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Graft failure following reduced-intensity cord blood transplantation for adult patients

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Received 7 July 2005; accepted for publication 8 September 2005

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Summary

We reviewed the medical records of 123 adult reduced-intensity cord blood transplantation (RI-CBT) recipients to investigate the clinical features of graft failure after RI-CBT. Nine (7·3%) had graft failure, and were classified as graft rejection rather than primary graft failure; they showed peripheral cytopenia with complete loss of donor-type haematopoiesis, implying destruction of donor cells by immunological mechanisms rather than poor graft function. Three of them died of bacterial or fungal infection during neutropenia. Two recovered autologous haematopoiesis. The remaining four patients underwent a second RI-CBT and developed severe regimen-related toxicities. One died of pneumonia on day 8, and the other three achieved engraftment. Two of them died of transplant-related mortality, and the other survived without disease progression for 9·0 months after the second RI-CBT. In total, seven of the nine patients with graft failure died. The median survival of those with graft failure was 3·8 months (range, 0·9–15·4). Graft failure is a serious complication of RI-CBT. As host T cells cannot completely be eliminated by reduced-intensity preparative regimens, we need to be aware of the difficulty in differentiating graft rejection from other causes of graft failure following RI-CBT. Further studies are warranted to establish optimal diagnostic and treatment strategies.

Keywords: graft failure, graft rejection, chimaerism, second transplantation, graft-*versus*-host disease.

Graft failure is a serious complication after allogeneic stem cell transplantation (allo-SCT) (Georges & Storb, 2000). It comprises two clinical entities: primary graft failure and graft rejection (Hows, 1991). Primary graft failure implies that donor-derived haematopoiesis has not been obtained during a specific time interval after transplantation. Graft rejection implies the complete destruction of donor cells by an immunological mechanism (Georges & Storb, 2000). Three treatment options are available for primary graft failure: administration of haematopoietic growth factor (Nemunaitis *et al*, 1990), a booster infusion of donor haematopoietic stem cells (Bolger *et al*, 1986; Davies *et al*, 1994), and an infusion of previously harvested autologous haematopoietic stem cells (Mehta *et al*, 1996). In the management of graft rejection, another immunosuppressive conditioning is necessary before the second infusion of haematopoietic stem cells (Georges & Storb, 2000). It is critical to differentiate graft rejection from other causes of graft failure since the treatment approaches are different according to the aetiology.

Some recent reports have demonstrated the feasibility of cord blood transplantation (CBT) using reduced-intensity regimens (RI-CBT) for adult patients with advanced haematological diseases (Barker *et al*, 2003; Miyakoshi *et al*, 2004). While graft failure is probably a significant complication in RI-CBT as well as in CBT using myeloablative preparative regimens (Laughlin *et al*, 2004; Rocha *et al*, 2004), little information is available and its optimal diagnostic procedures and treatments remain unknown. We investigated the clinical features and determined the incidence of graft failure following RI-CBT.

doi:10.1111/j.1365-2141.2005.05832.x

Patients and methods

Study patients

Between January 2002 and August 2004, 123 patients with haematological diseases or solid tumours underwent RI-CBT at Toranomon Hospital (Table I). All patients had incurable diseases by conventional treatments, and were not candidates for conventional allo-SCT because of the lack of a suitable sibling or unrelated donor, age over 50 years, and/or organ dysfunction (often attributable to previous intense chemotherapy and/or radiotherapy). The RI-CBT procedures have been reported previously (Miyakoshi *et al*, 2004). All patients provided written informed consent in accordance with the requirements of the Institutional Review Board.

Table I. RI-CBT patient characteristics.

Variable	Number
Age (years), median (range)	55 (17–79)
Sex (male/female)	70/53
Primary diseases	
Acute lymphoblastic leukaemia	18
Acute myeloid leukaemia	35
Chronic myeloid leukaemia	3
Adult T-cell leukaemia	15
Myelodysplastic syndrome	13
Malignant lymphoma	27
Multiple myeloma	4
Solid tumour	2
Aplastic anaemia	6
Risk of underlying diseases (high/low)	93/30
Preparative regimens	
Flud 125 mg/m^2 + L-PAM (80–140 mg/m^2) + TBI (2–8 Gy)	111
Flud 150 mg/m^2 + BU 8 mg/kg + TBI (4–8 Gy)	9
Flud 150 mg/m^2 + BU 8 mg/kg	1
Flud 150 mg/m^2 + L-PAM 140 mg/m^2	2
Number of infused nuclear cells, median (range), $\times 10^6$/kg	28 (17–52)
Number of infused CD34$^+$ cells, median (range), $\times 10^6$/kg	0·074 (0·001–0·33)
HLA match	
6/6	2
5/6	20
4/6	101
GVHD prophylaxis (cyclosporine alone/ tacrolimus alone)	89/34

Flud, fludarabine; L-PAM, melphalan; BU, busulphan; TBI, total body irradiation; GVHD, graft-*versus*-host disease.
The following conditions were defined as low/high risk: acute leukaemia in complete remission, chronic myelogenous leukaemia in chronic phase, malignant lymphoma in complete remission, multiple myeloma in complete remission, myelodysplastic syndrome in refractory anaemia (RA), and aplastic anaemia as low/high risk. All other conditions were considered as high risk.

