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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

該当なし

2. 実用新案登録

該当なし

3. その他

該当なし

Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kishi Y, Taniguchi S, et al.	Early central nervous system complications after reduced-intensity stem cell transplantation.	Biol Blood Marrow Transplant	10	561-568	2004
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Ⅳ. 研究成果の刊行物・別刷

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Early Central Nervous System Complications after Reduced-Intensity Stem Cell Transplantation

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ABSTRACT

To investigate clinical characteristics of early central nervous system (CNS) complications after reduced-intensity stem cell transplantation (RIST), we reviewed the medical records of 232 patients who had undergone RIST for hematologic diseases at our institutions between September 1999 and June 2003. All patients had received purine analog–based preparative regimens. Stem cell sources comprised granulocyte colony-stimulating factor–mobilized blood from HLA-identical or 1 locus–mismatched related donors (n = 151), unrelated bone marrow (n = 44), or unrelated cord blood (n = 37). Graft-versus-host disease prophylaxis incorporated cyclosporine with or without methotrexate. Diagnosis of CNS complications was based on clinical, radiologic, and microbiological findings. CNS complications occurred in 18 patients (7.8%), with a median onset of 22 days, and were infectious (n = 1), metabolic (n = 15), or cerebrovascular (n = 2). Symptoms included seizures (n = 7), visual disturbance (n = 2), headache (n = 8), nausea (n = 8), vomiting (n = 6), impaired consciousness (n = 16), and hemiparesis (n = 3). Complications improved promptly in 10 patients, and 8 patients died without improvement within 30 days. Multivariate analysis with logistic regression identified umbilical cord blood transplantation as a significant risk factor for early CNS complications (odds ratio, 14.5; 95% confidence interval, 3.7-56.9; $P < .0001$). CNS complications are a significant problem after RIST, particularly with umbilical cord blood. Limbic encephalopathy is an unrecognized subtype of neurotoxicity after umbilical cord blood transplantation.

KEY WORDS

Allogeneic hematopoietic stem cell transplantation • Graft-versus-host disease • Umbilical cord • Cyclosporine neurotoxicity • Limbic encephalopathy

INTRODUCTION

Research in the area of neurologic complications is limited with regard to allogeneic hematopoietic stem cell transplantation (allo-HSCT). Most studies have been either retrospective or reliant on autopsy records [1–6]. Prospective evaluation of this complication has been rare [7,8]. The incidence of neurologic complications has varied from 37% to 91%, and such complications have been the cause of death in 6% to 26% of patients [1,3,8]. These findings indicate that neurologic complications represent a significant problem in conventional myeloablative allo-HSCT.

Neurologic complications occur at 3 stages of allo-HSCT: (1) after the use of conditioning agents for marrow ablation, (2) during posttransplantation pan-

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cytopenia, or (3) after immunosuppressive therapies and graft-versus-host disease (GVHD) [1–3,9]. These complications are usually categorized into 4 groups: (1) infectious, (2) cerebrovascular, (3) metabolic, or (4) immune-mediated disorders. Among these 4 types of neurotoxicity, cerebrovascular disorders and central nervous system (CNS) infection before engraftment have represented significant problems in conventional allo-HSCT [1,4,8]. Whether GVHD can affect the CNS remains controversial [10], and neurotoxicity has thus been regarded as an early complication after allo-HSCT.

A new transplantation strategy using a nonmyeloablative preparative regimen—reduced-intensity stem cell transplantation (RIST)—was developed to decrease regimen-related toxicity while preserving adequate antitumor effects [11,12]. Different pioneering conditioning regimens for RIST have been investigated, such as those including purine analogs [11–13] and total body irradiation (TBI) combined with potent immunosuppressants [14]. Although early reports on RIST emphasized safety advantages [11,15], recent studies have revealed considerable toxicities associated with this type of transplantation [16,17]. Little information is available on CNS complications after RIST. We investigated early CNS complications after RIST with regard to incidence, characteristics, and risk factors.

PATIENTS AND METHODS

Patients

Medical records of all patients who underwent RIST for treatment of hematologic diseases at the National Cancer Center Hospital or Toranomon Hospital between September 1999 and June 2003 were reviewed. Subjects comprised 232 patients (143 men and 89 women) with a median age of 54 years (range, 15-73 years). Primary diseases consisted of acute myeloid leukemia (n = 63), chronic myelogenous leukemia (n = 15), acute lymphoblastic leukemia (n = 8), malignant lymphoma (n = 67), myelodysplastic syndrome (n = 42), adult T-cell leukemia/lymphoma (n = 17), multiple myeloma (n = 10), aplastic anemia (n = 8), and others (n = 2). Hematologic malignancies were refractory to cytotoxic chemotherapy in 142 patients and were in remission or sensitive to treatment in 81 patients. Underlying diseases were not malignant in the remaining 9 patients.

Transplantation Procedures

All patients had received purine analog–based preparative regimens comprising fludarabine/cyclophosphamide (n = 12) [18], fludarabine/busulfan (n = 139) [19], fludarabine/melphalan (n = 55) [20], cladribine/busulfan (n = 25) [13], and others (n = 1). Rabbit antithymocyte globulin and TBI (4-8 Gy) were added to preparative regimens in 50 and 65 patients, respectively.

Stem cell sources were HLA-identical or 1 locus–mismatched granulocyte colony-stimulating factor–mobilized peripheral blood (n = 151), unrelated bone marrow (n = 44), or unrelated umbilical cord blood (n = 37). GVHD prophylaxis was cyclosporine alone (3 mg/kg) in RIST from an HLA-identical related donor and reduced-intensity umbilical cord blood transplantation (RI-UCBT). Patients who received transplants from a 1 locus–mismatched related donor or a matched unrelated donor received cyclosporine and short-term methotrexate. Grade II to IV acute GVHD was treated with methylprednisolone 2 mg/kg/d in addition to cyclosporine.

Diagnostic Criteria for Early CNS Complications

Early CNS complications were defined as CNS toxicity occurring within 100 days of transplantation. Diagnosis of CNS complications was made by clinical, radiologic, or microbiological findings (or a combination of these). CNS complications were categorized into 4 groups: (1) infectious, (2) cerebrovascular, (3) metabolic, and 4) immune-mediated disorders. CNS complications that occurred after relapse or progression of underlying diseases were excluded from analysis. Diagnosis of cyclosporine encephalopathy was based on the typical radiologic findings, ie, symmetrical white matter lesions mainly localized in the occipital lobe. In the case of limbic encephalopathy, the diagnosis was based on selective involvement of the medial temporal lobe on magnetic resonance imaging (MRI). Diagnosis of cerebrovascular diseases was confirmed by neuroradiologic or postmortem studies (or both). Abnormalities on imaging were defined as areas of low white-matter attenuation on computed tomographic (CT) scans and as areas of T1-weighted hypointensity and T2-weighted hyperintensity on MRI.

End Points and Statistical Analysis

The primary end point of this study was incidence of early CNS complications after RIST. A secondary objective was to investigate characteristics and risk factors for such complications. The median follow-up of surviving patients was 17.5 months (range, 8.5-52.7 months).

