Preemptive therapy of human herpesvirus-6 encephalitis with foscarnet sodium for high-risk patients after hematopoietic SCT

Preemptive therapy of human herpesvirus-6 encephalitis with foscarnet sodium for high-risk patients after hematopoietic stem cell transplantation

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Running title: Preemptive therapy of human herpesvirus-6 encephalitis

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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 (HSCT). A prospective, multicenter study was conducted to assess the safety and efficacy of preemptive therapy with foscarnet sodium (PFA) for the prevention of HHV-6 encephalitis. Plasma HHV-6 DNA was measured thrice weekly from day 7 until day 36 after umbilical cord blood transplantation (UCBT) or HSCT from HLA-haploidentical relatives. PFA, 90 mg/kg/day, was started when HHV-6 DNA exceeded 5×10^2 copies/ml. Mild and transient adverse events were associated with PFA in 7 of 8 patients. Twelve of 15 UCBT recipients became positive for HHV-6 DNAemia, defined by greater than 1 x10^2 copies/ml of HHV-6 DNA in plasma. The virus exceeded 5×10^2 copies/ml in 7 patients, while none of the 5 HLA-haploidentical HSCT recipients became positive. One patient developed mild limbic encephalitis just after initial PFA administration. Preemptive PFA therapy is safe, but since HHV-6 DNAemia can abruptly develop before neutrophil engraftment in UCBT recipients, prophylactic PFA administration from day 7 or earlier after UCBT may be needed.

Keywords

Human herpesvirus-6, hematopoietic stem cell transplantation, alternative donors, limbic encephalitis, and foscarnet sodium.

Introduction

Umbilical cord blood (UCB) from unrelated donors has been successfully used as an alternative hematopoietic stem cell source for treatment of hematologic malignancies in patients who do not have HLA-matched bone marrow (BM) or peripheral blood stem cell (PBSC) donors. UCB transplantation (UCBT) has several advantages over BM transplantation (BMT) or PBSC transplantation (PBSCT) because of its rapid availability and lower risk of acute graft-versus-host disease (GVHD), even when there is a 1-3 HLA antigen mismatch (1-4). However, UCBT is associated with a higher risk of engraftment failure and more delayed immunological recovery than BMT and PBSCT (5-10).

Recently, human herpesvirus-6 (HHV-6) has been recognized as an important pathogen in allogeneic hematopoietic stem cell transplantation (HSCT) (11-14). The reactivation of HHV-6 occurs around the time of neutrophil engraftment and occasionally causes limbic encephalitis, which is characterized by a loss of short-term memory and abnormal hippocampal findings on magnetic resonance (MR) images (15). Although limbic encephalitis due to HHV-6 used to be a rare complication after conventional HSCT, it is becoming one of the most serious complications after HSCT as the number of UCBT increases (16, 17).

HHV-6, a causal virus of infantile exanthem subitum, latently infects almost all Japanese adults. HHV-6 DNA is detected in the plasma of 33% to 48% patients treated with HSCT but is undetectable in plasma from healthy individuals or from UCB (18, 19). HHV-6 DNA becomes detectable around day 9 or later after HSCT, and high HHV-6 DNA copy numbers are associated with development of BM suppression (20-22). Foscarnet sodium (PFA) is thought to be preferable to ganciclovir (GCV) as an anti-cytomegalovirus (CMV) drug used in the early post-transplant period because it has less BM toxicity than GCV (23), however, the safety of PFA administration early after HSCT has not been established in UCBT recipients. Ogata et al. (24) reported that the HHV-6 DNA copy number in the peripheral blood increased 100-fold within 3 to 4 days in some cases, and limbic encephalitis developed in UCBT recipients when the HHV-6 DNA copy number in plasma exceeded 1×10^4/ml. According to their report, once a week monitoring of HHV-6 DNA in plasma followed by preemptive administration of PFA was insufficient to prevent limbic encephalitis. More frequent monitoring of HHV-6 DNA such as three times a week, early after HSCT and preemptive administration of PFA based on positive results, defined as a low viral copy number (5×10^2 copy/ml), may help prevent HHV-6 encephalitis. To examine this hypothesis, we conducted a prospective, multicenter study of preemptive

therapy of HHV-6 infection with low-dose PFA for high-risk patients after HSCT. This study documented the safety and efficacy of the preemptive administration of PFA in the prevention of severe HHV-6 encephalitis.

