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

Yukiko Kishi,[1] Shigesaburo Miyakoshi,[2] Masahiro Kami,[1] Masayuki Ikeda,[3] Yuta Katayama,[1] Naoko Murashige,[1] Eiji Kusumi,[2] Koichiro Yuji,[2] Kazuhiko Kobayashi,[4] Daisuke Kato,[2] Tamae Hamaki,[1] Tomoko Matsumura,[2] Sung-Won Kim,[1] Shinichi Morinaga,[2] Shinichiro Mori,[1] Mineo Kanemaru,[5] Tatsuyuki Hayashi,[6] Yoichi Takaue,[1] Shuichi Taniguchi,[2] for the Tokyo Stem Cell Transplantation Consortium

[1]Hematopoietic Stem-Cell Transplantation Unit, the National Cancer Center Hospital, Tokyo, Japan; [2]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [3]Department of Neurology, the National Center Hospital for Mental, Nervous and Muscular Disorders, Tokyo, Japan; [4]Department of Hematology, JR Tokyo General Hospital, Tokyo, Japan; [5]Department of Internal Medicine, Higashijyujyo Hospital, Tokyo, Japan; [6]Department of Internal Medicine, Tokyo Metropolitan Police Hospital, Tokyo, Japan

Correspondence and reprint requests: Masahiro Kami, MD, Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan (e-mail: mkami@ncc.go.jp).

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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-

Y.K. and S.M. contributed equally to this article.

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)*	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 syndrome	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.
*Continuous infusion of cyclosporin was given at target levels of 250-350 ng/mL.

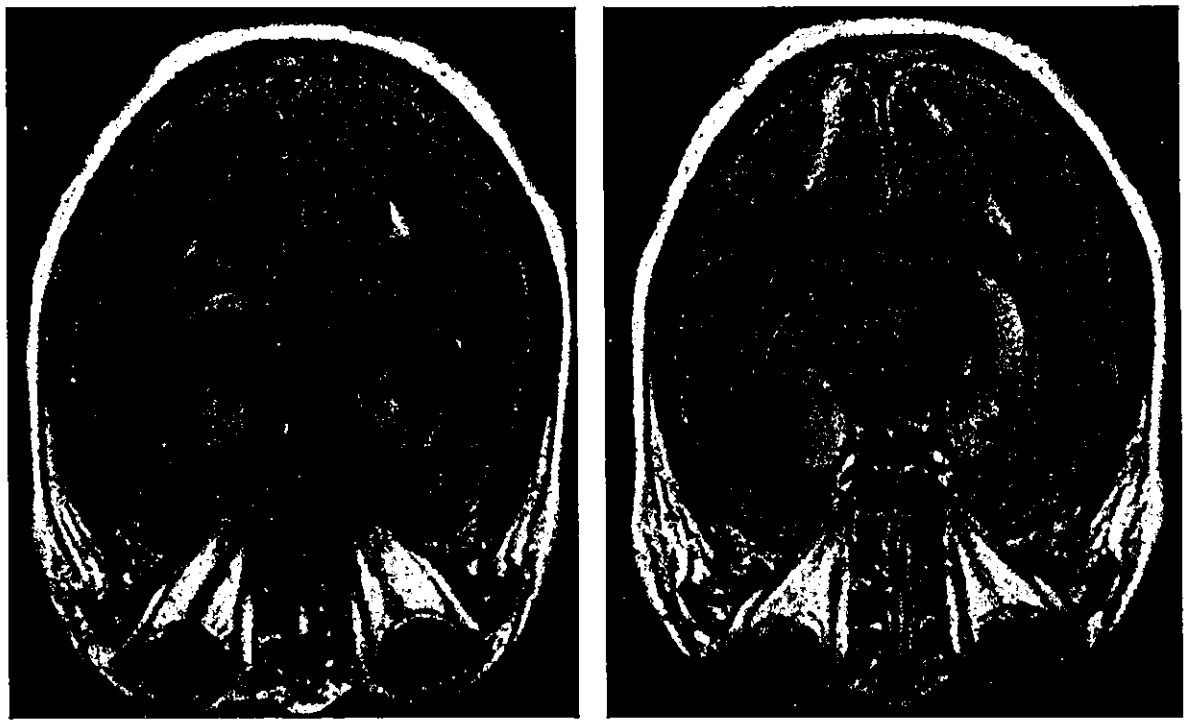

Figure 1. T2-weighted magnetic resonance image of the brain showing high-intensity signals in bilateral temporal lobes. The patient was diagnosed with limbic encephalopathy.

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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Regimen-related toxicity following reduced-intensity stem-cell transplantation (RIST): comparison between Seattle criteria and National Cancer Center Common Toxicity Criteria (NCI-CTC) version 2.0

M Sakiyama[1], M Kami[1], A Hori[1], O Imataki[1], T Hamaki[1], N Murashige[1], K Kobayashi[1], Y Kishi[1], R Kojima[1], S-W Kim[1], E Kusumi[2],K Yuji[2], S Miyakoshi[2], S Mori[1], R Tanosaki[1], S Taniguchi[2] and Y Takaue[1], for the Tokyo SCT Consortium

[1]*Hematopoietic Stem Cell Transplantation Unit, The National Cancer Center Hospital, Tokyo, Japan; and* [2]*Department of Hematology, Toranomon Hospital, Tokyo, Japan*

Summary:

Acute regimen-related toxicity (RRT) is minimal in reduced-intensity stem-cell transplantation (RIST). However, the Seattle RRT grading (Bearman *et al*), developed in the context of conventional-intensity transplantation, is frequently applied to RIST. We compared the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 with the Seattle criteria after RIST in 86 patients. RRT within 30 days of transplant graded by both sets of criteria were significantly associated with the outcome confirming the predictive value of both the systems. A total of 15 patients died of disease progression, and 12 of transplant-related mortality: RRT ($n=2$), graft-versus-host disease (GVHD) ($n=7$), infection ($n=1$), and others ($n=2$). GVHD-related deaths primarily resulted from infections after steroid treatment ($n=6$) and bronchiolitis obliterans ($n=1$). This study shows that NCI-CTC is appropriate in toxicity evaluation of RIST, and that its application to RIST enables a toxicity comparison between RIST and other types of cancer treatments. Since GVHD is a significant problem in RIST, modifications are required to evaluate immunological complications following RIST.

