative donor	52	27	
HLA	Match	42	19	0.31
	Mismatch	32	29	
Disease risk	High	46	29	0.13
	Low	28	14	
Graft source	Bone marrow	57	20	0.11
	Peripheral blood	17	35	
Preparative regimen (1)	Non-ATG containing	72	23	0.35
	ATG containing	2	50	
Preparative regimen (2)	Non-TBI regimen	62	25	0.53
	TBI regimen	12	17	
Acute GVHD	Grades 0–I	29	10	0.04
	Grades II–IV	45	32	
GVHD prophylaxis	CyA + MTX	63	19	0.08
	Tacrolimus + MTX	11	47	
Steroid at any doses	None	22	5	0.04
	Done	52	31	
Steroid at ⩾0.5 mg/kg	None	35	9	0.007
	Done	39	36	
Steroid at ⩾1 mg/kg	None	45	16	0.09
	Done	29	35	
Initial dose of ganciclovir	10 mg/kg	45	18	0.15
	5 mg/kg	29	32	
Multivariate analysis *Factors*	*Relative risk*	*95% CI*	*P-value*	
Steroids Use at ⩾0.5 mg/kg	4.60 (done *vs* none)	1.3–16.0	0.017	

Table 4 Characteristics of patients who developed high-grade CMV antigenemia

UPN	*Sex/age*	*Diagnosis*	*Preparative regimen*	*aGVHD grade*	*Steroid at start of GCV (mg)*	*Day 0 to 1st HG-CMV (days)*	*Duration of steroid before HG-CMV (days)*	*Peak value of CMV-Ag*	*Duration of GCV/FOS (days)*	*Negative conversion*	*Second HG-CMV*	*Time from first/second HG-CMV to disease (days)*	*CMV disease*	*Survival (months)*	*Cause of death*
1	M/25	ALL	TBI	–	PSL 30	66	18	115	40/–	+	–	–	–	7.8	Relapse
2	F/40	ALL	TBI	III	mPSL 50	40	6	65	22/–	+	–	–	–	43.0+	Alive
3	M/26	CML	TBI	IV	mPSL 200	49	32	298	20/15	+	+	43/3	Pneumonia, Retinitis	38.8+	Alive
4	M/31	CML	non-TBI	IV	PSL 70	45	7	70	21/23	+	–	57	Pneumonia	3.4	TMA
5	M/44	MDS	TBI	II	PSL 15	39	13	419	9/18	+	–	–	–	33.3+	Alive
6	M/57	CML	TBI	II	mPSL 120	36	17	66	22/–	+	–	–	–	28.5+	Alive
7	M/32	MDS	TBI	III	mPSL 35	216	180	50	15/–	+	+	37/2	Pneumonia, Colitis	8.3	IA
8	M/44	NHL	TBI	I	PSL 34	637	557	157	16/–	–	–	–	–	21.6	SIRS
9	M/35	AML	TBI	II	mPSL 90	40	17	56	23/–	+	–	–	–	24.6+	Alive
10	F/25	CML	TBI	III	mPSL 250	42	19	52	11/9	+	–	227	Gastritis	21.5+	Alive
11	F/38	AML	TBI	II	mPSL 40	53	14	95	23/–	+	+	303/101	Colitis	16.9+	Alive
12	M/28	NHL	TBI	II	mPSL 30	48	24	83	20/–	+	+	–	–	9.7	Relapse
13	F/33	AA	ATG	–	–	94	–	338	27/–	–	–	–	–	3.8+	Alive
14	F/60	AML	FB 16	III	mPSL 90	43	9	821	72/–	+	–	0	Colitis	3.8	Relapse
15	M/49	Pancreatic ca.	Gem+FB	III	mPSL 80	59	13	251	22/–	+	+	–	–	6.1+	Alive
16	M/56	NHL	non-TBI	II	PSL 25	157	15	186	35/–	–	–	12	Colitis	6.4+	Alive
17	M/33	AML	TBI	III	PSL 60	269	224	86	47/–	+	–	–	–	26.9+	Alive

UPN = unique patient number; aGVHD = acute graft-versus-host-disease; HG-CMV = high-grade cytomegalovirus; GCV = ganciclovir; FOS = foscarnet; AA = aplastic anemia; ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CML = chronic myelogenous leukemia; MDS = myelodysplastic syndrome; NHL = non-Hodgkin' lymphoma; ATL = adult T-cell leukemia/lymphoma; TBI = total body irradiation; Campath = alemtuzumab; ATG = antithymocyte globulin; FB = fludarabine and busulfan; Gem = gemcitabine; PSL = predonisolone; mPSL = methylpredonisolone; TMA = thrombotic microangiopathy; IA = invasive aspergillosis; SIRS = systemic inflammatory response syndrome.

those who did not (49.5 *vs* 4%, $P<0.001$, Figure 1). However, overall survival was equivalent between the two groups (59.5 *vs* 59.4% at 5 years, $P=0.79$, Figure 2). The direct causes of death in HG-antigenemia patients did not include CMV disease, but did include relapse in three, and thrombotic microangiopathy (TMA), invasive aspergillosis (IA), and systemic inflammatory response syndrome in one each.

