Table 3. Univariate and Multivariate Analyses of Risk Factors for Grade III-IV Acute GVHD

	Univariate Analysis			Multivariate Analysis		
Covariate	HR	95% CI	*P* value	HR	95% CI	*P* value
Recipient BMI						
18 ≤ BMI < 25 kg/m^2	1.00		.086*	1.00		.19*
BMI < 18 kg/m^2	0.99	0.73 to 1.35		1.01	0.72 to 1.43	
25 ≤ BMI < 30 kg/m^2	1.16	0.94 to 1.45		1.16	0.79 to 1.72	
30 ≤ BMI kg/m^2	1.56	0.90 to 2.71		1.42	0.70 to 2.87	
Recipient age, years						
< 30	1.00		.97*			
30 ≤ age <50	0.98	0.81 to 1.19				
≥ 50	1.00	0.79 to 1.25				
Donor age, years						
< 40	1.00		< .0001	1.00		< .0001
≥ 40	1.52	1.28 to 1.79		1.53	1.27 to 1.84	
Sex, donor/recipient						
Match	1.00		.057	1.00		.15
Male/female	1.21	0.99 to 1.49		1.13	0.90 to 1.42	
Female/male	1.23	1.00 to 1.51		1.24	0.99 to 1.56	
TNC (× 10^{-8}/kg)						
TNC < 3.0	1.00		.56*			
3.0 < TNC < 5.0	0.90	0.71 to 1.15				
5.0 < TNC	0.91	0.72 to 1.16				
Diagnosis						
Acute leukemia	1.00		.66			
Chronic leukemia	1.13	0.90 to 1.41				
MDS/MPD	0.99	0.78 to 1.24				
ML	0.98	0.77 to 1.26				
MM	0.71	0.35 to 1.43				
Blood type disparity						
M	1.00		.55			
IA	1.04	0.67 to 1.61				
MA	1.11	0.91 to 1.13				
MI	1.15	0.94 to1.40				
HLA disparity						
HLA allele match	1.00		.0002	1.00		< .0001
HLA 1 allele mismatch	1.36	1.13 to 1.64		1.43	1.18 to 1.74	
HLA 2 allele mismatch	1.57	1.10 to 2.24		1.57	1.07 to 2.30	
HLA 3 allele mismatch	1.49	0.48 to 4.66		1.47	0.47 to 4.60	
Conditioning regimen						
TBI for conditioning						
No	1.00		.49			
Yes	0.94	0.78 to 1.13				
Intensity of conditioning regimen						
Conventional	1.00		.29			
Reduced-intensity	1.11	0.91 to 1.36				
ATG for conditioning						
No	1.00		.26			
Yes	0.80	0.55 to 1.18				
GVHD prophylaxis						
CSP-based	1.00		.029	1.00		.02
TAC-based	0.93	0.79 to 1.10		0.81	0.67 to 0.97	
Others	1.78	1.09 to 2.91		1.27	0.71 to 2.28	
Comorbidity						
Liver dysfunction						
No	1.00		.95			
Yes	0.99	0.78 to 1.27				
Renal dysfunction						
No	1.00		.20			
Yes	1.32	0.87 to 1.99				
Heart dysfunction						
No	1.00		.26			
Yes	0.82	0.57 to 1.16				
Lung dysfunction						
No	1.00		.11	1.00		.20
Yes	1.39	0.93 to 2.07		1.36	0.86 to 2.16	

*The log-rank trend test was used for calculating P values.

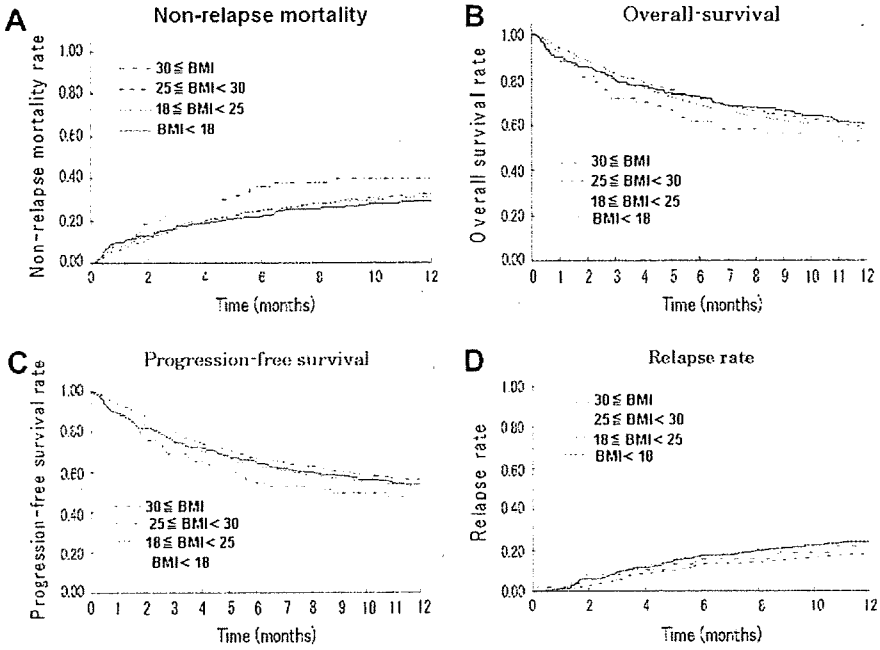

Figure 2. Probability of nonrelapse mortality (A), progression-free survival (B), overall survival (C), and relapse/progression (D).

weight (odds ratio = 1.9; 95% confidence interval [CI] = 1.1 to 3.2; $P = .02$). The incidence of liver dysfunction, including sinusoidal occlusive syndrome, was 19% in the low-BMI group, 20% in the normal-BMI group, 21% in the overweight group, and 25% in the obesity group; the differences were not statistically significant. The incidence of interstitial pneumonia, excluding obvious infectious diseases such as cytomegalovirus or *Pneumocystis jirovecii* pneumonia, was 13% in the low-BMI group, 13% in the normal-BMI group, 12% in the overweight group, and 15% in the obesity group; again, the differences are not statistically significant. The causes of death are given in Table 4. More infections and GVHD-related deaths were seen in the obesity group. If only early mortality is considered, then the nonrelapse mortality within 100 days was 17% in the low-BMI group, 18% in the normal-BMI group, 17% in the overweight group, and 25% in the obesity group. Obesity tended to be associated with greater early nonrelapse mortality, but this difference was not statistically significant ($P = .83$). The incidence of infection-related mortality within 100 days was 5%, 5%, 4% and 8%, respectively, in the 4 groups. Bacterial infection was the main cause of infection-related mortality, with 6 cases (40%) in the low-BMI group, 91 cases (67%) in the normal-BMI group, 17 cases (74%) in the overweight group, and 3 cases (60%) in the obesity group.

To investigate whether pretransplantation BMI had an additional impact on outcome in the patients who developed acute GVHD, we stratified the patients according to the grade of acute GVHD and analyzed the association between pretransplantation BMI and early nonrelapse mortality. We found that pretransplantation BMI had no additional impact on early nonrelapse mortality.

DISCUSSION

Both obesity and malnutrition are considered risk factors for complications, especially infectious diseases. To elucidate the impact of pretransplantation BMI on the clinical outcome, in this study we retrospectively reviewed the data of patients who underwent unrelated BMT, stratified according to recipient BMI, and found results similar to those reported previously [1,2]. The present study has an obvious limitation,

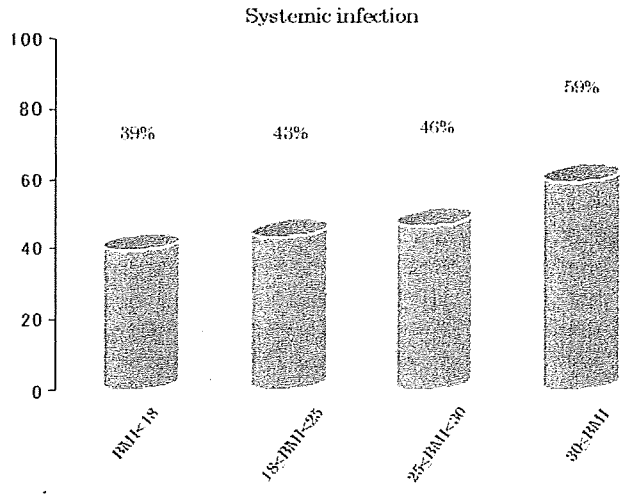

Figure 3. Incidence of systemic infections.

Table 4. Causes of Death

	BMI < 18 kg/m^2	18 ≤ BMI < 25 kg/m^2	25 ≤ BMI < 30 kg/m^2	30 kg/m^2 ≤ BMI
	(n = 295)	(n = 2906)	(n = 565)	(n = 61)
Relapse, n (%)	66 (22%)	609 (21%)	95 (17%)	8 (13%)
Nonrelapse mortality, (%)	97 (33%)	988 (34%)	206 (36%)	24 (39%)
Infection, n (%)	25 (8%)	276 (9%)	56 (10%)	8 (13%)
Bacterial, n	13	151	34	4
Fungal, n	3	37	7	1
Viral, n	4	39	5	1
Mixed, n	2	16	3	1
Others, n	3	33	6	1
Acute GVHD, n (%)	9 (3%)	78 (3%)	26 (5%)	4 (7%)
Chronic GVHD, n (%)	4 (1%)	45 (2%)	13 (2%)	2 (3%)
Graft failure, n (%)	2 (1%)	31 (1%)	6 (1%)	1 (2%)
Organ dysfunction, n (%)	47 (16%)	395 (14%)	83 (15%)	7 (11%)
Others, n (%)	10 (3%)	163 (6%)	22 (4%)	2 (3%)

lacking concise data regarding weight-based dose adjustment of chemotherapy, which is critical for analyzing the incidence of organ dysfunction. Dosing schemes for preparative chemotherapy regimens vary widely among transplantation centers. In addition, centers differ in their use of ideal body weight, actual body weight, and compensatory calculations that yield doses between the actual and ideal weights [19,20]. Another limitation of this study is that low prevalence of obesity in Japan makes the study's statistical power less reliable. For example, patients with morbid obesity (BMI > 35 kg/m^2), considered a significant comorbidity in a hematopoietic cell transplantation-specific comorbidity index, are quite rare in Japan [21]. Similar analyses need to be performed in Western countries to clarify the impact of obesity, especially morbid obesity, after allogeneic HSCT.

