reduced-intensity or nonmyeloablative allogeneic transplantation (15%).[8] Since 20% of RIST recipients had advanced malignant lymphoma,[8] approximately half of the patients with malignant lymphoma who underwent RIST in Japan were surveyed in this study.

Data were derived from questionnaires distributed to each participating center. Minimum data required for the inclusion of a patient in this study were age, histological diagnosis, prior treatment details, status at transplant, conditioning regimens, date of transplant, date of last follow-up, disease status at last follow-up, date of disease progression/death and causes of death. Information on rituximab use prior to RIST was not collected in this study.

Definition

Reduced-intensity regimens were defined as reported previously.[9,10] The upper limits of busulfan, melphalan, and TBI were 8 mg/kg, 140 mg/m^2, and 2 Gy, respectively, for consideration as reduced-intensity preparative regimens. Engraftment was defined as white blood cell counts $>1.0\times10^9$/l or absolute neutrophil counts $>0.5\times10^9$/l for two consecutive days. Graft-versus-host- disease (GVHD) was clinically diagnosed in combination with skin or gut biopsies. Acute and chronic GVHD were graded according to the established criteria.[11,12]

Histological diagnosis was based on institutional diagnosis. Discrepancies in nomenclature among centers were resolved according to the synonyms in the WHO classification.[13] Indolent, aggressive, and highly aggressive lymphomas were classified according to the report by Chan[14] with some modifications. Transformed low-grade lymphoma was classified into aggressive lymphoma. However, patients who had recurrent low-grade lymphoma rarely receive biopsy before transplant, and patients with transformed low-grade lymphoma might have been analyzed as low-grade lymphoma in this study. Adult T-cell leukemia/lymphoma was classified into a highly aggressive category, because its clinical course is aggressive and patients' median survival is as short as about 6 months. Chimerism was determined by short-tandem repeat PCR method or sex chromosome FISH, and disease status was evaluated with CT, MRI scan, bone marrow aspiration, or spinal tap in varying intervals from 1 month to 60 months according to each participating hospital's rule. Those with chemosensitive diseases included all patients who had shown a response to the last therapy prior to transplantation (partial remission (PR), complete remission (CR) unconfirmed, and CR); all the other patients were classified as having chemoresistant diseases. Progression-free survival (PFS) was measured as the time from the day of transplantation until disease relapse/progression or death from any causes. Both relapse and progression were defined as disease progression with transplantation-related deaths being censored. TRM is defined as all causes of deaths without disease progression at any time after transplant. RRT was defined as all nonhematological organ dysfunctions from day 0 to day 28, and were graded according to the Seattle criteria.[15]

Statistical analysis

The primary end point was 3-year PFS. Secondary end points included 3-year overall survival (OS), TRM, and disease progression rates. The cumulative incidences of progression and progression-free mortality were evaluated using the Gray's method,[16] considering each other's risk as a competing risk. OS and PFS were estimated using the Kaplan–Meier method. Potential confounding factors considered in the analysis were age, sex, donor types (an HLA-matched related donor and an alternative donor), stem cell sources (marrow, peripheral blood, and cord blood), performance status according to the Eastern Cooperative Oncology Group (ECOG) criteria,[17] serum levels of lactate dehydrogenase, intervals from diagnosis to transplantation, the number of prior chemotherapy regimens, history of autologous SCT, history of radiation, clinical stages, chemosensitivity, presence of extramedullary involvement (central nervous system, and marrow), presence of bulky mass, disease category (indolent, aggressive, highly aggressive), different conditioning regimens, and use of methotrexate as GVHD prophylaxis. Proportional hazard modeling was used to evaluate the influence of these factors on PFS and disease progression. The influence of the development of GVHD on PFS and disease progression was evaluated using the proportional hazard modeling treating the development of acute GVHD as a time-dependent covariate. Factors associated with at least borderline significance ($P<0.10$) in a univariate analysis were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. *P*-values of less than 0.05 were considered statistically significant.

Results

Patient characteristics and transplantation procedures

Patients' characteristics and transplantation procedures are shown in Table 1. None received *ex vivo* T-cell depleted transplantation.

Regimen-related toxicity

Information on RRT within 28 days of RIST was available in 106 patients and was graded according to Bearman's criteria (Table 2).

Engraftment

Four patients died before engraftment. None developed primary graft failure. Of the 108 patients who achieved primary engraftment, 91 patients were evaluable for chimerism. In all, 85 patients (93%) achieved complete donor-type chimerism within 100 days of transplant. Three subsequently achieved complete donor-type chimerism, one died of infection with mixed chimerism 164 days after transplant, and two remained alive with mixed chimerism (623 and 606 days after transplant). None received donor lymphocyte infusion (DLI) for engraftment.

Table 1 Patient characteristics and transplantation procedures

	Indolent lymphoma[a]	*Highly-aggressive*[b], *Aggressive lymphoma*[c]
Sex		
Male/female	21/24	41/26
Age		
Median (range)	48 (61–32)	50 (72–22)
Interval from diagnosis to transplantation (years)		
Median (range)	3.7 (0.1–15.1)	1.6 (0.3–12.1)
Numbers of prior chemotherapy regimens		
Median (range)	4 (1–15)	4 (1–14)
Prior local radiation therapy		
Yes/no	11/34	25/42
Previous history of HDT/ASCT		
Yes/no	10/35	30/37
Disease status at transplant		
CR/Non-CR/ND	1/40/4	6/56/5
I–II/III–IV/ND	9/31/5	12/44/11
Patients with bone marrow invasion	15	15
Patients with CNS invasion	2	9
Patients with bulky mass	6	4
Performance status at transplant		
0–1/2–4	40/3	50/14
Increased serum LDH level at transplant[d]		
Yes/no	19/26	34/33
Chemosensitivity at transplant		
Sensitive/ resistant	31/14	38/29
Conditioning regimens		
Fludarabine and busulfan	16	25
Fludarabine and cyclophosphamide	12	16
Fludarabine and melphalan	9	12
Fludarabine and 200 cGy total body irradiation	2	3
200 cGy total body irradiation	1	5
Other	5	6
GVHD prophylaxis		
Cyclosporin and methotrexate	16	25
Cyclosporin and mycofenolate mofetil	2	7
Cyclosporin alone	21	28
Tacrolimus and methotrexate	5	6
Tacrolimus alone	1	1
Use of anti-thymocyte globulin as preparative regimens		
Yes/no	9/36	9/58
Stem-cell sources		
Blood from an HLA-matched related donor	29	49
Blood from an HLA-mismatched related donor	3	5
Marrow from an HLA-matched related donor	1	5
Marrow from an HLA-matched unrelated donor	7	7
Mismatched cord blood	0	6

HDT/ASCT = high-dose therapy and autologous stem cell transplantation; CR = complete remission; ND = not described; LDH = lactate dehydrogenase; GVHD = graft-versus-host disease.
[a]Indolent lymphoma included follicular ($n=44$), marginal zone B-cell ($n=2$), small lymphocytic ($n=1$), lymphoplasmacytic ($n=1$), and cutaneous T-cell ($n=1$).
[b]Highly aggressive lymphoma included lymphoblastic ($n=3$), adult T-cell ($n=4$), and Burkitt ($n=2$).
[c]Aggressive lymphoma included diffuse large B-cell ($n=27$), peripheral T-cell, unspecified ($n=9$), mantle cell ($n=8$), NK-cell ($n=4$), anaplastic large cell ($n=4$), and angioimmunoblastic ($n=2$). Transformed low-grade lymphoma was treated as diffuse large B-cell lymphoma ($n=4$).
[d]Normal ranges of LDH were determined in each participating hospital.

Table 2 Regimen-related toxicity within 28 days according to the Bearman's criteria

Grade	*0*	*I*	*II*	*III*	*IV*
Mucosa	64	27	12	1	0
Central nervous system	99	0	1	4	0
Lung	93	3	4	4	1[a]
Kidney	84	13	3	4	0
Liver	74	15	14	1	1[b]
Bladder	100	4	0	0	0
Heart	95	3	5	1	0
Gut	74	20	6	4	0

[a]Idiopathic pneumonia syndrome.
[b]Hepatic veno-occlusive disease.

Graft-versus-host disease

Seven patients were not evaluated for acute GVHD, since four died before engraftment and three lacked the data regarding GVHD. In the remaining 105 patients, cumulative incidence of grade II–IV acute GVHD was 49% with a median onset of day 24 (range, 8–99). Of the 98 patients survived longer than 100 days after transplant, cumulative incidence of chronic GVHD was 59%.

Response to RIST

In all, 84 patients including 52 patients with chemosensitive diseases and 32 patients with chemoresistant diseases had measurable lesions prior to transplant, and were evaluated for response to RIST. A total of 72 patients (86%) responded to RIST (CR 63 and PR nine). As of February 2004, median duration of response was 22.5 months (range, 2.2–38.9). After initial response to RIST, primary disease recurred or progressed in four patients. Median interval between initial response and disease progression was 4.1 months (range, 1.4–11.2). Response to RIST was shown according to histological subtypes (Table 3). Five patients

Table 3 Response rates and outcomes of RIST according to histological subtypes

Chemosensitivity	*Indolent (n = 45)*		*Aggressive (n = 58)*		*Highly aggressive (n = 9)*	
	Sensitive	*Refractory*	*Sensitive*	*Refractory*[a]	*Sensitive*	*Refractory*
No. of patients	31	14	34	24	4	5
Response rate[b]	24/26 (92%)	11/11 (100%)	22/23 (97%)	11/17 (65%)	3/3 (100%)	1/4 (25%)
Progression after response	1	0	2	1	0	0
Progression-free survival at 3 years (%)	83	64	56	30	0	0
Total deaths	4	5	12	16	1	5
Causes of death						
Primary disease	1	0	3	6	0	3
GVHD	2	2	5	4	1	1
Infection	1	2	4	5	0	1
Other TRM	0	1	0	1	0	0

RIST = reduced intensity stem cell transplantation; GVHD = graft-versus-host disease; TRM = transplant-related mortality.
[a]Four patients with chemorefractory transformed low-grade lymphoma responded to RIST, and survived without disease progression with a median follow-up of 25.2 months (range, 16.1–32.4)
[b]Patients without measureable disease at transplant were excluded.