Clinical outcome of patients who did not develop HG-antigenemia

Two of the 57 patients without HG-antigenemia developed CMV disease during GCV treatment. One developed CMV retinitis with a maximal antigenemia level of eight positive cells, who died of acute respiratory distress syndrome of unknown cause. Another developed CMV colitis with a maximal antigenemia level of 31 positive cells, who eventually died of fungal and bacterial pneumonia. The direct causes of death in 41 patients without HG-antigenemia included relapse in 15, infection in nine (two bacterial, five fungal, and two viral), noninfectious pulmonary complications in six, acute GVHD in five, multiple organ failure in three, cardiac complications in two, and TMA in one.

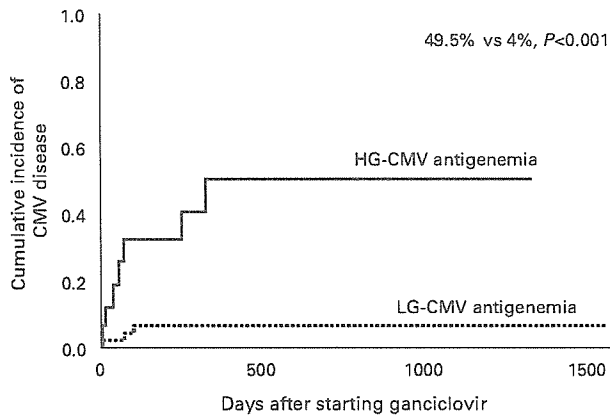

Figure 1 Cumulative incidence of CMV disease in patients who received preemptive administration of ganciclovir, grouped according to the antigenemia level. HG = high grade, LG = low grade.

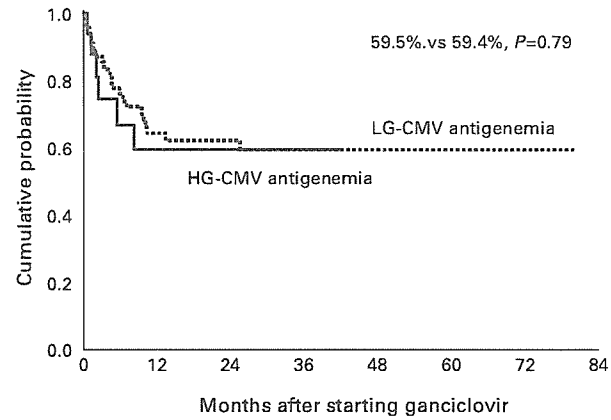

Figure 2 Overall survival of patients who received preemptive administration of ganciclovir grouped according to the antigenemia level.

Discussion

The use of systemic corticosteroids at $\geqslant 0.5$ mg/kg/day at the initiation of GCV was identified as an independent risk factor for HG-antigenemia. Although the cumulative incidence of CMV disease in patients with HG-antigenemia was significantly higher than that of patients with LG-antigenemia, overall survival was almost equivalent. We used a different threshold of CMV antigenemia to start GCV. By the use of this risk-adapted preemptive therapy, the incidence of HG-antigenemia and CMV diseases was not significantly different between those transplanted from an HLA-matched related donor and those transplanted from an alternative donor. Therefore, this risk-adapted approach could have overcome the risk due to the use of an alternative donor.

There has been no consensus on the cutoff point of HG-antigenemia. Therefore, we added an analysis by dividing the patients with a maximal antigenemia of less than 50 positive cells per two slides into low-LG- and intermediate-LG-antigenemia groups, defined by the maximal positive cells less than 10 and maximal positive cells between 10 and 49, respectively. One of 28 patients with intermediate-LG-antigenemia and one of 29 patients with low-LG-antigenemia developed CMV disease, respectively. The incidence of CMV disease in patients with intermediate-LG-antigenemia was significantly lower than in those with HG-antigenemia (4.3 *vs* 49.5%, $P=0.0015$), where as it was equivalent between low-LG-antigenemia patients and intermediate-LG-antigenemia patients (4.3% in both groups). Therefore, the cutoff value appeared to be appropriate.

The use of corticosteroids at the initiation of GCV was identified as an independent risk factor for HG-antigenemia, which agreed with the conclusion of previous studies that host immune status is most important for the response to GCV treatment.[6,14,15] We made the greatest efforts to reduce the dose of corticosteroids for patients who developed CMV reactivation, although it was difficult in patients with severe acute GVHD. In addition, a patient who received ATG as conditioning developed HG-antigenemia without corticosteroid. It has been reported that the use of ATG in the conditioning regimen leads to delayed immune recovery and is strongly associated with CMV infections.[15,16]

None of the 17 HG-antigemenia patients directly died of CMV diseases, leading the inference that a fatal outcome could be avoided with an optimal antiviral therapy. However, two patients died of TMA and IA, which might have been indirectly caused by CMV infection and/or its treatment, since CMV infection has been identified as a risk factor for TMA and IA.[17,18]

Studies that evaluated the relationship between CMV load and CMV disease have shown conflicting results. Some studies showed a significant correlation between a

high viral load and CMV disease or transplant-related death.[7,8] However, in a recent study by Nichols *et al*,[6] the antigenemia level during antiviral therapy did not correlate with either CMV disease or survival. In this study, HG-antigenemia was associated with a higher risk of CMV disease but not with inferior survival. The studies that have shown a significant association between CMV viral load and survival did not use a preemptive approach, but rather ganciclovir or foscarnet was administered after the detection of CMV-related symptoms. Thus, this discrepancy may show that preemptive therapy helped to improve outcome of high-risk patients. A major difference between Nichols's study and ours was the duration of ganciclovir administration. They administered ganciclovir until day 100 after HSCT, whereas we stopped ganciclovir when antigenemia became negative. The optimal duration of preemptive GCV is still controversial. In this study, the median duration of preemptive administration of GCV was 22 days, which was equivalent to that in previous studies.[4,12] However, the recurrence of HG-antigenemia was observed in 30% of patients after the discontinuation of GCV. Furthermore, in two of the three patients with CMV pneumonia, it developed only a median of 2.5 days after the detection of second HG-antigenemia. These patients had been persistently receiving high-dose corticosteroids for grade III or IV acute GVHD. Therefore, it might be better to extend the duration of GCV administration for patients who develop HG-antigenemia and who are still receiving high-dose corticosteroid.

