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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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KEY WORDS: Reduced-intensity stem cell transplantation, Chimerism, Busulfan

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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

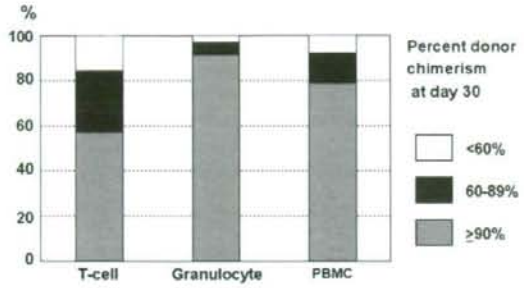

Figure 1. Distribution of chimerism status at day 30 after RIST.

(≥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 ($P < .01$).

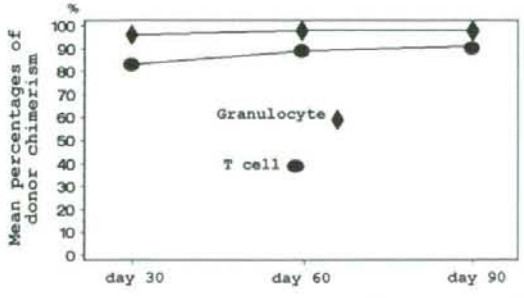

Figure 2. Kinetics of chimerism status after RIST (mean percentages of donor chimerism levels). Percent donor cell chimerism was significantly higher in granulocyte than T cell fraction throughout the entire course, and the mean values were, respectively, 96% versus 83%, 98% versus 89 %, and 98% versus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

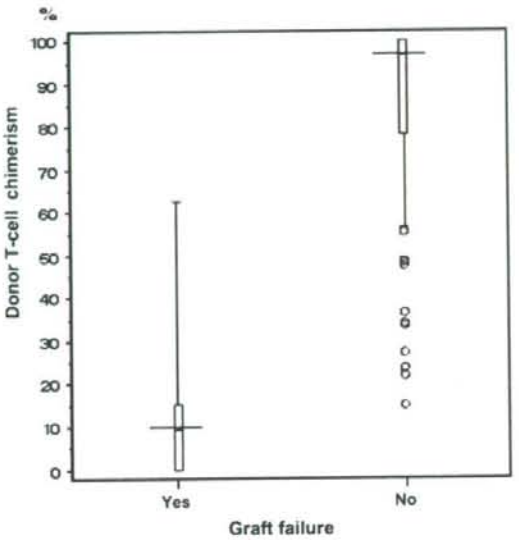

Figure 3. Donor T cell chimerism levels at day 30 in patients with or without subsequent graft failure. Five of the 117 patients (4%) who experienced graft failure had a significantly lower donor T cell chimerism level than the other engrafted patients (n = 112) (donor T cell chimerism, median 9% [range: 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

Outcome	Total (n=117)	T-cell chimerism at day 30 <60% (n=18)	T-cell chimerism at day 30 ≥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).

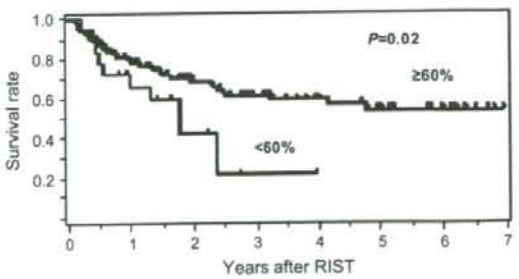

Figure 4. OS stratified according to donor T cell chimerism at day 30. OS was significantly better in patients who achieved 60% or more donor T cell chimerism at day 30 than in those who did not ($P = .02$).

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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CASE REPORT

Successful treatment of parainfluenza virus 3 pneumonia with oral ribavirin and methylprednisolone in a bone marrow transplant recipient

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Abstract We report a case of severe parainfluenza (PIV) 3 pneumonia in a hematopoietic stem cell transplant recipient that was successfully treated with oral ribavirin and methylprednisolone. A 42-year-old woman diagnosed with acute myelogenous leukemia (FAB M5a) in first complete remission underwent allogeneic bone marrow transplantation from an HLA-matched unrelated donor in May 2006. In July 2007, she developed PIV3 pneumonia. Her respiratory status progressively worsened and she required O_2 inhalation at 6 L/min. After an informed consent was obtained, oral ribavirin was initiated (16 mg/kg per day) for 1 week on July 31. By day 3 of treatment, the high-grade fever had disappeared. However, it recurred after ribavirin was discontinued. In addition, the patient's hypoxia continued to worsen, requiring O_2 inhalation at 9 L/min. To suppress the inflammatory reaction in the lung caused by PIV3 pneumonia, intravenous methylprednisolone (1,000 mg once a day for 3 days) was started along with high-dose oral ribavirin (16 mg/kg per day) on August 11. The patient showed dramatic clinical improvement, and oxygen inhalation was discontinued on September 3. Our case suggests that with concomitant effective anti-viral treatment, corticosteroids may suppress host inflammatory or immune reactions that lead to respiratory failure.

