HHV-6 AS A CAUSATIVE AGENT IN HEMATOLOGICAL MALIGNANCIES

Two human herpesvirus, Epstein-Barr virus (EBV) and HHV-8, are well-known as oncogenic agents. As HHV-6 strains were first isolated from patients with lymphoproliferative disorders,[1] pathogenetic roles for HHV-6 in the development of lymphoproliferative diseases have been a matter of continuous interest. A possible pathogenetic role for HHV-6 in lymphoproliferative diseases was first suggested by the ability of its DNA to transform established NIH 3T3 cells and human epidermal keratinocytes *in vitro*.[22,23] Kashanchi *et al.*[24] reported that HHV-6 genes encode transactivation proteins, one of which has been shown to possess transformative properties. However, transforming events after HHV-6 infection have not been confirmed *in vitro*, and no definitive association between HHV-6 and canceration have been provided *in vivo*. HHV-6 has therefore not yet been defined as an oncogenic pathogen.

Hodgkin lymphoma

Both genetic and environmental factors have been implicated in the pathogenesis of Hodgkin lymphoma.[25] EBV is present in the neoplastic cells of 20-40% of patients with Hodgkin lymphoma,[25] and has been shown to represent an oncogenic infectious agent for Hodgkin lymphoma.[26] Associations between HHV-6 and Hodgkin lymphoma were first suggested by Torelli *et al.*[27] in 1991. They showed higher anti-HHV-6 antibody titers in patients with Hodgkin lymphoma than in normal blood donors, and HHV-6 sequences were detected in 3 of 25 patients with Hodgkin lymphomas. Since then, many studies aimed at identifying the HHV-6 genome in pathologic specimens using polymerase chain reaction (PCR) have been reported (Table 1).[28-36] Frequencies of positive HHV-6 DNA appear to vary widely among these studies, and may depend on the differences in PCR sensitivity for each study. Variant B has been identified more frequently than variant A. However, it is important to note that positive PCR results do not necessarily indicate the presence of HHV-6 in neoplastic cells. HHV-6 is a ubiquitous pathogen, and remains in a latent state in various host cells including leukocytes. Altered immune status due to disease may thus induce HHV-6 reactivation. As a result, positive PCR results for HHV-6 may merely reflect latent infection or immunological dysfunction. In fact, Sumiyoshi *et al.*[28] found amplified HHV-6 DNA using PCR in 64.3% of patients with Hodgkin lymphoma but were unable to detect HHV-6 DNA using Southern blot analysis. They concluded that the presence of HHV-6 DNA shown by PCR was derived from latent infection. Histopathological analysis is a reliable method to demonstrate HHV-6 infection in neoplastic cells. A small number of studies have investigated HHV-6 antigen expression in lymphoid tissue.[29-31] These investigations found a lack of HHV-6 antigen expression in neoplastic cells and limited expression in Reed-Sternberg cells,[29-31] arguing against any major pathogenic role of the virus in lymphomagenesis.

The possibility remains that the virus infection is associated with the clinicopathological features in patients with Hodgkin lymphoma. Several studies have shown that the frequency of detecting HHV-6 DNA is higher in patients with nodular sclerosis (NS) subtype than with other subtypes.[27,34,36] Lacroix *et al.*[36] reported that patients with the NS subtype of Hodgkin lymphoma who were positive for HHV-6 in lymph nodes were younger than those showing negative results. They also showed that the prognosis in these patients was very good, and HHV-6 positivity can be considered as a predictor of good outcomes.

Non-Hodgkin lymphoma (NHL)

1) Angioimmunoblastic T-cell lymphoma (AITL)

Clinical presentations including high-fever, polyclonal gammopathy, or polymorphic histological appearances raise the possibility of a role for infectious agents in the pathogenesis of AITL. To date, EBV,[37,38] HHV-6, and HHV-8 have been reported to show associations with AITL. HHV-6 is found in 22-62.5% of AITL cases by PCR (Table 2).[28,31,34,38-41] However, neither EBV[37] nor HHV-6[31] has been found in malignant cells by histopathological analysis, suggesting a lack of direct causative roles in the development of AITL. Zhou *et al.*[38] reported simultaneous infection with both EBV and HHV-6 B only in specimens showing histological patterns I or II, and a tendency towards an inverse correlation between EBV and HHV-6 B viral loads. These findings suggest an association among EBV, HHV-6 B, and histological progression of AITL.

2) Non-Hodgkin lymphoma (other than AITL)

The *HHV-6* genome is detected in 22.2-62.1% of cases of NHL by PCR (Table 2).[28, 34, 35, 40, 43, 44] Similar to what was outlined in the section on Hodgkin lymphoma and AITL, these results do not necessarily indicate presence of HHV-6 in neoplastic cells. Negative results for the detection of HHV-6 DNA by Southern blot analysis[28] and a lack of HHV-6 antigen expression in neoplastic cells[31, 44] suggest that HHV-6 DNA shown by PCR was derived from latent infection.

