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Nutritional Support for Patients Suffering From Intestinal Graft-versus-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation

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Background: Patients who exhibit gastrointestinal (GI) involvement due to graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (SCT) are often recommended to withhold oral intake (NPO) to avoid further damage to the GI mucosa. However, it is possible that continuing oral intake could be beneficial in many patients compared to total parenteral nutrition (TPN).

Objective: The primary objective of this prospective study was to evaluate whether programmed step-ladder oral dieting (enteral nutrition; EN) is feasible and beneficial for these patients.

Methods: A total of 18 patients who exhibited GI-acute GVHD (stage I to III gut GVHD) after SCT received an EN dieting program, and changes in clinical and laboratory parameters were compared to those in a control cohort of 17 patients who were placed on NPO with TPN. Patients with GVHD were included prospectively and those with intestinal bleeding/obstruction, severe pancreatitis, and cytomegalovirus enterocolitis were excluded.

Results: None of the patients in the EN group experienced significant adverse events, including exacerbation of GI symptoms. Although there was no statistically significant difference in the volume or frequency of diarrhea or the time to complete dietary recovery, parameters including body weight and serum levels of total protein and albumin tended to improve faster in the EN group.

Conclusion: The EN diet is safely applicable to patients suffering from GI involvement by GVHD. Am. J. Hematol. 81:747–752, 2006. © 2006 Wiley-Liss, Inc.

Key words: graft-versus-host disease (GVHD); enteral nutrition; immunonutrition

INTRODUCTION

Graft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (SCT) that influences the ultimate prognosis of patients [1]. Gut involvement due to GVHD particularly impairs the host nutritional status and QOL due to long-lasting diarrhea and anorexia. Hence, effective supportive care of patients suffering from GVHD should include attention to intense nutritional support and bone mineral retention, since many receive concomitant steroid therapy. Additionally, normal intestinal architecture and functions are required to prevent biliary stasis, retarded bowel movement, bacterial translocation, and resultant systemic infection [2,3]. With the development of gut GVHD, patients are often recommended to withhold oral intake (NPO, "bowel rest") to avoid further damage to the gastrointestinal (GI) mucosa. However, this raises a serious concern since NPO care can induce atrophic deficit of the GI mucosa and resultant dysfunction

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TABLE I. Grade of Programmed EN Dieting

Step	Staple food (form of rice)	Side dishes (approved foods and cuisines)	Nutritive value
0	Liquid	Juice (without grain, without oranges), electrolytic supplement solution	500–2000 ml
1	Liquid	Water gruel, starch gruel, clear soup, consomme, juice, miso soup	Calories 300–350 kcal Protein 5–7 g Fat 1.5–2 g Dietary fiber 1.5 g
2	Mush	Potato, vegetables, canned fruits, vegetable juices, noodles, tofu, whitefish	Calories 600–650 kcal Protein 20–25 g Fat 5–8 g Dietary fiber 1.5–8 g
3	Rice gruel	Eggs, breads, banana, apple	Calories 900–1000 kcal Protein 30–35 g Fat 10–13 g Dietary fiber 8–9 g
4	Boiled rice	Blue-skinned fish, oil (~3 g/day)	Calories 1200–1300 kcal Protein 40–45 g Fat 15–20 g Dietary fiber 9–10 g
5	Boiled rice	Chicken (low fat), yogurt, oil (~8 g/day)	Calories 1500–1600 kcal Protein 60–65 g Fat 30–35 g Dietary fiber 12–13 g

Note: A patient-oriented stepped-up dieting program was gradually applied over six steps that varied with regard to the solidity, intensity, and acceptability by the patient.

of the GI system. Moreover, it has recently been reported that enteral nutrition (EN) was more effective than parenteral nutrition for the nutritional support of patients with an injured intestine due to trauma or an invasive operation [4,5]. Taken together, these findings suggest that the current patient management procedure that includes the interruption of oral feeding to enforce "bowel rest" in SCT patients suffering from GVHD should be critically reevaluated. Furthermore, EN, if tolerable, may be a preferred route for maintaining digestive and absorptive function as intact as possible.

In those suffering from GI involvement of GVHD, such evaluation becomes more complex since diarrhea is very often multifactorial and includes secretory dysfunction, osmotic factors, and rapid passage. Hence, the establishment of a standard care procedure remains very difficult. To address these concerns, we conducted a controlled cohort study to evaluate the benefit of different nutritional support measures for patients suffering from acute gut GVHD after SCT. Our clinical hypothesis was that a programmed and controlled scheduled oral nutritional support with EN is beneficial for patients who have mild to moderately progressing acute symptoms of gut GVHD.

PATIENTS AND METHODS

Patients

Seventy patients who were treated at the National Cancer Center Hospital from January 2001 to December 2003 and who developed GI symptoms by GVHD were involved in this prospective study. Forty among those eligible patients met the following inclusion criteria: (i) pathologically diagnosed GVHD with biopsied specimens, (ii) presented symptoms within 100 days after SCT, and (iii) clinically diagnosed as stage I to III gut GVHD and grade II to III acute GVHD according to the clinical grading criteria [6,7]. Patients who had intestinal tract bleeding, intestinal obstruction, or severe pancreatitis were excluded from this analysis, since these pathophysiologies are considered contraindications for EN. Additionally, patients with pathologically diagnosed cytomegalovirus enterocolitis were also excluded, and thus a total of 35 patients were left for this study.