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Reduced-intensity stem-cell transplantation (RIST) is associated with lower acute regimen-related toxicity (RRT).[1–6] The recognition and grading of toxicity caused by RIST is important in practice and in designing clinical trials.

After conventional-intensity stem-cell transplantation (CIST), patients can die of disease progression or complications of therapy. RRT is toxicity that is directly attributable to the conditioning regimen, but usually excludes graft-versus-host disease (GVHD), infection, and hemorrhage. It is often difficult to separate RRT from other toxicities. The Seattle group proposed a toxicity grading system specifically for allogeneic HSCT based upon a retrospective review of 195 patients who underwent CIST.[7] RRT in RIST is minimal and a significant proportion of morbidity and mortality is secondary to GVHD.[8] However, the Seattle criteria have been used to evaluate RIST.

The National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC ver. 2.0) has been widely used for development and evaluation of chemotherapeutic agents. If NCI-CTC ver. 2.0 can be applied to RIST, toxicity comparison between RIST and various other cancer treatments would be possible. We studied 86 patients who underwent RIST to see if NCI-CTC ver. 2.0 could be used to predict transplant-related mortality (TRM) and overall survival (OS) after RIST.

Patients and methods

Patients

The medical records of all of the patients ($n=86$) who underwent RIST at the National Cancer Center Hospital between January 1999 and April 2002 were reviewed. All patients and donors gave their written informed consent in accordance with the requirements of the Institutional Review Board of the National Cancer Center Hospital.

The median age was 51 years (range, 4–67). The underlying disease was AML ($n=26$), lymphoma ($n=21$), MDS ($n=11$), CML ($n=5$), ALL ($n=2$), other hematologic diseases ($n=3$), and solid tumors ($n=18$). The hematological malignancies were refractory to chemotherapy in 33 cases, and were in remission or sensitive to

Correspondence: Dr M Kami, Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; E-mail: mkami@ncc.go.jp
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treatment in the remaining 35 cases. All of the patients with solid tumors were refractory to conventional treatments.

Preparative regimens

The preparative regimens comprised busulfan 4 mg/kg daily for 2 days with fludarabine 25 mg/kg daily for 6 days ($n = 64$)[9] or cladribine 0.11 mg/kg daily for 6 days ($n = 22$).[5] Rabbit antithymocyte globulin (ATG, Thymoglobulin, IMTIX-SANGSTAT, Lyons, France) 2.5 mg/kg for 2 or 4 days and TBI (4 Gy) were added to the preparative regimen in 49 and three patients, respectively.

Stem-cell source

A total of 64 patients had an HLA-identical related donor and 17 had a one-locus mismatched related donor.[10] Peripheral blood was used for these 81 patients. Five patients received bone marrow from a matched unrelated donor (MUD).

Prophylaxis and treatment of GVHD

Patients who were transplanted from an HLA-identical related donor received cyclosporin alone (3 mg/kg). Those who were transplanted from an HLA-mismatched related donor or MUD received cyclosporin and short-course methotrexate.

The diagnosis of GVHD was made on clinical grounds in conjunction with biopsy of the skin and digestive tract. Acute and chronic GVHD were graded according to the consensus criteria.[11,12] Grade II–IV acute GVHD was treated with 2 mg/kg/day of methylprednisolone in addition to cyclosporin.

Management of infections

All of the patients stayed in reverse isolation in a laminar airflow-equipped room, and received prophylaxis with trimethoprim/sulfamethoxazole or pentamidine inhaler, ciprofloxacin, and fluconazole against *Pneumocystis carinii*, bacterial, and fungal infection, respectively. Herpes virus prophylaxis with acyclovir was also given as previously described.[13] CMV pp65 antigenemia was routinely monitored once a week. When antigenemia was detected, preemptive therapy with ganciclovir was initiated as previously reported.[14]

Toxicity grading

The Seattle criteria assess post transplant RRT in eight organs: the heart, bladder, kidneys, lungs, liver, mucosa, central nervous system (CNS), and gut. As the criteria exclusively assess RRT, they exclude adverse events attributable to GVHD and infection. Similarly, renal failure is excluded when it coincides with the administration of known nephrotoxic agents. RRT was graded with the Seattle criteria on the day of initiation of conditioning regimens and days 0, 7, 14, and 28 (and on day 100 for lungs) post transplant (Table 1).

NCI-CTC ver. 2.0 assesses more than 260 adverse events in 24 organ systems. To make a comparison, 16 items in NCI-CTC ver. 2.0 equivalent to those in the Seattle criteria were regraded. These included arrhythmia, cardiovascular dysfunction, hematuria, renal dysfunction/creatinine levels, lung toxicity, serum levels of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), weight gain/ascites, neurological dysfunction, mucositis, and diarrhea (Table 2). All of the observed adverse events were evaluated daily with NCI-CTC ver. 2.0 from the day of initiation of conditioning regimens until day 30 post transplant (and on day 100 for lungs).

Two or more independent physicians graded RRT based on the medical records. If there was discordance between their diagnoses, another physician (MK) made a final diagnosis.

Statistical analysis

The probability of OS was determined with the Kaplan–Meier method as of June 31, 2002. The median follow-up period after transplantation was 252 days (range, 82–1046 days). Surviving patients were censored on the last day of follow-up.

A univariate analysis using the χ^2 test and Mann–Whitney test were performed to identify the risk factors for RRT. A multivariable Cox proportional-hazards analysis was conducted to determine whether the development of RRT was independent of other clinical variables in predicting overall mortality. In RIST for solid tumors and malignant lymphoma, some lesions persist following preparative regimens. Sizes of these lesions frequently show a transient increase until the development of alloimmune responses. When patients die without disease progression, it is difficult to determine whether these deaths are attributable to disease progression or TRM. We therefore used overall mortality instead of nonrelapse deaths.