3) Adult T-cell leukemia (ATL)

HHV-6 can infect ATL cell lines.[45] HHV-6 has been effectively propagated in a T-cell line derived from a patient with ATL.[46] Persistent HHV-6 infection facilitates growth of ATL cells.[47] These *in vitro* findings suggest a possible pathogenic role for HHV-6 in ATL. Table 3 shows the results of HHV-6 DNA quantification in specimens from patients with ATL and other lymphoid malignancies using real-time PCR in

Table 1. HHV-6 infection in Hodgkin lymphoma

References	Detection method	Sample	No. of subjects	Positive rate for HHV-6	HHV-6 variant	Observations
Torelli *et al.* (1991)[27]	PCR	LN	Patients : 25	12 %	ND	All cases positive for HHV-6 (n = 3) belonged to the NS/ LD subtype.
			Controls[a] : 41	0 %		
Sumiyoshi *et al.* (1993)[28]	PCR	LN	Patients : 14	64. 3%	ND	
			Controls[b] : 56	98. 2%		
	Southern blot	LN	14	0 %		
Trovato *et al.* (1994)[29]	PCR	LN	15[c]	7 %	ND	
	ISH	LN	15	0 %		
Valente *et al.* (1996)[30]	PCR	LN	Patients : 52	73 %	2A&B/ 36B	
			Controls[b] : 19	68. 4%		
	ISH	LN	57	82. 4%		No Hodgkin or Reed-Sternberg cells were positive in any case.
	Southern blot	LN	NI	0 %		
Luppi *et al.* (1998)[31]	IHC	LN	14	14. 3%	ND	Early p 41 antigen was detected in Reed-Sternberg cells in two cases.
Schmidt *et al.* (2000)[32]	PCR	LN	88	13 %	8A/3B	
Shiramizu *et al.* (2001)[33]	PCR	LN	47[c]	0 %		
Collot *et al.* (2002)[34]	qPCR	LN	37	35. 1%	1A/12B	All Hodgkin lymphoma patients infected with HHV-6 presented with the NS subtype.
Hernández-Losa *et al.* (2004)[35]	PCR	LN	Patients : 20	40 %	ND	
			Controls[d] : 52	33 %		
Lacroix *et al.* (2007)[36]	qPCR	LN	86	79. 1%	5A/63B	HHV-6 genome was observed most often in the NS group (83. 6%). Among NS patients, $HHV\text{-}6^{-}/EBV^{+}$ patients were older than $HHV\text{-}6^{+}/EBV^{-}$ patients.

HHV-6, human herpesvirus 6 ; ISH, *in situ* hybridization ; IHC, immunohistochemistry ; qPCR, quantitative polymerase chain reaction ; LN, lymph node ; NI, not informative ; ND, not determined ; NS, nodular sclerosis ; LD, lymphocyte depletion ; EBV, Epstein-Barr virus
[a]non-Hodgkin lymphoma ; [b]benign lymphadenitis ; [c]pediatric Hodgkin lymphoma ; [d]normal donor spleen lymphocytes and reactive lymphadenitis

our institute. A relative high level of HHV-6 DNA was occasionally observed in specimens from ATL patients. However, whether high levels of HHV-6 DNA in pathogenic specimens reflect the presence of HHV-6 in ATL cells or HHV-6 reactivation from a latent state due to altered immune status remains uncertain.

Acute leukemia

Various hypotheses have been proposed concerning the involvement of infectious mechanisms in the development of acute leukemia. The role of HHV-6 in acute leukemia, particularly childhood acute lymphoblastic leukemia (ALL), has been a matter of continuous interest, but remains controversial. Ablashi *et al.*[48] found high levels of HHV-6 antibodies in a small group of children with ALL compared with normal subjects, but a sequential study[49] showed no significant differences in antibody titers between 50 patients with ALL and 50 sex-age matched blood donors. The largest serological case-control investigation[50] showed a slight but significant association between HHV-6 antibody titers and acute myeloid leukemia (AML) patients, while no significant association was found between HHV-6 antibodies and ALL. In 2002, however, Salonen *et al.*[51] found the presence of IgM antibodies in 40% of children with leukemia (n= 40) and high avidity of IgG compared with controls. The results again raise the possibility of a role for HHV-6 infection in childhood ALL. Bogdanovic *et al.*[52] analyzed HHV-6 and EBV DNA in Guthrie cards from children, but did not detect the DNA of these viruses in any samples from 54 subjects who later develop leukemia or 47 matched controls. These findings indicate that childhood ALL is unlikely to be associated with *in utero* infection by HHV-6.[52]

HHV-6 DNA was detected by PCR and *in situ* hybridization in the bone marrow cells of children with T-ALL[53] in 1991. However, Barozzi *et al.*[54] found that the presence of

