Graft-Versus-Host Disease

Fourteen of 31 patients (45%; 95% CI, 28–63%) who achieved primary engraftment developed grade II–IV acute GVHD: grade II (n=6) and grade III (n=8) (Fig. 1-A). Its median onset was day 26 (range, 12–90). Ten patients with grade II–IV acute GVHD received corticosteroids. The initial response to corticosteroid was CR in seven, partial response (PR) in two, and mixed response in one patient. No patients required second line immunosuppressive therapy for acute GVHD. Seven of the evaluable 26 patients (27%) developed chronic GVHD including extensive-type (n=2) and limited-type (n=5) (Fig. 1B). Neither acute nor chronic GVHD was fatal in any patients.

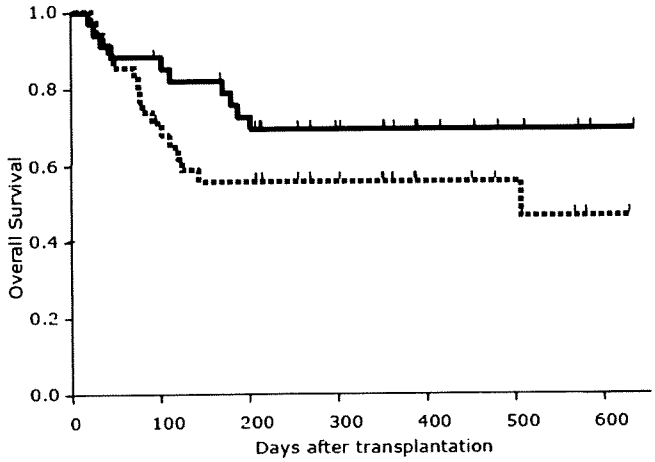

FIGURE 2. Probability of overall survival (OS) and event-free survival (EFS). As of December 2005, the median follow-up after RI-CBT for surviving patients was 12.1 months (range 3.1–21.1). The Kaplan-Meier probability of OS and EFS at 1 year were 70% and 55% (95% confidence interval: 38%–72%), respectively.

Infection

Twelve patients developed bacteremia (n=12). It was fatal in two patents. One patient developed disseminated tuberculosis, which was successfully treated by antitubercular drug (*11*). Reactivation of CMV was documented in 15 patients at a median of day 33 (range, day 17–87). Six patients developed CMV enterocolitis. It was successfully treated by ganciclovir or foscarnet. One developed viral hemorrhagic cystitis, which was successfully treated by vidarabine. Two patients developed encephalitis caused by human herpes virus 6. It was fatal in one patient despite foscarnet use.

Event-Free and Overall Survival

As of October 2005, the median follow-up after RI-CBT for surviving patients was 12.1 months (range 3.1–21.1). Five patients died due to disease progression. The Kaplan-Meier probability of EFS and OS at 1 year were 55% (95% CI, 38%–72%) and 70% (95% CI: 54%–85%), respectively (Fig. 2).

Prognostic Factors

Univariate analysis failed to identify any significant risk factors of OS, and multivariate analysis was therefore not conducted (Table 4).

Univariate analysis showed borderline significances between EFS and either risk of underlying diseases or HLA disparity; however, multivariate analysis failed to identify any prognostic factors of EFS (Table 3).

TABLE 4. Prognostic factors of overall and event-free survival

Univariate factors	Hazard ratio	95% CI	*P* value
Overall survival			
Age (years)	1.01	0.96–1.068	0.63
Sex (female vs. male)	1.99	0.42–9.39	0.38
Performance status (1 vs. 0)	4.15	0.88–19.56	0.072
Risk of underlying diseases (high vs. low)	3.20	0.41–25.31	0.27
Previous history of autologous stem-cell transplantation (yes vs. no)	1.68	0.36–7.93	0.51
Disparity of HLA-A, -B, -DR antigen (two- vs. one-antigen mismatched)	0.56	0.12–2.64	0.46
Number of infused nuclear cells	0.58	0.17–1.97	0.38
Number of infused CD34 positive cells	0.54	0.14–2.14	0.38
Event-free survival			
Age (years)	1.02	0.98–1.06	0.38
Sex (female vs. male)	0.57	0.21–1.55	0.27
Performance status (1 vs. 0)	2.35	0.81–6.79	0.11
Risk of underlying diseases (high vs. low)	2.76	0.63–12.18	0.18
Previous history of autologous stem-cell transplantation (yes vs. no)	1.00	0.23–4.44	1.00
Disparity of HLA-A, -B, -DR antigen (two- vs. one-antigen mismatched)	0.36	0.10–1.31	0.12
Number of infused nuclear cells	1.21	0.58–2.55	0.61
Number of infused CD34 positive cells	1.10	0.56–2.15	0.79
Multivariate factors		0.74–6.37	0.16
Performance status (1 vs. 0)	2.18	0.12–1.55	0.19
Disparity of HLA-A, -B, -DR antigen (one- vs. two-antigen mismatched)	0.43		

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TABLE 5. Clinical characteristics of patients treated with cyclosporine and tacrolimus

Variables	Cyclosporine[a]	Tacrolimus	*P* value
N	30	34	
Age, median years (range)	58.5 (20–70)	56.5 (22–68)	0.21
HLA matching (n)			
5/6	6	4	0.37
4/6	24	30	
Pre-engraftment immune reactions (%)	66.7	44.1	0.07
Grade II–IV acute GVHD (%)	37.5	45.2	0.57
Transplant-related mortality before day 100 (%)	26.7	11.8	0.13
Overall survival at 1 year (%)	26.4	69.5	0.02

[a] Patient characteristics were described in reference 7.

Clinical Impact of Cyclosporine and Tacrolimus as GVHD Prophylaxis

We summarized the clinical characteristics of the patients with between cyclosporine and tacrolimus in Table 5. The characteristics of patients with cyclosporine were previously described (*7*).

DISCUSSION

The present study suggests that GVHD prophylaxis using tacrolimus is feasible in adult RI-CBT recipients. Intensification of GVHD prophylaxis can suppress postCBT immune reactions including PIR and GVHD. PIR is an immune reaction before engraftment, which is frequently associated with fever, diarrhea, rash, and weight gain (*9*). We previously reported that 78% of the RI-CBT recipients given cyclosporine developed PIR (*9*), while 15 of the 34 patients (44%; 95% CI, 27–61%) developed it in the present study. PIR was less frequent in the present study than in RI-CBT with cyclosporine (*7*). Interestingly, it was treated supportively without corticosteroid in all the patients, while 66% of the patients with PIR were given corticosteroid than in RI-CBT with cyclosporine (*7*). Severity of PIR was milder in patients given tacrolimus than those given cyclosporine.

GVHD is the most significant concern in allo-SCT. The frequencies of grade II–IV acute GVHD in adult myeloablative CBT using mainly cyclosporine were 25–72% (*22–28*). We previously reported that 66% of adult RI-CBT recipients developed grade II–IV acute GVHD, when cyclosporine was used for GVHD prophylaxis (*9*). In the present study, 14 of 31 patients (45%; 95% CI, 28%–63%) developed grade II–IV acute GVHD. The incidence of acute GVHD was lower in RI-CBT using tacrolimus than that using cyclosporine. Tacrolimus has been shown stronger in its immunosuppressant effects than cyclosporine in randomized controlled studies (*29–31*) and in vitro studies (*32*). It is reasonable to assume that immunosuppression using tacrolimus might suppress acute GVHD in the present study. Alternatively, suppression of PIR with tacrolimus might have contributed to the prevention of acute GVHD following RI-CBT, since PIR can trigger GVHD (*9*).

The present study suggests the possibility that intensification of GVHD prophylaxis decreases TRM after RI-CBT, improving the prognosis. GVHD prophylaxis in adult CBT is mostly cyclosporine, and the TRM ranges 27–52% (*3, 4, 7, 8*). In contrast, TRM in the present study with tacrolimus was 12% (95% CI, 1–23%), which was much lower than those in previous reports (*3, 4, 7, 8*). The major causes of TRM after CBT are infections and GVHD (*3, 4, 7, 8, 10*). As none died of GVHD, intensification of immunosuppression may have reduced TRM. Additional immunosuppression such as steroids for PIR and GVHD may increase the risk for infections. Intense GVHD prophylaxis by tacrolimus probably reduced steroid use, and hence the risk of severe infections.