Clinical variables examined in a univariate analysis were entered in a backward, stepwise Cox proportional-hazards model to identify predictors of mortality. Variables with a P-value of less than 0.50 were entered into the model, and those with a P-value of less than 0.10 were retained. The P-values less than 0.05 were considered to be significant.

Results

Toxicity grading

Grade 3–4 toxicity by the Seattle criteria was observed in the lung (5%), CNS (2%), kidney (1%), and heart (1%) (Table 3). The maximal toxicity of grades 0, 1, 2, 3, and 4 was noted in eight (9%), 38 (44%), 35 (41%), four (5%), and one patient (1%), respectively.

Grade 3–4 toxicity by NCI-CTC ver. 2.0 was observed in all of the organs (Table 4): liver (31%), lung (21%), stomatitis (13%), gastrointestinal tract (9%), heart (6%), CNS (6%), kidney (2%), and bladder (1%). The maximal toxicity of grades 0, 1, 2, 3, and 4 was observed in two

Table 1 Regimen-related toxicity according to the Seattle criteria

Toxicity	*Grade 1*	*Grade 2*	*Grade 3*
Heart	Mild electrocardiogram abnormality, not requiring medical intervention; or noted heart enlargement on CXR with no clinical symptoms	Moderate electrocardiogram abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics	Severe electrocardiogram abnormalities with no or only partial response to medical intervention; or heart failure with no or only minor response to medical intervention: or decrease in voltage by more than 50%
Bladder	Macroscopic hematuria after 2 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days with subjective symptoms of cystitis not caused by infection	Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedures
Kidney	Increase in creatinine up to twice the baseline value	Increase in creatinine above twice baseline but not requiring dialysis	Requirement of dialysis
Lung	Dyspnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	CXR with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of PO_2 (>10% from baseline) but not requiring mechanical ventilation or >50% O_2 on mask and not caused by infection	Interstitial changes requiring mechanical ventilatory support or >50% oxygen on mask and not caused by infection or CHF
Liver	Mild hepatic dysfunction with 2.0 mg/dl <bilirubin <6.0 mg/dl or weight gain >2.5% and <5% from baseline, of noncardiac origin; or serum AST increase more than two-fold but less than five-fold from lowest preconditioning	Moderate hepatic dysfunction with bilirubin >6 mg/dl <20 mg/dl; or serum AST increase >five-fold from preconditioning; or clinical ascites or image-documented ascites >100 ml; or weight gain >5% from baseline of noncardiac origin	Severe hepatic dysfunction with bilirubin >20 mg/dl; or hepatic encephalopathy; or ascites compromising respiratory function
CNS	Somnolence but the patient is easily arousable and oriented after arousal	Somnolence with confusion after arousal; or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection	Seizures or coma not explained (documented) by other medication, CNS infection, or bleeding
Stomatitis	Pain and/or ulceration not requiring a continuous i.v. narcotic drug	Pain and/or ulceration requiring a continuous i.v. narcotic drug (morphine drip)	Severe ulceration and/or mucositis requiring preventive intubation; or resulting in documented aspiration pneumonia with or without intubation
GI toxicity	Watery stools >500 ml but <2000 ml every day not related to infection	Watery stools >2000 ml every day not related to infection; or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection	Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion

Grade IV regimen-related toxicity is defined as fatal toxicity.
CXR = chest X ray; i.v. = intravenous; CNS = central nervous system; GI = gastrointestinal; CHF = congestive heart failure.

Table 2 Regimen-related toxicity according to NCI-CTC version 2.0

Toxicity		*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*
Heart	Arrhythmia	Asymptomatic, not requiring treatment	Symptomatic, but not requiring treatment	Symptomatic and requiring treatment	Life-threatening (eg, arrhythmia associated with CHF, hypotension, syncope, shock)
	Ischemia/infarction	Nonspecific T-wave flattening or changes	Asymptomatic, ST- and T-wave changes suggesting ischemia	Angina without evidence of infarction	Acute myocardial infarction
	Left ventricular function	Asymptomatic decline of resting ejection fraction of >10% but <20% of baseline value; shortening fraction >24% but <30%	Asymptomatic but resting ejection fraction below LLN for laboratory or decline of resting ejection fraction >20% of baseline value; <24% shortening fraction	CHF responsive to treatment	Severe or refractory CHF or requiring intubation
Bladder	Hematuria	Microscopic only	Intermittent gross bleeding, no clots	Persistent gross bleeding or clots; may require catheterization or instrumentation, or transfusion	Open surgery or necrosis or deep bladder ulceration
Kidney	Renal dysfunction	Not defined	Not defined	Requiring dialysis, but reversible	Requiring dialysis and irreversible
	Creatinine levels	>ULN–1.5 × ULN	>1.5–3.0 × ULN	>3.0–6.0 × ULN	>6.0 × ULN
Lung	Hypoxia	Not defined	Decreased O_2 saturation with exercise	Decreased O_2 saturation at rest, requiring supplemental oxygen	Decreased O_2 saturation, requiring pressure support (CPAP) or assisted ventilation
	Pneumonitis/pulmonary infiltrates	Radiographic changes but asymptomatic or symptoms not requiring steroids	Radiographic changes and requiring steroids or diuretics	Radiographic changes and requiring oxygen	Radiographic changes and requiring assisted ventilation
Liver	Bilirubin	>ULN–1.5 × ULN	>1.5–3.0 × ULN	>3.0–10.0 × ULN	>10.0 × ULN
	Aspartate aminotransferase (AST)	>ULN–2.5 × ULN	>2.5–5.0 × ULN	>5.0–20.0 × ULN	>20.0 × ULN
	Alanine aminotransferase (ALT)	>ULN–2.5 × ULN	>2.5–5.0 × ULN	>5.0–20.0 × ULN	>20.0 × ULN
	Alkaline phosphatase (ALP)	>ULN–2.5 × ULN	>2.5–5.0 × ULN	>5.0–20.0 × ULN	>20.0 × ULN
	Weight gain/asites	2–5%	5–10%	⩾10% or as ascites	⩾10% or fluid retention resulting in pulmonary failure
CNS	Depressed level of consciousness	Somnolence or sedation not interfering with function	Somnolence or sedation interfering with function, but not interfering with activities of daily living	Obtundation or stupor; difficult; interfering with activities of daily living	Coma
Stomatitis	Stomatitis/pharyngitis	Painless ulcers, erythema, or mild soreness in the absence of lesions	Painful erythema, edema or ulcers but can swallow	Painful erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia
GI toxicity	Diarrhea	Increase of <4 stools/day over pretreatment	Increase of 4–6 stools/day, or nocturnal stools	Increase of ⩾7 stools/day or incontinence; or need for parenteral support for dehydration	Physiologic consequences requiring intensive care; or hemodynamic collapse