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Keywords Ribavirin · Parainfluenza virus · Pneumonia · Bone marrow transplantation

1 Introduction

Community-acquired respiratory virus infections are an important cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT) [1]. The best studied pathogens include respiratory syncytial virus (RSV), parainfluenza virus (PIV), and influenza virus [2]. PIV, an enveloped paramyxovirus containing single-stranded RNA, is classified into four serotypes. The latency period is 1–4 days [3, 4]. Only HSCT from an unrelated donor has been identified as a risk factor for infection with PIV [1]. In recipients of T-cell-depleted grafts, the degree of CD4 lymphopenia has been reported to increase the risk of all upper respiratory virus infections, including PIV [5]. Upper respiratory tract infection (URTI) is the predominant presentation. Progression to pneumonia seems to be less common than RSV. The most important risk factor for the progression from URTI to pneumonia is the use of corticosteroids and resulting lymphopenia [1, 2]. Thus, for PIV infection, host immunity seems to be a major determinant. Factors associated with a poor outcome after pneumonia include the presence of other infections and the use of ventilators. In these patients, PIV3 infections have been associated with a high mortality rate of up to 50% [1]. According to a few clinical studies, anti-viral treatment with ribavirin may improve the outcome of PIV infection in hematology patients. However, in the majority of the cases described, ribavirin has been administered in the aerosolized form, while only very few studies have addressed the use of ribavirin in the intravenous or oral form [1, 6–11]. Here, we report a case of severe PIV3

pneumonia in an HSCT recipient that was successfully treated with oral ribavirin and methylprednisolone.

2 Case report

A 42-year-old woman diagnosed with acute myelogenous leukemia (FAB M5a) in first complete remission underwent allogeneic bone marrow transplantation (BMT) from an HLA-matched unrelated donor in May 2006. The conditioning regimen comprised total body irradiation (12 Gy) and cyclophosphamide (120 mg/kg), and the graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and short-term methotrexate. She developed grade 1 acute GVHD that was well controlled with prednisolone. She had no chronic GVHD.

In July 2007 (14 months after BMT), while taking 0.5 mg of tacrolimus every other day, she presented with fever, nasal discharge and cough. Chest examination revealed no significant findings. Despite treatment with oral levofloxacin (300 mg per day), her cough and fever worsened. On admission to our hospital on July 28, she was hypoxic with an SpO_2 of 93% on room air, necessitating O_2 inhalation. Infiltrations in the right middle and left lower lung fields were seen on her chest radiograph. Computed tomography (CT) scans demonstrated ground-glass opacification throughout the right middle and left lower lobes (Fig. 1). Despite the treatment with broad-spectrum antibiotics, her respiratory state deteriorated. Bronchoscopic examination was performed. The bronchoalveolar lavage fluid (BALF) aspirate contained many neutrophils and was negative for bacterial or fungal culture. PCR examination of BALF was positive for PIV3 and negative for other bacterial, fungal, or viral pathogens, including *Pneumocystis carinii*, *Mycobacterium tuberculosis*, adenovirus, RSV, varicella-zoster virus, herpes simplex virus, and cytomegalovirus.

The patient was diagnosed with PIV3 pneumonia. At the onset of PIV3 pneumonia, the number of CD4 + T cells and CD8 + cells were 74/mm^3, 391/mm^3, respectively, indicating the poor recovery of her cellular immunity. The patient's respiratory state worsened progressively, requiring O_2 inhalation at 6 L/min. After an informed consent was obtained, oral ribavirin was initiated (16 mg/kg per day) for 1 week on July 31. Her fever improved gradually. However, it recurred after ribavirin was discontinued and her hypoxia worsened, requiring O_2 inhalation at 9 L/min. CT scans demonstrated diffuse interstitial lesions (Fig. 2). To suppress the inflammatory reaction in the lung of PIV3 pneumonia, intravenous methylprednisolone (1,000 mg once a day for 3 days) was started with high-dose oral ribavirin (16 mg/kg per day) on August 11. The patient showed marked clinical improvement, and oxygen inhalation was discontinued on September 3.

The chest radiographs and CT scans demonstrated resolving infiltrates (Figs. 3, 4). High-dose ribavirin therapy resulted in the complication of myelosuppression requiring red blood cell and platelet transfusion, and administration of granulocyte-colony stimulating factor (G-CSF). The serology showed an increase in the PIV3 antibody titer (tenfold

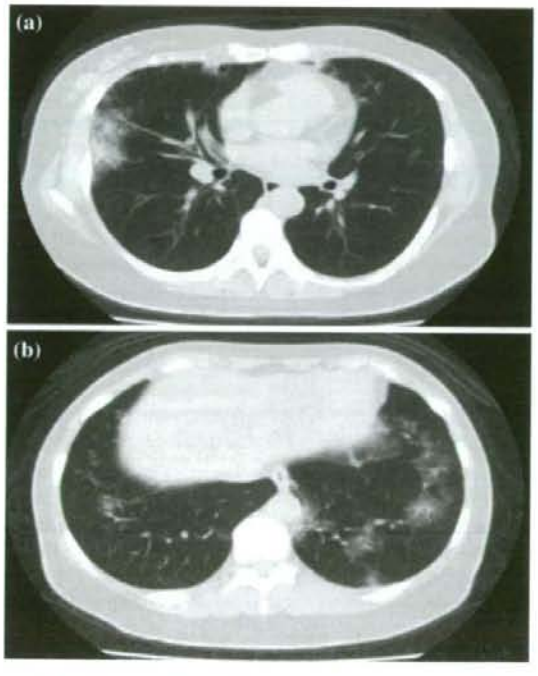

Fig. 1 Chest CT scans on admission demonstrating ground-glass opacification throughout the right middle (**a**) and left lower (**b**) lobes

