Safety of pre-engraftment prophylactic foscarnet administration after allogeneic stem cell transplantation

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Running title: Safety of prophylactic foscarnet after SCT

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Abstract

Human herpesvirus-6 (HHV-6) is a major cause of limbic encephalitis with a dismal prognosis after allogeneic hematopoietic stem cell transplantation (SCT). Since our previous trial of preemptive therapy with foscarnet sodium (PFA) failed to prevent HHV-6 encephalitis, we conducted a prospective study to examine the safety of prophylactic PFA administration and elucidate the changes in the plasma HHV-6 DNA levels in the early post-SCT period. Plasma HHV-6 DNA was measured thrice weekly from day 6. PFA, 90 mg/kg/day, was administered from days 7 to 21 after bone marrow or peripheral blood stem cell transplantation and to 25 after umbilical cord blood transplantation. Of the 10 patients enrolled, two dropped out of the study due to early death and a low glomerular filtration rate. Grade 3 or greater adverse events occurred in 9 of the 10 prophylactic PFA patients and in 7 of the 10 control patients who had clinical backgrounds similar to the study subjects and underwent SCT during the same period. Neurological disorders developed in none of the study subjects but in 4 of the 10 control patients, including 2 with HHV-6 encephalitis. HHV-6 reactivation occurred in 3 of 10 study subjects. The prophylactic PFA regimen was thus safe and it may reduce the risk of limbic encephalitis, but is not considered to be potent enough to prevent HHV-6 reactivation.

Keywords: Human herpesvirus-6; hematopoietic stem cell transplantation; limbic encephalitis; and foscarnet sodium.

Introduction

Human herpesvirus-6 (HHV-6) is a major cause of limbic encephalitis with a dismal prognosis after allogeneic hematopoietic stem cell transplantation (allo-SCT) (1). It was previously a rare complication after conventional allo-SCT, however, the incidence has been increasing as the number of alternative grafts, such as umbilical cord blood transplantation (UCBT), has increased (2). Currently, foscarnet sodium (phosphonoformic acid, PFA) and ganciclovir (GCV) are available in Japan for the treatment of HHV-6 infection, and PFA is thought to be preferable during the early post SCT period because of its lower toxicity for bone marrow (BM) (3). We previously conducted a prospective multicenter study to assess the safety and efficacy of preemptive therapy with PFA for the prevention of HHV-6 encephalitis, because the safety of PFA administration during neutrophil engraftment had not been established in UCBT recipients (4). This study revealed the safety of PFA administration beginning around day 15 after SCT or later; however, this strategy could not entirely prevent HHV-6-induced encephalitis. Based on these findings, we have started to consider prophylactic administration of PFA, but the safety of PFA administration before neutrophil engraftment after SCT has not yet been examined. We therefore designed a prospective institutional study to assess the safety of PFA administration in the early

post-SCT period. We herein present data on a matched-pair analysis comparing the patients who received the prophylactic PFA administration with those who did not receive such PFA treatment.

Patients and Methods

Endpoint of this study

This study was conducted primarily to assess the incidence of adverse events (AEs) associated with PFA administration in the early post-SCT period. The secondary endpoint was to follow the change of the amount of plasma HHV-6 DNA and to assess the efficacy of the prophylactic administration of PFA for the HHV-6 DNAemia.

Study design

Eligible patients were aged from 16 to 75 years with hematologic disorders refractory to conventional therapy and were considered to require allo-SCT from alternative grafts such as an HLA-mismatched relative, an unrelated donor, or UCBT due to the unavailability of an HLA-matched relative between February 2009 and March 2010 at Kanazawa University Hospital. The study was offered to all HCT recipients who met the inclusion criteria, and the patients who agreed to join this study were enrolled.