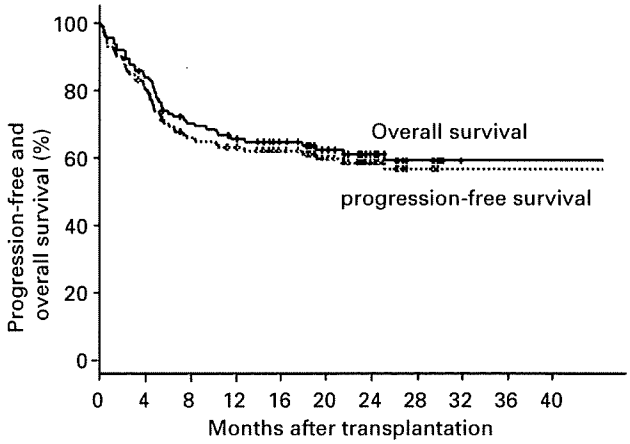

Figure 1 Overall survival (OS) and progression-free survival (PFS) following transplant. The 3-year OS and PFS were 59.0% (95% CI, 55.0–64.0%) and 56.5% (95% CI, 51.5–61.5%), respectively.

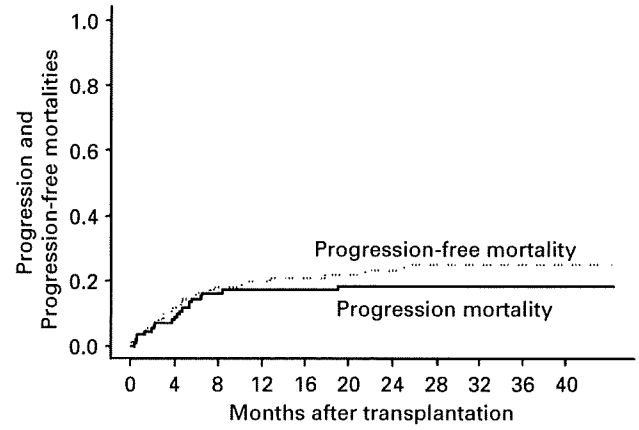

Figure 3 Cumulative incidences of disease progression mortality and transplant-related mortality (TRM). Cumulative incidences of disease progression mortality and TRM at 3 years were 18.3 and 25.2%, respectively.

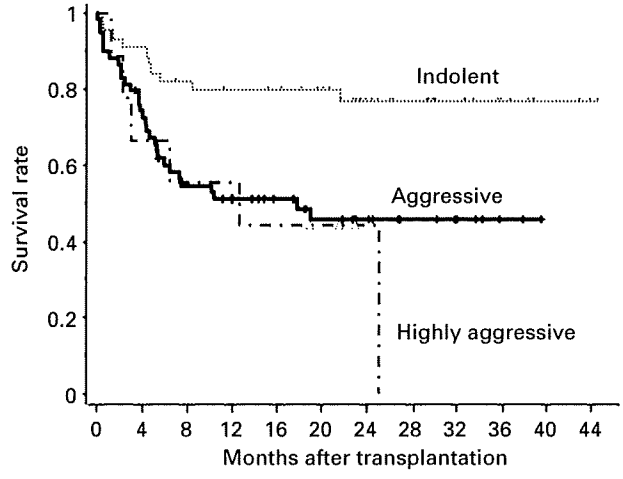

Figure 2 Overall survival (OS) following transplant according to the histological subtypes. The 3-year OS according to the histological subtypes was indolent 79% (95% CI, 67–91%), aggressive 48% (95% CI, 35–61%), and highly aggressive 0%; follicular 81% (95% CI, 69–92%), diffuse large B-cell 31% (95% CI, 13–49%), peripheral T-cell 56% (95% CI, 23–89%), and mantle cell 76% (95% CI, 45–100%).

received DLI for either disease progression or disease persistence following RIST. One showed objective disease response after DLI. The outcome in patients with CNS disease or whether they relapse in the CNS or outside the CNS was not collected.

OS, PFS and TRM

As of February 2004, 69 were alive with a median follow-up duration of 23.9 months (range, 3.4–44.5). The 3-year OS and PFS were 59.0% (95% CI, 55.0–64.0%) and 56.5% (95% CI, 51.5–61.5%), respectively (Figure 1). The 3-year OS according to the histological subtypes (Figure 2) was indolent 79% (95% CI, 67–91%), aggressive 48% (95% CI, 35–61%), and highly aggressive 0%; follicular 81% (95% CI, 69–92%), diffuse large B-cell 31% (95% CI, 13–49%), peripheral T-cell 56% (95% CI, 23–89%), and mantle cell 76% (95% CI, 45–100%). There was no difference in 3-year OS between T-cell and B-cell lymphomas ($P = 0.08$). The cumulative incidences of progression and progression-free mortality were 18.3 and 25.2%, respectively (Figure 3).

Since progression-free mortality was evaluated with relapse censored as a competing risk, it is apparently lower than an absolute incidence of 27%.

Primary causes of death were disease progression in 13, whereas 30 died without disease progression (Table 3) GVHD complicated with infection ($n=15$), infection ($n=13$), idiopathic pneumonia syndrome ($n=1$), and hepatic veno-occlusive disease ($n=1$). The causative organisms included Gram negative rods ($n=4$), Gram positive cocci ($n=4$), fungi ($n=3$), and unknown ($n=2$).

Prognostic factors for PFS

Results of univariate and multivariate analysis on relapse and PFS are shown in Tables 4 and 5, respectively. Three variables including history of any types of irradiation prior to RIST, CNS involvement at transplant, and absence of grade II–IV acute GVHD were adversely associated with disease progression (Table 4). Four variables including poor PS, short interval from diagnosis to transplant, nonmethotrexate-containing GVHD prophylaxis, aggressive-type histology were adversely associated with PFS (Table 5).

Discussion

Although the eligibility was decided according to different protocols at each participating hospital and the possibility of a selection bias cannot be excluded, this multicenter, retrospective analysis described the gross characteristics of RIST in Japan.

RRT has been a significant problem in allo-SCT for malignant lymphoma,[18–20] while only two patients (1.8%) died of RRT within 28 days of RIST. TRM was lower than those reported on conventional allo-SCT.[18–20] RIST might decrease RRT and provided better prognosis in short-term follow-up than conventional transplantation. The incidence of acute GVHD is lower in Japan than in Western countries because of the relative genetic homogeneity of the population;[21] however, 43 patients developed grade II to IV acute GVHD, which was fatal in 15 patients. The rate of acute GVHD was similar to those reported on myeloablative or reduced-intensity allo-SCT from Western countries.[19,20,22,23] The relatively high incidence of acute GVHD in the present study was probably associated with less intense GVHD prophylaxis in RIST than in conventional allo-SCT. The use of methotrexate beneficially affected PFS in our multivariate analysis. Additional methotrexate is probably beneficial especially in RIST because RIST recipients are elderly and with comorbidities, and GVHD is a higher risk of TRM.

A GVL effect is associated with GVHD in allo-SCT for hematologic malignancies.[3,24] While this trend is remarkable in acute leukemia,[3] it has been inconsistent in malignant lymphoma.[4,18,20,25] GVHD was associated with reduced disease progression; however, PFS was not improved in the present study. GVHD is sometimes fatal, and may offset patients' prognosis. Since the impact of GVHD on a GVL effect varies according to disease status and patients' conditions, management of GVHD should be tailored. Further studies are warranted to establish a proper GVHD prophylaxis.

Few reports are available on infections after RIST.[26–29] RIST seemed to be associated with less infections due to the shorter duration of neutropenia and less damage to mucosal barriers. However, we showed that opportunistic infection is the second leading cause of death in RIST. Most patients had received multiple courses of chemotherapy, and occult infections might have existed at RIST. These infections can be fatal in RIST recipients. Management of bacterial and fungal infections following RIST requires further investigation.

In the present study, PFS was significantly different according to histological subtypes (Figure 2), which is consistent with previous reports.[23,30] Indolent lymphoma has a low relapse rate, and the major causes of mortality are GVHD and infections (Table 3). Our study showed that chemotherapy-resistant indolent lymphoma can achieve good outcomes after RIST, and that the response to RIST is not associated with chemosensitivity before RIST (Table 3). These findings are comparable to previous reports.[19] RIST for indolent lymphoma needs to be reserved for those with advanced diseases, since RIST is associated with TRM. Intensification of GVHD prophylaxis and infection control may produce more promising results in RIST for indolent lymphoma.