Our findings demonstrate that obesity is associated with an increased risk of infectious disease compared with normal weight. Hyperglycemia, caused primarily by insulin resistance in obesity, can lead to increased incidence of infectious disease. As reported by Sheean et al. [22], hyperglycemia after HSCT may be a risk factor for infectious disease. Recently, Derr et al. [23] reported an association between hyperglycemia before a neutropenic period and increased risk of infectious diseases during a neutropenic period after HSCT. In our study, an increased incidence of acute GVHD was associated with an increased risk of infectious disease. On the other hand, low BMI, which suggests the presence of malnutrition, was not associated with an increased risk of infectious diseases or transplantation-related mortality, inconsistent with previous reports [10,11]. This could be because the incidence of acute GVHD was lower and the dose of TNC per body weight was higher in the low-BMI group. Even if we further divide the BMI < 18 kg/m^2 group into 3 subgroups (BMI < 16 kg/m^2, 16 ≤ BMI < 17 kg/m^2, and 17 ≤ BMI < 18 kg/m^2), we find no differences in the incidence of acute GVHD or infectious disease, or in clinical outcomes (data not shown). It is possible that in the Japanese population, BMI < 18 kg/m^2 may not directly reflect a malnutritional status.

Importantly, our findings also suggest an association between increased BMI and a significantly increased incidence of acute GVHD grade II-IV. This observation is based on multiple factors, and no single clear scientific explanation for it exists, but several mechanisms can be hypothesized. First, the dose of the conditioning regimen and GVHD prophylaxis could be improperly adjusted in obese patients, possibly leading to increased tissue damage or poorer GVHD prophylaxis and, ultimately, a higher incidence of acute GVHD. With regard to the conditioning regimen, the relapse rate was lower in the overweight and obese patients compared with the low-BMI and normal-BMI patients, but the incidence of regimen-related toxicity (ie, liver dysfunction and interstitial pneumonitis) did not differ significantly among these groups. With regard to GVHD prophylaxis, there might not have been any significant difference in drug exposure, because dose adjustment of the calcineurin inhibitor usually is done through serial monitoring of drug concentration. Second, the stem cell dose could influence the incidence of acute GVHD. But in this study, the stem cell dose was analyzed independently, and no association was found between stem cell dose and the incidence of acute GVHD. Third, there was an obvious selection bias in each group. For example, it is possible that obese patients may be less likely to find an unrelated donor with an adequate dose of cells for transplantation. While the donor search continued, the number of chemotherapy courses could increase, and the patient's general condition (including disease status and organ function) could become worse. Finally, even though there were no direct data regarding glucose levels in this study, obesity is likely associated with hyperglycemia [7-9], possibly resulting in elevated levels of several cytokines [24-27], inducing a vicious cycle [28-30]. Our group previously reported an association

between hyperglycemia during neutropenia and the development of acute GVHD [6], possibly due to the augmented production of cytokines stimulated by the conditioning regimen. Furthermore, recently it has become clear that adipocytokines, which are secreted mainly from adipocytes, play important roles in the control of immunity [31-33]. In particular, the level of leptin has been found to be proportional to body fat weight and to affect T regulatory cell (Treg) proliferation and function [34,35]. Thus, it could be hypothesized that in obese patients, a higher leptin level suppresses Treg activity, increasing the risk of acute GVHD. These mechanisms are based on the results of animal models, however, and await confirmation in human studies.

The clinical significance of our findings merits careful consideration, because pretransplantation BMI is one of the few factors that can be properly managed and corrected during the unstable, fast-moving pretransplantation period. On the other hand, malnutrition can be corrected by appropriate nutritional support, and obesity can be controlled through an appropriate diet and exercise program during chemotherapy. This study suggests that such a pretransplantation nutritional support program can improve clinical outcomes after allogeneic BMT.

In conclusion, this retrospective analysis of registration data found an association between pretransplantation obesity and increased risk of infectious disease, possibly leading to increased risk of mortality. Although body weight is affected by multiple clinical factors, the effect of obesity on clinical outcome, as suggested here, needs to be confirmed by a prospective study to identify better patient management approaches.

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ORIGINAL ARTICLE

A randomized controlled trial of plasma real-time PCR and antigenemia assay for monitoring CMV infection after unrelated BMT

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Preemptive therapy is the standard strategy for preventing CMV disease after allogeneic hematopoietic SCT. In this study, unrelated BMT recipients were randomly assigned to a plasma real-time PCR group or an antigenemia group to compare the value of these monitoring tools for CMV reactivation. Ganciclovir (GCV) was started at 5 mg/kg/day when PCR reached 300 copies per ml or when antigenemia reached three positive cells per two slides. A total of 88 patients were randomized into the antigenemia group ($n=45$) or the PCR group ($n=43$). A significantly higher number of patients reached the threshold in the antigenemia group than in the PCR group (73.3 vs 44.2%, $P=0.0089$). However, only three patients (one in the antigenemia group and two in the PCR group) developed early CMV disease. These patients exclusively had colitis and were successfully treated with GCV or foscarnet. The median number of antigenemia-positive cells at the start of GCV was 47 in the PCR group. These findings suggest that antigenemia assay with the current cutoff was too sensitive and led to unnecessary use of GCV. However, the appropriateness of the threshold may be different by the methodology used, and therefore, it is difficult to generalize.

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Keywords: CMV; antigenemia; real-time PCR; preemptive therapy

Introduction

Cytomegalovirus infection is a frequent complication after allogeneic hematopoietic SCT. Universal prophylaxis with ganciclovir (GCV) did not improve the transplantation outcome because of neutropenia caused by GCV.[1,2] Therefore, the initiation of GCV triggered by the detection of CMV reactivation is currently the standard strategy for preventing CMV disease.[3–5] A CMV antigenemia assay has been widely used to monitor CMV reactivation. However, the details of preemptive therapy still need to be clarified, including the threshold number of antigenemia-positive cells for deciding when to start GCV, the dose and duration of GCV and so on. We previously showed that a risk-adapted preemptive therapy, in which the cutoff number of antigenemia-positive cells for deciding when to start GCV was changed according to the risk for CMV disease, was appropriate in allogeneic SCT recipients, but the incidence of neutropenia was still high.[6] Therefore, in the next study, we evaluated the feasibility of preemptive therapy with low-dose GCV, and the findings showed that the initial dose of GCV could be safely decreased to 5 mg/kg.[7]

The PCR used to detect CMV DNA has also been investigated for its ability to monitor CMV reactivation.[8] PCR using whole blood samples might be too sensitive as a trigger for deciding when to start preemptive therapy compared with an antigenemia assay or PCR using plasma samples.[9,10] However, the recent development of real-time PCR has enabled the quantification of CMV DNA. Several studies have shown the feasibility of preemptive therapy guided by real-time PCR monitoring using either whole blood or plasma samples.[11–14] As for whole blood real-time PCR, Gerna *et al.* performed two randomized controlled trials of PCR and antigenemia, one in young patients (0–25 years old) and the other in older patients (20–67 years old).[12,13] They showed that a threshold value of 10 000

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copies per ml for determining when to start GCV by whole blood PCR significantly reduced the use of GCV compared with a threshold in which GCV is started at any level of positive antigenemia. However, the study included heterogeneous patients in terms of donor type, stem cell source and GVHD prophylaxis. In particular, antithymocyte globulin was used in approximately half of the patients, and this may have strongly affected the incidence of CMV reactivation and disease.[15,16] In addition, preemptive therapy guided by antigenemia assay could be more appropriately performed by using a cutoff based on the number of positive cells.

Therefore, we performed a randomized controlled trial of plasma real-time PCR with a cutoff of 300 copies per ml and an antigenemia assay with a cutoff of three positive cells per two slides in a homogenous population of unrelated BMT recipients who received GVHD prophylaxis with a calcineurin inhibitor and MTX.

Patients and methods

Patients

Patients were eligible for the study if they were between 20 and 55 years old, would undergo BMT without *in vivo* or *ex vivo* T-cell depletion from an HLA-matched unrelated donor using a myeloablative conditioning regimen and had a good performance status without significant organ dysfunction, as defined in the protocol. Either the donor, the recipient or both must have been seropositive for CMV. Prophylaxis against GVHD was limited to a combination of CYA and MTX, but a combination of tacrolimus and MTX was allowed after June 2002. Patients were enrolled before starting a conditioning regimen, but randomization was performed between day 10 and day 12 after transplantation to exclude patients who developed significant organ dysfunction early after transplantation. This study was approved by the institutional review board of each participating center and a written informed consent was obtained from each patient (UMIN-CTR C000000347).

CMV monitoring methods

Cytomegalovirus antigenemia assay was performed as described previously.[17] 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 MoAb C10/11 (Clonab CMV; Biotest, Dreieich, Germany) and reacted with goat alkaline phosphatase-labeled anti-mouse Ig (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan). Under a light microscopy, CMV-positive cells were counted and the results are presented as the sum of the number of positive cells per two slides.

Real-time PCR was performed using primers and a TaqMan probe for immediate early genes using serum samples.[18] Briefly, DNA extracted from 100 μl of plasma was subjected to PCR using TaqMan Universal PCR Master Mix (PE Biosystems, Foster City, CA, USA) and the PCR product was detected as an increase in the fluorescent intensity using ABI Prism 7700 (PE Biosystems). Real-time fluorescent measurements were taken and a threshold cycle (CT) value for each sample was calculated by determining the point at which the fluorescence exceeded 10 times the baseline fluorescence. A standard curve was constructed using the CT values obtained from serially diluted DNA extracted from a plasmid that contains the respective region of CMV. The CT values from the clinical samples were plotted on the standard curve and the copy number was calculated automatically using Sequence Detection System version 1.6 (PE Biosystems).

