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Outcomes of patients with acute leukaemia who relapsed after reduced-intensity stem cell transplantation from HLA-identical or one antigen-mismatched related donors

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Summary

The characteristics of relapse following reduced-intensity stem-cell transplantation (RIST) remain to be clarified. We reviewed the medical records of 19 patients with acute leukaemia [acute myeloid leukaemia (AML), 16; acute lymphoblastic leukaemia (ALL), 3] who relapsed after RIST from related donors using purine-analogue-based regimens. Their median age was 55 years (range, 29–65 years). Median interval between RIST and relapse was 4·9 months (range, 1·8–24·9 months). Three chose not to receive interventions. The remaining 16 patients received withdrawal of immunosuppression ($n = 3$), chemotherapy ($n = 2$), donor lymphocyte infusion ($n = 10$) and second transplantation ($n = 7$), alone ($n = 9$) or in combination ($n = 7$). Four are alive with a median follow-up of 27·6 months (range, 16·0–28·9 months); three in remission and one in relapse. The 2-year overall survival after relapse was 28·9%. Causes of death in 15 patients included progressive disease ($n = 7$), graft-*versus*-host disease ($n = 5$) and infections ($n = 3$). Cumulative incidences of relapse-related and non-relapse-related deaths at 2 years after relapse were 37% and 32% respectively. Two prognostic factors were identified on univariate analysis: age [$P = 0·017$; hazard ratio (HR), 1·16; 95% confidence interval (CI), 1·03–1·32], and ALL as underlying disease ($P = 0·011$; HR, 10·4; 95% CI, 1·73–62·4). Some AML patients who relapse after RIST achieve durable remission with allogeneic immunotherapy-based interventions; however they carry a significant risk of non-relapse mortality.

Keywords: graft-*versus*-host disease, graft-*versus*-leukaemia effect, donor lymphocyte infusion, second allogeneic transplantation, non-myeloablative haematopoietic stem cell transplantation.

The relapse of underlying haematological malignancies after allogeneic haematopoietic stem-cell transplantation (allo-SCT) is a significant problem. Adults with acute leukaemia who relapsed after allo-SCT had a median survival of 3–4 months if no treatment was given (Mortimer *et al*, 1989). Approaches to treating patients in relapse after allo-SCT include rapid tapering of immunosuppressive agents, donor lymphocyte infusion (DLI), re-induction chemotherapy and second transplantation. Standard chemotherapy sometimes results in complete remission (CR), but long-term disease-free survival (DFS) is unlikely, because of regimen-related toxicity (RRT) and recurrence (Frassoni *et al*, 1988). Although a second allograft produces sustained molecular remission in a proportion of patients, transplant-related mortality (TRM) is high with 100-d mortality rates of 25–50% and a DFS of 10% (Mrsic *et al*, 1992; Radich *et al*, 1993). Poor prognostic factors after second allo-SCT include an interval between the procedures of <1 year, resistance to re-induction chemotherapy, older age and poor performance status (Michallet *et al*, 2000). Immunotherapy, such as cessation of immunosuppressive agents and DLI, is beneficial for patients with early relapse or those with chronic myeloid leukaemia (CML). DLI can result in a high CR rate of 60% in CML; however, it is less effective in acute leukaemia with an estimated rate of CR of only 15%

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(Collins *et al*, 1997). Graft-*versus*-leukaemia (GVL) effects seem to be weak, or rapid growth of leukaemic clones exceeds an effective immune response, which manifests 5–6 weeks after DLI (Kolb *et al*, 1995; Collins *et al*, 1997).

A new strategy for transplantation, reduced-intensity stem-cell transplantation (RIST) (Slavin *et al*, 1998; Giralt *et al*, 2001), has been developed to reduce RRT while preserving an adequate GVL effect. It appears to be promising for a variety of haematological malignancies, if disease activity is controlled prior to transplant (Michallet *et al*, 2001). In contrast, most physicians believe that RIST is insufficient in controlling advanced haematological malignancies, and small pilot studies showed that RIST was unsuccessful for advanced haematological malignancies (Giralt *et al*, 1997; Nagler *et al*, 2000). Relapse is a significant concern in RIST; however, little is known of the prognosis of patients who relapse after RIST, or of the value of interventions aimed at re-inducing remission (Bethge *et al*, 2003). We investigated the clinical characteristics of patients with acute leukaemia who relapsed following RIST.

Patients and Methods

Data collection

We retrospectively reviewed the medical records of 19 patients who had a relapse of acute leukaemia of 111 patients who achieved morphological CR following RIST from a human leucocyte antigen-identical or one antigen-mismatched related donor at the National Cancer Centre Hospital and Toranomon Hospital between September 1999 and March 2003. All patients had acute leukaemia that was incurable with conventional treatments, and were considered inappropriate for conventional allo-SCT because of age >50 years and/or organ dysfunction. Transplantation procedures, supportive care and chimaerism analysis were reported previously (Saito *et al*, 2002; Hamaki *et al*, 2004). Bone marrow examination was performed 1 and 3 months after transplantation, or when relapse was suspected. Minimal residual disease (MRD) in bone marrow was monitored by flow cytometry, cytogenetics and reverse transcription-polymerase chain reaction (RT-PCR), when MRD markers were available. The intervention selected for relapsed acute leukaemia after RIST was based on patient condition. All patients and donors gave their written informed consent in accordance with the requirements of our Institutional Review Board.

