TABLE I. The Incidence of Graft Failure after Allogeneic Transplantation Using Fludarabine, Melphalan, and Alemtuzumab

Underlying disease	*n*	Donor R/U	Dose of alemtuzumab	Primary graft failure	Secondary graft failure
CLL	41	24/17	100 mg in 27 60 mg in 6 50 mg in 7 40 mg in 1	3	5
AML/MDS	76	35/41	100 mg	2	0
MM	25	0/25	100 mg	0	0
NHL	88	65/23	100 mg	1	3
HL	49	31/18	100 mg	0	0

Note. CLL, chronic lymphocytic leukemia; AML, acute myeloblastic leukemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; HL, Hodgkin's lymphoma; R, related donor; U, unrelated donor.

a potentially lympholytic level for approximately 2 months after transplantation [5].

The pharmacokinetics of alemtuzumab in the treatment of CLL are quite different. The peak blood concentration after the first infusion of alemtuzumab at 30 mg for refractory CLL patients showed a wide variation among patients [7]. In addition, the blood concentrations of alemtuzumab showed a modest negative correlation with the starting lymphocyte counts [7]. These results suggest that the pharmacokinetics of alemtuzumab in CLL patients were affected by the number of tumor cells. The alemtuzumab concentration tends to be lower when the patient has bulky tumor cells, since alemtuzumab may bind to tumor cells.

In a transplantation setting, only a cumulative dose of 100 mg or less, which is far lower than that in the treatment of CLL, is highly immunosuppressive and clinically effective for the prevention of graft rejection and GVHD [11]. However, the cumulative dose of 1.2 mg/kg (66 mg/body) might have been insufficient to prevent graft rejection in this patient with bulky CD52-positive residual tumor cells. On the other hand, previous transplantation studies have not pointed out any differences in the pharmacokinetics of alemtuzumab between patients with CD52-positive lymphoid malignancies and those with myeloid malignancies [5]. However, Delgado et al. recently reported the results of 41 consecutive allogeneic hematopoietic cell transplantation for CLL using fludarabine, melphalan, and alemtuzumab [12]. They showed a higher incidence of primary or secondary graft failure (8 of 41) than that in transplantation for the other hematological malignancies using the same regimen (Table I) [13–16]. The alemtuzumab concentrations in these patients were assumed to be lower than those in transplantation for the other hematological malignancies, because they had CLL and/or the dose of alemtuzumab was reduced in 14 of the 41 patients. These data further support our hypothesis that the low alemtuzumab concentration might have resulted in graft rejection in the current CLL patient.

In conclusion, a residual CD52-positive tumor may strongly affect the blood concentration of alemtuzumab. It may be worthwhile to decrease tumor cells before the conditioning regimen or to increase the dose of alemtuzumab in a conditioning to prevent graft rejection and GVHD. More data on the pharmacokinetics of alemtuzumab are needed to determine an optimal dose for HSCT, especially from a 2- or 3-locus-mismatched donor.

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Effect of Blood Cyclosporine Concentration on the Outcome of Hematopoietic Stem Cell Transplantation From an HLA-Matched Sibling Donor

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We retrospectively evaluated the effect of the blood cyclosporine (CsA) concentration on the outcome of allogeneic hematopoietic stem cell transplantation from an HLA-matched sibling donor in 171 patients who received a continuous infusion of CsA and short-course methotrexate to prevent graft-versus-host disease (GVHD). CsA was started at 3.0 mg/kg/day and the dose was adjusted to maintain the blood CsA concentration between 250 and 350 ng/ml. The actual dose of CsA averaged 1.9 mg/kg/day at the 3rd week after transplantation. The incidence of grade II–IV acute GVHD was 29.9%. Patient age and sex were identified as independent significant risk factors for acute GVHD. The CsA concentration during the 3rd week after transplantation most strongly affected the incidence of grade II–IV acute GVHD (RR 0.995 for an increase in CsA concentration by 1 ng/ml, $P = 0.037$) adjusted for other risk factors. The incidence of acute GVHD was significantly lower in patients with a 3rd-week CsA concentration higher than 300 ng/ml than in those with values between 200 and 300 ng/ml (20% vs. 35%, $P = 0.036$). We concluded that the blood CsA concentration at peri-engraftment period may be important in preventing acute GVHD. Am. J. Hematol. 81:838–844, 2006. © 2006 Wiley-Liss, Inc.

Key words: hematopoietic stem cell transplantation; cyclosporine; graft-versus-host disease; continuous infusion; blood concentration

INTRODUCTION

The combination of cyclosporine (CsA) and methotrexate (MTX) is widely used for the pharmacologic prevention of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation. However, the dose, target blood level, and schedule of administration vary among protocols [1]. Previously, we retrospectively compared two methods of CsA infusion: continuous infusion versus twice-daily infusions early after transplantation [2]. The incidence of grade II–IV acute GVHD was significantly higher in patients who received a continuous infusion of CsA, although these patients showed a significantly lower incidence of renal dysfunction. The actual dose of CsA in the first 4 weeks after

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transplantation, which was adjusted to maintain the blood CsA concentration between 250 and 400 ng/ml, was significantly lower in patients who received a continuous infusion of CsA. Therefore, this target concentration might be inappropriate. In this study, we retrospectively evaluated the effect of the blood CsA concentration on the incidence of acute GVHD and the other transplantation outcome in patients who received a continuous infusion of CsA and short-course MTX.

PATIENTS AND METHODS

Patients

We retrospectively analyzed the records of 171 adult patients who underwent allogeneic hematopoietic stem cell transplantation from an HLA-matched sibling donor using a GVHD prophylaxis regimen consisting of CsA and MTX for acute leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome at seven centers that participated in the Kanto Study Group for Cell Therapy (KSGCT) between 1995 and 2002.

Transplantation Procedure

The conditioning regimen was mainly a combination of cyclophosphamide (120 mg/kg) with either busulfan (16 mg/kg/day) or fractionated total body irradiation (12 Gy). CsA was started at 3 mg/kg/day as a continuous infusion on day 1. The whole blood CsA concentration was measured at least once a week by fluorescence polarization immunoassay with a specific monoclonal antibody [3]. The sensitivity of this assay was 25 ng/ml. The dose of CsA was adjusted to maintain the blood CsA concentration between 250 and 350 ng/ml. The route of CsA administration was converted to oral at a ratio of 1:2 or 1:3 when patients were able to tolerate oral intake at least 3 weeks after transplantation. MTX was administered intravenously at 10–15 mg/m^2/day on day 1 and 7–10 mg/m^2/day on days 3 and 6 at the discretion of each center. Bone marrow or peripheral blood stem cells were harvested from an HLA-matched sibling and administered without ex vivo manipulation.

Statistical Considerations

Standard-risk disease was defined as acute leukemia in first or second complete remission, chronic myelogenous leukemia in first or second chronic phase, and myelodysplastic syndrome without leukemic transformation, while others were considered high-risk diseases [4]. The primary endpoint was the incidence of grade II–IV acute GVHD, which was evaluated based on clinical and pathological findings among patients who achieved engraftment [5]. Disease-free survival (DFS) and the cumulative incidences of acute GVHD, relapse, and nonrelapse mortality were the secondary endpoints. DFS and the cumulative incidence of acute GVHD were calculated using the Kaplan-Meier method. The incidences of relapse and nonrelapse mortality were calculated using Gray's method considering each other event as a competing risk [6]. Potential confounding factors considered in the analysis were age, sex, performance status, disease risk, stem cell source, conditioning regimen, and prophylactic use of fluconazole and granulocyte-colony stimulating factor (G-CSF). Factors that showed at least borderline significance ($P < 0.15$) in univariate analyses were included in the multivariate analyses and then stepwisely deleted from the model. The blood CsA concentration was added in the final model. Finally, factors with P values less than 0.05 were considered significant. There were missing data in several factors as shown in Table I. Although the number of patients with missing data was small, it might have affected the results of statistical analyses.

TABLE I. Characteristics of the Patients

Donor	
Median age	39 (range 9–65)
Sex	Male 87/female 82 (N.D. 2)
Patient	
Median age	40 (range 14–58)
Sex	Male 102/female 69
Performance status	0/1/2/3 113/32/7/0 (N.D. 19)
Underlying disease	AML 66, AUL 2, ALL 30, CML 50, MDS 23
Disease risk	Standard 111, high 53 (N.D. 7)
Transplantation procedure	
Total body irradiation	Yes 109/No 62
Stem cell	Bone marrow 101/peripheral blood 70
Fluconazole prophylaxis	Yes 121/No 50
G-CSF	Yes 124/No 38 (N.D. 9)

N.D., not described.