Written informed consent was obtained from all subjects according to the Declaration of Helsinki, and this study protocol was approved by the institutional ethics committee (#5537). This trial was registered in the UMIN Clinical Trials Registry (UMIN-CTR; http://www.umin.ac.jp/ctr/index.htm) under identifier UMIN000001706. HLA matching was evaluated with molecular typing. Patients with high serum creatinine levels and/or a lower estimated glomerular filtration rate greater than grade 2, and/or other organ dysfunctions greater than grade 3 defined by the Common Terminology Criteria ver.3.0 for Adverse Events (CTCAE) of the National Cancer Institute, USA, were excluded. Regimens for preconditioning and prophylaxis for graft-versus-host disease (GVHD) were not specified. Peripheral blood samples were obtained every Monday, Wednesday, and Friday from day 6 to day 28 after BM transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT) from unrelated donors or HLA-mismatched relatives and day 6 until day 35 after UCBT. Frozen plasma samples were sent to SRL, Inc (Tokyo, Japan) to measure the amount of HHV-6 DNA using a real-time quantitative PCR method the following day (5) (6). This system could detect both HHV-6 variant A and variant B, but it could not distinguish between them. Prophylactic administration of PFA at 90 mg/kg/day was started from day 7 and continued for 14 days after BMT or PBSCT and was administered from day 7 for 18 days after UCBT. In case HHV-6

DNAemia developed during the PFA administration, PFA was extended until HHV-6 DNA became negative on 3 consecutive occasions. If a patient's creatinine clearance fell below 1.4 ml/min/kg, the PFA dose was reduced according to the manufacturer's instructions. The administration of other antiviral agents, such as acyclovir and GCV, was discontinued during the PFA administration period. AEs developing during the test period were evaluated by the CTCAE ver.3.0. The last 10 patients that received SCT by the end of March 2010 and met all of the criteria noted above except for PFA administration by day 35 after SCT, were selected as a control group for a matched-pair analysis to eliminate any possible bias. This group consisted of patients who were transplanted before and after the study period and those who refused to participate in the study.

Definition of HHV-6 encephalitis

The diagnosis of HHV-6 encephalitis was made by a neurologist based on a positive result for HHV-6 DNA in cerebrospinal fluid and the presence of neurologic symptoms such as paresthesia, confusion, seizure and consciousness disturbance (2). When cerebrospinal fluid was not available for the examination of HHV-6 DNA, the diagnosis

was made based on the findings compatible with limbic encephalitis according to the magnetic resonance (MR) imaging findings and the presence of HHV-6 DNAemia.

Statistical analysis

The following variables related to patients and their clinical data were compared among the groups using the Mann-Whitney U test: age, date of WBCs > 0.1×10^9/l, and date of neutrophils > 0.5×10^9/l. All *P* values were two-sided with values < 0.05 being considered statistically significant. These analyses were performed using JMP® ver. 7.0 (SAS Institute Inc., Cary, NC, USA).

Results

Patients' characteristics

A total of 10 patients were enrolled this study. The characteristics of these patients and the 10 patients enrolled as the control group are summarized in Table 1; the median age of the tested patients was younger than the control patients (40 years for prophylactic PFA patients vs. 51 years for control patients), but there were no significant differences between the groups. The underlying disease in the prophylactic PFA patients were 6 acute leukemias, 1 myelodysplastic syndrome (MDS) and 3 lymphomas, while there

were 5 acute leukemias, 2 MDS and 3 lymphomas in the patients of the control group. Approximately half of the patients had reached complete remission and received myeloablative conditioning prior to SCT in both of the groups. Most of the patients received BM grafts from an unrelated donor, except 2 SCT that were from mismatched siblings and 2 UCBT in the prophylactic PFA patients. Eight of the 10 patients in each group received cyclosporine for GVHD prophylaxis.

Toxicities of prophylactic PFA administration

Two patients dropped out of this study due to AEs; one patient died due to transplant-related complications (bacterial pneumonia) and another patient was grade 2 low glomerular filtration rate. Two patients required dose reduction of PFA because of creatinine increase (grade 2 in 1 patient and grade 1 in 1 patient). AEs graded greater than 3 by CTCAE were observed in 9 of the 10 patients who received prophylactic PFA and in 7 of the 10 patients in the control group (Table 2). Noticeable AEs in prophylactic PFA patients were infection and cellulitis; mortal pneumonia in 1 patient, bacteremia in 2 patients, bacterial cystitis in 1 patient and viral cystitis in 1 patient. These AEs were improved, except for the grade 5 pneumonia, after appropriate therapy. Renal dysfunction, a characteristic AE caused by PFA, occurred less frequently in the

PFA patients (2 with grade 2) than in the control group (grade 4 in 1 patient and grade 2 in 4 patients). The patient with grade 4 renal dysfunction developed uncontrollable aGVHD (grade 4) complicated by a critical infection, thus leading to the onset of drug-induced renal failure, due to the administration of antibiotics and cyclosporine. A non-infectious pulmonary complication (grade 3) developed in 1 PFA patient, who transiently required oxygen inhalation for 3 weeks and thus was withdrawn from the study. Of interest, disturbance of consciousness was not seen in any of the patients receiving prophylactic PFA administration, but was seen in 4 of the 10 patients in the control group, including 2 patients diagnosed with HHV-6 encephalitis.