Definition

Graft failure comprises two clinical entities: primary graft failure and graft rejection. We defined the former as the combination of peripheral cytopenia and marrow hypoplasia for >60 d of RI-CBT, with the existence of a donor-type haematopoiesis (mixed or complete donor chimaerism). The latter was defined as the complete loss of donor-type haematopoiesis occurring anytime after transplantation. Peripheral cytopenia was defined as an absolute neutrophil count (ANC) $<0{\cdot}5 \times 10^9$/l and platelet count $<20 \times 10^9$/l. Engraftment was defined as an ANC $> 0{\cdot}5 \times 10^9$/l with mixed or complete donor chimaerism for three consecutive days. Regimen-related toxicity (RRT) was evaluated by the Common Terminology Criteria for Adverse Events version 3·0 (CTCAE v3·0) (Trotti *et al*, 2003). Transplant-related mortality (TRM) was defined as any death without progression of underlying diseases.

Diagnosis and management of graft failure

Chimaerism was assessed using fluorescent *in situ* hybridisation in sex-mismatched donor-recipient pairs. In sex-matched pairs, polymerase chain reaction (PCR) for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10% (Thiede *et al*, 1999). Whole blood CD3$^+$ cells or marrow was assessed for chimaerism at the time of granulocyte engraftment. When engraftment was delayed, chimaerism was assessed before day 30. Patients with graft rejection generally underwent a second RI-CBT. When donor-type haematopoiesis was documented, granulocyte colony-stimulating factor (G-CSF) was given until day 60 or engraftment.

Statistical analysis

We used a univariate analysis to compare the differences between the patients with graft failure and those who achieved engraftment. *P*-values of <0·05 were considered significant.

Results

Engraftment

Ninety-six patients achieved engraftment at a median day 20 (range, 11–53 d). The other 27 patients had not achieved engraftment. One patient with adult T-cell leukaemia relapsed before engraftment (day 35). Graft failure was diagnosed in nine patients (7·3%). All of them were classified as having graft rejection. The other 17 patients died of TRM on a median of day 19 (range, 4–47). The causes of death included sepsis ($n = 12$), haemorrhage ($n = 2$), pneumonia ($n = 1$), invasive aspergillosis ($n = 1$) and multiple organ failure ($n = 1$).

Chimaerism was assessed before engraftment in 23 of 123 patients. Complete donor chimaerism was documented in 12 patients, of whom seven died before engraftment and five

achieved engraftment. Two patients had mixed chimaerism; one achieved engraftment and the other died before engraftment. The other nine had complete host chimaerism or graft rejection.

Clinical features of graft failure

Characteristics of the nine patients with graft failure and those who achieved engraftment are shown in Table II. There was no significant difference in patient characteristics between the two groups.

Of the nine with graft failure, three patients died of bacterial or fungal infection during neutropenia 28, 31, and 35 d after RI-CBT; two had sepsis and one had pneumonia.

Four patients underwent the second RI-CBT following fludarabine 125 mg/m^2, either melphalan 80 mg/m^2 or busulphan 8 mg/kg, and total body irradiation (TBI) 2–4 Gy. The median interval between the first and second RI-CBT was 46·5 d (range, 32–58 d). All of them developed grade 3–5 RRT. One died of pneumonia on day 8, and three achieved engraftment at median day 17 (range, 15–32 d) after the second RI-CBT. Of these, two died of TRM (thrombotic microangiopathy on day 57, and sepsis on day 160). The other patient survived without disease progression 9·0 months after the second RI-CBT.

Autologous haematopoiesis recovered in two patients. One patient finally died of disease progression 6·0 months after RI-CBT, and the other, who had refractory acute myeloid leukaemia, was alive in remission 15·4 months after RI-CBT.

As of May 2005, seven of the nine with graft failure had died, and the median survival of the nine patients was 3·8 months (range, 0·9–15·4 months).