RESULTS

Patients' Characteristics

The characteristics of the 171 patients and their donors are summarized in Table I. The underlying disease was acute myeloblastic leukemia in 66, acute unclassified leukemia in 2, acute lymphoblastic leukemia in 30, chronic myelogenous leukemia in 50, and myelodysplastic syndrome in 23. Bone marrow

was used in 101 patients, whereas 70 patients received peripheral blood stem cell graft. Fluconazole and G-CSF were prophylactically administered in 121 and 124 patients, respectively.

Risk Factors for Grade II–IV Acute GVHD

Engraftment was observed in 166 patients a median of 16 days (range 7–47 days) after transplantation. The incidence of grade II–IV acute GVHD was 29.9%, with a median onset of 21 days (range 3–37 days) after transplantation (see Fig. 1). The incidence of grade III–IV acute GVHD was 9.8%. In univariate analyses to evaluate the impact of potential confounding factors on the incidence of grade II–IV acute GVHD, patient age was identified as the only significant risk factor (Table II, A). Patient sex, stem cell source, and the total dose of MTX were borderline significant. All of these factors were included in the multivariate analysis using the backward stepwise selection and only two factors, patient age (relative risk [RR] 1.04 for an increase in age by 1 year, 95% confidence interval [CI] 1.00–1.07, $P = 0.01$) and patient sex (RR 0.54, 95% CI 0.29–1.00, $P = 0.05$), were identified as independent significant risk factors (Table II, B).

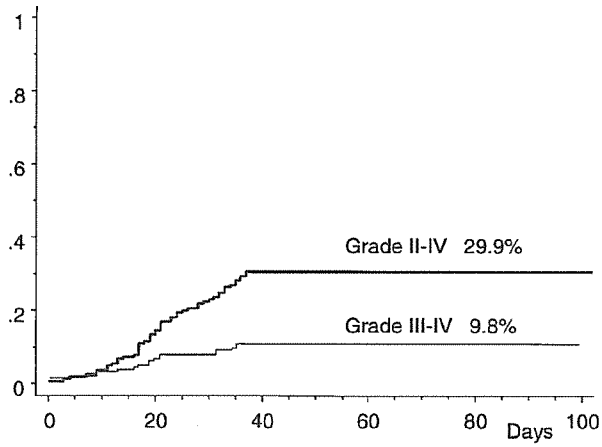

Fig. 1. The incidence of grade II–IV and grade III–IV acute GVHD.

Effect of CsA Concentration on the Incidence of Grade II–IV Acute GVHD

The dose of CsA was adjusted to maintain the blood CsA concentration between 250 and 350 ng/ml. This adjustment was successful and the mean CsA concentration was 375 ± 124, 310 ± 79, and 326 ± 130 ng/ml at the 1st, 2nd and 3rd week after transplantation, respectively (Fig. 2A). However, the actual dose of CsA was decreased every week and the mean dose of CsA was 2.5 ± 0.6, 2.0 ± 0.7, and 1.9 ± 0.7 mg/kg at the 1st, 2nd, and 3rd week after transplantation, respectively (Fig. 2B). In three of the four patients who developed early renal toxicity, CsA was stopped or the dose of CsA was decreased, but the mean dose of CsA was the same (1.80 ± 0.6 mg/kg) when we excluded patients who developed renal dysfunction within 21 days after

TABLE II. Univariate (A) and Multivariate (B) Analyses for the Incidence of Grade II–IV Acute GVHD

	Incidence (%)	Relative risk (95% CI)	*P* value
A. Univariate analysis			
Dichotomous variables			
Patient sex (male vs. female)	34 vs. 23		0.11
Patient PS (0 vs. 1–4)	30 vs. 30		0.89
Donor sex (male vs. female)	32 vs. 29		0.88
Sex mismatch (yes vs. no)	33 vs. 28		0.36
Disease risk (standard vs. high)	31 vs. 30		0.76
Total body irradiation (yes vs. no)	28 vs. 34		0.37
G-CSF (yes vs. no)	28 vs. 40		0.18
Stem cell (BM vs. PB)	35 vs. 23		0.12
Continuous variables			
Patient age		1.03 (1.00–1.06)	0.02
MTX dose		1.04 (0.99–1.09)	0.15
B. Multivariate analysis			
Patient age		1.04 (1.00–1.07)	0.01
Patient sex		0.54 (0.29–1.00)	0.05
C. Effect of cyclosporine concentration			
Cyclosporine concentration		0.995 (0.991–0.999)	0.023
1st week concentration		0.999 (0.996–1.001)	0.34
2nd week concentration		0.998 (0.994–1.002)	0.26
3rd week concentration		0.995 (0.991–1.000)	0.037

The effect of blood cyclosporine concentration was adjusted for independent significant risk factors (C).

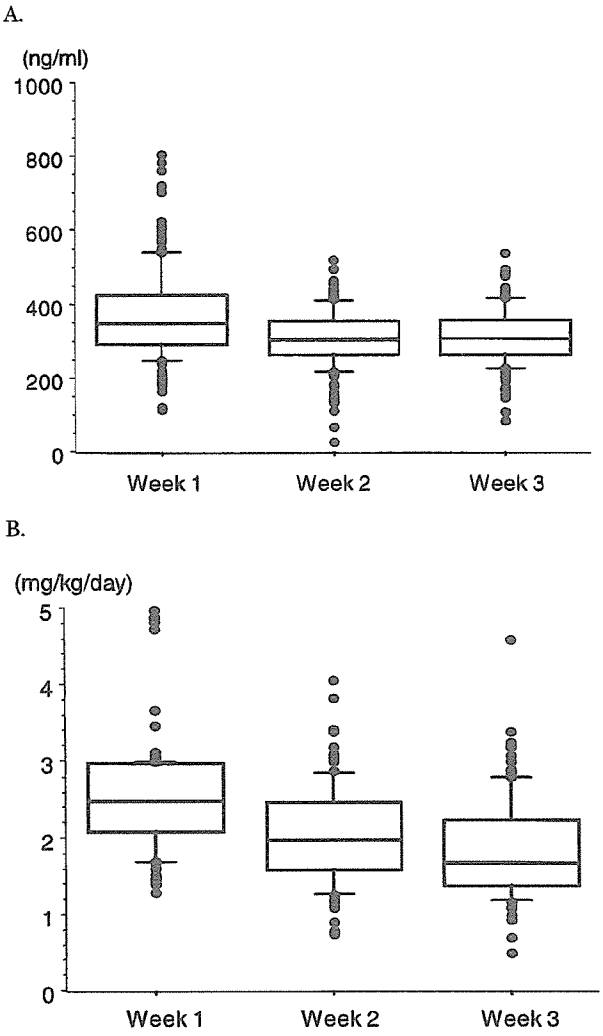

Fig. 2. A: Blood CsA concentration at the 1st, 2nd, and 3rd week after transplantation. B: The actual daily dose of CsA at the 1st, 2nd, and 3rd week after transplantation. The box-and-whisker plot shows 10, 25, 50, 75, and 90 percentile values. Outliers are indicated by dots.

transplantation. The ratio of the blood CsA concentration to the actual dose of CsA was not different between those who were receiving fluconazole and those who were not (194 vs. 189 [ng/ml]/[mg/kg], $P = 0.78$), which suggested that fluconazole did not significantly interact with the CsA concentration.

The mean CsA concentration in each patient within 3 weeks after transplantation significantly affected the incidence of acute GVHD (RR 0.995 for an increase in CsA concentration by 1 ng/ml, 95% CI 0.991–0.999, $P = 0.023$, Table II, C), adjusted for the two independent significant risk factors. Next, we evaluated the effect of the CsA concentration during the 1st, 2nd, and 3rd weeks after transplantation on the incidence of grade II–IV acute GVHD among patients who did not develop GVHD before each period. Only the CsA concentration during the 3rd week after transplantation significantly correlated with the incidence of acute GVHD (RR 0.995, 95% CI 0.991–1.000, $P = 0.037$). As shown in Fig. 3, the incidence of acute GVHD was different among the CsA-concentration groups during the 3rd week after transplantation (44% for patients with CsA concentration lower than 200 ng/ml, 35% for those with CsA concentration between 200 and 300 ng/ml, and 20% for those with CsA concentration higher than 300 ng/ml, $P = 0.035$). When we grouped patients with the 1st week CsA concentration lower than 300 ng/ml and the 3rd week CsA concentration higher than 300 ng/ml as "low–high" group and those with the 1st week CsA concentration higher than 300 ng/ml and the 3rd week CsA concentration lower than 300 ng/ml as "high–low" group, the incidence of grade II–IV acute GVHD was 23, 33, and 30% in the "low–high" group, the "high–low" group, and the others, respectively, although the difference was not statistically significant.