Univariate analysis with χ^2 and Mann-Whitney tests was performed to identify risk factors for CNS toxicity. Variables included age, sex, primary disease, disease status (refractory or sensitive to cytotoxic chemotherapy), and type of transplantation. We added multiple logistic regression analysis to assess the fractionated contribution of the above-mentioned potentially predictive factors. Variables that had a *P* value of

<.25 on univariate analysis were entered into the mixed-effects model. Those that contributed <10% to the overall ability of the model to influence serum levels of fluconazole were sequentially eliminated. The level of significance was set at $P < .05$.

RESULTS

Incidences and Types of CNS Complications after RIST

A total of 18 patients (7.8%) developed early CNS complications. Subtypes comprised infectious (invasive aspergillosis; n = 1), metabolic (n = 15; cyclosporine neurotoxicity, n = 4; limbic encephalopathy, n = 4; hemophagocytic syndrome, n = 1; leukoencephalopathy, n = 1; idiopathic, n = 5), and cerebrovascular (subdural hematoma, n = 1; subarachnoid hemorrhage, n = 1) complications. No patient was diagnosed with immune-mediated CNS toxicity.

Clinical and Laboratory Features at Onset of CNS Complications

Backgrounds of the patients who developed CNS complications are shown in Table 1. Except for a patient with aplastic anemia, the remaining 17 patients had refractory hematologic diseases.

Clinical and laboratory findings at the onset of CNS complications are shown in Table 2. The median onset was 22 days (range, 1-74 days). Seizures developed in 7 patients (generalized, n = 6; focal, n = 1). Other symptoms included headache (n = 8), nausea (n = 8), vomiting (n = 6), impaired consciousness (n = 16), and hemiparesis (n = 3). Two of 11 evaluable patients developed visual disturbance (blurred vision). Cyclosporine blood levels were higher than target levels (250-350 ng/mL) in 4 patients. Nine patients displayed fever at the onset of CNS complications, and 2 patients were receiving steroid therapy for acute GVHD. Concomitant conditions in the 15 patients with metabolic encephalopathy included systolic hypertension (>170 mm Hg) in 6 patients, diastolic hypertension (>100 mm Hg) in 6, hyponatremia in 8, hypomagnesemia in 6, and hypocholesterolemia in 4. Cerebrospinal fluid obtained from 5 patients showed normal levels of protein and cell counts. No pathogens such as bacteria, fungi, or viruses were cultured from cerebrospinal fluid.

Imaging Studies

Seventeen patients underwent cranial imaging studies: CT only in 6, MRI only in 4, and both CT and MRI in 7. Results are shown in Table 2. Of the 14 patients with metabolic encephalopathy who underwent imaging studies, 7 displayed some abnormal findings. Lesions were located bilaterally in the occipital lobes (n = 3), temporal lobes (n = 3), or periventricular white matter (n = 1). Three patients who had received UCBTs were diagnosed with limbic encephalopathy on the basis of imaging studies (Figure 1).

Treatment and Outcomes

Cyclosporine was continued (n = 4) or withheld (n = 14) for 1 to 14 days. Two patients received antihypertensive agents. Corticosteroids were used in 16 patients. In most patients, subsequent treatment with cyclosporine was well tolerated without recurrence of neurotoxicity.

Eight patients died within 30 days of developing CNS complications. Causes of death included disease progression (n = 1), subarachnoid hemorrhage (n = 1), GVHD (n = 3), and infection (n = 3). CNS complication was a primary cause of death in 2 cases (invasive aspergillosis, n = 1; subarachnoid hemorrhage, n = 1).

Risk Factors

In univariate analysis, the development of CNS complications was associated with the use of umbilical cord blood ($P < .0001$) and the status of underlying disease (chemorefractory hematologic diseases versus others; $P = .032$). Multivariate analysis showed that the use of umbilical cord blood was significantly correlated with CNS complications after RIST (odds ratio, 14.5; 95% confidence interval, 3.7-56.9; $P < .0001$).

DISCUSSION

In this study, CNS complications occurred in 7.8% of RIST recipients, and mortality with 30 days of its development reached 44%. These findings indicate that early CNS complications are a common and important problem in both RIST and conventional allo-HSCT [1,3,4,8]. However, significant differences existed in clinical characteristics of CNS complications between RIST and conventional myeloablative allo-HSCT.

The incidence of CNS complications was lower in RIST than in conventional allo-HSCT, in which 11% to 44% of patients develop such complications [2,6,7]. In conventional transplantation, the most common causes of CNS complications are cerebrovascular disease and infection after conventional transplantation [1,4,8], and these are mostly attributable to regimen-related toxicity [21,22] or profound myelosuppression before engraftment [1,3,4]. However, in RIST, regimen-related toxicities are minimal, and myelosuppression is short. Acute GVHD, as the most important complication in RIST [16], rarely affects the CNS [23]. RIST has, at the very least, improved the safety of allo-HSCT by decreasing the incidence of CNS complications.

Table 1. *Backgrounds of Patients Who Developed CNS Complications after RIST*

Patient No.	Type of CNS Complication	Age (y)	Sex	Primary Disease	History of CNS Involvement	No. of Chemotherapy Regimens before Transplantation	Preparative Regimen	GVHD Prophylaxis	Stem Cell Source
1	Cerebrovascular	57	M	ALL	Yes	1	Flu/BU/ATG	Cyclosporine	HLA-identical sibling
2	Cerebrovascular	32	F	Malignant lymphoma	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
3	Infectious	40	M	MDS	No	2	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
4	Metabolic	21	M	Aplastic anemia	No	1	Flu/BU/ATG	Cyclosporine	HLA-identical sibling
5	Metabolic	67	M	Malignant lymphoma	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
6	Metabolic	67	M	MDS	No	1	Flu/BU/TBI 4 Gy/ATG	Cyclosporine/Methotrexate	Matched unrelated donor
7	Metabolic	51	M	AML	No	2	Flu/BU/TBI 4 Gy	Cyclosporine	Umbilical cord blood
8	Metabolic	52	M	MDS	No	2	Flu/ATG	Cyclosporine	Mismatched related donor
9	Metabolic	49	M	ALL	No	1	Flu/BU	Cyclosporine	HLA-identical sibling
10	Metabolic	48	F	AML	Yes	3	Flu/BU/ATG	Cyclosporine/Methotrexate	Mismatched related donor
11	Metabolic	57	F	AML	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
12	Metabolic	66	M	Malignant lymphoma	No	2	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
13	Metabolic	63	M	MDS	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
14	Metabolic	54	M	AML	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
15	Metabolic	55	M	Malignant lymphoma	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
16	Metabolic	62	F	ATL	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
17	Metabolic	46	M	ATL	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood
18	Metabolic	54	F	ATL	No	1	Flu/Mel/TBI 4 Gy	Cyclosporine	Umbilical cord blood

AML indicates acute myeloblastic leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; ATL, adult T-cell leukemia/lymphoma; Flu, fludarabine; BU, busulfan; ATG, antithymocyte globulin; TBI, total body irradiation; CNS, central nervous system.