Clinical significance of prophylactic PFA administration and the development of HHV-6 DNAemia

We have summarized the clinical information of the patients, dividing them into 2 groups; patients who received prophylactic PFA administration and control patients (Table 3). No differences were observed between the 2 groups in terms of the period from SCT to achieving neutrophils > 0.5×10^9/l and WBCs > 0.1×10^9/l, and the development of acute GVHD.

Of the 10 patients who received prophylactic PFA administration, 3 patients developed HHV-6 DNAemia between day 18 and day 23 after SCT. The highest HHV-6 DNA copy number in these 3 patients was 6.4 x 10^3/ml. Two of the 3 patients (UPN 102 and UPN 110) were detected to have HHV-6 DNA during PFA administration, and the PFA treatment was extended until HHV-6 DNA disappeared from their plasma (Figure 1a). The other patient (UPN109) was observed to have HHV-6 DNAemia 3 days after cessation of PFA, and the HHV-6 DNA disappeared in 4 days with no additional PFA administration (Figure 1b). None of the patients who received prophylactic PFA administration developed neurological symptoms, thus suggesting the occurrence of HHV-6 encephalitis during the test period. However, 3 of 4 control patients who experienced neurological symptoms were positive for HHV-6 DNA in their plasma, including 2 patients diagnosed as having HHV-6 encephalitis (Table 3). The cerebrospinal fluid was examined in 2 of the 3 control patients who were positive for plasma HHV-6 DNA. One patient was positive (UPN C3) for HHV-6 DNA and the other was negative (UPN C15).

Figure 2 shows the MR images of a control patient (UPN C3) who developed limbic encephalitis; hallucination and short-term memory loss were observed on day 20, and a seizure following a coma occurred on day 22 after BMT. Only 1.2 x 10^2/ml of the

HHV-6 DNA was detected in the patient's plasma on day 26, which thus indicates a diagnosis of HHV-6 encephalitis at that time. However, the MR images on day 27 revealed symmetric enhancement of the limbic cortex on fluid-attenuated inversion-recovery (FLAIR) imaging (Figure 2A) and the positive result for HHV-6 DNA in cerebrospinal fluid on day 28 indicated that the etiology of these neurologic symptoms was HHV-6 encephalitis. The level of consciousness and the MR findings of the patients slowly improved (Figure 2B), but the short-term memory loss remained.

Discussion

The results of an *in vitro* study showed that PFA and cidofovir were superior to GCV for the treatment of HHV-6 infection (7, 8). PFA was selected as an anti-HHV-6 agent because cidofovir was not authorized in Japan and PFA had lower BM toxicity than GCV (9). A few papers have so far been published regarding the safety and efficacy of PFA for cytomegalovirus infection after SCT (3) (10) (11). However, most of the patients enrolled in these studies were administered PFA around day 30 or later after SCT, and the investigators were unable to evaluate the influence of PFA on hematopoiesis before neutrophil engraftment. In contrast to GCV, PFA administration is not usually associated with BM suppression; however, it is possible that it may

impair hematopoietic stem cell engraftment when it is used soon after transplantation. In addition, the administration of PFA in the early SCT period with intensive multidrug therapy might cause more severe renal dysfunction than conventional use. The current study showed that administration of PFA had little impact on hematopoiesis after SCT, suggested by the equivalence of WBCs and neutrophil recovery between the treatment and control groups. Also, the number of patients who developed renal dysfunction and electrolyte abnormalities as AEs in the PFA-treated group was not higher than in the control group, although 2 patients needed dose reduction of PFA, thus indicating the safety of PFA administration during this period. We considered that the higher frequency of infectious events in PFA-treated patients compared to the untreated patients might have been caused by the differences in the graft source for SCT; the PFA-treated patients received HLA-mismatched grafts and UCB, while all of the untreated patients were grafted with BM from an unrelated donor.