In conclusion, severe immunosuppression due to high-dose steroid increased the incidence of HG-antigenemia. HG-CMV antigenemia was associated with a significantly higher incidence of CMV disease, but had no influence on overall survival, since the progression of CMV disease to a fatal outcome could be prevented by antiviral treatment. However, the establishment of optimal preemptive therapies is needed in profoundly immunosuppressive patients who are receiving high-dose corticosteroid for severe GVHD.

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Cardiac complications after haploidentical HLA-mismatched hematopoietic stem cell transplantation using *in vivo* alemtuzumab

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Summary:

Alemtuzumab is a humanized monoclonal antibody directed against human CD52 with a strong lympholytic effect. We have performed unmanipulated hematopoietic stem cell transplantation (HSCT) from 2- or 3-locus-mismatched family donors in 14 patients using *in vivo* alemtuzumab. All achieved complete donor cell engraftment and grade III–IV acute graft-*versus*-host disease was observed in only one patient. However, eight of the 14 patients developed grade II–IV cardiac complications according to Bearman's criteria. Next, we retrospectively analyzed the records of 142 adult patients who underwent allogeneic HSCT from 1995 to 2004 to evaluate whether the use of alemtuzumab was an independent risk factor for cardiac complications. Among several factors that increased the incidence of grade II–IV cardiac complications with at least borderline significance, a multivariate analysis identified the cumulative dose of anthracyclines ($P = 0.0016$) and the use of alemtuzumab ($P = 0.0001$) as independent significant risk factors. All of the cardiac complications in the alemtuzumab group were successfully treated with diuretics and/or catecholamines. Patient selection and close monitoring of cardiac function may be important in HLA-mismatched HSCT using *in vivo* alemtuzumab.

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Keywords: cardiac complications; hematopoietic stem cell transplantation; HLA-mismatch; alemtuzumab

Alemtuzumab (Campath-1H) is a humanized IgG1 antibody that recognizes CD52, a glycoprotein antigen expressed on T, B, NK, and dendritic cells.[1] The addition of *in vivo* alemtuzumab to the conditioning regimen before HLA-matched allogeneic hematopoietic stem cell transplantation (HSCT) decreases the incidence of graft-*versus*-host disease (GVHD).[2,6] We thus conducted a study to evaluate the safety of unmanipulated HSCT from a 2- or 3-locus-mismatched family donor using alemtuzumab only *in vivo* and have shown that alemtuzumab is very effective for preventing GVHD.[7] However, eight of the 14 patients developed grade II–III cardiac complications according to Bearman's criteria.[8] We describe the clinical course of cardiac complications after HLA-mismatched HSCT using *in vivo* alemtuzumab. In addition, we report the results of retrospective analyses that evaluated whether the use of *in vivo* alemtuzumab was an independent risk factor for cardiac complications after adult allogeneic HSCT.

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Patients and methods

Transplantation procedure

The study to evaluate the safety of unmanipulated HSCT from 2- or 3-locus-mismatched family donors using *in vivo* alemtuzumab was started in March 2002 after approval by the ethics committee of the University of Tokyo Hospital. The transplantation procedure has been described in detail elsewhere.[7] Briefly, the conditioning regimen consisted of total body irradiation (TBI) at 2 Gy twice daily for 3 days and cyclophosphamide at 60 mg/kg/day for 2 days. The dose of cyclophosphamide was decreased to 20 mg/kg/day for 2 days, and etoposide at 40 mg/kg/day was added instead in a patient with impaired cardiac function due to anthracyclines. For elderly patients, a non-TBI containing regimen consisting of fludarabine at 30 mg/kg/day for 6 days and busulfan at 1 mg/kg four times daily for 4 days was used. After November 2003, we added TBI at 2 Gy twice daily on day −1 and decreased the dose of busulfan to 4 mg/kg/day for 2 days. Alemtuzumab was added to these regimens at 0.2 mg/kg/day for 6 days (days −8 to −3), following pretreatment with 1 mg/kg of methyl-prednisolone. Cryopreserved donor peripheral blood (PB) stem cells were infused on day 0 without *ex vivo* manipulation. GVHD prophylaxis was with cyclosporine A and short-term methotrexate. Cyclosporine was started on day −1 at a dose of 3 mg/kg/day by continuous infusion and the dose was adjusted to maintain a blood concentration between 250 and 350 ng/ml. Cyclosporine was changed to an oral form when it could be tolerated by the patient. Methotrexate was administered at 15 mg/m^2 on day 1, and 10 mg/m^2 on days 3, 6, and 11. For patients without acute GVHD, we

started to taper cyclosporine from day 30 by 10% per week and discontinued it on day 100.