ULN = upper limit of normal values; LLN = lower limit of normal value; WNL = within normal limits; CHF = congestive heart failure; GI = gastrointestinal.

Table 3 Toxicity grading using the Seattle criteria in 86 patients undergoing RIST

Grade	*0*	*1*	*2*	*3*	*4*
Gut	69	17	0	0	0
Stomatitis	47	29	10	0	0
Central nervous system	78	4	2	2	0
Liver	23	31	31	1	0
Lung	75	4	3	3	1
Kidney	51	31	3	1	0
Bladder	84	2	0	0	0
Heart	70	8	7	1	0
Maximal grades	8	38	35	4	1

Table 4 Toxicity grading using NCI-CTC ver. 2.0 in 86 patients undergoing RIST

Grades	*0*	*1*	*2*	*3*	*4*
Gut	50	18	10	7	1
Stomatitis	35	8	32	1	0
Central nervous system	74	5	2	2	3
Liver	5	27	27	22	5
Lung	56	12	0	17	1
Kidney	47	26	11	2	0
Bladder	61	23	1	1	0
Heart	72	6	3	3	2
Maximal grades	2	16	25	35	8

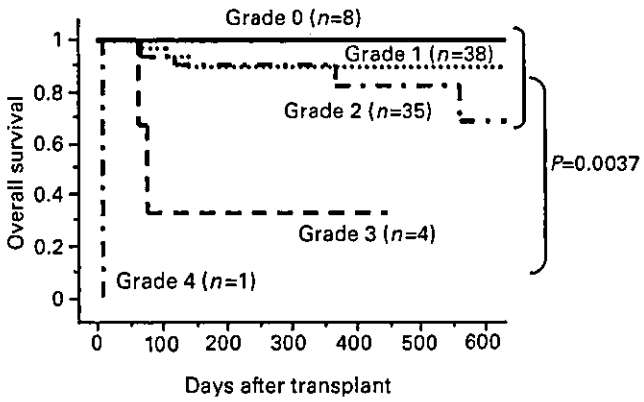

Figure 1 Overall survival evaluated by the Seattle criteria. Five patients had grade 3–4 toxicity in at least one organ, of whom three died (60%). The estimated 1-year OS was 25.0% (95% confidence interval (CI) 0.0–61.7%). In contrast, of the 81 patients with grade 0–2 toxicity in all the organ systems, eight died (9.9%). The estimated 1-year OS was 74.2% (95% CI, 64.2–84.2%). The one-year OS was significantly lower in the patients with grade 3–4 toxicity ($P = 0.0037$).

(2%), 16 (19%), 25 (29%), 35 (41%), and eight patients (9%), respectively.

The lung toxicity on day 100 using either the Seattle criteria or NCI-CTC ver. 2.0 was not maximal in any of the 86 patients.

There were 10 cases of the 'up-staging' of the toxicity from the Seattle criteria to NCI-CTC version 2.0: liver ($n = 6$), and kidney ($n = 4$).

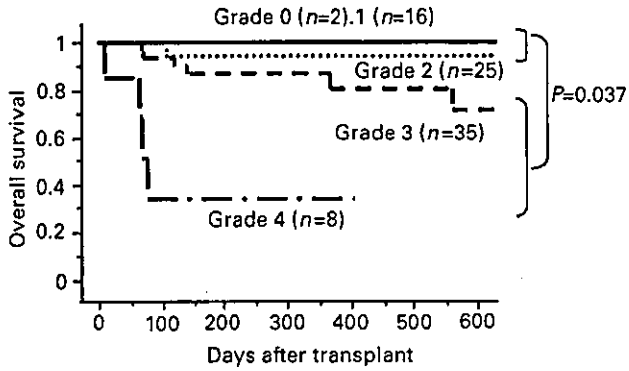

Figure 2 Overall survival evaluated by NCI-CTC version 2.0. In total, 43 patients had grade 3–4 toxicity in at least one organ, of whom 10 patients (23.2%) died of TRM. The estimated 1-year OS was 64.7% (95% CI, 50.2–79.2%). In contrast, of the remaining 43 patients with grade 2 toxicity in all the organ systems, one died of GVHD, resulting in 78.5% (95% CI, 64.8–92.2%) of estimated 1-year OS and 8.8% of TRM. The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P = 0.037$).

Table 5 Variables influencing the grades of regimen-related toxicity according to NCI-CTC ver. 2.0

Variables	*Grade 0–2 (n = 43)*	*Grade 3–4 (n = 43)*	*P-value*
Age			
Median (range)	53 (4–65)	50 (19–67)	0.459
Sex			
Male/female	26/17	31/12	0.362
Risk of primary diseases			
High/low	13/30	20/23	0.183
Preparative regimens			
Fludarabine-based/cladribine-based	34/9	30/13	0.459
ATG-containing yes/no	22/21	27/16	0.384
TBI-containing yes/no	1/42	2/41	0.999
GVHD prophylaxis			
Cyclosporine alone/ cyclosporine and methotrexate	38/5	32/11	0.165
Donors			
Related/unrelated	41/2	40/3	0.999
Matched/mismatched	37/6	32/11	0.278

ATG = anti-thymocyte globulin, TBI = total body irradiation, GVHD = graft-versus-host disease.
Any variables were significant on multivariate analysis.

