from non–KIR-L–mismatched donors. In contrast, another study failed to demonstrate the beneficial effect of KIR-L mismatches in CBT recipients [35]. Only 16% of the 218 patients analyzed in the former study received a GVHD prophylaxis regimen that included MMF [34], whereas 78% of the 257 patients treated in the latter study received MMF [34]. Although a number of factors are involved in the different outcomes between these 2 studies, the use of MMF and the resultant NK cell impairment might be one reason for the difference in the KIR-L–mismatch effect between the 2 studies. Thus, the possible negative effect of MMF on the GVL effect after SCT from KIR-L–mismatched donors merits examination in a prospective randomized study.

In conclusion, our in vitro study shows that MPA has a more potent inhibitory effect on NK cells than other immunosuppresants commonly used for GVHD prophylaxis. Thus, the combination of MTX + CI might be preferred over MMF + CI in terms of the retention of the NK cell–mediated GVL effect as a GVHD prophylaxis regimen.

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Impact of T Cell Chimerism on Clinical Outcome in 117 Patients Who Underwent Allogeneic Stem Cell Transplantation with a Busulfan-Containing Reduced-Intensity Conditioning Regimen

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Within the concept of reduced-intensity stem cell transplantation (RIST) there is a wide range of different regimens used, and little information is available on the clinical impact of chimerism status in patients conditioned with a busulfan-containing regimen. Therefore, we retrospectively reviewed lineage-specific chimerism and the subsequent clinical outcome in 117 patients (median age, 55 years; range: 29-68) who underwent busulfan-containing RIST. The conditioning regimen consisted of busulfan (oral 8 mg/kg or i.v. 6.4 mg/kg) and fludarabine (180 mg/m^2, n = 64) or cladribine (0.66 mg/kg, n = 53), with or without 2-4 Gy total-body irridiation (TBI) (n = 26) or antihuman T-lymphocyte immunoglobulin (ATG; 5-10 mg/kg; n = 31). Chimerism was evaluated with peripheral blood samples taken on days 30, 60, and 90 after transplantation by polymerase chain reaction (PCR)-based amplification of polymorphic short tandem repeat regions. The median follow-up of surviving patients was 1039 days (153-2535). The percent donor-chimerism was significantly higher in granulocyte than T cell fraction throughout the entire course, and the median (mean) values were, respectively, 100% (96%) versus 95% (83%), 100% (98%) versus 100% (89%), and 100% (98%) versus 100% (91%) at days 30, 60, and 90 after RIST. In a multivariate analysis, having received <2 types of chemotherapy regimens before RIST was the only factor that was significantly associated with low donor T cell chimerism (<60%) at day 30 (hazard ratio [HR]: 6.1; 95% confidence interval [CI], 2.1-18.4; $P < .01$). The median percentage of donor T cell chimerism at day 30 was 9% (0%-63%) in 5 patients who experienced graft failure, which was significantly lower than that (97%; 15%-100%) in the rest of the patients ($P < .01$). No correlation was found between the kinetics of T cell chimerism and the occurrence of acute or chronic GVHD (aGVHD, cGVHD). The stem cell source and the addition of TBI or ATG were not associated with the degree of T cell chimerism, overall survival (OS) or event-free survival (EFS). In a Cox proportional hazard model, low donor T cell chimerism of <60% at day 30 was associated with both poor OS (HR: 2.2; 95% CI, 1.1-4.5; $P = .02$) and EFS (HR: 2.0; 95% CI, 1.1-3.8; $P = .02$). In conclusion, we found that 43% of the patients retained mixed donor T cell chimerism (<90% donor) at day 30, whereas 92% achieved complete chimerism in granulocyte fraction. Low donor T cell chimerism of <60% at day 30 may predict a poor outcome, and a prospective study to examine the value of early intervention based on chimerism data is warranted.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) with a reduced-intensity conditioning (RIC) regimen has been increasingly used in patients with hematologic diseases who cannot be candidates for conventional HSCT because of age, medical comorbidities, or prior failed myeloablative SCT. Many different RIC regimens are currently in use, but most of them

incorporate fludarabine (Flu) as a background agent in combination with other drugs including cyclophosphamide (Cy) [1], melphalan (Mel) [2], busulfan [2,3], low-dose total body irradiation (TBI) [4], antithymocyte globulin (ATG) [3], and alemtuzumab [5].

RIC regimens have been investigated in the hope of reducing toxicity, whereas their engraftment potential and antileukemia effect rely mainly on the expansion of donor-derived cells and subsequent immune-mediated graft-versus-leukemia (GVL) effects [6,7]. In this setting, lineage-specific chimerism analysis to assess the origin of lymphohematopoietic cells becomes particularly important for identifying patients at risk for graft failure/rejection, graft-versus-host disease (GVHD), and relapse or progressive disease (PD) [4,8,9]. Because the posttransplantation chimerism status is based on a fine balance between the cytotoxicity or immunosuppressive potential of the regimen used and the recipient's reserve immunocompetence, each RIC regimen should be evaluated individually for chimerism kinetics [1,4,10-13].

Compared with a regimen that includes Flu and Me, it has been reported that the combination of Flu and i.v. Bu was associated with improved survival in patients transplanted in remission, which was more frequently associated with mixed chimerism [2]. However, very little information is currently available on the clinical impact of lineage-specific chimerism status in patients who are conditioned with a Bu-containing RIC regimen. Therefore, we examined the correlation between specific patterns of lineage-specific chimerism and subsequent clinical outcomes.

PATIENTS AND METHODS

Patients and Transplantation Procedures

We retrospectively reviewed the medical records of 117 patients who had various hematologic malignancies and underwent allogenic HSCT with Bu-containing RIC at our hospital from January 2000 to December 2006. The reasons for selecting RIC regimens included older patient age, medical comorbidities, and prior failed myeloablative SCT. The patients' characteristics are summarized in Table 1. The median age of the patients was 52 years (range: 29-68 years), and the hematologic malignancy included acute myelogenous leukemia (AML) (n = 23), AML evolving from a myelodysplastic syndrome (MDS) (n = 16), acute lymphoblastic leukemia (ALL) (n = 5), malignant lymphoma (n = 44), MDS (n = 16), chronic myelogenous leukemia (CML) (n = 9), chronic lymphocytic leukemia (CLL) (n = 1), multiple myeloma (MM) (n = 1), and atypical CML (n = 2).

The conditioning regimen consisted of Bu (oral 8 mg/kg or i.v. 6.4 mg/kg) and Flu (180 mg/m^2, n = 64) or cladribine (0.66 mg/kg, n = 53), with or without 2-4 Gy TBI (n = 26) or antihuman T-lymphocyte immunoglobulin (Fresenius Biotech GmbH, Germany) (ATG; 5-10 mg/kg, n = 31).

In Japan, only bone marrow is permitted as a stem cell source in transplantation from an unrelated healthy volunteer donor. In the setting of nonmyeloablative SCT from an unrelated donor, the sustained engraftment rate has been reported to be lower for recipients of bone marrow than for those given granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells (G-PBMC) [14]. Therefore, low-dose TBI was also added to the conditioning regimen in 25 of the 32 patients who underwent reduced intensity stem cell transplantation (RIST) from an unrelated bone marrow donor to facilitate engraftment. Recipients of HLA-mismatched grafts tended to receive ATG-containing conditioning regimens (20 of the 27 recipients of HLA-mismatched grafts [74%] versus 11 of the 90 recipients of HLA-matched grafts

Table 1. Association between patients characteristics and donor T-cell chimerism at day 30

Characteristics	Total (n=117)	T cell chimerism at day 30 <60% (n=18)	T cell chimerism at day 30 ≥60% (n=99)
Patient age, years			
Median (range)	55 (29-68)	57 (35-66)	54 (29-68)
<55	56 (48%)	6 (33%)	50 (51%)
≥55	61 (52%)	12 (67%)	49 (49%)
Diseases type			
Acute leukemia	44 (38%)	5 (28%)	39 (39%)
Lymphoma	46 (39%)	6 (33%)	40 (40%)
MDS/MPD	27 (23%)	7 (39%)	20 (20%)
Disease risk			
High	91 (78%)	15 (83%)	76 (77%)
Low	26 (22%)	3 (17%)	23 (23%)
No. of prior chemotherapy regimens			
≥2	77 (66%)	6 (33%)	71 (72%)
<2	40 (34%)	12 (67%)	28 (28%)
Donor			
Unrelated	32 (27%)	2 (11%)	30 (30%)
Related	85 (73%)	16 (89%)	69 (70%)
HLA			
Match	90 (77%)	15 (83%)	75 (76%)
Mismatch	27 (23%)	3 (17%)	24 (24%)
Stem cell source			
G-PBMC	81 (69%)	13 (72%)	68 (69%)
Bone marrow	36 (31%)	5 (28%)	31 (31%)
Conditioning regimen			
2CdA/Bu	24 (21%)	4 (22%)	20 (20%)
2CdA/Bu/ATG	18 (15%)	4 (22%)	14 (14%)
2CdA/Bu/TBI	11 (9%)	1 (6%)	10 (10%)
Flu/Bu	38 (32%)	8 (44%)	30 (30%)
Flu/Bu/ATG	11 (9%)	1 (6%)	10 (10%)
Flu/Bu/ATG/TBI	2 (2%)	0 (0%)	2 (2%)
Flu/Bu/TBI	13 (11%)	0 (0%)	13 (13%)