It remains unknown whether intensification of GVHD prophylaxis using tacrolimus might hamper graft-versus-malignancy (GVM) effects, since they are closely associated with GVHD (*33*). In the present study, the cumulative probability of relapse at 1 year was 37%, and 55% of the RI-CBT recipients survived without disease progression at 1 year after transplantation. Considering the patients' backgrounds in this study, these findings suggest that RI-CBT using tacrolimus carries a considerable GVM effects. Since the impact of GVHD on a GVM effect varies according to disease status and patients' conditions, management of GVHD should be tailored. Further studies are warranted to establish a proper GVHD prophylaxis following RI-CBT.

Adverse effects of tacrolimus were tolerable in RI-CBT recipients in the present study. Its major adverse effects include renal insufficiency, hyperglycemia, and hypertension. Renal insufficiency by tacrolimus has been reported higher in incidence than by cyclosporine (*34*). Despite the advanced age in most of our patients, renal insufficiency by tacrolimus was reversible. While another concern about tacrolimus may be graft failure with intense immunosuppression (*35*), incidence of engraftment in the present study was comparable to those in the previous reports (*3, 4, 7, 8*). This study has demonstrated that tacrolimus is feasible in RI-CBT for patients with advanced age.

While the present study suggested the possible improvement in TRM by intensification of GVHD prophylaxis, there are some problems to be discussed. First, it is a small-sized, retrospective study; unrecognized biases might have affected the results. Large-scale prospective evaluations are required. Second, the follow up was rather short. Little information is available concerning chronic GVHD and GVM effects. Longer follow-up observations are necessary to investigate them. Third, we did not investigate the postCBT immune reconstitutions in the recipients with tacrolimus. Evaluation of immune parameters such as

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CD4 and CD8 T cell might be worth investigating. The last optimal strategy should be established in the management of acute GVHD following RI-CBT. An appropriate protocol of tacrolimus use in RI-CBT for patients with advanced age remains unclear and requires further study.

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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

age) who were expected to survive beyond 100 days after HSCT were eligible. The eligibility criteria also included serum creatinine less than twice the upper normal limit, as well as serum total bilirubin less than 1.5 times, and aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltranspeptidase less than three times the upper normal limit. Left ventricular ejection fraction $\geqslant 50\%$ or arterial blood oxygen saturation $\geqslant 94\%$, and in adult patients a carbon monoxide lung diffusing capacity $\geqslant 60\%$, were required. Patients with arrhythmia, hypertension or diabetes mellitus that was difficult to control despite medication, severe cardiopulmonary or renal disease, chronic active hepatitis, liver cirrhosis, acute hepatitis, ascites more than 1 l, central nervous system disorders, active infection; positive hepatitis B surface antigen, hepatitis B core antibody, hepatitis C virus antibody or human immunodeficiency virus antigen/antibody; or prior HSCTs were all excluded. Patients were also required to have either BM available from an HLA-matched related or unrelated donor or G-CSF-mobilized PBSCs available from an HLA-matched related donor without T-cell depletion. The study was conducted in conformity with ICH-GCP and the Declaration of Helsinki. The protocol and informed consent forms were approved by each institution's Research Ethics Committee. All patients gave written informed consent prior to their participation in the study.

Conditioning regimen

The i.v. BU (KRN246; Kirin Pharma Co. Ltd., Tokyo, Japan) was given at 0.8 mg/kg through a central venous catheter for 2 h every 6 h at a total of 16 doses for 4 days on days −7 to −4. CY 60 mg/kg was administered through a central venous catheter for 3 h at a total of two doses for 2 days on days −3 and −2. After a rest on day −1, BM or G-CSF-mobilized PBSC without T-cell depletion was infused on day 0. A fixed-dose regimen for BU was calculated based on either the ideal body weight or actual body weight, whichever was less, for adults (18–55 years of age) and the actual body weight for children (over 5 and less than 18 years of age).

Supportive care

For seizure prophylaxis, phenytoin was administered at 5–10 mg/kg/day (upper limit of 300 mg/kg/day) in 2–3 divided doses starting from 2 days before initiation (day −9) to 48 h after completion of BU administration (day −2). G-CSF was administered on day 1 or 5 until engraftment. For patients undergoing allogeneic HSCT, GVHD prophylaxis consisted of CYA (3 mg/kg/day by continuous i.v. infusion from day −1 in related and 3–5 mg/kg/day in unrelated transplantation) and short-term methotrexate, that is, 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6 in related pairs or 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3, 6 and 11 in unrelated pairs. Mesna was administered at a dose equivalent to 120% of CY on days −3 and −2. Other supportive treatments including antiemetic administration, antibiotic treatment, transfusion support, GVHD treatment and VOD treatment were given according to the standards of each hospital.

Evaluation of clinical data

The efficacy variables were myeloablation, engraftment, relapse, overall survival (OS) and disease-free survival (DFS). The safety variables were non-relapse mortality and adverse events included convulsive seizure, VOD, acute GVHD and other organ toxicities. Engraftment was defined as an absolute neutrophil count of 0.5×10^9/l for three consecutive days. Engraftment failure was defined as the failure to reach an absolute neutrophil count of 0.5×10^9/l by day 28 after transplantation. OS was measured as the time from the day of transplantation until death from any cause, and DFS as the time from the day of transplantation until disease relapse or death from any cause. Relapse, OS and DFS were calculated using the Kaplan–Meier method.[23] non-relapse mortality was defined as any death without progression of the underlying disease. Patients were monitored daily for adverse events, hematology and transplant-related complications. After discharge, patients were followed weekly for adverse events and transplant-related complications, and monitored weekly for hematologic and biochemical data through 100 days after transplantation. The appearance of VOD by day 30 was evaluated based on any two of the major criteria as established by McDonald *et al.*[24] and Jones *et al.*[25] GVHD was graded according to the consensus criteria.[26,27] Kirin Pharma Co. Ltd. provided financial support for the medical costs associated with the conditioning regimen, including i.v. BU for enrolled patients, monitored source data and entered these data in a database. Statistical analysis was performed using SAS software (version 8.02; SAS Institute, Cary, NC, USA).

PK sampling and analysis

The objective of this study was to describe the PK characteristics of i.v. BU, with parameters including BU concentrations for the first and ninth administrations and the accumulation of i.v. BU. Plasma samples were collected from all patients at designated times, in conjunction with the first and ninth doses as follows: immediately before drug infusion and at 15, 30 and 45 min after the start of infusion, at 5 min before the end of infusion and at 15, 30, 60, 120, 180 and 240 min after completion of infusion. In addition, one sample was taken immediately before the 13th infusion and 5 min before its completion. The plasma was assayed using a gas chromatographic-mass spectrometric detection method.[10]

Plasma concentrations for first and ninth dose in individual subjects were analyzed by the non-compartmental method using WinNonlin (version 3.3; Pharsight Corp., Mountain View, CA, USA). The maximum plasma concentration (C_{max}) and the time to reach maximum plasma drug concentration (t_{max}) were observed values. The terminal half-life ($t_{1/2}$) was calculated as $\ln 2/k_{el}$, where k_{el} was the elimination rate constant, determined by log-linear regression of the terminal phase data points. The area under the plasma concentration–time curve from time 0 to infinity (AUC_{inf}) for the first dose was calculated as $AUC_{0-t} + C_t/k_{el}$, where AUC_{0-t} was the AUC from time 0 to the last detectable time, calculated using linear trapezoidal rule, and C_t was the plasma concentration at

the last detectable time. AUC at steady state (AUC_{ss}) for the ninth dose was calculated by the linear trapezoidal rule. Clearance (CL) was calculated as dose/AUC. Volume of distribution (V_z) was calculated as CL/k_{el}. CL and V_z were normalized to actual individual body weight (CL/ABW and V_z/ABW) on the day of dosing. Summary statistics were obtained for C_{max}, t_{max}, $t_{1/2}$, AUC, CL/ABW and V_z/ABW at the first and ninth dose. The AUC at dose 1 (AUC_{inf}) and dose 9 (AUC_{ss}) and the trough concentration ($C_{p,trough}$) and peak concentration ($C_{p,peak}$) at doses 9 and 13 were calculated and compared by preparing each plot.