Methods

In the study periods, two different nutritional intervention procedures were applied; patients who developed gut GVHD before July 2002 ($n = 17$) were treated with NPO and total parenteral nutrition (TPN) (C group), while the remaining patients who developed gut GVHD after July 2002 ($n = 18$) were treated by programmed GVHD dieting intervention (EN group). The patients were consecutively registered to our database at National Cancer Center Hospital, and this prospective study was approved by the IRB. The programmed EN dieting consisted of six steps with regard to solidity, intensity, and acceptability for intestinal digestion, as shown in Table I. Each food and nutrient was made more solid and dense

in a step-up manner, after the confirmation of stable symptoms that lasted for a minimum of 3 days. Each step of programmed EN dieting was suitably stepped down when intolerance or exacerbation of gut GVHD symptoms developed. Patients were made NPO with the appearance of significant abdominal symptoms (nausea, vomiting, and abdominal pain). Patients in the EN group only received oral intake without enteral tube feeding. On the other hand, the patients in group C were adequately allowed to eat according to their symptoms with TPN.

We evaluated "time to complete dietary recovery," which was defined as the duration from the start of nutritional management (stopping oral intake or start of programmed EN dieting) to the restoration of a normal diet with the recovery of nutritional parameters. Nutritional parameters evaluated in this study included (1) clinical symptoms, including volume and frequency of diarrhea, and body weight and (2) laboratory data, including total serum protein and albumin. Body mass index (BMI) was calculated as BMI = {height (m)}2/body wt (kg).

Statistical Analysis

Our clinical hypothesis was that a programmed and controlled schedule of nutritional support with oral intake (EN dieting) could be effective in the support of patients suffering from acute gut GVHD with mild to moderately progressing symptoms. We evaluated "the time to complete dietary recovery," which was defined as the duration from the start of nutritional management (stopping oral intake or start of EN dieting) to the recovery to normal diet, various enteral symptoms, and nutritional parameters. The time to complete dietary recovery is shown with a time–event cumulative curve, and the log-rank test was used to compare groups C and EN. Nutritional parameters are given as the mean of each group by time course, and the data in groups C and EN were compared by an analysis of variance (ANOVA). A P value of less than 0.05 was considered significant.

RESULTS

Patients' Characteristics

The patients' clinical backgrounds are summarized in Table II, which shows that there are no essential differences between groups C and EN. Older patients tended to receive a reduced-intensity regimen more often than a conventional regimen.

Safety of Programmed EN Dieting

Throughout the study, no severe adverse events associated with nutritional intervention were observed, indicating that our procedure with gradual stepped-up or -down dieting was safe. No severe infectious episodes were observed in each group. EN dieting had to be terminated early in 2 of 18 cases due to prolonged GI symptoms and exacerbation of an underlying malignant disorder. There were 4 censored cases in group C, mainly due to recurrence of the basic malignant disorder.

TABLE II. Patients' Characteristics

	EN group (N = 18)	C group (N = 17)
Age median (range)	53 (22–64)	53 (23–69)
Sex male/female	12/6	14/3
Disease		
AML	6	8
MDS	3	2
ALL	4	2
CML	3	1
NHL	1	2
ATL	1	1
Solid tumors	0	1
Transplantation source		
BM	1	3
PBSC	17	14
Transplantation regimen		
Conventional	5	7
Reduced intensity	13	10
Donor HLA typing		
Full match	14	14
1 locus mismatch	4	0
2 loci mismatch	0	3
GVHD prophylaxis		
CSP alone	10	8
CSP + MTX	6	4
CSP + ATG	2	2
Others	0	3
Gut GVHD stage		
1	5	9
2	7	3
3	6	5
GVHD grade		
II	6	8
III	12	9
Onset day of gut GVHD (mean of day)	74	68

Note: Patients who underwent SCT and developed gut GVHD were enrolled in this study. Patients who developed gut GVHD before July 2002 (n = 17) were treated with no oral intake (C group), while the EN group (n = 18) was treated by programmed GVHD dieting. AML, acute myelogeneous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogeneous leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T-cell leukemia; BM, bonemarrow; PBSC, peripheral blood stem cell; CSP, cyclosporine; MTX, methotrexate; ATG, anti-thymocyte globulin.

Efficacy of Programmed EN Dieting

Although there was a wide variation in each patient in diarrhea volume and frequency of diarrhea, we adapted ANOVA to evaluate whether there is a statistically significant difference between the two groups

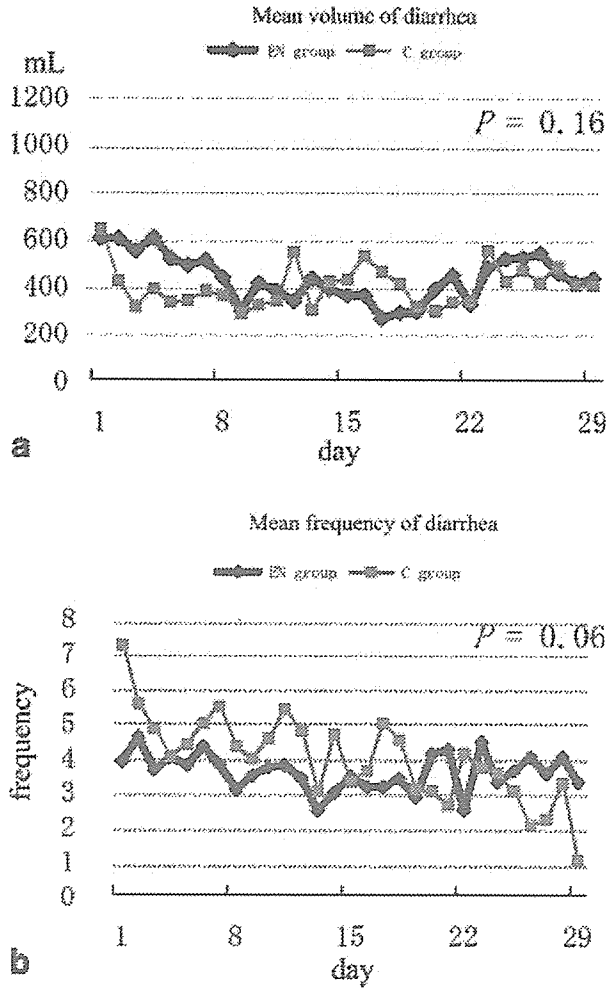

Fig. 1. Changes in mean volume and frequency of diarrhea. No difference was observed between the C and EN groups in the time-course of diarrhea as evaluated by volume ($P = 0.16$) (a) and frequency ($P = 0.06$) (b).