Preemptive therapy against CMV disease

Patients were randomly assigned to the antigenemia group or the PCR group using a random block design. Assignment was stratified by the institute, age and the presence or absence of GVHD at the time of randomization. CMV reactivation was monitored weekly by both the antigenemia assay and PCR in all patients, but only the results of the assigned monitoring method were returned to the physicians. Preemptive therapy with GCV was started at an induction dose of 5 mg/kg/day when three or more CMV-positive cells per two slides were detected in the antigenemia group and 300 or more CMV DNA copies per ml were detected in the PCR group. The dose of GCV was increased to 10 mg/kg/day when a rising CMV load was observed. The dose of GCV was decreased to 5 mg/kg/day when a declining CMV load was observed in patients who were receiving GCV at 10 mg/kg/day. A rising and declining CMV load was defined as an increase and decrease in the CMV load by 50% or more of the previous value, respectively. However, changes in antigenemia-positive cells by less than five cells per two slides and changes in the DNA copy number by less than 500 copies per ml were regarded as a stable CMV load. When the CMV load fell below the threshold to start GCV, the dose of GCV was decreased to 5 mg/kg/day, if the patient was receiving GCV at 10 mg/kg/day, and GCV was discontinued if the patient was receiving GCV at 5 mg/kg/day. The dose of GCV was adjusted according to the renal function.[19] CMV monitoring was continued until all of the following three requirements were fulfilled: (i) More than 100 days had passed after transplantation; (ii) More than 2 weeks had passed after the last administration of GCV; and (iii) Absence of the use of (methyl-)prednisolone at 0.5 mg/kg/day or more.[20]

Definition of CMV disease

All patients with symptoms compatible with CMV disease such as interstitial pneumonia, colitis and gastritis underwent extensive pathological and microbiological examination of biopsy specimens. The diagnosis of CMV disease was made by histopathological examination and immunochemical staining of biopsy specimens. However, CMV retinitis was diagnosed when CMV DNA was detected by PCR using aqueous humor samples associated with characteristic retinal changes by ophthalmoscopy. Early and late CMV diseases were defined as those occurring before and after day 100, respectively.

Statistical considerations

The primary end point of the study was the incidence of early CMV disease. We defined success as the absence of CMV disease before day 100. Noninferiority was predefined as a difference in the success rates between the antigenemia group and the PCR group of no more than 10 percentage points. On the basis of the assumption of a success rate of 95% in the PCR group and 90% in the antigenemia group, 39 patients in each treatment group were required to show noninferiority with an alpha error of 5% and a power of 80%, which permitted a 10% difference in the success rate. On the basis of the assumption of a 20% loss of patients between the enrollment and randomization, a total of 96 patients needed to be enrolled in this study. Comparisons for dichotomous and continuous variables between groups were performed with Fisher's exact test and *t*-test, respectively. Pearson's correlation coefficient was calculated to compare the results of the two monitoring methods after logarithmic transformation.

Results

Incidence of CMV reactivation and the use of GCV

A total of 96 patients were enrolled in the study between January 2002 and March 2007. Among these patients, eight patients were excluded because of the use of tacrolimus as GVHD prophylaxis in one, negative CMV Ab in both the donor and recipient in one and organ dysfunction after the conditioning regimen in six. Therefore, a total of 88 patients were randomized into the antigenemia group ($n=45$) or the PCR group ($n=43$) (Figure 1). There were no differences in age, sex, background disease, CMV serostatus, conditioning regimen or GVHD prophylaxis between the two groups (Table 1). In addition, the incidence of grade II–IV acute GVHD was similar (42 vs 47%, $P=0.67$).

Cytomegalovirus reactivation, defined as a detection of CMV at any level, was more frequently observed in the antigenemia group (40 of 45 patients, 88.9%) than in the

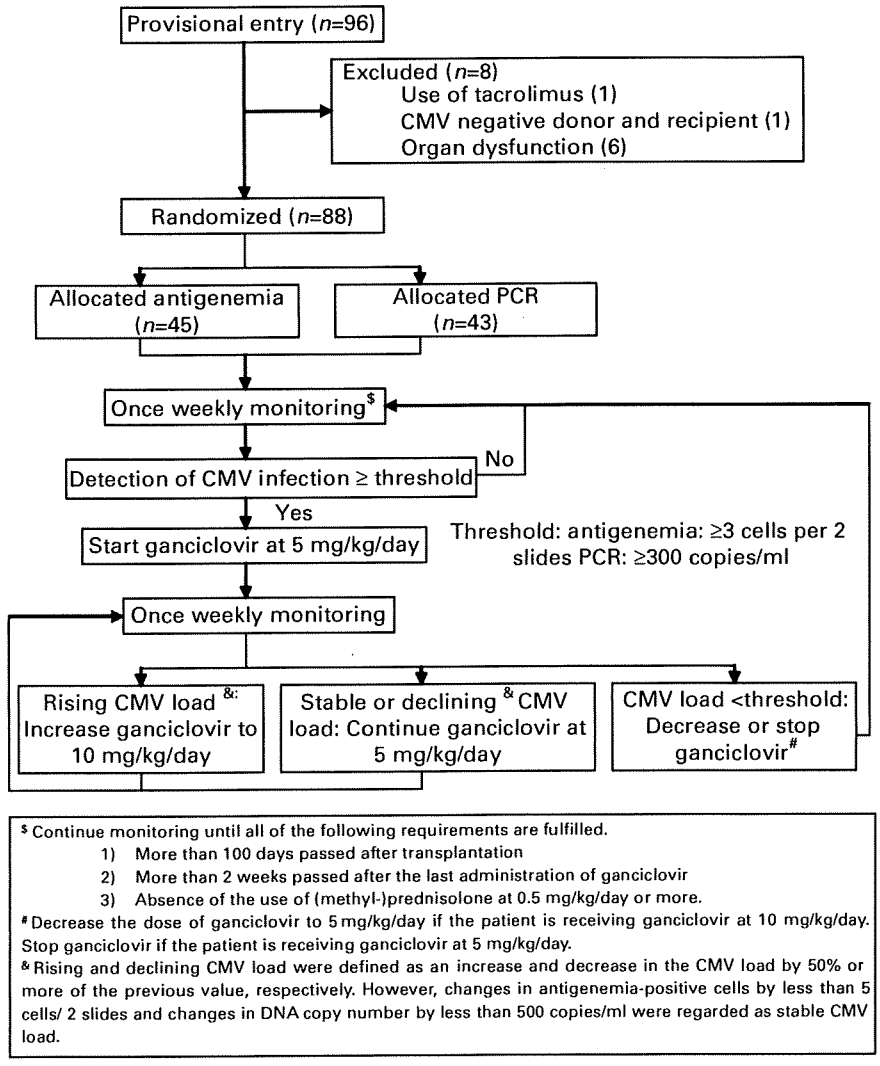

Figure 1 Design of the study.

PCR group (27 of 43 patients, 62.8%) ($P=0.0050$, Table 2). The probability of starting GCV was significantly higher in the antigenemia group than in the PCR group (73.3 vs 44.2%, $P=0.0089$, Figure 2). The results of PCR in the antigenemia group and those of the antigenemia assay in the PCR group were disclosed after the completion of the study. A good correlation was seen between the results of PCR and the antigenemia assay ($P<0.0001$, $r^2=0.38$, Figure 3). Of the 33 patients who received GCV in the antigenemia group, PCR and the antigenemia assay reached the threshold simultaneously in five patients and PCR reached the threshold before starting GCV in only four patients (Figures 4a and 5a). In the other 24 patients, the CMV DNA copy number was persistently below the threshold until GCV was started. On the other hand, in 11 of 19 patients who received GCV in the PCR group, the results of the antigenemia assay reached the threshold earlier in 11 patients and simultaneously in 7 patients (Figures 4b and 5b). The results of the antigenemia assay were persistently below the threshold until GCV was started in only one patient. The median number of antigenemia-positive cells at the start of GCV was 5 (range: 3–102) and 47 (range: 0–2921) in the antigenemia and PCR groups, respectively (Figure 6a, $P=0.0051$). The median CMV DNA copy number was negative (range: 0–4400) and 750 (range: 310–13000) in the antigenemia and PCR groups, respectively (Figure 6b, $P<0.0001$).

Among the 52 patients who received preemptive therapy with GCV at 5 mg/kg/day, only 13 and 7 patients in the antigenemia and PCR groups, respectively, experienced a rising CMV load and required dose-escalation to 10 mg/kg/day, suggesting that the initiation of GCV at 5 mg/kg was appropriate.

Table 1 Patient characteristics

	Antigenemia (n=45)	*PCR (n=43)*	P-*value*
Pre-transplantation factors			
Median age (range)	41 (20–55)	40 (20–53)	0.82
Sex (male/female)	25/20	24/19	>0.99
HLA mismatch	7 (16%)	9 (21%)	0.59
Background disease			
AML	17	18	
ALL	12	12	
CML	6	3	
MDS	5	7	
Others	5	3	0.57
Donor/recipient CMV status			
Pos./Pos.	28	26	
Pos./Neg.	5	4	
Neg./Pos.	8	6	0.74
Conditioning regimen			
TBI	39	36	
Non-TBI	6	7	0.77
GVHD prophylaxis			
CYA–MTX	25	25	
TAC–MTX	16	16	0.59

Abbreviations: MDS = myelodysplastic syndrome; Neg. = negative; Pos. = positive; TAC = tacrolimus.

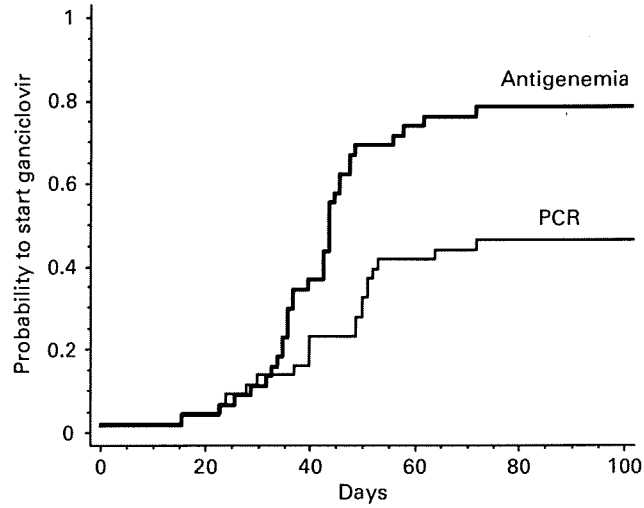

Figure 2 Days to start ganciclovir after transplantation.

Table 2 CMV-related events after engraftment

	Antigenemia (n=45)	*PCR (n=43)*	P-*value*
CMV reactivation[a]	40	27	0.0050
Start ganciclovir	33	19	0.0089
Duration of ganciclovir (days)	23.2 ± 19.4	20.8 ± 14.2	0.64
Total dose of ganciclovir (mg/kg)	140.8 ± 129.7	118.4 ± 91.2	0.51
Dose escalation to level II	13	7	>0.99
Neutropenia <500 per μl	5	3	>0.99
Stop ganciclovir because of neutropenia	1	0	>0.99
Increase in serum creatinine[b]	8	0	0.039
CMV disease			
Early (before day 100)	1	2	0.61
Late (after day 100)	0	1[c]	0.48

[a]Detection of antigenemia or DNA at any level.
[b]Increase in serum creatinine level by 0.5 mg per 100 ml or more from the baseline level.
[c]The patient developed early CMV disease, which was improved by ganciclovir. However, intestinal symptoms recurred after day 100 and CMV colitis was suspected because of positive antigenemia, although it was not confirmed by biopsy.