Table 2. HHV-6 infection in non-Hodgkin lymphoma

References	Detection method	Sample	No. of subjects	Positive rate for HHV-6	HHV-6 variant	Observations
AITL, AILD, or IBL						
Sumiyoshi *et al.* (1993)[28]	PCR	LN	Patients : 8	62.5%	ND	
			Controls[a] : 56	98.2%		
	Southern blot	LN	8	0 %		
Luppi *et al.* (1993)[39]	PCR	LN	12	58.3%	ND	
Luppi *et al.* (1998)[31]	IHC	LN	5	0 %		
Ohyashiki *et al.* (1999)[40]	PCR-ELISA	PB & LN	Patients : 3	100 %	3 B	Number of HHV-6 genomes in patients was high.
			Controls[b] : 23	43.4%	9B/2 unclassified	
Collot *et al.* (2002)[34]	qPCR	LN	5	20.0%	1 B	
Vrsalovic *et al.* (2004)[41]	PCR	LN	18	22.2%	ND	
Zhou *et al.* (2007)[38]	qPCR	LN	42	45.2%	Only HHV-6B was examined.	Simultaneous infection with EBV and HHV-6B was found in specimens with patterns I and II. A tendency toward an inverse correlation between EBV and HHV-6 B viral load was seen.
Mycosis fungosides						
Erkek *et al.* (2001)[42]	PCR	TT	92	1.1%	ND	
HIV-associated NHL						
Fillet *et al.* (1995)[43]	PCR	TT	27	44.4%	2A/1B/6A & B	
T-cell lymphoma						
Sumiyoshi *et al.* (1993)[28]	PCR	LN	Patients[c] : 33	57.6%	ND	
			Controls[a] : 56	98.2%		
	Southern blot	LN	33[c]	0 %		
Ohyashiki *et al.* (1999)[40]	PCR-ELISA	PB & LN	6	50 %	2 B/1 unclassified	
Collot *et al.* (2002)[34]	qPCR	LN	8[c]	25.0%	2 B	
B-cell lymphoma						
Sumiyoshi *et al.* (1993)[28]	PCR	LN	Patients : 29	62.1%	ND	
			Controls[a] : 56	98.2%		
	Southern blot	LN	29	0 %		
Ohyashiki *et al.* (1999)[40]	PCR-ELISA	PB & LN	10	20 %	1 B/1 unclassified	The HHV-6 viral load was low.
Collot *et al.* (2002)[34]	qPCR	LN	36	22.2%	1 A/7 B	
Any type						
Razzaque *et al.* (1996)[44]	PCR	LN	6	100 %	ND	
	IHC	LN	6	33.3%		
Luppi *et al.* (1998)[31]	IHC	LN	15	0 %		
Hernández-Losa *et al.* (2004)[35]	PCR	LN	Patients : 63	27 %	ND	
			Controls[d] : 52	33 %		

AITL, angioimmunoblastic T cell lymphoma ; AILD, angioimmunoblastic lymphadenopathy with dysproteinemia ; IBL, immunoblastic lymphadenopathy ; HIV, human immunodeficiency virus ; NHL, non-Hodgkin lymphoma ; IHC, immunohistochemistry ; qPCR, quantitative polymerase chain reaction ; LN, lymph node ; PB, peripheral blood ; TT, tumor tissue ; ND, not determined

[a]benign lymphadenitis ; [b]peripheral blood leukocyte from healthy volunteers ; [c]other than AITL or AILD ; [d]normal donor spleen lymphocytes and reactive lymphadenitis

Table 3. Quantification of HHV-6 DNA in patients with adult T-cell leukemia and other lymphoid malignancies (data from Oita University)

Disease	Sample	No. of subjects	No. of positive cases	Positive rate for HHV-6	HHV-6 DNA among positive samples (copies/μg)
ATL	LN	6	2	33.3%	180.7
					38.6
	PB	11	5	45.5%	2933.3
					107.7
					63.1
					35.0
					31.2
T-cell lymphoma	LN	2	0	0 %	
B-cell lymphoma	LN	12	2	16.7%	7.5
					6.3
Hodgkin lymphoma	LN	2	2	100 %	121.8
					4.5
Reactive lymphadenitis	LN	2	2	100 %	6.5
					4.5

ATL, adult T-cell leukemia; LN, lymph node; PB, peripheral blood

HHV-6 DNA is not frequent in patients with ALL compared to normal subjects. Seror *et al.*[55] recently analyzed HHV-6 DNA copy number by real-time PCR in bone marrow and peripheral blood from 36 children with ALL at diagnosis and during complete remission. Positive rates were 13.9% in leukemia samples and 34.1% in complete remission samples. Viral load was lower at diagnosis than at complete remission. Based on these findings, they concluded that HHV-6 may be unable to infect leukemia cells and reactivation may be observed during complete remission.

HHV-6 chromosomal integration and development of hematological malignancies

The unique form of HHV-6 persistence is characterized by integration of the viral DNA sequences into chromosomes. The incidence of chromosomal integration (CI) for HHV-6 is about 2% in the population of the United Kingdom.[56] Whether integrated HHV-6 is capable of replication or is associated with disease remains unclear. Daibata *et al.*[57] demonstrated integration of *HHV-6* genome in a Burkitt's lymphoma cell line. Furthermore, they showed chromosomal transmission of HHV-6 DNA in ALL.[58] These findings suggest the possibility of an association between chromosomally integrated HHV-6 and development of hematological malignancies. On the other hand, Hobacek *et al.*[59] recently reported the prevalence of HHV-6 CI among children with ALL or AML. Among 339 patients, 5 patients (1.5%) were confirmed with HHV-6 CI. They concluded that the prevalence of HHV-6 CI in childhood leukemia does not differ from that published for other patients or healthy populations.

HHV-6 AS AN INFECTIOUS AGENT IN HEMATOLOGICAL MALIGNANCIES

As described above, many studies have tried to establish links between HHV-6 infections and development of hematological malignancies, with discordant results. However, HHV-6 is increasingly being recognized as an opportunistic pathogen rather than a causal pathogen among clinical hematologists. Particularly in the field of stem cell transplantation (SCT), HHV-6 is now considered as an important pathogen linked to life-threatening encephalitis. On the other hand, the clinical syndrome of HHV-6 reactivation in patients with hematological malignancies who do not receive allogeneic SCT is not well defined.