In contrast, the outcomes of RIST for aggressive and highly aggressive lymphomas were poor.[23] Although allo-SCT has been considered ineffective for these lymphomas,[30] the present study showed that some can achieve remission after RIST (Table 3). However, the response rate of these lymphomas was not satisfactory in RIST for chemorefractory aggressive and highly aggressive lymphomas. Investigations are necessary to determine better timing and indications of RIST for these lymphomas. This study and others[31] revealed history of irradiation, central nervous system involvement and chemosensitivity at transplantation as significant prognostic factors (Table 4). These are useful to identify patients who would benefit from RIST. Another approach to improve the response rates of RIST for these lymphomas is intensification of preparative regimens as far as patients can tolerate without increasing RRT. Since the strength of GVL effect depends on the initial ratio between the number of tumor-specific immunocompetent cells in the graft and tumor cell burden of the recipient,[32] debulking of lymphoma cells by preparative regimens will be beneficial. The other problem in RIST for aggressive and highly aggressive lymphoma is the high rates of TRM. Most patients who achieved response after RIST remained progression-free (Table 3), suggesting a benefit of allogeneic immunity to suppress disease progression. Intensification of GVHD prophylaxis contributes to improve GVHD-related outcomes;[33–35] however, use of potent immunosuppressive agents might diminish a GVL effect,[35] and could increase the rate of serious infections.[34] Maintaining the fine balance between GVHD and GVL effects is important and frequently difficult in RIST for these lymphomas. Another promising approach is to reinforce a GVL effect without increasing GVHD. For example, monoclonal antibodies such as rituximab, tumor vaccines, and adoptive transfer of cytotoxic T-cells

Table 4 Univariate and multivariate analysis on progression

Factors	*Relative risk (95% confidence interval)*	*P-value*
Univariate		
Age		
per year	0.96 (0.92–1.00)	0.048[a]
Sex		
Male *vs* female	1.22 (0.50–2.95)	0.67
Performance status[b]		
2–4 *vs* 0–1	1.55 (1.02–2.33)	0.038[a]
Interval from diagnosis to transplant[c]		
Per year	0.88 (0.75–1.04)	0.14
Numbers of prior chemotherapy regimens[c]		
Per cycle	0.98 (0.85–1.14)	0.8
History of autologous transplant		
Yes *vs* no	1.52 (0.63–3.64)	0.35
History of radiation[d]		
Yes *vs* no	3.68 (1.51–8.95)	0.0041[a]
Clinical stage at transplant		
3–4 *vs* 1–2	1.28 (0.95–1.74)	0.11
Serum levels of LDH prior to transplant		
Elevated *vs* normal	1.95 (0.78–4.90)	0.15
Chemosensitivity		
Sensitive *vs* refractory	0.45 (0.19–1.07)	0.07
CNS involvement at transplant		
Yes *vs* no	7.27 (2.91–18.18)	<0.001[a]
Bone marrow involvement at transplant		
Yes *vs* no	0.49 (0.14–1.73)	0.27
Bulky disease at transplant[e]		
Yes *vs* no	3.13 (1.05–9.29)	0.040[a]
Histology		
Indolent	1	
Aggressive	4.15 (1.20–14.26)	0.024[a]
Highly aggressive	5.95 (1.22–29.10)	0.028[a]
Stem-cell sources		
Peripheral blood	1	
Bone marrow	0.64 (0.21–1.95)	0.44
Cord blood	1.28 (0.15–10.79)	0.82
Conditioning regimen		
Fludarabine and busulfan	1	
Fludarabine and cyclophosphamide	1.22 (0.27–5.4)	0.79
Fludarabine ane melphalan	3.19 (0.88–11.5)	0.077[f]
TBI based	4.02 (1.05–15.4	0.043[f]
Others	2.67 (0.61–11.7)	0.19
Methotrexate-containing GVHD prophylaxis		
Yes *vs* no	0.47 (0.18–1.21)	0.12
Grade II–IV acute GVHD		
II–IV/0–I	0.52 (0.19–1.45)	0.21
Multivariate		
History of radiation[d]		
Yes *vs* no	3.45 (1.12–10.0)	0.03[a]
CNS involvement at transplant		
Yes *vs* no	6.25 (2.08–20.0)	0.001[a]
Grade II to IV acute GVHD		
II–IV/0–I	0.28 (0.090–0.86)	0.026[a]

LDH = lactate dehydrogenase; CNS = central nervous system; GVHD = graft-versus-host disease.
[a]Statistically significant.
[b]Performance status was defined according to the Eastern Cooperative Oncology Group (ECOG) criteria.
[c]They were analyzed as a continuous variable.
[d]Any types of irradiation prior to RIST were included.
[e]When patients had at least one mass with its diameter longer than 10 cm, they were defined as cases with bulky disease.
[f]Flu/Mel and TBI entered a multivariate analysis and rejected in backward stepwise proportional-hazard modeling.

targeting minor histocompatibility antigens or tumor-specific antigens have been investigated.[36–38]

The risk of progression was significantly higher among patients with prior history of local radiation therapy (RT) than those who did not received RT (Table 4). RT is indicated when the patients have chemo-refractory disease, central nervous system involvement or bulky mass, which means that patients with a history of RT carry risk factors of poor outcomes.

The survival of patients with PS 0–1 was significantly longer than that with PS 2–4 (Table 5). PS is affected by age, infections, and aggressiveness of the diseases, and patients with poor PS carry the overlapping risk factors of poor outcomes. While RIST is considered feasible even for patients with worse PS than is conventional stem-cell transplantation, the present study showed that the poor PS is also a risk factor of poor RIST outcomes. The time from diagnosis to RIST also affected the outcomes; our univariate and multivariate analyses showed significant differences in PFS. The observations are comparable to the results by van Besien.[18]

While the present study provided novel information on RIST for advanced lymphoma, we need to take its limitations into consideration. It is a small-sized, retrospective study; unrecognized biases might have affected the results. However, it demonstrated that many patients with advanced lymphoma can survive after RIST. These observations provide a rationale for continuing our clinical trials on RIST for malignant lymphoma, focusing on minimizing toxicities, preventing GVHD, and controlling infectious complications. It is imperative to establish optimal preparative regimens and management of GVHD to enhance a GVL effect and to reduce TRM. Although the present study showed that patients with chemotherapy-resistant indolent lymphoma can achieve durable remission after RIST, we cannot yet conclude that RIST improves the prognosis. Despite progressive improvement of safety, the risk of significant TRM limits the widespread application of allo-SCT for malignant lymphoma. Without evidence of efficacy, most physicians considered this risk too high to justify studies of allo-SCT. Phase III clinical trials

Table 5 Univariate and multivariate analysis on progression-free survival

Factors	*Relative risk (95% confidence interval)*	*P-value*
Univariate		
Age	1.00 (0.97–1.03)	0.78
Sex		
Male *vs* female	0.80 (0.44–1.45)	0.47
Performance status[a]		
2–4 *vs* 0–1	1.99 (1.49–2.66)	<0.0001[b]
Interval from diagnosis to transplant[c]		
Per year	0.83 (0.73–0.94)	0.004[b]
Numbers of prior chemotherapy regimens[c]		
Per cycle	0.93 (0.82–1.06)	0.26
History of autologous transplant		
Yes *vs* no	1.69 (0.95–3.03)	0.077
History of radiation		
Yes *vs* no	1.57 (0.87–2.84)	0.14
Clinical stage at transplant		
3–4 *vs* 1–2	1.30 (0.99–1.72)	0.064
Serum levels of LDH prior to transplant		
Elevated *vs* normal	1.90 (1.04–3.49)	0.38
Chemosensitivity		
Sensitive *vs* refractory	0.35 (0.20–0.63)	0.0004[b]
CNS involvement at transplant		
Yes *vs* no	2.39 (1.11–5.15)	0.026[b]
Bone marrow involvement at transplant		
Yes *vs* no	0.91 (1.46–1.80)	0.79
Bulky disease at transplant		
Yes *vs* no	1.97 (0.83–4.66)	0.12
Histology		
Indolent	1	
Aggressive	3.04 (1.48–6.24)	0.0024[b]
Highly aggressive	1.25 (0.52–3.00)	0.62
Stem-cell sources		
Peripheral blood	1	
Bone marrow	1.47 (0.68–3.17)	0.32
Cord blood	0.66 (0.14–3.11)	0.6
Conditioning regimen		
Fludarabine and busulfan	1	
Fludarabine and cyclophosphamide	0.64 (0.29–1.37)	0.25
Fludarabine ane melphalan	0.80 (0.35–1.85)	0.6
TBI based	0.58 (0.22–1.52)	0.27
Others	0.87 (0.31–2.41)	0.79
Methotrexate-containing GVHD prophylaxis		
Yes *vs* no	0.33 (0.17–0.64)	0.0009[b]
Grade II–IV acute GVHD		
II–IV/0–I	0.89 (0.45–1.73)	0.72
Multivariate		
Performance status[a]		
2–4 *vs* 0–1	1.83 (1.32–2.53)	0.0003[b]
Interval from diagnosis to transplant[c]		
Per year	0.86 (0.74–0.99)	0.04[b]
Methotrexate-containing GVHD prophylaxis		
Yes *vs* no	0.26 (0.13–0.54)	0.0002[b]
Histology		
Indolent	1	
Aggressive	2.69 (1.17–6.15)	0.019[b]
Highly aggressive	1.89 (0.69–5.18)	0.21

LDH = lactate dehydrogenase; CNS = central nervous system; GVHD = graft-versus-host disease.
[a]Performance status was defined according to the Eastern Cooperative Oncology Group (ECOG) criteria.
[b]Statistically significant.
[c]They were analyzed as a continuous variable.

comparing RIST with standard chemotherapy are warranted. However, these trials are frequently problematic, considering that therapeutic approaches are different between transplant and chemotherapy, and that the standard therapies for some subtypes such as mantle cell and peripheral T-cell lymphoma are dismal. Registry multicenter data such as in this study will allow for a reasonable analysis of the role of RIST in advanced lymphoma.

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Appendix

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Allografting

Comparison between reduced intensity and conventional myeloablative allogeneic stem-cell transplantation in patients with hematologic malignancies aged between 50 and 59 years

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Summary:

To evaluate the efficacy of reduced-intensity stem-cell transplantation (RIST), we retrospectively compared outcomes of 207 consecutive Japanese patients aged between 50 and 59 years with hematologic malignancies who received RIST ($n=70$) and conventional stem-cell transplantation (CST) ($n=137$). CST recipients received total body irradiation (TBI)-based or busulfan/cyclophosphamide-based regimens. RIST regimens were purine analog-based ($n=67$), 2 Gy TBI-based ($n=2$), and others ($n=1$). Most CST recipients (129/137) received calcineurin inhibitors and methotrexate as graft-versus-host (GVHD) prophylaxis, while 32 RIST recipients received cyclosporin. In all, 23 CST and five RIST recipients died without disease progression within 100 days of transplant. Grade II to IV acute GVHD occurred in 56 CST and 38 RIST recipients. There was no significant difference in overall survival (OS) and progression-free survival between CST and RIST. On multivariate analysis on OS, five variables were significant: preparative regimens (CST *vs* RIST) (hazard ratio = 1.92, 95% confidence interval, 1.25–2.97; $P=0.003$), performance status (2–4 *vs* 0–1) (2.50, 1.51–4.16; $P<0.001$), risk of underlying diseases (1.85, 1.21–2.83; $P=0.004$), acute GVHD (2.57, 1.72–3.84; $P<0.001$), and CML (0.38, 0.21–0.69; $P=0.002$). We should be careful in interpreting results of this small-sized retrospective study; however, reduced regimen-related toxicity might contribute to better survival in RIST. The low relapse rates following RIST suggest a strong antitumor activity through allogeneic immunity.
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Allogeneic hematopoietic stem-cell transplantation (autologous stem-cell transplantation (allo-SCT)) is a therapeutic option for advanced hematologic malignancies. A small but significant proportion of these patients can be cured with allo-SCT.[1] Conditioning regimens have been developed to maximize dose intensity, escalating the dose-limiting toxicity in nonhematopoietic tissues.[2] Conventional stem-cell transplantation (CST) using a myeloablative preparative regimen is associated with severe regimen-related toxicities (RRT), resulting in high nonrelapse mortality (NRM) especially for old patients.[3] NRM tends to be higher in patients with refractory or advanced diseases, who have been treated heavily, compared with those who have achieved remission.[3] Considering that high-dose chemotherapy followed by allo-SCT is ineffective for these patients,[4] and that intensification of preparative regimens usually leads to severe RRT and high NRM,[5] it remains unknown whether myeloablative preparative regimens are beneficial to improve survival of patients with advanced chemorefractory leukemia.