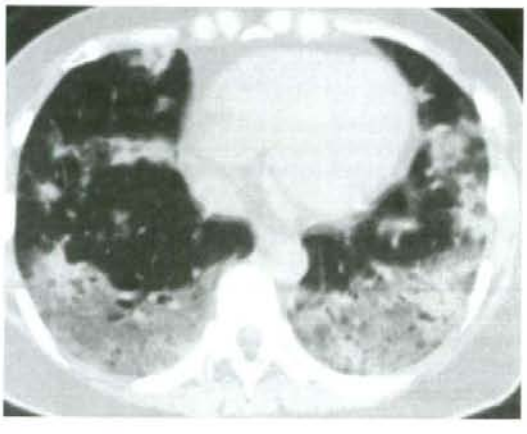

Fig. 2 Chest CT scan before initiating methylprednisolone demonstrating diffuse interstitial lesions

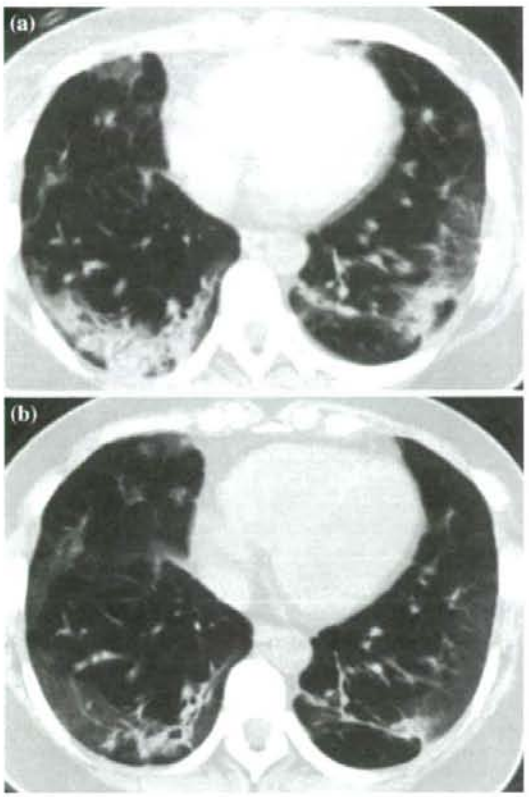

Fig. 3 Chest CT scans 2 (**a**) and 4 (**b**) weeks after initiating methylprednisolone demonstrating amelioration of interstitial lesions

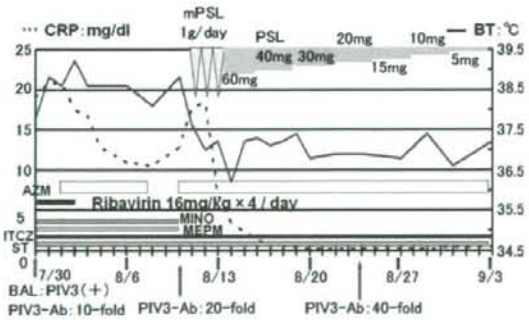

Fig. 4 Clinical course of treatment with oral ribavirin and methylprednisolone. *mPSL* methylprednisolone, *AZM* azithromycin, *MINO* minocycline, *MEPM* meropenem, *ITCZ* itraconazole, *ST* sulfamethoxazole/trimethoprim, *BAL* bronchoalveolar lavage, *PIV3* parainfluenza virus 3, *PIV3-Ab* parainfluenza virus 3 antibody titer

on admission, 20-fold 2 weeks after admission, and 40-fold 4 weeks after admission), and no evidence of any other agent. The patient was discharged on October 16, and she remained well 4 months after discharge.

3 Discussion

This is the first case report to document the successful treatment of PIV3 lower respiratory tract infection with combined high-dose oral ribavirin and corticosteroid therapy in an HSCT recipient. PIV3 infection in an immunocompromised patient can be fatal, and the prognosis is poor especially in HSCT recipients with lower respiratory tract infections. In this specific patient group, the mortality rate has varied between 30 and 50% [1]. In a retrospective analysis, neither aerosolized ribavirin nor immunoglobulin led to improved outcomes for PIV3 pneumonia or a reduction of viral shedding following PIV3 pneumonia [1, 7]. The use of systemic ribavirin has only been reported in case observations. The association of high-dose steroid treatment and lymphopenia with progression to disease suggests host immunity to be a major determinant. In this context, reduction of immunosuppressive therapy is desired when possible [1, 2, 12, 13].