Acute leukemia (n=44): acute myelogenous leukemia (AML; n=23), AML evolving from a myelodysplastic syndrome (n=16), and acute lymphoblastic leukemia (ALL; n=5); Lymphoma (n=46): malignant lymphoma (44), chronic lymphocytic leukemia (CLL; n=1) and multiple myeloma (MM; n=1); MDS/MPD (n=27): MDS n=16 and MPD including chronic myelogenous leukemia (n=9) and atypical CML (n=2); G-PBMC indicates granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells; 2CdA, cladribine; Bu, busulfan; Flu, fludarabine; ATG, antihuman T-lymphocyte immunoglobulin; TBI, total-body irradiation.

[12%]). Prophylaxis for GVHD consisted of cyclosporin (CsA) alone (n = 55), Cyclosporin with short-term methotrexate (sMTX) (n = 38), tacrolimus alone (n = 13), or tacrolimus with sMTX (n = 11).

In 81 of the 117 patients, the source of stem cells was G-PBMC from a related donor, which contained a mean of 3.3×10^6 $CD34^+$ cells/kg (range: 1.5-7.0 $\times 10^6$ $CD34^+$ cells/kg) and 8.7×10^7 $CD3^+$ cells/kg (range: 6.4-86.1 $\times 10^7$ $CD3^+$ cells/kg). The other 36 patients received related (n = 4) or unrelated (n = 32) bone marrow, which contained a mean of 2.9×10^8 total nucleated cells (TNC)/kg (range: 0.97-6.53 $\times 10^8$ TNC/kg).

A total of 9 patients received donor lymphocyte infusion (DLI), mainly after day 90, and all of them received DLI for relapse of disease. There was no patient who received DLI for low donor T cell chimerism.

Informed consent was obtained according to the Declaration of Helsinki.

Definitions

Graft failure was defined as (1) failure of absolute neutrophil count (ANC) to surpass 500 /mm^3 at day 30 after HSCT or (2) decrease in ANC <100 /mm^3 at 3 determinations after the initial engraftment or (3) absence of donor T cells (<5%) before relapse, disease progression, second HSCT, or death. The diagnosis and clinical grading of acute and chronic GVHD (aGVHD, cGVHD) were performed according to established criteria [15-17]. Complete remission (CR) was defined as according to the International Workshop Criteria in AML [18] and lymphoma [19] patients. Low disease risk was defined as AML or ALL in first CR, MDS-refractory anemia, and CML in first chronic phase. All other diagnoses were classified as high risk.

Chimerism Analysis

We assessed donor-recipient chimerism by the polymerase chain reaction (PCR)-based amplification of a polymorphic short tandem repeat region. Chimerism was evaluated using peripheral blood samples on days 30, 60, and 90 after transplantation. Samples were separated using Ficoll-hypaque into mononuclear cells and a precipitate that included red blood cells and granulocytes. Mononuclear cells were further separated into CD3-positive and -negative fractions with immunomagnetic beads (CD3 Magnetic Particles-DM, BD Pharmingen, San Diego, CA). Granulocytes were collected by lysing red blood cells in the precipitate. Briefly, DNA was extracted from selected cells using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Multiplex PCR was performed using primer sets (AmpFISTR Identifiler Kit, Applied Biosystems, Foster City, CA). Five-color fluorescence detection was performed on an ABI 3100-Avant Genetic Analyzer (Applied Biosystems). For each STR allele, the area under the curve for the corresponding signal was automatically processed using GeneScan 3.7 software (Applied Biosystems). The percentage of donor cells was calculated as (area signal donor)/(area signal donor + area signal recipient). The range of the error of chimerism was regarded as 5% at our laboratory (Heike et al., unpublished data).

Statistical Analysis

The chi-square test, Fisher's exact test, and Pearson correlation coefficients were used to evaluate the association of percent donor chimerism with various clinical factors such as patient age at the time of RIST (with 55 years as a cutoff), disease type (acute leukemia, MDS/myeloproliferative disease [MPD], lymphoma), disease risk (high, low), stem cell source (G-PBMC, bone marrow), serologic HLA matching (match, mismatch), and conditioning with TBI (yes, no) or ATG (yes, no).

Overall survival (OS) was defined as the time between stem cell infusion to death from any cause. Event-free survival (EFS) was defined as the time from stem cell infusion to graft failure, PD, or nonrelapse mortality (NRM), whichever occurred earlier. OS and EFS were estimated by the Kaplan-Meier method [20]. The log-rank test and the generalized Wilcoxon test were used to compare the probabilities of survival after HSCT over time across patient subgroups. Multiple Cox regression models were used for multivariate risk factor analysis for OS and EFS. Clinical factors evaluated in the OS and EFS analyses were donor T cell chimerism at day 30 (with 60% as a cutoff), patient age at the time of RIST, disease type, disease risk, stem cell source, HLA matching, and conditioning. Logistic regression models were used for multivariate risk factor analysis for low donor T cell chimerism (<60%) at day 30. Clinical factors evaluated for the risk of low donor T cell chimerism at day 30 were number of prior chemotherapy regimens (≥2, <2) and donor type in addition to the variables mentioned above. We considered 2-sided *P*-values of <.05 to be statistically significant. Statistical analyses were performed with SAS version 8.2 (SAS Inc., Cary, NC).

RESULTS

Kinetics of Chimerism

Whereas 43% of the patients retained mixed donor chimerism (<90% donor) in the T cell fraction, 92% achieved complete chimerism (≥90%) in the granulocyte fraction at day 30 after RIST (Figure 1). In the peripheral blood mononuclear cell (PBMC) fraction, 72% of the patients achieved complete chimerism

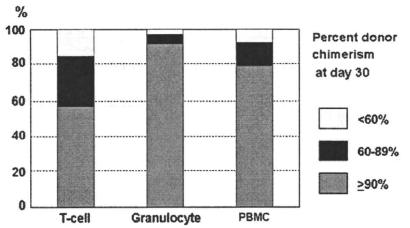

Figure 1. Distribution of chimerism status at day 30 after RIST.

(≥90%). The percent donor-chimerism was significantly higher in granulocyte than T cell fraction throughout the entire course, and the median (mean) values were, respectively, 100% (96%) versus 95% (83%), 100% (98%) versus 100 % (89%), and 100% (98%) versus 100% (91%) at days 30, 60, and 90, respectively after RIST (Figure 2).

In univariate and multivariate analyses (Table 2), having received <2 types of chemotherapy regimens before RIST was the only factor that was significantly associated with low donor T cell chimerism (<60%) at day 30 (hazard ratio [HR]: 6.1; 95% confidence interval [CI], 2.1-18.4; $P < .01$). Non-TBI regimens and related donor also tended to be associated with lower donor T cell chimerism.

Graft Composition and Donor Chimerism

By examining the impact of graft composition of G-PBMC on donor chimerism, we found that increases in TNC and $CD3^+$ T cells contents paralleled the increase in donor T cell chimerism at day 30 ($P < .03$ and $P < .05$, respectively). The same relationship was observed between $CD34^+$ cell contents and granulocyte chimerism ($P = .06$). In patients who received bone marrow, a higher number of TNC infused was associated with a higher level of donor T cell chimerism at day 30 ($P < .01$).

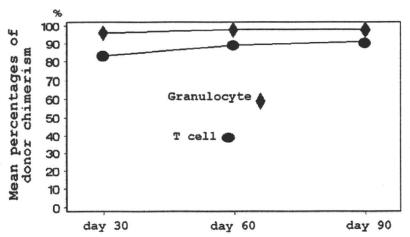

Figure 2. Kinetics of chimerism status after RIST (mean percentages of donor chimerism levels). Percent donor cell chimerism was significantly higher in granulocyte than T cell fraction throughout the entire course, and the mean values were, respectively, 96% versus 83%, 98% versus 89 %, and 98% versus 91% at days 30, 60, and 90 after RIST.