Discussion

This study demonstrated that graft failure is a significant problem in RI-CBT, and is associated with a high mortality. It is important to differentiate graft rejection from other causes of graft failure, given that there are different treatment options for these conditions. Genetic markers have been used to make the distinction in allo-SCT using myeloablative preparative regimens, as they permit the determination of whether the marrow cells, peripheral blood neutrophils, and T cells are of donor or host origin (Georges & Storb, 2000). However, such a strategy may not be helpful in the diagnosis of graft failure following RI-CBT, where host T cells are not completely eliminated by preparative regimens. Our chimaerism study suggested that considerable amounts of host T cells might remain even in patients with graft failure because of poor graft function. Thus, we cannot technically rule out graft rejection in patients with primary graft failure after reduced-intensity stem cell transplantation (RIST). This is the dilemma in treating graft failure after RI-CBT.

Incidence of graft failure in our patients was comparable with that in myeloablative CBT (Laughlin *et al*, 2001). The high incidence of graft failure might be because of the features of cord blood rather than the conditioning regimens. The infused cell dose was reportedly a major determinant of neutrophil recovery (Laughlin *et al*, 2001; Wagner *et al*, 2002). However, the infused cell dose in the patients with graft failure was not significantly different from those in the patients who engrafted. The observations suggest that other risk factors might be associated with the development of graft failure rather than infused cell dose in our patients. However, it remains unknown whether the conventional risk factors for graft failure after allo-SCT, such as virus infections (Georges & Storb, 2000), could significantly affect graft failure in RI-CBT. Future studies are warranted.

The treatment of graft failure after RI-CBT has not been established. In cases of poor graft function that is unresponsive to haematopoietic growth factors, re-transplantation of another cord blood unit is necessary as a haematopoietic stem cell booster is unavailable in CBT. The selection of conditioning regimens is difficult. Most physicians believe that a conditioning regimen may be unnecessary for patients with primary graft failure because of poor graft function (Davies *et al*, 1994), while some conditioning regimens are essential to suppress graft rejection because of an immunological mechanism (Storb *et al*, 1987; Kernan *et al*, 1989). It is difficult to differentiate the two types of graft failure following RI-CBT. Since the important factor affecting the prognosis is opportunistic infection during prolonged neutropenia (McCann *et al*, 1994), our primary goal in the second RI-CBT was to ensure engraftment. We, therefore, adopted a conditioning regimen to suppress residual host T cells despite the possibility of increasing RRT.

While less toxic, immunosuppressive conditioning regimens, such as cyclophosphamide and antithymocyte globulin (ATG), are recommended in re-transplantation for graft rejection after conventional allo-SCT (Storb *et al*, 1987), the use of ATG in RIST and RI-CBT is controversial (Fujisaki *et al*, 2004; Kusumi *et al*, 2004). ATG strongly suppresses host immunity as well as donor immune cells in the graft, as it remains in the recipient's circulation because of its long half-life (Bunn *et al*, 1996). Furthermore, ATG in RI-CBT may increase the risk of graft failure (Kusumi *et al*, 2004). Due to this consideration, we avoided ATG and selected agents with short half-lives, such as fludarabine and melphalan, in addition to TBI. Consequently, three of the four patients with graft failure achieved engraftment, and the other died of early infection. These findings suggest that re-transplantation is a promising treatment for graft failure after RI-CBT. However, two patients died of TRM within 100 d of the second RI-CBT. Three of the four patients who underwent the second RI-CBT developed grade 3–5 RRT, and all three engrafted patients developed graft-*versus*-host disease (GVHD). The safety of the second RI-CBT needs to be improved. Since the conditioning regimen for the second RI-CBT included melphalan and TBI, which have significant mucous membrane toxicity (Sarosy *et al*, 1988), this may have contributed to the RRT. Indications

Table II. Characteristics of patients with graft failure and primary engraftment.

Variable	Graft failure	Primary engraftment
Number of patients	9	96
Age (years), median (range)	57 (17–68)	55 (20–79)
Day of diagnosis, median (range)	22 (15–43)	20 (11–53)
Risk of underlying diseases (high/low)	6/3	71/25
Primary diseases		
Acute lymphoblastic leukaemia	2	13
Acute myeloid leukaemia	3	30
Chronic myeloid leukaemia	0	3
Adult T-cell leukaemia	0	10
Myelodysplastic syndrome	1	11
Malignant lymphoma	1	20
Multiple myeloma	0	4
Solid tumours	0	1
Aplastic anaemia	2	4
Preparative regimens		
Flud + L-PAM 80 mg/m^2 + TBI 2 Gy	0	5
Flud + L-PAM 80 mg/m^2 + TBI 4 Gy	7	78
Flud + L-PAM 80 mg/m^2 + TBI 8 Gy	0	2
Flud + BU 8 mg/kg + TBI 4 Gy	2	6
Flud + BU 8 mg/kg + TBI 8 Gy	0	1
Others	0	4
Number of infused nuclear cells, median (range), $\times 10^6$/kg	27 (22–34)	28 (17–52)
Number of infused CD34$^+$ cell, median (range), $\times 10^6$/kg	0·066 (0·031–0·16)	0·08 (0·017–0·33)
HLA matching		
6/6	1	1
5/6	1	16
4/6	7	79
GVHD prophylaxis (cyclosporine alone/tacrolimus alone)	8/1	67/29
Treatment		
Second RI-CBT	4	NA
Supportive cares	5	NA
Outcomes		
Engraftment after second RI-CBT*	3	NA
Autologous marrow recovery	2	NA
Death without engraftment	4	NA