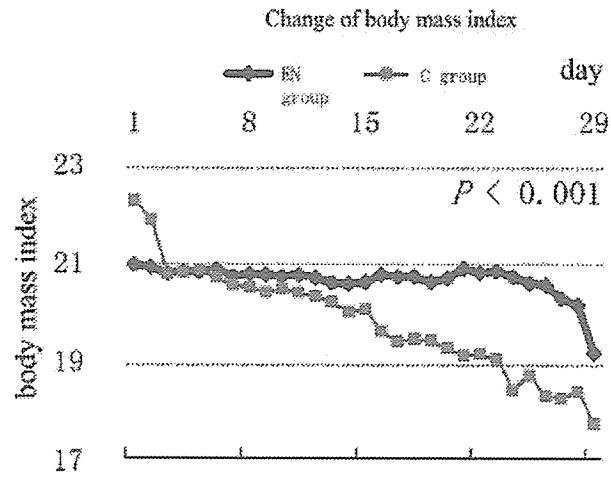

Fig. 2. Changes in BMI. The mean changes in BMI, with the first evaluation as a control, were compared between the two groups. A slower decrease in body weight tended to be observed in the EN group, while patients retained their BMI significantly better in the EN group than in the C group ($P < 0.001$). BMI was calculated as BMI = {height (m)}^{2}/body wt (kg).

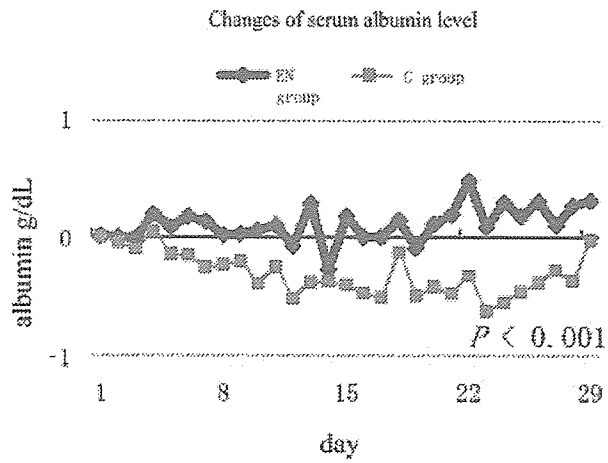

Fig. 3. Changes in albumin as nutritional parameter. One of the nutritional parameters, albumin (Alb), was evaluated between the C and EN groups. In the EN group, patients maintained significantly more stable levels of Alb ($P < 0.001$).

($P = 0.16$ and 0.06, respectively, Figure 1a and b). The mean body weight values in each group were compared by considering the absolute changes after adjusting by the value at the initial evaluation. In comparing the two groups, the decrease in body weight after the start of nutritional management was more obvious in group C than in group EN but this difference was not statistically significant ($P = 0.09$), since there was a wide interpatient variation. On the other hand, the change in BMI was significantly different between the two groups (Figure 2, $P < 0.001$).

Nutritional status was also estimated by laboratory parameters, including serum levels of total protein and albumin (Alb), which were determined as absolute changes by adjusting by the value at the first evaluation at the starting point of nutritional management, and a significantly slower decrease was noted in the EN group ($P < 0.001$) (Figure 3). These nutritional parameters remained higher in group EN than in group C. During the study period, no patient actually met with stopping rules mentioned above and consequently, the total number of days for NPO was not evaluated. The time to complete dietary recovery was compared between the two groups. While 38 days were required for the

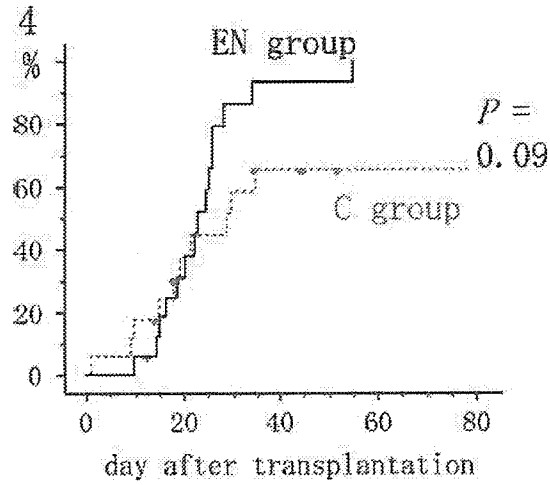

Fig. 4. Time to complete dietary recovery. The number of days required for return to a normal diet was 38 days in group C, while it was 31 days in group EN, with no statistically significant difference (P = 0.09).

recovery to a normal diet in group C, 31 days were required in group EN (Figure 4).

DISCUSSION

Since Weisdorf et al. reported that central venous parenteral nutritional support improved long-term survival in patients who underwent bone marrow transplantation (BMT) [8], intravenous TPN has been widely used in SCT. However, it has not yet been confirmed which procedure, enteral or parenteral nutrition, can provide more effective and safer nutritional support. In this study, we considered that the patients in the EN group may have preserved nutritional parameters better than the other group and ate sooner, although no differences were found in the time to complete dietary recovery. A clinical study group at Johns Hopkins University randomized BMT patients into two groups to receive different types of nutritional support, TPN or EN, and they did not observe any differences in nutritional parameters between the two groups [9]. In their study, patients who received TPN were allowed to eat anything they liked, while those with EN had few chances to receive TPN treatment. Moreover, those who had been receiving TPN were allowed to take oral intake and thus were not on strict NPO. Additionally, in our study, the two groups of patients were evaluated in different study periods, and there was a significant difference in the modality of the supportive measures. These points make a direct and strict comparison between the TPN and EN groups very difficult and unreliable. These biases, which are inherent to studies in this field, also existed in our study, which might explain why we failed to detect significant differences in clinical benefits.