Methods

Endpoint of this study

This study was conducted primarily to assess the incidence of adverse events (AEs) associated with PFA administration until day 36 after HSCT because the safety of using PFA early after HSCT has not been established. The secondary endpoint was to assess the efficacy of preemptive administration of PFA in preventing the development of limbic encephalitis, as well as in reducing the amount of plasma HHV-6 DNA.

Study design

Eligible patients were aged from 16 to 75 years with hematologic disorders refractory to conventional therapy and were considered to require UCBT or HLA 1-haplotype matched HSCT (haploidentical HSCT) from relatives due to the unavailability of an HLA-identical relative or a suitable unrelated donor. Informed consent was obtained

from all subjects according to the Declaration of Helsinki, and this study protocol was approved by the institutional ethics committee (No. 5434). This trial was registered to UMIN Clinical Trials Registry (UMIN-CTR; http://www.umin.ac.jp/ctr/index.htm) under identifier UMIN000001346. HLA matching was evaluated with molecular typing. Patients with high serum creatinine levels and/or 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 GVHD prophylaxis were not specified. The serum HHV-6 IgG titer before transplantation was determined by immunofluorescence assay. Peripheral blood samples were obtained on every Monday, Wednesday, and Friday from day 7 to day 36 after HSCT, and frozen plasma samples were sent to SRL, Inc (Tokyo, Japan) to measure the amount of HHV-6 DNA using a real-time polymerase chain reaction (PCR) method on the following day (25, 26). Administration of PFA, 90 mg/kg/day, was started on the day when the amount of plasma HHV-6 DNA exceeded 5×10^2 copies/ml. The PFA dose was increased to 180 mg/kg/day when the plasma HHV-6 DNA copy number increased to more than 1×10^5/ml or when symptoms suggestive of encephalitis appeared. PFA was discontinued when the plasma HHV-6 DNA was negative on 3 consecutive

occasions. If the patients' creatinine clearance fell below 1.4 ml/min/kg, the PFA dose was reduced according to the manufacturer's instructions.

Statistical analysis

The following variables related to patients and their clinical data were compared among the groups using Fisher's exact probability test or the Mann-Whitney U test: gender (male vs. female), HHV-6 IgG titer before HSCT, intensity of the conditioning regimen (myeloablative vs. reduced-intensity conditioning), prophylactic regimens for GVHD (cyclosporine-based vs. tacrolimus-based), transplanted cell number, type of HSCT (UCBT vs. haploidentical HSCT), date of WBCs > 0.1×10^9/l, date of neutrophils > 0.5×10^9/l, date of developing HHV-6 DNAemia (defined as the state characterized by the presence of HHV-6 DNA greater than 1×10^2 copies/ml in plasma), duration of HHV-6 DNAemia, and development of CMV antigenemia (positive vs. negative). All *P* values were two-sided with values less than 0.05 being considered statistically significant. These analyses were performed using JMP® ver. 7.0 (SAS Institute Inc.).

Results

Patients' characteristics

A total of 21 patients was enrolled from 4 different institutions between September 2007 and February 2009. The characteristics of the patients are summarized in Table 1; their median age was 51 years (range, 18 - 72 years). Eight patients received myeloablative preparative regimens, and 13 patients received reduced-intensity conditioning before HSCT. Sixteen patients received a UCB graft, while 5 patients were grafted with PBSCs from HLA-haploidentical donors. GVHD prophylaxis regimens were cyclosporine in 5 patients and tacrolimus in 16 patients. All patients were seropositive for CMV without CMV disease before HSCT. One patient (UPN 2) who received PBSCT developed primary graft rejection due to anti-HLA class I antibodies specific to a donor's mismatched allele and received second transplantation with UCB. This patient was re-registered as UPN 4. One UCBT patient (UPN 16) was excluded from the analysis because of early death (on day 4) after HSCT due to hepatic failure associated with primary biliary cirrhosis.