RI-CBT, reduced-intensity cord blood transplantation; GVHD, graft-*versus*-host disease; Flud, fludarabine; L-PAM, melphalan; BU, busulphan; TBI, total body irradiation; NA, not applicable.

The following conditions were defined as low/high risk: acute leukaemia in complete remission, chronic myelogenous leukaemia in chronic phase, malignant lymphoma in complete remission, multiple myeloma in complete remission, myelodysplastic syndrome in refractory anaemia (RA), and aplastic anaemia as low risk. All other conditions were considered as high risk.

*One patient died of thrombotic microangiopathy on day 57, and one patient died of sepsis because of gram-positive rods on day 160.

and dosage of such agents need further investigation. GVHD prophylaxis could be intensified, as GVHD tends to be severe after the second transplantation (Davies *et al*, 1994).

The present study showed that graft failure is a significant complication of RI-CBT; however, it has several limitations that should be discussed. First, this is a small retrospective study, and it might have unrecognised biases. Secondly, it should be noted that 17 patients died of TRM before engraftment. Considering the results of chimaerism analysis showing complete donor chimaerism in the evaluable seven patients, we cannot deny the possibility that poor graft function might have caused delayed engraftment, leading to TRM. The present study might have underestimated the risk of graft failure, especially that because of poor graft function. While we believe that this study will provide valuable information on this fatal complication, further large-sized studies are warranted to establish its optimal management.

Optimal procedures to reduce the risk of graft failure in RI-CBT have not been established. Establishment of optimal conditioning regimens would be beneficial. Physicians are concerned that immunosuppressive agents, such as ATG and alemtuzumab, in conditioning regimens might suppress donor immune cell function in the graft (Fujisaki *et al*, 2004; Kusumi *et al*, 2004; Rao *et al*, 2005). In contrast, a high rate of

engraftment has been reported in CBT using ATG (Staba *et al*, 2004). Further studies on the use of these agents are warranted. Barker *et al* (2005) recently reported a high rate of engraftment in myeloablative CBT using double cord blood units. This approach might be useful in RI-CBT as well. *Ex vivo* expansion of cord blood (Shpall *et al*, 2002; Ballen, 2005), and combination of cord blood and haploidentical bone marrow (Fernandez *et al*, 2003) might be worth investigating in RI-CBT.

Conflicts of interest

None of the authors have any conflict of interest.

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Bone Marrow Transplantation (2005) 36, 205–213

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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.
Bone Marrow Transplantation (2005) **36**, 205–213. doi:10.1038/sj.bmt.1705027; published online 6 June 2005

Keywords: graft-versus-host disease; graft-versus-lymphoma effect; nonmyeloablative hematopoietic stem cell transplantation; indolent lymphoma; aggressive lymphoma

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

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Received 1 March 2005; accepted 4 April 2005; published online 6 June 2005

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.

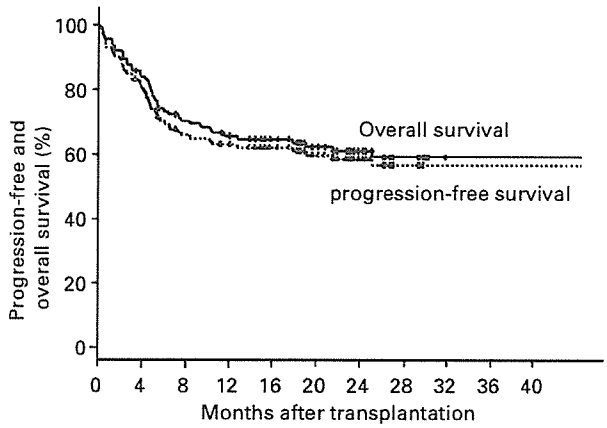

Figure 1 Overall survival (OS) and progression-free survival (PFS) following transplant. The 3-year OS and PFS were 59.0% (95% CI, 55.0–64.0%) and 56.5% (95% CI, 51.5–61.5%), respectively.