Definition

Diagnosis of acute leukaemia was based on the World Health Organization classification (Brunning *et al*, 2001a,b). Treatment responses were evaluated according to Cheson *et al* (2003). CR was defined as morphological complete remission: patients achieved the morphological leukaemia-free state and had an absolute neutrophil count $>1{\cdot}0 \times 10^9$/l. Recovery of platelets of $\geq 100 \times 10^9$/l was not required.

Graft-*versus*-host disease (GVHD) was diagnosed by clinical judgment as well as skin or digestive tract biopsies to support the clinical diagnosis. Acute and chronic GVHD were graded according to the consensus criteria (Sullivan *et al*, 1991; Przepiorka *et al*, 1995).

Endpoints and statistical analysis

The aims of this study were (i) to describe clinical characteristics of relapse following RIST, and (ii) to identify its prognostic factors. The probability of overall survival was calculated using the method of Kaplan and Meier. Overall survival was defined as the duration of survival between the first relapse after RIST and either death or last follow-up. Cumulative incidences of relapse-related and non-relapse-related mortality were calculated as reported previously (Gooley *et al*, 1999). An initial analysis comparing potential prognostic factors was carried out using the log-rank test. Acute GVHD was included as a time-dependent covariate. Multivariate analysis was not conducted because of the small number of patients. $P < 0{\cdot}05$ were considered significant.

Results

Patient characteristics

Nineteen patients relapsed after RIST. Their backgrounds are shown in Table 1. Clinical characteristics of relapse after first RIST are shown in Table 2. All 19 patients had achieved morphological remission after first RIST, while platelets counts had not normalized ($>100 \times 10^9$/l) in four patients (case 6, 12, 16 and 19). In all 19 patients, MRD analyses using cytogenetics and flow cytometry were negative at morphological remission after first RIST. MRD was monitored by RT-PCR in two patients (*AML1-MTG8* and *E2A/PBX1* in cases 6 and 18 respectively). In these patients, the chimaeric transcripts had been positive at morphological remission after first RIST.

Treatment of relapse

Treatment of relapse was heterogeneous and varied depending on the individual patients' condition (Table 2).

No treatment

Three patients (cases 4, 11 and 14) chose not to receive further intervention after relapse; one patient (case 11) is currently alive in non-remission without any intervention, and the remaining two (cases 4 and 14) died of underlying disease.

Intervention

The other 16 patients received the firstline treatments. At diagnosis of relapse, three patients (cases 5, 17 and 18) who were still receiving immunosuppression had the drugs discon-

Table I. Characteristics of patients (n = 19) who relapsed after RIST.

Age (years) [median (range)]	55 (29–65)
Sex (male/female)	15/4
Reasons for RIST	
Age >50 years/organ dysfunction	17/2¶
Numbers of cytotoxic chemotherapies prior to first RIST	5 (0–7)
Diagnosis at first RIST	No. of patients
Acute lymphoblastic leukaemia	
Second complete remission	3
Acute myeloid leukaemia	
Second complete remission	2
Induction failure	4
Relapse	8
Myelodysplastic syndrome§	
Refractory anaemia	1
Refractory anaemia with excess blasts	1
Conditioning regimen	
Fludarabine/busulphan*	15
Fludarabine/melphalan†	2
Cladribine/busulphan‡	2
Graft-*versus*-host disease prophylaxis	
Ciclosporin	19
Donor (matched sibling/one-antigen mismatched related)	13/6
History of GVHD prior to relapse (0-I/II-IV)	16/3
Interval between RIST and relapse (months)	4·9 (1·8–24·9)

*The preparative regimen comprised fludarabine 30 mg/m^2 for 6 d and busulphan 4 mg/kg for 2 d. Three patients received rabbit ATG (Thymoglobulin; Imtix-Sangstat, Lyons, France) 2·5 mg/kg for two consecutive days.

†The preparative regimen comprised fludarabine 30 mg/m^2 for 6 d and melphalan 80 mg/m^2 for 1 d.

‡The preparative regimen comprised cladribine 0·11 mg/kg for 6 d and busulphan 4 mg/kg for 2 d. Two received rabbit ATG (Thymoglobulin; Imtix-Sangstat, Lyons, France) 2·5 mg/kg for two consecutive days.

§These two patients are those described in ‡ above.

¶The complications included renal dysfunction and hepatic dysfunctions.

tinued. Two patients (cases 17 and 18) received secondary intervention (chemotherapy and DLI) following rapid tapering of cyclosporine. The chemotherapy regimen comprised cytarabine and idarubicin. The other patient (case 5) refused to receive secondary intervention, and died 9·0 months after relapse.

The remaining 13 patients received one or more of the following treatments, based on the physicians' discretion after consideration of their general status, aggressiveness of the underlying disease and presence of comorbidity.

Two patients (cases 1 and 2) received re-induction chemotherapy comprising cytarabine and idarubicin. Both patients underwent secondary interventions including second RIST from the same donor and DLI, and achieved durable remission.

Eight patients (cases 7–10, 13, 15, 16 and 19) received DLI from their original donors. The median number of DLI was one (range, 1–3). The median dose of lymphocytes transfused was 0·8 × 10^8/kg (range, 0·4–1·4 × 10^8/kg). Two patients (cases 9 and 10) achieved durable remission. Another two (cases 8 and 15) and one patient (case 19) died of acute GVHD and infection during myelosuppression respectively. The other three patients (cases 7, 13 and 16) did not achieve remission after DLI, and underwent second RIST as secondary intervention. The stem cell sources were granulocyte colony-stimulating factor-mobilized peripheral blood (case 7), marrow from a matched unrelated donor (case 13), and umbilical cord blood (case 16). One patient (case 13) achieved durable remission. Two patients (cases 7 and 16) died of septicaemia and progressive disease respectively.