Toxicities of preemptive PFA administration

In 15 patients who received UCB grafts, AEs graded greater than 3 by CTCAE, were observed in 7 of the 8 patients (88%) treated by PFA and 4 of the 7 patients (57%) not

treated with PFA (Table 2a). Most of these AEs associated with PFA treatment were electrolyte abnormalities such as hypernatremia, hypokalemia, and hypomagnesemia (Table 2b). These abnormalities improved promptly after appropriate fluid therapy. Severe renal dysfunction did not develop in any of the PFA-treated patients, though grade 2 renal dysfunction, such as a low glomerular filtration rate, was observed in 2 PFA-treated patients. Other grade 3 AEs included a transient rise in the aspartate aminotransferase level requiring no treatment and a systemic skin rash that disappeared after the administration of 100 mg hydrocortisone succinate. Four patients were dropped out of this study and died; 2 patients developed HHV-6 DNAemia not requiring PFA treatment, and the other 2 patients remained negative for HHV-6 DNA during the observation period. The causes of death were hepatic veno-occlusive disease, bacteremia leading to pulmonary alveolar hemorrhage, thrombotic microangiopathy, and bacteremia. Both attending physicians and central reviewers judged that there was no relationship between PFA administration and the causes of death in all 4 cases.

Development of HHV-6 DNAemia

Of 15 UCBT recipients, 12 (80%) developed HHV-6 DNAemia. The HHV-6 DNA copy number exceeded 5×10^2/ml in 7 of the 11 patients; 1 patient (UPN 19) was

erroneously treated with PFA when the HHV-6 copy number was less than 5×10^2/ml and was therefore excluded from this analysis. On the other hand, all 5 haploidentical HSCT recipients remained negative for HHV-6 DNA in their plasma during the observation period ($P < 0.004$). Therefore, further analyses focused on UCBT recipients. When the clinical characteristics were compared between patients positive for HHV-6 DNAemia (n = 12) and those who did not develop HHV-6 DNAemia (n = 3), on univariate analysis there were no significant differences in age, sex, HHV-6 IgG titer (tested in 11 cases), intensity of conditioning regimens, transplanted cell number, GVHD prophylactic regimens, date of WBCs > 0.1×10^9/l, and date of neutrophils > 0.5×10^9/l (Table 3). Table 4 shows the comparison of the characteristics of HHV-6 DNAemia between patients who eventually required PFA due to an increase in HHV-6 DNA copy number to greater than 5×10^2 /ml and those who did not require PFA treatment after HSCT. In 6 of 7 treated patients, the HHV-6 DNA copy number was greater than 5×10^2 copies/ml at the time when HHV-6 DNA was detected for the first time (median, 2.4×10^3 copies/ml). On the other hand, the HHV-6 DNA copy number was significantly lower in untreated patients at the time of the first HHV-6 DNA detection than in treated patients (median, 1.1×10^2 copies/ml, $P = 0.01$). HHV-6 DNAemia developed significantly later in patients who did not eventually require PFA

treatment than in patients who required PFA treatment (median, day 22 vs. day 17, $P < 0.02$). The duration of HHV-6 DNAemia was significantly shorter in untreated patients than in the PFA-treated patients (median, 2 days vs. 9 days, $P < 0.008$).

Relationship between the time for neutrophil engraftment and that for the development of HHV-6 DNAemia

Figure 1 illustrates the changes in the HHV-6 copy number in 7 patients who required PFA administration. These patients achieved neutrophils $> 0.5 \times 10^9$/l and WBCs $> 0.1 \times 10^9$/l on day 13-33 (median, day 22) and day 10-20 (median, day 14), respectively. HHV-6 DNAemia developed prior to the day of neutrophils $> 0.5 \times 10^9$/l in 5 of 7 patients and WBCs $> 0.1 \times 10^9$/l in 3 of 7 patients, indicating that HHV-6 DNAemia occurs much earlier than neutrophil engraftment in UCBT recipients.