Table 2. *Clinical, Laboratory, and Radiologic Characteristics at the Onset of CNS Complications*

				Clinical Findings							Laboratory Findings							Radiologic Examination			
Patient No.	Type of CNS Complication	Cause	Onset (day)	Impaired Consciousness	Seizures	Visual Disturbance	Fever (>38.0°C)	Blood Pressure (Systolic; mm Hg)	Blood Pressure (Diastolic; mm Hg)	Cyclosporine (ng/mL)[a]	Creatinine (mg/dL)	Hemoglobin (g/dL)	Na (mEq)	K (mEq)	Mg (mEq)	T-Chol (mg/dL)	CT	T2-Weighted MRI	Electroencephalogram	Outcomes
1	Cerebrovascular		16	No	No	No	No	170	87	386	0.6	9.0	139	4.3	1.5	NA	NA	Subdural hematoma	NA	Improved
2	Cerebrovascular		40	Yes	Yes	Yes	Yes	152	98	NA	1.6	7	137	3.5	1.3	217	Brain edema, subarachnoid hemorrhage	NA	NA	Dead
3	Infection		68	Yes	No	No	No	108	64	NA	1.2	7.5	136	3.4	0.6	140	Mass in the parietal lobe	NA	NA	Dead
4	Metabolic encephalopathy	Cyclosporine	8	No	No	No	Yes	142	74	316	0.8	6.5	140	4.0	1.5	NA	NA	Bilateral parietal and occipital lobes	NA	Improved
5	Metabolic encephalopathy	Limbic encephalopathy	22	Yes	Yes	No	No	170	108	219	0.3	8.2	124	3.5	1.2	143	NA	Bilateral temporal lobes	NA	Improved
6	Metabolic encephalopathy	Cyclosporine	22	Yes	Yes	Not evaluable	Yes	182	100	266	1.2	7.5	141	2.5	1.8	NA	Low-density area in the bilateral occipital lobes	NA	NA	Improved
7	Metabolic encephalopathy	Cyclosporine	22	Yes	Yes	Not evaluable	No	120	80	348	1.5	6.5	139	4.8	1.3	107	Normal	Bilateral occipital lobes	NA	Improved
8	Metabolic encephalopathy	Cyclosporine	7	Yes	Yes	Not evaluable	Yes	170	78	342	0.8	4.1	138	3.6	1.3	145	Normal	NA	NA	Improved
9	Metabolic encephalopathy	Hemophagocytic syndroma	46	Yes	No	Yes	No	130	64	NA	0.7	9.7	139	4.1	NA	110	NA	Normal	NA	Dead
10	Metabolic encephalopathy	Leukoencephalopathy	12	Yes	No	No	Yes	110	56	NA	1.1	6.5	134	4.0	NA	NA	Normal	Bilateral frontal and parietal lobes (periventricular area)	NA	Dead
11	Metabolic encephalopathy		20	Yes	No	No	No	154	100	584	0.8	8.2	130	2.9	NA	157	Normal	NA	NA	Dead
12	Metabolic encephalopathy		13	Yes	No	Not evaluable	Yes	190	120	511	1.5	8.1	130	4.2	NA	162	Normal	Normal	Normal	Improved
13	Metabolic encephalopathy	Limbic encephalopathy	24	Yes	No	No	No	158	85	68	0.8	9.7	131	4.3	NA	NA	Normal	Bilateral temporal lobes	Diffuse slow waves	Improved
14	Metabolic encephalopathy		22	Yes	Yes	Not evaluable	Yes	168	86	416	0.8	7.8	134	3.9	1.3	107	Normal	Normal	Diffuse slow waves	Dead
15	Metabolic encephalopathy		41	Yes	No	No	Yes	180	120	52	NA	NA	NA	NA	NA	NA	Normal	NA	Diffuse slow waves	Dead
16	Metabolic encephalopathy	Limbic encephalopathy	26	Yes	Yes	Not evaluable	Yes	174	98	37	2.4	7.4	127	3.4	1.4	130	Normal	Bilateral temporal lobes	Spike wave in frontal lobes	Dead
17	Metabolic encephalopathy		18	Yes	No	Not evaluable	No	150	100	156	0.4	9.7	113	3.7	NA	NA	Normal	Normal	Diffuse slow waves	Improved
18	Metabolic encephalopathy		74	Yes	No	No	No	130	80	NA	2.2	12.1	119	5.4	NA	NA	NA	NA	NA	Improved

NA indicates not applicable; T-chol, total cholesterol.
[a]Continuous infusion of cyclosporin was given at target levels of 250-350 ng/mL.

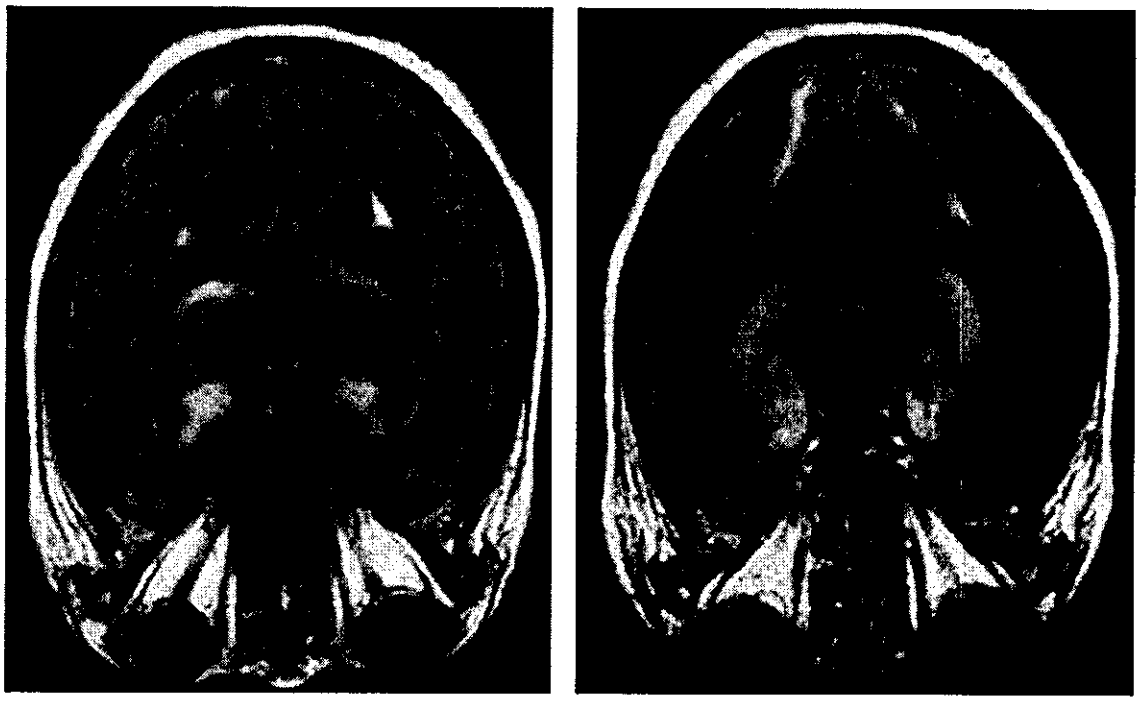

Figure 1. T2-weighted magnetic resonance image of the brain showing high-intensity signals in bilateral temporal lobes. The patient was diagnosed with limbic encephalopathy.