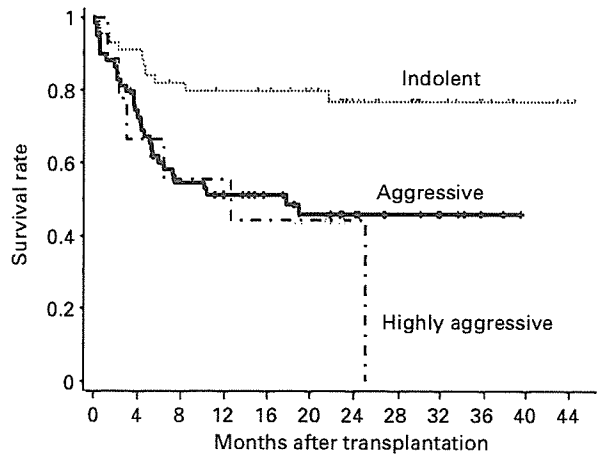

Figure 2 Overall survival (OS) following transplant according to the histological subtypes. The 3-year OS according to the histological subtypes was indolent 79% (95% CI, 67–91%), aggressive 48% (95% CI, 35–61%), and highly aggressive 0%; follicular 81% (95% CI, 69–92%), diffuse large B-cell 31% (95%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

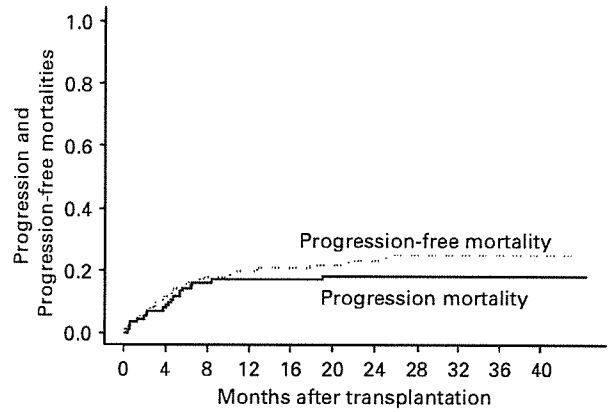

Figure 3 Cumulative incidences of disease progression mortality and transplant-related mortality (TRM). Cumulative incidences of disease progression mortality and TRM at 3 years were 18.3 and 25.2%, respectively.

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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Biology of Blood and Marrow Transplantation 11:314-318 (2005)

1083-8791/05/1104-0010$30.00/0
doi:10.1016/j.bbmt.2005.01.012

Reduced-Intensity Unrelated Cord Blood Transplantation for Patients with Advanced Malignant Lymphoma

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Received December 24, 2004; accepted January 31, 2005

ABSTRACT

We report the results of reduced-intensity unrelated cord blood transplantation (RI-UCBT) in patients with advanced malignant lymphoma. Twenty patients (median age, 46.5 years; range, 27-66 years) underwent RI-UCBT with a preparative regimen consisting of fludarabine 125 mg/m^2, melphalan 80 mg/m^2, and 4 Gy of total body irradiation. The median infused total cell dose was 2.75 × 10^7/kg (range, 2.3-3.4 × 10^7/kg). Graft-versus-host disease (GVHD) prophylaxis was composed of cyclosporine or tacrolimus alone. Fifteen patients achieved primary neutrophil engraftment after a median of 20 days. Eight patients developed grade II to IV acute GVHD, and 2 developed chronic GVHD. Of the 16 patients with evaluable disease, 10 achieved a complete response. Primary disease recurred in 1 patient, and transplant-related mortality within 100 days occurred in 8 of 20 patients. The estimated 1-year probability of progression-free survival was 50%. These data suggest that RI-UCBT is a feasible option for patients with refractory lymphoma who lack an HLA-matched donor.

KEY WORDS

Non-Hodgkin lymphoma • Reduced-intensity stem cell transplantation • Cord blood transplantation

INTRODUCTION

Allogeneic stem cell transplantation is a curative treatment for advanced or chemorefractory malignant lymphoma [1,2]. The therapeutic benefits are attributable to myeloablative radiochemotherapy and the graft-versus-malignancy effect [3], whereas severe regimen-related toxicity limits the efficacy of allogeneic hematopoietic stem cell transplantation to young patients without comorbidities. Reduced-intensity stem-cell transplantation (RIST) with a nonmyeloablative preparative regimen has been developed to decrease regimen-related toxicity while preserving an adequate antitumor effect. RIST may be a curative treatment for heavily pretreated elderly patients with malignant lymphoma [1,4-9]. Umbilical cord blood from unrelated donors has been used as an alternative stem cell source [10-13], and there have been a few reported cases of cord blood transplantation for refractory lymphoma [8,14-18]. However, the results in adult lymphoma patients of treatment with reduced-intensity unrelated cord blood transplantation (RI-UCBT) remain unclear. We analyzed the outcome of RI-UCBT in patients with relapsed or refractory lymphoma.