Effect of preemptive PFA administration

PFA, 90 mg/kg/day, was administered to 7 patients whose HHV-6 DNA copy number exceeded 5×10^2 copies/ml from day 15 to day 20 (median, day 17) after UCBT. The amount of HHV-6 DNA in the plasma decreased on the next day of PFA administration in 4 of the 7 patients, while 3 other patients required 3-4 days until the

copy number decreased (Figure 1, red arrows). In 2 patients, the HHV-6 DNA copy numbers exceeded 1×10^4/ml (UPN 1 and UPN 21), a level that predicts the development of limbic encephalitis, on the next day of first PFA administration with no symptoms suggestive of encephalitis, and the HHV-6 DNA copy number decreased 3-4 days after PFA administration. Of 15 patients who achieved neutrophil engraftment after HSCT, 4 of 7 patients who did not receive PFA developed CMV antigenemia within 60 days after HSCT, while no patients treated with PFA developed CMV antigenemia ($P < 0.03$).

Limbic encephalitis developed in 1 patient (UPN 9) who received preemptive PFA administration. This patient showed an increase in the HHV-6 DNA copy number on day 17 after UCBT (Figure 2). When the first PFA administration was started at 8 pm on day 18 by 3 hours' intravenous drip infusion, there were no neurological symptoms. The patient was found to be unconscious at his bedside around 11 PM during the PFA infusion. A magnetic resonance study revealed asymmetric enhancement of the limbic cortex on fluid-attenuated inversion-recovery (FLAIR) imaging. Cerebrospinal fluid was not examined because of the patient's low platelet count. Electroencephalography showed periodic, lateralized, epileptiform discharges and sharp-and-slow-wave complexes, a finding compatible with HHV-6 encephalitis.

Five days after increasing the PFA dose from 90 mg/kg/day to 180 mg/kg/day, the patient's consciousness level improved. The patient achieved WBCs > 0.1×10^9/l and neutrophils > 0.5×10^9/l on day 19 and day 26, respectively. Currently, he is an outpatient with mild motor neuropathy but no impaired memory.

Discussion

PFA has been used for the treatment of HHV-6 encephalitis (27-29), however, the safety of initiating the administration of PFA before neutrophil engraftment has not been established. Although PFA administration is not usually associated with BM suppression, it may impair UCB engraftment when it is used soon after transplantation. Moreover, PFA frequently causes renal dysfunction. Two papers described the effectiveness and safety of low-dose PFA treatment for CMV infection after HSCT (23, 30), but little is known about the toxicity and the efficacy of its administration in the early period after HSCT so far because most patients received PFA treatment on day 30 or later. Previous studies documented that HHV-6 DNAemia developed as early as day 15 after UCBT (17). One of our patients (UPN 21) developed HHV-6 DNAemia, with a copy number of 7.3×10^3/ml on day 10 after UCBT. Therefore, it is necessary

to confirm the safety of the use of anti-HHV-6 agents before neutrophil engraftment. The current study revealed that low-dose PFA could be administered to UCBT recipients with acceptable toxicities even in the very early period after HSCT. Greater than grade 3 AEs occurred in 7 of 8 PFA-treated patients and 4 of 7 PFA-untreated patients, and there appears to be a tendency toward a higher incidence of severe AEs in PFA-treated patients than in untreated patients. However, the total number of greater than grade 3 AEs that occurred in the 8 PFA-treated patients was 8, similar to the 6 in the 7 untreated patients. A skin rash developed on day 17 and day 20, just before starting PFA treatment in 2 patients. Because it disappeared quickly with PFA treatment, the rash may have been associated with HHV-6 reactivation (31, 32).

Several risk factors have been identified for HHV-6 reactivation after HSCT. These include younger age, treatment with steroid, low-titers of anti-HHV-6 IgG before HSCT, and development of GVHD (14, 24, 33, 34). The use of transplantation from alternative graft sources, such as HLA-mismatched BM and UCB, is another risk factor for HHV-6 reactivation (24, 34-36). In the present study, UCBT was more associated with HHV-6 reactivation than haploidentical PBSCT. Of note, none of the patients who enrolled in this study developed acute GVHD and required corticosteroid treatment as a result, except for short-acting corticosteroids given to ameliorate fever

or allergic symptoms. It is conceivable that PFA administration with this dosage may mitigate the secretion of inflammatory cytokines such as IFN-γ or TNF-α from immune cells by inhibiting HHV-6 reactivation. This possibility needs to be examined in a prospective study involving a larger number of patients.