Analyses of risk factors for cardiac complications

After we observed grade II–IV cardiac complications according to Bearman's criteria in eight of the 14 patients who underwent HLA-mismatched HSCT using *in vivo* alemtuzumab, we retrospectively analyzed the records of all adult patients who had undergone allogeneic HSCT for the first time between June 1995 and June 2004 at the University of Tokyo Hospital. In the following statistical analyses, we included 142 patients for whom standard 12-lead electrocardiogram (ECG) and ultrasound cardiography (UCG) within 3 months before transplantation were available. We routinely performed these procedures on all patients before conditioning. Among them, 14 patients underwent 2- or 3-locus-mismatched HSCT using *in vivo* alemtuzumab, whereas the remaining 128 patients underwent HLA-matched or 1-locus-mismatched HSCT using standard GVHD prophylaxis with cyclosporine or tacrolimus combined with methotrexate. Cyclophosphamide at more than 100 mg/kg was used in 107 patients and TBI was used in 95 patients. The stem cell source was bone marrow (BM) in 94 patients and PB stem cells in 48.

Cardiac complications observed within 28 days after HSCT were considered regimen-related toxicities and grouped according to Bearman's criteria.[8] Potential confounding factors considered in the statistical analyses were age, sex, status of underlying disease, previous cardiac disease, smoking, serum ferritin level, cumulative dose of anthracyclines, irradiation involving the heart, heart rate, blood pressure, QT interval, QT dispersion, left ventricular ejection fraction (LVEF) evaluated by UCG, dose of cyclophosphamide in the conditioning regimen, use of TBI, use of alemtuzumab, stem cell source (BM or PB), serological/genotypical HLA-mismatch, and donor type (related or unrelated). Details of the methods used to measure these parameters have been described previously.[9] For univariate analyses, continuous variables in the two groups were compared using the unpaired *t*-test or the Mann–Whitney *U*-test, whereas categorical variables were compared using the Fisher's exact test. Factors associated with at least borderline significance ($P<0.10$) on univariate analysis were subjected to multivariate analysis using logistic regression. *P*-values of less than 0.05 were considered statistically significant.

Results

HLA-mismatched HSCT using in vivo *alemtuzumab*

In total, 14 patients underwent 2- or 3-locus-mismatched HSCT using *in vivo* alemtuzumab. There were eight males and six females with a median age of 49.5 years (range 27–60). Nine patients had active disease at transplantation. Eight received a TBI-based regimen, while six received a fludarabine-based regimen. The median number of CD34+ and CD3+ cells in the graft was 5.1×10^6 cells/kg (range 4.3–7.7) and 2.5×10^8 cells/kg (range 1.0–7.1), respectively. All patients achieved donor cell engraftment and complete donor-type chimerism with a median duration to neutrophil recovery $>500/mm^3$ of 18.5 days and platelet recovery $>20\,000/mm^3$ of 18.0 days. Only two patients developed grade II–IV acute GVHD; one patient each with grade II and III, respectively. Infection-related death was observed in one patient who died of cytomegalovirus pneumonitis on day 98. Grade II–IV regimen-related toxicities according to Bearman's criteria were observed as follows: stomatitis in nine patients, renal toxicity in two, liver toxicity in one, and cardiac toxicity in eight (57.1%), mainly with congestive heart failure (Table 1). Six patients developed grade II cardiac toxicity diagnosed by an increased cardiothoracic ratio, which was detected by routine X-ray and required the use of diuretics. Grade III cardiac toxicities were observed in two patients who responded poorly to diuretics and required catecholamines. One of them showed markedly decreased left ventricular function (EF of 24%) on UCG. Another patient had paroxysmal supraventricular tachycardia. Treatment with catecholamines resolved the symptoms in both patients. There were no long-term sequelae except that one patient with grade III cardiac toxicity showed persistent LV dysfunction on UCG.

Retrospective analyses to identify risk factors for cardiac complications

Of the 142 patients included in the retrospective analysis, 23 (16.2%) and 10 (7.0%) patients developed grade II–IV and III–IV cardiac complications, respectively, within 28 days after transplantation. The median onset of cardiac complication was 13.5 and 4.5 days after HSCT in patients who received alemtuzumab and those who did not, respectively ($P=0.02$; Figure 1). Seven died of cardiac causes a median of 3 days after the onset of cardiac complications, but all the cardiac complications in the alemtuzumab group were successfully treated with diuretics and/or catecholamines.

Univariate analyses to evaluate the impact of possible confounding factors on the incidence of grade II–IV cardiac complications identified eight factors with a *P*-value less than 0.10: smoking history ($P=0.036$), serum ferritin level ($P=0.033$), cumulative dose of anthracyclines ($P=0.001$), heart rate ($P=0.084$), EF ($P=0.070$), serological HLA-mismatch ($P=0.054$), genetic HLA-mismatch ($P=0.087$), and the use of alemtuzumab ($P=0.0002$) (Table 2a).

Among these, only the cumulative dose of anthracyclines (odds ratio 1.003, 95% confidence interval (CI) 1.001–1.005, $P=0.0016$) and the use of alemtuzumab (OR 12.1, 95% CI 3.3–44.1, $P=0.001$) were identified as independent significant risk factors on multivariate analysis (Table 2b).