We used to routinely advise patients to stop oral intake with the development of gut GVHD. Thereafter, they were encouraged to drink or eat gradually, since it has been suggested that inadequate nutritional support further deteriorates gut GVHD symptoms. To establish clearly defined subjective guidelines, we conducted this interventional cohort study. We found that both controlled and uncontrolled EN can be administered safely. Since the time to complete dietary recovery was almost comparable in the two groups, the results suggest that any EN program is acceptable and does not harm or degrade the QOL of patients suffering from GVHD. If this is confirmed, a restricted diet would not be necessary for those with moderately symptomatic gut GVHD. Nevertheless, the evaluation of nutritional parameters in this study suggested that controlled EN did a better job of maintaining body weight and serum nutritional status, compared to the results in the NPO group. The random administration of food intake may be inadequate compared to scheduled dieting, which attempts a gradual build-up of intestinal mucosa by the comprehensive supply of nutrients including glucose, protein, fat, fiber, etc. This may have a secondary advantage of keeping the mucosal barrier intact and preventing bacterial translocation through the GI tract.

Nevertheless, since the cause of diarrhea is multifactorial, it is inherently difficult to assess the effectiveness of and standardize nutritional intervention procedures. In the literature, four pathologies have been reported to be contraindications for EN since they cause undesirable bowel movement, i.e., presence of gastrointestinal bleeding, intestinal obstruction, severe pancreatitis, and intestinal perforation. The pathophysiology of diarrhea associated with gut GVHD includes osmotic and secretory diarrhea. Hypertonic EN is considered to further deteriorate symptoms of diarrhea. Hence, it is reasonable to suggest that dietary foods in EN adequately maintain an isotonic status as well as nutritional status to improve immunologic function. An intact GI system is vital for maintaining normal immune functions, and a novel concept of nutrition support, "immunonutrition," has been introduced, which focuses on the maintenance of the comprehensive biological protection system against external pathogens to maintain normal immune function [10]. Clinical benefits of immunonutrition, including improvement of nutritional parameters, decreased risk of infection, and shorter duration of hospitalization, have been reported in patients in the perioperation period and in those who required care in the ICU [11,12]. However, currently a precise evaluation

of the efficacy of each component of immunonutritional agents is difficult [13], and controversy still exists regarding the value of immunonutrition after SCT. This study did not evaluate this proposed immunonutrition, and to accomplish this in SCT practice, prospective monitoring of immune parameters would be required.

The serum level of albumin can be significantly affected by many variables including diarrhea associated with GVHD and, hence, would not be a very good marker for the evaluation of protein status in the HSCT population. However, in our experience, serum albumin decreased after SCT to suggest the possibility of the use in the estimation of patient's nutrition status at least for a short period of follow-up, when referring to the general description in the guideline by American Society for Parenteral and Enteral Nutrition, i.e., "low serum levels indicate which hospitalized patients are at increased risk of morbidity and mortality" [14].

In conclusion, the current study is hampered by preexisting biases including a small number of studied patients, a cohort analysis in different periods, and a lack of adequate measures for data evaluation. Nevertheless, it appears that patients supported by programmed EN experienced no exacerbation of gut GVHD symptoms, with a suggested benefit of enhanced maintenance of nutrition status. Further study is warranted to prospectively evaluate the value of various nutrients including arginine, o-3 fatty acid, and nucleic acid [13] and various clinical outcomes including the cost, complications, and QOL in an attempt to improve the nutritional and immune status of transplanted patients.

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Hyperacute GVHD and emergence of peripheral CD3+CD56+ T cells and activated natural killer cells are useful markers for early diagnosis of post-transplant hemophagocytic syndrome

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Hemophagocytic syndrome (HPS) is a cytokine-related disorder characterized by sustained high-grade fever, pancytopenia, hepatomegaly, coagulopathy and hemophagocytosis in the marrow, spleen, or lymph nodes.[1] The development of HPS is related to underlying immune dysregulation and inappropriate activation of macrophages. In adults, viral, bacterial, and fungal infections and/or malignant lymphoma are associated with HPS.[1]

HPS is a rare complication following stem-cell transplantation (SCT), and occasional case reports only have been published.[2,3] Besides infection and hematologic malignancies, immune reactions particularly hypercytokinemia following SCT have been associated with HPS. Although some patients with early-onset HPS respond to corticosteroids, HPS is often fatal. At present, its clinical characteristics remain unknown. We report a patient who developed early-onset HPS after allogeneic SCT. A 30-year-old man with chemorefractory acute myeloid leukemia was referred to our hospital for allogeneic SCT in November 2003. After cytoreduction using cytarabine, he underwent peripheral blood SCT from his one-locus-mismatched sister in January 2004. The preparative regimen consisted of busulfan 4 mg/kg/day for 4 days and cyclophosphamide 60 mg/kg for 2 days. Graft-versus-host disease (GVHD) prophylaxis was tacrolimus 0.03 mg/kg/day. Methotrexate was omitted from GVHD prophylaxis to enhance a graft-versus-leukemia effect. The numbers of infused CD34+ cells and CD3+ cells were 2.6×10^6/kg and 3.2×10^8/kg, respectively.