Two groups have documented the favorable results of prophylactic GCV administration for HHV-6 infection after neutrophil engraftment (37, 38). In the present study, 5 of the 7 patients whose DNA copy number later exceeded 5×10^2/ml became positive for HHV-6 DNA prior to reaching neutrophils > 0.5 x10^9/l, indicating the necessity of other strategies for preventing HHV-6 infection, because GCV is associated with BM suppression. The present study showed that the earlier the reactivation occurred, the more the HHV-6 viral load increased; the viral load increased within 48 hours after the negative test to a level greater than 5×10^2/ml. Thus, it may be important to ensure that the test result for HHV-6 DNA measurement can be returned on the day of blood sampling, or at least by the following morning, in order to make the preemptive approach successful. However, most hospitals are unable to get results in this short of time. It is possible that prophylactic PFA administration before the time of leukocyte recovery would be a more reasonable approach rather than preemptive PFA administration following the identification of HHV-6 DNA.

To prevent limbic encephalitis, what needs to be treated with PFA is HHV-6 DNAemia that occurs before the rise in the leukocyte count. Therefore, prophylactic administration of PFA from day 7 or earlier to day 20 may be a more reasonable approach than preemptive PFA administration guided by HHV-6 DNA detection to prevent limbic encephalitis in UCBT recipients. The efficacy of such prophylactic administration of PFA after UCBT is now being examined in a prospective study.

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Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Relationship between the time for neutrophil engraftment and the time for the development of DNAemia in patients treated with PFA. Changes in the HHV-6 DNA copy number in each patient are shown. The horizontal axis shows days after the time for neutrophil engraftment ($> 0.5 \times 10^9$/l, (A)) and the time for leukocytes $> 0.1 \times 10^9$/l (B). The arrows indicate when PFA was started.

Figure 2. Clinical course of a patient who developed HHV-6 limbic encephalopathy. (A) MR imaging of the patient. A high intensity area is observed at the cingulate gyrus, left insula, left hippocampus, and the gyrus parahippocampalis. (B) The patient's clinical course.