Results

Patient characteristics

Thirty Japanese patients were registered in this prospective trial between July 2002 and October 2003. The disease characteristics and status at transplantation are given in Table 1. The median age of the patients was 30 years (range, 7–53 years). The median body mass index (BMI) was 22.65 (14.4–29.1), and the mean BMI was 22.32 ± 3.47. There were no patients with moderate or severe obesity (BMI <30). The diseases were AML in 13 patients (43%), ALL or CML in chronic phase in five patients each (17%), non-Hodgkin lymphoma (NHL) in four patients (13%) and MDS in three patients (10%). In total, 11 of the 12 patients with AML were in CR. Four of the five patients with ALL were in CR. Three patients with MDS included refractory anemia, refractory anemia with excess blasts and refractory anemia with excess blasts in transformation. Four patients with NHL included diffuse large B-cell lymphoma in CR ($n=2$), primary refractory peripheral T-cell lymphoma ($n=1$) or suspected extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue in CR ($n=1$). One patient with AML who was in remission at registration was subsequently withdrawn from protocol treatment due to onset of cardiac myopathy on day −3, and CY was changed to fludarabine. Owing to an additional protocol violation, this patient was excluded from the objective group in the analysis.

Table 1 Patient characteristics

Variables	n *(%)*	
	Allogeneic HSCT (n=28)	*Autologous HSCT (n=2)*
Patient age (years) (range, median)	7–53, 30	48–50, 49
5–17	3 (11)	0
18–49	20 (71)	1 (50)
50–55	5 (18)	1 (50)
Gender		
Men	18 (64)	2 (100)
Women	10 (36)	0
Disease		
AML	12 (43)	1 (50)
ALL	5 (18)	0
CML	5 (18)	0
Myelodysplastic syndrome	3 (11)	0
Non-Hodgkin lymphoma	3 (11)	1 (50)
Disease status		
CR, CP, RA	23 (82)	2 (100)
NR, RAEB, RAEB-t	5 (18)	0
Prior chemotherapy	26 (93)	2 (100)
Prior radiotherapy	2 (7)	0
Source of stem cells		
BM	18 (64)	0
Peripheral blood cells	10 (36)	2 (100)
Related or unrelated donor		
Related	19 (68)	NA
Unrelated	9 (32)	NA
Cell dose infused		
Nucleated (× 10^8/kg, median, range)	2.6 (0.7–4.4)	NA
CD34 positive (× 10^6/kg, median, range)	2.7 (2.1–6.3)	2.9 (2.7–3.1)

Abbreviations: CP = chronic phase; HSCT = hematopoietic SCT; NA = not applicable; NR = non-remission; RA = refractory anemia; RAEB = refractory anemia with excess of blasts; RAEB-t = refractory anemia with excess of blasts in transformation.

Engraftment

Twenty-eight patients (97%) achieved engraftment at a median of 14 days (range, 9–20 days) and 11 days after allogeneic and autologous HSCT, respectively (Table 2). One patient who received unrelated BMT for CML had graft failure. No secondary engraftment failure was observed.

Toxicity and complications

All adverse events were those that are commonly observed in HSCT and no characteristic events related to i.v. BU were observed. None of the patients had to interrupt i.v. BU treatment because of adverse events. The number of observed adverse events was 714 in 27 patients who received allogeneic HSCT and 19 in two patients who received autologous HSCT. The most frequent adverse events in the 27 allogeneic HSCT patients were vomiting and nausea in 20 patients each (74%), anorexia in 19 patients (70%), stomatitis and diarrhea in 18 patients each (67%) and headache in 17 patients (63%; Table 2). Both of the autologous HSCT patients showed stomatitis, vomiting, catheter-related infection, anorexia and dysgeusia. No seizures were observed, and with regard to other neuropsychological profiles, seven patients experienced mild dysgeusia, one moderate systemic burning sensation, one severe tremor, one severe mood change and one severe insomnia in an allogeneic setting. With regard to cardiovascular profiles, one patient experienced mild cardiac failure and the other developed moderate cardiomyopathy due to CY in the allogeneic setting, as described above. This patient had completed i.v. BU administration for 4 days and CY once. When the patient complained of chest discomfort, the heart rate was 101 beats/min, and her electrocardiography showed ST depressions in leads II, III, aVF and V_1–V_6 1 h after the completion of the first dose of CY, which made suspected diagnosis of CY-induced cardiomyopathy. The signs and symptoms subsided shortly, and the second dose of CY on day −2

Table 2 Regimen-related toxicity, engraftment, GVHD and death

Outcome	*Allogeneic HSCT (n=28) (%)*	*Autologous HSCT (n=2) (%)*
Toxicity		
Vomiting	21 (75)	2 (100)
Nausea	21 (75)	1 (50)
Anorexia	19 (68)	2 (100)
Stomatitis	18 (64)	2 (100)
Diarrhea	18 (64)	0 (0)
Headache	18 (64)	0 (0)
Seizure	0 (0)	0 (0)
VOD	1 (4)	0 (0)
	Allogeneic HSCT (n=27) (%)	*Autologous HSCT (n=2) (%)*
Engraftment	26 (96)	2 (100)
Median (days)	14	11
Range (days)	9–20	11
Graft failure	1 (4)	0 (0)
Acute GVHD	13 (48)	—
Grade I	4 (15)	—
Grade II	5 (19)	—
Grade III	2 (7)	—
Grade IV	2 (7)	—
Chronic GVHD	16 (59)	—
Death	8 (30)	0 (0)
Relapse	4 (15)	0 (0)
Non-relapse	4 (15)	0 (0)

Abbreviations: HSCT = hematopoietic SCT; VOD = venoocclusive disease.

was substituted by fludarabine with no subsequent complications.

One patient who received allogeneic HSCT was diagnosed with mild VOD on day 1 based on two diagnostic criteria,[24,25] which resolved on day 3. In another patient, elevated total bilirubin and body weight gain were found on days 60–69, and this was not confirmed to be VOD based on these criteria. Opportunistic infection occurred in 16 of 27 patients (59%), with a median onset of day 113 (range, 7–399). Pulmonary complications occurred in 7 of 27 patients (26%), with a median onset of day 149 (range, 65–335).

GVHD

Acute GVHD occurred in 13 of the 27 patients (48%) who received allogeneic HSCT; four (15%) had grade I, five (19%) grade II and two each (7%) grades III or IV (Table 2). Acute GVHD was documented in 7 of the 19 patients (37%) who received related transplantation (six had grades II–IV), and in six of the eight patients (75%) who received unrelated transplantation (three patients had grades II–IV). Acute GVHD occurred with a median onset of day 45 (range, 7–98). Chronic GVHD occurred in 16 of 27 patients (59%) with a median onset of day 133 (range, 39–239).

Causes of death

Four patients (15%) died of non-relapse causes (Table 2). One patient who received allogeneic HSCT died of multi-

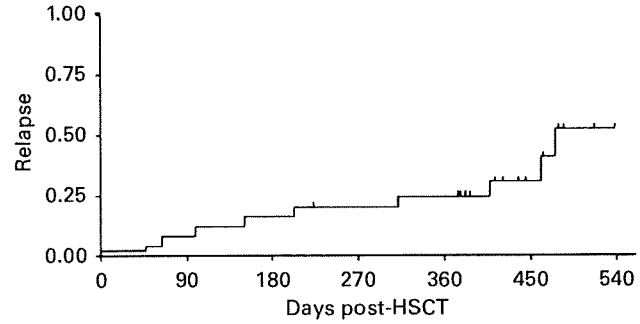

Figure 1 Disease relapse after i.v. BU and CY prior to allogeneic hematopoietic SCT in patients with leukemia and lymphoma.

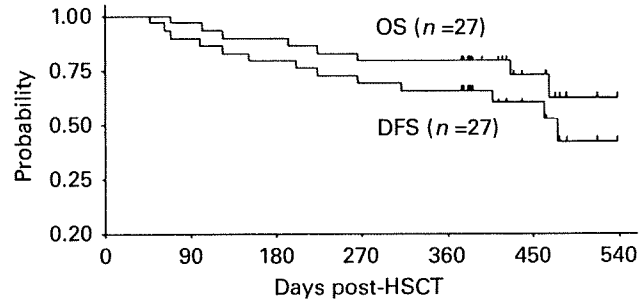

Figure 2 Overall survival and disease-free survival after i.v. BU and CY prior to allogeneic hematopoietic SCT in patients with leukemia, myelodysplastic syndrome and lymphoma.

organ failure due to aggravated GVHD on day 69. Three patients who received allogeneic HSCT died of chronic GVHD on day 223, hepatic failure due to unknown reasons on day 266 (with extensive chronic GVHD and methicillin-resistant *staphylococcus aureus* (MRSA) pneumonia) and pneumonia due to adenovirus and cytomegalovirus on day 124. Four patients (15%) died of relapse.