His clinical course was uneventful until day 3, when he developed a high-grade fever. Lacy blanching erythema and diarrhea appeared on day 4. White blood cell (WBC) count was 100/mm^3 on the day. Hyperacute GVHD was suspected. Painful hepatomegaly, weight gain, and jaundice appeared on day 7, and hepatic veno-occlusive disease (VOD) was diagnosed. When renal dysfunction developed on day 7, we switched tacrolimus to methylprednisolone 0.5 mg/kg. On day 8, the WBC increased to 1600/mm^3 including 90% lymphocytes (Figure 1). Double X signals were shown by sex chromosome fluorescence *in situ* hybridization in 100% of the peripheral leukocytes. Fluorescence-activated cell sorter (FACS) showed that 44, 36 and 8% of the lymphocytes were CD3−CD56+, CD3+CD56−, and CD3+CD56+, respectively (Figure 1), and that 31% of cytoplasmic CD3+ cells expressed NKp46, a specific marker of activated natural killer cells (NK cells).[4] While the skin rash and diarrhea improved, his renal and respiratory functions deteriorated. Chest radiographs revealed bilateral pulmonary edema and pleural effusions. Serum creatinine increased to 5.9 mg/dl. We suspected worsening hyperacute GVHD and VOD, and increased the dose of methylprednisolone to 2.0 mg/kg. He achieved neutrophil engraftment on day 10, when the WBC was 5700/mm^3. The numbers of neutrophils, surface CD3+, and CD20+ cells were 4500, 780, and 70/mm^3, respectively. Despite intensive immunosuppression, he rapidly deteriorated and died of multiple-organ failure on day 11. Throughout his clinical course, the spleen was not palpable. Repeated blood cultures failed to detect any organisms.

Post-mortem examination showed normocellular marrow with an increase in activated macrophages. In the liver, fibrous obliteration of terminal hepatic venules, dilatation of centrilobular sinusoids, and necrosis of zone 3 hepatocytes were evident. These findings were consistent with VOD. No findings suggested hepatic involvement by acute GVHD. A massive infiltration of activated macrophages was present in the liver, while neither CD3+ nor CD56+ cells were observed. Serum cytokine levels were: interferon gamma 0.2 IU/ml (normal range: <0.1 IU/ml), interleukin (IL) −2<0.8 U/ml (normal range: <0.8 U/ml), IL-6 467 pg/ml (normal range: <4.0 pg/ml), tumor necrosis factor alpha 7 pg/ml (normal range: <5.0 pg/ml), and macrophage colony-stimulating factor 10 000 pg/ml (mean levels in healthy volunteers, 670 pg/ml).

Hyperacute GVHD preceded the onset of HPS in this patient, which is an immunological syndrome overlapping with engraftment syndrome, capillary leak syndrome, and periengraftment clinical abnormalities.[5] The previously reported patients with HPS following allo-SCT, and our patient had high-grade fever, skin eruption, and diarrhea in common; these are typical manifestations of hyperacute GVHD,[2,3] although the possibility of toxicity from the preparative regimen cannot be excluded. The negative post-mortem findings for GVHD in our patient cannot exclude the diagnosis of hyperacute GVHD because it had resolved before death and because the pathological findings of hyperacute GVHD are not always identical with those of conventional GVHD.[5,6] High-dose corticosteroid is usually effective for hyperacute GVHD. However, this persisted despite corticosteroids in this patient and finally resulted in fatal HPS, although the time from the onset of hyperacute GVHD to steroid initiation might have influenced the outcome. Since this patient had a one-locus-mismatched related donor and GVHD prophylaxis with tacrolimus and no methotrexate,[6] he was at high risk of hyperacute GVHD. Severe hyperacute GVHD might have aggravated cytokine dysregulation, contributing to the development of HPS. This hypothesis is consistent with previous observations, suggesting that a severe alloimmune response resulted in HPS.[2]

It should be noted that the patient developed lymphocytosis with CD3+CD56+ T cells. The number of activated NK cells expressing NKp46 was also elevated in the peripheral blood. These findings are comparable with