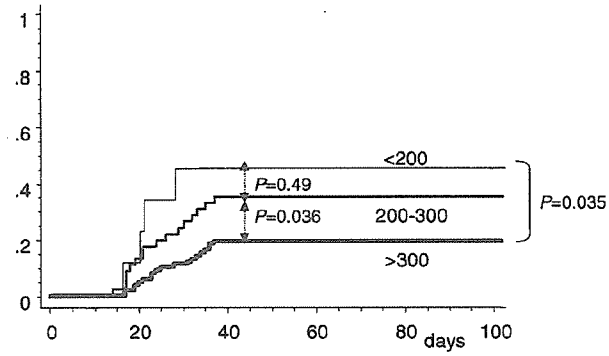

Fig. 3. Incidence of grade II–IV acute GVHD grouped according to the blood CsA concentration (ng/ml) during the 3rd week after transplantation among patients who did not develop GVHD before the period.

Effect of CsA Concentration on Transplantation Outcome

DFS for the entire population was 56% at 3 years after transplantation. Patient age, performance status, and disease risk were identified as independent significant factors for DFS (Table III). We evaluated the effect of the CsA concentration on DFS adjusted for these factors and did not find a significant effect of CsA concentration on DFS (RR 1.00, 95% CI 0.994–1.004, $P = 0.57$). The cumulative incidences of relapse and non-relapse mortality at 3 years were 24 and 20%, respectively. The blood CsA concentration did not significantly affect the incidence of these events after adjusting for other significant risk factors (RR 0.998, 95% CI 0.993–

TABLE III. Univariate (A) and Multivariate (B) Analyses for Disease-Free Survival (DFS)

	3-year DFS	Relative risk (95% CI)	*P* value
A. Univariate analysis			
Dichotomous variables			
Patient sex (male vs. female)	55 vs. 59		0.42
Patient PS (0 vs. 1–4)	61 vs. 41		0.02
Donor sex (male vs. female)	60 vs. 52		0.24
Sex mismatch (yes vs. no)	58 vs. 54		0.43
Disease risk (standard vs. high)	67 vs. 30		<0.0001
Total body irradiation (yes vs. no)	58 vs. 56		0.95
G-CSF (yes vs. no)	64 vs. 54		0.62
Stem cell (BM vs. PB)	59 vs. 53		0.47
Continuous variables			
Patient age		1.02 (1.00–1.04)	0.07
MTX dose		1.00 (0.96–1.04)	0.96
B. Multivariate analysis			
Patient PS (0 vs. 1–4)		0.57 (0.33–0.97)	0.04
Patient age		1.03 (1.00–1.05)	0.03
Disease risk (standard vs. high)		0.41 (0.25–0.68)	0.0005
C. Effect of cyclosporine concentration			
Cyclosporine concentration		1.00 (0.994–1.004)	0.57

The effect of the blood cyclosporine concentration was adjusted for independent significant risk factors (C).

TABLE IV. Univariate (A) and Multivariate (B) Analyses for the Incidence of Relapse

	Incidence	Relative risk (95% CI)	*P* value
A. Univariate analysis			
Dichotomous variables			
Patient sex (male vs. female)	21 vs. 28		0.35
Patient PS (0 vs. 1–4)	19 vs. 35		0.07
Donor sex (male vs. female)	25 vs. 23		0.64
Sex mismatch (yes vs. no)	19 vs. 29		0.28
Disease risk (standard vs. high)	21 vs. 40		0.06
Total body irradiation (yes vs. no)	26 vs. 21		0.40
G-CSF (yes vs. no)	26 vs. 14		0.29
Stem cell (BM vs. PB)	22 vs. 27		0.51
Continuous variables			
Patient age		1.00 (0.97–1.03)	0.87
MTX dose		0.99 (0.94–1.04)	0.71
B. Multivariate analysis			
Disease risk (standard vs. high)		2.31 (1.14–4.66)	0.02
C. Effect of cyclosporine concentration			
Cyclosporine concentration		0.998 (0.993–1.003)	0.41

The effect of the blood cyclosporine concentration was adjusted for independent significant risk factors (C).

1.003, $P = 0.41$ for relapse adjusted for disease risk and RR 0.998, 95% CI 0.991–1.005, $P = 0.57$ for nonrelapse mortality adjusted for patient sex, sex mismatch, and patient age, Tables IV and V).

Renal Toxicity

Renal dysfunction, defined as an elevation of the serum creatinine level above 2.0 × baseline, was observed in 18.9% of the patients, with a median onset of 36 days (range 5–151 days) after transplantation. Among them, only four developed renal dysfunction within the first 3 weeks after transplantation and thus we could not evaluate the effect of the blood CsA concentration on the incidence of renal dysfunction.

DISCUSSION

The results of this study clearly demonstrated the importance of the blood CsA concentration in the

TABLE V. Univariate (A) and Multivariate (B) Analyses for the Incidence of Nonrelapse Mortality (NRM)

	Incidence	Relative risk (95% CI)	*P* value
A. Univariate analysis			
Dichotomous variables			
Patient sex (male vs. female)	24 vs. 13		0.06
Patient PS (0 vs. 1–4)	20 vs. 24		0.55
Donor sex (male vs. female)	15 vs. 25		0.11
Sex mismatch (yes vs. no)	27 vs. 14		0.05
Disease risk (standard vs. high)	18 vs. 30		0.19
Total body irradiation (yes vs. no)	18 vs. 22		0.33
G-CSF (yes vs. no)	20 vs. 22		0.23
Stem cell (BM vs. PB)	19 vs. 21		0.74
Continuous variables			
Patient age		1.04 (1.01–1.08)	0.02
MTX dose		1.01 (0.95–1.07)	0.77
B. Multivariate analysis			
Patient sex (male vs. female)		2.46 (1.08–5.60)	0.03
Sex mismatch (yes vs. no)		2.46 (1.19–5.12)	0.02
Patient age		1.05 (1.01–1.08)	0.01
C. Effect of cyclosporine concentration			
Cyclosporine concentration		0.998 (0.991–1.005)	0.57

The effect of the blood cyclosporine concentration was adjusted for independent significant risk factors (C).

incidence of acute GVHD, especially during the 3rd week after transplantation from an HLA-matched sibling donor. The median time to engraftment was 16 days and thus the blood CsA concentration during the peri-engraftment period may be important for preventing acute GVHD [7]. The incidence of acute GVHD was significantly lower in patients with a 3rd-week CsA concentration higher than 300 ng/ml than in those with values between 200 and 300 ng/ml. Although a target range around 300 ng/ml is widely used as daily practice, it may be worthwhile to explore a higher target range, since the actual dose of CsA was decreased to 1.9 mg/kg/day and only four patients developed renal dysfunction within the first 3 weeks after transplantation. In this regard, we considered that the results of previous studies that compared the continuous infusion of CsA and tacrolimus as GVHD prophylaxis should be interpreted with caution [8–10]. The comparison between CsA at higher-dose and tacrolimus at less-toxic dose is warranted to evaluate their true efficacy to prevent GVHD.

Elevating the target concentration of CsA may result in an increased incidence of relapse. In this study, we did not observe a relationship between the CsA concentration and relapse incidence. However, the CsA concentration was kept at a significantly lower level in high-risk patients than in standard-risk patients (319 ng/ml vs. 346 ng/ml, $P = 0.03$), probably to enhance a graft-versus-leukemia effect, and this might have biased the effect of the CsA concentration on the incidence of relapse. In fact, we previously showed that the incidence of relapse was significantly lower after the continuous infusion of CsA with this low target CsA concentration when compared with twice-daily infusions, which resulted in better DFS in high-risk patients [2]. Therefore, we are currently evaluating the feasibility and efficacy of the continuous infusion of CsA with a target CsA concentration of between 450 and 550 ng/ml only in standard-risk patients. When the dose of CsA is adjusted to this target concentration, we can expect that the actual dose is maintained around 3 mg/kg [11].

In conclusion, the blood CsA concentration significantly affected the incidence of grade II–IV acute GVHD after allogeneic hematopoietic stem cell transplantation from an HLA-matched sibling donor. The CsA concentration during the 3rd week after transplantation appeared to be most important. Therefore, the CsA concentration at peri-engraftment period should be monitored carefully to prevent acute GVHD.

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EXTENDED REPORT

A phase I-II trial of autologous peripheral blood stem cell transplantation in the treatment of refractory autoimmune disease

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Objectives: To carry out a phase I-II trial to elucidate the feasibility and efficacy of high dose cyclophosphamide (CY) supported by autologous peripheral blood stem cell transplantation (PBSCT) in the treatment of severe and refractory autoimmune disease (AD).