In contrast to conventional allo-HSCT, the incidence of metabolic encephalopathy is increased with RIST. In this study, 15 of 18 CNS complications were metabolic. Of these patients, 4 were diagnosed with cyclosporine encephalopathy on the basis of typical clinical and imaging findings. The incidence of cyclosporine encephalopathy was 1.7% after RIST, which is comparable to that after conventional allo-HSCT in young patients [24]. The median onset was 15 days (range, days 7-22). Three patients displayed seizures and altered mental status that improved after discontinuation of cyclosporine. Blood levels for cyclosporine were normal in all of the 4 patients. Risk factors for cyclosporine encephalopathy have been reported [24,25], and hypertension (2/4), hypocholesterolemia (1/2), and hypomagnesemia (3/4) were observed in our study. These findings are comparable to previous reports on cyclosporine neurotoxicity [24,25]. The growing use of RIST has increased the chance of cyclosporine being administered to elderly patients. Our study does not support the hypothesis that cyclosporine neurotoxicity increases in elderly patients, but further investigation of the safety issues for cyclosporine is warranted. General management such as blood pressure control and electrolyte replacement may be important in preventing adverse effects of cyclosporine.

No findings in the remaining 11 patients with metabolic encephalopathy suggested cyclosporine encephalopathy. However, it should be noted that all 11 patients received a fludarabine-based preparative regimen and that fludarabine has a considerable neurotoxicity [26–32]. These findings suggest that fludarabine might have contributed to the development of CNS toxicity in this study. Except for 1 patient with leukoencephalopathy and hemophagocytic syndrome–related CNS complications, the other 10 patients had undergone UCBT. The incidence of CNS complications after RI-UCBT was 24%. Cord blood as a stem cell source was an independent risk factor in multivariate analysis (odds ratio, 14.5; 95% confidence interval, 3.7-56.9; $P < .0001$). Few studies on CNS complications after myeloablative UCBT have been reported. This complication is possibly characteristic of RI-UCBT. All 10 patients developed altered mental status, including 3 with generalized seizures. Brain imaging in 3 patients showed abnormal signals around the hippocampus, whereas images were normal in the other 6 patients. Hippocampal encephalopathy in the 3 patients involved both white and gray matter and was thus distinct from leukoencephalopathy. Similar findings after RI-UCBT have recently been reported [33]. Although an association with tacrolimus administration has been suggested, none of our patients received tacrolimus, thus indicating other causes. Possibilities include infection, regimen-related toxicity, and immune reaction associated with the use of cord blood. Eight patients who developed metabolic encephalopathy after RI-UCBT had received fludarabine, melphalan, and TBI as a preparative regimen.

This has a higher intensity than most reduced-intensity regimens and might have caused CNS toxicities.

Conversely, CNS complications do not represent a significant concern in bone marrow or peripheral blood transplantation with similar reduced-intensity regimens. Because adult RI-UCBT recipients receive a relatively low dose of $CD34^+$ cells, it would raise the concern that there might have been delayed engraftment, leading to an increase in subclinical or undetected CNS viral infections. However, this possibility seemed unlikely. In RI-UCBT with fludarabine, melphalan, and intermediate-dose TBI as a preparative regimen and cyclosporine as GVHD prophylaxis [34], the median day of neutrophil engraftment was 17.5 days. This is comparable to RIST with granulocyte colony-stimulating factor–mobilized blood [11,13]. Furthermore, neither cerebrospinal findings nor blood cultures identified CNS infection in our study, and no patient had GVHD at the onset of CNS complications. Because 4 of the 10 patients who underwent RI-UCBT died soon after the development of CNS complications, symptoms might represent an early manifestation of a systemic disorder predisposing for multiple organ dysfunction syndrome, increasing the risk of transplant-related mortality [35]. However, the association of CNS complications with engraftment is noteworthy in RI-UCBT. We did not use antithymocyte globulin or corticosteroids for preparative regimens or GVHD prophylaxis, respectively, although these practices have been commonly used in previous studies on UCBT [36]. Both agents display strong immunosuppressive properties. The fluid accumulation often observed during this period may have accentuated the tendency for brain edema to develop, as seen in patients with renal decompensation. In RI-UCBT with our regimens [34], the cumulative incidence of complete donor chimerism at day 60 was 93%, and the median time to complete donor chimerism was 22 days. Grade II to IV acute GVHD occurred in 27% of patients. Approximately 60% of RI-UCBT recipients had a noninfectious fever before engraftment (median onset, day 9). Manifestations included a high-grade fever, eruption, and diarrhea, and corticosteroids were effective for ameliorating these reactions. These findings suggest that they might be associated with a cytokine storm induced by massive proliferation of cells with a unique cytokine profile and that the CNS toxicity was attributable to these immune responses. We therefore treated the CNS toxicity with corticosteroids. Because CNS toxicity is associated with considerable morbidity and mortality, optimal preventive measures for CNS complications after RI-UCBT should be established. Intensification of GVHD prophylaxis, such as with methotrexate, might prove beneficial for this purpose.

This investigation was a retrospective study based on medical records. Pathologic examinations were not used in most patients, and diagnosis of CNS complications was established on the basis of clinical and radiologic findings. Mild neurotoxicity associated with allo-HSCT was likely neglected, and incidences might have been underestimated in this study. Compared with autopsy studies, approximately half of the patients with neurologic complications had been diagnosed during life [4]. Further prospective evaluation is warranted to clarify incidences and clinical characteristics for CNS complications after RIST and to establish optimal preventive and therapeutic measures.

In conclusion, we have demonstrated that CNS complications are a common and frequently fatal complication after RIST, particularly after the use of umbilical cord blood. Metabolic encephalopathy is the most common subtype of CNS complication after RIST, and it frequently manifests as limbic encephalopathy in RIST with umbilical cord blood.