Three patients (cases 3, 6 and 12) underwent second RIST as first intervention. All the three patients tolerated transplantation procedures. One (case 3) achieved durable remission, but died of chronic GVHD. The other two patients (cases 6 and 12) did not achieve remission; one (case 6) was alive in relapse 22·3 months after second RIST, and the other (case 12) died of disease progression.

Responses and survival

Six of the 19 patients (cases 1–3, 9, 10, 13) achieved complete morphological remission after first and/or second interventions. The association between GVHD and response was evaluable in 11 patients. All the four patients with acute GVHD (cases 1, 2, 9, 10) achieved CR, while five of the seven patients without GVHD showed progressive disease (cases 3, 5, 6, 12, 13, 16, 18).

In these six patients, duration of CR following the interventions was longer than that from the first RIST to relapse (Table 2). Four of the 19 patients were alive at a median follow-up of 27·6 months (range, 16·0–28·9 months); three in CR, and one in relapse. The 2-year overall survival rate after relapse was 28·9% (95% confidence interval; 7·3–50·5%) (Fig. 1).

Causes of deaths

Causes of death in 15 patients included progressive disease (n = 7), acute GVHD (n = 3), chronic GVHD (n = 2), and infections (n = 3; Table 2).

Cumulative incidences of relapse-related and non-relapse-related deaths at 2 years after post-transplant relapse were 37% and 32% respectively.

Prognostic factors

Results of univariate analysis on overall survival are shown in Table 3.

Discussion

The present study shows that some patients with relapsed acute myeloid leukaemia (AML) after RIST can achieve remission

Table II. Outcomes of relapse following RIST.

Case	Age (years)	Sex	Underlying disease	Disease status at 1st RIST	Grade II-IV acute GVHD after 1st RIST	Chimaerism analysis after 1st RIST (% of donor type)			Haematologic findings at relapse after 1st RIST			Interval between RIST and relapse (months)	Intervention		Response	Acute GVHD after intervention	Survival after relapse (months)	Outcomes/ cause of death
						Day 30	Day 60	Day 90	Blast in marrow (%)	Leucocytes in peripheral blood ($\times 10^9$/l)	Blast in peripheral blood (%)		First	Second				
1	55	F	AML	Relapse	Absent	100	Not performed	Not performed	44	2·4	0	10·9	Chemotherapy	SCT*	CR	Grade 4	15·3	Acute GVHD
2	29	M	AML	Relapse	Absent	100	100	100	7	3·8	4	15·7	Chemotherapy	DLI	CR	Grade 2	30·6	Leukaemia
3	58	M	AML	2nd remission	Absent	100	100	100	32	3·9	0	4·5	SCT*	None	CR	None	23·8	Chronic GVHD
4	53	M	AML	Induction failure	Absent	45	Not performed	95	56	3·2	4	17·4	None	None	PD	NA	11·3	Leukaemia
5	65	M	AML	2nd remission	Absent	100	56	Not perfomed	26	3·2	1	2·6	Tapering of ciclosporin	None	PD	None	9·0	Leukaemia
6	55	F	AML	Relapse	Grade 2	82	92	100	59	5·3	39	7·5	SCT*	None	PD	None	27·8+	Alive in relapse
7	57	M	AML	Relapse	Absent	100	100	85	63	4·8	5	12·4	DLI	SCT*	ND†	None	2·5	Septicaemia
8	51	M	AML	Relapse	Grade 2	100	88	100	15	2·2	0	4·9	DLI	None	ND†	Grade 4	1·1	Acute GVHD
9	52	M	AML	Induction failure	Absent	Not performed	Not performed	Not performed	48	1·4	5	4·0	DLI	None	CR	Grade 3	16·0+	Alive in remission
10	49	M	MDS	RA	Absent	100	100	100	9	3·1	0	24·9	DLI	None	CR	Grade 2	30·2	Chronic GVHD
11	64	F	MDS	RAEB-2	Absent	88	82	92	59	3·7	10	14·8	None	None	PD	NA	27·4+	Alive in relapse
12	56	M	AML	NR	Absent	65	0	Not performed	6	2·7	0	2·5	SCT*	None	PD	None	4·9	Leukaemia
13	53	F	AML	Relapse	Absent	Not performed	Not performed	not performed	30	14·1	0	6·0	DLI	UBMT‡	CR	None	28·9+	Alive in remission
14	58	M	ALL	2nd remission	Absent	100	100	Not performed	22	7·6	3	7·9	None	None	PD	NA	0·5	Leukaemia
15	55	M	ALL	2nd remission	Grade 2	100	Not performed	Not performed	54	27·6	7	2·8	DLI	None	ND†	Grade 4	1·8	Acute GVHD
16	54	M	AML	Induction failure	Absent	88	100	100	Dry tap	3·6	8	3·3	DLI	UCBT§	PD	None	5·6	Leukaemia
17	54	M	AML	Relapse	Absent	100	90	Not performed	8	8·9	1	2·9	Tapering of ciclosporin	SCT*	ND*†	None	3·0	Invasive aspergillosis

18	63	M	ALL	2nd remission	Absent	100	Not performed	Not performed	87	3·9	9	3·1	Tapering of ciclosporin	DLI	PD	None	1·8	Leukaemia
19	60	M	AML	Relapse	Absent	100	66	Not performed	5	1·6	1	1·8	DLI	None	ND†	None	0·7	Pneumonia