Methods: Peripheral blood stem cells (PBSCs) were mobilised during haematological recovery after relatively high dose CY (2 g/m^2) for 2 days, followed by administration of granulocyte colony stimulating factor. After collecting PBSCs—more than 2×10^6 CD34+ cells/kg—by apheresis, CD34+ cells were immunologically selected and cryopreserved. Eight patients were enrolled—five had systemic sclerosis (SSc) alone, one had SSc with systemic lupus erythematosus, one amyopathic dermatomyositis (ADM), and one Wegener's granulomatosis (WG). All of the patients were treated with high dose CY (50 mg/kg) for 4 days and autologous PBSCT.

Results: Haematopoietic reconstitution was rapid and sustained. Toxicity due to the regimen included various infections such as pneumonia, sepsis, cystitis, herpes zoster, and acute heart failure. However, there was no treatment related mortality. Encouraging results were obtained after autologous PBSCT. Sclerosis of the skin was markedly improved in all of the patients with SSc. Interstitial pneumonia (IP), evaluated by PaO_2, serum KL-6 levels, and pulmonary high resolution computed tomography, improved significantly. In a patient with ADM, severe and progressive IP also improved markedly. In a patient with WG, the size of the left orbital granuloma decreased substantially, resulting in reduction of the exophthalmos.

Conclusions: These observations suggest that high dose CY with autologous PBSCT is feasible and may be effective in the treatment of severe and refractory AD.

Although most patients with autoimmune disease (AD) have a relapsing, remitting or smouldering disease, some of them are damaged severely or fatally from the uncontrolled disease progression, and conventional treatments are not effective. The concept of high dose immunosuppressive treatment and autologous haematopoietic stem cell transplantation (HSCT) for AD is based on the finding that HSCT is effective for animal models of AD,[1–3] and that patients receiving autologous HSCT for treatment of malignant diseases can achieve long term remission of coincidental AD.[4–6]

Autologous HSCT as a treatment for AD was initiated in 1996, and more than 800 patients with AD have been treated.[7] Clinically significant responses were found in two thirds of the patients who received HSCT, and treatment related mortality (TRM) was reported to be relatively high (9%) in the early period until 2000.[8] The mechanism for inducing remission in AD is based not only on the eradication of autoreactive lymphocytes by an immunoablative pretransplant conditioning regimen but also on the correction of a dysregulated immune balance by newly developed lymphocytes derived from the haematopoietic stem cells transplanted.[9]

Many reports have examined the clinical results of autologous HSCT for AD. However, few studies provided detailed information about the effect of autologous HSCT on interstitial pneumonia (IP), which is often associated with AD. In the study by McSweeney *et al*, 19 patients with systemic sclerosis (SSc) were treated with high dose immunosuppression followed by autologous HSCT, resulting in no significant changes in carbon monoxide transfer factor (T_{LCO}) or vital capacity (VC) at 12 months after autologous HSCT.[10] We carried out a phase I-II trial to elucidate the feasibility and efficacy of high dose cyclophosphamide (CY) supported by autologous peripheral blood CD34 selected stem cell transplantation (PBSCT) in patients with severe and refractory AD. We report encouraging results obtained in eight patients, suggesting that high dose CY with autologous PBSCT may be effective for treatment of AD complicated by IP.

Abbreviations: A-aDO_2, alveolar-arterial oxygen tension difference; AD, autoimmune disease; ADM, amyopathic dermatomyositis; ATG, antithymocyte globulin; CY, cyclophosphamide; HRCT, high resolution computed tomography; G-CSF, granulocyte-colony stimulating factor; HSCT, haematopoietic stem cell transplantation; IP, interstitial pneumonia; mRSS, modified Rodnan skin score; NHL, non-Hodgkin's lymphoma; NIH-CTC, National Cancer Institute-Common Toxicity Criteria; PaO_2, arterial oxygen pressure; PBSCs, peripheral blood stem cells; PBSCT, peripheral blood stem cell transplantation; SSc, systemic sclerosis; TBI, total body irradiation; T_{LCO}, carbon monoxide transfer factor; TRM, treatment related mortality; VC, vital capacity; WG, Wegener's granulomatosis

Table 1 Patient profile

Patient No	Diagnosis	Sex	Age (years)	PS	mRSS	Major disorders associated with AD	VC/TLco (%)	Auto-antibody	Prior treatment	Follow up (months)
1	SSc+SLE	F	54	2	16	IP, digital ulcer	58/51	Anti-Scl-70	St, CY	33
2	SSc	M	55	2	15	IP	65/47	Anti-Scl-70	St	25
3	SSc	M	58	2	31	IP	63/44	–	St	22
4	SSc	F	54	1	26	IP	73/60	Anti-Scl-70	St	20
5	SSc	F	53	1	28	IP	74/29	Anti-Scl-70	St	17
6	SSc	F	48	2	32	IP	77/25	Anti-Scl-70	St, CY, CsA	13
7	ADM	F	54	2		IP	50/50	–	St, CY, CsA	31
8	WG	M	21	1		Exophthalmos		Anti-PR-3	St, CY, CsA	16

PS, performance status; mRSS, modified Rodnan skin score for systemic sclerosis; AD, autoimmune disease; F, female; M, male; SSc, systemic sclerosis; IP, interstitial pneumonia; ADM, amyopathic dermatomyositis; WG, Wegener's granulomatosis; PR3, proteinase 3; St, corticosteroids; CY, cyclophosphamide; CsA, ciclosporin A.

PATIENTS AND METHODS

Protocol

The protocol of this phase I-II clinical trial was approved by the ethics committee of Kyushu University Hospital. Written informed consent was obtained from all patients.

Patients and eligibility

Patients aged between 16 and 65 years were eligible at the time of pretransplant evaluation. Patient eligibility depended on a diagnosis of AD. All of the patients were followed up for at least 12 months after transplantation for the evaluation of treatment outcomes.

Patients with SSc were eligible when they had severe diffuse SSc that had rapidly developed over the previous 4 years. They also had to have at least one of the following: (*a*) pulmonary involvement including VC or TLco <70% predicted or arterial oxygen pressure (Pao_2) at room temperature below 70 mm Hg and evidence of interstitial lung disease defined by pulmonary high resolution computed tomography (HRCT); (*b*) cardiac disease, which was reversible congestive heart failure or significant arrhythmia; and (*c*) renal involvement such as hypertension, persistent urine analysis abnormalities, microangiopathic haemolytic anaemia, and renal insufficiency.

Patients with limited scleroderma were considered eligible when progressive and life threatening IP was present.

Patients with amyopathic dermatomyositis (ADM) were eligible when they had the following criteria: (*a*) clinical diagnosis of ADM by the criteria reported[11]; (*b*) progressive and life threatening IP that was refractory to conventional immunosuppressive treatment.

Table 2 Apheresis and CD34+ selection in eight patients

Characteristic	Median (range)
Apheresis	
Number of apheresis/patient	2 (1–4)
Total cells $\times 10^9$	22.3 (8.7–90.4)
CD34+ (%)	2.06 (0.11–4.72)
CD34+ $\times 10^7$	36.8 (10.1–161.1)
CD34+ $\times 10^6$/kg	7.61 (2.06–35.80)
CD34+ selection	
Total cells $\times 10^7$	33.4 (11.6–95.0)
Total cells $\times 10^6$/kg	5.11 (2.34–21.11)
CD34+ $\times 10^7$	28.0 (10.1–94.3)
CD34+ $\times 10^6$/kg	4.90 (2.10–21.10)
CD3+ $\times 10^5$	5.78 (0–15.00)
CD3+ $\times 10^4$/kg	0.91 (0–2.95)
Purity (%)	96.4 (87.0–99.3)
Yield (%)	75.7 (58.6–100.0)

Patients with Wegener's granulomatosis (WG) were eligible when they had the following criteria: (*a*) clinical diagnosis of WG by the criteria reported[12]; (*b*) vasculitis or granuloma causing severe organ damage that was refractory to conventional immunosuppressive treatment.

Exclusion criteria

Patients were excluded from the study when they had uncontrolled arrhythmia, heart failure with left ventricular ejection fraction (LVEF) <50%, mean pulmonary artery pressure >50 mm Hg, TLco <20% predicted, and creatinine clearance below 40 ml/min/m^2.