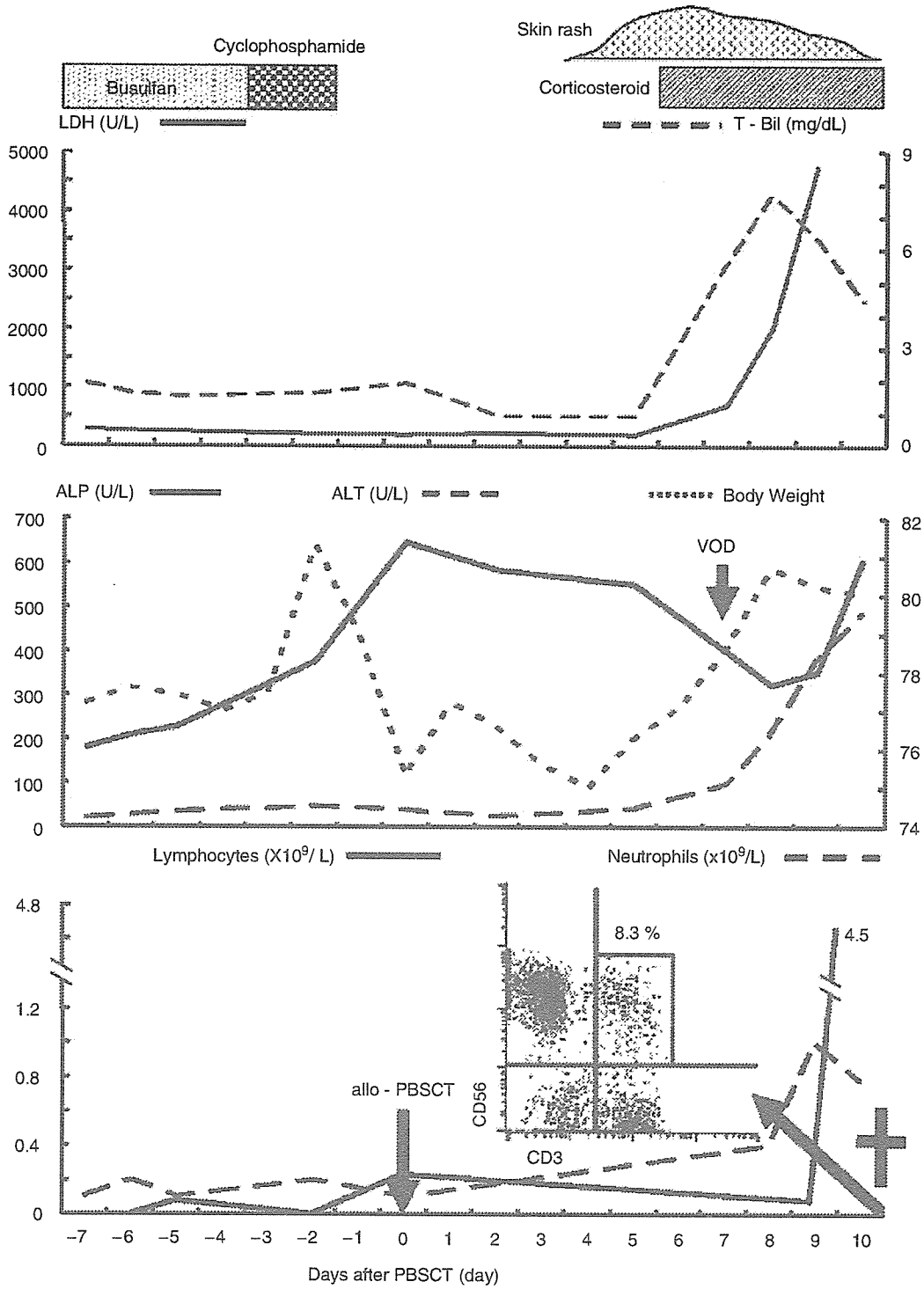

Figure 1 Clinical course of this patient. On day 8, the WBC increased to 1600/mm^3 including 90% lymphocytes. Double X signals were shown by sex chromosome fluorescence *in situ* hybridization in 100% of the peripheral leukocytes. FACS showed that 44, 36, and 8% of the lymphocytes were CD3−CD56+, CD3+CD56−, and CD3+CD56+.

our previous case report.[7] While CD3+CD56+ T cells have been described in both murine and human tissues,[8,9] their clinical significance has not been fully clarified. Some of these lymphocytes are classified into cytokine-induced killer (CIK) cells, which are a unique population of cytotoxic T-lymphocytes with higher proliferative and

cytolytic activities in comparison to the reported CD3−, CD56+ lymphokine-activated killer cells, which are essentially activated NK cells.[10] Since CIK cells can be generated *in vitro* using exogenous IL-2, IL-7, or IL-12, cytokine storm associated with severe hyperacute GVHD might have promoted the generation of CIK cells, causing HPS and severe organ damages in this patient. Alternatively, an increase in CD3+CD56+ lymphocytes and NK cells might have reflected activated immune reactions, and these cells might not have caused severe organ damages. At present, the exact mechanisms of these immune reactions remain unknown, and further investigation is required to clarify the clinical significance. Post-mortem examination, which failed to show infiltration of CD3+CD56+ T cells in the liver, supports the latter hypothesis. The clinical course of this patient suggests that the development of hyperacute GVHD, and proliferation of CD3+CD56+ T cells and activated NK cells with NKp46 in the peripheral blood are useful markers for early diagnosis of HPS.

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bjh research paper

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	5	3·9	1·6	9	1	3·1	1·8	Tapering of ciclosporin	DLI	PD	None	1·8	Leukaemia
19	60	M	AML	Relapse	Absent	100	66	Not performed									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.

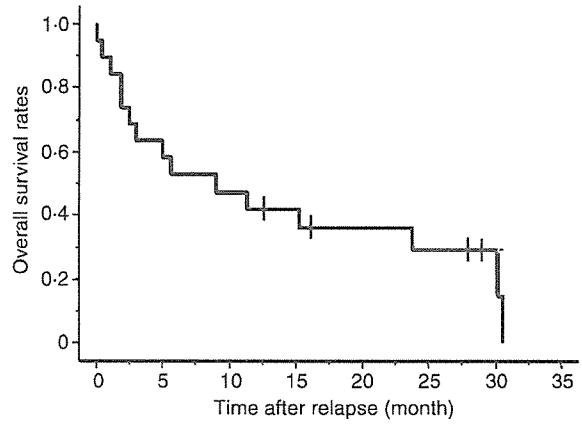

Fig 1. Probability of survival for 19 patients who relapsed after reduced-intensity stem-cell transplantation. The 2-year overall survival rate after relapse was 28·9% (95% confidence interval; 7·3–50·5%).

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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Allogeneic hematopoietic stem cell transplantation with a reduced-intensity conditioning regimen for treatment of metastatic renal cell carcinoma: single institution experience with a minimum 1-year follow-up

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Objective. **The aim of this study was to evaluate the safety and efficacy of allogeneic hematopoietic stem cell transplantation with a reduced-intensity conditioning regimen (RIST) for interferon-α–refractory metastatic renal cell carcinoma (RCC).**

Patients and Methods. **Of 26 patients referred to the National Cancer Center Hospital for possible RIST between June 2000 and April 2002, an HLA-identical relative was identified for 12 patients. Nine patients underwent RIST. The conditioning regimen consisted of fludarabine 180 mg/m^2 or cladribine 0.66 mg/kg, plus busulfan 8 mg/kg and rabbit antithymocyte globulin 5 mg/kg. Graft-vs-host disease (GVHD) prophylaxis was cyclosporine alone.**

Results. **All patients achieved engraftment without grade III to IV nonhematologic regimen-related toxicity. All patients achieved complete donor-type chimerism without donor lymphocyte infusion by day 60. Four patients developed acute GVHD, and four developed chronic GVHD. One patient (11%) achieved partial response. As of July 2003, six patients were alive at median follow-up of 681 days. The actuarial overall survival rate was 89% at 1 year and 74% at 2 years. The overall survival rate tended to be higher in the 12 patients with a matched donor than in the other 14 patients without a matched donor ($p = 0.088$).**

Conclusion. **Our RIST procedure is feasible without severe toxicity. The efficacy of RIST for RCC should be confirmed in phase II/III clinical trials.** © **2004 International Society for Experimental Hematology. Published by Elsevier Inc.**

Allogeneic hematopoietic stem cell transplantation (HSCT) has been established as a standard therapy for various hematologic malignancies [1]. In allogeneic HSCT, malignant cells are eradicated through 1) myeloablation by irradiation or cytotoxic agents in the pretransplant conditioning regimen, and 2) an immunologic antitumor effect mediated by donor-derived immune competent cells [graft-vs-leukemia (GVL) effect] [1]. Although complete myeloablation once was considered essential for the engraftment of infused donor cells, recent investigations have proven that intense immunosuppression is sufficient for durable engraftment [2,3].