Relapse and survival

Relapse occurred in 9 of the 23 evaluable allogeneic HSCT patients with leukemia and lymphoma (39%). None of the 23 evaluable patients had central nervous system relapse. The relapse rates at days 100 and 365 were 18% (95% confidence interval (CI), 0–38%) and 26% (95% CI, 8–45%), respectively (Figure 1). The median day of relapse was day 202 (range, 46–476).

OS at days 100 and 365 in allogeneic HSCT was 96% (95% CI, 88–100%) and 78% (95% CI, 62–94%), respectively, with the median follow-up of 413 days (range, 69–537 days) (Figure 2). The median day of death in eight allogeneic HSCT patients was day 208 (range, 69–467). DFS at days 100 and 365 in allogeneic HSCT was 81% (95% CI, 63–99%) and 63% (95% CI, 45–81%), respectively (Figure 2). The two autologous HSCT patients were alive disease-free at day 365.

PK analysis

Intensive PK sampling was assessed at doses 1 and 9 of i.v. BU, and peak and trough levels were obtained at dose 13. Although these analyses were completed in all 30 patients,

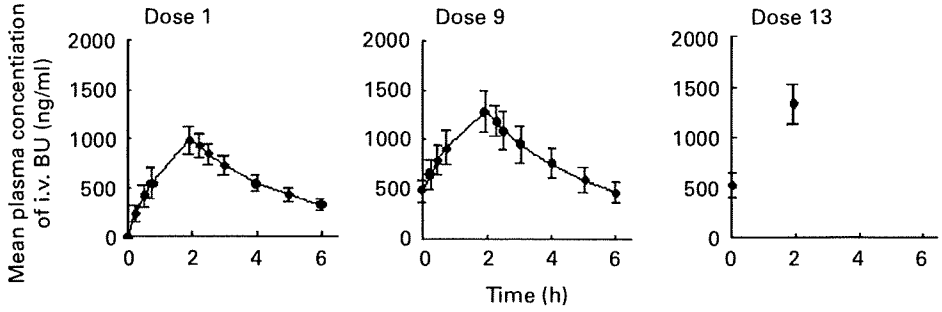

Figure 3 Pharmacokinetic results of i.v. BU at doses 1, 9 and 13 ($n = 30$).

data from one patient were excluded from the objective analysis group as noted above. All PK parameters for dose 1 were obtained from 29 patients. For dose 9, all PK parameters except for C_{max} and t_{max} were obtained from 28 patients because the last sample for one patient was collected after initiation of the next dose (Figure 3). The documented plasma concentration of i.v. BU increased over the 2-h period of infusion, with C_{max} observed in the last 5 min, and this was followed by a rapid decrease. The profile of trough and peak levels was essentially the same between doses 9 and 13.

The resulting parameters are listed in Table 3. The mean AUC for doses 1 and 9 was 1171 μmol min/l (coefficient of variation (CV) = 19%) and 1242 μmol min/l (CV = 17%), and the mean C_{max} was 994 ng/ml (CV = 12%) and 1311 ng/ml (CV = 15%), respectively. The mean CL/ABW was 2.66 ml/min/kg (CV = 17%) and 2.46 ml/min/kg (CV = 15%), respectively. V_z/ABW was 0.60 l/kg (CV = 9%) and 0.60 l/kg (CV = 11%), respectively. The AUC of the initial dose was below 1500 μmol min/l in 27 patients (90%), and this was within the range of 900–1350 μmol min/l in 21 of the 29 patients (72%).

The AUC for doses 1 and 9 are compared in Figure 4, which supports both intra- and interpatient predictability and consistency. In the patient who developed VOD, the AUC for doses 1 and 9 was 1102 and 1181 μmol min/l, respectively, whereas for the remaining patients without VOD, it was 1173 μmol min/l (CV = 19%) and 1244 μmol min/l (CV = 17%).

Pediatric patients

A 7-year-old girl with AML in first remission received allo-BMT from a matched unrelated donor. Her body weight and BMI were 17.8 kg and 14.4, respectively. Her AUC was 963.9 μmol min/l. Her regimen-related toxicities were grade 3 vomiting and grade 2 acute hemorrhagic gastritis and hypoalbuminemia. She is alive without graft failure or relapse.

A 13-year-old boy with CML in first chronic phase received allo-BMT from a matched unrelated donor. His body weight and BMI were 46.7 kg and 18.8, respectively. His AUC was 932.6 μmol min/l. His regimen-related toxicities were grade 4 anorexia and grade 2 fatigue and vomiting. He did not achieve engraftment by day 28, and he soon received a second allo-BMT from a mismatched

Table 3 Pharmacokinetics of i.v. BU ($n = 30^a$)

	C_{max} (ng/ml)	$t_{1/2}$ (h)	AUC (μmol min/l)	CL/ABW (ml/min/kg)	V_z/ABW (l/kg)
Dose 1					
Mean	999	2.64	1171	2.67	0.596
Median	997	2.66	1144	2.65	0.596
s.d.	124	0.41	216	0.44	0.054
Maximum	1320	3.52	1698	3.72	0.716
Minimum	796	1.97	811	1.94	0.483
Dose 9					
Mean	1317	2.86	1247	2.46	0.601
Median	1315	2.82	1198	2.36	0.605
s.d.	192	0.37	205	0.36	0.068
Maximum	1720	3.59	1686	3.05	0.786
Minimum	964	2.27	889	1.80	0.466

Abbreviations: ABW = actual body weight; AUC = area under the plasma concentration–time curve; CL = clearance; C_{max} = maximum plasma concentration; s.d. = standard deviation; $t_{1/2}$ = terminal half-life; t_{max} = time to observed maximum plasma concentration from dosing; V_z = volume of distribution.

[a]For dose 9, all PK parameters except for C_{max} and t_{max} were obtained from 29 patients because the last sample for one patient was collected after initiation of the next dose.

For dose 1, AUC_{inf} is shown; for dose 9, AUC_{ss} for the 6-h dosing interval is presented.

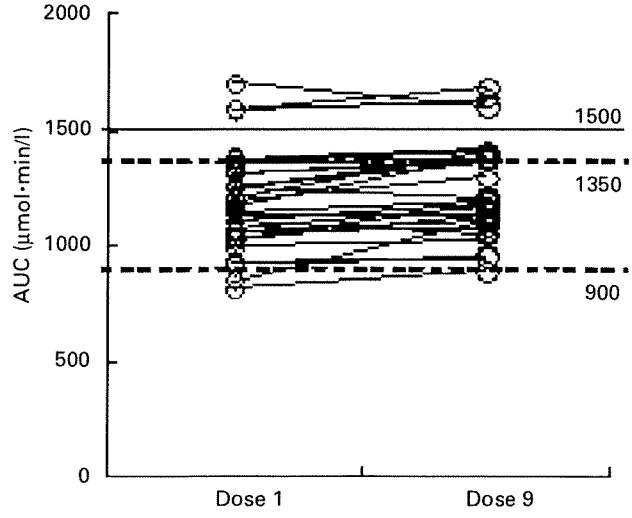

Figure 4 Individual patient area under the plasma concentration–time curve (AUC) values of i.v. BU at doses 1 and 9 ($n = 29$).

related donor. He is alive without graft failure or relapse after the second transplant.

A 17-year-old woman with AML in first relapse received allo-BMT from a matched unrelated donor. Her body weight and BMI were 43.2 kg and 17.3, respectively. Her AUC was 902.7 μmol min/l. Her regimen-related toxicities were grade 4 thrombocytopenia, grade 3 febrile neutropenia and grade 2 nausea, vomiting and stomatitis. She died of disease progression on day 193.