Association between toxicity grading and survival following RIST

A total of 27 patients died: 16 of disease progression (19%) and 11 of TRM (13%). The 11 patients who died of TRM had the maximal toxicity of grade 2 ($n = 1$), grade 3 ($n = 6$), and grade 4 ($n = 4$) by NCI-CTC ver. 2.0, which was also graded with the Seattle criteria to be grade 1 ($n = 3$), grade 2 ($n = 5$), grade 3 ($n = 2$), and grade 4 ($n = 1$). The causes of TRM were GVHD/steroid-related infection ($n = 6$), GVHD (bronchiolitis obliterans) ($n = 1$), infection ($n = 2$), and

Table 6 Regimen-related toxicities (RRT) in studies reported previously

Authors/Reference	*n*	*Preparative regimens*	*Age years*	*Primary diseases*	*GVHD prophylaxis*	*RRT*				*Transplant-related mortality (TRM)*
						Criteria	*III–IV*	*III*	*IV*	
Carella *et al*[15]	15	Flu/CY[a]	34 (19–60)	HD, NHL	CSP/MTX	Details not described				Not described
Childs *et al*[3]	19	Flu/CY[b]	48 (37–65)	RCC	CSP	Details not described				2/19 (11%)
Giralt *et al*[16]	86	Flu/Mel[c] ($n=78$)	52 (22–70)	AML/MDS, CML, ALL/ lymphoma	FK506/MTX	Bearman's criteria	19	13	6	38.0%
		Clad/Mel[d] ($=8$)					12	5	7	88.0%
Khouri *et al*[4]	6	Flu/CY[a]	62 (51–71)	CLL	FK506/MTX	Details not described	0%	0%	0%	
	4	PFA[e]	55 (47–61)	Intermediate-grade lymphoma or in Richter's transformation						
	5	Flu/CY[f]	50 (47–57)	Low-grade lymphoma						
Khouri *et al*[17]	20	Flu/CY[a] or Flu/CY[f]	51 (31–68)	Follicular lymphoma	FK506/MTX	Bearman's criteria	0%[g]	0%	0%	3/20 (15%)
Slavin *et al*[2]	26	Flu/BU/ATG	33.5 (1–61)	Acute leukemia, chronic leukemia, NHL, MDS, MM, and genetic diseases	CSP	WHO criteria				4/26 (15%)
Nagler *et al*[18]	23	Flu/BU/ATG	41 (13–63)	HD, NHL	CSP	Bearman's criteria	17%	13%	4%	7/23 (30%)
McSweeney *et al*[6]	45	TBI 200 cGy	56 (31–71)	ALL, AML, CLL, CML, HD, MM, NHL, WM, CLL, MDS	CSP/MMF	Details not described[i]	0%	0%	0%	3/56 (5%)[h]
This study	86	Clad or Flu/ BU ± ATG[j]	51 (4–67)	AML, MDS, CML, ALL, lymphoma, TLBL, ATL, solid tumor	CSP or CSP/ MTX	Bearman's criteria	6%	5%	1%	11/86 (13%)
						NCI-CTC ver. 2.0	50%	41%	9%	

HD = Hodgkin's lymphoma; NHL = non-Hodgkin's lymphoma; RCC = renal cell carcinoma; AML = acute myeloid leukemia; MDS = myelodysplastic syndrome; CML = chronic mylogenous leukemia; ALL = acute lymphocytic leukemia; CLL = chronic lymphocytic leukemia, MM = multiple myeloma; WM = Waldenstrome's macroglobulinemia.

[a]Flu/CY: fludarabine 30 mg/m^2 with cyclophosphamide 300 mg/m^2 daily for 3 days.

[b]Flu/CY: fludarabine 25 mg/m^2 given daily for 5 days and cyclophosphamide 60 mg/kg for 2 days.

[c]Flu/Mel: fludarabine 25 mg/m^2 given daily for 5 days and melphalan 90 mg/m^2 or 70 mg/m^2 for 2 days.

[d]Clad/Mel: cladribine 12 mg/m^2 given daily for 5 days and melphalan 90 mg/m^2 or 70 mg/m^2 for 2 days.

[e]PFA: cisplatin 25 mg/m^2 daily for 4 days; fludarabine 30 mg/m^2; and cytarabine 500 mg/m^2 daily for 2 days.

[f]Flu/CY: fludarabine 30 mg/m^2 given daily for 5 days and cyclophosphamide 1000 mg/m^2 for 2 days.

[g]Moderate VOD was observed in two and was severe in two subjects.

[h]Four with disease progression died from DLI-induced GVHD and infections and three died of transplantation complications without disease progression.

[i]No patient experienced regimen-related painful mucositis, severe nausea and vomiting, pulmonary toxicity, cardiac toxicity, hemorrhagic cystitis, or new-onset alopecia.

[j]Flu/BU/ATG: fludarabine 30 mg/m^2 for 6 consecutive days, oral busulfan 4 mg/kg/day for 2 days, and anti-T-lymphocyte globulin 10 mg/kg/day for 4 days.

others ($n=2$). Maximal grades of GVHD in fatal cases were IV ($n=1$), III ($n=1$), II ($n=3$), and I ($n=2$).

Figure 1 demonstrates the association between the maximal toxicity of the Seattle criteria and OS. Five patients had grade 3–4 toxicity in at least one organ, of whom three died (60%). Estimated 1-year OS was 25.0% (95% confidence interval (CI) 0.0–61.7%). In contrast, of the 81 patients with grade 0–2 toxicity in all the organ systems, eight died (9.9%). The estimated 1-year OS was 74.2% (95% CI, 64.2–84.2%). The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P=0.0037$).