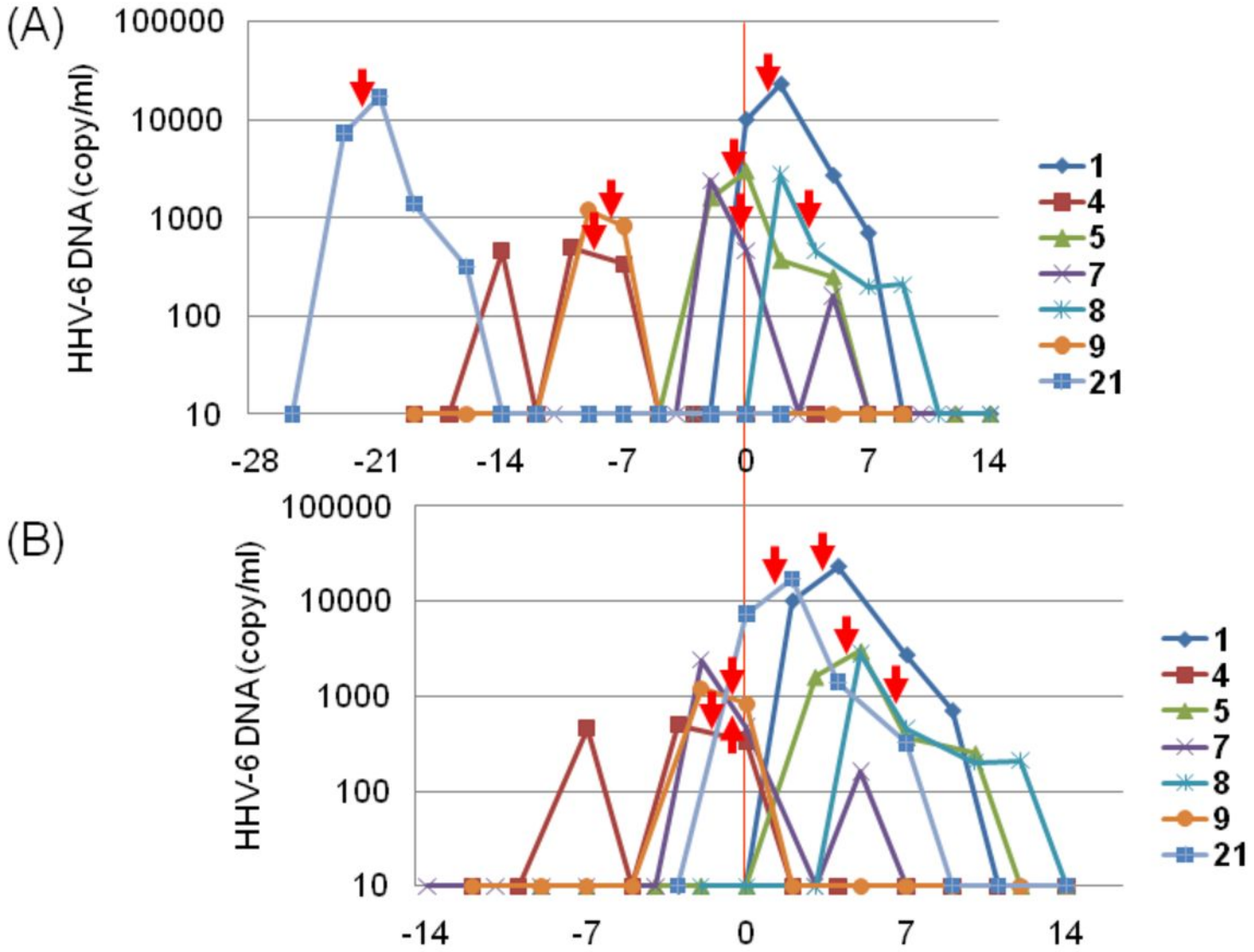
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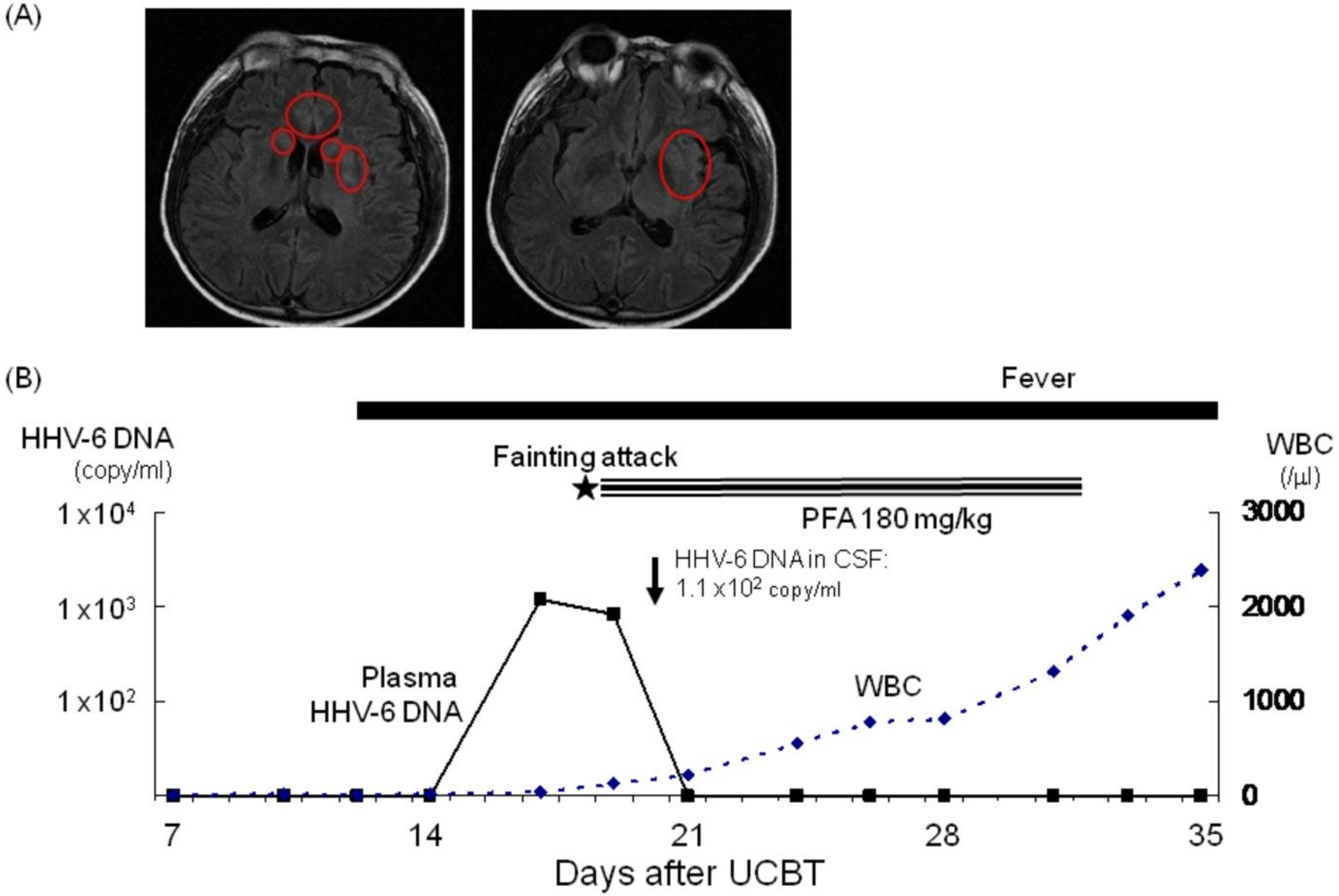
Figure 1.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(B)
Fever
HHV-6 DNA
(copy/ml)
Fainting attack
WBC
(/μl)
1 x10⁴
PFA 180 mg/kg
3000
HHV-6 DNA in CSF:
1.1 x10² copy/ml
1 x10³
2000
Plasma
HHV-6 DNA
WBC
1 x10²
1000
0
7
14
21
28
35
Days after UCBT