Discussion

It has been reported that a high steady-state concentration of BU causes toxicities including VOD,[5–10] whereas a low steady-state concentration leads to graft rejection[10–15] or relapse/progression of the disease.[11] Targeted dose adjustment of BU to maintain the overall systemic exposure within a proper range may reduce these risks.[4–7,14,15] Although it has been reported that there are ethnic differences in PK for a wide range of drugs,[28] this has not been seriously examined with i.v. BU. Therefore, we conducted this drug bioavailability study in a Japanese population. The data obtained were compared with those published mostly overseas. In this study, all observed treatment-related toxicities were as expected, with a low incidence of severe complications. One patient was clinically diagnosed with VOD. This patient showed body weight gain, liver enlargement and right upper abdominal pain, but had no jaundice. As his body weight returned to the baseline within 2 days, this could have been due to overhydration. One patient who developed graft failure had CML and underwent unrelated BMT following interferon therapy, all of which are well-known risks of graft failure.[10,29] The incidence of relapse and the survival rate in this study were similar to those in previous studies.[11,19]

In studies with an oral preparation of BU, it was unclear whether plasma levels of BU correlate with severe regimen-related toxicities.[4,6–8,11] In the pivotal study for US approval of i.v. BU, plasma levels of BU exceeded 1500 μmol min/l in two of the five patients who developed VOD,[19] whereas in our study there was no case of VOD in three patients who had a level over 1500 μmol min/l. This may suggest an ethnic difference in the PK of BU. On the other hand, a population pharmacokinetic analysis of i.v. BU is rare.[30] Our earlier small-scale study revealed high inter- and intrapatient consistency for i.v. BU pharmacokinetics.[22] However, the value of therapeutic drug monitoring remains crucial. Our study demonstrated no essential difference in PK analysis from earlier published Western data,[19] and this supports the notion that racial factors may not seriously influence the bioactivity of i.v. BU.

Acknowledgements

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ORIGINAL ARTICLE

Oral valganciclovir as preemptive therapy is effective for cytomegalovirus infection in allogeneic hematopoietic stem cell transplant recipients

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Abstract Between March 2007 and January 2008, the safety and efficacy of oral valganciclovir (VGC) preemptive therapy for cytomegalovirus (CMV) infection was evaluated in ten consecutive patients who received allogeneic hematopoietic stem cell transplantation (HSCT). Patients were screened once or twice per week after engraftment using CMV pp65 antigenemia assay. When more than 2 CMV antigen-positive cells per 50,000 leukocytes were detected, preemptive therapy with oral VGC was initiated at a dose of 900 mg twice daily for 3 weeks. Nine patients (90%) completed the 3-week VGC treatment except for one patient who developed febrile neutropenia. There was no other significant toxicity. CMV antigen-positive cells were rapidly decreased in all nine patients and became undetectable by the end of the VGC treatment. None of the patients developed CMV disease. CMV infection relapsed in four of the ten patients (40%) after the VGC treatment. These observations suggest that preemptive therapy with VGC is effective for preventing CMV disease in allogeneic HSCT patients. Further studies with a large number of patients will be necessary to determine the optimal initial- and maintenance-dose of VGC.

Keywords Allogeneic hematopoietic stem cell transplantation · Cytomegalovirus infection · Preemptive therapy · Valganciclovir

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1 Introduction

Despite improvement in the treatment of cytomegalovirus (CMV) infection and CMV disease with ganciclovir (GCV) and/or foscarnet, CMV disease is still a major cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT) [1–4]. Major risk factors for CMV disease include CMV seropositivity before transplantation, development of graft-versus-host disease (GVHD), unrelated donor transplantation, and T cell depleted transplantation [3, 5–7]. In addition, new transplantation modalities such as nonmyeloablative conditioning regimens consisting of intensive immunosuppression increase the risk of late-onset CMV infection and CMV disease [2, 8]. Therefore, extended prevention of CMV disease may be required, especially for high-risk recipients, not only those within 100 days after HSCT but also those in the later period after HSCT [8–10]. Currently, the prevention of CMV disease involves general prophylaxis and preemptive therapy. Preemptive therapy is based on the early detection of CMV infection by virus surveillance, by monitoring with either CMV antigenemia assay or PCR techniques and followed by immediate treatment with anti-CMV drugs

[4, 11–13]. Intravenous GCV (IV-GCV) and/or foscarnet are commonly used for preemptive therapy and are effective for decreasing the incidence of early CMV disease [11, 13, 14]. However, these antiviral treatments are given intravenously and often require hospitalization, as well as high costs and IV-related complications.

Valganciclovir hydrochloride (VGC) is an oral valine-ester GCV prodrug with a tenfold higher bioavailability than oral GCV, and it is rapidly hydrolyzed to GCV after oral administration. VGC and IV-GCV have similar efficacy in the treatment of CMV retinitis in HIV-infected patients and in preemptive CMV treatment in solid organ (heart, renal, and renal-pancreas) transplant patients [15–19]. Recently, several studies have shown the efficacy of VGC for preemptive therapy in allogeneic HSCT patients [20–23]. We evaluated the safety and efficacy of oral VGC as preemptive therapy for CMV reactivation in ten allogeneic HSCT patients.

2 Patients and methods

2.1 Patients

This was a prospective multicenter study with VGC. The study patients were adults who had received an allogeneic bone marrow or peripheral blood stem cell transplant. Patients were eligible when they screened for CMV infection using CMV pp65 antigenemia assay and more than two CMV antigen-positive cells were detected. Patients unable to take oral medication, and those who impaired renal function (serum creatinine level >2.0 mg/dL) were ineligible. Patients, who developed CMV disease, had received antiviral agents other than acyclovir and who developed more than stage 2 gastrointestinal GVHD were also ineligible. Ten consecutive patients who received allogeneic HSCT at Kyushu University Hospital and Hamanomachi General Hospital between March 2007 and January 2008 were included in the study (Table 1). This study was approved by Institutional Review Board of each institute and a written informed consent was obtained from each participating patient.

Eight patients had acute myeloid leukemia, one had myelodysplastic syndrome, and one had non-Hodgkin's lymphoma. The median age of the patients at the time of transplantation was 56 years (range 33–63). They received bone marrow grafts from an HLA-matched sibling donor ($n = 1$), a matched unrelated donor ($n = 8$), or an HLA-1 locus mismatched unrelated donor ($n = 1$). All of the patients were CMV seropositive before transplantation. Nine patients received myeloablative preparative regimens including total body irradiation/cyclophosphamide (Cy) in five patients and busulfan (BU)/Cy in four patients.

Table 1 Patient characteristics

Number of patients	10
Median age, years (range)	56 (33–65)
Diagnosis	
Acute myeloid leukemia	8
Myelodysplastic syndrome	1
Non-Hodgkin's lymphoma	1
Stem cell source	
HLA-identical sibling bone marrow	1
HLA-matched unrelated bone marrow	8
HLA-mismatched unrelated bone marrow	1
CMV serologic status	
Donor + /Recipient +	9
Donor −/Recipient +	1
Preparative regimens	
TBI/Cy	5
Bu/Cy	4
Flu/Bu/TBI	1
GVHD prophylaxis	
Taclorimus + MTX	9
CSP + MTX	1
Acute GVHD prior to CMV reactivation	
Grade I	1
Grade II	7
Grade III	2
PSL treatment at the time of starting VCG	8

Bu busulfan, *CMV* cytomegalovirus, *CSP* cyclosporine, *Cy* cyclophosphamide, *Flu* fludarabine, *GVHD* graft-versus-host disease, *TBI* total body irradiation, *MTX* methotrexate, *PSL* prednisolone, *VGC* valganciclovir

The remaining patient received a fludarabine-based reduced-intensity conditioning regimen. GVHD prophylaxis consisted of taclorimus/short-term methotrexate (MTX) ($n = 9$) or cyclosporine/short-term MTX ($n = 1$). Patients who developed grade II–IV acute GVHD were given methylprednisolone (mPSL) or prednisolone (PSL) at a dose of 1 or 2 mg/kg. Acyclovir was administered orally (1,000 mg/day) or intravenously (500 mg/day) from days −7 to 35 as a prophylaxis against herpes simplex infection.

2.2 CMV antigenemia assay

CMV antigenemia assay was determined as previously described [7, 24]. In brief, peripheral blood leukocytes isolated from 3 mL of EDTA-treated blood were applied to slides by centrifugation and fixed with cold acetone. The slides were stained using a direct immunoperoxidase technique that employed the peroxidase-conjugated monoclonal antibody HRP-C7 (Teijin, Tokyo, Japan) against the CMV pp65 antigen. CMV antigen-positive cells were counted under a light microscope and the results were

expressed as the number of CMV antigen-positive cells per 50,000 leukocytes.