Table 2. Factors affecting low donor T cell chimerism (<60%) at day 30

	Univariate analysis		Multivariate analysis	
Characteristics	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Patient age, years				
<55	1			
≥55	2.04 (0.71 - 5.87)	0.19		
Disease type				
Lymphoma	1			
MDS/MPD	2.33 (0.69 - 7.87)	0.17		
Acute leukemia	0.86 (0.24 - 3.03)	0.81		
Disease risk				
Low	1			
High	1.51 (0.40 - 5.69)	0.54		
No. of prior chemotherapy regimens				
≥2	1		1	
<2	5.07 (1.73-14.83)	<0.01	6.08 (2.01–18.41)	<0.01
Stem cell source				
G-PBMC	1			
Bone marrow	0.84 (0.28 - 2.57)	0.77		
Donor				
Unrelated	1		1	
Related	3.48 (0.75-16.08)	0.11	4.21 (0.86–20.49)	0.08
HLA				
Match	1			
Mismatch	0.63 (0.17 - 2.34)	0.49		
TBI				
No	1		1	
Yes	0.17 (0.02 - 1.38)	0.10	0.13 (0.02–1.05)	0.06
ATG				
No	1			
Yes	1.08 (0.35 - 3.32)	0.89		

Association between Donor T Cell Chimerism at Day 30 and RIST Outcome

Graft failure

The median (mean) percentage of donor T cell chimerism at day 30 was 9% (18%) (0%-63%) in 5 patients who experienced graft failure, which was significantly lower than those in the other patients (97% [86%], 15%-100%, $P < .01$), as shown in Figure 3. Day 30 T cell chimerism below 60% was associated with a significantly increased risk of graft failure (Table 3). Among the 5 patients who experienced graft failure, 4 had achieved complete donor chimerism at day 30 when evaluated in the granulocyte fraction.

Whereas 4 of the 5 patients (80%) who experienced graft failure received HLA-mismatched grafts, 23 of the 112 patients (21%) who did not experience graft failure received HLA-mismatched grafts ($P = .01$). In a multivariate analysis, however, neither day 30 T cell chimerism below 60% nor HLA mismatch was associated with an increased risk of graft failure. Among 18 patients with <60% donor T cell chimerism at day 30, HLA mismatch was significantly associated with an increased risk of grafts failure (3 of 3 who received HLA-mismatched graft versus 1 of 15 who received HLA-matched grafts, $P = .005$). In contrast, HLA mismatch was not associated with an increased risk of graft failure in 99 patients with 60% or more donor T cell chimerism at day 30 (1 of 24

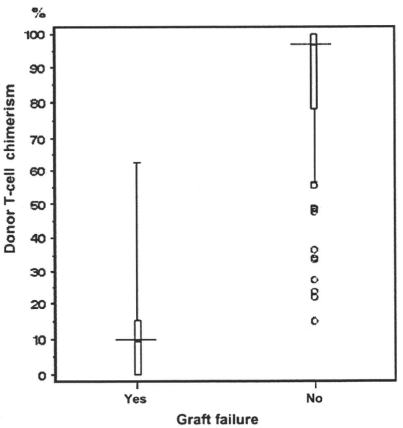

Figure 3. Donor T cell chimerism levels at day 30 in patients with or without subsequent graft failure. Five of the 117 patients (4%) who experienced graft failure had a significantly lower donor T cell chimerism level than the other engrafted patients (n = 112) (donor T cell chimerism, median 9% [range: 0%-63%] versus 97% [range: 15%-100%], respectively) ($p < .01$). Horizontal lines, median; boxes, 25-75 percentile; vertical lines, 10-90 percentile; circles, individual data outside the 10-90 percentile.

who received HLA-mismatched grafts versus 0 of 75 who received HLA-matched grafts, $P = .24$).

GVHD

Grade II-IV aGVHD occurred in 54 patients (46%), and cGVHD occurred in 63 patients (64%). No correlation was found between the kinetics of T cell chimerism and the occurrence of aGVHD or cGVHD, as shown in Table 3.

Table 3. Association between donor T-cell chimerism at day 30 and clinical outcome

		T-cell chimerism at day 30		
Outcome	Total (n=117)	<60% (n=18)	≥60% (n=99)	P
Graft failure				
No	112 (96%)	14 (78%)	98 (99%)	<0.01
Yes	5 (4%)	4 (22%)	1 (1%)	
Acute GVHD				
0-I	64 (55%)	11 (61%)	53 (54%)	
II-IV	53 (45%)	7 (39%)	46 (46%)	0.55
Chronic GVHD*				
No	36 (36%)	7 (50%)	29 (34%)	0.25
Yes	63 (64%)	7 (50%)	56 (66%)	
NRM (at 1 year)	11.0%	11.1%	10.9%	0.26
PD (at 1 year)	27.3%	22.6%	28.1%	0.45
OS (at 1 year)	78.0%	65.7%	80.3%	0.02
EFS (at 1 year)	61.8%	55.6%	62.8%	0.02

GVHD indicates graft-versus-host disease; NRM, non-relapse mortality; PD, relapse or progressive disease; OS, overall survival; EFS, event-free survival;
*Proportion of patients with chronic GVHD was assessed among 99 evaluable patients.

NRM and PD

Nineteen patients experienced NRM, with a 1-year probability of 11% (Table 3). No correlation was found between T cell chimerism at day 30 and the incidence of NRM.

PD was observed in 39 patients, with a 1-year probability of 27% (Table 3). No correlation was found between T cell chimerism at day 30 and the incidence of PD.

Cause of death

Among the 18 patients who had <60% donor T cell chimerism at day 30, 7 (39%) died of PD and 4 (22%) died of NRM, including bacteria sepsis (n = 2), pneumonitis (n = 1), and secondary carcinoma (n = 1). In contrast, among the remaining 99 patients who achieved 60% or more donor T cell chimerism, 21 (21%) died of PD and 15 (15%) died of NRM, including pneumonitis (n = 8), sepsis (n = 3), hemorrhage (n = 1), GVHD (n = 1), cerebral infarction (n = 1), and unknown cause (n = 1).

OS and EFS

Seventy patients (60%) are currently alive at a median follow-up of 1040 days after RIST (range: 153-2535). The 1-year probabilities of OS and EFS among all of the patients were 78% and 62%, respectively. As shown in Figure 4, OS was significantly better in patients who achieved 60% or more donor T cell chimerism at day 30 than in those who did not ($P = .02$). In a Cox proportional hazard model, low T cell donor chimerism (<60%) at day 30 was associated with poor OS (HR: 2.2; 95% CI, 1.1-4.5; $P = .02$) and EFS (HR: 2.0; 95% CI, 1.1-3.8; $P = .02$) adjusted for other significant prognostic factors (Table 4). In addition, high-risk disease and patient age (≥55 years) were associated with an increased risk of poor EFS (HR: 2.4; 95% CI, 1.2-5.0; $P = .02$, HR: 1.8; 95% CI, 1.1-3.0; $P = .03$, respectively) (Table 4).

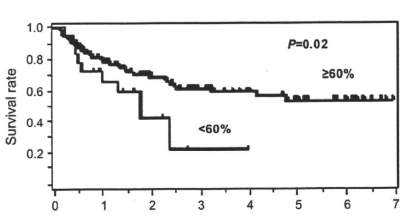

Figure 4. OS stratified according to donor T cell chimerism at day 30. OS was significantly better in patients who achieved 60% or more donor T cell chimerism at day 30 than in those who did not ($P = .02$).

Table 4. Multivariate analysis: factors associated with clinical outcome

Outcome	Variable	Harzard ratio	95% CI	P
OS				
	Donor T-cell chimerism at day 30			
	≥60%	1		
	<60%	2.25	1.13–4.47	0.02
EFS				
	Donor T-cell chimerism at day 30			
	≥60%	1		
	<60%	2.05	1.10–3.81	0.02
	Patients age, years			
	<55	1		
	≥55	1.80	1.07–3.04	0.03
	Disease risk			
	Low	1		
	High	2.44	1.19–5.01	0.02

Clinical factors evaluated in the OS and EFS analyses were donor T-cell chimerism at day 30 (with 60% as a cutoff), patient age at the time of RIST, disease type, disease risk, stem cell source, HLA matching and conditioning.

DISCUSSION

In this retrospective study of RIST with Bu, we showed that 43% of the patients retained mixed donor T cell chimerism (<90%), whereas 92% achieved complete chimerism in the granulocyte fraction, which was consistent with previously published observational studies in RIST [4,10,11,13,21]. Furthermore, we showed that low donor T cell chimerism of <60% at day 30 predicted poor OS and EFS, which suggests that the kinetics of T cell chimerism are important after Bu-containing RIST.