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Featured Article

Successful Engraftment After Reduced-Intensity Umbilical Cord Blood Transplantation for Adult Patients with Advanced Hematological Diseases

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ABSTRACT

Purpose: **The purpose of this research was to evaluate the feasibility of reduced-intensity unrelated cord-blood transplantation (RI-UCBT) in adult patients with advanced hematological diseases.**

Experimental Design: **Thirty patients (median age, 58.5 years; range, 20–70 years) with advanced hematological diseases underwent RI-UCBT at Toranomon Hospital between September 2002 and August 2003. Preparative regimen composed of fludarabine 25 mg/m^2 on days −7 to −3, melphalan 80 mg/m^2 on day −2, and 4 Gy total body irradiation on day −1. Graft-*versus*-host disease prophylaxis was composed of cyclosporin alone.**

Results: **Twenty-six patients achieved primary neutrophil engraftment after a median of 17.5 days. Median infused total cell dose was 3.1 × 10^7/kg (range, 2.0–4.3 × 10^7/kg). Two transplant-related mortalities occurred within 28 days of transplant, and another 2 patients displayed primary graft failure. Cumulative incidence of complete donor chimerism at day 60 was 93%. Grade II-IV acute graft-*versus*-host disease occurred in 27% of patients, with median onset 36 days. Primary disease recurred in 3 patients, and transplant-related mortality within 100 days was 27%. Estimated 1-year overall survival was 32.7%. Excluding 7 patients with documented infection, 19 patients displayed noninfectious fever before engraftment (median onset, day 9). Manifestations included high-grade fever, eruption, and diarrhea. The symptoms responded well to corticosteroid treatments in 7 of 13 treated patients.**

Conclusion: **This study demonstrated the feasibility of RI-UCBT in adults.**

INTRODUCTION

Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) is a curative treatment for refractory hematological malignancies. The therapeutic benefits are attributable to myeloablative radiochemotherapy and graft-*versus*-leukemia effects (1), whereas the severe regimen-related toxicity (RRT; Ref. 2) limited allo-HSCT to young patients without comorbidities.

Reduced-intensity stem-cell transplantation (RIST) using a nonmyeloablative preparative regimen has been developed to decrease RRT, whereas preserving adequate antitumor effects (3–5). Different pioneering conditioning regimens for RIST have been investigated, such as those including purine analogs (3–6) and total body irradiation (TBI). Although RIST has been attempted in various diseases (5, 6), suitable preparative regimens with adequate immunosuppression have yet to be established.

Although allo-HSCT from an HLA-identical sibling is promising, only 30% of the patients have an HLA-identical sibling donor. The value of unrelated cord-blood transplantation (UCBT) was confirmed for pediatric patients (7, 8). It has seen recent application in adult patients (9). Whereas the potential graft-*versus*-leukemia effects by cord-blood (CB) without severe graft-*versus*-host disease (GVHD; Ref. 10) has been reported, current questions include whether CB provides a sufficient number of stem cells for adults and suitable graft-*versus*-leukemia effects.

Reduced-intensity (RI)-UCBT (11, 12) represents a promising treatment for advanced hematological malignancies. Wagner *et al.* (12) reported recently the feasibility of RI-UCBT for pediatric patients. However, the feasibility in adult patients remains unclear. We report 30 adult patients with advanced hematological diseases who underwent RI-UCBT after fludarabine, melphalan, and 4 Gy TBI since October 2003 at our institution.

PATIENTS AND METHODS

Study Patients and Donors. Thirty patients with hematological diseases underwent RI-UCBT at Toranomon Hospital between September 2002 and August 2003. All of the patients had hematological disorders that were incurable with conventional treatments and were considered inappropriate for conven-

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tional allo-HSCT due to the lack of an HLA-identical sibling or a suitable unrelated donor, age >50 years old and/or organ dysfunction (generally attributable to previous intense chemo- and/or radiotherapy).

All of the patients provided written informed consent in accordance with the requirements of the Institutional Review Board.

HLA Typing and Donor Matching. An unrelated donor was searched through the Japan Marrow Donation Program (13) for patients without an HLA-identical sibling donor. When no appropriate donor was identified, the Japan Cord Blood Bank Network (14) was searched. CB units, which were ≥4 of 6 HLA-antigen matched and contained at least 2×10^7 nucleated cells/kg of recipient body weight before freezing were used. CB units were not depleted of T lymphocytes.

Preparative Regimen. The preparative regimen was composed of fludarabine 25 mg/m^2 on days −7 to −3, melphalan 80 mg/m^2 on day −2, and 4 Gy TBI in 2 fractions on day −1.

Supportive Cares. All of the patients were managed in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis carinii* prophylaxis. Fluoroquinolone and fluconazole were administered for prophylaxis of bacterial and fungal infections, respectively. Prophylaxis of herpes virus infection with acyclovir was also given (15). Neutropenic fever was managed according to the guidelines (16, 17). Cytomegalovirus (CMV) pp65 antigenemia was monitored once a week. If positive results were identified, preemptive therapy with foscarnet was initiated. Hemoglobin and platelet counts were maintained at >7 g/dl and $>10 \times 10^9$/liter, respectively, with in-line filtered and irradiated blood transfusions.

Management of GVHD. GVHD was clinically diagnosed in combination with skin or gut biopsies after engraftment or attainment of 100% donor chimerism. Acute and chronic GVHD were graded according to the established criteria (18, 19).

GVHD prophylaxis was a continuous infusion of cyclosporin 3 mg/kg from day −1 until the patients tolerated oral administration. It was tapered off from day 100 until day 150. If grade II-IV acute GVHD developed, 1 mg/kg/day of prednisolone was added to cyclosporin and tapered from the beginning of clinical response.

Chimerism Analysis. Chimerism was assessed using fluorescent *in situ* hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, PCR for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10% (20).

Whole blood and CD3-positive cell chimerism was assessed at the time of granulocyte engraftment. When engraftment was delayed, chimerism was assessed on day 30. For those who died before engraftment, chimerism was assessed at least once during life.

Engraftment. Engraftment was defined as WBC counts $> 1.0 \times 10^9$/liter or absolute neutrophil counts $> 0.5 \times 10^9$/liter for 2 consecutive days. Granulocyte colony stimulating factor (Filgrastim) 300 μg/m^2/day was administered i.v. from day 1 until neutrophil engraftment.

Graft failure was defined as peripheral cytopenia and marrow hypoplasia occurring later than day 60, without detection of donor markers by cytogenetic and/or molecular techniques.

Table 1 Patient characteristics (n = 30)

Age (y), median (range)	58.5 (20–70)
Weight (kg), median (range)	52 (38–75)
Male/female	16/14
Diagnosis	
Malignancy	
Acute myeloid leukemia	14
Myelodisplastic syndrome	1
Acute lymphoblastic leukemia	3
Adult T-cell leukemia	5
Plasma cell leukemia	1
Chronic myeloid leukemia	1
Malignant lymphoma	1
Benign	
Severe aplastic anemia	4
Disease status at transplantation (malignancy)	
Remission	1
Refractory to previous chemotherapy	25

RRT and Transplantation-Related Mortality (TRM). RRT was defined as any nonhematological organ dysfunction from day 0 to day 28 and was graded according to the Bearman's criteria (2). TRM was defined as death without the primary disease progression.

Endpoints and Statistical Analysis. Primary end points were composed of the rates of durable engraftment and TRM within day 100. Secondary end points were the rates of RRT, acute and chronic GVHD, infections, event-free survival (EFS), and overall survival (OS).

Acute GVHD was analyzed for engrafted patients. Chronic GVHD was analyzed for patients who survived ≥100 days.