Discussion

Generally, cardiac complications are uncommon after treatment with alemtuzumab.[10–13] However, a high incidence of cardiac complications was reported in four of

Table 1 Cardiac complications in patients who underwent HLA 2- or 3-locus-mismatched hematopoietic stem cell transplantation using *in vivo* alemtuzumab

(a) No.	*Age/sex*	*Disease*	*Anthracycline dose (mg/m²)*	*TBI (Gy)*	*Engraftment (day)*	*Onset (day)*	*Bearman grade*	*Treatment*	*Outcome*
1	44/F	Ph+ALL CR1	90	12	17	10	II, Mild CHF	Diuretics	Resolved
2	27/F	ALL CR2	794	12	16	20	II, Mild CHF	Diuretics	Resolved
3	56/F	Ph+ALL NR	491	(—)	20	13	II, Mild CHF	Diuretics	Resolved
4	45/M	AML PIF	186	12	18	14	II, Mild CHF	Diuretics	Resolved
5	41/F	ALL CR1	310	12	29	15	III, Severe CHF & arrhythmia	Diuretics & catecholamines	Resolved
6	57/M	AML PIF	140	4	20	14	II, Mild CHF	Diuretics	Resolved
7	54/M	MDS NR	0	4	12	6	II, Mild CHF	Diuretics	Resolved
8	33/F	AML NR	587	12	43	8	III, Severe CHF	Diuretics & catecholamines	Resolved

(b) No.	*CTR before HSCT (%)*	*CTR at onset (%)*	*EF before HSCT (%)*	*EF at onset (%)*	*BW before HSCT (kg)*	*BW at onset (kg)*	*Oximetry at onset (%)*	*ECG findings other than sinus tachycardia*
1	52.4	56.0	66	—	61.1	62.0	98	—
2	43.3	47.8	56	47	46.4	48.8	98	—
3	46.0	54.1	70	—	45.6	48.0	97	—
4	39.8	48.3	58	—	65.6	68.7	96	—
5	43.0	51.4	63	—	44.5	49.6	95	PSVT
6	49.0	55.3	55	—	56.1	58.3	92	—
7	45.1	51.0	65	—	67.3	69.2	92	—
8	41.4	57.9	55	24	55.4	60.0	96	—

TBI = total body irradiation; CR = complete remission; PIF = primary induction failure; NR = not in remission after relapse; onset = onset of cardiac complications; CHF = congestive heart failure.

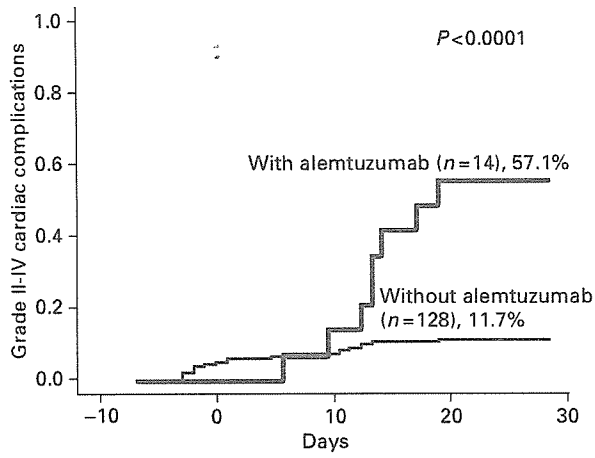

Figure 1 Cumulative incidence of grade II–IV cardiac complications according to Bearman's criteria, grouped according to the use of *in vivo* alemtuzumab in the conditioning regimen.

eight patients who received alemtuzumab for mycosis fungoides or Sézary syndrome.[14] The expression of CD52 was not observed on cardiac myocytes and, thus, cytokine-release syndrome after alemtuzumab infusion was considered to be responsible for the cardiac complications,[14] because inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6) have been reported to be responsible for the development and progression of heart failure.[15,16]

The frequent cardiac complications in our HLA-mismatched HSCT study could not be explained solely by alemtuzumab, since cardiac complications were infrequently mentioned in HLA-matched HSCT with reduced-intensity conditioning.[2,6] Therefore, cardiac complications in our series might have resulted not only from alemtuzumab but also from the intensive conditioning and/or increased cytokine secretion associated with the engraftment of HLA-mismatched donor cells. In fact, the median duration to cardiac complications after HSCT was significantly longer in patients who received alemtuzumab and most of the cardiac complications after allogeneic HSCT using alemtuzumab were observed in the peri-engraftment period (Table 1), when the secretion of various cytokines is known to increase.[17]

We monitored cardiac function after HSCT by daily measurements of body weight, pulse oximeter oxygen saturation and weekly chest X-ray. Thus, we were able largely to avoid fatal cardiac complications after HSCT using alemtuzumab. However, two patients developed grade III cardiac complications that required catecholamine support. An evaluation of the value of possibly more sensitive markers, such as plasma brain natriuretic peptide level, is thus warranted.[18] The use of diuretics may be sufficient if cardiac complications can be detected at an early stage.