A new strategy for transplantation using a reduced-intensity stem-cell transplantation (RIST) or nonmyeloablative preparative regimen has been developed to reduce RRT while preserving an adequate antileukemia effect.[4–6] This strategy decreases the risk of NRM and allows

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transplantation in elderly patients or those with organ dysfunction. RIST appears to be promising for a variety of hematologic diseases, if disease activity is controlled prior to transplant.[7] Most physicians believe that RIST is insufficient in controlling advanced hematologic malignancies, and that intensification of preparative regimens is required to improve their prognosis. Small pilot studies showed that RIST had been unsuccessful for advanced hematologic malignancies,[5,8] yet, efficacy of RIST has not been fully evaluated. Few comparative studies have been reported between RIST and CST for hematologic malignancies.[9]

Patients older than 50 years are regarded as candidates for RIST, yet, patients younger than 60 years frequently undergo CST. Either RIST or CST is offered to patients aged between 50 and 59 years according to doctors' preferences or based on patients' conditions. To evaluate the efficacy of RIST for hematologic malignancies in the elderly patients, we retrospectively compared the outcomes of 207 consecutive patients aged between 50 and 59 years with hematologic malignancies who had received either RIST ($n=70$) or CST ($n=137$).

Patients and methods

Data collection

We conducted a nation-wide retrospective survey of 207 adult Japanese patients aged between 50 and 59 years who received allo-HSCT from an HLA-identical sibling for the treatment of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS) from February 1998 to November 2002 in 55 participating hospitals. Patients with a history of previous transplantation were excluded from this study.

All the CST and RIST recipients who were eligible in this study were included in each hospital. In Japan, approximately 2000 transplants are performed annually. The types of transplantations are autologous (40%), myeloablative allogeneic (45%), and reduced intensity or nonmyeloablative allogeneic transplantation (15%).[10] RIST recipients are generally treated as clinical studies in Japan. Most patients were incurable with conventional treatments and were considered inappropriate for conventional allo-SCT because they were age >50 years old and/or due to organ dysfunction (generally attributable to previous intensive chemo- and/or radiotherapy).

Data from participating centers were derived from questionnaires distributed to each center. Minimum data required for the inclusion of a patient in this study were age, performance status (PS) according to the Eastern Cooperative Oncology Group (ECOG) criteria before conditioning, medical complications at transplant, diagnosis of underlying diseases, treatment prior to allo-HSCT, disease status at transplant, preparative regimens, GVHD prophylaxis, date of transplant, date of follow-up, disease status at follow-up, development of acute and/or chronic GVHD, date of acute and/or chronic GVHD, date of disease progression/death, and causes of death. We have not collected information on the types of chronic GVHD (limited *vs* extensive).

Definition

Reduced-intensity regimens were defined as reported previously.[11,12] The upper limits of busulfan, melphalan, and TBI were 8, 140 mg/m^2, and 2 Gy for consideration as reduced-intensity preparative regimens. Neutrophil recovery was defined as an absolute neutrophil count of more than 0.5×10^9/l for two consecutive days. Patients were divided into two groups based on their disease status at transplant. Low-risk patients were defined as those with acute leukemia in first remission, CML in chronic phase, and myelodysplastic syndrome refractory anemia. The others were classified into the high-risk group. NRM was defined as death without progression of the underlying disease. Overall survival (OS) was defined as the duration of survival between transplant and either death or last follow-up. Progression-free survival (PFS) was defined as the duration of survival after transplant without disease progression, relapse, and death.

End points and statistical analysis

The primary end points were 2-year OS and PFS. The secondary end points included NRM within 100 days and 1-year of transplant, incidence of acute GVHD, and relapse rates. These end points were compared between CST and RIST recipients. For the analysis of OS and PFS, patients were stratified according to the risk of the underlying disease.

OS and PFS were determined using the Kaplan–Meier method. The last follow-up was on 1st August 2003. Median follow-up of surviving patients was 26.6 months (range, 9.5–63.6). Surviving patients were censored on the last day of follow-up. Acute GVHD was analyzed in patients who achieved initial engraftment. Cumulative incidence of acute GVHD, relapse rates, and NRM was calculated using Gray's method, considering each other event as a competing risk.[13]

Clinical characteristics were compared between CST- and RIST recipients using Fisher's exact test or the Mann–Whitney test. A multivariate Cox proportional hazards model was used to identify independent and significant prognostic factors on OS. The variables entered in each analysis were patient age, sex, primary disease, their risks, PS, and type of preparative regimens (CST *vs* RIST). Acute and/or chronic GVHD was included as a time-dependent covariate. A significance level of 5% was set as the limit for inclusion in the model. Prognostic factors, significant at $P<0.05$ in the stepwise proportional model analysis, were considered to be of importance in influencing survival.

Results

Patient characteristics and transplantation procedures

Types of transplants were CST ($n=137$) and RIST ($n=70$). Patient characteristics and transplantation procedures are shown in Table 1. Between the two groups, there were significant differences in age, sex, types of stem cells, presence of infectious complications at transplant, and PS.

Table 1 Characteristics of patients

Variables	*CST (n = 137)*	*RIST (n = 70)*	*P-value*
Pretransplant factors			
Age			
Median (range)	52 (50–59)	57 (50–59)	<0.01*
Sex			
Male/female	93/44	35/35	0.012*
Underlying diseases			
AML	56 (41%)	33 (47%)	0.42
ALL	27 (20%)	8 (11%)	
CML	34 (25%)	16 (23%)	
MDS	20 (15%)	13 (19%)	
Risk of underlying diseases[a]			
Total: low/high	63/74	25/45	0.18
AML: low/high	19/37	7/26	
ALL: low/high	14/13	5/3	
CML: CP/BC/AP	19/3/4	12/3/2	
MDS: RA/RAEB/RAEB in T/CMMoL	0/0/0/1	1/1/1/1	
Stem cells[b]			
Peripheral blood/bone marrow	57/80	68/2	<0.01*
Complications			
Cardiac impairment	5	3	0.72
Liver dysfunction	10	6	0.78
Respiratory dysfunction	6	6	0.22
Infection	9	11	0.028*
Performance status (PS)			
0–1/2–4	123/12	54/13	0.033*
Sex mismatch			
Donor→Recipient; F→M	35	12	0.17
Transplantation procedures			
Conditioning regimen	12 Gy TBI-based	74 (54%)	
BU/CY-based	51 (37%)		
TBI/BU/CY	12 (9%)		
Cladribine-based		6 (9%)	
Fludarabine-based		61 (87%)	
2 GY TBI-based		3 (4%)	
GVHD prophylaxis			
CSP	3 (2%)	32 (46%)	
CSP + sMTX	124 (91%)	23 (33%)	
FK506 + sMTX	5 (4%)	8 (11%)	
Others	5 (4%)	7 (10%)	

*Statistically significant.
[a]We divided the risk of transplantation into two groups. The low-risk group was as follows: acute myeloid or lymphoid leukemia in first remission, chronic myelogeneous leukemia in chronia phase, and myelodysplastic syndrome refractory anemia.
[b]Four patients were infused both peripheral and bone marrow.
CST = conventional stem cell transplantation; RIST = reduced-intensity stem cell transplantation; TBI = total body irradiation; CY = cyclophosphamide; BU = busulfan; 2-CdA = cladribine; Flu = fludarabine; Mel = melpharan; CSP = cyclosporine; sMTX = short-term methotrexate; AML = acute myeloid leukemia; ALL = acute lymphoid leukemia; CML = chronic myelocytic leukemia; MDS = myelodysplastic syndrome; RA = refractory anemia; RAEB = refractory anemia with excess blasts; RAEB in T = refractory anemia with excess blasts in transformation; CMMoL = chronic myelomonocytic leukemia.

RIST recipients had poorer characteristics than CST recipients.

All the CST recipients received either TBI-based or busulfan/cyclophosphamide-based regimens. RIST regimens were purine analog based ($n = 67$), and 2 Gy TBI based ($n = 3$).

Most CST recipients (129/137) received a combination of calcineurin inhibitors (cyclosporin or tacrolimus) and short-term methotrexate as GVHD prophylaxis, while 32 of the 70 RIST received cyclosporin alone as GVHD prophylaxis (Table 1).

Engraftment

Six CST recipients (9%) died of NRM before engraftment. Neutrophils did not decrease below 0.5×10^9/l in 6 RIST recipients (9%). The other 131 CST recipients (96%) and 64 RIST recipients (91%) achieved primary neutrophil engraftment. The median intervals between transplant and neutrophil engraftment were 15 days (range, 5–27) and 12 days (range, 9–30) in CST and RIST, respectively.

Secondary graft failure developed in three patients (CST 2 and RIST 1) 3–9 months after transplant. All the three patients died of infectious complication during neutropenia.