Figure 2 demonstrates the association between the maximal toxicity of NCI-CTC ver. 2.0 and OS. In all, 43 patients had grade 3–4 toxicity in at least one organ, of whom 10 patients (23.2%) died of TRM. The estimated 1-year OS was 64.7% (95% CI, 50.2–79.2%). In contrast, of the remaining 43 patients with grade 2 toxicity in all the organ systems, one died of GVHD, resulting in 78.5% (95% CI, 64.8–92.2%) of estimated 1-year OS and 8.8% of TRM. The 1-year OS was significantly lower in the patients with grade 3–4 toxicity ($P=0.037$).

Variables influencing RRT

No variables were found to be associated with RRT of NCI-CTC ver. 2.0 by univariate (Table 5) or multivariate analysis.

Variables influencing overall survival

Patients who survived longer than 30 days were included in this analysis. Multivariate analysis showed that survival was significantly different between unrelated *vs* related donors (hazard ratio 7.5, 95% CI 1.7–32.8, $P=0.0074$), HLA-mismatched *vs* matched (hazard ratio 3.8, 95% CI 1.1–12.9, $P=0.0295$), and the maximal toxicity grade 3–4 *vs* grade 2–3 of NCI-CTC ver. 2.0 within day 30 post transplant (hazard ratio 3.0, 95% CI 1.2–7.3, $P=0.0177$).

Discussion

Evaluation of RRT after RIST is not uniform. As a result, toxicity grades vary among studies (Table 6) (2–4, 6, 15–18). Our study shows that both the Seattle criteria and NCI-CTC ver. 2.0 are significantly associated with outcome, and have predictive value.

The prognosis of grade 3 by the Seattle criteria is comparable to that of grade 4 by NCI-CTC ver. 2.0, and the prognosis of grade 2 by the Seattle criteria is comparable to that of grade 3 by NCI-CTC ver. 2.0 (Figures 1 and 2). However, neither criteria can offer a cutoff to predict death since the sensitivity and specificity are insufficient; the threshold of $\leqslant$grade 2 by Seattle criteria and $\leqslant$grade 3 by NCI-CTC ver. 2.0 would be sensitive but not specific to predict TRM, whereas the threshold of $\geqslant$grade 3 by the Seattle criteria and $\geqslant$grade 4 by NCI-CTC ver. 2.0 would be specific but not sensitive. These findings suggest that these criteria need to be modified for use in RIST.

There are two types of complications associated with allogeneic HSCT. One is the organ toxicity directly caused by preparative regimens. The other is immunological complications, represented by GVHD. When anti-T-cell antibodies are included in conditioning regimens, the frequency of GVHD is decreased[19,20] showing that GVHD is influenced by the types of preparative regimens. Given the fact that GVHD is the most common cause of nonrelapse death after RIST,[8] GVHD should be considered in the safety evaluation of conditioning regimens.

Another common complication after RIST is early progression of the underlying malignancy. This phenomenon could potentially be considered a consequence (and therefore toxicity) of the reduced intensity of the conditioning regimen.

Another consideration is the follow-up duration in evaluating immunological complications following RIST. The duration of observation after chemotherapy is usually 30 days. In contrast, the onset of GVHD can be delayed. The period of 30 days of observation is not long enough to evaluate the safety of RIST. Although the day 100 TRM has been used in RIST, it is not sufficient in evaluation of the immunological complications. We propose that TRM until day 200 should be used in the evaluation criteria for RIST-related toxicity.

Our study shows that both the Seattle criteria and NCI-CTC ver. 2.0 are useful in evaluating toxicity of RIST. Prospective studies are required to establish a proper toxicity grading system for RIST.

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Review Article

Reduced-intensity Hematopoietic Stem Cell Transplantation (RIST) for Solid Malignancies

Masahiro Kami, Atsushi Makimoto, Yuji Heike and Yoichi Takaue

Department of Medical Oncology, Hematopoietic Stem Cell Transplantation Unit, The National Cancer Center Hospital, Tokyo, Japan

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Review Article

Reduced-intensity Hematopoietic Stem Cell Transplantation (RIST) for Solid Malignancies

Masahiro Kami, Atsushi Makimoto, Yuji Heike and Yoichi Takaue

Department of Medical Oncology, Hematopoietic Stem Cell Transplantation Unit, The National Cancer Center Hospital, Tokyo, Japan

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Reduced intensity stem cell transplantation (RIST) is a new approach of stem cell transplantation, which has shown promising features as reported in multiple phase I and II studies. Elderly patients, who are not eligible for conventional myeloablative hematopoietic stem cell transplantation (HSCT), are now treatable with RIST. It has also reduced regimen-related toxicity and provided better prognosis in short-term follow-up than conventional HSCT. Among solid tumors, metastatic renal cell carcinoma was found to respond well to RIST. Clinical studies are currently being conducted to evaluate the efficacy of RIST in other types of solid tumors. However, the mechanism of graft-versus-host disease (GVHD) and graft-versus-tumor (GVT) effects remains unclear. More knowledge on the mechanism is crucial to enhance the antitumor effect and to improve the prognosis further.

Key words: graft-versus-tumor effects – graft-versus-host disease – renal cell carcinoma – allogeneic hematopoietic stem cell transplantation – breast cancer

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AS AN IMMUNE THERAPY

Allogeneic hematopoietic stem cell transplantation (allo-SCT) for the treatment of hematological malignancies was originally based upon the effect of a myeloablative preparative regimen. A preparative regimen using high-dose chemoradiotherapy would suppress the host's immune response and eradicate the residual tumor cells. Marrow was infused to restore hematopoiesis (1). In combination with preceding induction and consolidation cytotoxic chemotherapy, myeloablative preparative regimens followed by allo-SCT were supposed to eradicate the residual underlying diseases.