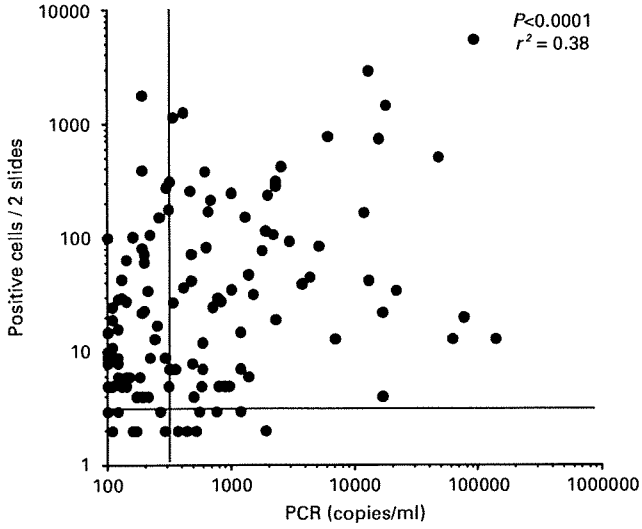

Figure 3 Correlation between the number of positive cells in the antigenemia assay and copy number by PCR.

CMV diseases

Early CMV disease was diagnosed in 1 of the 45 patients (2.2%) in the antigenemia group and 2 of the 43 patients (4.7%) in the PCR group ($P=0.61$). These patients exclusively developed CMV colitis. Another patient in the PCR group showed characteristic retinal changes and was presumptively treated with GCV, although CMV infection was not detected in either the aqueous humor or the peripheral blood. The 95% confidence interval for the difference in the success rate was −10.1 to 5.2%, and thus was just outside the predefined lower limit of −10%. However, as shown in Table 3, the development of CMV disease in the PCR group could not be avoided even if these patients were assigned to the antigenemia group, as either the antigenemia assay and PCR reached the threshold simultaneously (UPN32) or the antigenemia assay did not reach the threshold before the diagnosis of CMV disease (UPN35). All of these patients were successfully treated with GCV or foscarnet, although one patient (UPN35) showed the recurrence of colitis after day 100. None of the other patients developed late CMV disease.

Adverse events during preemptive therapy

The mean duration of preemptive therapy with GCV and the mean total dose of GCV was 23.2 ± 19.4 days and 140.8 ± 129.7 mg/kg in the antigenemia group and 20.8 ± 14.2 days and 118.4 ± 91.2 mg/kg in the PCR group ($P=0.64$ and $P=0.51$), respectively. Neutropenia with a neutrophil count of <500 per μl was observed in 5 of the 33 patients in the antigenemia group and 3 of the 19 patients in the PCR group ($P>0.99$). Only one patient in the antigenemia group required a discontinuation of GCV because of neutropenia. The total dose of GCV was higher in patients who developed neutropenia, but this difference was not statistically significant (163.8 ± 82.5 vs 126.9 ± 121.4, $P=0.42$).

An increase in the serum creatinine level by at least 0.5 mg per 100 ml was observed in 8 of the 33 patients in the antigenemia group and in none of the 19 patients in the PCR group ($P=0.039$). The total dose of GCV was significantly higher in patients who developed renal impairment (255.0 ± 198.0 vs 106.0 ± 45.5, $P=0.0004$).

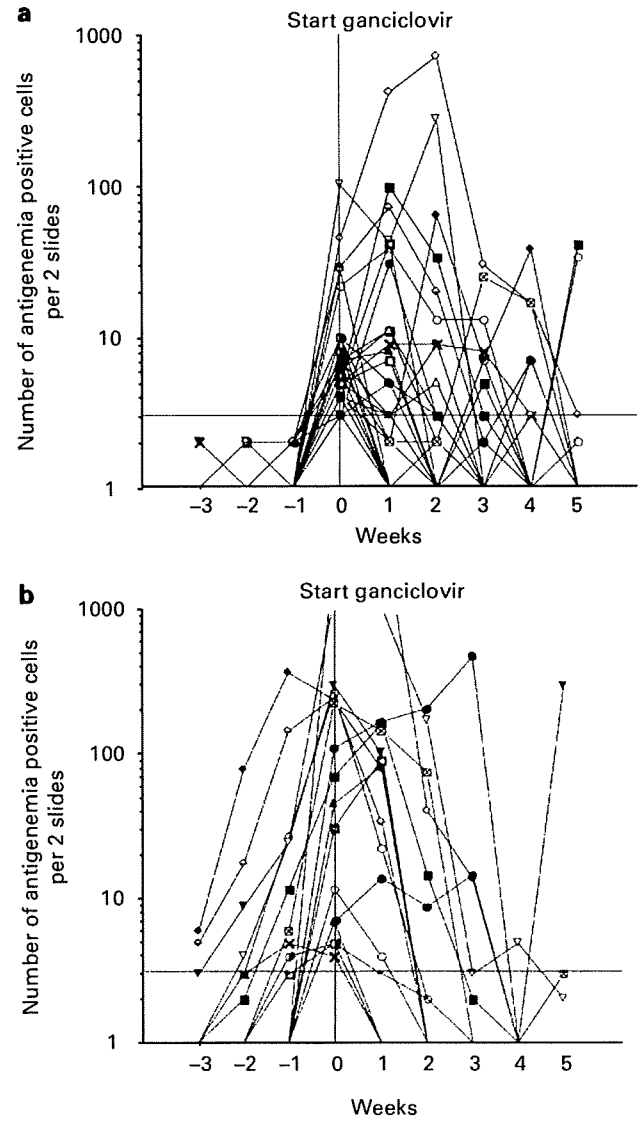

Figure 4 Serial changes in the number of antigenemia-positive cells in patients who received preemptive therapy in the antigenemia group (**a**) and in the PCR group (**b**). Week 0 represents the day ganciclovir was started.

Discussion

In this randomized controlled trial, we compared plasma real-time PCR with a cutoff at 300 copies per ml and an antigenemia assay with a cutoff at three positive cells per two slides as a trigger for deciding when to start preemptive therapy with GCV after unrelated BMT. GCV was used significantly less frequently in the PCR group. A comparison of the number of antigenemia-positive cells and the CMV DNA copy number at the start of GCV treatment clearly revealed that plasma PCR was significantly less sensitive than the antigenemia assay, at least with the current cutoff values. Although the 95% confidence

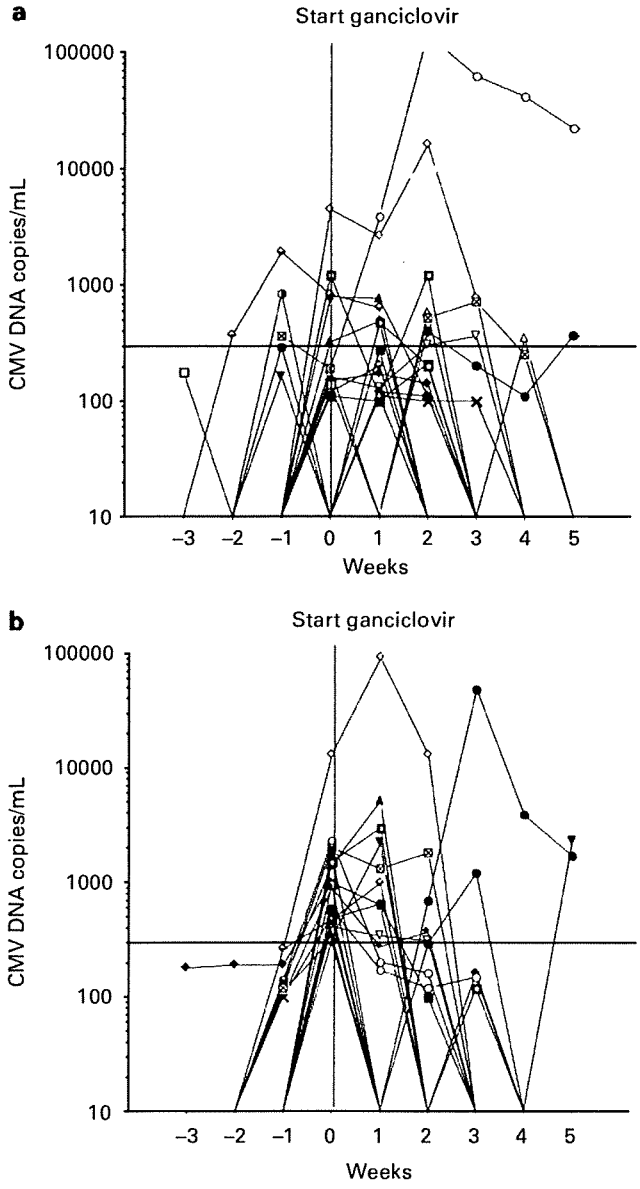

Figure 5 Serial changes in CMV DNA copy number in patients who received preemptive therapy in the antigenemia group (**a**) and in the PCR group (**b**). Week 0 represents the day ganciclovir was started.