Compared with aerosolized ribavirin, intravenous administration of the drug is easy, although potentially inferior in clinical efficacy [1, 2, 8, 14]. However, there are reports showing that the intravenous form of ribavirin is effective in other respiratory virus infections, such as adenovirus or RSV pneumonia and pneumonitis [2, 15–18].

In Japan, the intravenous form of ribavirin is not commercially available, but the oral form of the drug has been approved for the treatment of hepatitis C virus infection.

In the current report, oral ribavirin in combination with corticosteroids was successful in treating PIV3 pneumonia [19]. The dose and duration of oral ribavirin for the treatment of PIV3 pneumonia after HSCT have not been established. It is reported that the bioavailability of oral ribavirin is approximately 50% [20–23].

Corticosteroids have proven to be clinically useful in the treatment of *P. carinii* pneumonia with hypoxemia and the treatment of children with croup [24–27]. However, in HSCT patients with PIV URTI, corticosteroids are a risk factor for progression to lower respiratory tract infection [1]. On the other hand, some researches of antiviral therapy combined with steroid on viral pneumonia except PIV pneumonia in immunocompetent adults have been reported [28]. According to the study, the only combination of acyclovir and steroid against varicella zoster virus pneumonia was associated with a better outcome; however, the dosage and duration of steroid therapy for all of the viral pneumonia were heterogeneous in the study. Therefore the effectiveness of the combination of antiviral and steroid is not certain, and the authors suggested that future randomized clinical studies were necessary. The mechanism of corticosteroids for the treatment of viral pneumonia is not clarified. The mechanism of lung injury is considered not specifically a virally mediated lung injury, but rather an

abandoned host immune response to the virus. For viral pneumonia, the role of steroids is supposed to be similar to that in *P. carinii* pneumonia and miliary tuberculosis; both infections cause T-cell mediated responses. Corticosteroids directly inhibit both T cell function and neutrophil adherence to epithelial cells [29]. In our case, the use of oral ribavirin had limited efficacy, and a dramatic clinical improvement was observed following the introduction of corticosteroids with oral ribavirin.

Our case suggests that with concomitant effective antiviral treatment, corticosteroids may suppress host inflammatory or immune reactions that lead to respiratory failure.

In conclusion, we describe a case of severe PIV 3 pneumonia in an HSCT recipient successfully treated with oral ribavirin and methylprednisolone. The optimal use of oral ribavirin for the treatment of PIV pneumonia remains to be determined.

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Case report

Disseminated tuberculosis following second unrelated cord blood transplantation for acute myelogenous leukemia

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Abstract: Here we report the case of a 43-year-old Japanese woman with acute myelogenous leukemia who underwent 2 unrelated cord blood transplantations (UCBT), terminating in fatal disseminated tuberculosis (TB). The patient did not achieve remission despite intensive chemotherapy, and subsequently underwent UCBT with a standard conditioning regimen. However, engraftment was not achieved. Fifty days after the first UCBT, the patient underwent a second UCBT with a reduced-intensity conditioning regimen. She developed a pre-engraftment immune reaction, which responded well to prednisolone, and engraftment was documented. However, 50 days after the second UCBT, the patient presented with high fever and developed pneumonia despite antibiotic and antifungal treatments. Thereafter, *Mycobacterium tuberculosis* was detected in blood cultures and specimens of bronchoalveolar lavage, thus indicating disseminated TB. Despite anti-tuberculous treatment, she died on day 85. TB should always be considered as a possible diagnosis when treating febrile immunocompromised patients.

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Key words: tuberculosis; disseminated; cord blood; transplantation; AML

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Unrelated cord blood transplantation (UCBT) has been established as an alternative to allogeneic hematopoietic stem cell transplantation (allo-HSCT). UCBT can provide potential advantages in the form of rapid availability and a lower risk of graft-versus-host disease (GVHD), thus permitting less stringent human leukocyte antigen (HLA) matching (1). Furthermore, reduced-intensity conditioning (RIC) regimens for allo-HSCT can be undertaken as alternatives to conventional myeloablative conditioning regimens to decrease regimen-related toxicity while preserving anti-tumor effects (2). Therefore, the use of UCBT with RIC has been increasing recently for patients who do not have an HLA-matched donor, but have a history of prior transplantation or comorbid organ conditions to preclude the use of conventional allo-HSCT (3). However, despite several advantages and considerable progress in supportive care, opportunistic infections still remain a major cause of morbidity and mortality due to delayed or impaired immune reconstitution after UCBT (3).

Mycobacterium tuberculosis is a common pathogen worldwide. It infects one-third of the world's population, and is especially endemic in East Asia including Japan (4, 5). Recent reports on tuberculosis (TB) following HSCT have shown that this is a significant problem in endemic countries (6, 7). Here, we describe a case of disseminated TB manifesting early after a second UCBT with RIC for refractory acute myelogenous leukemia (AML). Immunologic immaturity of the infused UCB cells could result in a lack of tuberculous granulation formations, leading to fatal disseminated TB early after UCBT. It should be noted that if patients receiving HSCT have unexplained fever, tests to detect *M. tuberculosis* should be performed immediately to improve the outcome in these patients.