In conclusion, although *in vivo* alemtuzumab is very effective for preventing GVHD even in HLA-mismatched HSCT, the use of *in vivo* alemtuzumab along with myeloablative conditioning for HLA-mismatched HSCT may increase the incidence of reversible cardiac compli-

Table 2 Risk factors for grade II–IV cardiac complications according to Bearman's criteria

(a) *Univariate*	*Grade II–IV cardiac complications*		*P-value*
	Positive (n = 23)	*Negative (n = 119)*	
Sex (male)	52.2%	69.7%	0.145
Age (>40 years)	39.1%	54.6%	0.254
Disease status (high)	60.9%	58.0%	0.113
History of cardiac disease	17.4%	7.6%	0.228
Smoking	21.7%	47.1%	0.036
Ferritin level (log(ferritin))	2.804	2.514	0.033
Cumulative dose of anthracyclines	350.8	175.5	0.001
History of radiation involving heart	0%	2.5%	0.999
Vital sign			
Heart rate (beats/min)	80.83	75.45	0.084
Systolic blood pressure (mmHg)	107.2	110.9	0.356
Diastolic blood pressure (mmHg)	67.7	68.5	0.720
ECG			
ECG abnormality	17.4%	13.4%	0.743
QT interval (ms)	386.3	379.9	0.447
QTc interval (ms)	444.6	424.7	0.105
QT dispersion (ms)	47.6	52.1	0.343
QTc dispersion (ms)	55.1	57.9	0.603
Echocardiography			
Echocardiographic EF (%)	61	64.6	0.070
Regimen			
Cyclophosphamide >100 mg/m^2	60.9%	78.2%	0.111
TBI	78.3%	64.7%	0.236
Alemtuzumab	34.8%	5.0%	0.0002
Stem cell			
Peripheral blood	56.5%	70.6%	0.225
Donor			
Unrelated donor	30.4%	42.0%	0.358
HLA matched (serological level)	39.1%	19.3%	0.054
HLA matched (genetic level)	47.8%	28.6%	0.087

(b) *Multivariate*	*Odds ratio (95% CI)*	*P-value*
Cumulative dose of anthracyclines (mg/m^2)	1.003 (1.001–1.005)	0.0016
Alemtuzumab	12.1 (3.3–44.1)	0.0001

cations. Patient selection and close monitoring of cardiac function are important during such transplants, especially in patients who have received a high cumulative dose of anthracyclines. Cardiac complications after alemtuzumab should be manageable by early detection and treatment.

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A Prospective Trial to Evaluate the Safety and Efficacy of Pravastatin for the Treatment of Refractory Chronic Graft-Versus-Host Disease

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This prospective study evaluates the safety and efficacy of pravastatin for the treatment of chronic graft-versus-host disease (GVHD). We included 18 patients with refractory chronic GVHD. Oral pravastatin was started at 10 mg/day, and the dose was increased up to 40 mg/day in 4 weeks. This maximum dose was administered over 8 weeks. There were no severe adverse events caused by pravastatin. A clinical response was observed in the skin score in two patients, mouth score in five patients, eye score in two patients, liver score in three patients, platelet count score in one patient, and weight loss in two patients. The overall response rate was 28%. Immunophenotypic analyses showed that T-helper (Th)1 cells were dominant in all but one patient before treatment and that the Th1/Th2 ratio tended to be lower in the responders than in the nonresponders. A randomized controlled trial is warranted to evaluate the efficacy of pravastatin against chronic GVHD.

Keywords: Chronic graft-versus-host disease, pravastatin, treatment.

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Chronic graft-versus-host disease (GVHD) is one of the major complications after allogeneic hematopoietic stem-cell transplantation and develops in 25% to 80% of allogeneic transplant recipients (*1–3*). Corticosteroids and cyclosporine are most widely used to treat chronic GVHD, but they have demonstrated limited efficacy.

Pravastatin is a lipid-lowering agent that inhibits 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Recently, the immunosuppressive effect of statins has been highlighted in both clinical and laboratory studies. Pravastatin reduced the incidence of graft rejection after cardiac and kidney transplantation (*4, 5*). Statins also prevented islet allograft rejection in a mouse model (*6*). Two distinct molecular mechanisms of the immunosuppressive effect of statins have recently been proposed. First, statins suppress the induction of major histocompatibility complex-II expression by interferon-gamma on human endothelial cells and macrophages (*7*). Second, statins selectively inhibit the molecular association between leukocyte function antigen-1 and intercellular adhesion molecule-1 (*8*). With these data, we performed a prospective clinical trial to evaluate the safety and efficacy of pravastatin for the treatment of chronic GVHD.

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Patients aged between 20 and 70 years who had refractory pathologically proven chronic GVHD were eligible for the study. Refractory chronic GVHD was defined as chronic GVHD that was not improved by first-line treatment with corticosteroids at more than 0.5 mg/kg or cyclosporine at a therapeutic blood level for at least 2 weeks, or that showed progression during the tapering of first-line treatment. Patients had to demonstrate good hepatic and renal function as defined by serum bilirubin less than 85.5 μmol/L (5 mg/dL), alanine aminotransferase less than 500 IU/L, and serum creatinine less than 176.8 μmol/L (2.0 mg/dL). Patients with myopathy or who were receiving fibrates were excluded to avoid rhabdomyolysis. All of the patients provided their written informed consent. This study was approved by the institutional review board at each participating institution.

Pravastatin was started orally at 10 mg/day. The dose was increased to 20 mg/day after 2 weeks and finally to 40 mg/day after 2 additional weeks with close monitoring for adverse events. The maximum dose was continued over 8 weeks, unless grade 3 or 4 adverse events attributable to pravastatin were observed. Immunosuppressive agents that were being taken at study entry were continued at the same dose. However, once the dose of these immunosuppressive agents was increased or other immunosuppressive agents were added, the patient was withdrawn from the study and considered a nonresponder.