Although many clinicians have recognized the risk of HHV-6 encephalitis, strategy for preventing HHV-6 encephalitis have not been established. Two previous reports showed the efficacy of prophylactic GCV from the time of neutrophil engraftment (12) (13); but only 1 UCBT recipient was included in this study. Ogata et

al. has documented that a preemptive approach consisting of HHV-6 DNA measurement in peripheral blood once a week and starting PFA administration in cases where the HHV-6 DNA exceeded 1.0 x10^4/ml was insufficient (14). Our previous study, consisting of HHV-6 DNA measurement in peripheral blood three times per week from day 7 after SCT and starting PFA administration when the amount of HHV-6 DNA increased to greater than 5 x 10^2/ml, also failed to prevent encephalitis, and it was concluded that prophylactic PFA administration from day 7 or earlier until day 20 after SCT would be necessary (4). The current results shows that the 90 mg/kg dose of prophylactic PFA beginning from day 7 after SCT is safe with tolerable AEs, and that it could be effective for the prevention of HHV-6 encephalitis. The development of HHV-6 DNAemia could not be entirely prevented, but all 3 positive cases had less than 1.0 x10^5/ml of HHV-6 DNA, a level that indicates the development of limbic encephalitis (15). Therefore, these levels of HHV-6 DNA observed in patients who received PFA prophylaxis, which were lower than described in previous reports, might therefore reflect the efficacy of prophylactic PFA administration. Meanwhile, 1 of the 2 control patients who developed limbic encephalitis showed a significantly high copy number of HHV-6 DNA (> 1.0 x10^5/ml), which was characteristic of HHV-6 encephalitis; however, the other case showed only 1.2 x

10^2/ml of HHV-6 DNA in his plasma. These two cases may differ due to the timing of the examination of HHV-6 DNA, because the HHV-6 DNA copy number dramatically changed within a couple of days (4, 15) . The current study examined the plasma HHV-6 just after the appearance of the initial neurologic symptoms in the former case, but 6 days after the initial symptoms in the latter case, which may have missed the peak of the HHV-6 DNA.

In the current study, the reactivation of HHV-6 occurs around the time of neutrophil engraftment; however, all 3 PFA-treated cases positive for HHV-6 DNA in the plasma developed on and after neutrophil engraftment, despite the fact that 5 of 7 UCBT patients developed HHV-6 DNAemia on and before neutrophil engraftment in our previous study. These results suggest that the prophylactic PFA administration may delay the development of HHV-6 DNAemia for a few days, signifying that its development is avoided in the early period after SCT, which might lead to decreased transplant-related mortality.

The current study was a small trial designed to evaluate the safety of prophylactic PFA administration early after SCT before neutrophil engraftment. The results achieved the primary endpoint. This strategy may reduce the risk of limbic encephalitis, though it may not be able to entirely prevent HHV-6 reactivation.

Although our present study failed to establish sufficient clinical evidence because of the shortage of cases, our results are thus considered to provide the basis for the design of a large-scale prospective study with many patients, to compare the efficacy and safety between preemptive therapy and prophylactic therapy by PFA, to prevent HHV-6 disease in allo-SCT recipients.

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Figure legends

Figure 1. Development of HHV-6 DNAemia in patients during (a, UPN 102) and after (b, UPN 109) PFA administration. The columns represent copy numbers of HHV-6. WBC, white blood cell count; PFA, phosphonoformic acid (foscarnet).

Figure 2. Magnetic resonance images of the brain of the patient who developed limbic encephalitis (UPN C3). Both of the images were taken by fluid-attenuated inversion-recovery (FLAIR) at the level of the basal ganglia. A; day 27 after SCT, B; day 38 after SCT.