Peripheral blood stem cell (PBSC) mobilisation, CD34 cell selection, and autologous PBSCT

PBSCs were mobilised during haematological recovery after relatively high dose CY (2 g/m^2) for 2 days followed by administration of recombinant human granulocyte-colony stimulating factor (G-CSF, filgrastim; Kirin Brewery, Tokyo, Japan) at a dose of 10 μg/kg as previously described.[13] After collecting PBSCs to obtain 2×10^6 CD34+ cells/kg or more by apheresis, CD34+ cells were positively selected using immunomagnetic beads with an anti-CD34 monoclonal antibody (CliniMACS, Miltenyi Biotec, Germany). Mobilisation of PBSCs was repeated when 2×10^6 CD34+ cells/kg were not obtained. For pretransplant conditioning, high dose CY (50 mg/kg) was given for 4 days from day −5 to −2. After transplantation of frozen-thawed CD34+ cells on day 0, G-CSF was administered from day 1. Acyclovir (250 mg/day, from day 1 to 18), ciprofloxacin (600 mg/day from day −7 to −1), fluconazole (400 mg/day from day −7 to 14, 200 mg/day from day 15 to 100), trimethoprim-sulfamethoxazole (1920 mg/day, from day −14 to −2 and 1920 mg/day, twice a week from day 30 to 100) were prophylactically given as previously described.[14]

Treatment outcome

The modified Rodnan skin score (mRSS) was used to evaluate the improvement of skin sclerosis in patients with SSc.[14] Arterial blood gas at room temperature, a pulmonary function test, pulmonary HRCT, and serological examinations were used to evaluate the effect of high dose CY on IP. HRCT scans were graded and scored blinded according to the relative amount of ground glass opacity and reticular infiltrates as follows: 1 = pure ground glass; 2 = ground glass more than reticular; 3 = ground glass equals reticular; 4 = reticular more than ground glass; 5 = pure reticular.[15] The lower grade indicates more active inflammation in this system. Regimen related toxicity was determined and graded according to the National Cancer Institute-Common Toxicity Criteria (NIH-CTC) version 2. Cytomegalovirus antigenaemia was determined as previously described.[16]

Table 3 Number of reinfused cells and haematological recovery

Patient No	Number of reinfused CD34+ cells ($\times10^6$/kg)	Number of reinfused CD3+ cells ($\times10^4$/kg)	ANC >0.5×10⁹/l (days)	Platelet >50×10⁹/l (days)	Interval between PBSC harvest and PBSCT (days)
1	8.4	0.33	9	20	27
2	4.9	0.27	9	10	64
3	2.2	2.95	10	12	39
4	2.1	1.71	11	16	87
5	7.2	13.00	13	9	51
6	4.0	2.35	11	11	355
7	4.9	0.50	8	10	31
8	5.0	0.52	13	11	50

ANC, absolute neutrophil count; PBSC, peripheral blood stem cell; PBSCT, peripheral blood stem cell transplantation.

Statistical analysis

Wilcoxon's signed rank test was used for statistical analysis of the data.

RESULTS

Patients

Eight patients (three male, five female) with a median age of 54 years (range 21–58) were studied (table 1). Patients 1–6 were diagnosed as diffuse SSc. Patient 1 had had systemic lupus erythematosus (SLE) for 22 years and SSc for 2 years. She had progressive IP and severe digital ulcers due to SSc while the SLE was inactive. Patients 2, 3, 5, 6 (SSc), and 7 (ADM) also developed severe and progressive IP. Patient 4 had mild IP. Patients 3, 4, 5, and 6 showed severe skin sclerosis. Patient 3 had been in complete remission of non-Hodgkin's lymphoma (NHL) for 1 year and he was considered to be eligible. Patient 8 (WG) presented with severe exophthalmos due to a granuloma, which was 18 mm in diameter and located in the upper lateral region of the left orbit affecting the superior rectus muscle. He needed monthly steroid pulse therapy to prevent further growth of the granuloma. Eastern Cooperative Oncology Group performance status[17] was <3 in all patients. Anti-Scl-70 antibody was positive in 5/6 patients with SSc. CY and ciclosporin A were used in four and three of the patients, respectively. All patients were treated with corticosteroids, and the median duration of follow up was 21 months (range 13–33). Results are reported as of February 2005.

PBSC mobilisation and CD34+ cell selection

PBSCs were collected by apheresis after CY plus G-CSF-induced mobilisation in all patients as previously described.[13] The median of the total number of CD34+ cells collected was 7.61×10^6/kg (range 2.06–35.80) after apheresis (table 2). CD34+ cell selection was performed using CliniMACS. Purity and yield of the CD34+ cells selected were 96.4% (range 87.0–99.3) and 75.7% (range 58.6–100), respectively. Mobilisation was repeated in patient 4 because an insufficient number of CD34+ cells (2×10^6/kg) were collected after the initial mobilisation.

Autologous PBSCT

All the patients received autologous transplantation of frozen-thawed CD34+ cells after pretransplant conditioning with high dose CY. The median numbers of CD34+ and CD3+ cells infused were 4.92×10^6/kg (range 2.1–8.4) and 1.17×10^4/kg (range 0.27–13.0), respectively (table 3). All the patients achieved rapid haematopoietic engraftment. Median days to an absolute neutrophil count >0.5×10⁹/l and a platelet count >50×10⁹/l were 10.5 (range 8–13) and 11.5 (range 9–20), respectively. The interval between PBSC harvest and PBSCT was a median of 50.5 days (range 27–355).

Toxicity

Patients 1, 6, and 7 developed post-transplant infections and showed grade 3–4 toxicity according to NCI-CTC (table 4). Patient 1 also developed pneumonia of unknown cause and adenoviral cystitis, and patient 6 showed positive blood cultures for *Streptococcus mitis* in addition to adenoviral cystitis. Adenoviral cystitis was successfully treated with cidofovir. Patient 7 had positive blood cultures for *Listeria monocytogenes*. Four patients developed herpes zoster with grade 2–3 toxicity around 12 months after transplantation. Five patients showed cytomegaloviral antigenaemia. Epstein-Barr titres were not checked in this study. Patient 1 had ventricular arrhythmia, and patient 4 showed ST depression in ECG during intravenous administration of CY. Patient 6 developed acute heart failure, requiring temporary intubation

Table 4 Toxicity (NCI)

Patient No	Infection	CMV antigenaemia	Cardiovascular	Haemorrhage	Pulmonary	Gastrointestinal	Hepatic
1	Pneumonia (3) cystitis, adenovirus (3)	+ (2)	VPCs (3)	–	–	Nausea (2)	Raised transaminase (1)
2	–	–	–	GI bleeding (3)	–	(1)	(1)
3	HZ (3)	+ (1)	–	–	–	(2)	(1)
4	HZ (3)	+ (1)	Cardiac ischaemia (2)	–	–	(2)	(2)
5	HZ (3)	–	–	–	Hypoxia (3)	(1)	–
6	Sepsis, *Strep. mitis* (3), cystitis, adenovirus (3)	+ (1)	CHF (4)	–	–	(2)	(3)
7	Sepsis, *Listeria monocytogenes* (3), HZ (2)	+ (2)	–	–	–	(2)	(2)
8	–	–	–	–	–	(1)	(2)

NCI, National Cancer Institute; CMV, cytomegalovirus; HZ, herpes zoster; *Strep*, *Streptococcus*; VPC, ventricular premature capture; GI, gastrointestinal; CHF, congestive heart failure; (number), grade of toxicity.

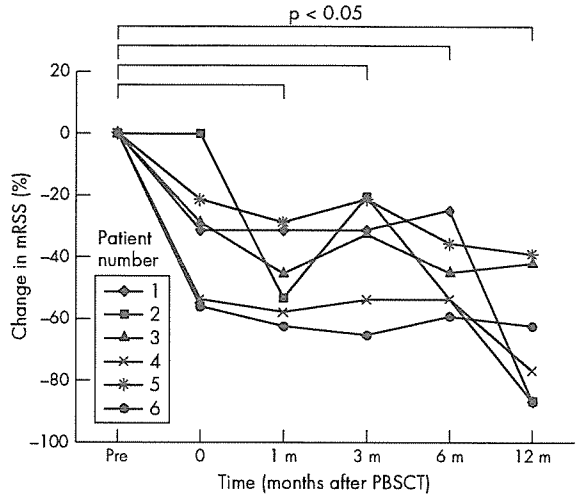

Figure 1 Evaluation of the modified Rodnan skin score (mRSS). The serial skin score data are presented for six patients with SSc. The proportional change from baseline measurement was calculated for each patient at each available time point. The x axis is not drawn to scale. Data obtained before mobilisation and just before conditioning are shown as "Pre" and "0" respectively.

after mobilisation. Patient 2 was complicated by grade 3 bleeding from an intestinal ulcer due to a non-steroidal anti-inflammatory drug during mobilisation. Patient 5 showed hypoxia due to transient worsening of IP shortly after administration of G-CSF. All the patients had grade 1–2 nausea and seven patients showed grade 1–3 hepatic toxicity. Twelve months after autologous PBSCT, patient 3 experienced a relapse of the NHL, which was successfully treated to reinduce complete remission by chemotherapy including rituximab. All the patients are alive with performance status 1 or 2.