The incidences and severity of adverse events attributable to pravastatin were evaluated according to the National Cancer Institute Common Toxicity Criteria, Version 2.0. To evaluate the efficacy of pravastatin, chronic GVHD was graded at study entry according to Akpek's prognostic model (*9*). Response was evaluated every 2 weeks for 12 weeks after the initiation of treatment as an intent-to-treat basis. Response in individual organs was defined as follows: A marked response was a change from Akpek's code 2 or 3 to code 1, a

good response was a change from code 3 to code 2, and no response was no change in code or progression. An overall response to treatment was defined as a marked or good response in at least one organ, without progression in any other organs. We planned to include 18 patients with target and lower response rates of 40% and 10% and alpha and beta errors of 5% and 10%, respectively.

The trough blood concentrations of cyclosporine or tacrolimus and the peak plasma concentration of pravastatin were measured every 2 weeks to evaluate interaction between pravastatin and these immunosuppressants. Immunologic changes were evaluated at weeks 2, 4, 8, and 12 by quantification of the CD4/CD8 ratio, the T-helper (Th)1/Th2 ratio, and the expression of human leukocyte antigen-DR on T cells, B cells, and monocytes. Immunologic data were compared between responders and nonresponders using a repeated measures analysis of variance after logarithmic transformation.

Eighteen patients with a median age of 44 years (range 20–68 years) were included in the study. There were 14 men and 4 women. The underlying disease was acute myeloblastic leukemia in seven, chronic myeloid leukemia in four, non-Hodgkin's lymphoma in three, acute lymphoblastic leukemia in two, myelodysplastic syndrome in one, and aplastic anemia in one. Thirteen and five patients received grafts from a related or an unrelated donor, respectively. Ten of them demonstrated chronic GVHD of progressive onset. All patients but one demonstrated extensive chronic GVHD before starting pravastatin, and nine patients were receiving prednisolone. The grade of chronic GVHD at study entry according to Akpek's prognostic model is shown in Table 1. Seven patients, 10 patients, and 1 patient were grouped into the low-, intermediate-, and high-risk groups, respectively.

Pravastatin was well tolerated, and no patients developed grade 3 or 4 adverse events attributable to pravastatin. Treatment was discontinued in three patients between 14 and 41 days after starting pravastatin because of unrelated causes, including painful oral chronic GVHD, infection, and interstitial pneumonitis. According to each organ, a response was observed in the skin score in two patients, mouth score in five patients, eye score in two patients, liver score in three patients, and platelet count score in one patient (Table 1). An overall response was seen in five patients (28%). Pravastatin did not act through the interaction with cyclosporine or tacrolimus, because an increase in these blood levels was not observed after the administration of pravastatin (data not shown). The serum pravastatin concentration on day 42 was not different between responders and nonresponders (median 157.5 ng/mL vs. 253.1 ng/mL, $P=0.53$). The serum total cholesterol level significantly decreased from 6.37 mmol/L (standard deviation [SD] 1.79) before treatment to 5.67 mmol/L (SD 1.40, $P=0.0095$) and 4.77 mmol/L (SD 1.99, $P=0.0001$) on days 14 and 84 after starting pravastatin, respectively. The initial cholesterol response (ratio between cholesterol level on day 14 and before treatment) was significantly better in GVHD responders (0.78 vs. 0.95, $P=0.029$).

The Th1/Th2 ratio before the administration of pravastatin was greater than 1.0 in all but one patient. The Th1/Th2 ratio at study entry tended to be lower in responders than in nonresponders and became even lower after pravastatin treatment in responders, but not in nonresponders, although these differences were not statistically significant (Fig. 1, $P=0.22$). The CD4/CD8 ratio and the expression of human leukocyte antigen-DR on T cells, B cells, and monocytes did not change after treatment (data not shown).

This study demonstrated that pravastatin at 40 mg/day can be safely administered in patients with refractory chronic GVHD, including those taking cyclosporine. The overall response of 28% was similar to that with other alternative salvage treatments including tacrolimus, mycophenolate mofetil, thalidomide, and so on (*3*). However, considering the safety profile of pravastatin, it may be worthwhile for patients with chronic GVHD, especially in those with a coexisting infection that precludes severely immunosuppressive treatments. We chose pravastatin among many statins because it is hydrophilic and was considered to be less likely to cause rhabdomyolysis than other lipophilic statins (*10*, *11*). However, atorvastatin, lovastatin, and simvastatin have stronger in vitro immunosuppressive effects than pravastatin, and thus they may also have greater in vivo effects against chronic GVHD (*7*, *8*).

There is some controversy whether human chronic GVHD is a Th1 or Th2 disease. The immunophenotypic analyses in this study clearly showed that Th1 cells were dominant in patients with chronic GVHD. The efficacy of statin against rheumatoid arthritis, a Th1 disease, has been demonstrated clinically (*12*). In a mouse model of chronic and relapsing

TABLE 1. Severity of chronic graft-versus-host disease in each organ and the response to pravastatin

Each organ	Severity code before treatment				Response to treatment				
	1	2	3	NE	Marked	Good	NC	PD	NE
Performance status	16	2	0	0	0	0	18	0	0
Skin and fascia	6	6	4	2	1	1	13	2	1
Mouth	5	11	2	0	3	2	12	1	0
Eye	7	8	3	0	2	0	13	2	1
Liver enzyme	4	5	9	0	2	1	13	2	0
Thrombocytopenia	14	1	3	0	0	1	15	2	0

Overall response	Responder	5 (28%)
	Nonresponder	13

NE, not evaluable; NC, no change; PD, progressive disease.