Case report

In August 2007, a 43-year-old Japanese woman was referred to our hospital because of hematologic abnormalities. Bone marrow aspiration and cytogenetic analysis revealed AML of the M2-subtype according to the French–American–British classification, with *t*(6, 9) abnormality. She did not achieve hematologic remission despite the induction of chemotherapy with idarubicin and cytosine arabinoside. In October 2007, she underwent UCBT with HLA mismatch at 2 loci. Cord blood cells (2.15×10^7 nucleated cells/kg and 1.32×10^5 CD34-positive cells/kg) were infused after a conditioning regimen including total body irradiation (TBI) (12 Gy) and cyclophosphamide (120 mg/kg). Prophylaxis for GVHD consisted of cyclosporine and short-term methotrexate. Hematopoietic recovery was delayed, and engraftment failure was confirmed by chimerism analysis using DNA amplification of polymorphic short tandem repeats of bone marrow cells on day 26.

Following this, in December 2007, 50 days after the first UCBT, she underwent the second UCBT from another donor with HLA mismatch at 2 loci. Cord blood cells (2.43×10^7 nucleated cells/kg and 1.03×10^5 CD34-positive cells/kg) were infused following a RIC regimen including fludarabine (125 mg/kg) and melphalan (80 mg/kg), and GVHD prophylaxis with cyclosporine and mycophenolate mofetil. On day 26, after the second UCBT, she presented with high fever, skin eruptions, and weight gain. She was diagnosed with a pre-engraftment immune reaction (8), which responded well to treatment with prednisolone (1 mg/kg daily). The patient became afebrile and engraftment was documented on day 33. On day 50, she developed high fever again, and chest computed tomography (CT) scan demonstrated homogenous amorphous opacification with air bronchograms in the right upper lobe, and an absence of the typical cavitary consolidation of TB (Fig. 1). The lesions were considered to be bacterial or fungal infections based on consultation with the radiologists and infectious disease control team. Despite the administration of broad-spectrum antibiotics and antifungal drugs, the high fever persisted. On day 55, Ziehl–Neelsen staining and polymerase chain reaction analysis of bronchoalveolar lavage specimens exhibited positivity for *M. tuberculosis* and negativity for other bacteria, fungi, *Pneumocystis jirovecii*, and cytomegalovirus. The subsequent blood culture was positive for *M. tuberculosis*, indicating that she suffered from disseminated TB. She had no prior history of TB. Transbronchial lung biopsy could not be performed because of an extremely low platelet count. Anti-tuberculous therapy with isoniazid, rifampicin, ethambutol, and pyrazinamide was started immediately. However, her respiratory state gradually worsened. Progression of hypoxia could not be controlled and finally she died on day 85. Postmortem examination was not permitted.

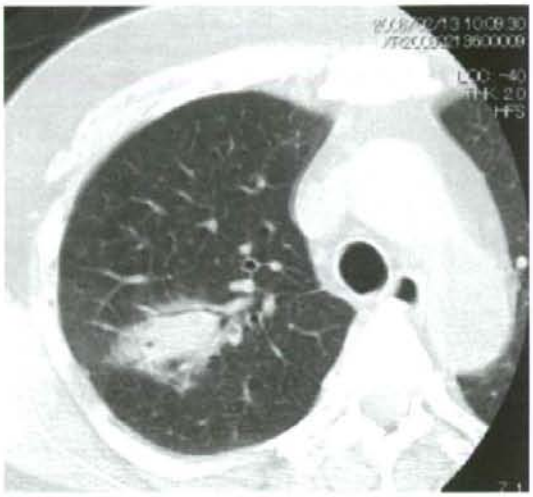

Fig. 1. Computed tomography image on day 50 showing consolidation in the right upper lobe, and homogenous amorphous opacification with air bronchograms, but not showing the typical cavitary consolidation of tuberculosis.

Discussion

The high incidence of TB in HSCT recipients is considered to be due to the severe immunodeficiencies that these patients suffer (6, 9). According to recent studies, TB occurred in about 0.4% of HSCT recipients; the incidence was reported as 0.13% in autologous and 0.57% in allo-HSCT, whereas mortality rates from TB amounted to approximately 0% in autologous and 30% in allogeneic patients, indicating that allo-HSCT presented a higher risk for the occurrence and greater severity of TB (10). Chronic GVHD, immunosuppressive therapy, TBI, and T-cell depletion are all risk factors contributing to the development of TB following allo-HSCT (6, 10, 11). Our patient underwent UCBT twice at a short interval. Conditioning regimens included TBI in the first UCBT and potent immunosuppressive agents such as fludarabine and melphalan were included in the RIC regimen for the second UCBT. In addition, the patient developed pre-engraftment immune reaction, which required prednisolone administration. Prolonged immunosuppression and damage to alveolar macrophages by TBI might have contributed to the onset of fatal TB (12).