NRM

In all, 23 CST (17%) and five RIST recipients (7%) died of NRM within 100 days of the transplant. Cumulative incidences of 100 days NRM following CST and RIST were 16% (95% confidence interval (CI), 10–22%) and 7% (95% CI, 1–14%), respectively ($P = 0.040$). As of August 2003, 46 CST (34%) and 16 RIST recipients (23%) died of NRM. The median onset of NRM following CST and RIST was day 95.5 (range, 2–967) and day 254 (range, 49–724), respectively. Cumulative incidences of 1-year NRM following CST and RIST were 31% (95% CI, 23–39%) and 15% (95% CI, 6–23%), respectively ($P = 0.0062$, Figure 1). Primary causes of NRM following CST and RIST are

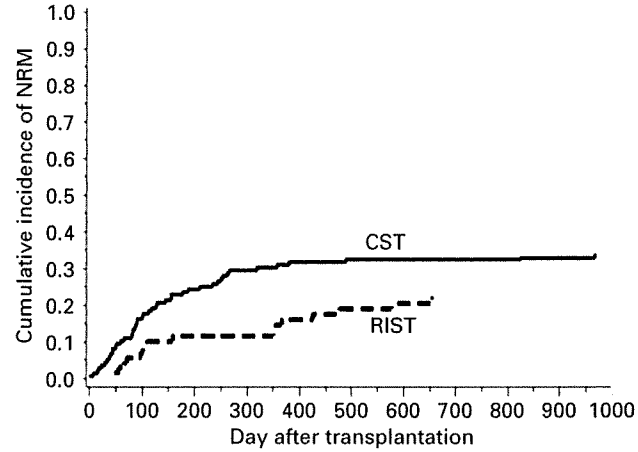

Figure 1 Cumulative incidences of NRM following CST and RIST. Cumulative incidences of NRM following CST and RIST were 31% (95% CI, 23–39%) and 15% (95% CI, 6–23%), respectively.

shown in Table 2. NRM attributable to RRT occurred in 12 and one patient following CST and RIST, respectively.

Graft-versus-host disease

A total of 130 CST and 68 RIST recipients were evaluable. There was no difference in the cumulative incidences of grade II–IV acute GVHD between CST and RIST (Figure 2).

In CST, grade II–IV and grade III–IV acute GVHD occurred in 56 (43%) and 24 patients (18%), respectively. The median onset of grade II–IV acute GVHD was day 23 (range, 3–146 days). GVHD was fatal in 13 of the 56 patients. Of the 104 patients who survived longer than 100 days, 60 patients (58%) developed chronic GVHD.

In RIST, grade II–IV and grade III–IV acute GVHD developed in 38 (56%) and 16 (24%), respectively. The median onset of grade II–IV acute GVHD was day 44 (range, 7–109). GVHD was fatal in 11 of the 38 patients. Of the 57 patients who survived longer than 100 days, 37 (65%) developed chronic GVHD.

Survival

As of August 1, 2003, median follow-ups of surviving patients following CST and RIST were 31.6 months (range, 9.5–63.6) and 20.3 months (range, 9.5–38.4), respectively. Disease-specific outcomes are shown in Table 3.

In all, and low-risk patients, significant differences were not observed in OS between CST and RIST ($P=0.25$, $P=0.69$) (Figures 3 and 4). Among the high-risk patients, there was a significant difference between the two groups ($P=0.044$). The 2-year OS following CST and RIST was 27 and 37%, respectively (Figure 5). There was no significant difference in PFS between CST and RIST among all and low-risk patients ($P=0.39$, $P=0.77$). Among high-risk patients, there was a trend toward better PFS after RIST ($P=0.063$). The 2-year PFS following CST and RIST was 30 and 56%, respectively.

Underlying diseases relapsed in 38 CST and 23 RIST recipients. There was no significant difference in the cumulative incidence of 1-year relapse rates between the two groups; CST 24% (95% CI, 17–32%) and RIST 29% (95% CI, 19–40%) ($P=0.21$, Figure 6).

Risk factors

A univariate analysis revealed that CML ($P<0.0001$), risk of underlying diseases ($P=0.0002$), PS ($P<0.0001$), and

Table 2 Causes of deaths

	CST	*RIST*
Relapse	28	16
Graft-versus-host disease	13	11
Infection	4	0
Bacteria	5	0
Virus	4	1
Fungi		
Idiopathic pulmonary syndrome	5	0
Thrombotic microangiopathy	5	1
Hepatic venoocclusive disease	2	0
Secondary malignancy	2	1
Cardiac failure	1	1
Cerebral infarction	1	0
Others	4	1

CST = conventional stem cell transplantation; RIST = reduced-intensity stem cell transplantation.

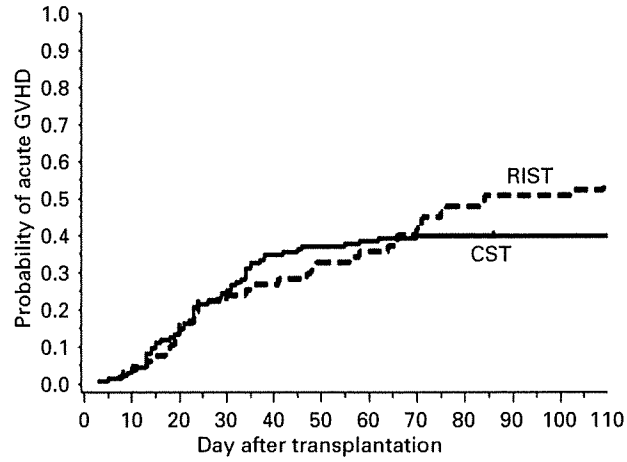

Figure 2 Cumulative incidences of grade II–IV acute GVHD. There was no difference in the cumulative incidences of grades II–IV acute GVHD between CST and RIST.

Table 3 Disease-specific outcomes

Underlying disease	*Type of transplant*	*Number of patients*	*Nmber of patients who died of TRM*	*Number of patients who developed disease progression*	*2-year overall survival*[a]
AML	CST	56	19	20	38.7 (25.8–51.6)
	RIST	33	8	12	69.3 (53.4–85.2)
ALL	CST	27	11	10	33.3 (15.5–51.1)
	RIST	8	2	3	50.0 (15.3–84.7)
MDS	CST	34	8	5	45.0 (23.2–66.8)
	RIST	16	5	3	53.8 (26.8–80.8)
CML	CST	20	8	3	73.4 (58.5–88.3)
	RIST	13	1	5	93.3 (80.8–100)

[a]Each column denotes a rate of 2-year overall survival and its 95% confidence interval.
AML = acute myeloid leukemia; ALL = acute lymphoid leukemia; MDS = myelodysplastic syndrome; CML = chronic myelocytic leukemia; TRM = transplant-related mortality; CST = conventional stem-cell translantation; and RIST = reduced intensity stem cell transplantation.

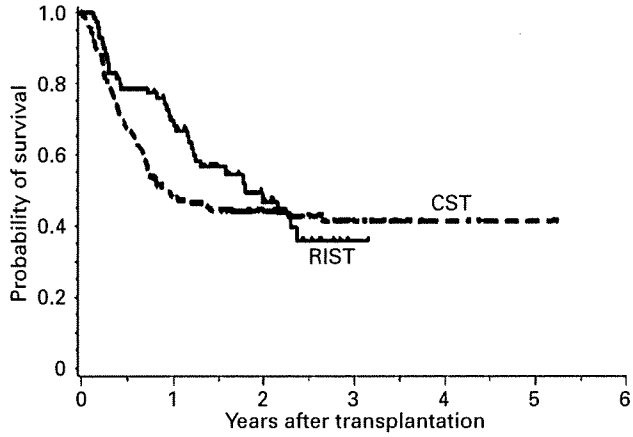

Figure 3 Overall survival (OS) following CST and RIST in all patients. There was no significant difference in OS between CST and RIST ($P=0.25$).

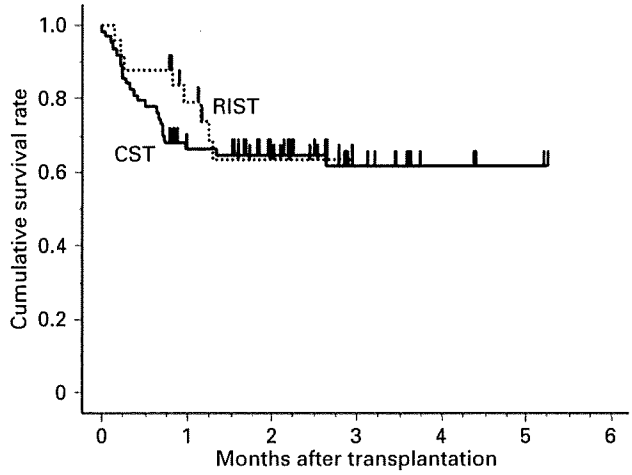

Figure 4 OS following CST and RIST in patients with low-risk diseases. There was no significant difference in OS between CST and RIST ($P=0.69$).

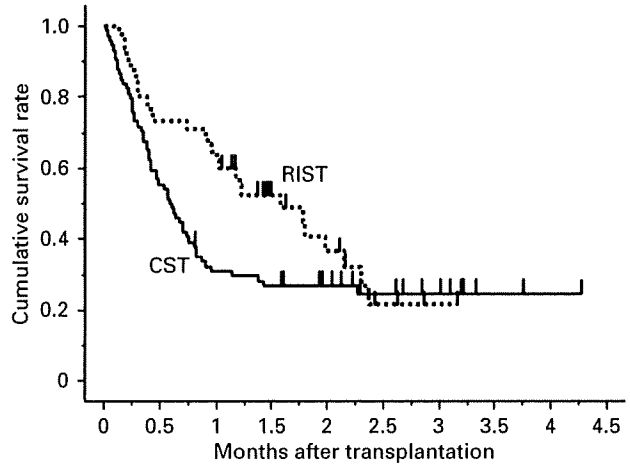

Figure 5 OS following CST and RIST in patients with high-risk diseases. There was a significant difference in OS between CST and RIST ($P=0.044$). The 2-year OS following CST and RIST were 27 and 37%, respectively.