HHV-6 reactivation in allogeneic SCT

Overall, HHV-6 has been shown to reactivate in 40-50% of patients undergoing SCT.[60-66] Most HHV-6 infections are due to reactivation of HHV-6 type B.[63,67] HHV-6 appears most frequently around 2-6 weeks after SCT,[61,63,65,66,68] and onset of HHV-6 reactivation is concentrated around 0-9 days after neutrophil engraftment.[65] HHV-6 can reactivate to high levels within a week,[65,69] but duration of HHV-6 reactivation is usually short (Fig. 1).[65] Younger age,[63] underlying diseases,[63] sex mismatch,[63] HLA mismatch,[65,66] steroid treatment,[63,65] unrelated transplants,[64,65] cord blood transplantation,[66,70] and low anti-HHV-6 IgG titer before transplantation[66] have been identified as risk factors associated with HHV-6 reactivation. Steroid administration[65] and cord blood transplantation[70] are also associated with higher-

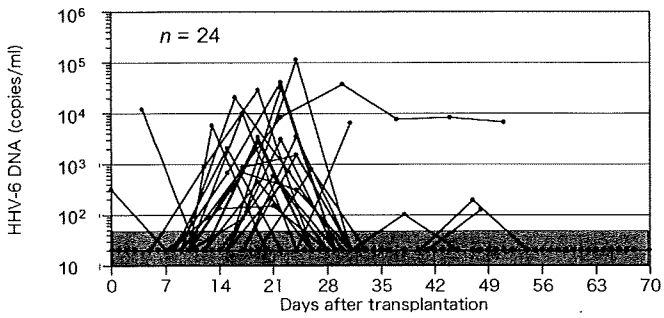

Fig. 1. Kinetics of human herpesvirus (HHV)-6 DNA loads in plasma among patients who had received allogeneic stem cell transplantation and showed positive results for HHV-6 DNA by polymerase chain reaction. Taken from [Reference 65].

Table 4. Clinical manifestations potentially associated with human herpesvirus 6 in stem cell transplantation

Observed disease	References
Pyrexia	60, 71
Rash	61, 71-74
Acute GVHD	63, 64, 72, 75
Delayed platelet engraftment	60, 63, 65, 68, 76
Myelosuppression	60, 64, 76
Encephalopathy	63, 65, 66, 68, 69, 77-87
Lung disease	64, 88-90
Gastrointestinal disease	64, 91
All-cause mortality	63

GVHD, graft-*versus*-host disease

level HHV-6 reactivation. Cord blood transplant recipients thus display a higher risk of HHV-6 infection in terms of both incidence and level.

To date, many studies have shown the significance of HHV-6 as a pathogen for various complications after SCT (Table 4).[60, 61, 63-66, 68, 69, 71-91] Due to the significant incidence and poor prognosis, HHV-6 encephalitis is thought to represent the most important complication associated with HHV-6.

HHV-6 encephalitis in SCT

Diagnostic criteria for HHV-6 encephalitis have yet to be established, but HHV-6 encephalitis is generally defined as : the presence of neurological symptoms ; positive PCR results for HHV-6 in cerebrospinal fluid ; and the absence of other identified etiologies of encephalitis.[81] Retrospective surveillance by a Japanese group has reported an incidence of 0.98%.[81] Five epidemiological studies that monitored HHV-6 viral load have shown associations between HHV-6 reactivation and development of central nervous system (CNS) dysfunction.[63, 65, 66, 68, 69] Incidences ranged from 3.6% to 8.0%. Vu *et al.*[84] reported an incidence of 11.6% in patients receiving alemtuzumab-supported conditioning. The high incidence of HHV-6 encephalitis among patients receiving cord blood transplant is becoming a major concern in Japan.

A retrospective analysis of 23 patients with HHV-6 encephalitis in Japan[86] revealed that most cases of HHV-6 encephalitis developed in patients who had received transplants from alternative donors including unrelated donor or cord blood, and more than half had received steroid treatment, with onset of encephalitis beginning at a median of day 22 after SCT. Symptoms included coma/impaired consciousness (91%), loss of short-term memory (73%) and seizures (70%). Magnetic resonance imaging (MRI) revealed abnormal findings within the temporal lobes in 73% of patients. Zerr[80] reviewed 48 recipients with HHV-6 encephalitis who had previously been described in the literature and found similar results, with 84% of patients receiving mismatched related or unrelated transplantation. Onset of encephalitis began on a median of day 24. Symptoms were characterized by short-term memory loss, depressed consciousness, confusion, disorientation and seizure. MRI showed abnormal findings in 70% of patients, most commonly within the medial temporal lobes (limbic encephalitis). Fig. 2 shows MRI findings in a patient who developed HHV-6 encephalitis after SCT.

The limbic system seems to be an exclusive target of HHV-6.[65, 69, 77, 78, 80, 82, 83, 86] Using immunohistochemical methods, several investigators have found that HHV-6 displays tropism for hippocampal astrocytes in recipients who developed encephalitis.[77, 78, 82] The pathogenic mechanisms underlying HHV-6 encephalitis, however, have not been well defined. HHV-6 encephalitis develops concomitant to peak HHV-6 DNA levels in plasma,[65] and higher levels of HHV-6 DNA in peripheral blood are associated with the development of CNS dysfunction.[65, 87] These findings suggest direct destruction of the CNS by HHV-6. However, not all patients with high HHV-6 load in peripheral blood develop CNS dysfunction,[87] suggesting that additional factors are required for progression to encephalopathy. A recent report showed higher levels of plasma interleukin-6 before HHV-6 reactivation are associated with progression to HHV-6 encephalitis.[87]

The prognosis of HHV-6 encephalitis is poor. A retrospective study in Japan[86] showed sequelae in about half of patients despite receiving antiviral treatment. Zerr[80] reported that 19 of 44 patients with HHV-6 encephalitis who had been previously described in the literature were left with neurological compromise or died of encephalitis. These observations indicate that the efficacy of antiviral treatments appears insufficient once HHV-6 encephalitis has developed.