Consistent with other reports, we found that the induction of complete chimerism in T cell fraction after a Bu-containing regimen was rather slow, and granulocyte engraftment was earlier than T cell engraftment compared to patients who received RIC regimens containing a combination of Flu and Mel [10]. When the combination of Cy and Flu was used for RIST conditioning, full donor chimerism was achieved earlier in T cells than in myelogenous cells [1,22]. Interestingly, when alemtuzumab was used in a RIC regimen, 58% retained mixed donor chimerism at day 90 after RIC [13]. This may be because of the fact that alemtuzumab remained in the peripheral circulation long after RIST, which suppressed not only host but also donor lymphocytes. Based on these reports, we suspected that a Cy-containing regimen suppresses host granulocytes less intensely than a Bu-containing regimen, whereas a Mel-containing regimen suppresses host lymphocytes more intensely than a Bu-containing regimen.

The only significant variable associated with a lower level of donor T cell chimerism at day 30 was having received <2 regimens of chemotherapy pretransplant in our results. This result was consistent with previous reports [4,10]. When a patient is treated with RIST, such as our low-dose Bu-containing regimen, prior chemotherapy may facilitate the achievement of higher levels of donor T cell chimerism by decreasing the recipient immunocompetence.

In previous reports there has been some controversy regarding whether there are any differences in the levels of donor T cell chimerism after RIST with or without low-dose TBI [11,13]. In our study with Bu-containing regimens, regimens that included additional low-dose TBI tended to offer higher donor T cell chimerism in a multivariate analysis. However, there was no correlation between ATG-conditioning regimens and donor T cell chimerism at day 30, which was consistent with other regimens [13]. This might be because of the lower dose of ATG (Fresenius, 5-10 mg/kg) in our regimens compared to other studies that utilized the same ATG preparation (Fresenius, 40-90 mg/kg) [23,24]. Alternatively, this might be simply because of the small number of patients who received ATG in our study.

In previous reports, recipients of G-PBMC after RIST showed higher percentages of donor T cell chimerism than those who received bone marrow [4,25], which was not confirmed in our study. With regard to regimens that include Bu, no previous large-scale study has analyzed the correlation between the type of stem cell source and T cell engraftment. When low-dose Bu is contained in the RIC regimen, the stem cell source may no longer influence the level of T cell chimerism. Alternatively, this may be because of the fact that most of the bone marrow recipients in our study also received an additional 2-4 Gy TBI. There was a trend toward a decreased risk of low donor T cell chimerism in recipients of unrelated grafts, although the difference was not significant. We speculate that a lower probability of low donor T cell chimerism might be because of the addition of low dose TBI for patients who underwent unrelated HSCT.

Patients who received G-PBMC showed an increase in TNC and $CD3^+$ T cells that paralleled an increase in donor T cell chimerism at day 30 after RIST in our study. The same relationship was observed between $CD34^+$ cell contents and granulocyte chimerism. Baron et al. [26] reported that higher numbers of donor T cells and $CD34^+$ progenitor cells in the grafts were associated with higher levels of day 28 donor T cell chimerism. Similarly, Carvallo et al. [22] reported that higher levels of $CD34^+$ progenitor cells in the grafts were associated with higher levels of donor myeloid chimerism early after RIST.

In this study, donor T cell chimerism levels of below 60% early after RIST were significantly associated with an increased risk of graft failure. It has been reported that patients with <50% donor T cell chimerism early after nonmyeloablative HSCT were more likely to have graft failure than those with more than

50% donor T cell chimerism [4]. After Bu-containing RIC, Mattsson et al. [21] reported that 2 of the 8 patients who had >50% recipient T cells on day 28 had graft failure or rejection, whereas this was not seen in any of the 22 patients with <50% recipient T cells. Lower donor natural killer NK-cell chimerism after Bu-containing RIST was associated with an increased risk of graft failure [4,27]. Although significant associations of low donor T cell chimerism and HLA mismatch with graft failure disappeared in our multivariate model, our data suggested that HLA mismatch was an important predictor of graft failure only in patients with <60% donor T cell chimerism at day 30. The current study demonstrated that patients at high risk of graft failure could be identified by chimerism analysis at day 30 in T cell fractions, but not in granulocyte fractions, and that chimerism analysis at day 30 after Bu-containing RIST may allow early interventions aimed at reversing graft failure.

Our results suggest that low donor T cell chimerism of <60% at day 30 may predict a poor outcome, although levels of donor T cell chimerism were not associated with NRM PD. In our study, the levels of donor T cell chimerism were not associated with aGVHD or cGVHD, although some reports have stated that donor T cell chimerism was associated with the risk of GVHD [1,4,13,19,28]. It is still controversial whether or not achievement of complete donor T cell chimerism is needed to improve OS and reduce the relapse risk in patients who undergo RIST. Baron et al. [9] suggested that the assessment of donor chimerism levels helps to identify patients who are at higher risk of relapse after nonmyeloablative HSCT. High donor chimerism levels among immune competent cells including T cells and NK cells might be a surrogate for a high graft-versus-tumor effect, and a fractionated chimerism analysis may be useful for detecting and quantifying minimal residual disease after RIST. In a small case series of Bu-containing RIST, mixed donor chimerism was associated with an increased risk of relapse and a worse prognosis [12,29]. In contrast, among patients who underwent RIST that contained Flu, Bu, and alemtuzumab, those who showed mixed donor chimerism beyond day 100 were associated with an improved OS and a lower incidence of GVHD and NRM, without any effect on the relapse risk [13]. Further studies are needed to determine whether the achievement of complete chimerism after RIST is beneficial with less risk of PD and/or more risk of NRM.

In conclusion, within the limitations of a retrospective study, we found that the percentage of donor chimerism was significantly higher in granulocyte than T cell fraction throughout the entire course after Bu-containing RIST. Low donor T cell chimerism of <60% at day 30 may predict a poor outcome, and a prospective study to examine the value of early intervention based on chimerism data is warranted.

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BRIEF ARTICLES

ASBMT
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High Incidence of Human Herpes Virus 6-Associated Encephalitis/Myelitis following a Second Unrelated Cord Blood Transplantation

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Human herpes virus (HHV)6-associated limbic encephalitis and/or myelitis is one of the life-threatening central nervous system complications following allogeneic hematopoietic stem cell transplantation (HSCT). Recent reports have shown significant correlations of these complications with unrelated cord blood transplantation (UCBT). We retrospectively analyzed 228 allogeneic HSCT recipients in our single institution; 13 patients (5.7%) were diagnosed with HHV6-associated encephalitis/myelitis. This complication was documented in 8 of 51 UCBT recipients (15.7%) and 5 of 177 recipients (2.8%) transplanted with bone marrow or peripheral blood stem cells, indicating a higher incidence of this complication occurring in UCBT recipients ($P = .0005$). In addition, HHV6-associated encephalitis/myelitis occurred more frequently in recipients who underwent 2 or more HSCTs (7 of 59 recipients [11.9%]), compared to those who received only 1 HSCT (6 of 169 recipients [3.6%], $P = .018$). Of note, the incidence of this complication increased to 28.6% (6 of 21 recipients), when the analysis was restricted to a second or more UCBT recipients. All 13 patients presented preengraftment immune response prior to the onset of encephalitis. Two patients manifested typical symptoms at the onset of HHV6-associated encephalitis/myelitis, such as memory dysfunction, disorientation, and consciousness disturbance. However, 4 patients presented only with dysesthesia and pruritus, described as typical manifestations of patients with calcineurin-inhibitor-induced pain syndrome (CIPS), and the remaining 7 showed both symptoms, indicating that CIPS-like symptoms might be manifestations of HHV6-associated myelitis. Thus, physicians should be alert to this rare but often fatal complication, particularly for those who receive 2 or more HSCTs using UCB.

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KEY WORDS: Human herpes virus-6, Encephalitis, Myelitis, Cord blood, Second transplantation

INTRODUCTION

Human herpes virus (HHV)6 causes roseola infantum in infants, acute nonspecific febrile illness in younger children, and can give rise to an infectious mononucleosis-like syndrome in adults [1]. HHV6 can be reactivated under conditions of severe immunosuppression, and is recognized as an opportunistic and potentially life-threatening pathogen for allogeneic hematopoietic stem cell transplantation (HSCT) recipients [2-8]. Many investigators have reported various clinical manifestations of HHV6 reactivation in the central nervous system (CNS), such as short-term memory dysfunction, disorientation, consciousness disturbance, hyponatremia [6,9], or diabetes insipidus [10]. Diagnosis of these complications in the CNS can be made using specific techniques, such as abnormal high-intensity signals in bilateral temporal lobes (limbic area) on T2-weightned and/or fluid-attenuated inversion recovery sequences from magnetic resonance imaging (MRI), and detection of HHV6 DNA in the cerebrospinal fluid (CSF) by the polymerase chain reaction (PCR) method.