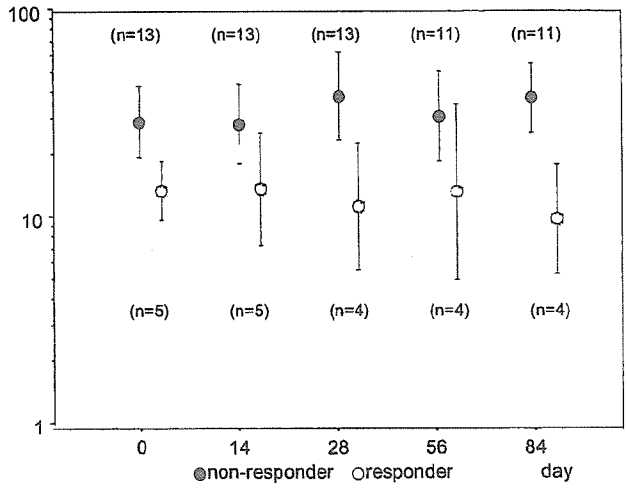

FIGURE 1. Serial changes in the T-helper (Th)1/Th2 ratio in responders and nonresponders. Data are shown as geometric mean and standard error.

experimental autoimmune encephalomyelitis, oral atorvastatin promoted a Th2 bias and reversed paralysis through the inhibition of STAT4 phosphorylation and the induction of STAT6 phosphorylation (*13*). Although we did not find a statistically significant association between the Th1/Th2 ratio and the response to pravastatin, pravastatin might have ameliorated chronic GVHD by inducing a Th2 shift.

In conclusion, our experience suggests that pravastatin may be safe and effective for the treatment of refractory chronic GVHD. However, a double-blind, randomized, controlled trial is needed to evaluate its true efficacy against refractory chronic GVHD.

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Graft-versus-host disease

Increased incidence of acute graft-versus-host disease with the continuous infusion of cyclosporine A compared to twice-daily infusion

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Summary:

We retrospectively compared the incidence of acute graft-versus-host disease (GVHD) before and after September 1999, when we changed the mode of cyclosporine A (CsA) administration from twice-daily infusions (TD) ($n = 58$) to continuous infusion (CIF) ($n = 71$). The incidence of grade II–IV acute GVHD in the CIF group (56%) was significantly higher than that in the TD group (27%, $P = 0.00022$). Multivariate analysis identified only two independent significant risk factors for the development of grade II–IV acute GVHD; CIF of CsA (relative risk 2.59, 95% CI 1.46–4.60, $P = 0.0011$) and the presence of HLA mismatch (2.01, 95% CI 1.15–3.53, $P = 0.014$). The incidence of relapse was significantly lower in the CIF group when adjusted for disease status before transplantation (0.41, 95% CI 0.18–0.95, $P = 0.038$), which resulted in better disease-free survival in high-risk patients (43 *vs* 16% at 2 years, $P = 0.039$), but not in standard-risk patients (72 *vs* 80%, $P = 0.45$). CIF of CsA with a target level of 250–400 ng/ml may not be appropriate for GVHD prophylaxis in standard-risk patients.

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Keywords: hematopoietic stem cell transplantation; cyclosporine A; graft-versus-host disease; continuous infusion

Cyclosporine A (CsA) is a mainstay of treatment in the pharmacologic prevention of graft-versus-host disease (GVHD), and is usually combined with methotrexate (MTX). However, the dose, target blood level, and schedule of administration vary among protocols.[1] In particular, it has not been assessed whether CsA should be administered as a continuous infusion (CIF) or as twice-daily infusions (TD) in the early period after transplantation when patients cannot tolerate an oral intake. In September 1999, we changed the mode of CsA administration from TD to CIF, without major changes to other transplantation procedures. The aim of this study was to evaluate the impact of these two different modes of administration on the incidence of acute GVHD.

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Patients and methods

Patients

We retrospectively analyzed the records of adult patients who underwent allogeneic hematopoietic stem cell transplantation for the first time between June 1995 and May 2000 using a GVHD prophylaxis regimen consisting of CsA and MTX. During that time, this combination was the standard regimen for GVHD prophylaxis in our center, but CsA alone was used for patients who were at a very high risk for relapse, and a combination of tacrolimus and MTX was used for patients who had received a graft from an unrelated donor with at least one allele or antigen mismatch. Those who received a T-cell-depleted graft and those who received a reduced-intensity conditioning regimen or a conditioning regimen that included ATG or CAMPATH1-H were excluded. Otherwise, consecutive patients were included in the study. The data for 129 patients were analyzed. There were 95 males and 34 females with a median age of 38 years (range 18–60). Bone marrow (BM) was exclusively used in unrelated transplants, whereas 13 related donors chose a collection of G-CSF-mobilized peripheral blood stem cells (PBSC) rather than a BM harvest. BM was additionally harvested from poor mobilizers.

Transplantation procedure

Conditioning 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). GVHD prophylaxis was with CsA and short-term MTX (10–15 mg/m^2 on day 1 and 7–10 mg/m^2 on days 3 and 6, and optionally on day 11), with a starting dose of CsA of 3 mg/kg/day. Before September 1999, CsA was administered as a 4 h infusion twice daily in equally divided doses. The dose of CsA was adjusted to maintain the trough blood CsA concentration between 150