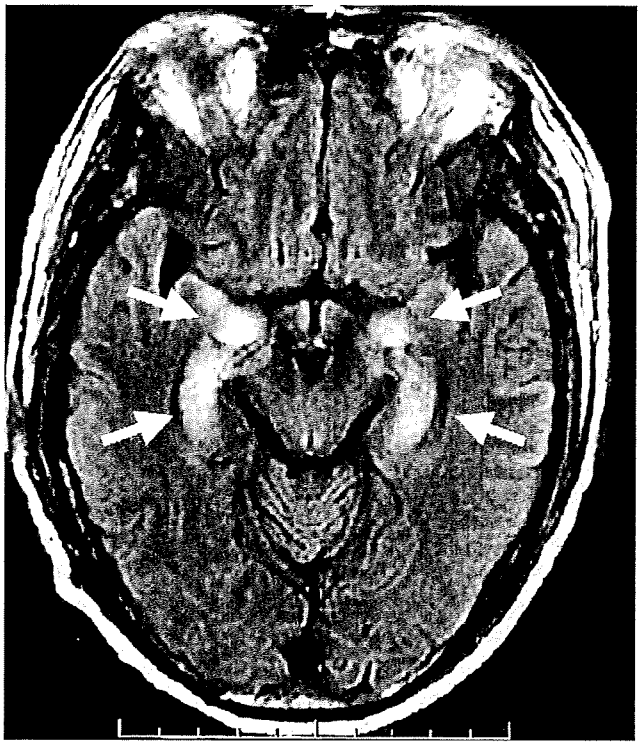

Fig. 2. T 2-weighted fluid-attenuated inversion recovery imaging in a patient who developed human herpesvirus 6 encephalitis. *Arrows* indicate signal hyperintensities in the region of the limbic system. Taken from [Reference 65].

HHV-6 infection in patients with hematological malignancies who do not receive allogeneic SCT

A few case reports have described patients who developed HHV-6-associated complications, including thrombotic microangiopathy after autologous SCT[92,93] or HHV-6 encephalitis in patients with ATL.[94] However, few epidemiological studies have examined the incidence or significance of HHV-6 reactivation in patients with hematological malignancies who do not receive allogeneic SCT. Yoshikawa *et al.*[61] found no cases of HHV-6 viremia among patients receiving autologous SCT. Chemaly *et al.*[95] reported 11 of 37 patients with leukemia displayed positive HHV-6 DNA in whole blood specimens. However, that study specifically examined severely immunosuppressed patients with leukemia at risk of mold infection, and the results may therefore not be applicable to the general leukemia population. The clinical significance of HHV-6 reactivation in each hematological malignancy or each therapy should be clarified in the future.

CONCLUSIONS AND FUTURE INVESTIGATIONS

Many studies have tried to establish links between HHV-6 infection and development of hematological malignancies, with discordant results. Interpretation of positive PCR results for HHV-6 in pathologic specimens is complicated by the ubiquitous nature of HHV-6 and its abilities to remain in a latent state, reactivate under altered immune status, and integrate into host chromosomal DNA. Examinations of HHV-6 antigen expression in tumor tissue would improve the interpretation of results. To date, however, relatively few studies[29-31] have focused on HHV-6 expression on neoplastic cells, and no evidence has been found for the involvement of HHV-6 in neoplastic cells. More large-scale studies using histopathological methods might identify a pathogenic role for HHV-6 in a subset of lymphoproliferative disorders.

Despite the lack of HHV-6 infection in neoplastic cells, HHV-6 infection may be associated with the clinical course for NS-type Hodgkin lymphoma[36] and with pathological features for AITL.[38] These findings suggest HHV-6 infection of normal lymphocytes in tumor tissue affects the histological progression or prognosis in a subset of lymphomas. The ability of HHV-6 to modulate the production of and response to cytokines and chemokines[38,96,97] may be associated with such behaviors. Further in-depth examinations may identify complementary roles for HHV-6 in the pathogenesis or progression of lymphoma.

HHV-6 is now recognized as a well-known pathogen in the field of allogeneic SCT. About half of SCT recipients experience HHV-6 reactivation. The most important, life-threatening complication associated with HHV-6 reactivation appears to be encephalitis. The pathogenic roles of HHV-6 have not been well clarified but may include direct or immune-mediated destruction of the CNS. Further exploration of the pathogenic roles of HHV-6 in the development of encephalitis may contribute to the development of effective preventative methods and the improvement of prognosis. The efficacy of anti-viral therapy against developed HHV-6 encephalitis appears insufficient, and the establishment of strategies for appropriate pre-emptive or prophylactic methods against HHV-6 encephalitis represents an important challenge for SCT physicians.

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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.

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 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 ($\times 10^8$/kg, median, range)	2.6 (0.7–4.4)	NA
CD34 positive ($\times 10^6$/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.

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.