2.3 Definition of CMV infection and CMV disease

A positive test for CMV antigenemia was defined as the presence of one or more CMV antigen-positive cells per 50,000 leukocytes. CMV infection was considered in patients with a positive test for CMV antigenemia. CMV disease was diagnosed according to published recommendations [25]. Patients with clinical manifestations of CMV disease, such as interstitial pneumonia and gastroenteritis in the presence of CMV infection, were examined histopathologically and immunochemically from biopsy specimens.

2.4 Preemptive therapy with VGC for CMV infection

Monitoring with CMV antigenemia assay was performed at least once per week after engraftment until day 100 after HSCT and once every other week thereafter. Preemptive therapy with VGC for CMV infection was initiated at the time of the first detection of more than two CMV antigen-positive cells per 50,000 leukocytes. VGC was administered orally at a dose of 900 mg twice daily for 3 weeks. The dose was adjusted for patients with impaired renal function according to the manufacturer's recommendation. Acyclovir for the prophylaxis against herpes simplex infection was discontinued when VGC treatment was started. Supplemental immunoglobulin was administered only when a total IgG level was less than 400 mg/dL.

2.5 Endpoints and definitions

The primary endpoint was the rate of complete response of the VGC preemptive therapy to the CMV infection. The efficacy of VGC was monitored weekly using a CMV antigenemia assay. A complete response was defined as the conversion from positive to negative CMV antigenemia test results at the completion of the treatment. Patients who persistently showed positive test results for CMV antigenemia after 3 weeks of preemptive therapy or developed CMV disease during the period of preemptive therapy were considered a treatment failure.

The secondary endpoints included the safety of preemptive therapy, the incidence of CMV disease during VGC treatment, and the incidence of a recurrent CMV reactivation after the completion of VGC treatment. The patients were monitored with the CMV antigenemia assay for 5 weeks after the completion of the VGC treatment. At least once per week, a safety analysis was conducted. The analysis included the monitoring of blood counts, liver and renal function tests, and documenting other unexpected side effects. The incidence of CMV disease was evaluated for the entire period of the study. The incidence of recurrent reactivation of CMV infection after the VGC preemptive therapy was based on the conversion from negative CMV antigenemia to positive CMV antigenemia test results with more than two CMV antigen-positive cells per 50,000 leukocytes during the 5-week follow-up period.

3 Results

3.1 CMV infection and VGC preemptive therapy

Forty-seven patients received allogeneic bone marrow/peripheral blood stem cell transplants at these two institutes during the study period. Thirty-one patients showed positive CMV antigenemia test results after transplantation. Ten patients were enrolled into this study, but the remaining 21 patients were not enrolled mostly by their inability to take oral medication. Ten enrolled patients were given preemptive therapy with VGC for CMV infection (Table 1). All patients were CMV seropositive before transplantation, and nine donors were also CMV seropositive. In these patients, more than 2 CMV antigen-positive cells per 50,000 leukocytes were detected after a median of 69 days (range 22–252) following transplantation. The median number of CMV antigen-positive cells at the initiation of VGC therapy was 5 per 50,000 leukocytes (range 3–59). All of the patients developed acute GVHD prior to CMV infection after a median of 23 days (range 11–135). The severity of acute GVHD was grade I in one patient, grade II in seven, and grade III in two. Eight patients received mPSL or PSL for the treatment of acute GVHD. Preemptive therapy with VGC was started within five days after the detection of CMV antigen-positive cells. Nine patients completed 21 days of VGC treatment, whereas one patient failed to complete the therapy because of the development of grade 4 neutropenia and subsequent febrile neutropenia. Patients were followed at least 5 weeks after the completion of VGC preemptive therapy. The median follow-up was day 122 (range 41–355).

3.2 Response to VGC preemptive therapy

All patients showed negative test results for CMV antigenemia within 3 weeks after the initiation of the VGC treatment. In nine patients, CMV antigen-positive cells became negative within 2 weeks (Fig. 1). The remaining patient, who had 60/50,000 CMV antigen-positive cells at the time of initiation of VGC treatment, took 3 weeks to clear CMV antigen-positive cells. None of the patients required other anti-CMV agents. None of the patients developed CMV disease during the preemptive therapy or

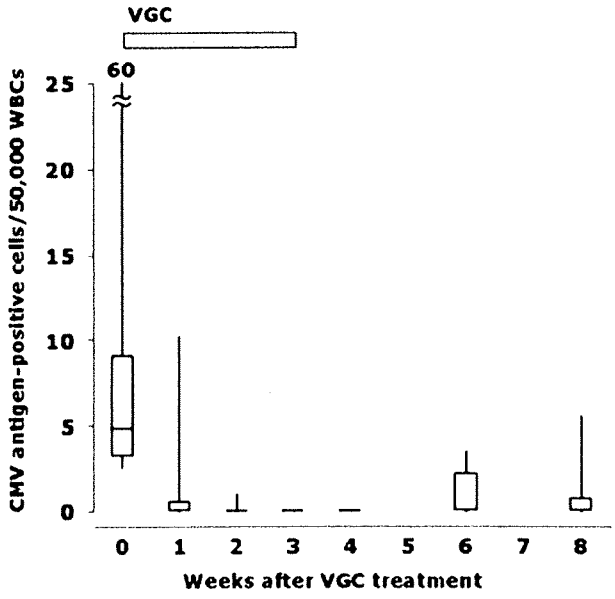

Fig. 1 Time course of the number of cytomegalovirus (CMV) antigen-positive cells after valganciclovir treatment. CMV antigenemia was reduced during treatment with valganciclovir. The *box plots* display the median, the 25th and 75th percentiles (*box*), and the smallest and largest values (*longitudinal line*). One patient discontinued valganciclovir on day 18 due to grade 4 neutropenia

in the subsequent 5 weeks after the completion of the VGC treatment.

CMV infection relapsed in four of the ten patients within 3–5 weeks after the completion of the preemptive VGC therapy. These four patients were successfully treated with IV-GCV.

3.3 Toxicity

Nine patients completed a 21-day course of VGC treatment, but one patient discontinued VGC due to grade 4 neutropenia. Due to impaired renal function (serum creatinine level, 1.68 mg/dL), this patient received a reduced VGC dose of 450 mg once per day for the first week. Renal function improved with the reduced dose, and the VGC dosage was increased to 450 mg twice per day in the second week of treatment. However, this patient developed grade 4 neutropenia (absolute neutrophil counts 0.17×10^9/L) after 17 days of treatment and then developed febrile neutropenia. The VGC was discontinued, and the patient immediately received granulocyte-colony stimulating factor (G-CSF) and antibiotic therapy. Neutrophil counts recovered to more than 1.0×10^9/L, and neutropenia resolved after five days. Recurrent CMV reactivation was not observed in this patient during the follow-up period. None of the patients developed thrombocytopenia (platelet count $<30 \times 10^9$/L)(Fig. 2).

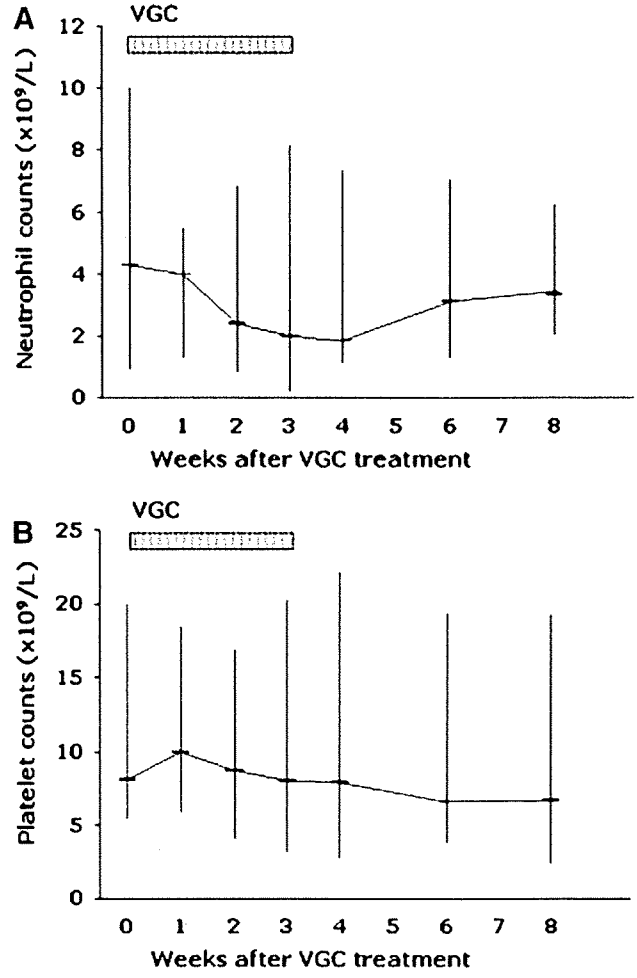

Fig. 2 Time course of neutrophils and platelets during valganciclovir treatment. Time course of neutrophil (**a**) and platelet numbers (**b**) during treatment with valganciclovir. The bar graph displays the median (*horizontal line*), and the smallest and largest values (*longitudinal line*). One patient discontinued valganciclovir on day 18 due to grade 4 neutropenia