Figure 2.

Table 1. Patients characteristics

	n=21	
Age at HSCT (years)	18-72 (Median: 51)	
Gender	M:F=11:10	
Diagnosis	n	
AML	9	
refractory		3
1CR		1
1CR → rej		1
1rel		1
2CR or worse		3
ALL, 2CR	2	
Acute Leukemia, mixed phenotype	3	
2CR		1
refractory		2
CML, 2CP	1	
T-PLL, refractory	1	
MDS	4	
RAEB-1		1
RAEB-2		3
NHL, 1rel	1	
Graft	n	
PBSC	5 1.7-14.6 x10^6/kg (Median: 3.5)	
UCB	16* 1.4-3.0 x10^7/kg (Median: 2.7)	
Regimen		
Myeloablative, TBI base	8	
RIC	13*	
Flu＋α (except 3 cases below)		8*
Flu＋ATG＋α		3
Flu＋L-PAM＋Campath		1
TBI＋VP16＋CY		1
GVHD prophylaxis		
CyA base	5	
FK506 base	16*	

* Exclude 1 case from further analysis because of early death after transplantation.

Abbraviations: ATG; antithymocyte globulin, ALL; acute lymphoblastic leukemia, AML; acute myeloid leukemia, Campath; alemtuzumab, CB; cord blood, CML; chronic myelogenous leukemia, CP; chronic phase, CR; complete remission, CY; cyclophosphamide, CyA; cyclosporine A, FK506; tacrolimus, Flu; fludarabine, GVHD; graft-versus-host disease, L-PAM; melphalan, MDS; myelodysplastic syndrome, NHL; non-Hodgkin's lymphoma, PBSC; peripheral blood stem cell, PLL; prolymphocytic leukemia, rej; rejection, RAEB; refractory anemia with excess blasts, rel; relapse, RIC; reduced intensity conditiong, TBI; total body irradiation, VP16; etoposide.