Generally, TB lesions are more commonly restricted to lungs; however, in some cases, disseminated TB can occur (6, 10, 11). The patient in our report initially presented with disseminated TB, despite having no prior history of TB. Only one report documents cases of TB following UCBT (13). The group in Toranomon, Japan, reported that TB was diagnosed in 3 out of 113 (2.7%) adult patients, and all 3 manifested disseminated TB at diagnosis (13). Despite anti-tuberculous treatment, 2 patients died immediately. This report, together with our case, suggests that UCBT recipients might have a higher risk for disseminated TB and a higher mortality rate than transplantation recipients having another stem cell source. Under steady-state conditions, T cells and macrophages secrete interferon-gamma and tumor necrosis factor-alpha, which are crucial mediators of protection against the onset of *M. tuberculosis*-mediated granuloma (14–16). However, cord blood T cells and macrophages represent a naive and immunologic immature cell population to be primed for defense against infections including TB (17). The Toranomon group also reported that biopsy specimens collected from UCBT recipients with TB revealed necrosis without granulation in any organ (13). In our case, CT images showed consolidation reflecting bacterial or fungal infections, but did not show the typical cavitary consolidation of TB. Demirkazik et al. (18) reported that in the evaluation of febrile immunocompromised patients on the basis of CT findings, pulmonary fungal infection and *P. jirovecii* pneumonia could be identified with great accuracy, but not TB. Therefore, the lack of tuberculous granulation formation due to the immaturity of cord blood cells can make the diagnosis of TB difficult based on the CT findings, and can result in the development of fatal disseminated TB following UCBT.

We described a UCBT patient who developed disseminated TB. In endemic areas including Japan, TB should be considered in HSCT patients with fever of unknown origin, and precise tests for *M. tuberculosis* detection should be conducted immediately. In addition, even if patients have had no past history of TB, prophylactic anti-tuberculous therapy could be considered in the event of severe immunosuppressive conditions.

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CASE REPORT

Additional acquisition of t(1;21)(p32;q22) in a patient relapsing with acute myelogenous leukemia with *NUP98-HOXA9*

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Abstract We report a 29-year-old Japanese male with acute myelogenous leukemia (AML)-M4 with a cryptic t(7;11)(p15;p15), in which a chimeric *NUP98-HOXA9* fusion was detected by polymerase chain reaction analysis and a chromosomal analysis showed 46,XY. The patient received intensive chemotherapy and underwent autologous stem cell transplantation, and remission was confirmed by the disappearance of *NUP98-HOXA9*. However, 6 months after transplantation, the patient relapsed; *NUP98-HOXA9* was detected again and karyotypic analysis revealed 46,XY, t(1;21)(p32;q22). Fluorescent in situ hybridization (FISH) analysis using an *AML1-ETO* translocation dual probe, showed that the 21q22 breakpoint involved *AML1* locus. A retrospective FISH analysis showed that t(1;21) was absent at onset. This is the first reported case with AML who had a cryptic t(7;11)(p15;p15), and additionally acquired t(1;21)(p32;q22) at relapse.

Keywords AML · t(1;21) · t(7;11) · *NUP98-HOXA9* · Clonal evolution

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1 Introduction

A significant proportion of acute myelogenous leukemia (AML) cases are characterized by a variety of recurrent chromosomal abnormalities that serve as the most important diagnostic and prognostic markers. The progression of AML is frequently accompanied by a cytogenetic clonal evolution with the appearance of additional chromosomal abnormality.

Translocation t(7;11)(p15;p15), which generate a chimeric *NUP98-HOXA9* fusion gene, is one of the rare chromosomal abnormalities found in myeloid malignancies, such as AML-M2 or M4 subtype [1–3]. This translocation has been frequently observed in Asians, and the prognosis of patients with this aberration is poor [4–6]. In contrast, *AML1* gene at 21q22 is the most frequent target of chromosomal rearrangement observed in acute leukemia and includes t(8)(q22;q22) in AML-M2, t(3;21)(q26;q22) in therapy-related AML and in the blast crisis of chronic myelogenous leukemia, and t(12;21)(p13;q22) in B-cell lymphocytic leukemia [7–9]. To our knowledge, t(1;21)(p32;q22) has only been described in one patient with AML-M4 [10].

Here, we report a patient with AML-M4 presenting 46,XY with a cryptic t(7;11)(p15;p15) detected by polymerase chain reaction analysis (PCR) for *NUP98-HOXA9* at the initial diagnosis. This patient obtained remission after autologous peripheral blood stem cell transplantation (auto-PBSCT) that was confirmed by the disappearance of *NUP98-HOXA9*. At the recurrence of AML-M4, *NUP98-HOXA9* was identified again, and a chromosomal analysis showed 46,XY, t(1;21)(p32;q22). This is the first patient with AML who had a cryptic t(7;11)(p15;p15), and additionally acquired t(1;21)(p32;q22) at relapse.