Table 2 Adverse events other than hematological toxicities related to valganciclovir

Adverse events		No. of cases
Gastrointestinal		
Diarrhea	Grade 1	1/10
Hepatic		
AST/ALT	Grade 1	3/10

None of the patients experienced renal toxicity during the VGC treatment. Three patients developed grade 1 liver dysfunction, and one patient had grade 1 diarrhea (Table 2). However, none of these complications required discontinuation of the VGC.

4 Discussion

Effective preemptive therapy with IV-GCV reduced the incidence of early CMV disease to 5–10%; however, the risk of late CMV disease beyond day 100 after transplantation has increased over the past few years. Therefore, extended CMV monitoring beyond day 100 is currently recommended, especially in high-risk patients [2, 8]. There is a need for an effective oral anti-CMV drug that can be used for outpatient care. Oral VGC could be a useful alternative to IV-GCV in patients who require preemptive therapy for CMV infection. This study demonstrated the efficacy and safety of preemptive VGC therapy for CMV infection after allogeneic HSCT. There are four published studies that have shown the safety and the efficacy of VGC as preemptive therapy after allogeneic HSCT [20–23]. Although dosage and duration of the drug varied between studies, VGC therapy resulted in a rapid decrease of the viral load in all of the patients. In this study, we administered a dose of 900 mg twice daily for 3 weeks, and corroborated the efficacy and the tolerability of preemptive VGC therapy.

We demonstrated that VGC at a dose of 900 mg twice per day was effective and resulted in a rapid clearance of CMV antigen-positive cells in all patients. No CMV disease developed during the preemptive therapy or the subsequent 5 weeks after the completion of treatment. VGC was well tolerated as 90% of the patients completed the entire treatment course. However, four of the ten patients developed a recurrent CMV reactivation after the discontinuation of VGC treatment, and they were all successfully treated with IV-GCV. Because a guideline for preemptive VGC therapy has not been established for patients that have received allogeneic HSCT, further studies will be necessary to determine the optimal initial- and maintenance-dose of VGC.

We, and four other groups, have obtained good results with VGC starting-doses of 900 mg twice per day [20–23]. This dose was based on observations from previous pharmacokinetics studies in HIV-infected patients and liver transplant recipients. A VGC dose of 900 mg results in an area under the concentration-time curve for GCV similar to that of 5 mg/kg IV-GCV [26, 27], which is the recommended standard dose for preemptive CMV therapy [28, 29]. One of the concerns of using VGC after allogeneic HSCT is the absorption of oral VGC in patients suffering from severe gastrointestinal GVHD. Recently, Einsele et al. [30] conducted a randomized crossover clinical trial of IV-GCV and VGC in patients with or without intestinal GVHD. The results showed that patients without intestinal GVHD who took VGC were exposed to more GCV when compared to those administered IV-GCV. This was also true in patients with grade I and II intestinal GVHD. Thus, VGC may be as effective even in patients developing a mild form of intestinal GVHD as in patients without intestinal GVHD. However, a higher exposure of VGC may increase the toxicity of the drug, and the absorption of VGC was not evaluated in patients with severe intestinal GVHD. Recently, Candoni et al. [22] examined the efficacy of a lower dose of VGC. Preemptive therapy with 900 mg/day VGC was as effective for clearing CMV antigen-positive cells and preventing CMV disease as the standard dose of 1800 mg/day. These findings suggest that the initial dose of VGC could be reduced to 900 mg/day as preemptive therapy in low-risk patients.

The effective duration for preemptive VGC therapy is currently unclear. In the previous studies, patients received VGC for 2 weeks and then it was either discontinued or continued at a maintenance dose of variable duration dependant upon a negative CMV test result. Different from previous studies, we continued an initial dose of VGC for 3 weeks. The dosage and duration of VGC therapy likely affects the incidence of hematological toxicity such as neutropenia. In a study by Busca et al. [21], in which VGC was administered at a dose of 1,800 mg/day for 2 weeks, followed by 900 mg/day for an additional 2 weeks, 4 of the 15 patients failed to complete the 3-week scheduled therapy due to neutropenia and/or thrombocytopenia. In our study, only one of the ten patients failed to complete treatment. Thus, hematologic toxicity may be a significant problem after a 3 week treatment with VGC.

In our study, four of the ten patients treated with VGC developed recurrent CMV reactivation 3–5 weeks after the discontinuation of VGC. This was somewhat similar to the 10–53% recurrence rates in previous studies [20–23]. Thus, careful monitoring after the completion of VGC therapy is recommended. We continued an initial dose of VGC for 3 weeks. However, when considering hematological toxicity and frequent recurrence of CMV antigenemia, the duration of treatment and/or maintenance should be decided by monitoring CMV.

As previously reported [20–23], we found neutropenia to be the main toxic effect of VGC. One patient, who had impaired renal function before the preemptive therapy that required a dose reduction, discontinued the drug on day 17 due to grade 4 neutropenia. In high-risk patients, especially outpatient should be closely monitored, although any other toxicity profile different from IV-GCV was not observed in this study.

Our study demonstrated that the oral VGC preemptive therapy at a dose of 900 mg daily seemed to be as effective as conventional IV-GCV at a dose of 10 mg/kg daily to clear CMV antigen-positive cells. However, as shown in Fig. 1, CMV antigen-positive cells seem to decrease in numbers much faster after VGC treatment than those observed after standard dose of IV-GCV treatment.

Furthermore, hematological toxicities were considerable. Although pharmacokinetic data was not available in this study, these observations coincide with the previous pharmacokinetic study in HSCT recipients that showed the exposure of GCV after administration of 1800 mg daily VGC was significantly higher compared with 10 mg/kg IV-GCV even in patients without gastrointestinal GVHD [30]. Careful monitoring of neutrophil counts will be useful to improve the safety of VGC in HSCT recipients, especially with reduced renal function. Kanda et al. [14] showed the efficacy of response-oriented preemptive therapy using a low initial dose of IV-GCV that resulted in a successful reduction of the total dose of IV-GCV and decreased hematological toxicities. A lower dose of VGC could be also used as preemptive therapy by close CMV monitoring. Similar studies with a large number of patients will be required to define the optimal treatment schedule for preemptive VGC therapy.

Despite a limited number of patients, our results suggest that oral VGC is an effective alternative to IV-GCV for preemptive therapy to prevent CMV disease in allogeneic HSCT patients. Studies with a larger number of patients will be necessary to assess the efficacy and long-term effect of this preemptive therapy.

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LETTER TO THE EDITOR

Unexpectedly high AUC levels in a child who received intravenous busulfan before stem cell transplantation

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A 6-year-old boy underwent a craniotomy with subtotal resection of the cerebellar vermian tumor. Microscopic examination showed medulloblastoma. Brain magnetic resonance imaging after resection did not show residual disease. He subsequently received four cycles of ICE (ifosfamide, cisplatin, and etoposide) chemotherapy followed by whole craniospinal (24 Gy) and local (30 Gy) radiation therapy. He eventually received tandem high-dose chemotherapy. Conditioning regimens for the first and the second were carboplatin (1200 mg/m^2) + thiotepa (750 mg/m^2) and busulfan 1.1 mg/kg/dose intravenous every 6 h for total 16 doses (17.6 mg/kg) + melphalan 70 mg/m^2 once daily intravenous for 2 days (140 mg/m^2), respectively. Autologous PBSC were infused after high-dose chemotherapy. The numbers of CD-34-positive cells were 5.2×10^6/kg in the first and 7.0×10^6/kg in the second, respectively. Engraftment after the second autologous PBSCT was achieved on day 11. Brain magnetic resonance imaging on day 25 of the second autologous PBSCT showed no evidence of recurrence.