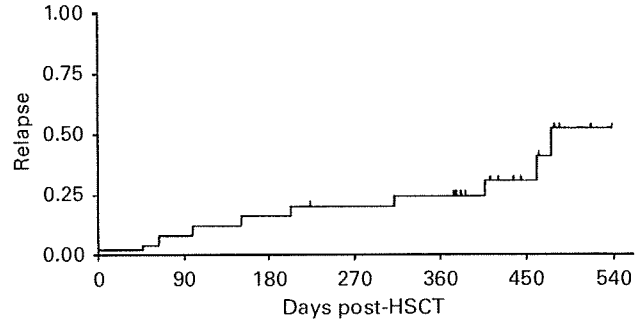

Figure 1 Disease relapse after i.v. BU and CY prior to allogeneic hematopoietic SCT in patients with leukemia and lymphoma.

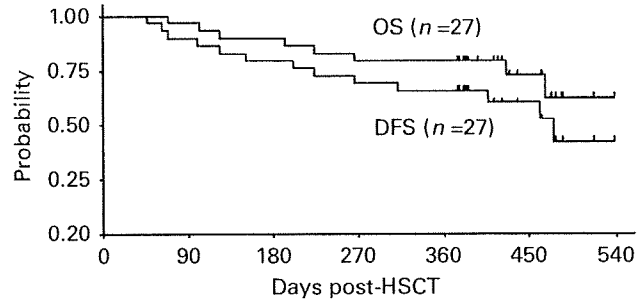

Figure 2 Overall survival and disease-free survival after i.v. BU and CY prior to allogeneic hematopoietic SCT in patients with leukemia, myelodysplastic syndrome and lymphoma.

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-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,

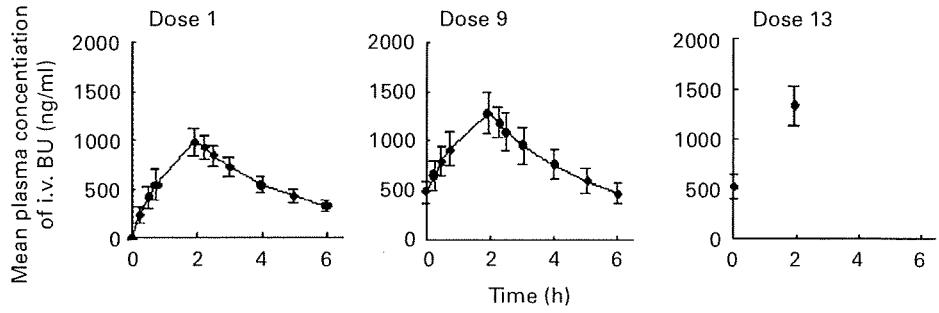

Figure 3 Pharmacokinetic results of i.v. BU at doses 1, 9 and 13 ($n = 30$).

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 0.601/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.

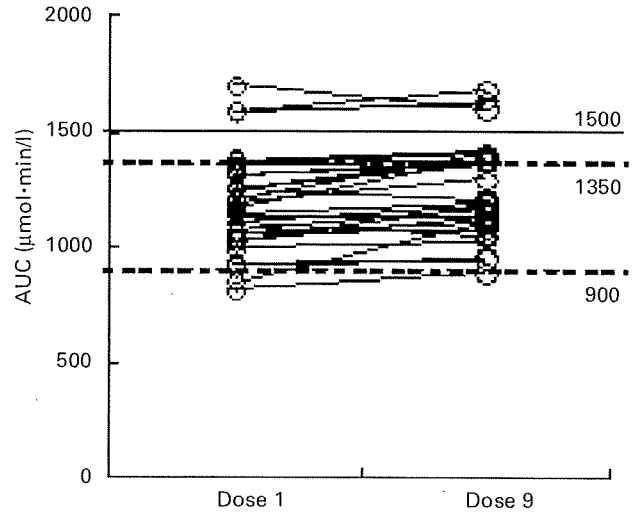

Figure 4 Individual patient area under the plasma concentration–time curve (AUC) values of i.v. BU at doses 1 and 9 ($n = 29$).

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.

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Possible Association between Obesity and Posttransplantation Complications Including Infectious Diseases and Acute Graft-versus-Host Disease

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Both obesity and malnutrition are considered risk factors for complications after bone marrow transplantation (BMT). To elucidate the impact of pretransplantation body mass index (BMI) on clinical outcome, we performed a retrospective cohort study with registration data from the Japan Marrow Donor Program (JMDP). Between January 1998 and December 2005, a total of 3935 patients received unrelated BMT through the JMDP; of these, 3827 patients for whom pretransplantation height and weight data were available were included in the study. Patients were stratified according to pretransplantation BMI values (low BMI: BMI < 18 kg/m^2, n = 295; normal BMI: $18 \leq$ BMI < 25 kg/m^2, n = 2906; overweight: $25 \leq$ BMI < 30 kg/m^2, n = 565; obese: 30 kg/m$^2 \leq$ BMI, n = 61). In a univariate analysis, pretransplantation BMI was associated with a significantly greater risk of grade II-IV acute graft-versus-host disease (GVHD; $P = .03$). Multivariate analysis showed that pretransplantation BMI tended to be associated with an increased risk of grade II-IV acute GVHD ($P = .07$). Obesity was associated with an increased risk of infection compared with normal BMI (odds ratio = 1.9; 95% confidence interval = 1.1 to 3.2; $P = .02$). Our findings demonstrate a correlation between pretransplantation BMI and posttransplantation complications. Although BMI depends strongly on multiple factors, the effect of obesity on clinical outcome should be evaluated in a prospective study.