RIST, reduced intensity stem cell transplantation; AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukaemia; SCT, stem-cell transplantation; CR, complete remission; PD, progressive disease; ND, not determined; UCBT, umbilical cord blood transplantation; SCT, stem cell transplantation; UBMT, unrelated bone marrow transplantation; DLI, donor lymphocyte infusion.
*Donors and preparative regimens were same as the first transplantation.
†These patients died of infection or GVHD during neutropenia following DLI or chemotherapy. We were not able to determine the responses to interventions for post-transplant relapses.
‡The patient was transplanted from a matched unrelated donor following fludarabine 30 mg/m^2 for 6 d, busulphan 4 mg/kg for 2 d and 4 Gy total body irradiation.
§The patient underwent umbilical cord blood transplantation following fludarabine 25 mg/m^2 for 6 d, melphalan 80 mg/m^2 and 4 Gy total body irradiation.

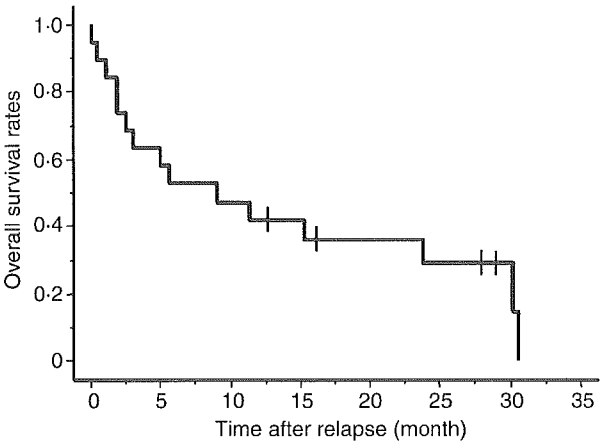

Fig 1. Probability of survival for 19 patients who relapsed after reduced-intensity stem-cell transplantation. The 2-year overall survival rate after relapse was 28·9% (95% confidence interval; 7·3–50·5%).

Table III. Univariate analyses on overall survival.

Factor	Relative risk (95% confidence interval)	*P*-value
Age	1·16 (1·03–1·32)	0·017*
Sex (female *versus* male)	0·17 (0·022–1·34)	0·093
Interval from diagnosis to transplant (months)	0·91 (0·81–1·02)	0·10
HLA disparity (Matched *versus* mismatched)	1·73 (0·37–8·06)	0·48
Use of ATG at first RIST (ATG *versus* non-ATG)	1·52 (0·50–4·68)	0·46
Underlying disease (Lymphoid *versus* myeloid)	10·4 (1·73–62·4)	0·011*
Disease status at first RIST (Non-remission *versus* remission)	0·63 (0·21–1·94)	0·42
Grade I-IV acute GVHD (absent/present)	1·39 (0·38–5·01)	0·72

*Statistically significant
ATG, antithymocyte globulin; RIST, reduced intensity stem cell transplantation; GVHD, graft-*versus*-host disease.

and even long-term survival. Seven patients (cases 1–3, 6, 9, 10 and 13) achieved remission after relapse following RIST. The remission duration after secondary interventions was longer than a year and longer than the duration between the first RIST and relapse. This observation supports that the interventions after relapse has improved the outcomes. In contrast to those who achieved long-term survival with currently available interventions, patients with acute lymphoblastic leukaemia (ALL) and older patients had poor outcomes. The three patients with ALL who underwent RIST in the second CR died 2·8–7·9 months after relapse. As ALL probably has low susceptibility to allogeneic immunity, long-term survival cannot be expected after relapse following RIST as well as

conventional myeloablative allo-SCT (Kolb *et al*, 1995; Slavin *et al*, 1995; Collins *et al*, 1997). In the present study, six of the eight patients who survived longer than 12 months after relapse following RIST were younger than 55 years old. The outcomes of older patients are poor, probably because of the high biological malignancy of leukaemia at advanced ages and because of the reduced tolerance to GVHD and chemotherapies. Further investigations are necessary to improve the treatment outcome in these patients.

The appropriate intervention for the relapsed leukaemia after RIST has not been established. The primary physicians decide the treatment according to the conditions of the primary malignancies and performance status of the patients. Of the seven patients who survived longer than 1 year after the secondary interventions (cases 1–3, 6, 9, 10 and 13), two received chemotherapies and four underwent a second RIST. As it is unlikely that the long-term remission was maintained solely by the effects of chemotherapies and conditioning regimens before RIST, allogeneic immunity must have contributed to suppression of AML progression. While two underwent a second RIST from a different donor, it should be noted that five patients achieved long-term remission after the second RIST or DLI from the same donor as in the first RIST. The outcomes contrast with the observation that some AML patients who relapse after conventional myeloablative allo-SCT can achieve remission by secondary interventions, such as DLI, but the remission is short. Although the reason is unclear, the delay in the manifestation of GVHD/GVL effects after RIST, compared with conventional myeloablative allo-SCT, may partly explain the difference. The median onset of GVHD was 2 months after RIST with our conditioning regimens, which was 1 month later than that after conventional allo-SCT (Nakai *et al*, 2003). As the tumour reduction by the conditioning regimens for RIST is limited and allogeneic immunity manifests late after RIST compared with conventional allo-SCT, the probability of early relapse may be high after RIST. When AML relapses after RIST, leukaemic cells have not been exposed enough to allogeneic immunity and may not be resistant to allogeneic immunity. While the duration from conventional allo-SCT to relapse is associated with the prognosis, that is not necessarily true of RIST (Mortimer *et al*, 1989; Levine *et al*, 2002). The GVL effects of DLI for AML manifest 1 month later. As a GVL effect plays a crucial role in reducing the risk of relapse after RIST for AML and myelodysplastic syndrome (Martino *et al*, 2002), DLI from the identical donor may be promising for slowly progressive AML and/or in cases where AML progression can be suppressed by chemotherapies or the conditioning regimen for RIST.