Reduced-intensity hematopoietic stem cell transplantation (RIST), which is expected to work mainly through a GVL effect rather than myeloablation, is associated with less regimen-related toxicity (RRT) compared to conventional HSCT [2,4]. Fludarabine and cladribine (2-chlorodeoxyadenosine), purine analogues with intense immunosuppressive

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but moderate myelosuppressive activities, have been commonly used as backbone agents in many current RIST regimens [2,4,5].

Renal cell carcinoma (RCC) is highly resistant to systemic therapy with hormonal and chemotherapeutic agents [6,7]. Although some patients show a durable response to immunotherapy with interferon-α (IFN-α) or interleukin-2 (IL-2), its response rate usually remains around 10–20% [6,7]. When they become refractory to IFN-α, the prognosis is uniformly grim, and no effective salvage therapies have been established [8]. Recently, the allogeneic immune-mediated antitumor effect has been proven to work against some solid tumors [graft-vs-tumor (GVT) effect] [9–13], including RCC [14–19]. Childs et al. [14] reported their treatment results in 19 patients with metastatic RCC using allogeneic HSCT; the response rate was 53%. However, there have been large differences in the conditioning regimen, immune regulatory maneuver after transplantation, and patients' backgrounds among studies on allogeneic HSCT against RCC [14–19]. In particular, differences in patient selection criteria and ethnic considerations [20,21] make it difficult to compare these reports. A suitable regimen and procedure for allogeneic RIST against metastatic RCC remain to be established.

To obtain additional information on the feasibility and efficacy of RIST against metastatic RCC, we report the results of our Japanese phase I study on RIST against metastatic RCC. The patients were followed for a minimum of 1 year.

Patients and methods

Patients

Patients with measurable metastatic RCC that was refractory to treatment with IFN-α and who had an HLA-identical or one antigen-mismatched healthy related donor were eligible to participate in the phase I protocol. Between September 1999 and October 2002, 26 patients with metastatic RCC who were referred to our hospital underwent HLA typing for donor screening. The median number of relatives examined per patient was 2 (range 1–14). HLA-matched donors were available in 12 patients, but 3 donor/recipient pairs were found to be ineligible for transplantation during further examinations. The remaining 9 patients were enrolled in this study, which was approved by the Institutional Review Board of National Cancer Center Hospital in Tokyo, Japan. Written informed consent was obtained from all patients/donors.

Eligibility criteria were as follows: 1) younger than 70 years; 2) life expectancy of at least 6 weeks; 3) Karnofsky performance score ≥70% or Eastern Cooperative Oncology Group score ≤2; 4) satisfactory cardiac function as evidenced by ejection fraction ≥45% by echocardiogram, diffusion capacity ≥40%, forced expiratory volume ≥50%, and PaO_2 in room air ≥60 mmHg; 5) serum bilirubin ≤2.5 mg/dL and serum aspartate aminotransferase <3 times upper reference limit; and 6) serum creatinine ≤2.0 mg/dL or creatinine clearance ≥50 mL/min. Patients were excluded if they had active infection, cardiac insanity including unstable angina pectoris and heart failure, uncontrolled diabetes mellitus, or a mental disorder that required treatment. Although nephrectomy was not a condition of enrollment, all of the patients had, in fact, previously undergone radical nephrectomy.

Stem cell collection

All patients received granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cell (PBSC). Donors were injected with G-CSF at 5 μg/kg subcutaneously twice daily starting 3 days before the first collection of PBSC until the end of collection. Leukapheresis was performed daily until $>3.0 \times 10^6$ $CD34^+$ cells/kg of recipient body weight were collected. Collected cells were then cryopreserved using standard techniques. Cryopreserved PBSC were thawed and infused after the conditioning regimen was completed on day 0.

Conditioning regimen and transplantation procedures

The conditioning regimen in the first three patients consisted of cladribine 0.11 mg/kg/day by 2-hour intravenous infusion for 6 days (days −8 to −3), oral busulfan 4 mg/kg for 2 days (days −4 and −3), and rabbit antithymocyte globulin (ATG, Thymoglobulin; IMTIX-SANGSTAT, Lyon, France) 2.5 mg/kg by 12-hour intravenous infusion for 2 days (days −2 and −1). In the remaining six patients (no. 4–9), cladribine was replaced by fludarabine at 30 mg/m^2/day because cladribine was no longer available. Graft-vs-host disease (GVHD) prophylaxis consisted of cyclosporine alone, initiated on day −1 at a dose of 3 mg/kg/day by continuous intravenous infusion to maintain serum levels of 250 ng/mL. This was changed to an oral form when it could be tolerated. Withdrawal of cyclosporine was started on day 30 and completed by day 100 in the absence of acute GVHD. However, in the three most recently treated patients, cyclosporine was discontinued on day 45 in an attempt to induce a GVT effect earlier. Methylprednisolone (1–2 mg/kg) was added for patients who developed grade II to IV acute GVHD [22].

Engraftment and supportive care

Engraftment was defined as the first of 2 consecutive days with an absolute neutrophil count (ANC) of 0.5×10^9/L or more. Patients received G-CSF at a dose of 5 μg/kg/day by intravenous injection from day 6 after transplant until engraftment. Packed platelets and red blood cells were transfused to maintain the platelet level above 20×10^9/L and the hemoglobin level above 8 g/dL. All blood products were irradiated and filtered before transfusion.