2 Case report

In April 2007, a 29-year-old Japanese male presented with a history of fever for 2 weeks, and was referred to our hospital. Hemoglobin was 6.2 g/dL; platelet count, 30×10^9/L; and white blood cell count, 155×10^9/L with 69% blasts, 5% myelocytes, 2% metamyelocytes, 10% neutrophils, 9% monocytes, and 5% lymphocytes. Bone marrow aspiration showed a markedly hypercellular bone marrow with 73% medium-sized blastic cells containing moderately irregular nuclei and prominent nucleoli (Fig. 1a). The blasts stained positive for myeloperoxidase and chloroacetate esterase. Flow cytometric analysis showed that the blasts were positive for CD13, CD33, CD34, CD38, and HLA-DR with negative lymphoid markers. By cytogenetic analysis, all of 20 metaphases examined were 46,XY (Fig. 2a). Leukemia blasts were positive for *NUP98-HOXA9*, but negative for other transcripts by reverse transcriptase PCR (RT-PCR) analysis [11]. Because *NUP98-HOXA9* is known to be generated by t(7;11)(p15;p15) translocation, the patient was diagnosed with AML-M4 with a cryptic t(7;11). The patient started induction chemotherapy with idarubicin and cytosine arabinoside and obtained complete remission (CR). Following two courses of intensive chemotherapy, consisting of high-dose cytosine arabinoside with mitoxantrone or etoposide, the patient underwent auto-PBSCT in September 2007, as previously described [12]. The patient attained CR in the absence of *NUP98-HOXA9*.

In March 2008, leukocytosis and thrombocytopenia were documented. Bone marrow examination revealed an increase in myeloblasts (51% of the marrow differential count). The myeloblasts morphologically resembled those at the initial diagnosis (Fig. 1b), and they were positive for CD13, CD33, CD34, CD38, and HLA-DR; the same phenotype as that observed at the first presentation. *NUP98-HOXA9* was detected again by RT-PCR analysis, and the karyotype had evolved to 46,XY, t(1;21)(p32;q22) in 16 of 20 cells and 46,XY in four cells (Fig. 2b). We diagnosed the disease as a relapse of AML-M4. Following the re-induction chemotherapy, the patient underwent allogeneic bone marrow transplantation from an unrelated donor in May 2008. After transplantation, he has maintained hematological CR; a karyotype analysis confirmed cytogenetic CR and *NUP98-HOXA9* was undetectable.

Since 21q22 breakpoint suggested possible involvement of the AML1 locus, fluorescent in situ hybridization (FISH) analyses, using an *AML1/ETO* translocation dual probe, were performed on bone marrow samples at the initial diagnosis and at relapse. At relapse, FISH analysis revealed

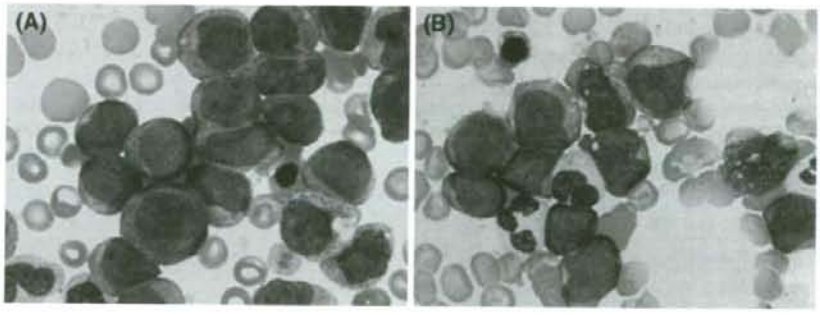

Fig. 1 Leukemia cells in patient's bone marrow on admission (**a**) and recurrence of AML (**b**)

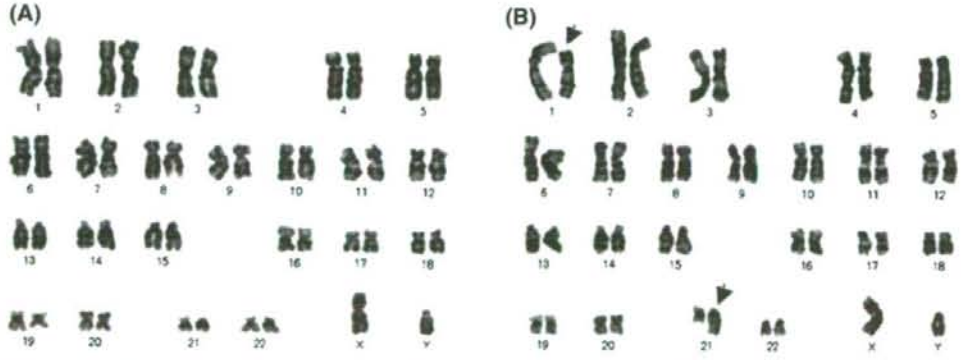

Fig. 2 G-banded karyotype of the bone marrow cells at the initial diagnosis, 46,XY (20/20 cells) (**a**) and at the relapse of AML, 46,XY,t(1;21)(p32;q22) (16/20 cells) (**b**). *Arrows* indicate rearranged chromosomes

that 119 of 200 interphases had an expected *AML1* signal on the normal chromosome 21 homolog, two normal *ETO* signals on the chromosome 8 homologs, and a reduced *AML1* split signal on the derivative chromosome 1 (der(1)t(1:21)) and the derivative chromosome 21(der(21)t(1;21)) homologs (Fig. 3b). We also retrospectively analyzed the presence of t(1;21) in the bone marrow cells collected at onset. FISH analyses disclosed that all interphases had normal *ETO* and *AML1* signals on the chromosome 8 and 21 homologs, demonstrating that t(1;21)(p32;q22) was absent at onset, but had been acquired on relapse (Fig. 3a).