On day 64 of the second autologous PBSCT, he developed sudden dyspnea with 90% oxygen saturation in room air. A chest X-ray film showed a ground-glass appearance in both lungs. Chest computed tomography scan also showed bilateral areas of ground-glass opacities, and no centrilobular micronodule. Aspergillus, candida, and cryptococcal antigen for the serum were negative. The β-D-glucan level was significantly elevated (234.4 pg/ml). Intravenous cotrimoxazole therapy was initiated, because pneumocystis pneumonia was thought to have caused pneumonia based on the clinical course. He was placed on mechanical ventilation and methylprednisolone pulse therapy (30 mg/kg × 3 days) was started on day 72. His respiratory condition improved quickly with these treatments and mechanical ventilation was discontinued on day 77. Although polymerase chain reaction for *Pneumocystis jirovecii* in bronchoalveolar lavage fluid was negative, we diagnosed the cause of his pulmonary disorder had been pneumocystis pneumonia. His respiratory condition deteriorated again on day 82. Despite the second course of methylprednisolone pulse and continued cotrimoxazole therapy, he was placed on mechanical ventilation again on day 85 (Figure 1a). No pathogenic bacterial, fungal, or viral agents, including cytomegalovirus were identified by microbiological cultures and serological studies. The β-D-glucan level was also decreased to 33.4 pg/ml. Although various antibiotics, ganciclovir, antifungal drugs, and cotrimoxazole therapy were continued, his pulmonary oxygenation became worse day-by-day. He died on day 133 of the second autologous PBSCT because of respiratory failure (Figure 1b).

An autopsy was performed with the consent of his parents. Microscopically, organizing diffuse alveolar damage was seen, and nuclear enlargement, hyperchromasia, and pleomorphism were seen along alveolar and bronchial epithelium. This cytologic atypia was seen not only in the lung, but also in the urothelium of the renal pelvis. There was no evidence of bacterial, fungal, or viral infection. These pathological findings were consistent with busulfan-induced lung disease. Organizing diffuse alveolar damage is the most common manifestation of busulfan lung toxicity and is associated with bronchiolar and alveolar epithelium atypia.[1] This cytologic atypia is often seen extrapulmonary sites, including urinary bladder, breast, and uterine cervix.[1] The incidence of pulmonary toxicity after high-dose oral busulfan therapy before stem cell transplantation has been reported to be 3.6%.[2] Corticosteroids are effective for treating this disease to various degrees. Although some patients improve, others progress and die.[1]

It has become evident posthumously that busulfan areas under the drug plasma concentration–time curve (AUC) levels at the 1st and 9th doses were significantly elevated (2353 μM × min, 2347 μM × min) (target AUC; 900–1500 μM × min). Busulfan concentrations for the 1st and 9th doses and the accumulation of intravenous busulfan in plasma were assayed using a high-performance liquid chromatography system (Figure 2).[3] Plasma concentrations were analyzed by the non-compartmental method using WinNonlin (version 5.2.1; Pharsight Corp., Mountain View, CA, USA). The AUC from time 0 to infinity (AUC_{inf}) for the 1st dose and at steady state (AUC_{ss}) for the 9th dose was calculated using linear trapezoidal rule. The relationship between high busulfan AUC levels and the occurrence of busulfan-induced lung disease has not been established,[4] although high busulfan AUC levels are commonly associated with hepatic veno-occlusive disease.[5]

The cause of high busulfan AUC levels in our patient is still unclear. He did not have the distinct liver or renal disorder at the time of busulfan administration. Busulfan is metabolized in the liver through conjugation with glutathione by glutathione *S*-transferase (GST) enzymes.[6] GSTA1 is the predominant isoform of GST, which catalyzes the conjugation of busulfan with glutathione. Polymorphisms in GSTA1 are thought to be associated with alterations in the pharmacokinetics of busulfan.[7]

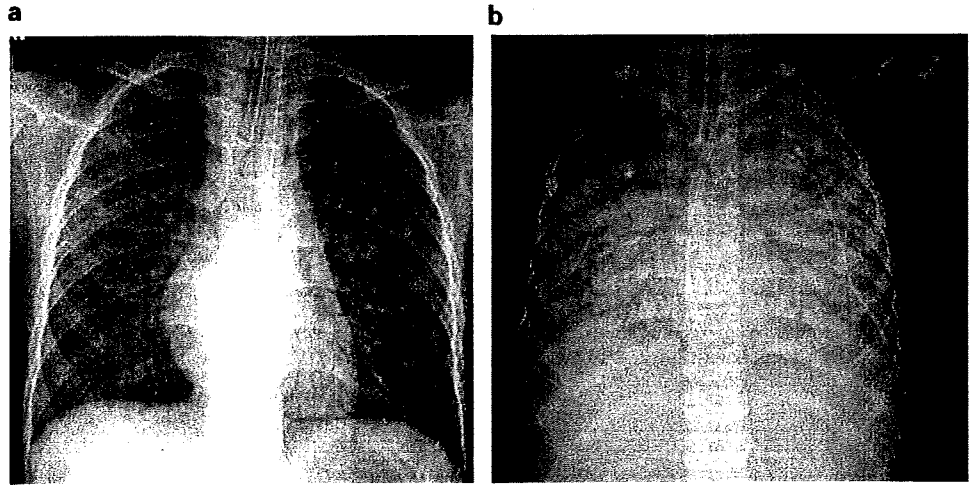

Figure 1 Chest X-ray films. (**a**) (On day 85) bilateral lung fields showed a ground-glass appearance. (**b**) (On day 133) bilateral lung fields showed marked radiopacity and air bronchograms.

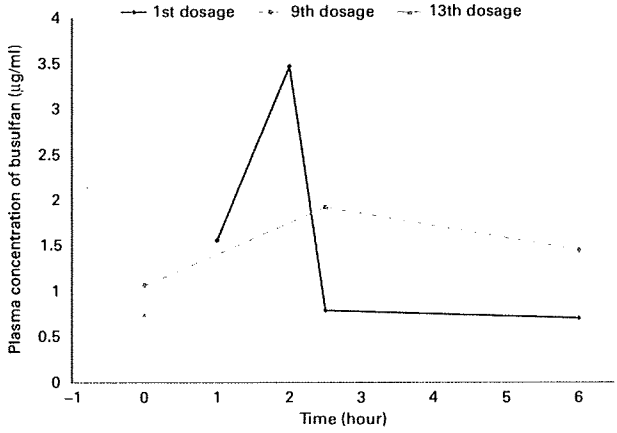

Figure 2 Plasma concentration of busulfan. Peak level of plasma concentration of busulfan is very high (1st dose). Busulfan clearance is poor (before 13th dose).

Johnson *et al.*[7] reported that the GSTA1*B variant had a 2.6-fold higher busulfan AUC level than other variants after intravenous busulfan exposure in the pediatric population. Our patient's genotype of the promoter region of GST A1 by DNA sequencing was GSTA1*A diplotype (-567T, -69C, -52G) which is thought to be more active than the GSTA1 *A/*B. Nevertheless, busulfan AUC levels were significantly elevated. This may indicate that polymorphisms other than GSTA1 polymorphisms may affect busulfan metabolism.

Intravenous busulfan should have a much more predictable pharmacokinetic profile than oral busulfan. Treatment with a fixed dose of 0.80 mg/kg intravenous busulfan achieved the target AUC level (900–1500 μM × min) in 80% of adult patients.[8] The remaining 20% were very close to achieving the target level.[8] A recent European study showed that 91% of children achieved target AUC levels by weight-based dosing.[9] They concluded that this weight-based dosing in children is sufficient without therapeutic drug monitoring and dose adjustment. Our patient received intravenous busulfan according to his body weight, as in the European study. However, his busulfan AUC levels were higher than has been reported elsewhere in the literature.[9,10] To avoid unexpectedly high busulfan AUC levels, therapeutic drug monitoring and dose adjustment should be recommended for all patients who are treated with high-dose busulfan. When therapeutic drug monitoring is not applicable, test dosing of intravenous busulfan before high-dose therapy would be preferable.

Conflict of interest

The authors declare no conflict of interest.

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