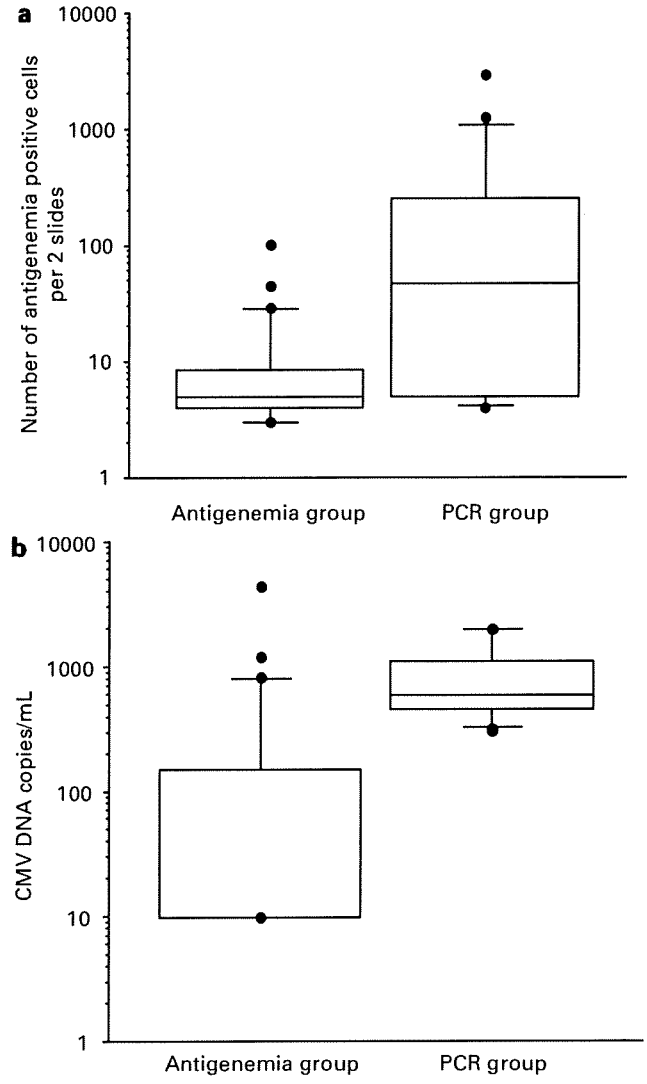

Figure 6 The number of antigenemia-positive cells (**a**) and the CMV DNA copy number at the start of preemptive therapy (**b**), grouped according to the randomization arm. The box-and-whisker plot shows 10, 25, 50, 75 and 90 percentile values. Outliers are indicated by dots.

interval for the difference in the successful prevention rate was just outside the predefined lower limit of −10%, and therefore, we could not show the noninferiority of the PCR group, the incidence of CMV disease was limited to two patients even in the PCR group. In addition, prevention of CMV pneumonia, the main aim of preemptive therapy, was completely achieved in both groups. These findings suggest that an antigenemia assay with a cutoff of three positive cells per two slides was too sensitive and resulted in the unnecessary use of GCV.

The unnecessary use of GCV may be reduced if the cutoff value for the antigenemia assay is increased. The antigenemia assay has already been shown to be not sensitive enough for detecting gastrointestinal involvement by CMV even with a low threshold.[21] In this study, the median number of antigenemia-positive cells at the start of GCV treatment was 47 in the 19 patients who received preemptive therapy in the PCR group. Figure 7 shows the serial changes in the number of antigenemia-positive cells in the patients of the PCR group who developed positive antigenemia that reached the threshold, but who did not receive GCV at that time. In about half of the patients, antigenemia spontaneously became negative without GCV treatment. On the other hand, seven patients developed high-grade antigenemia of over 100 positive cells per two slides. However, GCV was started when the number of positive cells was 260 (median, range: 73–1262 cells) and none of these patients developed CMV disease. Although patients who developed grade II–IV acute GVHD or who received steroid at 0.5 mg/kg or higher experienced high-grade antigenemia more frequently than those who did not

develop grade II–IV acute GVHD and did not receive steroid (Figures 7a and b), the use of GCV was comparable (54.5 vs 40%, $P=0.67$). Thus, although it is difficult to determine the appropriate cutoff value for the antigenemia assay, we thought that it may be worth trying to apply a cutoff value of 20 positive cells per two slides, which we are already safely using in allogeneic hematopoietic SCT from an HLA-matched sibling donor,[20] to transplantation from an unrelated donor.

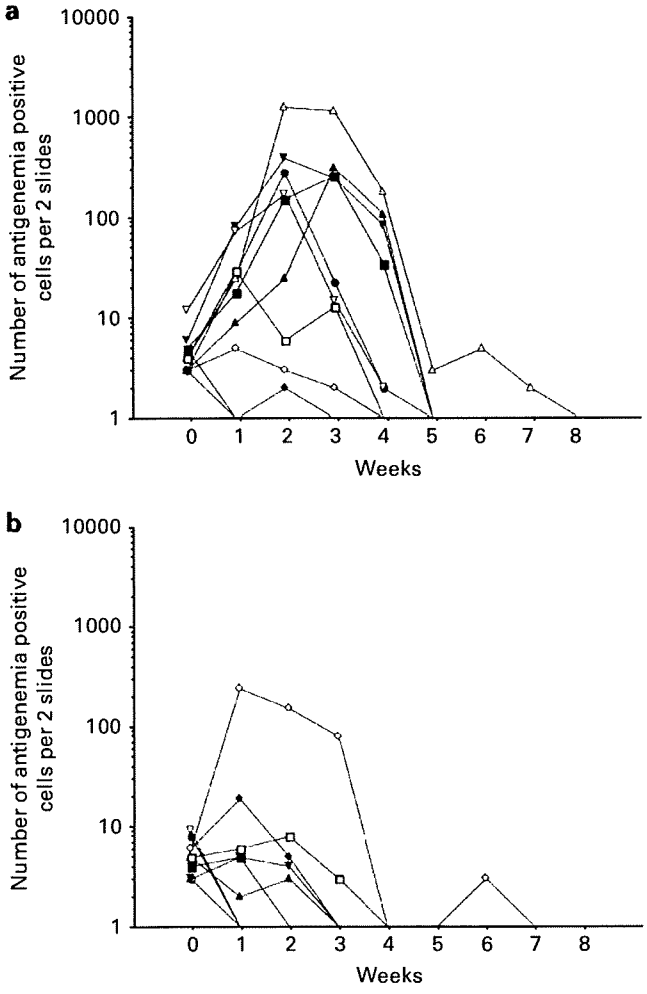

Figure 7 Serial changes in the number of antigenemia-positive cells in the PCR group patients who developed positive antigenemia that reached the threshold, but who did not receive ganciclovir. (**a**) Patients who developed grade II–IV acute GVHD or who received steroid at 0.5 mg/kg or more. (**b**) Patients who did not develop grade II–IV acute GVHD and did not receive steroid.

Although Boeckh *et al.*[3] reported a 14% incidence of early CMV disease using the same cutoff as in the current study, the incidences of positive antigenemia at any level and three or more positive cells per two slides were similar to those in this study (79 and 70% in Boeckh's study and 89 and 73% in the current study). Therefore, the higher incidence of early CMV disease probably resulted from the high incidence (35%) of grade III–IV acute GVHD in their study rather than from the difference in the method used for the antigenemia assay, as acute GVHD is one of the strongest risk factors for CMV disease.

Nevertheless, it is important to note that the sensitivity and specificity of these assays vary depending on the methodology used.[9,22–24] In fact, the unexpected differences in the sensitivities of the two assays in this study could be explained by the difference in the methodology used in the antigenemia assay. The cutoffs used for the antigenemia assay and real-time PCR were determined based on our previous study in which HRP-C7 Ab was used in the antigenemia assay.[18] In this study, however, we used C10/C11 Ab in the antigenemia assay, as this Ab has been used worldwide. Although we did not believe that there are clinical differences between these two antigenemia assays,[6,7,20] we should have tested the correlation between the results of plasma PCR and the antigenemia assay using C10/C11 Ab. Fortunately, the unexpected difference in the sensitivity in these assays contributed to the finding that the antigenemia assay with the current cutoff was too sensitive as a trigger for deciding when to start preemptive therapy. These data are valid only when the same methodology is used, and standardization of the methods is warranted.[25,26]

In conclusion, CMV colitis could not be completely prevented by the current preemptive strategy using the peripheral blood samples, but CMV pneumonia was completely prevented in both groups. The initiation of GCV at 5 mg/kg/day was confirmed to be safe, provided the CMV load continues to be monitored. Plasma PCR with a cutoff at 300 copies per ml seemed to be appropriate for monitoring CMV reactivation after transplantation. The cutoff number of positive cells should be raised above that used here when using an antigenemia assay. However, the appropriateness of the threshold of these assays may be different on the basis of the methodology and patient background, such as the risk of GVHD, and therefore, it is difficult to generalize.

Table 3 CMV load in patients who developed CMV disease

Age/sex	*Acute GVHD*	*Onset/affected organ of CMV disease*		*−3 weeks*	*−2 weeks*	*−1 week*	*Onset*
UPN32 38/M (PCR group)	Grade II	Day 56/colitis	PCR	(−)	260	13 000[a]	93 000
			Ag	(−)	(−)	2921	5467
UPN35 36/M (PCR group)	Grade II	Day 46/colitis	PCR	(−)	(−)	(−)	(−)
			Ag	0	0	2	12
UPN70 38/M (Antigenemia group)	Grade II	Day 50/colitis	PCR	(−)	(−)	110	100
			Ag	2	(−)	5[a]	99

[a]Preemptive therapy was started.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Outcome of 93 patients with relapse or progression following allogeneic hematopoietic cell transplantation

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Relapse/progression after allogeneic hematopoietic cell transplantation (allo-HCT) remains the major cause of treatment failure. In this study, the subsequent clinical outcome was overviewed in 292 patients with leukemia/myelodysplastic syndrome who received allo-HCT. Among them, 93 (32%) showed relapse/progression. Cohort 1 was chosen to receive no interventions with curative intent (n = 25). Cohort 2 received reinduction chemotherapy and/or donor lymphocyte infusion (n = 48), and Cohort 3 underwent a second allo-HCT (n = 20). Sixty-three patients received reinduction chemotherapy, and 27 (43%) achieved subsequent complete remission (CR). The incidence of nonrelapse mortality (NRM) was similar among the three cohorts (4, 15, and 5%). The 1-year overall survival (OS) after relapse was significantly better in patients with a second HCT (58%) than in others (14%, Cohorts 1 and 2; P < .001). However, the 2-year OS did not differ between the two groups, which suggests that it is difficult to maintain CR after the second HCT. Multivariate analysis showed that reinduction chemotherapy, CR after intervention, second HCT, and longer time to post-transplant relapse were associated with improved survival. In conclusion, for patients with relapse after allo-HCT, successful reinduction chemotherapy and a second HCT may be effective for prolonging survival without excessive NRM. However, effective measures to prevent disease progression after a second HCT clearly need to be developed. Am. J. Hematol. 84:815–820, 2009. © 2009 Wiley-Liss, Inc.

Introduction

Relapse or progression of leukemia occurring after allogeneic hematopoietic cell transplantation (allo-HCT) remains the major cause of post-transplantation mortality, with a median postrelapse survival of 1.6–6 months when aggressive intervention is suspended [1–6]. The optimal treatment strategy for these patients has not yet been established. Although some patients can be reinduced into complete remission (CR) with conventional chemotherapy, only a few become long-term survivors while maintaining conventional chemotherapy [4–6], and the benefit of donor lymphocyte infusion (DLI) for acute leukemia is limited [1,3,7].

Several studies have shown that a second allo-HCT improved survival after relapse and represents a potential therapeutic option, which may increase the duration of leukemia-free survival (6–25 months) [1,6,8–14]. However, this is associated with a high rate of nonrelapse mortality (NRM) (24–75%) [8–13,15]. In many studies, the results regarding a second HCT are generally represented by heterogeneous cohorts of patients or series with relatively few patients carrying variable backgrounds. Furthermore, most studies have not compared the outcome of a second HCT with that of other interventions in the modern treatment era.