Recent reports have suggested that HHV6 reactivation is more likely to occur in recipients of unrelated cord blood transplantation (UCBT) than in those who

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receive allogeneic bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT) [6,11,12]. These findings may be because of the absence of primed HHV6-specific T cells and the immunologic immaturity of UCBT [11-14]. Because of the increasing preference for UCBT procedures because of the rapid availability of stored transplantable units, we should pay more attention to HHV6-associated encephalitis/myelitis for UCBT procedures.

In this study, we described 13 patients with HHV6-associated encephalitis/myelitis among 228 allogeneic HSCT recipients. We found a higher incidence of HHV6-associated encephalitis/myelitis for UCBT recipients (8 of 51, 15.7%) than for BMT and PBSCT recipients (5 of 177, 2.8%, $P = .0005$). In addition, the incidence of HHV6-associated encephalitis/myelitis increased to 28.6% for recipients who received 2 or more UCBTs (6 of 21). We also identified the factors associated with the onset of HHV6-associated encephalitis/myelitis in this study.

PATIENTS AND METHODS

Patients

The medical records of all patients who underwent allogeneic HSCT at the Kyushu University Hospital between January 2002 and October 2009 were reviewed. A total of 228 patients (136 men, 92 women) (median age = 47 years), were studied. Patients' characteristics are listed in Table 1. Primary diseases included myelodysplastic syndrome (MDS)/acute myelogenous leukemia (AML) (n = 92), chronic myelogenous leukemia (CML) (n = 13), acute lymphoblastic leukemia (ALL) (n = 31), malignant lymphoma (ML) (n = 57), aplastic anemia (AA) (n = 14), and others (n = 21). A total of 101 cases were considered early state defined as follows: acute leukemia (AML and ALL) in remission; CML in chronic phase (CP); MDS classified as refractory anemia (RA) or RA with ringed sideroblasts. All others (n = 127) were considered as nonearly state. This study was approved by the institutional review board of Kyushu University Hospital.

Transplantation Procedures

A total of 133 patients had received conventional preparative regimens comprised of either total body irradiation (TBI)/cyclophosphamide (Cy) (n = 91), or busulfan (Bu)/Cy (n = 42) (Table 1). The remaining 95 cases had received purine analog-based reduced-intensity conditioning (RIC) regimen comprised of either fludarabine (Flu)/Cy (n = 16), Flu/Bu (n = 48), or Flu/melphalan (n = 31). Low-dose TBI (2-4 Gy), antithymocyte globulin (ATG), and alemutuzumab were administered in 49, 3, and 3 cases, respectively. The sources of stem cells were related granulocyte colony-stimulating factor (G-CSF)-mobilized PB (n = 71), related BM (n = 15), unrelated BM (n = 91), or unrelated CB (n = 51). HLA-matching varied from haploidentical (3/6) to identical (6/6). A total of 59 recipients had received 2 or more HSCTs as listed in Table 1: the source of stem cells for the first transplant were 24 autologous and 35 allogeneic ones. The reasons for a second or more HSCT were disease relapse (n = 50) and graft failure (n = 9). Graft-versus-host-disease (GVHD) prophylaxis was as follows: 20 received calcineurin-inhibitor (CI) alone; 193 received CI plus short-term methotrexate (MTX); and 15 received CI plus mycophenolate mofetil (MMF).

Diagnosis of HHV6-Associated Encephalitis/Myelitis

HHV6-associated encephalitis/myelitis was directly proved when HHV6 viral DNA was detected in the CSF, as previously reported [15]. For those patients who were unable to undergo lumbar puncture because of severe thrombocytopenia or a deteriorated general condition, we diagnosed HHV6-associated encephalitis/myelitis if they satisfied more than 2 of the following 3 criterion: (1) typical clinical manifestations; (2) detection of HHV6 viral DNA in PB, previously reported as a predictive marker for developing HHV6-associated encephalitis/myelitis [16]; or (3) limbic encephalopathy based on the selective involvement of the medial temporal lobe on MRI. Routine monitoring of HHV6 viral load of PB by PCR was not performed in this study.

Statistical Analysis

The aim of this study is to clarify the factor correlated with developing HHV6-associated encephalitis/myelitis. For univariate comparisons, we examined categoric variables, including age, sex, underlying diseases, disease status, conditioning regimen, stem cell source, HLA matching, GVHD prophylaxis, and prior HSCT using the chi-square test. The incidence of HHV6-associated encephalitis/myelitis is generally very low. The risk of type 1 error has been known to increase when the event per variable is <10; we narrowed an analysis to 1 risk factor selected by −2 log likelihood method to avoid it. Odds ratios were calculated using logistic regression analysis. Survival following allogeneic HSCT was measured from the date of stem cell infusion until the date of death. The survival period was calculated using the Kaplan-Meier method. P-values < .05 were considered to indicate statistical significance. All statistical analyses used SPSS 17.0 program (SPSS Japan Inc., Tokyo, Japan).

Table 1. Characteristics of the 228 Patients

Characteristics	Total	HHV6 Encephalitis/Myelitis Yes (n = 13)	HHV6 Encephalitis/Myelitis No (n = 215)	*P*-value
Age, median (range)	47 (16-68)	48 (29-65)	47 (16-68)	NS
Sex (male/female)	136/92	10/3	126/89	NS
Underlying disease				NS
MDS/AML	92	7	85	
CML	13	0	13	
ALL	31	1	30	
ML	57	4	53	
AA	14	1	13	
Others	21	0	21	
Disease status at transplantation				NS
Early*	101	4	97	
Nonearly	127	9	118	
Conditioning regimen				.038
Conventional	133	4	129	
Reduced-intensity	95	9	86	
Stem cell source				.0005
Related PB	71	2	69	
Related BM	15	0	15	
Unrelated BM	91	3	88	
Unrelated CB	51	8	43	
Times and sources of HSCT				.018
First	169	6	163	
non-UCB	139	4	135	
UCB	30	2	28	
Second / first source	55	6	49	
non-UCB / Auto	18	1	17	
/ non-UCB	14	0	14	
/ UCB	4	0	4	
UCB / Auto	4	0	4	
/ non-UCB	7	4	3	
/ UCB	8	1	7	
Third / second source	4	1	3	
Non-UCB / Auto	1	0	1	
/ non-UCB	1	0	1	
UCB/ non-UCB	2	1	1	
HLA matching				.012
≤4/6	52	6	46	
5/6	47	4	43	
6/6	129	3	126	
GVHD prophylaxis				<.0001
CI alone	20	2	18	
CI plus sMTX	193	5	188	
CI plus MMF	15	6	9	
IgG-antibody for HHV6 (titer)				NS
negative	0	0	0	
×10-×20	45	3	42	
×40-×80	125	7	118	
×160-	26	1	25	
unknown	32	2	30	

CI indicates calcineulin-inhibitor, including cyclosporine and tacrolimus; NS, not significant; CML, chronic myelogenous; MDS/AML, myelodysplastic syndrome/acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; AA, aplastic anemia; MTX, methotrexate; MMF, mycophenolate mofetil; PB, peripheral blood; BM, bone marrow; CB, cord blood, HSCT, hematopoietic stem cell transplantation; UCB, unrelated CB; GVHD, graft-versus-host disease; non-UCB included related PB, related BM, and unrelated BM.

*Early stage is defined as: acute leukemia in remission; CML in chronic phase; MDS classified as refractory anemia or refractory anemia with ringed sideroblasts; lymphoma in remission. All others are considered nonearly stage.

RESULTS

Incidence of HHV6-Associated Encephalitis/Myelitis

In our series, a total of 13 patients among 228 allogeneic HSCT recipients (5.7%) developed HHV6-associated encephalitis/myelitis after allogeneic HSCT. Median onset was 23 days after transplantation, in line with the previous multicenter retrospective research in Japan [17]. HHV6 viral DNA was detected in CSF samples of 9 patients, whereas the remaining 4 were diagnosed with HHV6-associated encephalitis/myelitis by satisfying the criteria for clinical features and MRI findings (n = 1), clinical features and PCR assay of PB (n = 2), or all 3 criteria (n = 1). There was no statistical difference among the underlying diseases. A previous report has demonstrated a close association between a lower titer of anti-HHV6 IgG (<×40) and development of HHV6 reactivation [14], however, in our study, there was no significant relationship between them (Table 1).