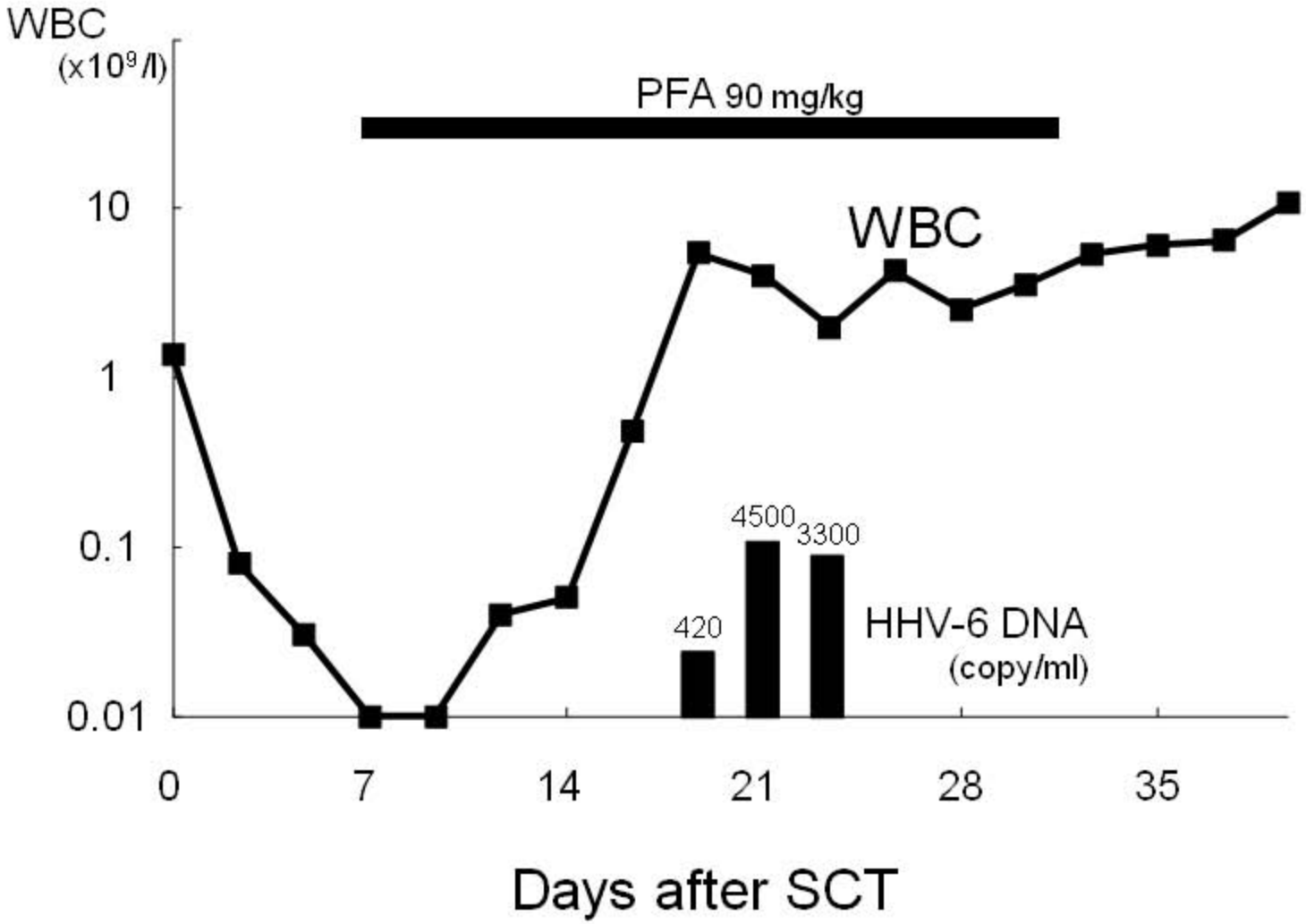

WBC
(x10⁹/l)
PFA 90 mg/kg
10
1
0.1
0.01
WBC
4500
3300
420
HHV-6 DNA
(copy/ml)
0
7
14
21
28
35
Days after SCT