To identify the factors that influence the outcome of patients with relapse after various salvage therapies, including second HCT, we performed a retrospective single-center analysis of consecutive 292 patients.

Patients and Methods

Patients. Between January 2000 and December 2006, a total of 292 patients with leukemia or myelodysplastic syndrome (MDS) underwent allo-HCT at the National Cancer Center Hospital. Recipients of haploidentical transplants from related donors and patients aged 15 or under were not included in this study. The characteristics of the patients and transplantations are summarized in Table I. The underlying diseases were AML (n = 142), MDS (n = 73), CML (n = 34), and ALL (n = 43). The median age at the initial HCT was 50 years (range: 16–68). Of the 292 patients, 148 received an initial HCT with myeloablative conditioning (cyclophosphamide plus fractionated TBI or busulfan), and the remaining 144 received reduced-intensity conditioning (RIC; fludarabine- or cladribine-based).

Definitions. Relapse/progression after transplantation was defined as the presence of or increase in leukemic blasts as detected by morphology either in bone marrow or peripheral blood. Detection of minimal residual disease by flow cytometry, PCR, or decreasing donor chimerism did not constitute evidence of recurrence in the absence of morphological abnormalities. CR was defined as normocellular bone marrow with less than 5% blasts along with the absence of blasts in the peripheral blood [16]. Postrelapse overall survival (OS) was measured from the date of relapse or progression to the time of death or censored date of last contact. Withdrawal of immunosuppression (WIS) was defined as the cessation of immunosuppression at the diagnosis of relapse or progression. Chemotherapy was categorized into two groups: reinduction chemotherapy and less-intensive chemotherapy intended for palliative treatment. Disease-specific reinduction chemotherapy included high-dose cytarabine, idarubicin + cytarabine, aclarubicin + low-dose cytarabine [17,18], and other remission-induction therapies for myeloid and lymphoid leukemia. Imatinib mesylate for CML, all-trans retinoic acid or arsenic trioxide for acute promyelocytic leukemia (APL), gemtuzumab ozogamicin for CD33-positive AML, and intrathecal chemotherapy alone for isolated central nervous system (CNS) relapse were also included in the reinduction chemotherapy group. Less-intensive chemotherapy included oral hydroxyurea, cytarabine or 6-mercaptopurine, and the sole intravenous administration of aclarubicin or vincristine, which are not thought to be intensive enough to achieve remission, but are aimed at palliation. NRM was defined as death from toxicities related to therapy without disease recurrence.

Interventions were categorized into three cohorts: Cohort 1, WIS or less-aggressive chemotherapy; Cohort 2, reinduction chemotherapy and/or DLI; Cohort 3, second allo-HCT.

Statistical analysis. Data were retrospectively reviewed and analyzed as of August 2007. The primary endpoint of the study was OS following relapse/progression. OS was estimated by the Kaplan-Meier method.

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Conflict of interest: Nothing to report.

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The log-rank test and generalized Wilcoxon test were used to compare the probabilities of survival over time across patient subgroups. Multiple cox regression models were used for multivariate risk-factor analysis for OS following relapse/progression. The clinical factors evaluated were diagnosis, patient age at the initial HCT, gender, conditioning in the initial HCT (myeloablative or RIC), donor in the initial HCT (HLA-matched related or others), disease status at the initial HCT, interval from the initial HCT to relapse/progression, interventions that were chosen after relapse (Cohorts 1–3), and the response to the initial intervention. We considered two-sided *P*-values of <0.05 to be statistically significant. Statistical analyses were performed with the SPSS statistics and SAS version 8.2 (SAS, Cary, NC).

TABLE I. Patient and Transplanation Characteristics

Characteristics	All patients	Relapsed patients %[a]
No. of patients	292	93 (32)
Age, year, median (range)	50 (16–68)	47 (16–68)
Diagnosis[b]		
AML	142	57 (40)
MDS	73	13 (9)
CML	34	5 (4)
ALL	43	18 (13)
Gender		
Male	173	49 (35)
Female	119	44 (31)
Matched related donor		
Yes	125	44 (31)
No	167	49 (35)
Conditioning regimen		
Myeloablative		
TBI-based	90	38 (27)
BU/CY-based	58	21 (15)
RIC	144	34 (24)
Stem cell source		
BM	125	37 (26)
PBSC	149	49 (35)
CB	18	7 (5)
Disease status at first HCT		
CR	150	42 (30)
non-CR	142	51 (36)
GVHD prophylaxis		
CSP-based	243	77 (54)
TAC-based	49	16 (11)

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoid leukemia; TBI, total body irradiation; BU/CY, busulfan/cyclophosphamide; RIC, reduced-intensity conditioning; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; CR, complete remission; GVHD, graft-versus-host disease; CSP, cyclosporin; TAC, tacrolimus.

[a] The percentage shown here indicates the proportion fo relapsed patients among each category.

[b] MDS overt leukemia was categorized into AML.

Results

Relapse or progression

The characteristics of all patients and relapsed patients are shown in Table I. Overall, 93 of the 292 patients (32%) relapsed or progressed at a median of 154 days (range; 15–1,211) after the initial HCT (AML, $n = 57$; MDS, $n = 13$; CML, $n = 5$; ALL, $n = 18$). The interval from the initial HCT to relapse/progression was less than 100 days in 34 patients, 100 days to 1 year in 39 patients, and more than 1 year in 20 patients.

TABLE II. Outcomes of Interventions after Relapse

Therapy	*n*	CR (%)	NRM (%)	OS after relapse, day, median, (range)
Total	93	34 (37)	9 (10)	184 (5–1456)
No aggressive Tx	25	1 (4)	1 (4)	61 (5–245)
No therapy	7	0	0	56 (22–166)
WIS alone	10	1	1	60 (5–245)
Less- int. CTx	8	0	0	74 (12–203)
Chemotherapy/DLI	48	18 (38)	7 (15)	194 (19–1,456)
Reinduction CTx	31	9 (29)	2 (6)	167 (19–1,456)
CTx + DLI	14	7 (50)	4 (29)	194 (52–1,254)
DLI alone	3	2 (67)	1 (33)	240 (32–243)
second HCT	20	15 (75)	1 (5)	502 (66–997)

CR, complete remission; NRM, nonrelapse mortality; OS, overall survival; Tx, therapy; WIS, withdrawal of immunosuppression; Less-int. CTx, less-intensive chemotherapy; DLI, donor lymphocyte infusion; HCT, hematopoietic cell transplantation.

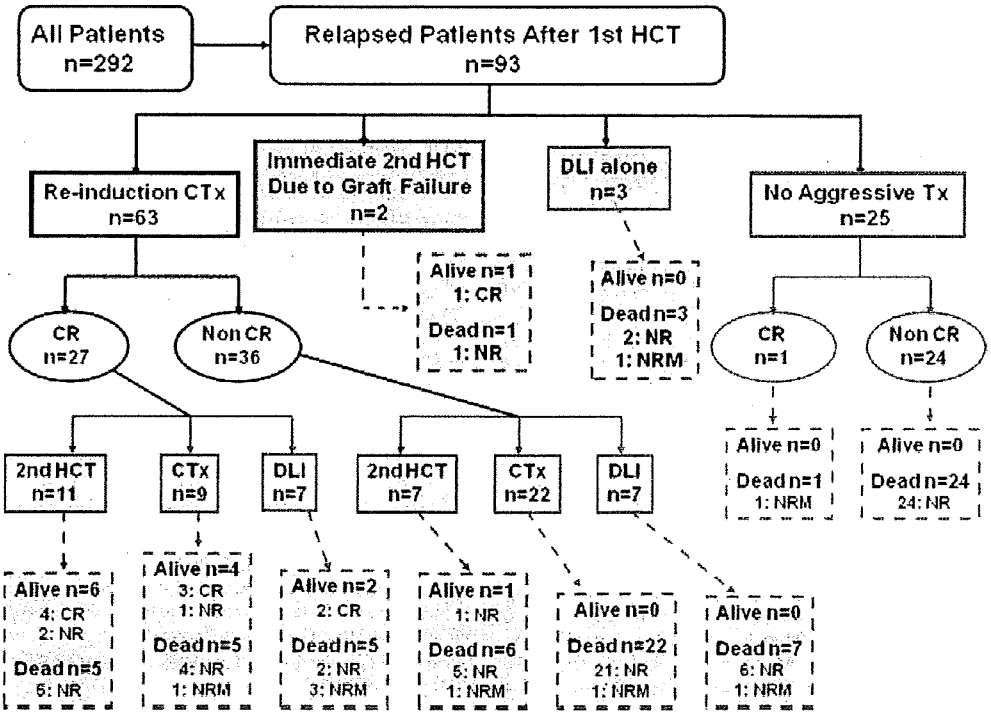

Figure 1. Summary of interventions after relapse. Abbreviations: HCT, hematopoietic cell transplantation; CTx, chemotherapy; Tx, therapy; CR, complete remission; DLI, donor lymphocyte infusion; NR, nonremission; NRM, nonrelapse mortality. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE III. Patient Characteristics of Intervention Group

Characteristics	No aggressive Tx (%)	CTx and/or DLI (%)	Second HCT (%)	P
Total no. of patients	25	48	20	
Diagnosis				0.053
AML	10 (40)	32 (67)	15 (75)	
MDS	7 (28)	3 (6)	3 (15)	
CML	2 (8)	3 (6)	0 (0)	
ALL	6 (24)	10 (21)	2 (10)	
Age				0.333
<50	11 (44)	28 (58)	13 (65)	
≥50	14 (56)	20 (42)	7 (35)	
Matched related donor				0.143
Yes	8 (32)	27 (56)	9 (45)	
No	17 (68)	21 (44)	11 (55)	
Disease status at first HCT				0.105
CR	7 (28)	26 (54)	9 (45)	
non-CR	18 (72)	22 (46)	11 (55)	
Time from first HCT to relapse				0.938
≥100 days	16 (64)	31 (65)	12 (60)	
<100 days	9 (36)	17 (35)	8 (40)	

Tx, therapy; CTx, chemotherapy; HCT, hematopoietic cell transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoid leukemia; CR, complete remission.

Interventions after relapse/progression

After the diagnosis of relapse or progression, the need for salvage therapy was determined at a multiprofessional conference, at which the clinical circumstances and the opinions of physicians and patients were weighed. The various therapeutic options used after the diagnosis of relapse are summarized in Table II and Fig. 1.