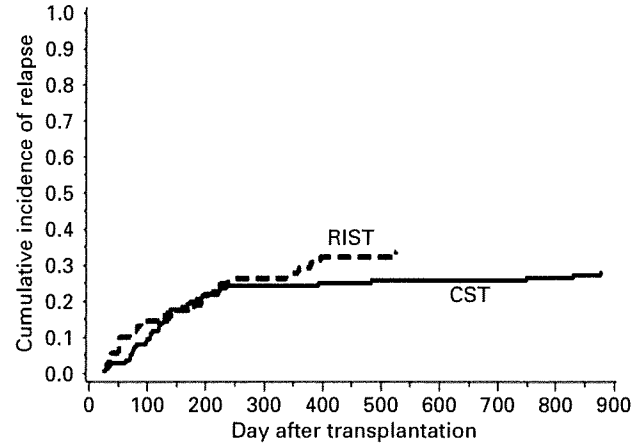

Figure 6 Cumulative incidences of relapse following RIST and CST. There was no significant difference in cumulative incidences of relapse between RIST and CST.

Table 4 Risk factors for overall survival following allogeneic hematopoietic stem-cell tranplantation

	Hazard ratio	*95% confidence interval*	*P-value*
Factors			
Univariate analysis			
Pretransplant factors			
Sex: Female	0.85	0.58–1.25	0.40
Age: 56–59 *vs* 50–51 years	1.11	0.72–1.70	0.63
Donor: female to male recipient	1.22	0.80–1.88	0.35
Disease	1.00		0.0002
CML	0.29	0.15–0.58	
ALL	1.30	0.73–2.31	
AML	0.91	0.55–1.51	
Risk of underlying diseases; high	2.30	1.58–3.37	<0.0001*
PS: 2–4	3.49	2.16–5.64	<0.0001*
Preparative regimen; CST	1.26	0.85–1.88	0.25
Posttransplant factor			
Grade II–IV acute GVHD; presence	2.58	1.76–3.79	<0.0001*
Variables			
Multivariate analysis			
Preparative regimen; CST *vs* RIST	1.92	1.25–2.97	0.003*
PS; 2–4 *vs* 0–1	2.50	1.51–4.16	<0.001
Disease; CML	0.38	0.21–0.69	0.002
Risk of underlying diseases; high	1.85	1.21–2.83	0.004
Grade II–IV acute GVHD; presence	2.57	1.72–3.84	<0.001*

*Statistically significant.
AML = acute myeloid leukemia; CML = chronic myelogeneous leukemia; MDS = myelodysplastic syndrome; ALL = acute lymhoid leukemia; PS = performance status; CST = conventional stem-cell transplantation; GVHD = graft-versus host disease.

development of GVHD ($P<0.001$) were significant risk factors for OS (Table 4). On multivariate analysis, five variables were significant: preparative regimens (CST *vs* RIST) (hazard ratio (HR) = 1.92, 95% CI, 1.25–2.97; $P=0.003$), PS (2–4 *vs* 0–1) (HR = 2.50, 95% CI,

1.51–4.16; $P<0.001$), risk of underlying diseases (HR = 1.85, 95% CI, 1.21–2.83; $P=0.004$), development of grade II–IV acute GVHD (HR = 2.57, 95% CI, 1.72–3.84; $P<0.001$), and CML (HR = 0.38, 95% CI, 0.21–0.69; $P=0.002$).

Discussion

This study suggests that patients with hematologic malignancies aged between 50 and 59 years can achieve remission following RIST as well as CST. There was no significant difference in OS and PFS between RIST and CST (Figure 3). Follow-up of this study was too short to draw a definite conclusion; however, short-term survivals tended to be better in RIST recipients than in CST recipients in the high-risk group (Figure 5). These situations were in contrast to the low-risk group, in which OS and PFS were similar between the two groups (Figure 4). Myeloablative preparative regimens might have been intolerable for high-risk elderly patients. Patients with more progressive diseases might have received CST rather than RIST.

Most physicians believe that it is difficult to control advanced hematologic malignancies with RIST.[5,7] Yet, feasibility of myeloablative preparative regimens has not been fully investigated in patients aged between 50 and 59 years. It is questionable whether intensification of preparative regimens is beneficial for controlling advanced or chemoresistant hematologic malignancies in these patients, because patients with high-risk hematologic malignancies frequently have organ damage due to repeated cytotoxic chemotherapies prior to transplantation.[14] These patients are at high risk of NRM.[15,16] As shown in this study, a myeloablative preparative regimen is not necessarily beneficial in allo-HSCT for elderly patients with high-risk hematologic diseases. In contrast, patients aged between 50 and 59 years in good physical condition are able to tolerate a high-dose preparative regimen. Variables such as CML, low-risk underlying disease, and good PS were independent good prognostic factors for OS. We should tailor preparative regimens considering the patient's condition and risk of the underlying disease.

There are two types of complications associated with allo-HSCT. One is RRT, which often occurs within 30 days of transplantation.[3] The other is GVHD, which is frequently complicated with infections.[14,17] In the present study, there was a significant difference in NRM attributable to RRT between CST and RIST (16 *vs* 7%, $P=0.04$). Reduced-intensity regimens cause less organ damage, contributing to less NRM. These findings were comparable to previous reports.[4,16,18]

GVHD is the most significant concern after allo-HSCT. This study confirmed the previous studies on GVHD following RIST.[19,20] There was no significant difference in the incidence of GVHD between CST and RIST (43 *vs* 56%), and onset of GVHD was delayed in RIST compared with CST. Mortality of GVHD was similar between CST and RIST (23 *vs* 29%). Development of grade II to IV acute GVHD was an independent poor prognostic factor for OS (HR = 2.57, 95% CI, 1.72–3.84; $P<0.001$). These findings demonstrate that GVHD is a significant complication following RIST as well as CST, and that its optimal management awaits further investigation. Balancing GVHD and GVL effects is a delicate issue in allo-HSCT. The augmentation of GVHD prophylaxis may hamper GVL effects, and malignant cells cannot be eradicated by reduced-intensity conditioning alone. Augmentation of GVL effects such as prophylactic donor lymphocyte infusion, vaccination, and administration of cytotoxic T-cells[21] may be beneficial to control residual leukemia without increasing regimen-related mortality. At present, allo-HSCT recipients received uniform GVHD prophylaxis irrespective of the risk of underlying diseases and the patient's condition. In the future, management of GVHD should be optimized considering the risk of the underlying disease and patient conditions.

Relapse is another concern in RIST. This study did not show significant differences in relapse rates between CST and RIST (Figure 6). The unexpectedly low relapse rates following RIST suggest that it has a strong antitumor activity through allogeneic immunity. Augmentation of allogeneic immunity without increasing the intensity of the arative regimen is promising for controlling advanced hematological malignancies. However, late relapse might increase following RIST due to the lack of reduction of leukemic cells by the preparative regimen. It is too early to draw definite conclusions about the incidence of late relapse following RIST based on the results of this study, since allo-HSCT recipients have a considerable risk of relapse within 3 years of transplant[22] and median follow-up of surviving patients was only 26.7 months. Long-term follow-up is required to clarify the prognosis of RIST recipients.

This is a small-sized retrospective study, and we should be careful in interpreting results. The most important was a difference in patient backgrounds between CST and RIST recipients. To minimize unrecognized biases, patients enrolled in this study were limited to those aged between 50 and 59 years who had leukemia or MDS. Yet, RIST recipients were significantly older, and their disease status and PS were significantly worse than CST recipients. These variables influence survival following RIST[7,23] as well as CST.[24–26] Furthermore, there was a wide difference in GVHD prophylaxis between CST and RIST. Most RIST recipients received cyclosporin alone. Short-term methotrexate, and cyclosporin or tacrolimus were given to CST recipients. The median follow-up of surviving patients enrolled in this study was 26.6 months, and thus too short, requiring further observation. Considering these facts, it is difficult to make an accurate comparison between reduced-intensity and myeloablative preparative regimens in this study. We are now planning a prospective randomized study to compare RIST with CST for hematologic malignancies.

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Appendix

This study was conducted at the following institutions under the auspices of the following investigators in Japan: Tanimoto E Tetsuya (Kyusyu University Graduate School of Medical Sciences, Fukuoka), Iida H (Meitetsu Hospital, Aichi), Matsue K (Kameda General Hospital, Chiba), Kato K (Hamanomachi Hospital, Fukuoka), Shinagawa K (Okayama University Medical School, Okayama), Abe Y (Kyusyu University Graduate School of Medical Sciences, Fukuoka), Nakajyo T (Kanazawa University Graduate School of Medicine, Kanazawa), Uike N (National Kyushu Cancer Center, Fukuoka), Okamoto S (Keio University School of Medicine, Tokyo), Hirabayashi N (Nagoya Daini Red Cross Hospital, Aichi), Komatsu T (Tsukuba Memorial Hospital, Ibaraki), Tamaki S (Yamada Red Cross Hospital, Mie), Izumi Y (Kokura Memorial Hospital, Fukuoka), Karasuno T (Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka), Yamane T (Osaka City University, Osaka), Ashida T (Kinki University Hospital, Osaka), Wakita A (Nagoya City University Graduate School of Medical Science, Aichi), Furukawa T (Niigata Uniersity Medical Hospital, Niigata), Teshima H (Osaka City General Hospital, Osaka), Yamashita T (National Defense Medical College Hospital, Saitama), Miyazaki Y (Kansai Medical University Hospital, Osaka), Kobayashi Y & Taniwaki M (Kyoto Prefectural University of Medicine, Kyoto), Kobayashi H (Nagano Red Cross Hospital, Nagano), Ito T (Nihon University School of Medicine, Tokyo), Ishida Y (Iwate Medical University Hospital, Iwate),

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Reduced-Intensity Allogeneic Hematopoietic Stem-Cell Transplantation as an Immunotherapy for Metastatic Colorectal Cancer

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Background. Allogeneic stem-cell transplantation (allo-SCT) can induce curative graft-versus-leukemia reactions for hematologic malignancies through allogeneic immunity. Because the gastrointestinal tract is a target of graft-versus-host disease (GvHD), colorectal cancer might be a candidate for allo-SCT.