Figure 1a

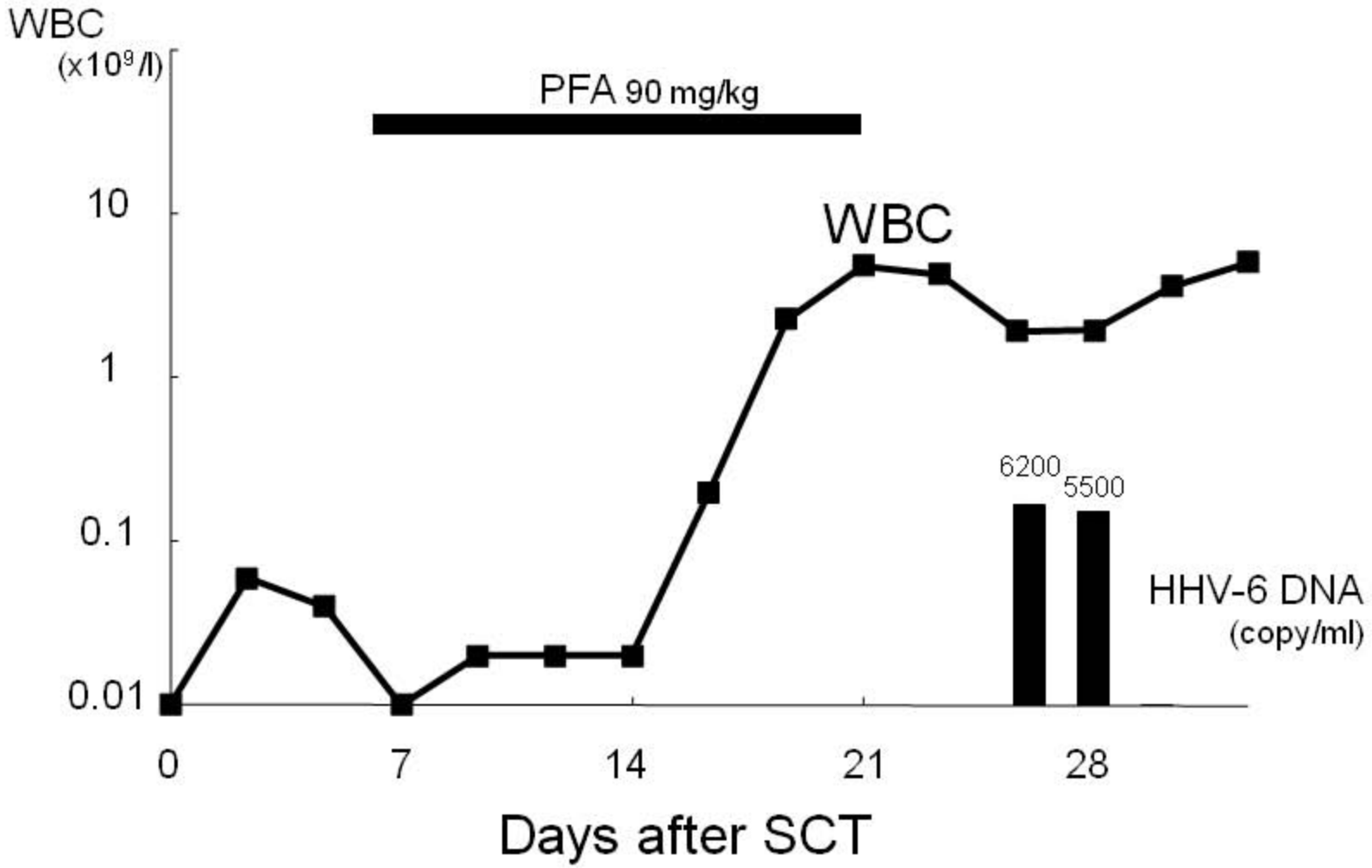
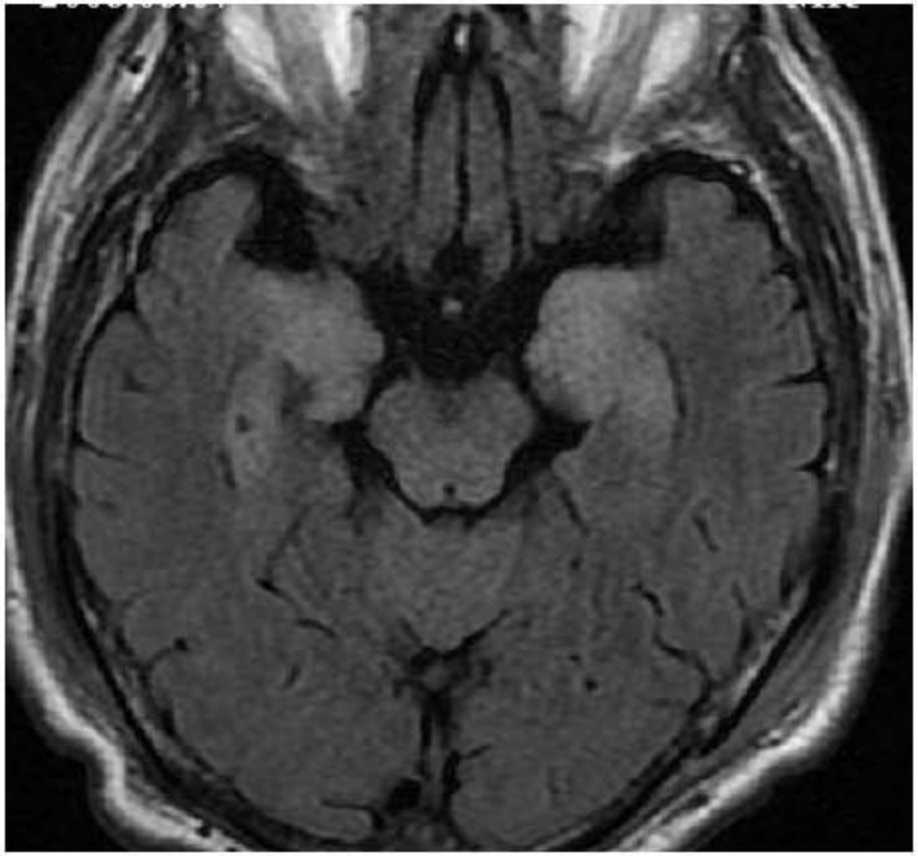