EFS was defined as the duration of survival after transplantation without disease progression, relapse, graft failure, or death. The probabilities of OS and EFS were shown by the Kaplan-Meier method as of January 31, 2004. Surviving patients were censored on the last day of follow-up. Cox regression analysis was used to determine the effect of various variables on OS.

RESULTS

Patient Characteristics. Median age was 58.5 years (range, 20–70 years), and median weight was 52 kg (range, 38–75 kg; Table 1). All of the patients were CMV-seropositive.

The malignancies of 25 patients were refractory to cytotoxic chemotherapies except acute myeloblastic leukemia (n = 1) in first CR. The remaining 4 patients had transfusion-dependent severe aplastic anemia.

CB Characteristics. Twenty-four and 6 patients received 4 of 6 and 5 of 6 HLA-antigen-matched CB, respectively. Twenty-one patient CB pairs were sex-mismatched. Median infused total nucleated cell dose and CD34-positive cell dose before freezing were 3.1×10^7/kg (range, $2.0–4.3 \times 10^7$/kg) and 0.74×10^5/kg (range, $0.17–2.5 \times 10^5$/kg), respectively.

Engraftment. Twenty-six patients [87%; 95% confidence interval (95% CI), 75–99%] achieved primary neutrophil engraftment, among whom median day of engraftment was 17.5 days (range, 10–54 days; Fig. 1). Their engraftment was durable

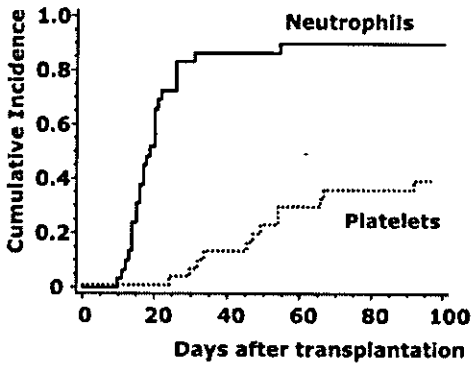

Fig. 1 Engraftment of neutrophils and platelets. Twenty-six (87%; 95% confidence interval, 75–99%) and 16 patients (40%; 95% confidence interval, 25–57%) achieved primary neutrophil and platelet engraftment, respectively.

Table 2 Neutrophil engraftment, chimerism, and overall survival

Neutrophil engraftment Variable	n	% (95% CI)[a]	*P*
Total cell dose			
≧3 × 10^7/kg	16	94% (82–100%)	
<3 × 10^7/kg	14	79% (57–100%)	0.25
HLA disparities			
HLA 5/6 match	6	67% (29–100%)	
HLA 4/6 match	24	92% (81–100%)	0.24
100% Donor chimerism			
Total cell dose			
≧3 × 10^7/kg	16	100%	
<3 × 10^7/kg	14	86% (67–100%)	0.63
HLA disparity			
HLA 5/6 match	6	83% (54–100%)	
HLA 4/6 match	24	96% (88–100%)	0.31
Overall survival			
Total cell dose			
≧3 × 10^7/kg	16	54% (24–83%)	
<3 × 10^7/kg	14	52% (6.6–87%)	0.70
HLA disparities			
HLA 5/6 match	6	63% (20–100%)	
HLA 4/6 match	24	51% (20–81%)	0.60

[a] CI, confidence interval.

without requiring readministration of Filgrastim. Two patients died of TRM within 28 days of transplant. Primary graft failure occurred in the remaining 2 patients, who underwent second RI-UCBT with the same preparative regimen and GVHD prophylaxis and achieved neutrophil engraftment and complete donor chimerism. No patients experienced a decrease in neutrophil <0.5 × 10^9/liter during the follow-up.

Platelet counts >20 × 10^9/liter were achieved by 16 patients (40%; 95% CI, 25–57%) on a median day of 39 days (range, 25–95 days). No other patient achieved platelet recovery until the last day of follow-up.

No significant association was found between neutrophil engraftment and either infused cell dose or HLA disparity (Table 2).

Chimerism Analysis. Chimerism data were obtained from all of the 30 patients. Cumulative incidence of complete donor chimerism at day 60 was 93% (95% CI, 84–100%), and median time to complete donor chimerism was 22 days (range, 13–56 days; Fig. 2). The 2 patients who died of TRM within 28 days had complete donor chimerism before neutrophil engraftment. All of the surviving patients were monitored for chimerism every 3 months, followed the cyclosporine tapering schedule from day 100 to day 150, and maintained complete donor chimerism during the follow-up even after the discontinuation of immunosuppressants.

No significant association was identified between complete donor chimerism and either infused cell dose or HLA disparity (Table 2).

RRT and TRM. Four patients (13%) developed grade III RRT. No patient had grade IV RRT. The most commonly involved organs were the gut and kidney (Table 3).

TRM within 100 days of RI-UCBT was 27%. Primary causes of death were interstitial pneumonitis (n = 2), acute GVHD (n = 2), gastrointestinal bleeding (n = 1), acute heart failure (n = 1), limbic encephalopathy (n = 1), and sepsis (n = 1).

GVHD. Grade II-IV and III-IV acute GVHD occurred in 27% (95% CI, 11–43%) and 23% (95% CI, 7.4–39%) of the patients, respectively. Median onset of grade II-IV acute GVHD was day 36 (range, day 17–66; Fig. 3).

Of the 13 patients who survived >100 days, 3 (23%) developed chronic GVHD.

Infection. Twelve patients developed infections: bacteremia (n = 8), invasive aspergillosis (n = 3), and pulmonary tuberculosis (n = 1). Nine of them had been treated with

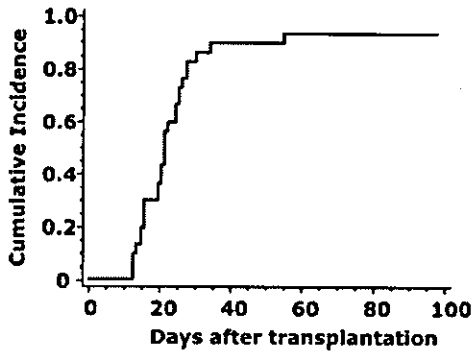

Fig. 2 Achievement of complete donor chimerism. Cumulative incidence of complete donor chimerism at day 60 after reduced-intensity unrelated cord-blood transplantation (*RI-UCBT*) was 93% (95% confidence interval, 84–100%), and median time to complete donor chimerism was day 22 (range, day 13–56).

Table 3 Regimen-related toxicity within 28 days (Bearman's score)

Score	Diarrhea	Kidney	CNS[a]	Liver	Lung
Grade 0	18	18	26	22	27
Grade 1	8	5	0	3	2
Grade 2	4	6	1	4	0
Grade 3	0	1	3	1	1
Grade 4	0	0	0	0	0

[a] CNS, central nervous system.