Table 1. *Patient Characteristics*

Patient No.	Age (y)/Sex	Histology	Stage	Previous Regimens/Previous Auto-HSCT	Previous Radiotherapy	Remission Status at RI-UCBT	Cord Blood Cell Dose (× 107/kg)	HLA Disparities	GVHD Prophylaxis
1	27/M	ALCL	IV	6/No	No	CR2	2.39	4/6	FK
2	32/F	DLBCL	IV	1/No	No	CRI	2.71	4/6	CSP
3	33/M	DLBCL	IV	3/No	Yes	PD	2.78	4/6	CSP
4	40/M	DLBCL	III	6/No	Yes	PD	2.27	4/6	FK
5	40/M	PTCL	IV	1/No	No	PR	3.4	4/6	CSP
6	41/F	DLBCL	IV	3/No	No	PD	3.2	4/6	FK
7	45/M	DLBCL	III	4/Yes (once)	Yes	PD	2.56	4/6	CSP
8	46/F	FL	IV	1/No	No	CR2	2.83	4/6	CSP
9	47/M	Nasal NK/T	II	1/yes (once)	Yes	PD	3	4/6	CSP
10	48/M	DLBCL	IV	2/No	No	PD	3.28	4/6	FK
11	48/F	FL	IV	5/No	No	PD	2.71	4/6	CSP
12	52/F	DLBCL	IV	3/No	No	PD	2.81	4/6	FK
13	55/M	DLBCL	III	4/No	Yes	PD	2.56	4/6	CSP
14	57/M	DLBCL	III	1/No	No	PD	2.9	4/6	CSP
15	57/F	DLBCL	IV	4/Yes (once)	Yes	PD	2.59	6/6	CSP
16	59/M	DLBCL	IV	3/Yes (once)	Yes	PD	2.26	4/6	FK
17	66/F	IVL	IV	2/No	No	PD	2.69	4/6	CSP
18	28/F	NS	IV	5/Yes (twice)	No	CR3	3.28	4/6	CSP
19	32/F	NS	IV	3/No	Yes	PD	2.83	4/6	FK
20	47/M	NS	III	7/Yes (once)	Yes	PD	2.34	4/6	CSP

ALCL indicates anaplastic large-cell lymphoma; auto, autologous; CR, complete remission; CSP, cyclosporine; DLBCL, diffuse large B-cell lymphoma; FK, tacrolimus; FL, follicular lymphoma; HSCT, hematopoietic stem cell transplantation; IVL, intravascular large B-cell lymphoma; Nasal NK/T, extranodal natural killer/T-cell lymphoma, nasal type; NS, nodular sclerosis classic Hodgkin lymphoma; PD, progression of disease; PR, partial remission; SCT, stem cell transplantation; PTCL, peripheral T-cell lymphoma, unspecified.

METHODS

Between September 2002 and April 2004, 20 adult patients with refractory lymphoma were treated with RI-UCBT at Toranomon Hospital, Japan. Biopsy-confirmed histologic diagnosis was based on the synonyms in the World Health Organization classification [19]. Of the 17 non-Hodgkin lymphoma patients, 2 had indolent lymphoma, and the other 15 had aggressive lymphoma (Table 1). Eligible patients had disease refractory to primary chemotherapy, had relapsed after first-line conventional chemotherapy or autologous transplantation, were considered inappropriate for conventional allogeneic hematopoietic stem cell transplantation because of the lack of an HLA-identical sibling or a suitable unrelated donor, were >50 years old, and/or had organ dysfunction. Those with chemosensitive diseases included all patients who had shown a response to the last therapy before transplantation (partial remission [PR] and complete remission [CR]); all other patients were classified as having chemoresistant diseases growing through multiple chemotherapy regimens (disease progression [PD]). All of the patients provided written informed consent in accordance with the requirements of the institutional review board. Data analysis was performed on December 1, 2004. The preparative regimen was composed of fludarabine 25 mg/m^2 daily for 5 days, melphalan 80 mg/m^2 daily for 1 day, and 4 Gy of total body irradiation in 2 fractions for 1 day. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin A 3 mg/kg alone (n = 13) or tacrolimus 0.03 mg/kg alone (n = 7) from day −1 until the patients tolerated oral administration. The dose was tapered off from day 100 until 150. Patients were treated in laminar airflow rooms. All patients received prophylaxis with sulfamethoxazole or sulfadoxine against *Pneumocystis carinii* infection. Acyclovir and fluconazole prophylaxis were routinely used. Red blood cell and platelet transfusions were given to maintain hemoglobin levels >8 g/dL and platelet counts >10 × 10^9/L. Blood products were irradiated. Neutropenic patients received broad-spectrum intravenous antibiotics for the management of febrile neutropenia. Filgrastim 5 μg/kg/d was administered subcutaneously from day +1 until the neutrophil count was at least 1000/μL for 3 consecutive days. The cord blood unit was selected according to the number of nucleated cells per recipient's weight and HLA compatibility (HLA-A and -B by serology and HLA-DRB1 by high-resolution DNA typing). The chimerism status after RI-UCBT was determined by fluorescence in situ hybridization with a Y chromosome probe for sex-mismatched RI-UCBT or by polymerase chain reaction DNA typing of HLA antigens for HLA-mismatched RI-UCBT. Graft failure was defined as peripheral cytopenia and marrow hypoplasia occurring later than day 30, without detection of donor markers by cytogenetic or molecular techniques. The probability of overall and progression-free survival was estimated by the Kaplan-Meier method. Responses to transplantation were defined according to