WBC
(x10⁹/l)
PFA 90 mg/kg
10
1
0.1
0.01
WBC
6200
5500
HHV-6 DNA
(copy/ml)
0
7
14
21
28
Days after SCT

Figure 1b

A.



B.

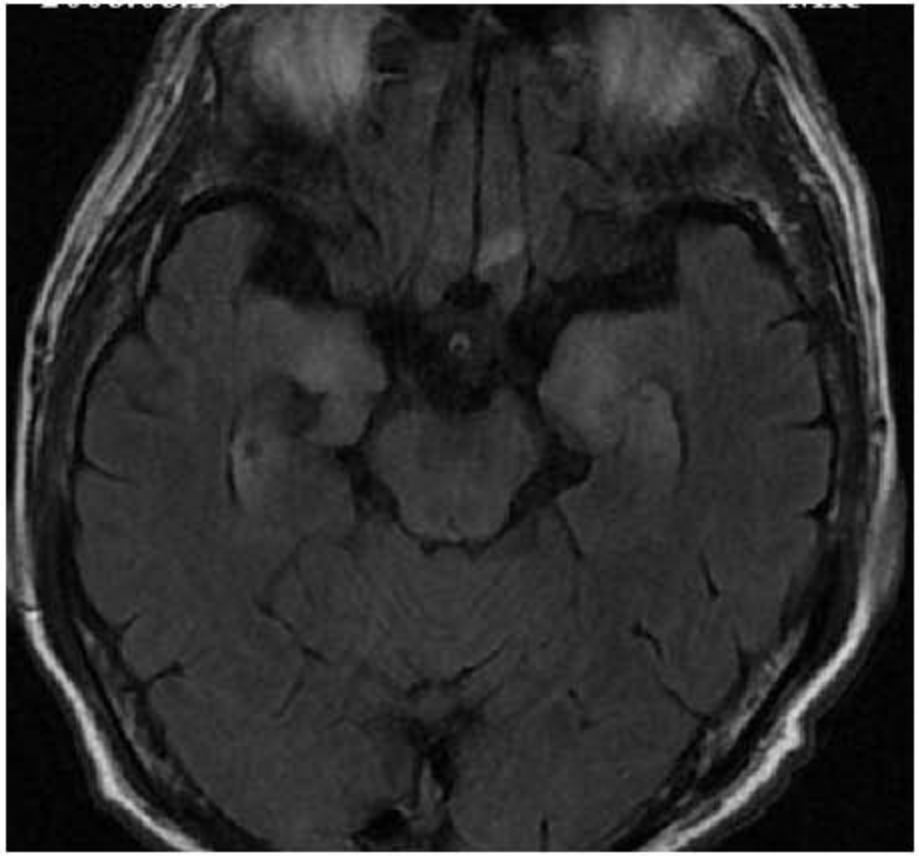


Figure 2

Table 1. Patients characteristics

	Age, median (range)	Gender	Diagnosis	Disease status at SCT	Regimen	Graft source	GVHD prophylaxis
Patients who received PFA prophylaxis (n=10)	40 (18-59)	Male 6 Female 4	AML 6 CMML 1 ATL 1 HL 1 NHL 1	CR 4 non-CR 6	MA 4 RIC 6	R-PBSC 1 R-BM 1 UR-BM 6 CB 2	CsA 8 FK506 2
detail of HHV-6 DNA positive patients							
UPN 102	*21*	*F*	*AML*	*CR*	*MA*	*UR-BM*	*CsA*
UPN 109	*50*	*F*	*CMML*	*non-CR*	*MA*	*UR-BM*	*CsA*
UPN 110	*22*	*M*	*AML*	*non-CR*	*RIC*	*CB*	*CsA*
Control patients (n=10)	52 (32-59)	Male 6 Female 4	AML 2 ALL 2 AL biphenotypic 1 RAEB 1 RCMD 1 NHL 3	CR 6 non-CR 4	MA 4 RIC 6	UR-BM 10	CsA 8 FK506 2