Seven of 59 (11.9%) patients who underwent 2 or more HSCTs developed HHV6-associated encephalitis/myelitis, which was significantly more frequent than in the 6 of 169 (3.6%) patients who received a first HSCT (P = .018). A high incidence of HHV6-associated encephalitis/myelitis was also found in patients who received UCBT compared to those who received a non-UCBT (8 of 51, 15.7% versus 5 of 177, 2.8%: P = .0005). Moreover, the incidence of HHV6-associated encephalitis/myelitis increased to 28.6% (6 of 21), when the analysis was restricted to those patients who received a second or more UCBT, which was significantly more frequent than those who received a second non-UCBT (1 of 38; P = .003). Patients who received HSCTs from HLA-mismatched donors developed HHV6-associated encephalitis/myelitis more frequently than those with HLA-matched donors (10 of 99, 10.1% versus 3 of 129, 2.3%; P = .012). This may reflect the fact that most UCBT recipients received transplants using HLA-mismatched cord blood. We also found a relatively high incidence of HHV6-associated encephalitis/myelitis in those patients who underwent Flu-based RIC (9 of 95) or CI and MMF for the prevention of GVHD (6 of 15), as UCBT recipients preferentially received RIC and GVHD prophylaxis with CI and MMF in our institution.

Clinical Features of HHV6-Associated Encephalitis/Myelitis

Clinical and laboratory findings for the 13 patients at the onset of HHV6-associated encephalitis/myelitis are shown in Table 2. The median onset of clinical symptoms was 23 days (range: 14-614 days). Symptoms occurred within 10 days of their neutrophil engraftments (>0.5 × 10^9/L) in 10 patients, whereas

the remaining 3 patients developed clinical manifestations more than 10 days after their engraftments. Prior to the onset of HHV6-associated encephalitis/myelitis, immunosuppressive treatments had commenced for 7 patients which comprised of >1 mg/kg of methylprednisolone against GVHD or hemophagocytic syndrome (HPS).

The cardinal features of HHV6-associated encephalitis are well documented: short-term memory dysfunction, disorientation, consciousness disturbance, and seizures [2-7]. In our study, these typical symptoms were found in 2 of the 13 patients, whereas 4 developed only systemic pruritus with no apparent skin rash and intermittent pain in the extremities, which were similar to the reported manifestations of CI-induced pain syndrome (CIPS) [18,19]. Both encephalitis and CIPS-like symptoms were found in the remaining 7 patients.

Positive results for head MRI scans (limbic encephalitis) were observed in 7 of 9 patients (77.8%) who developed encephalitis-type symptoms, which were not found in the 4 patients who only had CIPS-like symptoms; only 1 presenting with CIPS-like symptoms had positive results for spinal MRI. Interestingly, all patients who developed HHV6-associated encephalitis/myelitis had noninfectious fevers before their engraftments known as a "preengraftment immune reaction" (PIR) or "hyperacute GVHD" [13,20], and 3 of them subsequently developed HPS (shown in Table 2).

HHV6 viral DNA was detected in CSF samples of all 9 patients who were assessed. The viral copy levels varied from 2×10^2 to 2×10^5 copies/μL (Table 2): however, these did not correlate with the degree of sequelae or the presence of definite abnormal findings on MRI.

Treatment and Outcomes

Nine of 13 patients were treated with antiviral combination therapy using gancyclovir (GCV; 5-10 mg/kg/day) and foscarnet (FCV; 60-180 mg/kg/day). Monotherapy with FCV was initiated for 2 patients, ACV for 1, and GCV for the other. Clinical features were relieved in all but 1 patient treated with GCV plus FCV. However, mild neurologic sequelae remained in all 3 survivors, such as memory impairment, CIPS-like dysesthesia, dysuria, or dyschezia (Table 2).

Ten patients died thereafter; the causes included HHV6-associated encephalitis (n = 1), disease progression (n = 4), GVHD (n = 2), infection (n = 2), and donor-derived secondary MDS (n = 1). In our study, the 5-year survivals after HSCT were 17.3% ± 14.6% for the patients with HHV6-associated encephalitis/myelitis, and 37.2% ± 3.9% for the patients without this complication. Although no significant difference in survival was noted ($P = .31$), patients with HHV6-associated encephalitis/myelitis tended to have poorer outcomes, as previously reported [21,22].

Risk Factors for HHV6-Associated Encephalitis/Myelitis

From the univariate analysis using logistic regression, HHV6-associated encephalitis/myelitis were strongly associated with the stem cell source (CBT versus others; $P < 0.001$). Multivariate analysis confirmed that a second or more UCBT was the only independent risk factor for HHV6-associated encephalitis (odds ratio = 13.58; 95% confidence interval = 3.45-53.5; $P < .001$) (Table 3).

DISCUSSION

A second HSCT may be the only way to ensure survival in some patients with hematologic malignancies that relapse after the first HSCT. UCB has become increasingly preferred as the source of graft for a second HSCT because of the rapid availability of stored transplantable units. However, using UCB presents an increased risk of graft failure and infectious complications. HHV6-associated encephalitis/myelitis is one of the most life-threatening CNS complications after UCBT. In this study, HHV6-associated encephalitis/myelitis occurred more frequently for UCBT recipients (15.7%) compared to BMT and PBSCT recipients (2.8%), and the incidence of HHV6-associated encephalitis/myelitis increased to 28.6% for recipients who received 2 or more UCBTs, although this observation should be interpreted with caution and confirmed in a prospective study because of the limited number of patients. We should also pay attention to the possibility that this result might reflect the selection bias; the patients requiring a second UCBT had more aggressive disease than those who require second non-UCBT and were, by nature, more susceptible to various infections including HHV6 reactivation. In addition to the absence of primed HHV6-specific T cells with UCBT, the high incidence of HHV6-associated encephalitis/myelitis after 2 or more UCBTs may be explained by a prolonged immune deficiency caused by multiple HSCTs as well as by HLA mismatches between donors and recipients [11-14]. CMV antigen tests were positive in 10 of 13 patients with HHV6-associated encephalitis/myelitis (data not shown), which might also suggest the impaired immune function as well. In our study, the all-cause mortality within 100 days for second or more UCBT recipients who developed this complication reached 50%, indicating that HHV6-associated encephalitis/myelitis is a common serious problem, especially following a second or more UCBT.

Several reports have found that PIR often preceded engraftment and that HPS frequently co-occurred in those UCBT recipients who were treated with RIC

Table 2. Clinical, Laboratory, and Radiographic Characteristics of HHV6-Associated Encephalitis/Myelitis

Patient	Prior HSCTs			Prior						
	Graft Source	(Indication of Current HSCT)	Conditioning	PIR / HPS	Onset (Day)	Types of Clinical Manifestations	HHV6 Viral Load in CSF/PB (Copies/μL)	Localization on MRI	Antiviral Treatment	Outcome
1	1st	RPBSC	–	Conv	+/–	614	encephalitis	2×10^4 / N.A	limbic	GCV	survive with memory impairment
2	1st	RPBSC	–	Conv	+/+	24	combined	N.A / 7×10^3	limbic	GCV + FCV	dead by relapsed AML
3	1st	URBM	–	Conv	+/–	61	encephalitis	2×10^3 / N.A	None	GCV + FCV	dead by GVHD
4	1st	URBM	–	Conv	+/–	19	combined	2×10^5 / N.A	limbic	GCV + FCV	dead by GVHD
5	1st	UCB	–	RIC	+/–	23	combined	2×10^4 / N.A	limbic	GCV + FCV	dead by sepsis
6	1st	UCB	–	RIC	+/–	19	CIPS-like	5×10^4 / N.A	None	FCV	survive with CIPS-like dysesthesia
7	2nd	URBM	Auto (relapse)	RIC	+/–	22	combined	N.A / 4×10^5	None	GCV + FCV	dead by sepsis
8	2nd	UCB	UBM (rejection)	RIC	+/–	35	combined	N.A / negative	limbic	ACV	dead by HHV6 encephalitis
9	2nd	UCB	RPBSC (relapse)	RIC	+/–	27	combined	9×10^3 / 3×10^3	limbic	GCV + FCV	dead by donor-derived MDS
10	2nd	UCB	UCB (relapse)	RIC	+/+	19	combined	2×10^7 / 1×10^4	limbic	GCV + FCV	dead by relapsed AML
11	2nd	UCB	UBM (relapse)	RIC	+/+	23	CIPS-like	N.A / 8×10^3	None	FCV	dead by relapsed AML
12	2nd	UCB	UBM (relapse)	RIC	+/–	14	CIPS-like	1×10^3 / 8×10^3	None	GCV + FCV	survive with CIPS-like dysesthesia
13	3rd	UCB	Auto / UBM (relapse)	RIC	+/–	17	CIPS-like	2×10^3 / N.A	None	GCV + FCV	dead by relapsed AML

RPBSC indicates related peripheral blood stem cell; UCB, unrelated cord blood; URBM, unrelated bone marrow; Conv, conventional; RIC, reduced-intensity conditioning; PIR, preengraftment immune reaction; HPS, hemophagocytic syndrome; CIPS, calcineurin-induced pain syndrome; CSF, cerebrospinal fluid; N.A, not assessed; FLAIR, fluid-attenuated inversion recovery; ACV, acyclovir; GCV, ganciclovir; FCV, foscavir; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; AML, acute myelogenous leukemia; MRI, Magnetic resonance imaging; PB, peripheral blood.