At the diagnosis of relapse or progression, 70 patients had been receiving immunosuppression (median days after initial HCT, 125; range 15–705) and 63 of them had it withdrawn before receiving any other therapies.

After the diagnosis of relapse or progression, 63 patients received reinduction chemotherapy with disease-specific regimens, which included imatinib mesylate (CML, $n = 4$), all trans-retinoic acid and arsenic trioxide (APL, $n = 1$), gemtuzumab ozogamicin (AML, $n = 3$), and intrathecal chemotherapy alone for isolated CNS relapse (AML, $n = 3$; ALL, $n = 1$; CML, $n = 1$). Overall, 27 of the 63 patients who received reinduction chemotherapy achieved CR (43%). Among the 27 patients who achieved CR, 18 proceeded to DLI ($n = 7$) or second HCT ($n = 11$). The remaining nine received no further therapy other than chemotherapy; three patients with CNS relapse were in remission, and the remaining six patients subsequently progressed. Among the 36 patients who did not achieve CR, 14 proceeded to DLI ($n = 7$) or second HCT ($n = 7$), and the remaining 22 did not receive further treatment because of various reasons (disease progression, $n = 15$; infection and/or graft-versus-host disease (GVHD), $n = 4$; refusal, $n = 3$). Two other patients proceeded to second HCT directly after disease relapse with concomitant graft failure.

To compare the outcomes of the interventions after relapse/progression, we divided the 93 patients into three cohorts according to the intervention, that is, no aggressive therapy (Cohort 1, $n = 25$), reinduction chemotherapy and/or DLI without second HCT (Cohort 2, $n = 48$), and second HCT (Cohort 3, $n = 20$). There were no significant differences among the three groups in clinical characteristics such as patient age at the initial HCT, diagnosis, donor in the initial HCT, disease status at the initial HCT, and interval from the initial HCT to relapse (Table III).

No aggressive therapy (Cohort 1)

Among the 93 patients who relapsed, 25 (27%) received no aggressive therapy with curative intent other than WIS or less-intensive chemotherapy, mostly because of comorbidities and/or refractoriness of leukemia/MDS. Among the 10 patients who received WIS alone, only one achieved CR, but this patient subsequently died of bronchiolitis obliterans. All of the remaining eight patients who were given less-intensive chemotherapy alone and seven who received no therapy after relapse/progression died of disease progression without achieving CR. The median OS of the patients in Cohort 1 was 61 days after relapse/progression and the cause of death was primarily disease progression.

Reinduction chemotherapy and/or DLI without second HCT (Cohort 2)

Of the 63 patients who received reinduction chemotherapy after relapse, 45 patients did not receive a second HCT; these 45 patients with or without subsequent DLI and three other patients who received DLI without preceding chemotherapy were placed in Cohort 2.

Overall, 16 (36%) of the 45 patients achieved CR as the best response after reinduction chemotherapy. All three patients with isolated CNS relapse were alive in remission, whereas 11 of 13 patients who had marrow relapse eventually relapsed.

After reinduction chemotherapy, 14 patients (AML, $n = 9$; MDS, $n = 1$; ALL, $n = 3$; CML, $n = 1$) received DLI from the same donor as in the initial HCT. The initial CD3-positive cell dose of DLI ranged from 0.03 to 161 $\times$ 10^8/kg (median: 2.9 $\times$ 10^8/kg), and the number of courses of DLI was one to four, which were chosen according to the donor source or the disease status of patients at the discretion of physicians. Although the remission rate of patients who received DLI after chemotherapy was 50%, the incidence of NRM was also rather high (29%, GVHD with or without infection). The median OS of patients who received DLI after relapse/progression was 194 days (range: 52–1,254), which was similar to that of patients without DLI (167 days, range; 19–1,456).

Among the three patients who received DLI without preceding chemotherapy (AML, 1; MDS, 2), two achieved CR but all of them eventually died: one with toxicity and two with disease progression.

Second HCT (Cohort 3)

Table IV summarizes the profiles of 20 patients who underwent a second HCT. The median age at the initial HCT was 38 years (21–66 years) and 65% of the patients were younger than 50 years. The median time from the initial HCT to relapse/progression was 152 days (range: 21–1,211), and the median interval between the initial HCT and the second HCT was 325 days (range: 126–1,310). Six patients received HCT from the same donor as in the initial HCT (HLA-matched related donor, $n = 5$; unrelated bone marrow donor, $n = 1$), and the remaining 14 received the second HCT from a different donor (unrelated bone marrow donor, $n = 7$; cord blood, $n = 6$; haploidentical related do-

TABLE IV. Characteristics of Second Transplantation

Characteristics	No of patients second HCT (%)
Total	20
Age	
<50	13 (65)
≥50	7 (35)
Diagnosis	
AML	15 (75)
MDS	3 (15)
CML	0 (0)
ALL	2 (10)
Gender	
Male	9 (45)
Female	11 (55)
Time from first HCT to relapse	
<100 days	8 (40)
≥100 days	12 (60)
Time from first HCT to second HCT	
<1 year	12 (60)
≥1 year	8 (40)
Donor for first/second HCT	
Same	6 (30)
MRD-MRD	5
UBM-UBM	1
Different	14 (70)
UBM-UBM	4
MRD/CB-UBM	3
MRD/UBM-CB	6
Other	1
Conditioning for first/second HCT	
Myeloablative	8 (40)
Myeloablative-RIC	7 (35)
RIC-RIC	5 (25)
Stem cell source	
BM	8 (40)
PBSC	6 (30)
CB	7 (35)
Remission at second HCT	
No	9 (45)
yes	11 (55)
GVHD prophylaxis	
CSP-based	8 (40)
TAC-based	3 (15)
Others	3 (15)
GVHD	
No	10 (50)
Yes	10 (50)

HCT, hematopoietic cell transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoid leukemia; MRD, matched-related donor; UBM, unrelated bone marrow; CB, cord blood; RIC, reduced-intensity conditioning; PBSC, peripheral blood stem cell; CSP, cyclosporin; TAC, tacrolimus.

nor, $n = 1$). Among the 15 patients who had received myeloablative conditioning for the initial HCT, eight received myeloablative conditioning and seven received RIC for the second HCT. The remaining five patients received both HCT with RIC. Although the 1-year OS after relapse was better in patients who received myeloablative conditioning for the second HCT than in patients who received RIC (100 vs. 37%, $P = 0.015$), patients who received myeloablative conditioning for the second HCT were younger and had a longer interval between the initial and the second HCT than those who received RIC ($P < 0.001$ and $P = 0.006$, respectively). There was no difference in OS between patients who received a second HCT from the same donor and those who had a different donor (1-year OS: 44 vs. 60%, $P = 0.48$).

Two patients underwent immediate HCT after relapse with concomitant graft failure. Among the other 18 patients who received reinduction chemotherapy before the second HCT, 11 had achieved CR at the second HCT and seven were not in CR. Four of the nine patients with nonremission disease at the second HCT, including two patients who did not receive reinduction chemotherapy, subsequently achieved CR; only one of the nine patients is currently alive in CR.

Of the 20 patients who underwent a second HCT, eight are alive with a median follow-up after relapse of 335 days (range: 181–997); five are in CR and three have recurrent disease.

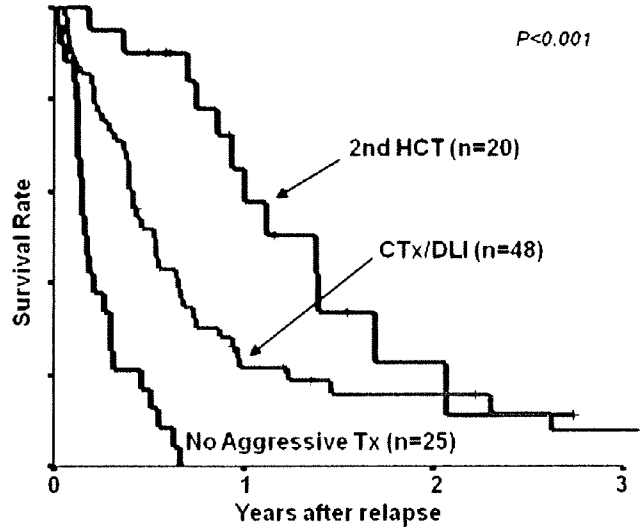

Figure 2. Overall survival. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

GVHD was newly diagnosed or interpreted to progress after the second HCT in 10 of the 20 patients. The median OS after relapse in patients with GVHD after the second HCT was 422 days (range: 181–997), and all of these patients achieved CR as a best response. The median OS after relapse for the remaining 10 patients without GVHD was 314 days (range: 66–757), and five of them failed to achieve CR as a best response.

Comparison of CR, NRM, and OS after relapse following the initial HCT

The median OS after the development of relapse in the 93 patients who had relapse/progression was 184 days (range: 5–1,456). Overall, 15 patients (16%) are currently alive with a median follow-up of 346 days (range; 33–1,456 days), and 10 of these patients are still in CR. Among the 78 patients who died, 69 died of disease progression and nine died of NRM (10%). The causes of NRM were GVHD and/or infection in eight (Cohort 1, one patient; Cohort 2, seven patients), and one early death after the second HCT with hepatic failure, which accounts for the one case of NRM for second HCT (Table II).

We compared the rate of CR, NRM, and OS after relapse among the three different cohorts (Table II). As the maximum response, the probabilities of achieving CR were 4% in Cohort 1, 38% in Cohort 2, and 75% in Cohort 3. The NRM rates were 4, 15, and 5% for each group, respectively. The median duration of remission after achieving CR was 177 days (range, 17–1,167). The median OS after relapse/progression in patients who underwent a second HCT (Cohort 3, 502 days) was significantly longer than those in Cohort 1 (61 days) and Cohort 2 (194 days, $P < .001$, Fig. 2). The 1-year OS after relapse was significantly better in patients with a second HCT (Cohort 3) than in the other patients (Cohorts 1 and 2) (58 vs. 14%). However, there was no significant difference in the 2-year OS, which suggests that it is difficult to maintain CR after a second HCT.

A multivariate analysis showed that CR after intervention (HR 3.83, 95% CI 2.06–7.11, $P < .001$), reinduction chemotherapy (HR 2.83, 95% CI 1.65–4.86, $P < .001$), a second HCT (HR 3.02, 95% CI 1.58–5.79, $P < .001$), and a longer time from the initial HCT to relapse (HR 1.99, 95% CI 1.21-3.28, $P = 0.007$) were associated with an improved OS after relapse/progression (Table V). Diagnosis, patient age at initial HCT, gender, conditioning regimen, or donor in the initial HCT and DLI were not significant factors.