CLINICAL OUTCOME

SSc

Figure 1 shows post-transplant changes in the mRSS for patients with SSc. A decline in skin score is considered significant if it is >25% of the baseline or >10% of the maximum skin score. When this definition is used, 6/6 (100%) patients showed significant improvement. The mean skin scores at 1, 3, 6, and 12 months post-transplant were significantly less than those before mobilisation ($p<0.05$). Five out of six patients showed an improvement in the skin score after mobilisation before pretransplant conditioning, although it was not statistically significant. Reincreases in skin score were not seen in any of the three patients who were followed up for 18 months or more after autologous PBSCT (data not shown).

To investigate the effect of autologous PBSCT on IP, blood gas analysis, a pulmonary function test, and pulmonary HRCT were performed at 3 and 12 months after transplantation. Figure 2A shows that Pao_2 was significantly increased from the median value of 66.5 mm Hg (range 51–88.7) before transplantation to 78.3 mm Hg (69.7–102) and 83.2 mm Hg (72.6–93.2) at 3 and 12 months after transplantation, respectively. Improvement of alveolar-arterial oxygen tension difference ($A\text{-}aDo_2$) was also seen in four patients at 12 months (fig 2B). The VC was improved in four and five patients at 3 and 12 months, respectively (fig 2C). Improvement of TLCO was seen in only one patient (fig 2D). Serum levels of KL-6, a marker for IP,[18] significantly decreased from the median value, 1823 U/ml (range 1080–2988) before transplantation to 890 U/ml (740–1962) and 989 U/ml (532–1273) at 3 and 12 months after transplantation, respectively (fig 2E). The ground glass opacity markedly regressed in all of the patients, although reticular infiltrates remained essentially unaffected after transplantation (fig 3), resulting in significant improvement of pulmonary HRCT grading from the median value of 2.5 (range 2–3) before transplantation to 4 (range 3–4) at 12 months after transplantation (fig 2F).

ADM

Skin lesions had resolved by conventional immunosuppressive treatment before mobilisation. Pao_2 was increased from 65.6 mm Hg before transplantation to 87.8 and 83.9 mm Hg at 3 and 12 months after transplantation, respectively. VC increased from 52.6% to 59.3% and 74.1% of the predicted value at 3 and 12 months, respectively. KL-6 decreased from 3280 IU/ml before transplantation to 1020 and 425 IU/ml at 3 and 12 months after transplantation, respectively. Both the ground glass opacity and the reticular infiltrates were markedly improved in pulmonary HRCT at 12 months post-transplant. The clinical course of this case has been described in detail elsewhere.[19]

WG

The size of the left orbital granuloma markedly decreased, resulting in an improvement of the exophthalmos, and regrowth of the granuloma has not been seen. Monthly steroid pulse therapy was not necessary to maintain this remission state. A serum level of proteinase 3 (PR3)-anti neutrophil cytoplasmic antibodies (ANCA) decreased from 72 IU/ml before transplantation to 39 IU/ml at 3 months after transplantation. However, it increased again to 157 IU/ml 12 months after transplantation.

DISCUSSION

In this study, we demonstrated that high dose CY with autologous PBSCT was feasible and effective in the treatment of refractory AD. For patients with SSc, we first showed that high dose CY and autologous PBSCT had favourable effects not only on skin sclerosis but also on IP. We thought our patient with WG was probably the first to have been treated with high dose CY and autologous PBSCT. However, during the revision of this manuscript a similar case was reported in the *Annals*.[19a]

We used a combination of high dose CY and G-CSF to mobilise a sufficient number of PBSC without the disease flare, although G-CSF alone was able to mobilise PBSC.[10] Flares of AD when G-CSF is used have been reported in rheumatoid arthritis,[20] multiple sclerosis,[21] and SSc.[22] In the European trial for SSc, the use of CY+G-CSF (84% of the cases) was preferred rather than G-CSF alone (10.7%).[23] In our trial, one patient had to repeat the mobilisation because an insufficient number of PBSC were obtained by the initial mobilisation. In another study, one of 12 PBSC mobilisations failed with the same protocol and autologous bone marrow transplantation was subsequently performed instead.[24]

We used immunological selection of CD34+ cells from PBSC harvests to minimise the risk of reinfusing autoreactive lymphocytes.[25] The selection device (CliniMACS) permitted good yield and purity of CD34+ cells with few contaminated T cells. In a study of patients with malignancy and concomitant AD, a high rate of recurrent AD was seen when unmanipulated autografts were used.[26] In the European phase I-II trial for SSc, 47/55 (85%) patients received CD34+ selection.[23] On the other hand, a randomised trial of 31 patients with rheumatoid arthritis comparing T cell depleted *v* unmanipulated autologous PBSCT after high dose CY (200 mg/kg) without additional T cell purging agents failed to

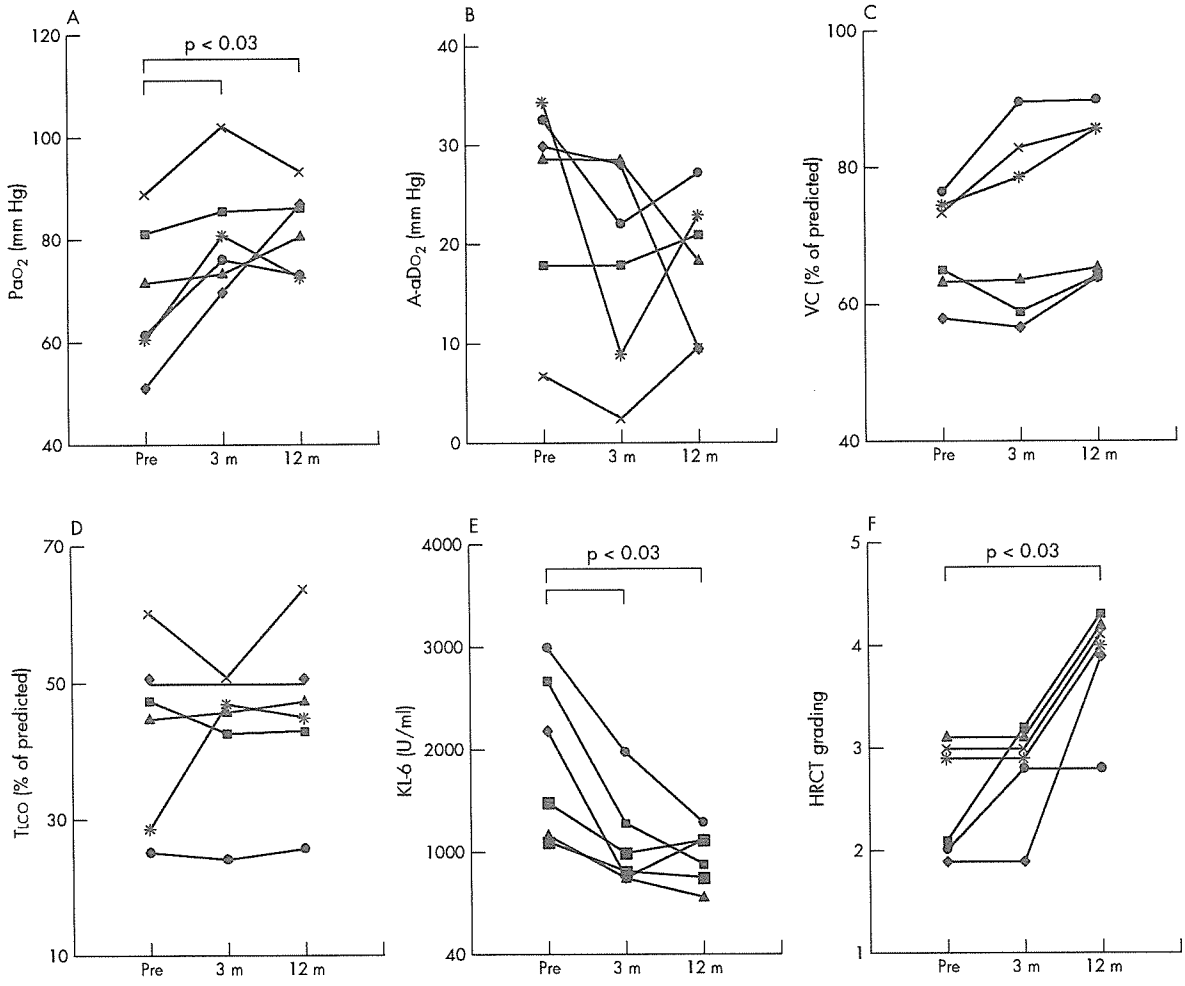

Figure 2 Evaluation of variables associated with IP in six patients with SSc before mobilisation, and at 3 and 12 months after autologous PBSCT. (A) Pa_{O_2} at room temperature; (B) $A\text{-}aD_{O_2}$; (C) VC; (D) T_{LCO}; (E) KL-6; (F) pulmonary HRCT grading. The x axis shows time. Data obtained before mobilisation and just before conditioning are shown as "Pre" and "0" respectively. m, month.

demonstrate significant differences between the two groups.[27] The usefulness of T cell depletion should also be investigated carefully in patients with other AD.