(RIC-UCBT), and that PIR and HPS were characterized by symptoms possibly induced by hypercytokinemia [23-25]. In the setting of RIC-UCBT, an HLA disparity as well as residual recipients' immuno competent cells may augment allo-immune mediated events, such as pro-inflammatory cytokine storms, which result in the development of PIR and/or HPS [6,23-25]. Our study also demonstrated that RIC-UCBT recipients developed PIR more frequently (23 of 31, 74.2%) than UCBT recipients who underwent conventional conditioning (5 of 20, 25%). Several investigators have also found a higher incidence of HHV6-associated encephalitis/myelitis that has been documented for RIC-UCBT recipients with complications like PIR or HPS [6,25]. In fact, in our study, all 13 patients with HHV6-associated encephalitis/myelitis had suffered from PIR prior to the onset of encephalopathy, and 3 of them subsequently developed severe HPS. In contrast, among 43 UCBT recipients who did not develop HHV6-associated encephalitis/myelitis, PIR and HPS were observed less frequently in 20 (46.5%) and 0 (0%), respectively, indicating that the development of HHV6-associated encephalitis/myelitis might be closely associated with PIR or HPS. In line with our observations, Ogata et al. [26] recently reported that episodes of PIR or GVHD were closely correlated with the development of HHV6-associated encephalitis. In addition, they also demonstrated a significant increase of serum interleukin (IL)-6 levels, particularly in those patients preceding HHV6 reactivation and progression to encephalopathy, suggesting that a high IL-6 level might be critical for the development of HHV6-associated encephalitis/myelitis. Several reports previously demonstrated high levels of IL-6 in the sera and cerebral spinal fluids of patients with aseptic meningitis/encephalitis, indicating the possible participation of IL-6 in the pathogenesis of meningitis/encephalitis [27-29]. The contributions of IL-6 to the development of meningitis/encephalitis may include epithelial and endothelial injuries, increased permeability in the blood-brain barrier, or apoptosis in the cerebrum resulting from a hyper immune reaction [26,30,31]. We also observed increased levels of IL-6 in the majority of the patients with PIR (unpublished data). Thus, PIR or HPS may augment excessive immune reactions when combined with these observations, such as high IL-6 secretion, which partially contribute to the subsequent development of HHV6-associated encephalitis/myelitis.

CIPS is a newly established disease entity that is characterized by severe pain, typically in the lower limbs. CIPS is hypothesized to result from calcineurin-induced vascular changes that disturb bone perfusion and permeability, which leads to intraosseous vasoconstriction and BM edema [18,19]. This rare syndrome has been described in organ transplant recipients [32-35], where CIs must be

Table 3. Results of a Logistic Regression Analysis

		Univariate		Multivariate (stepwise)	
Characteristics		Odds Ratio (95% Confidence Interval)	*P*-Value	Odds Ratio (95% Confidence Interval)	*P*-Value
Age		1.000 (0.954-1.048)	.988		
Sex	male versus female	1.799 (0.464-6.982)	.396		
Underlying disease	lymphoid malignancy versus others	1.312 (0.388-4.443)	.662		
Disease status	nonearly versus early	1.385 (0.393-4.876)	.612		
Conditioning regimen	Reduced intensity versus myeloabrative	2.491 (0.707-8.777)	.155		
Stem cell source	non-CB	1(referrence)	–	1(referrence)	–
	CB (previous HSCT −)	1.052 (0.119-9.313)	.964	1.052 (0.119-9.313)	.964
	CB (previous HSCT +)	13.58 (3.447-53.52)	<.001	13.58 (3.447-53.52)	<.001
HLA	mismatched versus matched	1.933 (0.542-6.889)	.309		
GVHD prophylaxis	CI alone	1(referrence)	–		
	CI plus sMTX	0.197 (0.035-1.107)	.065		
	CI plus MMF	2.800 (0.427-18.38)	.283		

GVHD indicates graft-versus-host disease; CB, cord blood; HSCT, hematopoietic stem cell transplantation; MTX, methotrexate; MMF, mycophenolate mofetil; CI, calcineulin-inhibitor.

maintained at a high concentration, whereas several cases of CIPS have been reported for patients undergoing HSCT [18,19,36-39]. Moreover, in our study, 11 of 13 patients with HHV6-associated encephalitis/myelitis developed CIPS-like dysesthesia and/or pruritus. Although CI concentrations in these patients were maintained within the targeted ranges and reduction in concentrations was ineffective, HHV6 DNA was directly detected in CSF samples and administration of antiviral agents improved their symptoms, indicating the relation of the symptoms to the reactivation of HHV6. We found that 7 cases that developed CIPS following HSCT have been reported to date [18,19,36-39]; 3 developed CIPS after UCBT, and 4 developed CIPS after a second HSCT: 2 for UCBT and 2 for BMT, respectively. In addition, Kida et al. [38] showed that no possible cases of CIPS were observed among 189 allogeneic BMT and 65 allogeneic PBSCT recipients, whereas 2 of 34 cases that underwent UCBT developed CIPS. As shown in our study, patients who receive UCBT or a second HSCT have a high risk of occurrence of HHV6-associated encephalitis/myelitis. Based on these observations, we hypothesize that HHV6 reactivation might contribute to aggravation of CIPS, and therefore, CIPS might be one of the early manifestations of HHV6-associated encephalitis/myelitis. HHV6 reactivation, localized in the posterior horn of the spinal cord, where signals for both itch and pain sensations are transmitted via c-fibers or Aδ-fibers [40], may cause distinctive manifestations like CIPS. Indeed, all 4 of the cases who only developed CIPS-like dysesthesia and/or pruritus had positive results for HHV6 in their CSF samples, but not for head MRI (limbic encephalitis), and only 1 case with MRI scanning of the spine (case #13) showed multiple abnormal T2-high lesions. Because HHV6-associated encephalitis/myelitis and CIPS can often occur at 2 to 4 weeks posttransplantation, it is important to make a differential diagnosis of these 2 syndromes.

In summary, our results have clearly shown that 2 or more RIC-UCBT presents a high risk for the development of HHV6-associated encephalitis/myelitis, although there are the limitations of the retrospective single center analysis. Earlier diagnosis of and intervention for this complication with combination antiviral therapy may provide prolonged survival and neurologic benefits. Therefore, transplantation physicians should be aware that CIPS-like dysesthesia and pruritus might be early manifestations that correlate with the reactivation of HHV6, especially for patients who develop myelitis. These "high-risk" recipients can be potential candidates for prophylactic administration of antiviral agents in future investigations.

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Allogeneic stem cell transplantation for adult Philadelphia chromosome negative acute lymphocytic leukemia: comparable survival rates but different risk factors between related and unrelated transplantation in first complete remission

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Allogeneic stem cell transplantation for adult Philadelphia chromosome–negative acute lymphocytic leukemia: comparable survival rates but different risk factors between related and unrelated transplantation in first complete remission

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To identify factors to improve the outcomes of related and unrelated allogeneic stem cell transplantations (allo-SCT) for Philadelphia chromosome–negative acute lymphocytic leukemia (Ph⁻ ALL) in the first complete remission (CR1), we retrospectively analyzed 1139 Ph⁻ ALL patients using the registry data, particularly the details of 641 patients transplanted in CR1. Overall survival was significantly superior among patients transplanted in CR1, but no significant difference was observed between related and unrelated allo-SCTs (related vs unrelated: 65% vs 62% at 4 years, respectively; $P = .19$). Among patients transplanted in CR1, relapse rates were significantly higher in related allo-SCT compared with unrelated allo-SCT, and multivariate analysis demonstrated that less than 6 months from diagnosis to allo-SCT alone was associated with relapse. On the other hand, nonrelapse mortality (NRM) was significantly higher in unrelated allo-SCT compared with related allo-SCT, and multivariate analysis demonstrated that 10 months or longer from diagnosis to allo-SCT, human leukocyte antigen mismatch, and abnormal karyotype were associated with NRM. In conclusion, our study showed comparable survival rates but different relapse rates, NRM rates, and risk factors between related and unrelated allo-SCTs. After a close consideration of these factors, the outcome of allo-SCT for adult Ph⁻ ALL in CR1 could be improved. (*Blood.* 2010;116(20):4368-4375)

Introduction

The indication of allogeneic stem cell transplantation (allo-SCT) for Philadelphia chromosome–negative acute lymphocytic leukemia (Ph⁻ ALL) is still controversial.[1,2] As for related allo-SCT, one prospective study suggested that related allo-SCT for Ph⁻ ALL in first complete remission (CR1) could provide the most potent antileukemic therapy and considerable survival benefits.[3] As for unrelated allo-SCT, the largest retrospective study of Ph⁻ ALL patients in CR1 showed worse overall survival (OS) rates because of higher incidences of nonrelapse morality (NRM) than those in related allo-SCT,[4] whereas another reported that there were no differences in OS rates and NRM rates between related and unrelated allo-SCTs for adult ALL in CR1.[5] These data indicated that unrelated allo-SCT could also be a treatment option for adult Ph⁻ ALL patients in CR1 if NRM rates were low enough, although it is not yet routinely performed.