Methods. Four patients with metastatic colorectal cancer underwent reduced-intensity stem-cell transplantation (RIST) in the National Cancer Center Hospital between July 2002 and February 2003. Three patients received transplants from an human leukocyte antigen (HLA)-identical related donor, and the remaining patient received selected CD34-positive cells from a two-loci HLA-mismatched donor. The basis of preparative regimen was busulfan 4 mg/kg for 2 days and fludarabine 25 mg/kg for 6 days.

Results. All the patients tolerated the preparative regimen and achieved engraftment without significant toxicities. All developed acute or chronic GvHD. Although serum levels of CA19–9 and carcinoembryonic antigen were transiently elevated after RIST in all the patients, the levels subsequently decreased below the levels from before RIST in all but one patient. Three had measurable lesions before RIST, one achieved partial response, and the others stable disease, which was durable for 120 and 60 days. Three patients died; the causes of death were progressive disease, GvHD, and accident. Postmortem examination was obtained for two patients; in one patient, the peritoneal metastatic lesions macroscopically disappeared, and in the other patient, the supraclavicular lymph node disappeared while the other measurable lesions remained stable.

Conclusions. All the patients showed some evidence suggesting the presence of a graft-versus-tumor effect for colorectal cancer, which should be confirmed in a future prospective trial.

Keywords: Graft-versus-tumor effect, Graft-versus-host disease, Allogeneic immunity, Fludarabine, Carcinoembryonic antigen.

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A new strategy of allogeneic hematopoietic stem-cell transplantation (allo-SCT) using a reduced-intensity preparative regimen (reduced-intensity stem-cell transplantation [RIST]) was developed to decrease regimen-related toxicity (RRT) while preserving an adequate antitumor effect (*1*, *2*). Different pioneering conditioning regimens for RIST have been investigated: those including purine analogs (*1–3*) and total body irradiation (TBI) combined with potent immunosuppressants (*4*). Because clinical studies on RIST have focused in hematologic malignancies, limited information is available on solid tumors (*5*), including renal cell carcinoma (RCC) (*6*), breast (*7*, *8*), lung (*9*), ovarian (*10*), and colon cancer (*11*).

Because the epithelium is the target of graft-versus-host disease (GvHD), any types of carcinoma arising from the epithelial tissues such as keratinocytes, fibroblasts, exocrine glands, hepatobiliary trees, and gastrointestinal tract are theoretically susceptible to a graft-versus-tumor (GvT) effect. Murine models have provided some evidence for an allogeneic immune-mediated antisolid-tumor effect (*12*). Porter et al. (*13*) conducted a phase I clinical trial to determine whether a GvT effect could be observed after primary donor lymphocyte infusion (DLI) without stem-cell support in patients with primary cancers. Three of the four patients with acute GvHD and late chimerism responded to DLI. Eibl et al. (*14*) demonstrated that allogeneic T cells collected during GvHD could mediate a cytotoxic effect against breast cancer cell lines. Childs et al. (*15*) reported the results of 19 patients who underwent RIST for metastatic RCC. Seven patients achieved complete response and seven partial response (PR). The tumor response was associated with the development of GvHD. These results suggest that a GvT effect does exist in RIST for a variety of solid tumors, although long-term prognosis remains unknown.

Colorectal cancer is the second cause of cancer death (*16*), and prognosis for patients with unresectable and refractory to chemotherapy metastasis is poor. Development of novel therapeutic strategy is required. Because the gastrointestinal tract is the common target of GvHD, colorectal cancer might be sensitive to allogeneic immunity. We report four

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patients who underwent RIST for metastatic colorectal cancer.

PATIENTS AND METHODS

Patients

Between July 2002 and February 2003, four patients with colorectal cancer underwent RIST. All the patients had progressive metastatic disease, which was refractory to any conventional anticancer therapies including fluorouracil and irinotecan. Peripheral blood stem-cells were mobilized by subcutaneous injection of granulocyte colony-stimulating factor, 10 μg/kg, and harvested with the target cell dose of greater than 2.0×10^6/kg CD34+ cells.

The RIST program was approved by the Institutional Review Board of the National Cancer Center Hospital. A written informed consent was obtained from all the patients and donors.

Conditioning and Donors

The basis of preparative regimen was busulfan 4 mg/kg for 2 days and fludarabine 30 mg/kg for 6 days. Patient 1 received additional TBI 4.0 Gy in two fractions. Because a human leukocyte antigen (HLA)-identical donor was not available for patient 1, he underwent RIST from his two-loci mismatched brother-in-law with CD34+ cell selection. The number of simultaneously infused CD3+ cells was 2.5×10^4/kg. The remaining three patients had HLA-identical related donors: a sibling (patient 2 and 4) and an offspring (patient 3). Patient 2 received additional rabbit antithymocyte globulin (ATG) 2.5 mg/kg for 2 days to ensure durable engraftment. Since October 2002, ATG was omitted from the preparative regimen in the protocol of RIST for solid tumors, and ATG was not given to patient 3 and 4.

Engraftment and Management of GvHD

Recipient-donor chimerism in peripheral blood mononuclear cells (PBMC), T cells, and granulocytes were analyzed monthly after transplantation using polymerase chain reaction of informative short tandem repeat (*17*). GvHD prophylaxis was intravenous cyclosporine 3 mg/kg or oral cyclosporine 6 mg/kg from day −1. Because the incidence of acute GvHD is approximately 10% in RIST from an HLA-matched sibling after an ATG-containing regimen in our institution (*18*), we tapered cyclosporine early and rapidly over a 2-week period to enhance a GvT effect. The diagnosis of GvHD was made in concert with biopsy of the skin or the gastrointestinal tract. Acute and chronic GvHD were graded according to the consensus criteria (*19*, *20*). Grade II to IV acute GvHD was treated with 2 mg/kg per day of methylprednisolone in addition to cyclosporine.

Supportive Measures

All the patients stayed in reverse isolation in a laminar airflow-equipped room and received prophylaxis with trimethoprim/sulfamethoxazole or pentamidine inhaler and ciprofloxacin against *Pneumocystis carinii* and bacterial infection, respectively. Fluconazole was administered for antifungal prophylaxis with a dose ranging from 200 to 400 mg/day. Herpes virus prophylaxis with acyclovir was also given as previously described (*21*). Cytomegalovirus pp65 antigenemia was routinely monitored once a week. When antigenemia was detected, preemptive therapy with ganciclovir was initiated as previously reported (*22*).

Evaluation of Tumor Response

All patients underwent computed tomography (CT) scanning before RIST and monthly after RIST to evaluate the tumor response. Serum levels of CA19–9 and carcinoembryonic antigen (CEA) were monitored weekly using chemiluminescent enzyme immunoassays (Lumipulse CA19–9-N, and Lumipulse CEA-N, Fujirevio, Tokyo, Japan, respectively).

Three patients (patient 2, 3, and 4) had measurable lesions before RIST, and tumor response was defined according to the response evaluation criteria in solid tumor (RECIST) (*23*) in these patients. Postmortem examination was available in the other two patients (patient 1 and 4).

RESULTS

Engraftment, Regimen-Related Toxicities, and GvHD

All the patients tolerated the preparative regimens with minimum RRTs. The neutrophil count reached 0.5×10^9/L on day 10 in patients 1, 3, and 4 and on day 12 in PATIENT 2. On day 30, chimerism analysis in PBMC showed full donor-type chimerism in all the patients. Grade II to IV acute GvHD developed in 3 patients, on median of 23 (20–47) days after RIST. Patient 1 developed grade IV acute GvHD after DLI to induce a GvT effect. The patient died of multiorgan failure caused by acute GvHD on day 62. The signs of GvHD in the remaining two patients spontaneously disappeared without additional immunosuppressant. Among two patients who survived over 100 days, both had chronic extensive GvHD.

Tumor Responses and Outcomes

Tumor responses were shown in Table 1. Although serum levels of CA19–9 and CEA transiently elevated after RIST in all the patients, the levels subsequently decreased below the levels before RIST in all but one patient (patient 4). Three patients had measurable lesions before RIST; one (patient 3) achieved PR, one (patient 2) stable disease (SD), which was durable for 120 days, and one (patient 4) progressive disease (PD).

Three patients died; the causes of death were GvHD (patient 1), PD (patient 2), and accident (patient 4). Postmortem examination was obtained for two patients; in patient 1, the peritoneal metastatic lesions macroscopically disappeared while microscopically detectable lesions remained. In patient 4, the supraclavicular lymph node metastasis disappeared while the other measurable lesions remained stable.

CASE PRESENTATION

Patient 1

A 44-year-old man underwent RIST from his two-loci mismatched brother-in-law in July 2002 for the treatment of metastatic rectal cancer. He had peritoneal metastasis, and no measurable lesions were documented before RIST. After preparative regimen consisting of fludarabine, busulfan, and 4 Gy TBI, he received CD34-positive stem cells.

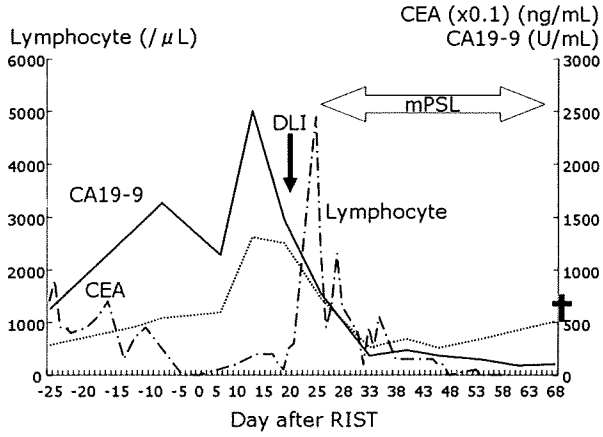

FIGURE 1. Clinical courses of patient 1. After engraftment and donor lymphocyte infusion (DLI), serum levels of tumor markers declined, and necropsy revealed that residual tumors in the peritonea shrunk. CEA, carcinoembryonic antigen; methylprednisolone, mPSL; RIST, reduced-intensity stem-cell transplantation.