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KEY WORDS: Obesity, Allogeneic transplantation, Infection, Acute graft-versus-host disease

INTRODUCTION

Both obesity and malnutrition are considered risk factors for complications and increased relapse and nonrelapse mortality in hematopoietic stem cell transplantation (HSCT). An inferior outcome after allogeneic HSCT has been reported in obese adult patients in both allogeneic [1,2] and autologous HSCT [3-5]. Furthermore, our group recently reported that hyperglycemia during the neutropenic period is associated with an increased risk of acute graft-versus-host disease (GVHD) and subsequent nonrelapse mortality [6]. Obesity obviously is associated with an increased risk of hyperglycemia [7], which can lead to an inferior outcome after allogeneic HSCT. Recently, obesity was reported to be associated with low-grade systemic inflammation and was identified as a possible risk factor for autoimmune diseases [8-10]. Alternatively, malnutrition has been reported to be associated with an increased risk of early death after allogeneic HSCT [11,12]. Several reports have noted an association between malnutrition and a high incidence of infectious disease in conventional chemotherapy settings [13-15].

Although we can speculate that these infectious complications may be associated with nonrelapse mortality in HSCT, there is currently no agreement

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regarding a suitable target range of pretransplantation body mass index (BMI) for clinical management. Previous studies have included various kinds of stem cell sources, and some have included HSCT with T cell depletion. The aim of the present study was to retrospectively evaluate the impact of pretransplantation BMI on the clinical outcome after unrelated bone marrow transplantation (BMT) for hematologic malignancies, using registration data from the Japan Marrow Donor Program (JMDP). The results should provide insight into how to better manage nutritional support for patients undergoing HSCT.

PATIENTS AND METHODS

A total of 3935 patients with various hematologic malignancies underwent BMT through the JMDP between January 1998 and December 2005. Data from 3827 of these patients for whom pretransplantation height and weight data were available were included in the present study. Patient characteristics are summarized in Table 1. The median patient age was 39 years (range, 18 to 72 years), and diagnoses included acute myeloid leukemia (AML; n = 1165), acute lymphoblastic leukemia (ALL; n = 755), myelodysplastic syndrome/myeloproliferative disease (MDS/MPD; n = 597), malignant lymphoma (ML; n = 500) and chronic ML (CML; n = 576), other leukemia (n = 69), multiple myeloma (n = 71), and other (n = 94). Standard risk included acute leukemia in first complete remission (CR1), CML in first chronic phase, MDS in refractory anemia, and lymphoma in CR1. The rest of the patients were categorized as a high-risk group. Bone marrow was the sole stem cell source for transplantation. Total body irradiation (TBI) was used in 2849 patients. GVHD prophylaxis included cyclosporine (CSP)-based (n = 1520) and tacrolimus (TAC)-based regimens (n = 2155), or other combinations (n = 152), with the addition of low-dose antithymocyte globulin (ATG) in 205 patients. Alleles at the HLA-A, -B, and -DRB1 loci were identified by high-resolution DNA typing. The median follow-up period was 565 days. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and the study design was approved by the JMDP's Institutional Review Board.

The study's primary endpoints were nonrelapse mortality at 100 days and 1 year, overall survival at 1 year, and progression-free survival at 1 year. For nonrelapse mortality, an event was death without disease progression after BMT. For overall survival, an event was death from any cause after BMT. For progression-free survival, an event was disease progression or death after BMT. Secondary endpoints were the incidence of infection (bacterial, viral, fungal, and others); incidence of lung organ toxicity including interstitial pneumonia, adult respiratory distress syndrome, bronchiolitis obliterans, pulmonary hemorrhage, and others, excluding pneumonia with obvious infectious diseases; and incidence of hepatic toxicity, including veno-occlusive disease and drug toxicity. Acute GVHD was classified as grade 0, I, II, III, or IV according to established criteria [16]. The probability of acute GVHD, nonrelapse mortality rate, overall survival, progression-free survival, and relapse rate were estimated using the Kaplan-Meier method. Death without acute GVHD was treated as censoring in the analysis of acute GVHD, and death without progression was treated as censoring in the analysis of relapse. Dichotomous variables between groups were compared using the χ^2 test, and survival times were compared using the log-rank test. An order-restricted version of the log-rank test (a log-rank trend test) was used to test ordered differences between the estimated survival curves. Multivariate analyses were performed using a logistic regression model or a Cox proportional hazards model, as appropriate. The following covariates were included in the univariate analysis: BMI (BMI < 18 kg/m^2, $18 \leq$ BMI < 25 kg/m^2, $25 \leq$ BMI < 30 kg/m^2, and 30 kg/m$^2 \leq$ BMI), sex (donor–recipient pairs), patient age (age < 30 years, $30 \leq$ age < 50 years, age ≥ 50 years), donor age (age < 40 years, age ≥ 40 years), type of disease, risk of leukemia relapse (standard vs high), conditioning (TBI-based vs non–TBI-based), GVHD prophylaxis (CSP-based vs TAC-based), genotypic HLA match versus HLA mismatch, ABO match versus mismatch (major mismatch vs minor mismatch vs major/minor mismatch vs match), cell dose in the graft (dose $< 3.0 \times 10^8$/kg, $3.0 \leq$ dose $< 5.0 \times 10^8$/kg, $\geq 5.0 \times 10^8$/kg), and use of ATG/antilymphocyte globulin (ALG) (ATG/ALG vs no ATG/ALG). All P values were 2-sided. A P value $< .05$ was considered statistically significant.