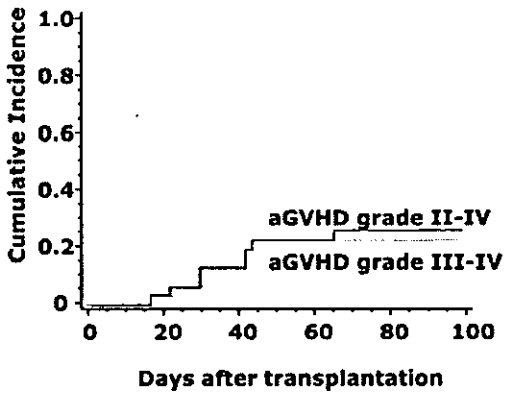

Fig. 3 Development of acute graft-*versus*-host disease (GVHD). Grade II-IV and III-IV acute GVHD developed in 27% (95% confidence interval, 11–43%) and 23% (95% confidence interval, 7.4–39%) of the patients, respectively. Median onsets of grade II-IV and III-IV acute GVHD were day 36 (range, day 17–66) and day 30 (range, day17–44), respectively.

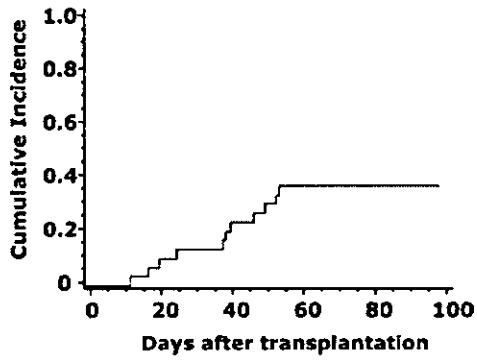

Fig. 4 Development of cytomegalovirus reactivation. Reactivation of cytomegalovirus was documented in 11 patients (37%) on a median of day 40 (range, day 13–55).

corticosteroids at the onset of infections. Reactivation of CMV was documented in 11 patients (37%) on a median of day 40 (range, day 13–55; Fig. 4). Eight of them had been treated with corticosteroids at the onset of CMV antigenemia. None of them developed CMV-related diseases. One patient developed hemorrhagic cystitis with adenovirus and BK virus infection.

Pre-Engraftment Noninfectious Fever. Seven patients with documented infection before engraftment were excluded from the analysis of pre-engraftment reaction (Table 4). Eighteen patients developed noninfectious fever before neutrophil engraftment (Fig. 5). Noninfectious high-grade fever often coexisted with eruption, diarrhea, and weight gain, starting on a median of day 9. Pathological examination of eruption from 8 patients revealed nonspecific inflammatory reactions and was not compatible with GVHD.

Survival. As of January 2004, a total of 11 patients remained alive. Median follow-up of the survivors and all of the enrolled patients were 238 days (range, 169–485) and 125 days (range, 26–485), respectively. Primary diseases recurred in 3 patients. Estimated 1-year OS and EFS were 32.7% (95% CI, 14.3–51.1%; Fig. 6) and 22.2% (95% CI, 5.9–38.5%; Fig. 7), respectively. Neither cell dose nor HLA disparity was associated with OS (Table 2).

DISCUSSION

Because CB contains a small amount of hematopoietic stem cells and stem cell boost or donor lymphocyte infusion is not available after UCBT, graft failure has been a major concern in adult UCBT. The present study demonstrated the feasibility of RI-UCBT for adult patients, in addition to pediatric patients (21). In this study, 26 of the 30 patients (87%) achieved durable engraftment, and 28 patients achieved complete donor chimerism by day 60, including 2 patients who died before engraftment. Interestingly, 4 patients with severe aplastic anemia, which has been associated with a high incidence of graft rejection (22), achieved complete chimerism after our reduced-intensity regimen. These findings suggest that the combination of fludarabine, melphalan, and low-dose TBI might be more immunosuppressive than conventional myeloablative regimens, creating niche for CB to engraft. Alternatively, CB may exert a strong graft-*versus*-host effect, making room for stable engraftment of stem cells.

Delayed hematopoietic recovery and infection during neutropenia are the significant concerns in adult UCBT. Laughlin *et*

Table 4 Characteristics of pre-engraftment reaction (n = 23)

Temperature	
38.0–38.9°C	2
39.0–39.9°C	10
≥40.0°C	7
Day of peak body temperature	9 (5–12)
Serum levels of CRP[a] (mg/dl)	13.8 (0.5–18.9)
Day of peak serum levels of CRP	10 (8–16)
Diarrhea	11
Eruption	10
Jaundice	5
Use of corticosteroid	13
Good response to corticosteroid	7

[a] CRP, C-reactive protein.

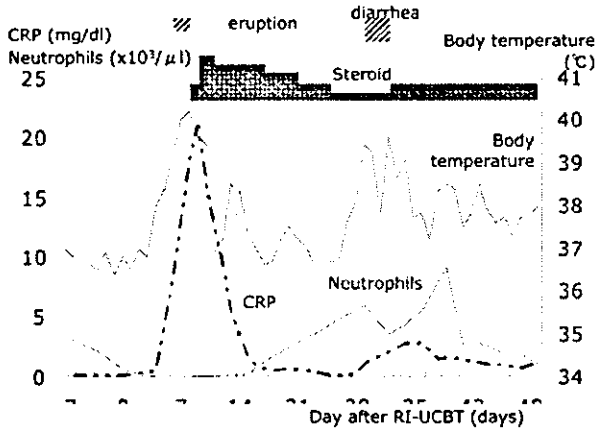

Fig. 5 Clinical course of a patient who developed pre-engraftment fever. Immune-reactions display two peaks, at around day 9 and day 18.

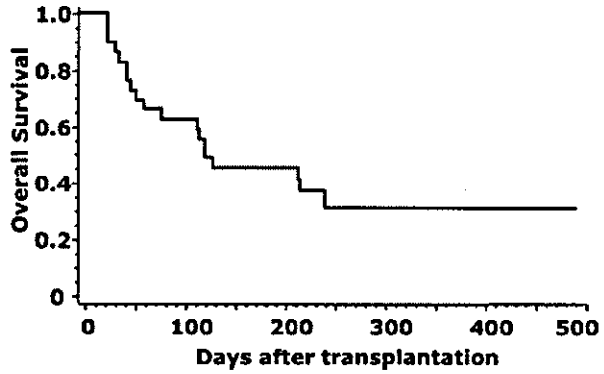

Fig. 6 Probability of overall survival after reduced-intensity unrelated cord-blood transplantation. Estimated 1-year overall survival was 32.7% (95% confidence interval, 14.3–51.1%).

al. (23) reported neutrophil recovery in 90% of patients by a median of 27 days after UCBT, which was significantly delayed compared with allo-HSCT. The delay has been attributed to the limited cell dose in the reports on myeloablative UCBT. The median nucleated cell dose in our study (3.1×10^7/kg) was greater than those in some reports from Western countries (2.1×10^7/kg; Ref. 9). The low median body weight (52 kg) in the Japanese population may favor neutrophil engraftment, whereas our results showed no association between the cell dose and engraftment in the small sample size. In the present study, median time to engraftment was 17.5 days (range, 10–54 days), which was much faster than that reported in previous studies on myeloablative UCBT (7–9). Our results were comparable with the report on adult RI-UCBT by Barker *et al.* (21). Their results showed neutrophil engraftment on a median of 26 days after busulfan/fludarabine/TBI 2 Gy and 9.5 days after cyclophosphamide/fludarabine/TBI 2 Gy. Whereas the reason for the difference remains unclear, these findings suggest that fludarabine-based reduced-intensity regimens enable rapid and stable engraftment.