Six of the eight patients had infectious episodes. Viral infections were more common than bacterial infections. Other toxicity included cardiac toxicity of CY and temporary exacerbation of IP by G-CSF. Patient 6 developed acute heart failure, requiring temporary intubation after mobilisation, which might be due to the combination of viral myocarditis and cardiac toxicity of CY. She recovered from heart failure and received autologous PBSCT 1 year later. Patient 3 had a relapse of NHL. The relation between autologous PBSCT and the relapse of NHL was not clear because autologous PBSCT was also used for treatment of NHL. There was no TRM in our study, whereas an early European study and the study by McSweeney *et al* reported that the overall TRM was 9% and 15.8%, respectively.[8 10] One of the most important toxicities was cardiotoxicity, possibly related to direct CY toxicity and hyperhydration. The patient selection was reported to be important to reduce TRM[23] and a full cardiological assessment before transplantation was recommended by the European group.[24] Careful patient selection, especially in the light of cardiac function evaluation, which is often underestimated in patients with severe rheumatic diseases, may have allowed us to avoid TRM.

Significant improvement in the skin score of >25% after autologous PBSCT occurred in all of the patients with SSc. In the European study and the study by McSweeney *et al*, it occurred in 69% and 100% of the patients transplanted, respectively.[10 29] The mechanism for the effect of autologous PBSCT on skin sclerosis may be due to intensive immunosuppression and immune reconstitution. In the European study and the study by McSweeney *et al*, 35% and 0% of the patients with an initial response relapsed during 20 and 14.7 months of median follow up after transplantation, respectively.[10 23] A longer follow up is necessary to assess the response duration of skin sclerosis in our trial.

Improvement of IP has been demonstrated in patients with SSc and ADM, whereas pulmonary function remained unaffected in other studies.[10 23 29] In this study, Pa_{O_2}, KL-6, and pulmonary HRCT grading were significantly improved, while T_{LCO} values showed no significant change. KL-6 is a high molecular weight glycoprotein recently identified in humans as MUC1 mucin. It is a useful marker in the evaluation of disease activity not only of idiopathic pulmonary fibrosis but also of IP associated with collagen vascular

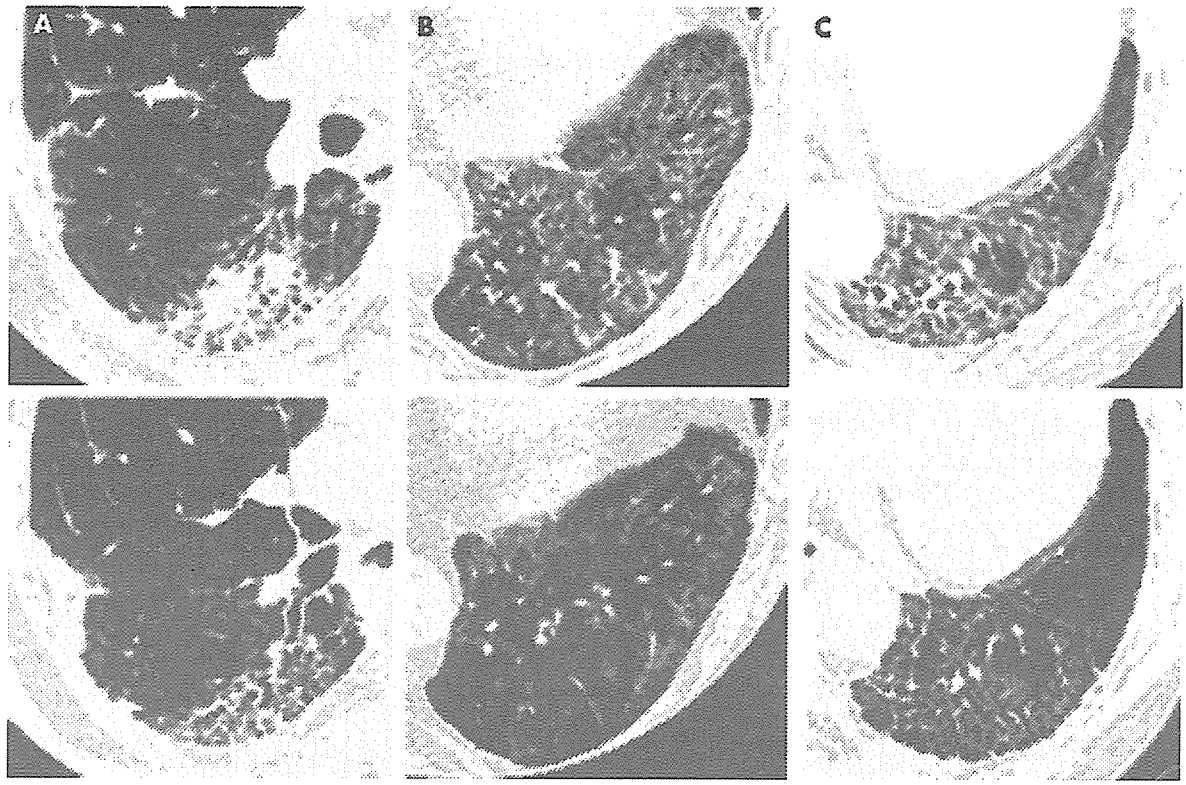

Figure 3 Pulmonary HRCT in patients with SSc. Upper panel, before mobilisation; lower panel, 12 months after autologous PBSCT. (A) Patient 1; (B) patient 2, (C) patient 4.

disease.[30] VC was increased in 5/6 patients with SSc, although it was not statistically significant. McSweeney *et al* treated 19 patients with SSc with high dose CY, total body irradiation (TBI), and antithymocyte globulin (ATG) followed by autologous PBSCT. They reported a significant decrease in the TLCO values at 3 months but not at 12 months and no significant change in the VC at 3 or 12 months after autologous PBSCT.[10]

Because IP was not evaluated with respect to Pao_2, KL-6, or pulmonary HRCT grading in previous studies, the improvement of IP might have been undetectable. Selection biases of patients may be another reason; we might have selected patients with more active IP without honeycombing, whereas more patients with inactive and stable IP might have been selected in other studies. A different treatment regimen, especially the use of TBI, might be responsible for the different results. It is reasonable to suppose that high dose CY and autologous PBSCT could provide favourable effects on the IP of patients with SSc because intravenous pulse CY was reported to be effective for IP in patients with collagen vascular diseases including SSc.[15 31] In this study, improvement of TLCO was not seen, as it was in previous studies, despite the improvement of KL-6 and pulmonary HRCT grading. Because TLCO reflects not only interstitial lesions but also microvascular lesions of the lung,[32] periphery distributed microvascular impairment of the lung due to SSc may not have improved after autologous PBSCT compared with the improvement of interstitial lesions, resulting in the absence of an improved TLCO.

In this study, a patient with WG receiving autologous PBSCT was described. In the European study, three patients with WG receiving autologous PBSCT were listed, but the treatment outcome was not described.[8 33] In our case, G-CSF in combination with CY did not cause a disease flare, and high dose CY with autologous PBSCT produced long term remission for more than 16 months.

We did not incorporate ATG into the conditioning regimen. Although ATG is believed to be useful for deleting the residual T cells and is often used in the other settings,[10 23 29] its usefulness has not been fully proved. Because we obtained significant clinical responses and considerable susceptibility to infections when treating with CY alone, ATG did not seem to be necessary. We did not incorporate TBI for a similar reason. Although our conditioning regimen was less intense than high dose CY with ATG and/or TBI, initial clinical responses were comparable, at least at the 12 month follow up. It is important to look at the response duration of our regimen in comparison with those of more immunosuppressive regimens. A randomised controlled trial will be necessary to assess the usefulness of ATG and/or TBI.

Most of the patients showed an improvement in disease activity after high dose CY and G-CSF for PBSC mobilisation before pretransplant conditioning, as shown in a previous study.[34] Haematopoietic stem cells express high levels of aldehyde dehydrogenase, an enzyme responsible for cellular resistance to CY. Hence, high dose CY should have strong effects on fully differentiated and aggressive autoreactive lymphocytes and allow immune reconstitution by newly developed lymphocytes from CY resistant haematopoietic stem cells.[35]

In conclusion, the present phase I-II study demonstrated that high dose CY with autologous PBSCT is feasible and effective in the treatment of refractory AD. We first demonstrated the clinical effects of high dose CY with autologous PBSCT on IP of SSc and on granuloma of WG. A prospective study with longer follow up time and more

patients will be necessary to assess the efficacy of this treatment modality in AD.