TABLE V. Univariate and Multivariate Analysis of risk Factors for OS after Relapse

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Diagnosis			–	–
CML	1.00			
AML	2.03 (0.62–6.65)	0.241		
ALL	2.54 (0.71–9.00)	0.150		
MDS	3.39 (0.94–12.24)	0.062		
Age			–	–
<50	1.00			
≥50	1.53 (0.98–2.41)	0.063		
Gender			–	–
Male	1.00			
Female	0.92 (0.59–1.43)	0.701		
Conditioning			–	–
Myeloablative	1.00			
RIC	1.34 (0.84–2.12)	0.216		
Donor			–	–
MRD	1.00			
Others	1.26 (0.80–1.97)	0.322		
Disease Status at first HCT				
Standard	1.00			
High	1.23 (0.70–2.12)	0.465		
Time from first HCT to relapse				
≥100 days	1.00		1.00	
<100 days	1.74 (1.09–2.79)	0.020	1.99 (1.21–3.28)	0.007
Reinduction CTx				
Yes	1.00		1.00	
No	3.79 (2.24–6.40)	<.001	2.83 (1.65–4.86)	<.001
CTx Intensity			–	–
Reinduction	1.00			
Less Intensive	4.44 (2.00–9.88)	<.001		
DLI			–	–
Yes	1.00			
No	1.00 (0.57–1.72)	0.968		
Second HCT				
Yes	1.00		1.00	
No	2.89 (1.55–5.38)	<.001	3.02 (1.58–5.79)	<.001
CR after Interventions				
Yes	1.00		1.00	
No	3.54 (2.06–6.09)	<.001	3.83 (2.06–7.11)	<.001

OS, overall survival; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; MDS, myelodysplastic syndrome; RIC, reduced-intensity conditioning; MRD, matched-related donor; HCT, hematopoietic cell transplantation; CTx, chemotherapy; DLI, donor lymphocyte infusion; CR, complete remission.

Discussion

With this retrospective single-center survey in which we compared the outcomes of interventions for relapse/progression after allo-HCT, we showed that a second HCT significantly improved the remission rate and survival. In contrast to previous reports (8–13, 15), NRM after a second HCT was observed in an acceptable percentage of patients (5%), even though 40% of the patients received myeloablative conditioning regimen for the second HCT.

As salvage interventions for leukemia/MDS relapsing after allo-HCT, chemotherapy, DLI either alone or in combination, and second HCT have been considered with different degrees of success. Consistent with reports from other groups [1,4–6], we found that patients who did not undergo intensive chemotherapy had significantly shorter survival. Even though 43% of the patients who were given reinduction chemotherapy achieved CR, all of the relapsed patients who did not receive further intervention eventually relapsed unless relapse is isolated to CNS, and all but one patient died. Prior reports have also suggested that, instead of a certain probability of obtaining remission with reinduction chemotherapy, subsequent relapse is frequently observed and the prognosis is poor when further immunotherapy is suspended [1,4,6,19].

Although DLI has been recognized as an effective treatment for relapsed CML, the efficacy of DLI for relapsed acute leukemia is rather discouraging [3,7,20–22]. Although the remission rate has been reported to be 15–42%, the survival rate has not improved (3-year OS less than 20%), mostly because of a high incidence of uncontrolled GVHD (10–50%). In our cohorts, survival was not improved by adding DLI after chemotherapy, although half of the patients had achieved transient remission. The incidence of NRM after DLI was 29%, which was mostly explained by GVHD. Compared to DLI, a second HCT yielded an even better remission rate and lower NRM in our cohort, which could be respectively explained by the efficacy of the use of conditioning radiochemotherapy and GVHD prophylaxis in the second HCT.

In our data, a second HCT significantly improved the remission rate and survival compared to other interventions, as proven by a multivariate analysis. Although Arellano et al. [1] indicated that immunotherapy including a second HCT was effective compared to chemotherapy or supportive care, other reports that compared interventions after relapse following initial HCT failed to show the advantage of a second HCT [2,6,22]. Prior reports that focused on a second HCT have also expressed concerns about the negative impact of NRM, which has ranged from 24 to 75% (8–13, 15). In contrast, our data revealed a 5% incidence of NRM after a second HCT, which led to improved OS. This unexpectedly low incidence of NRM may reflect the advances in GVHD prophylaxis and supportive care over the past several years. Another possible explanation would be a selection bias of fitter patients that led to less NRM after the second HCT, although there were no significant differences in available characteristics of patients in each intervention group.

Concerning the conditioning regimen for the second HCT, we found that patients who received myeloablative conditioning had a better OS than patients who received

RIC. Eapen et al. [9] indicated the importance of a tumor-killing effect of myeloablative conditioning for the second HCT compared to RIC. Other groups also reported a superior outcome of TBI-based myeloablative conditioning in the second HCT [8,11]. On the other hand, several recent reports have shown that RIC offers a toxicity-reducing benefit in the second HCT [10]. In our cohort, patients who received myeloablative conditioning for the second HCT were younger and had a longer interval from the initial HCT to the second HCT, which could reflect a selection bias in the choice of myeloablative conditioning. Therefore, myeloablative conditioning for the second HCT could be considered beneficial for selected patients.

Consistent with several previous reports, we demonstrated that remission status [4,6,8–12,14,22,23], the use of reinduction chemotherapy [2,6], and a longer interval from the initial HCT to relapse [1,2,4,8–12,14,15,19,22–24] were associated with improved OS after relapse by multivariate analysis. Most prior reports have shown that an interval of 6 months or longer was associated with better OS. We found that patients who relapsed after 100 days following the initial HCT had better OS. However, relapses after intervals of 6 months or 1 year were not significantly associated with improved OS (data not shown).

Prior reports have also suggested that the development of GVHD after a second HCT [2,7–9,13,15,24] and the use of a different donor for the second HCT were associated with a better outcome after the second HCT [10]. Our data showed that both the remission rate and OS tended to be improved in patients who developed newly diagnosed GVHD after the second HCT. However, the use of a different donor for the second HCT did not appear to offer any advantage. Nevertheless, the small number of patients who received a second HCT in our study limits our ability to draw definite answers.

Although the 1-year OS after the second HCT was significantly better than that with other interventions (58 vs. 14%), there was no significant difference in 2-year OS (22 vs. 10%). The substantial decline in the survival curve in the second HCT group after 1 year from relapse was clearly related to recurrence of the underlying diseases. Previous reports also showed a decline in survival in the later period (<30% at 3–5 years from the second HCT) and a substantial relapse rate after the second HCT (>40%) [9–11]. This evidence suggests the need for the effective management of disease recurrence after the second HCT.

Our study is limited by several inherent selection biases. Most importantly, this is a retrospective study that compared the outcomes of interventions that were chosen at the discretion of physicians, although there were no significant differences in patient characteristics among the three cohorts. For example, patients who successfully received intensive intervention such as a second HCT had to survive long enough after relapse to be able to undergo adequate salvage chemotherapy with a rather controlled disease and less comorbidity. Other limitations include the small number of patients, a short follow-up period, and other transplant variables that may have affected the outcomes. Nevertheless, the present data in a consecutive-case series from a single center that reviewed various interventions after relapse allowed us to identify the factors that influenced the prognosis of patients with relapse/progression after allo-HCT.

In summary, these observations may have important implications for the selection of interventions in patients who relapse after allo-HCT. Our data indicated that reinduction chemotherapy with curative intent is required for prolonged survival, if feasible. However, when CR is not available with chemotherapy, long-term survival may be unlikely even with a second HCT. The second HCT may produce improved survival without excessive toxicity. However, the substantial incidence of a later relapse after the second HCT was revealed to be a major concern. Further studies are warranted to identify innovative post-transplant strategies to reduce disease recurrence, including immunotherapy such as a vaccination strategy.

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ORIGINAL ARTICLE

Busulfex (i.v. BU) and CY regimen before SCT: Japanese-targeted phase II pharmacokinetics combined study

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To evaluate the toxicity and efficacy of an i.v. preparation of BU (12.8 mg/kg), combined with CY (120 mg/kg), a prospective study was performed on 30 Japanese patients (median age, 30 years) with hematologic malignancies undergoing hematopoietic SCT (28 allogeneic transplants from an HLA-matched donor and 2 autologous transplants). There were no significant toxicities, and all but one patient showed evidence of granulocyte engraftment at a median of 14 days for allogeneic and 11 days for autologous transplantation. Grades II–IV acute and chronic GVHD occurred in 9 (9/27, 33%) and 16 patients (16/27, 59%), respectively. Non-relapse mortality at days 100 and 365 was 3 and 17%, respectively. The pharmacokinetics of i.v. BU showed close inter- and intrapatient consistency; the area under the plasma concentration–time curve of the first administration remained at less than 1500 μmol min/l in 27 of the 29 patients (93%), and between 900 and 1350 μmol min/l in 22 patients (73%). As all of the profiles overlap with data from non-Japanese patients, we conclude that racial factors may not seriously influence the bioactivity of i.v. BU.

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Introduction

In hematopoietic SCT (HSCT), high-dose BU has been widely used, mostly in combination with CY.[1] To overcome the disadvantage of oral BU including gastrointestinal absorption,[2–16] i.v. BU was recently introduced into clinical use.[17–20] The initial experience with i.v. BU showed satisfactory dose assurance with reliable predictability of pharmacokinetics without dose adjustment.[19] Hence, it is very probable that its use reduces the incidence of various risks at transplantation such as hepatic venoocclusive disease (VOD), as shown by Kashyap *et al.*[21]

Nevertheless, drug profiles of i.v. BU preparation have not been fully evaluated in different races, who may have different pharmacokinetics. As part of our pivotal study in Japan, we conducted a phase II study with pharmacokinetic analysis of a combined i.v. BU and CY (BU/CY) regimen administered before allogeneic or autologous HSCT. A population pharmacokinetic analysis suggested that i.v. BU pharmacokinetics show high inter- and intrapatient consistency.[22] This study with the same population further focused on complete pharmacokinetic profiles with additional clinical and safety data.

Patients and methods

Eligibility criteria

Patients with acute leukemia, CML, MDS or malignant lymphoma were eligible for this study. Patients aged 5–55 years with a Lansky Performance Status >70 (over 5 and less than 16 years of age) or an Eastern Cooperative Oncology Group Performance Status ⩽2 (16–55 years of