The present study showed that interventions for relapsed acute leukaemia following RIST carry a significant risk of TRM; five and three patients died of GVHD and infection respectively. Of particular note is that four of the seven patients who underwent second RIST died of TRM. These findings were in contrast to previous reports (Bethge *et al*, 2003; Feinstein *et al*, 2003). In the report by the Seattle group on the outcomes of relapsed haematological malignancies after non-myeloablative stem-cell transplantation (NST) using 2 Gy total body irradiation with or without fludarabine, 46 of 66 patients who underwent interventions after relapse died: 41 of progressive disease and five of TRM. The Seattle researchers also reported that the rate of TRM was 6% in patients who received NST as second allo-SCT (Feinstein *et al*, 2003). TRM in their studies (Bethge *et al*, 2003; Feinstein *et al*, 2003) was much lower than that in our study, although the comparison of these studies with different patient characteristics is not appropriate. TRM after interventions for patients with relapsed acute leukaemia after RIST is high, at least partly because the conditioning regimens for RIST are more intense than those for NST. Our study suggests that control of GVHD and management of infection are important to improve prognosis of those patients with acute leukaemia who relapse after RIST. Intensification of GVHD prophylaxis using potent immunosuppressive agents will contribute to improving GVHD-related outcomes (Kottaridis *et al*, 2000; Nakai *et al*, 2003); however, use of these agents might diminish a GVL effect, and could increase the rate of relapse and infections (Chakraverty *et al*, 2002). It should be noted that responses to interventions for relapse after allo-SCT are frequently associated with the development of GVHD (Luznik & Fuchs, 2002; Bethge *et al*, 2003). Further studies are warranted to establish a strategy which enhances a GVL effect without causing GVHD.

Although this study is hampered by its small size and heterogeneity of patients' background, the results are still informative. It demonstrated that some patients with relapsed AML after RIST can survive with allogeneic immunotherapy. These observations provide a rationale for continuing our clinical trials on this treatment for relapsed AML, which should be modified to focus on minimizing toxicities, preventing GVHD and enhancing a GVL effect. There were no significant differences in prognosis between patients who were given DLI alone and those who underwent second RIST. Considering the high TRM of second RIST, we should be careful in choosing RIST as intervention for relapsed acute leukaemia after RIST.

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Allografting

Reduced-intensity hematopoietic stem-cell transplantation for malignant lymphoma: a retrospective survey of 112 adult patients in Japan

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Summary:

We conducted a nation-wide survey of 112 adult Japanese patients who underwent reduced-intensity stem cell transplantation (RIST) from 1999 to 2002. Underlying diseases included indolent ($n=45$), aggressive ($n=58$) and highly aggressive lymphomas ($n=9$). Median age of the patients was 49 years. A total of 40 patients (36%) had relapsed diseases after autologous stem cell transplantation and 36 patients (32%) had received radiotherapy. RIST regimens were fludarabine-based ($n=95$), low-dose total body irradiation-based ($n=6$) and others ($n=11$). Cumulative incidences of grade II–IV acute graft-versus-host disease (GVHD) and chronic GVHD were, respectively, 49 and 59%. Cumulative incidences of progression and progression-free mortality were 18 and 25%, respectively. With a median follow-up of 23.9 months, 3-year overall survival rates were 59%. A multivariate analysis identified three significant factors for progression, which are history of radiation (relative risk (RR) 3.45, confidential interval (CI) 1.12–10.0, $P=0.03$), central nervous system involvement (RR 6.25, CI 2.08–20.0, $P=0.001$) and development of GVHD (RR 0.28, CI 0.090–0.86, $P=0.026$). RIST may have decreased the rate of transplant-related mortality, and GVHD may have induced a graft-versus-lymphoma effect. However, whether or not these potential benefits can be directly translated into improved patient survival should be evaluated in further studies.
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Allogeneic stem cell transplantation (allo-SCT) is a curative treatment for advanced malignant lymphoma.[1,2] Initially, the benefit of allo-SCT was thought to be largely dependent on the intensity of the conditioning regimen prior to transplantation. Recently, an additional benefit of allo-SCT is derived from an allogeneic graft-versus-malignancy (GVM) effect that reduces the likelihood of disease relapse following transplantation.[3–6] With high regimen-related toxicity (RRT) and treatment-related mortality (TRM), high-intensity, myeloablative conditioning regimens are being replaced by reduced-intensity or nonmyeloablative conditioning regimens. The preliminary data suggest improved survival rates due to decreased TRM.[7] Reduced-intensity stem cell transplantation (RIST) is potentially a curative treatment for heavily pretreated, elderly patients; however, little information is available regarding the outcomes of RIST for malignant lymphoma. We retrospectively analyzed the outcome of RIST. The purpose of this study was to elucidate the treatment-related toxicity of RIST and to evaluate the impact of a potential graft-versus-lymphoma (GVL) effect.