However, it was found that allogeneic cells were responsible for immunological responses against tumor cells. This is called a graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect (2). Evidence supporting this hypothesis includes (i) lower incidences of relapse in patients receiving allo-SCT than in those receiving autologous SCT (3); (ii) higher risk of relapse in patients receiving syngeneic SCT (4); and (iii) lower risk of relapse in patients with acute and/or chronic graft-versus-host disease (GVHD) than those without these conditions (5). Furthermore, GVL or GVT effects were found to be mediated by lymphocytes, especially T cells, based on the clinical findings of (i) higher risk of relapse after T-cell depletion than non-depleted SCT (6); and (ii) therapeutic effects of donor lymphocyte infusion (DLI) (7). In particular, chronic myeloid leukemia (CML) responds well to DLI, and most patients with CML who relapse following allo-SCT can achieve remission with DLI (8). Based on these findings, allo-SCT is now regarded as one of the available immune therapies.

REDUCED-INTENSITY STEM CELL TRANSPLANTATION (RIST)

The high-dose chemotherapy and radiation used as preparative regimen for allo-SCT are associated with a considerable morbidity and mortality (9). This approach has therefore been restricted to young patients without co-morbidities. The majority of patients with hematological malignancies are ineligible for high-dose chemotherapy or radiotherapy because of their old age and co-morbidities. Although allo-SCT is the most powerful treatment for refractory hematological malignancies, only a small proportion of these patients have the opportunity to undergo this treatment.

For reprints and all correspondence: Masahiro Kami, Department of Medical Oncology, Hematopoietic Stem Cell Transplantation Unit, the National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan. E-mail: mkami@ncc.go.jp

Recently, a new strategy for transplantation using a reduced-intensity or non-myeloablative preparative regimen has been developed to reduce regimen-related toxicity (RRT) while preserving adequate antitumor effects (10–14). Various regimens with different intensity can be categorized roughly into two intensity groups: (i) reduced-intensity regimens which retain a certain degree of RRT and require hospitalization; and (ii) minimally myelosuppressive regimens which rely on post-grafting immunosuppression to permit engraftment (15,16). The aim of post-grafting immunosuppression is to control GVHD and to suppress residual host-versus-graft (HVG) effects that would impede engraftment.

These reduced-toxicity regimens are frequently termed 'non-myeloablative' and 'reduced-intensity' regimens. At present, a variety of preparative regimens have been developed. Both myelosuppression and immunosuppression vary widely among them. According to a working definition, a truly non-myeloablative regimen should allow prompt hematopoietic recovery (within 28 days of transplantation) without stem cell rescue, and mixed chimerism usually occurs upon engraftment (15,16). These regimens do not ablate host immunity and depend on the activity of donor T cells to achieve engraftment. The regimen of 2 Gy total body irradiation (TBI) with or without fludarabine reported by the Seattle Transplantation Team (12) is classified as a truly non-myeloablative regimen. In contrast, autologous hematopoietic recovery does not occur without stem cell support after the other regimens such as fludarabine/busulfan and fludarabine/cyclophosphamide, and they are termed reduced-intensity preparative regimens.

PRECLINICAL MODEL OF NON-MYELOABLATIVE SCT

The Seattle Transplantation Team reported the results of preclinical canine studies on non-myeloablative SCT. The researchers considered that two immunological barriers must be overcome in the setting of allo-SCT (17). One is the GVHD, and the other is the rejection or HVG reaction. Both reactions are mediated by T lymphocytes, suggesting that immunosuppressive agents given after allo-SCT to control GVHD might modulate HVG reactions. The latter feature would allow minimization of the high-dose chemotherapy given before allo-SCT for host suppression.

Animal models demonstrated a dose–response relationship between TBI and engraftment (18). In random-bred dogs, a single fraction of 920 cGy TBI, corresponding to 1500 cGy fractionated TBI, resulted in engraftment of dog leukocyte antigen (DLA)-identical littermate marrow in all cases. When the dose was decreased by 50%, the majority of dogs rejected their grafts. At the reduced dose, the addition of post-grafting prednisone did not enhance engraftment, while cyclosporin given for 5 weeks led to engraftment in all of the animals. When the TBI dose was decreased further to 200 cGy, cyclosporin only allowed engraftment for 3–4 months, after which the grafts were rejected. The combination of methotrexate and cyclosporin resulted in engraftment in two out of five animals, but the rest rejected. A combination of mycophenolate mofetil (MMF) and cyclosporin given for 4 and 5 weeks after transplantation was evaluated for its effect on engraftment. The regimen was capable of both controlling GVHD and preventing graft rejection by suppressing a GVH reaction, with 11 of 12 dogs demonstrating stable engraftment of marrow from DLA-identical littermates (19).

They further investigated whether the major role of TBI is to create marrow space or to provide host immunosuppression (20). They irradiated the central lymph node chain from the neck to the upper abdomen with 450 cGy before allo-SCT, and administered MMF and cyclosporin after allo-SCT. At 6 weeks post-transplant, donor cells were present in non-irradiated marrow spaces, suggesting that radiation was not essential to create marrow space for engraftment. After 1 year, DLI was given to the animals and recipient cells disappeared within 9 weeks. These findings indicate that engraftment might be accomplished by blocking HVG reactions and inducing the GVH reaction, and that high-dose cytotoxic chemotherapy and radiotherapy could be eliminated from the preparative regimens.

RATIONALE OF ALLO-SCT FOR SOLID TUMORS

Several findings justify allo-SCT for solid tumors: (i) GVT effects can target tissue-specific polymorphic antigens which are not derived from hematopoietic lineages; (ii) some solid tumors are sensitive to immunotherapy, such as renal cell carcinoma (RCC), melanoma and ovarian cancer; (iii) antigens restricted to the tumor could stimulate tumor-specific allo-immunity in contrast to defective T cells in the tumor-bearing host; and (iv) in theory, all carcinomas arising from epithelial tissues such as keratinocytes, fibroblasts, exocrine glands, hepatobiliary trees and the gastrointestinal tract, which are targets of acute and chronic GVHD, should be susceptible to a GVT effect.