His clinical courses was uneventful until day 21, when we added 4.0×10^6/kg CD3+ cells to improve immune recovery. The patient developed maculopapular rash, watery diarrhea, and jaundice on day 23. Based on the histopathologic examination of the skin, a diagnosis of grade IV acute GvHD was made. We initiated steroid-pulse therapy, but his condition deteriorated rapidly. He finally died of multiorgan failure caused by acute GvHD on day 62. Postmortem examination showed that the metastatic lesions in the peritoneum disappeared, whereas residual adenocarcinoma cells were observed by histopathologic examination.

Serum levels of CEA and CA19–9 increased from 45.7 ng/mL and 1388 U/mL before transplant to 131.0 ng/mL and 2507 U/mL on day 15, respectively. After DLI, serum levels of both values decreased rapidly (Fig. 1).

Patient 2

A 52-year-old woman underwent RIST from an HLA-identical sibling in August 2002 for metastatic rectal cancer. The metastatic lesions involved the liver and the both lungs. The liver lesions were measurable targets (Fig. 2). Because she had allergic reaction to the contrast agents on day 30, she received CT after day 60 without contrast agents. The patient had not developed acute GvHD, and we tapered off cyclosporine from day 35 until day 49 to enhance a GvT effect. Her clinical courses were uneventful until day 90, when she developed mucositis caused by chronic GvHD. To further augment the GvT effect, we withheld any immunosuppressive agents. The oral lesions had subsided spontaneously until day 120. Sequential abdominal CT scans failed to show progression until day 120 (Fig. 2). However, repeated CT scan on day 150 revealed an extensive progression of the metastatic lesions in the liver and the pleura (Fig. 2). She died of disease progression on day 172. An autopsy was denied by her family. The best and final responses were SD and PD, respectively. The duration of SD was 120 days.

Serum levels of CEA and CA19–9 were increased with transient decrease after engraftment until chronic GvHD developed and continued to decrease while the oral lesions persisted. Their serum levels were inversely associated with the severity of chronic GvHD (Fig. 3).

Patient 3

A 59-year-old woman underwent RIST from her HLA-identical offspring in October 2002. She presented metastatic

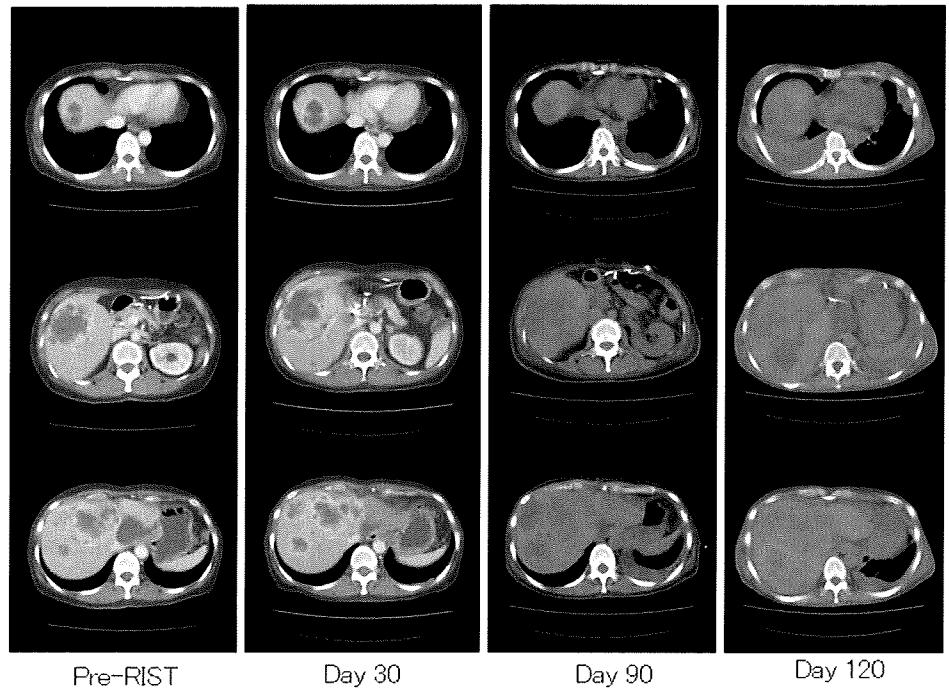

FIGURE 2. Changes of the metastatic lesions of the liver of patient 2. The metastatic lesions in the lung remained stable till day 120. However, metastatic lesions expanded with carcinogenic pleuritis.

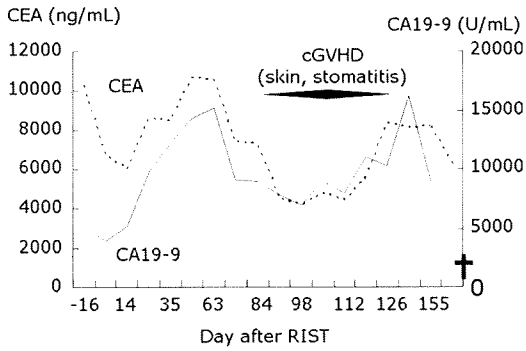

FIGURE 3. Clinical courses of patient 2. Serum levels of CEA and CA19–9 were inversely associated with the severity of chronic graft-versus-host disease (cGvHD).

lesions in the liver and the lungs. The pulmonary lesions were defined as measurable targets. After engraftment on day 10, she developed maculopapular rash on the trunk on day 20, which was histopathologically diagnosed as grade II acute GvHD. Mild but significant disease progression was detected on the follow-up CT scan on day 30 (Fig. 4). Because the cutaneous GvHD had resolved spontaneously by day 45, we started tapering of cyclosporine from day 50. The follow-up CT scans of the chest on day 60 showed a significant decrease in the size of target lesions. Thereafter, lung metastatic lesions progressed gradually again after the disappearance of acute GvHD, and donor lymphocyte containing 6.4×10^7/kg CD3+ cells were infused on day 181. She developed extensive chronic GvHD involving the skin and the lung, and size of the metastatic lesions decreased again. As of March 2004 (17 months after RIST), the best and final responses are both PR. Serum levels of CEA and CA19–9 were inversely associated with the development of GvHD (Fig. 5).

Patient 4

A 52-year-old woman with advanced colon cancer received RIST from an HLA-identical sibling in January 2003. Her multiple metastatic lesions included the liver, the lung, the peritoneal, multiple lymph nodes, and the residual colon. Liver metastases and lymph nodes were defined as measurable lesions. New metastatic lesions appeared in the lung on day 60, and the best response was PD. We tapered cyclosporine rapidly to enhance a GvT effect. With the development of grade II skin GvHD on day 38, the metastatic lesion of the supraclavicular lymph node decreased in size, and serum levels of CEA and CA19–9 decreased (Table 1). She was stable until day 88, when she accidentally fell on the floor. She suffered from fatal head injury. An autopsy revealed complete disappearance of the metastatic lesion in the supraclavicular lymph node. The other lesions were stable in size. The final response was PD.

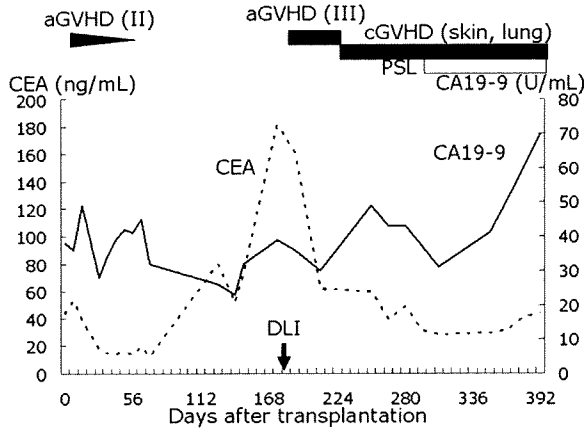

FIGURE 4. Evaluation of metastatic lesions in the lung of patient 3. Repeated computed tomography scans of the chest showed that metastatic lesions shrunk after the development of GvHD.

DISCUSSION

Allo-SCT has a considerable risk of transplant-related mortality. Development of optimal preparative regimens for colorectal cancer is an important issue for future clinical trials. With RIST procedure, all of the four patients achieved durable engraftment within 30 days of transplant. These findings suggest that our reduced-intensity regimen is sufficient to assure engraftment in RIST for metastatic colorectal cancer. Because most patients with advanced colorectal cancer are heavily treated with chemotherapeutic agents such as fluorouracil and irinotecan (*24*), the risk of graft rejection will be low compared with other solid tumors, which are rarely treated by cytotoxic agents.

RRT should be critically evaluated in developing allo-SCT. All of our patients had been treated heavily by surgery or cytotoxic chemotherapy before RIST. However, we demonstrated that RRT was mild in all the organs and that all the patients tolerated the procedure well. Grade 3 to 4 toxicity according to the Bearman's criteria (*25*) were not observed in any patients. We omitted the use of methotrexate to enhance a GvT effect. Because methotrexate causes mucosal damages, this might have contributed to the amelioration of gastrointestinal damages in this study. Our experience indicates that fludarabine/busulfan-based preparative regimens are safe and tolerable for patients with advanced colorectal cancer.

Concerning the efficacy of RIST for colorectal cancer, all the patients showed some evidence of antitumor effects. Serum levels of CEA and CA19–9 decreased significantly, either after engraftment (patient 1) or development of GvHD (patient 2 and 3). There might be a debate as to whether serum levels of CEA and CA19–9 can be reliable surrogate markers of treatment response (*26*). However, one patient (patient 3) achieved durable PR (*23*). In another patient (patient 1), postmortem examination showed marked reduction of the tumor size. Although patient 4 had supraclavicular lymph node, this lesion disappeared after development of acute GvHD. Although different conditioning regimens and GvHD prophylaxis methods were used in this study, these findings indicate that allo-SCT is promising for metastatic colorectal cancer.

We have demonstrated the feasibility of allo-SCT for colorectal cancer; however, there are some problems to be discussed. First, the precise mechanism of the GvT effect on solid tumors including colorectal cancer remains unknown. Disease regression associated with cyclosporine withdrawal, complete donor chimerism, and GvHD provides evidence