Patients and methods

Data collection

We conducted a nation-wide retrospective survey of 112 adult Japanese patients who underwent RIST from 1999 to 2002 in 32 participating hospitals. All of the RIST recipients who were eligible in this study were included in each hospital. In Japan, approximately 2000 transplants are performed annually. The types of transplantation are autologous (40%), myeloablative allogeneic (45%), and

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 $>1.0 \times 10^9$/l or absolute neutrophil counts $>0.5 \times 10^9$/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.

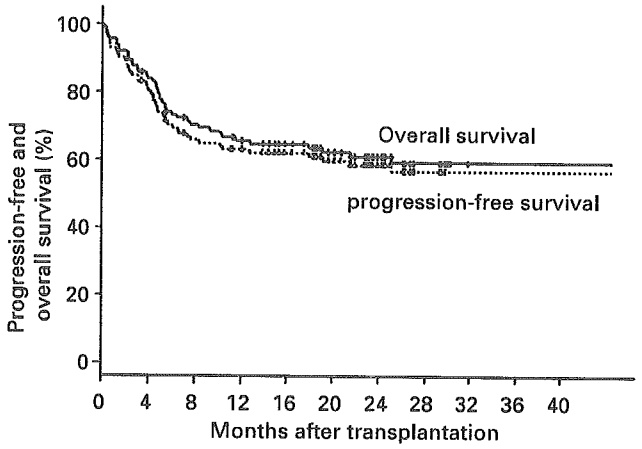

Figure 1 Overall survival (OS) and progression-free survival (PFS) following transplant. The 3-year OS and PFS were 59.0% (95% CI, 55.0–64.0%) and 56.5% (95% CI, 51.5–61.5%), respectively.

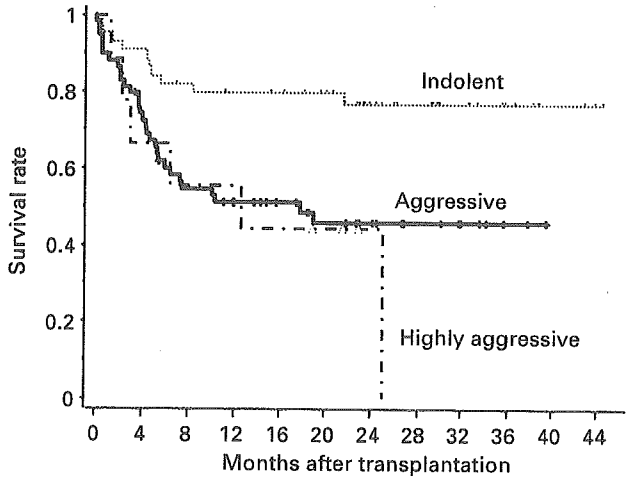

Figure 2 Overall survival (OS) following transplant according to the histological subtypes. The 3-year OS according to the histological subtypes was indolent 79% (95% CI, 67–91%), aggressive 48% (95% CI, 35–61%), and highly aggressive 0%; follicular 81% (95% CI, 69–92%), diffuse large B-cell 31% (95%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

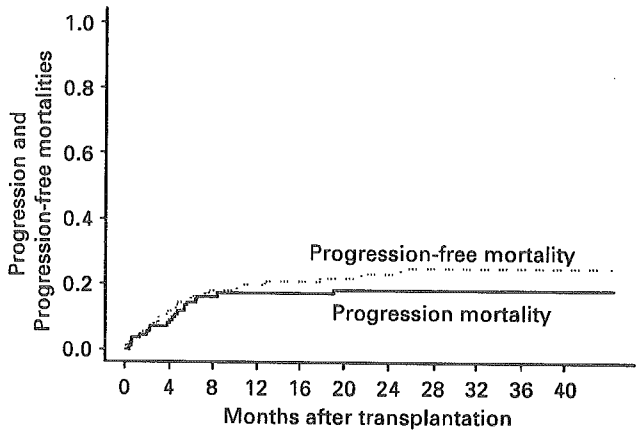

Figure 3 Cumulative incidences of disease progression mortality and transplant-related mortality (TRM). Cumulative incidences of disease progression mortality and TRM at 3 years were 18.3 and 25.2%, respectively.

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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Conditioning regimens

Reduced-intensity stem-cell transplantation for adult acute lymphoblastic leukemia: a retrospective study of 33 patients

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Summary:

Efficacy of reduced-intensity stem-cell transplantation (RIST) for acute lymphoblastic leukemia (ALL) was investigated in 33 patients (median age, 55 years). RIST sources comprised 20 HLA-identical related donors, five HLA-mismatched related, and eight unrelated donors. Six patients had undergone previous transplantation. Disease status at RIST was first remission ($n=13$), second remission ($n=6$), and induction failure or relapse ($n=14$). All patients tolerated preparatory regimens and achieved neutrophil engraftment (median, day 12.5). Acute and chronic graft-versus-host disease (GVHD) developed in 45 and 64%, respectively. Six patients received donor lymphocyte infusion (DLI), for prophylaxis ($n=1$) or treatment of recurrent ALL ($n=5$). Nine patients died of transplant-related mortality, with six deaths due to GVHD. The median follow-up of surviving patients was 11.6 months (range, 3.5–37.3 months). The 1-year relapse-free and overall survival rates were 29.8 and 39.6%, respectively. Of the 14 patients transplanted in relapse, five remained relapse free for longer than 6 months. Cumulative rates of progression and progression-free mortality at 3 years were 50.9 and 30.4%, respectively. These findings suggest the presence of a graft-versus-leukemia effect for ALL. RIST for ALL is worth considering for further evaluation.