3 Discussion

This is the first reported AML patient with *NUP98-HOXA9*, who additionally acquired t(1;21)(p32;q22) at relapse. Appearance of t(1;21)(p32;q22) might have been responsible for the relapse of AML after auto-PBSCT. Because of the cryptic t(7;11) translocation, we could not confirm the simultaneous presence of t(1;21) and t(7;11) in each of the leukemic cells, since FISH analysis for *NUP98-HOXA9* was not available in our hands. However, relapse is usually thought to arise from the original clone when the chromosomal abnormalities disclose the same ones at diagnosis and relapse, even though minor clonal variations occur. Therefore, we thought that the residual leukemic clone with *NUP98-HOXA9* acquired t(1;21)(p32;q22) additionally through clonal evolution, and resulted in a relapse of AML.

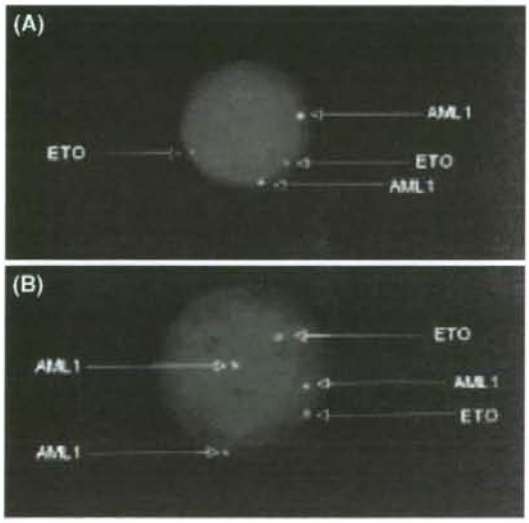

Fig. 3 FISH analysis of bone marrow cells at the initial diagnosis (**a**) and at relapse (**b**) using an *AML1/ETO* translocation dual probe. Interphase FISH with the *AML1/ETO* probe reveals a *red* signal on a normal chromosome 8 and a *green* signal on a normal chromosome 21. **a** Two *green AML1* and *red ETO* signals were present in cells at the initial diagnosis indicating no cryptic gene rearrangement associated with *AML1* gene on 21q22. **b** At relapse three *green AML1* signals disclosed an expected *AML1* signal on the normal chromosome 21 homolog, and two reduced *AML1* split signals on der(1)t(1:21) and der(21)t(1;21)

Chromosomal translocations generate chimeric fusion proteins that mediate a block in differentiation through deregulation of hematopoietic transcription factors. In animal models, the loss of function of transcription factors leads to impaired differentiation, but a single transcription factor gene mutation is not sufficient to cause acute leukemia [13]. Therefore, a novel two-hit model of AML emphasized a clear coordination between inactivating mutations of transcription factors and mutations affecting receptor tyrosine kinases [14, 15]. *AML1* is a novel transcription factor essential for the regulation of hematopoiesis, and is also the most frequently targeted chromosomal rearrangement in acute leukemia [7]. *AML1*-associated leukemia-fusion proteins may cause the dominant negative properties over the wild type of *AML1* and impair differentiation of normal hematopoiesis and contribute to leukemogenesis [7, 9]. Translocation t(1;21)(p32;q22) involving the *AML1* locus and an unknown locus on 1p32, has been reported in only one case of AML-M4 [10]; therefore, the role of this abnormality on the pathogenesis of AML still remains unclear. In contrast, recent studies have revealed the functions of *HOXA9, NUP98*, and chimeric *NUP98-HOXA9* fusion in hematopoiesis. *HOXA9* is a member of the homeobox family, that plays an important regulatory role in the self-renewal of hematopoietic stem cells [16], whereas *NUP98* is a component of the nuclear pore complex that facilitates mRNA export from the nucleus [17]. Enforced expression of *NUP98-HOXA9* in murine bone marrow resulted in a myeloproliferative disease progressing to AML by 8 months [18, 19], and transduction of *NUP98-HOXA9* fusion genes into human $CD34^+$ cells conferred a proliferative advantage and enhanced self-renewal [20]. *NUP98-HOXA9* mainly confers a proliferative or survival advantage for progression to acute leukemia. In addition, Auewarakul et al. [21] reported the coexistence of *AML1* mutation in an AML patient with t(7;11)(p15;p15), and they suggested that abnormalities in more than one transcription factor could together initiate and propagate the leukemic transformation without mutations of tyrosine kinases. Thus, in this case t(1;21) and t(7;11) translocations might have cooperated to induce leukemia relapse through impaired differentiation caused mainly by *AML1*-associated fusion proteins, and enhanced proliferation caused by *NUP98-HOXA9*.

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