Table 2. Adverse events in patients who received UCB grafts

a. Number of cases who develop adverse events greater than grade 2 / 3

	Greater than Grade 2	Greater than Grade 3
Patients with PFA treatment (n = 8)	8	7
Patients without PFA treatment (n = 7)	4	4

Table 2. Adverse events in patients who received UCB grafts

b. Number of events who develop adverse events greater than grade 2 / 3

	Patients with PFA treatment (n = 8)	Patients without PFA treatment (n = 7)
Grade 2		
Laboratory	1	2
Hepatobiliary	3	1
Renal	1	0
Dermatology	1	0
Grade 3		
Laboratory	5	2
Hepatobiliary	1	3
Renal	0	1
Dermatology	1	0
Grade 4		
Neurology	1	0

These adverse events were graded by the Common Terminology Criteria ver. 3.0 for Adverse Events (CTCAE) of the National Cancer Institute, USA. One patient who erroneously treated with PFA at under predetermined threshold was included in this analysis.
Abbraviation: PFA; foscarnet sodium, UCB; unrelated cord blood.

Table 3. Comparison of clinical features between HHV-6 DNAemia positive and negative patients in UCBT recipients

	Positive for HHV-6 (n = 12)	Negative for HHV-6 (n = 3)
Age, range (median)	32 - 72 (52)	14 - 64 (21)
Gender (M : F)	6 : 6	2 : 1
HHV-6 IgG titer, range (median)	20 - 1280 (80)	20 - 320 (20)
Regimen (Myeloablative vs. RIC)	5 : 7	1 : 2
GVHD prophylaxis (CyA vs. FK506)	5 : 7	0 : 3
Number of transplanted cells, range (median)	1.6 - 3.5 (2.7)	1.4 - 2.3 (2.1)
Date of WBCs > 0.1 x 10^9/l, range (median)	10 - 23 (16)	10 - 20 (15)
Date of neutrophils > 0.5 x 10^9/l, range (media	13 - 33 (27)	25

Abbraviations: CyA; cyclosporine A, FK506; tacrolimus, GVHD; graft-versus-host disease, HHV-6; human herpes virus-6, RIC; reduced intensity conditiong, UCBT; umbilical cord blood transplantation.

Table 4. Characteristics of HHV-6 DNAemia-positive UCBT recipients and their HHV-6 DNAemia

UPN	Age (years)	Gender	Day of WBC>100 ($x10^9$/l)	Day of neutrophils>0.5 ($x10^9$/l)	Day from UCBT until development of HHV-6 DNAemia	HHV-6 DNA copy number at development of HHV-6 DNAemia (/ml)	HHV-6 DNA copy number at peak (/ml)	Duration of HHV-6 DNAemia (day)
Patients who required PFA (n=7)								
1	50	M	13	15	15	1.0×10^4	2.3×10^4	9
4	40	F	20	27	17	4.6×10^2	5.0×10^2	7
5	60	F	17	22	20	1.6×10^3	3.0×10^3	9
7	54	M	14	21	19	2.4×10^3	2.4×10^3	7
8	72	F	10	13	15	2.8×10^3	2.8×10^3	9
9	49	M	19	26	17	1.2×10^3	1.2×10^3	4
21	57	M	10	33	10	7.3×10^3	1.7×10^4	9
Patients who did not require PFA (n=4)								
11	32	M	17	24	31	1.0×10^2	1.0×10^2	2
12	68	F	12	17	21	1.0×10^2	3.1×10^2	7
17	56	F	22	NA	22	1.1×10^2	1.1×10^2	1
18	37	M	23	NA	21	1.6×10^2	1.6×10^2	2

One patient who was erroneously treated with PFA at under the predetermined threshold was excluded in this analysis.
Abbreviations: HHV-6, human herpes virus-6; NA, not achieved; PFA, foscarnet sodium; UCBT, umbilical cord blood transplantation.