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Allogeneic stem-cell transplantation (allo-SCT) represents a curative option for acute lymphoblastic leukemia (ALL). Myeloablative therapy with high-dose radiochemotherapy or chemotherapy alone followed by allo-SCT is the most powerful method for eradicating leukemic cells. Patients who experience relapse are candidates for allo-SCT, since few patients who have relapsed are cured using conventional chemotherapies.[1] In adult patients with ALL in second complete remission (CR2), most studies have indicated a disease-free survival (DFS) rate of approximately 30%.[2,3] In contrast, allo-SCT for adult patients with ALL in first complete remission (CR1) is controversial, since allo-SCT is associated with significant transplant-related mortality (TRM). Whether allo-SCT is beneficial for ALL in CR1 remains unclear.[4,5] Some patients with certain high-risk factors, including specific cytogenetic abnormalities, should be offered allo-SCT in CR1.[6]

Elimination of leukemic cells following allo-SCT is attributable to two processes: the direct effect of chemo-radiotherapy; and graft-versus-leukemia (GVL) effects. The role of GVL effects is less defined in ALL. Some case reports have demonstrated durable remission following donor lymphocyte infusion (DLI),[7,8] and several studies have shown that a GVL effect associated with graft-versus-host disease (GVHD) contributes to a reduction in ALL relapse after allo-SCT.[9–11] However, these case reports and small studies might have suffered from patient selection bias. Two retrospective studies have suggested that GVL effects for ALL are weaker than those for myeloid

malignancies.[9,12] In a study of 44 ALL patients treated using DLI, only three achieved remission ⩾1 year, and 3-year overall survival (OS) was 13%.[13] DLI showed only limited benefit in the treatment of recurrent ALL after allogeneic CST.

A new strategy for transplantation using reduced-intensity conditioning for stem-cell transplantation (RIST) has been developed to reduce regimen-related toxicities while preserving antileukemic effects.[14] This strategy decreases the risk of nonrelapse mortality and allows transplantation in elderly patients or those with organ dysfunction. Since RIST is based on a GVL effect, usefulness for ALL has not yet been clarified. Most prospective studies on RIST have included small numbers of patients with ALL, ranging from 1 to 9.[14–19] Five studies have reported using ⩾10 patients with ALL.[20–24] Two retrospective multicenter studies focused on RIST for ALL.[21,22] Arnold *et al*[21] reported that four of 22 patients were alive and disease-free at 5–30 months after RIST, and that seven died of TRM. In all four patients who survived following RIST, underlying diseases were in CR at the time of transplant. Martino *et al*[22] summarized the features of patients from four prospective studies, including patients with advanced ALL. OS and TRM were 31 and 23%, respectively. Relapse rate was 33% in patients transplanted in CR, and 60% in those with overt disease.

The present study describes our experience of 31 patients with ALL who were treated using RIST.

Patients and methods

Diagnosis and classification of clinical subtypes of ALL

ALL was diagnosed on the basis of cytology, karyotype, and immunophenotyping of marrow cells. Patients had received induction/consolidation chemotherapy in accordance with local protocols.

Patient characteristics

A total of 33 patients with ALL underwent RIST at 18 transplant centers between October 2000 and November 2003. Patients who were not candidates for conventional myeloablative transplantation were considered for RIST. All patients satisfied one or more of the following inclusion criteria: age greater than 50 years, prior autologous transplantation with high-dose chemotherapy, and non-hematologic organ dysfunction. They all gave informed consent. Patient characteristics are shown in Table 1.

Preparatory regimen and transfused stem cells

Transplantation procedures are shown in Table 1. Preparatory regimens comprised fludarabine(Flu)/busulfan with ($n=3$) or without rabbit anti-thymocyte globulin (ATG) ($n=9$), or with total body irradiation (TBI) at 4–8 Gy ($n=2$), or with both ATG and TBI ($n=1$),[14,17] Flu/melphalan ($n=8$),[15] another fludarabine-based regimen ($n=4$),[25] cladribine-based regimen ($n=2$), TBI at 2 Gy ($n=2$),[16] and others ($n=2$). Either T-cell depletion or CD34-positive cell selection was performed in all patients. The median number of transfused cells was 3.9×10^6 $CD34^+$ blood stem cells/kg (range, 0.98–7.5×10^6 cells/kg) or 3.2×10^8 marrow-nucleated cells/kg (range, 0.43–5.3 cells/kg).

Table 1 Patient characteristics

Variables	*n*
Median age; range (years)	55; 17–68
Sex; Male/female	11/22
Performance status[a]; 0–2/3–4	29/3
Lineage; T/B	1/32
Cytogenetics	
Normal	10
t(9:22)(q34;q11)	14
t(1;19)(q23;p13.3)	1
Hypodiploid	1
Others	5
Not available	2
Disease status	
Complete remission first/second	13/6
Refractory primary	5
First/second/third relapse	4/4/1
Indication for RIST[b]	
Age	19
Organ dysfunction	9
Previous transplantation	6
Poor performance status	3
Heavily treated	1
Infection	4
Donor	
HLA-identical related	20
HLA-mismatch related	5
Unrelated	8
Stem cells; Marrow/blood	9/24
Preparative regimens	
Fludarabine/busulfan and related	15
Fludarabine/melphalan	8
Other fludarabine-based	4
Cladribine-based	2
Others	4
GVHD prophylaxis	
Cyclosporin alone	8
Cyclosporin/methotrexate	17
Tacrolimus/methotrexate	3
Others	5

[a]Performance status defined according to ECOG criteria. Information on PS was unavailable for one patient.
[b]Nine patients displayed two indications for RIST.