Abbreviations: AL, acute leukemia; ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; ATL, adult T-cell leukemia; BM, bone marrow; CB, umbilical cord blood; CMML, chronic myelomonocytic leukemia; CR, complete remission; CsA, cyclosporine A; FK506, tacrolimus, GVHD, graft-versus-host disease, HHV-6, humanherpes virus-6; HL, Hodgkin's lymphoma; MA, myeloablative, NA, not achieved; NHL, non-Hodgkin's lymphoma; PBSC, peripheral blood stem cell, PFA; foscarnet sodium; R, related; RAEB, refractory anemia with excess blasts; RCMD, refractory anemia with multilineage dysplasia; RIC, reduced intensity conditioning; SCT, stem cell transplantation; UR, unrelated; UCBT, umbilical cord blood transplantation.

Table 2. Number of adverse events

	Patients received PFA prophylaxis (n = 10)	Control patients (n = 10)	
Dropout patients	2*	NA	* : including 1 patient with grade 5 infection and 1 patient with grade 2 low glomerular filtration rate.
Patients death during the test period	1	0	
Adverse events			
Grade 2	10 patients	10 patients	
Number of events			
Electrolyte abnormality	1	1	
Hepatobiliary	3	4	
Renal	2*	4	* : including 1 patient dropped out of the study.
Hypoalbuminemia	6	7	
Diarrhea	0	2	
Greater than Grade 3	9 patients	7 patients	
Number of events			
Electrolyte abnormality	5 (1)	5 (2)	
Hepatobiliary	3	4 (1)	
Renal	0	1 (1)	
Cystitis	2	0	
Infection	3 (3*)	1 (1)	* : including 1 patient dropped out of the study (grade 5).
Pneumonitis	1	0	
Impaired consciousness	0	4 (2)	

The number in parentheses show the number of Grade 4 adverse events.
Abbreviation: PFA, foscarnet sodium.

Table 3. Comparison of clinical features between PFA treated and untreated (control) patients.

	Date of WBCs >0.1 x10^9 /L, median (range)	Date of neutrophils >0.5 x10^9 /L, median (range)	Severity of acute GVHD	CMV antigenemia	Duration of HHV-6 DNAemia (days)	Plasma HHV-6 DNA copy number at peak (/mL)	HHV-6 encephalopathy
Patients who received PFA prophylaxis (n=10)	13 (11-18)	17 (13-21)	Grade IV 1 Grade II 2 Grade I 2 no GVHD 4 NA 1	0	NA	NA	0
details of HHV-6 DNA positive patients							
UPN 102	*14*	*18*	*Grade IV*	*(-)*	*18～24*	*4.5 x10^3*	*(-)*
UPN 109	*16*	*19*	*(-)*	*(-)*	*23～27*	*6.4 x10^3*	*(-)*
UPN 110	*13*	*16*	*(-)*	*(-)*	*23～26*	*4.7 x10^2*	*(-)*
Control patients (n=10)	15 (11-20)	17 (13-24)	Grade IV 1 Grade II 3 Grade I 1 no GVHD 5	1	ND	ND	2
details of HHV-6 DNA positive patients							
UPN C3	*12*	*16*	*Grade II*	*(-)*	*NE*	*1.2 x10^2*	*(+)*
UPN C7	*11*	*13*	*Grade IV*	*(-)*	*NE*	*9.6 x10^5*	*(+)*
UPN C15	*17*	*19*	*(-)*	*(-)*	*NE*	*9.8 x10^3*	*(-)*

Abbreviations: CMV, cytomegalovirus, GVHD, graft-versus-host disease, HHV-6, human herpes virus-6; NA, not achieved; NE, not examined; PFA, foscarnet sodium; UCBT, umbilical cord blood transplantation.