Before clinical trials were initiated, murine models have provided some evidence for a GVT effect (21,22). Among animals inoculated with mammary adenocarcinoma cells, the recipients of allo-SCT showed better survival than did those of syngeneic SCT (21). Further studies provided evidence that murine mammary adenocarcinoma cells expressed minor histocompatibility antigens (mHas) that could be targeted by alloreactive donor T cells in the setting of allogeneic but not autologous bone marrow transplantation (23). Prigozhina et al. demonstrated in animal models that effective eradication of tumor cells as well as leukemic cells can be achieved following allo-SCT using non-myeloablative preparative regimens (24).

The earliest clinical evidence supporting the existence of a GVT effect in a solid tumor was observed in a patient with metastatic breast carcinoma undergoing fully myeloablative SCT for relapsed acute myeloid leukemia. The incidental regression of a metastatic lesion of breast carcinoma raised the possibility of a responsible GVT effect (25). Regression of liver

metastasis in association with severe acute GVHD was reported in a woman transplanted for metastatic breast carcinoma. The researchers demonstrated that allogeneic T cells collected during GVHD and cultivated were able to mediate a cytotoxic effect against breast cancer cell lines (26), suggesting that disease regression resulted from donor T cells targeting broadly expressed mHas. Since then, similar anecdotal reports have been published concerning a possible GVT effect in lung cancer (27), ovarian cancer (28), colon cancer (29), neuroblastoma (30), pancreas cancer (31,32) and ependymoma (33). Porter et al. conducted a phase I clinical trial to determine whether a GVT effect could be observed after primary DLI without stem cell support in patients with primary cancers (34). Three of four patients with acute GVHD and late chimerism responded to primary DLI. These findings indicate that the GVT effect does occur in the setting of allo-SCT for solid tumors.

CLINICAL TRIALS FOR SOLID TUMORS

Metastatic Renal Cell Cancer (RCC)

In 1997, Childs et al. initiated a clinical trial to evaluate GVT effects in metastatic RCC (35). Chemotherapy is ineffective in the majority of cases and does not prolong survival. However, RCC has a distinct nature from that of other solid tumors. There is increasing evidence that they may be susceptible to T-cell immune responses. Biopsy of spontaneously regressing lesions has shown tumor-infiltrating lymphocytes with predominant $CD8^+$ T cells exhibiting major histocompatibilty complex (MHC) class I restricted cytotoxicity against autologous tumor targets (36). Furthermore, unlike most solid tumors, RCC is susceptible to cytokines such as interleukin-2 (IL-2) and interferon-α (37), suggesting that T cells represent the principle effector.

Childs' group treated 19 patients with metastatic RCC (35). The preparative regimen consisted of fludarabine 25 mg/m^2 for 5 days and cyclophosphamide 60 mg/kg for 2 days. Cyclosporin, used to prevent GVHD, was withdrawn early in patients with mixed T-cell chimerism and/or disease progression. Patients without response received up to three courses of DLI. At the time of the last follow-up, nine of the 19 patients were alive 287–831 days after transplantation (median follow-up, 402 days). Two died of transplantation-related causes, and eight from progressive disease. In 10 patients, metastatic disease regressed: three had a complete response, and seven had a partial response. The patients who had a complete response remained in remission 27, 25 and 16 months after transplantation. Results of this clinical trial were updated in 2002 (38). Clinical response is significantly associated with the development of GVHD. There is a 4–6 month interval between transplantation and development of a GVT effect, and patients with rapidly progressive diseases are unlikely to benefit from RIST. Disease response was observed most commonly in patients with pulmonary metastases of clear-cell histology without other organ involvement. Some patients who had failed to respond to interferon-α prior to transplantation achieved responses following administration of a low dose of this agent after transplantation.

After the first report on RIST for RCC, several phase I/II studies have been reported (Table 1) (39–44). Response rates varied widely from 0 to 57%, but it should be noted that some responses were reported in seven of the nine studies. While long-term prognosis remains unknown, response to allo-SCT has been confirmed in some independent studies. Rini et al. described regression of primary kidney tumors, a rare event among responders to cytokine-based therapy (39). According to a European retrospective survey (45), allo-SCT was used in <20 cases of solid tumors until 1997; since then it increased to 159 in 2002, mainly for RCC.

We also initiated a phase I clinical trial on RIST for metastatic RCC (46). From June 2000 to April 2002, nine patients received peripheral blood stem cell transplantation from a

Table 1. Clinical trials on RIST for metastatic renal cell carcinoma

Reference	Donor	No. of patients	Preparative regimen	GVHD prophylaxis	Response rates
Childs et al. (35)	An HLA-identical or one locus-mismatched related donor	19	CY/Flu	CSP	53%
Childs and Barrett (38)	HLA-identical and one locus-mismatched related	52	CY/Flu	CSP	48%
Rini et al. (39)	An HLA-identical sibling	12	CY/Flu	Tacrolimus and MMF	33%
Bregni et al. (40)	An HLA-identical sibling	7	CY/Flu	CSP and MTX	57%
Blaise et al. (42)	An HLA-identical sibling	25	ATG/BU/Flu	CSP	4%
Ueno et al. (43)	An HLA-identical related or matched unrelated donor	15	Melphalan/Flu	Tacrolimus and MTX	27%
Pedrazzoli et al. (41)	An HLA-identical sibling	7	CY/Flu	CSP and MTX	0%
Hentschke et al. (44)	An HLA-identical related or matched unrelated donor	10	2 Gy TBI/Flu*	CSP and MMF	0%
Nakagawa et al. (46)	An HLA-identical sibling	9	ATG/BU/Flu	CSP	11%

CY, cyclophosphamide; Flu, fludarabine; CSP, cyclosporin; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, anti-thymocyte globulin; BU, busulfan; TBI, total body irradiation.
*Recipients receiving transplants from unrelated donors were given thymoglobulin.