RESULTS

Patient Characteristics

Table 1 gives the BMI distribution of the study group. Patients were classified into 4 groups based on pretransplantation BMI values according to consensus weight designations from the World Health Organization [17] and the National Heart Lung and Blood Institute Expert Panel [18], as follows: low BMI (BMI < 18 kg/m^2; n = 295), normal BMI ($18 \leq$ BMI < 25 kg/m^2; n = 2906), overweight ($25 \leq$ BMI < 30 kg/m^2; n = 565), and obesity (30 kg/m$^2 \leq$ BMI; n = 61). The prevalence of obesity was quite low compared with that in previous reports from Western countries [1-4]. Significant differences in patient characteristics were observed with regard to age, sex disparity, total nucleated cells (TNCs) per body weight, and primary disease. The low-BMI group

Table 1. Patient Characteristics

	n (%)				
	BMI < 18 kg/m^2	18 ≤ BMI <25 kg/m^2	25 ≤ BMI < 30 kg/m^2	30 kg/m^2 ≤ BMI	
	(n = 295)	(n = 2906)	(n = 565)	(n = 61)	*P* value
Recipient age, years					
< 30	116 (39)	734 (25)	90 (16)	14 (23)	< .0001
30 ≤ age < 50	121 (41)	1473 (51)	322 (57)	36 (59)	
> 50	58 (20)	699 (24)	153 (27)	11 (18)	
Donor age, years					
< 40	217 (74)	2099 (72)	385 (68)	38 (62)	.27
≥ 40	75 (25)	741 (25)	162 (29)	18 (30)	
Sex, donor/recipient					
Match	181 (61)	1833 (63)	374 (66)	39 (64)	< .0001
Male/female	70 (24)	519 (18)	87 (15)	9 (15)	
Female/male	43 (16)	495 (17)	87 (15)	9 (15)	
TNC ($\times 10^{-8}$/kg)					
TNC < 3.0	12 (4)	323 (11)	160 (28)	31 (51)	< .0001
3.0 ≤ TNC< 5.0	60 (20)	1085 (37)	267 (47)	16 (26)	
5.0 ≤ TNC	187 (63)	1191 (41)	78 (14)	1 (2)	
Year of transplantation					
1998	12 (4)	83 (3)	11 (2)	0 (0)	.18
1999	21 (7)	248 (9)	39 (7)	0 (0)	
2000	26 (9)	363 (12)	81 (14)	7 (11)	
2001	43 (15)	398 (14)	74 (13)	7 (11)	
2002	47 (16)	409 (14)	72 (13)	14 (23)	
2003	50 (17)	404 (14)	87 (15)	10 (16)	
2004	40 (14)	463 (16)	88 (16)	12 (20)	
2005	56 (19)	538 (19)	113 (20)	11 (18)	
Diagnosis					
Acute leukemia	186 (63)	1469 (51)	304 (54)	29 (48)	.02
CR1/CR2/>CR2	81/33/65	594/301/541	113/79/107	7/4/18	
Chronic leukemia	30 (10)	449 (15)	84 (15)	13 (21)	
CP1/CP2/AP/BC	16/5/5/3	251/66/65/53	53/8/12/11	5/3/3/2	
MDS/MPD	37 (13)	462 (16)	87 (15)	11 (18)	
RA/RAEB/others	7/12/10	99/155/166	25/33/20	7/3/1	
ML	35 (12)	400 (14)	62 (11)	4 (7)	
CR/>CR	10/19	138/230	24/33	1/3	
MM	5 (2)	56 (2)	10 (2)	0 (0)	
CR/>CR	1/1	10/33	1/7	0/0	
Disease stage*					
Standard	110 (37)	1034 (36)	202 (36)	19 (31)	.67
High	158 (54)	1686 (58)	324 (57)	38 (62)	
Blood type disparity					
Match	146 (49)	1477 (51)	276 (49)	28 (46)	.98
IA	8 (3)	103 (4)	18 (3)	2 (3)	
MA	71 (24)	650 (22)	127 (22)	13 (21)	
MI	65 (22)	586 (20)	121 (21)	14 (23)	
HLA disparity					
HLA allele match	185 (63)	1660 (57)	342 (61)	36 (59)	.01
HLA allele mismatch	70 (24)	857 (29)	149 (26)	18 (30)	
1 allele mismatch	59 (20)	728 (25)	118 (21)	11 (18)	
2 allele mismatch	10 (3)	116 (4)	31 (5)	6 (10)	
3 allele mismatch	1 (0)	13 (0)	0 (0)	1 (2)	
Conditioning regimen					
Conventional	235 (80)	2308 (79)	443 (78)	52 (85)	.25
Reduced-intensity	59 (20)	539 (19)	105 (19)	5 (8)	
TBI for conditioning					
No	80 (27)	654 (23)	146 (26)	12 (20)	.14
Yes	214 (73)	2188 (75)	402 (71)	45 (74)	
ATG for conditioning					
No	268 (91)	2670 (92)	517 (92)	55 (90)	.21
Yes	23 (8)	155 (5)	25 (4)	2 (3)	
GVHD prophylaxis					
CSP-based	137 (46)	1141 (39)	226 (40)	16 (26)	.19
TAC-based	153 (52)	1651 (57)	312 (55)	39 (64)	
Others	3 (1)	48 (2)	7 (1)	2 (3)	
Comorbidity					
Liver dysfunction					
No	239 (81)	2436 (84)	481 (85)	49 (80)	.78
Yes	41 (14)	360 (12)	66 (12)	7 (11)	

(Continued)