Although the analyses of the outcome of allo-SCT alone have some biases, such as excluding death during chemotherapy, and there may be potential differences in the baseline characteristics of patients between related and unrelated allo-SCTs, the comparison of transplantation outcomes and risk factors between related and unrelated allo-SCTs for adult Ph⁻ ALL could indicate strategies to improve transplantation outcomes for this disease. We particularly focused on allo-SCT in CR1 because this is the area of controversy.

Methods

Collection of data and data sources

The recipients' clinical data were provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP). Both JSHCT and JMDP collect recipients' clinical data at 100 days after allo-SCT. The patient's data on survival, disease status, and long-term complications, including chronic graft-versus-host disease (GVHD) and second malignancies, are renewed annually by follow-up forms. More than 99% of unrelated allo-SCT in Japan was captured in the JMDP database, and approximately 75% of related allo-SCT was captured in the JSHCT database. This study was approved by the data management committees of JSHCT and JMDP. Informed consent was obtained from both recipients and donors in accordance with the Declaration of Helsinki.

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Patients

Data of 1976 patients who underwent their first allo-SCT for Ph⁻ ALL between 1993 and 2007 were available in the registration database of JSHCT and JMDP. Excluding 662 patients whose age was 15 years or younger, 67 patients without data of GVHD prophylaxis and the interval from diagnosis to allo-SCT, 22 patients who underwent 2 or more human leukocyte antigen (HLA) loci mismatched related allo-SCT, and 86 patients who received reduced-intensity conditioning regimens, we analyzed 1139 adult Ph⁻ ALL patients (499 related and 640 unrelated). We particularly analyzed details of 641 patients transplanted in CR1, according to donor types (310 related and 331 unrelated). All but 4 patients were donated from Japanese donors harvested in Japanese harvest centers. Only bone marrow grafts were used in unrelated allo-SCT because peripheral blood stem cell donation from unrelated donors is not yet approved in Japan. HLA high-resolution molecular typing methods were performed for HLA-A, -B, -Cw, and -DRB1 for all patients in JMDP. Donor and recipient pairs were considered matched when HLA was matched at -A, -B, and -DRB1 loci in related allo-SCT and at -A, -B, -Cw, and -DRB1 loci in unrelated allo-SCT. Mismatches were defined as at least one disparity of these loci.

Definition

Neutrophil recovery was defined by an absolute neutrophil count of at least 0.5×10^9/L for 3 consecutive days; platelet recovery was defined by a count of at least 50×10^9/L without transfusion support. Acute and chronic GVHD was diagnosed and graded according to consensus criteria.[6,7] Relapse was defined as hematologic leukemia recurrence. NRM was defined as death during continuous remission. For analyses of OS, failure was death from any cause, and surviving patients were censored at the date of last contact. The date of allo-SCT was the starting time point for calculating all outcomes. Patients were classified at diagnosis by the Japan Adult leukemia Study Group (JALSG) risk stratification: low risk was defined as less than 30 years at diagnosis and white blood cell count less than 30 000/μL at diagnosis, high risk as 30 years or more at diagnosis and white blood cell count 30 000/μL or more at diagnosis, and intermediate risk as other.[8] To determine the cut-off for the upper limit of tolerability by age, we analyzed the cumulative incidence of NRM by categorizing the patients' age every 5 years. Because NRM rates of 45- to 49-year-old and 50-year-old or older categories showed higher incidences compared with other categories, we determined the best cut-off point as 45 years old.

Statistical analysis

The 2-sided χ^2 test was used for categorical variables. OS rates were estimated by the Kaplan-Meier method, and P values were calculated using a log-rank test.[9,10] Cumulative incidences of relapse, NRM, and GVHD were calculated by the Gray method.[11,12] Death without relapse was considered as a competing event for relapse, and relapse as a competing event for NRM. Univariate and multivariate analyses were performed using Cox proportional hazard regression model.[13] A significance level of P less than .05 was used for all analyses.

Results

Patient characteristics

Of 1139 patients, 641 received allo-SCT in CR1 (310 related and 331 unrelated), 199 in subsequent remission (56 related and 143 unrelated), and 299 in nonremission (133 related and 166 unrelated). The characteristics of the patients transplanted in CR1 are shown in Table 1. The frequencies of HLA mismatched donor and tacrolimus-based GVHD prophylaxis were higher, and the interval from diagnosis to allo-SCT was longer among patients who underwent an unrelated allo-SCT than among those who underwent a related allo-SCT. There was no significant difference in the age at allo-SCT, the white blood cell counts at diagnosis, JALSG risk stratification, and year of allo-SCT between related and unrelated allo-SCTs.

Survival

Median follow-up periods among survivors were 47.7 months (range, 1.3-162 months). OS rates at 4 years were 64% in CR1, 39% in subsequent CR, and 16% in non-remission ($P < .0001$). Although OS rates were significantly different among disease stages at allo-SCT, there were no significant differences in OS rates at 4 years between related and unrelated allo-SCTs in any disease stage (related vs unrelated: 65% vs 62% in CR1, $P = .19$; 44% vs 38% in subsequent CR, $P = .66$; and 17% vs 16% in non-remission, $P = .59$; respectively; Figure 1). There was no statistical difference in OS rates and NRM rates over transplantation years (data not shown). Among 641 patients transplanted in CR1, JALSG risk stratification did not have a significant impact on the OS after allo-SCT (68% in low risk, 62% in intermediate risk, and 58% in high risk, at 4 years, respectively; $P = .31$). To address our main issue, we performed the following analyses among patients transplanted in CR1 according to donor types.

Among 310 patients who underwent a related allo-SCT in CR1, multivariate analysis showed that age at allo-SCT and less than 6 months from diagnosis to allo-SCT were significant risk factors for OS. Among 331 patients who underwent an unrelated allo-SCT in CR1, multivariate analysis showed that abnormal karyotype [except for t(4;11) and t(1;19)], HLA mismatch, and 10 months or longer from diagnosis to allo-SCT were significant risk factors for OS (Table 2).

Relapse and NRM among patients transplanted in CR1

The cumulative incidence of relapse was significantly higher in patients who underwent a related allo-SCT compared with those who underwent an unrelated allo-SCT (related vs unrelated: 32% vs 22% at 4 years, $P = .03$; Figure 2A). Multivariate analyses according to donor type showed that less than 6 months from diagnosis to allo-SCT alone was associated with relapse among 310 patients who underwent a related allo-SCT in CR1, whereas only abnormal karyotype [except for t(4;11) and t(1;19)] was associated with relapse among 331 patients who underwent an unrelated allo-SCT in CR1 (Table 3).

The cumulative incidence of NRM was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 14% vs 27% at 4 years, $P = .0002$; Figure 2B). Multivariate analyses according to donor type showed that age only 45 years or older at allo-SCT was associated with NRM among 310 patients who underwent a related allo-SCT in CR1, whereas abnormal karyotype [except for t(4;11) and t(1;19)], HLA mismatch, and 10 months or longer from diagnosis to allo-SCT were associated with NRM among 331 patients who underwent an unrelated allo-SCT in CR1 (Table 4).

Acute and chronic GVHD among patients transplanted in CR1

The cumulative incidence of grade II-IV acute GVHD was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 30% vs 42% at day 100; $P = .0003$). The cumulative incidence of grade III-IV acute GVHD was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 7% vs 16% at day 100; $P = .0006$).