Definition

Reduced-intensity regimens were defined as reported previously.[26,27]

The day of neutrophil engraftment was defined as the first of three consecutive days on which absolute neutrophil count was >500 cells/μl. The day of platelet engraftment was defined as the first of seven consecutive days on which the platelet count was >20 000/μl without platelet transfusion.

The diagnosis of GVHD was based on clinical evidence with histologic confirmation whenever possible. Acute GVHD within the first 100 days after transplantation was graded according to standard criteria.[28,29] Patients who survived at least 100 days were evaluated for chronic GVHD. Chronic GVHD was graded as limited (localized skin or single organ involvement) or clinically extensive.

TRM was defined as death without progression of underlying disease. OS was defined as duration of survival between transplant and either death or last follow-up. Progression-free survival (PFS) was defined as duration of survival after transplant without disease progression, relapse, or death. When ALL recurred before engraftment, the underlying disease was considered to have progressed on the day of transplant.

Study endpoints

The major end point was 1-year PFS following RIST. Secondary end points included incidence of relapse, nonrelapse mortality, incidence and severity of GVHD, engraftment, complications, frequency of DLI, and OS. Patients were considered to have died of nonrelapse cause if no evidence of disease relapse or progression was apparent. Data were analyzed as of March 1, 2004.

Statistical analysis

Cumulative incidences of progression and progression-free mortality were evaluated using Gray's method, considering each other risk as a competing risk.[30] OS and PFS were estimated using the Kaplan–Meier methods. Potential confounding factors considered in the analysis were age, sex, donor type (HLA-matched related donor *vs* alternative donor), stem cell source (bone marrow (BM) *vs* peripheral blood stem cells (PBSC)), HLA-mismatch, disease status, conditioning regimen, and development of grades II–IV acute GVHD. To evaluate the influence of these factors on PFS, proportional hazard modeling was used, treating the development of acute GVHD as a time-dependent covariate. Factors associated with at least borderline significance ($P<0.10$) on univariate analyses were subjected to multivariate analysis using backward stepwise proportional-hazard modeling. Values of $P<0.05$ were considered statistically significant.

Results

Engraftment

Neutrophil counts did not decrease below 500/μl in one patient, while autologous blasts recovered in two patients. The remaining 30 patients achieved neutrophil engraftment within a median interval of 12.5 days (range, 8–26 days). Among 31 patients with neutrophil recovery, two patients never experienced thrombocytopenia and three patients died without platelet engraftment. The other 26 patients achieved platelet engraftment within a median of 13 days (range, 8–50 days).

GVHD and other complications

Two patients died within 100 days of transplant. Causes of death were diffuse alveolar damage and hemophagocytic syndrome.

A total of 15 patients (45%) developed grades II–IV acute GVHD. Maximal ratings were grade II ($n=9$), III ($n=3$), or IV ($n=3$). Acute GVHD was fatal in five patients. Of the 25 evaluable patients (64%) who survived longer than 100 days, 16 developed chronic GVHD.

Infections were documented in seven patients. Causative organisms included methicillin-resistant *Staphylococcus aureus* ($n=3$), *Escherichia coli* ($n=1$), *Pseudomonas aeruginosa* ($n=1$), *Candida tropicalis* ($n=1$), and *Aspergillus* species ($n=1$). Infections were fatal in four patients.

Noninfectious complications other than GVHD occurred in eight patients, and comprised: hepatic veno-occlusive disease ($n=2$); chronic subdural hematoma ($n=1$); hemophagocytic syndrome ($n=1$); interstitial pneumonitis ($n=1$); pleural effusion ($n=1$); idiopathic pulmonary syndrome ($n=1$); and engraftment syndrome ($n=1$).

A total of 21 patients died, and disease progression was absent in nine of these cases. Causes of death included GVHD ($n=6$), idiopathic pulmonary syndrome ($n=1$), hemophagocytic syndrome ($n=1$), and sepsis ($n=1$). The remaining 12 patients died after leukemic progression, with eight deaths due to progressive disease, and other four attributable to complications associated with DLI (acute GVHD $n=2$) or second RIST (thrombotic microangiopathy, $n=2$; hepatic veno-occlusive disease, $n=2$).

Response to RIST

In all, 12 of 14 patients transplanted during relapse achieved durable remission (Table 2). In the other two patients, ALL recurred immediately after neutrophil engraftment. In seven patients with t(9:22)(q34;q11), minimal residual disease was monitored with reverse transcriptase-polymer chain reaction assay (RT-PCR) using bcr-abl-specific primers. Molecular remission was confirmed in four of the seven patients (Patients 4, 13, 26, and 27), with durations of 4.2, 4.6, 11.1, and 27.6 months, respectively.

OS and PFS

As of March 2004, 12 patients were alive in CR ($n=9$) and relapse ($n=3$) with a median follow-up of 11.6 months (range, 3.5–37.3 months). Probability of 2-year PFS and OS was 18.6% (95% confidence interval (CI), 2.4–34.9%) and 29.7% (95% CI, 11.7–47.7%), respectively (Figure 1). The median duration of overall survival was 177 days (range, 40–1119 days). Cumulative rates of progression and progression-free mortality at 3 years were 50.9 and 30.4%, respectively (Figure 2).

Actuarial 1-year PFS rates were 30.6% (95% CI, 7.7–53.5%) for the 19 patients transplanted in CR1/CR2 and 28.6% (95% CI, 4.9-52.2%) for the 14 patients transplanted in relapse or induction failure ($P=0.26$) (Figure 3).