Patients received antibacterial and antifungal prophylaxis consisting of oral ciprofloxacin 600 mg/day and fluconazole 200 mg/day, beginning 3 days before the start of the conditioning regimen. As prophylaxis against *Pneumocystis carinii* pneumonia, sulfamethoxazole/trimethoprim was given for at least 14 consecutive days (1600 mg of sulfamethoxazole and 320 mg of trimethoprim daily) before transplantation and was resumed after engraftment on a 2-day per week schedule. As prophylaxis against herpes simplex virus infection and varicella zoster virus reactivation, acyclovir was given 1000 mg/day orally or 750 mg/day intravenously from days −7 to 35, followed by long-term low-dose (400 mg/day) oral administration until the end of immunosuppressive therapy [23]. All patients received cytomegalovirus (CMV) high-titer intravenous immunoglobulin 5 g weekly for the first 3 months after transplantation. A CMV antigenemia assay with C7-HRP monoclonal antibody (Teijin, Tokyo, Japan) was performed at least once per week, and antigenemia-guided preemptive therapy with ganciclovir was performed as previously described [24].

Assessment of chimerism

Chimerism assay was performed with peripheral blood $CD3^+$ cells or mononuclear cells by the short tandem repeat method on days 30, 60, 90, and 120 after transplantation, and every 60 days thereafter, as described previously [4]. Complete donor-cell type chimerism was defined as 90% or more donor-type DNA. Donor lymphocyte infusion (DLI) was planned when patients failed to achieve complete donor chimerism after discontinuation of cyclosporine.

Treatment for progressive disease following RIST

DLI or low-dose subcutaneous IFN-α therapy was planned for patients with persistent or progressive disease in the absence of GVHD after discontinuation of cyclosporine.

Outcome measures

The primary endpoint was achievement of sustained engraftment with the induction of complete donor-type chimerism, without death during the first 100 days. All deaths within the first 100 days of transplant were defined as failure regardless of the cause of death, because it often is difficult to distinguish between transplantation-related mortality and death due to progressive disease during the early posttransplant period. RRT and acute and chronic GVHD were evaluated according to standard criteria [25–27]. Treatment response was evaluated monthly after transplantation according to the Response Evaluation Criteria in Solid Tumors (RECIST) [28]. Briefly, if the longest diameters of measurable lesions were reduced by 30% or more for at least 4 weeks compared with those before RIST, the patients were determined as partial remission (PR). If the diameters showed 20% or greater increase compared with the smallest diameters or if new lesions appeared, the patients were determined as progressive disease (PD). Patients were determined as stable disease (SD) when they did not met either PR or PD criteria. We also evaluated the duration of SD.

Statistical analysis

The characteristics of the patient groups were compared using Fisher's exact test or Mann-Whitney U-test. The actuarial survival rate was calculated by the Kaplan-Meier method. To calculate the survival of transplanted cases, the date of transplantation was defined as day 0 of the survival period. To compare the survival of transplanted and nontransplanted patients or patients with and without an HLA-matched donor, the date of HLA typing was defined as day 0 of the survival period. Differences between survival rates were calculated using Wilcoxon's log rank analysis. $p < 0.05$ were considered significant.

Results

Transplantation, engraftment, and chimerism analysis

Characteristics of the enrolled patients and transplantation outcomes are given in Tables 1 and 2, respectively. All nine patients received a stem cell graft from an HLA-matched sibling donor. Engraftment was achieved a median of 10.5 days after transplantation (range 10–11). Six patients did not develop a platelet count $<20 \times 10^9$/L, and the other three achieved an unsupported platelet count $>20 \times 10^9$/L on a median of 11 days (range 9–11). Three patients received transfusion of packed red blood cells with a median of 12 units during the first month (range 10–18), and seven patients received packed platelets with a median of 24 units during the first month (range 10–100). Complete donor chimerism was achieved without additional DLI by day 30 (n = 6) or day 60 (n = 3).

Toxicities

Transplantation-related adverse events are listed in Table 3. No acute phase nonhematologic RRT of grade III/IV was observed. Most patients could maintain oral intake throughout the transplantation course, except for patient 8

Table 1. Patient characteristics

Patient no.	Age/sex	Histology of primary tumors	Prior surgeries	Prior treatments	Days from nephrectomy to transplantation	Metastatic organs	
						No.	Sites
1	29/M	Granular + spindle	Nephrectomy	IFN-α	734	4	Liver, lung, skin, renal fossa
2	38/M	Clear + granular	Nephrectomy, lung resection	IFN-α, IL-2	1282	2	Lung, LN
3	32/F	Papillary	Nephrectomy, LN dissection	IFN-α	1045	1	Lung
4	48/F	Clear	Nephrectomy, parotidectomy	IFN-α, IL-2, tegafur	3746	5	Lung, bone, salivary gland, LN, renal fossa
5	59/M	Clear + granular + spindle	Nephrectomy	IFN-α, tegafur	1083	2	Lung, pleura
6	35/M	Clear + granular	Nephrectomy, liver resection	IFN-α, IL-2, tegafur, XRT (bone)	322	4	Lung, liver, bone, LN
7	25/F	Papillar	Nephrectomy	IFN-α, tegafur, XRT (bone)	62	3	Liver, bone, LN
8	47/F	Clear	Nephrectomy	IFN-α, TAE (kidney)	3722	5	Lung, pancreas, kidney, adrenal gland, LN
9	61/M	Clear + granular + spindle	Nephrectomy, lung resection	IFN-α, XRT (bone)	427	1	Bone

clear = clear cell carcinoma; granular = granular cell carcinoma; IFN-α = interferon-α; IL-2 = interleukin-2; LN = lymph node; papillary = papillary carcinoma; spindle = spindle cell carcinoma; TAE = transarterial embolization; XRT = radiation therapy.