Table 2. *Outcomes*

Patient No.	Neutrophils >5 × 10^8/L, d	Platelets >20 × 10^9/L, d	Acute GVHD Grade (Organ Involvement and Stage)	Chronic GVHD (Organ Involvement and Type)	Status post RI-UCBT (d)	Cause of Death
1	20	34	0	None	CR, 242+	
2	20	35	0	NE	Dead, 50, RL	Disease Progression
3	NE	NE	NE	NE	Dead, 15, NE	Pulmonary bleeding
4	17	36	III (skin 2, liver 0, gut 2)	None	PR, 292+	
5	16	40	0	Extensive (skin, lung, de novo)	CR, 352+	
6	NE	NE	NE	NE	Dead, 10, NE	Sepsis
7	14	36	III (skin 2, liver 0, gut 0)	None	CR, 471+	
8	23	72	III (skin 2, liver 0, gut 0)	None	CR, 482+	
9	NE	NE	NE	NE	Dead, 18, NE	Sepsis
10	23	39	0	None	CR, 306+	
11	33	NE	IV (skin 3, liver 3, gut 3)	NE	Dead, 74, CR	Sepsis
12	22	NE	0	NE	Dead, 40, CR	CMV pneumonia
13	GF	GF	NE	NE	Dead, 44, NE	Sepsis
14	NE	NE	NE	NE	Dead, 22, NE	Sepsis
15	12	26	0	None	CR, 392+	
16	18	29	II (skin 3, liver 0, gut 0)	None	CR, 213+	
17	14	57	III (skin 3, liver 0, gut 3)	NE	Dead, 73, CR	GVHD
18	13	43	I (skin 1, liver 0, gut 0)	None	CR, 406+	
19	25	48	III (skin 1, liver 0, gut 2)	Extensive (skin, gut, quiessent)	CR, 317+	
20	24	40	III (skin 0, liver 0, gut 2)	None	Dead, 113, CR	Pneumonia

CR indicates complete remission; GF, graft failure; GVHD, graft-versus-host disease; NE, not evaluable; PR, partial remission; RL, relapse.

the recommendations of the international working group [20].

RESULTS

The characteristics of the 20 patients and cord blood units are shown in Table 1. Nineteen (95%) of the 20 patients had advanced-stage disease at diagnosis (stage III/IV) and an International Prognostic Index score ≥2. Fifteen (75%) patients had refractory disease. Six patients (30%) had already experienced a treatment failure with front-line autologous transplantation. The median age of the patients was 46.5 years (range, 27-66 years), the median weight was 56 kg (range, 44-75 kg), and the median number of cryopreserved nucleated cells was 2.75 × 10^7/kg (range, 2.27-3.40 × 10^7/kg). Fifteen (75%) patients who underwent transplantation had a sustained engraftment as defined by neutrophil counts >0.5 × 10^9/L and an untransfused platelet count >20 × 10^9/L for at least 3 consecutive days (Table 2). Primary graft failure occurred in 1 patient, and autologous recovery was not observed. The median time to recovery to an absolute neutrophil count of 0.5 × 10^9/L was 20 days (range, 12-33 days), and the median time to achieve platelets >20 × 10^9/L was 39 days (range, 26-72 days). All patients with neutrophil engraftment showed full donor chimerism. Grade II to IV acute GVHD occurred in 8 of 15 evaluable patients, and chronic GVHD occurred in 2 of 11 evaluable patients. As for disease response, both patients with indolent lymphoma, 8 of 15 patients with aggressive lymphoma, and all 3 patients with Hodgkin lymphoma achieved CR after transplantation. A total of 16 patients had measurable disease at transplantation, and disease was reassessed at regular intervals after transplantation. The maximal response was CR in 10 patients and PR in 1. Among the CR patients, the median time to CR was 88 days (range, 32-220 days). Three of 4 patients in CR at RI-UCBT remain in sustained remission, and 1 has died of PD. One patient in PR at RI-UCBT achieved CR. Five of the 15 patients in PD at RI-UCBT achieved CR, 1 achieved PR, and 9 died of nonrelapse causes (sepsis/pneumonia, n = 7; acute GVHD, n = 1; pulmonary bleeding, n = 1). The cumulative incidence of nonrelapse mortality at 100 days was 41% (95% confidence interval, 19%-63%). At a median follow-up of 334.5 days (range, 213-482 days), 10 of the 20 patients were alive: 9 in CR and 1 in PR. The estimated 1-year overall and progression-free survival rates were both 50% (95% confidence interval, 28%-72%; Figure 1). Total nucleated cell dose HLA disparities, disease status, and preceding therapies were not associated with differences in survival (data not shown).