TRM within 100 days was 27% in this study, which is lower than those reported on myeloablative UCBT (Refs. 7, 9, 24; 32–51% in pediatric patients and 56–63% in adults). Given the relatively old age (median, 58.5 years) and advanced stages of the primary diseases, our reduced-intensity preparative regimen probably decreased TRM. Our TRM within 100 days is comparable with that of 28% in adult RI-UCBT by Barker *et al* (21).

All of the patients tolerated our preparative regimen without grade IV RRT (Bearman's criteria; Ref. 2). Four patients developed grade III RRT with common involvements of the gut, kidney, and liver (Table 3). We used melphalan, which has dose-limiting toxicities of the gut and liver (25). These remained mild without hepatic veno-occlusive disease. Because renal toxicities of fludarabine, busulfan, and TBI 4 Gy are reportedly minimal, the high incidence of renal toxicity might be attributable to concomitant administration of nephrotoxic agents such as cyclosporin and antibiotics. Elderly patients might be susceptible to RRT. We plan to investigate optimal dosages of cyclosporin in RIST for elderly patients. Because TBI, even at a low dose, sometimes causes significant late toxicities in the lung (22), long-term follow-up is required.

Little information on GVHD after RI-UCBT is available. In the present study, the incidences of grade II-IV and III-IV acute GVHD and chronic GVHD were 27%, 23%, and 23%, respectively, whereas some reported those to be 33–44%, 11–22%, and 0–25%, respectively, in myeloablative UCBT (7, 8, 26). There are no significant differences in the incidences of GVHD between myeloablative UCBT and RI-UCBT. This is similar to the GVHD incidences in myeloablative allo-HSCT and RIST (27). Median onset of acute GVHD was 36 days (range, 17–66 days) in the present study, which was comparable with that of myeloablative UCBT (7, 8, 26). In contrast, the achievement of complete donor chimerism and the onset of acute GVHD are delayed in RIST compared with myeloablative allo-HSCT (27, 28). CB might have a potential of intense graft-*versus*-host effect, allowing niche for early engraftment. The characteristics of GVHD after RI-UCBT remain to be investigated, including different organ involvements and response to immunosuppressive treatment.

Interestingly, 20 patients developed inflammatory reactions before engraftment (Table 4). These reactions included noninfectious high-grade fever, eruption, diarrhea, and jaundice, starting on a median of day 9. Because the reactions preceded engraftment (median, day 17.5), we speculated that some form of immune reaction that is not categorized as acute GVHD occurs after RI-UCBT without achieving engraftment. The pre-engraftment fever has been reported on rare occasions in previous reports of UCBT and might be similar to those observed after haploidentical transplantations. Antithymocyte globulin and corticosteroids, which have strong immunosuppressive properties, were commonly used in previous studies on UCBT (9), whereas neither was used in the present study. Immunosuppressive treatment with corticosteroids was effective for the pre-engraftment fever. These findings support that immune-mediated reactions after UCBT might manifest easily with the present regimen. The doubling time of cultured CB $CD34^+$ cells is 7–10 days, which is several hundred-fold faster than that of cultured adult marrow cells (29). Mononuclear cells from CB display a unique cytokine profile such as comparable levels of

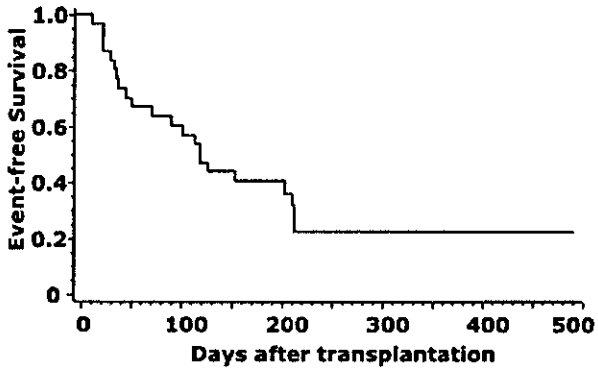

Fig. 7 Probability of event-free survival after reduced-intensity unrelated cord-blood transplantation. Estimated 1-year event-free survival was 22.2% (95% confidence interval, 5.9–38.5%).

interleukin (IL) 2, IL-6, and tumor necrosis factor α, reduced levels of IFN-γ and IL-10, and complete absence of IL-4 and IL-5 (30, 31). Pre-engraftment fever is possibly attributable to a cytokine storm induced by massive proliferation of cells with a unique cytokine profile. Another possibility is homeostasis-driven proliferation of naive T cells in highly immunosuppressed individuals, as demonstrated in murine models (32, 33). This reaction is reportedly associated with cytotoxic cytokines (32, 33). Fever as a transient response to contamination with maternal blood or cells during CB collection cannot be excluded (34). Reactivation of human herpesvirus 6 might be associated with this complication (35). If pre-engraftment fever exerts some antitumor effects, it is reasonable that patients with advanced and chemorefractory hematological diseases achieved long-term remission after RI-UCBT in the present study.

Infection is a common and significant problem in myeloablative UCBT (8, 9, 24), but little is known in RI-UCBT. The present study demonstrated that infection is also problematic in RI-UCBT. Twelve patients developed infection in this study, 9 of whom had been on corticosteroid therapy. Eight of 11 patients with CMV antigenemia had received corticosteroids. Delayed immunological reconstitution with or without GVHD, pre-engraftment fever, and corticosteroids may be risk factors for infection. Appropriate management of GVHD and pre-engraftment fever warrants additional investigation.

One-year OS was 35% in the present study, showing that some patients with advanced hematological malignancies can achieve durable remission after RI-UCBT. Contrary to our prediction, primary diseases recurred only in 3 patients. The candidates for RI-UCBT have extremely poor prognosis with conventional salvage chemotherapy. These findings suggest that RI-UCBT exerts strong antitumor activity and is promising for patients with refractory hematological malignancies without an HLA-identical sibling or an unrelated donor. In contrast, it is premature to apply RI-UCBT to low-risk diseases.

In conclusion, our study demonstrated the feasibility of RI-UCBT for adult patients with advanced hematological diseases, although the limitations included the small sample size and short follow-up. If CB is feasible for adults as an alternative stem cell source, RI-UCBT may become the choice of treatment for patients with advanced hematological diseases that are incurable with conventional treatments. RI-UCBT is particularly appealing for patients who require urgent treatments. Although RI-UCBT is currently associated with a high TRM, this study provided a rationale for continuing our clinical trials. Additional investigations need to focus on minimizing adverse effects including RRT, GVHD, and pre-engraftment immune reactions, whereas preserving graft-*versus*-leukemia effects.

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