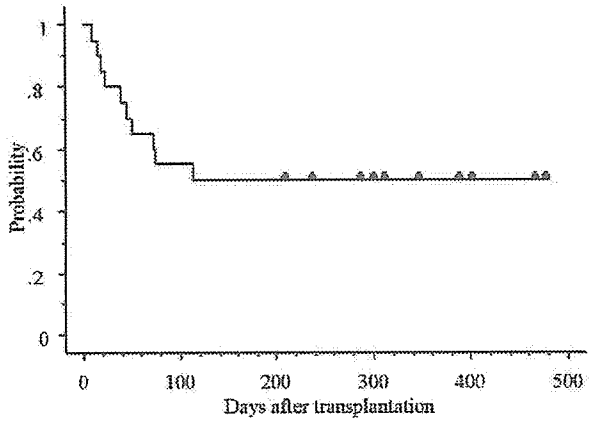

Figure 1. Overall survival (OS) and progression-free survival (PFS) after transplantation. The estimated 1-year OS and PFS were both 50.0% (95% CI, 28.1%-71.9%).

DISCUSSION

The results of our trial of RI-UCBT in patients with lymphoma whose disease recurred after a previous treatment are encouraging. The rapid availability of a unit of cord blood may be a particular advantage for lymphoma patients who require urgent transplantation, and cord blood can be an acceptable alternative stem cell source. Our patients were heavily pretreated and included chemoresistant and aggressive histologic types of disease whose outcome after RIST is reported to be very poor, with median survival rates from 19% to 32% at 1 year [21]. Despite our low-intensity conditioning regimen, a high rate of clinical remission was observed. This suggests that RI-UCBT does exert a strong allogeneic graft-versus-lymphoma effect. The median of 100 days between RI-UCBT and the appearance of a maximal response might be consistent with the time required for the activation and expansion of antitumor cytotoxic T cells.

The transplant-related mortality of 41% at day 100 in this study was higher than that reported for RIST in other studies [11,13,21-23]. Patient age, prior repeated therapies, disease status [24], and prolonged immunosuppression [25] would have played a role in the high transplant-related mortality after RI-UCBT. The proportion of deaths related to infection has recently been reported to be higher soon after cord blood transplantation than after bone marrow transplantation [12]. Additional strategies to promote engraftment and prevent early infectious complications should be developed, and this treatment strategy should be investigated among patients with less advanced diseases.

In conclusion, although our patient cohort was small and the observation period was limited, our result suggests that RI-UCBT is feasible for patients with refractory lymphoma who lack a suitable donor and require urgent treatment. RI-UCBT is associated with high transplant-related mortality, and this provides a rationale for a clinical study, which should be modified to focus on minimizing toxicities, controlling infectious complications, and enhancing any graft-versus-leukemia effect.

ACKNOWLEDGMENTS

We are indebted to the medical and nursing staff of the Department of Hematology at the Toranomon Hospital for taking care of the patients and to the Chushikoku CBB, Kanagawa CBB, Hokkaido CBB, Hyogo CBB, Metro Tokyo Red Cross CBB, Tokai CBB, Tokai University CBB (Cord Blood Bank), and Tokyo CBB for processing the cord blood units. This study was supported by a grant-in-aid of the Ministry of Health, Labor and Welfare, Japan.

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Erratum

In the March issue of *BBMT* the article by Drs. Lamb and Lopez (T cells: a new frontier for immunotherapy? *Biol Blood Marrow Transplant.* 2005;11:161-168) included an editing error. The phrase "guanosine monophosphate" was inadvertently inserted in place of "good manufacturing product". The correct passage is as follows: "Techniques are currently being developed for good manufacturing product–compatible clinical scale *ex vivo* expansion of T cells. The first clinical trials are expected within the next 6 to 12 months."