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Myeloablative allogeneic hematopoietic stem cell transplantation for non-Hodgkin lymphoma: a nationwide survey in Japan

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We retrospectively surveyed the data of 233 patients who underwent myeloablative allogeneic hematopoietic stem cell transplantation (allo-HSCT) for non-Hodgkin lymphoma (NHL). Donors were HLA-matched relatives in 154 patients (66%) or unrelated volunteers in 60 (26%). Ninety patients (39%) were in complete remission. One hundred ninety-three (83%) received a total body irradiation (TBI)–based regimen, and 40 (17%) received a non–TBI-based regimen. Acute graft-versus-host disease (GVHD) occurred in 155 (67%) of the 233 evaluable patients; grade II to IV in 90 (39%), and grade III to IV in 37 (16%). Treatment-related mortality (TRM) was observed in 98 patients (42%), and 68% of them were related to GVHD. In a multivariate analysis, chemoresistance, prior autograft, and chronic GVHD were identified as adverse prognostic factors for TRM. Relapse or progression of lymphoma was observed in 21%. The 2-year overall survival rates of the patients with indolent (n = 38), aggressive (n = 111), and lymphoblastic lymphoma (n = 84) were 57%, 42%, and 41%, respectively. In a multivariate analysis, chemoresistance, prior autograft, and prior radiotherapy were identified as adverse prognostic factors for overall survival. Although myeloablative allo-HSCT represents an effective therapeutic option for patients with NHL, more work is still needed to decrease TRM and relapse. (Blood. 2006;108:382-389)

Introduction

Hematopoietic stem cell transplantation (HSCT) for patients with non-Hodgkin lymphoma (NHL) has been mainly focused on an autograft strategy. High-dose therapy with autologous HSCT (auto-HSCT) can increase remission rates and possibly prolong disease-free survival and overall survival (OS) in patients with chemotherapy-sensitive NHL at relapse.[1] This is also effective as first-line therapy for those with advanced aggressive lymphoma.[2] Nevertheless, relapse is a frequent cause of treatment failure after auto-HSCT.[1,3]

Allogeneic HSCT (allo-HSCT) has several advantages over auto-HSCT, because the former can avoid the reinfusion of malignant cells and can also be associated with a graft-versus-lymphoma (GVL) effect, which might reduce the risk of relapse. Most physicians believe that a small fraction of patients with end-stage aggressive lymphoma can still achieve prolonged lymphoma-free survival with the application of allo-HSCT. However, the high incidence of treatment-related mortality (TRM) (up to 55%) associated with allogeneic HSCT with a myeloablative regimen has prevented the wider application of this strategy.[4-8] Several reports on allo-HSCT for refractory or advanced lymphoma, as well as studies comparing auto- versus allo-HSCT for NHL, have been published over the past decade.[8-10] However, most of these studies were small and nonrandomized, and incorporated patients who had heterogeneous backgrounds. Thus, the role of allo-HSCT in the treatment of NHL remains controversial. Moreover, the outcome of allo-HSCT in each histologic subtype has not been fully determined. Previous studies have suggested that allo-HSCT improves the prognosis of patients with advanced follicular lymphoma (FL),[7,10,11] whereas few reports have been published on its benefit in aggressive lymphoma.[12,13] In particular, there has been very little information available on subtypes, including mantle-cell lymphoma[11,14]; peripheral T-cell lymphoma, unspecified (PTCL)[15]; natural killer (NK) cell lymphoma[16]; and anaplastic large cell lymphoma.

The application of reduced-intensity stem cell transplantation (RIST) or "nonmyeloablative" HSCT has been reported to decrease

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TRM.[17-19] Additionally, the recent development of supportive treatments may have decreased the risk of TRM and facilitated the application of allo-HSCT to NHL.[20] Therefore, we conducted a retrospective nationwide survey on Japanese patients with NHL who had undergone conventional allo-HSCT to establish a benchmark of myeloablative allo-HSCT in the treatment of NHL.

Patients, materials, and methods

Data sources

This survey collected the data of 233 consecutive patients who received myeloablative allo-HSCT for NHL between 1990 and 2001 in 56 participating hospitals. Data were derived from questionnaires distributed to each hospital. Additional questionnaires were sent to confirm the follow-up data, including the occurrence of graft-versus-host disease (GVHD). The indications for allo-HSCT were left to the discretion of each institution. The patients included in this study received a conditioning regimen with an intensity that was equivalent to that of total body irradiation (TBI) plus cyclophosphamide or busulfan plus cyclophosphamide. Patients who had previously received monoclonal antibody therapy or T-cell–depleted transplantation, those younger than 14 years, and those who received RIST were not included. Additionally, those with adult T-cell leukemia/lymphoma were excluded because their clinical course differed from that of other types of lymphoma. The minimum data required for the inclusion of a patient in this study were age, sex, histologic diagnosis, prior treatment details, status at transplantation, donor information, conditioning regimen, date of transplantation, therapy-related complications, date of last follow-up, disease status at follow-up, date of disease progression/death, and cause of death. Approval was obtained from the institutional review board. Informed consent was provided according to the Declaration of Helsinki.

Definitions

The initial institutional histologic diagnosis was further reviewed by a pathologist (K. Takeuchi) using the WHO classification.[21] Briefly, NHL was divided into 3 clinical subtypes: indolent, aggressive, and lymphoblastic lymphoma. Indolent lymphoma included all grades of FL and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Aggressive lymphoma included all lymphomas except for indolent and lymphoblastic lymphoma. Transformed indolent lymphoma and Burkitt lymphoma were classified as aggressive lymphoma. Furthermore, because most of the patients were evaluated before publication of the WHO classification, this analysis only included those who had tumors that formed lesions, such as T-cell lymphoblastic lymphoma (T-LBL), and all other patients who had features of leukemia were excluded. Those with chemosensitive disease included all patients who had shown a response to the last chemotherapy prior to transplantation (partial remission [PR], complete remission [CR] unconfirmed, and CR), whereas chemoresistant disease included those with primary refractory disease or refractory relapse prior to transplantation. Acute and chronic GVHD was graded according to the consensus criteria.[22,23] Patients who survived 100 days were evaluable for the assessment of chronic GVHD. OS was measured as the time from the day of transplantation until death from any cause, and progression-free survival (PFS) was the time from the day of transplantation until disease progression (PD)/relapse or death from any cause. Patients who died from transplantation-related causes were classified as TRM regardless of their disease status.

Statistical analysis

OS and PFS were calculated using the Kaplan-Meier method.[24] Surviving patients were censored on the last day of follow-up, in July 2002. The associations among patient-, disease-, and transplantation-related factors and OS were assessed by using univariate and multivariate Cox proportional hazards models. The associations between these factors and TRM were assessed by using univariate and multivariate logistic models. The variables analyzed included age, clinical subtype, histologic diagnosis, chemosensitivity, history of autograft or radiotherapy, years of transplantation, donor, source of stem cells, TBI-containing regimen, GVHD prophylaxis, and acute and chronic GVHD. Acute GVHD was treated as a time-dependent covariate in the Cox model. Stepwise variable selection at a significance level of .05 was used to identify covariates associated with outcomes. TRM and disease progression/relapse were calculated by using cumulative incidence. The statistical analysis was performed with the SAS 8.2 program package (SAS Institute, Cary, NC).

Results

Patients' characteristics

The patients' characteristics are listed in Table 1. All patients were younger than 60 years at the time of transplantation, with a median age of 31 years. Thirty-eight patients (16%) had indolent lymphoma, 111 (48%) had aggressive lymphoma (diffuse large B-cell, n = 44; PTCL, n = 22; extranodal NK/T-cell, n = 19; anaplastic large cell, n = 7; mantle cell, n = 5; Burkitt, n = 4; angioimmunoblastic T cell, n = 2; blastic NK cell, n = 2; hepatosplenic T-cell, n = 2; subcutaneous panniculitis like T cell, n = 2; mycosis fungoides with visceral dissemination, n = 2), and 84 (36%) had lymphoblastic lymphoma. Ninety patients (39%) were in CR, 38 (16%) were in PR, 42 (18%) were in primary refractory, and 63 (27%) had refractory relapse at the time of allo-HSCT. Ninety patients (39%) had received 4 or more chemotherapy regimens before allo-HSCT. Forty patients (17%) had received prior autograft, and 81 (35%) had received prior radiotherapy. One hundred fifty-four patients (66%) received a transplant from a human leukocyte antigen (HLA)–matched related donor, 19 (8%) from a 1-antigen–mismatched related donor, 43 (19%) from a matched unrelated donor, and 17 (7%) from a 1-antigen–mismatched unrelated donor. One hundred fifty-nine (68%) patients received bone marrow (60 from an unrelated donor) and 70 (30%) received granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood. One hundred ninety-three patients (83%) received TBI-based myeloablative regimens, including TBI 12 Gy plus cyclophosphamide (n = 60); a combination of TBI, cyclophosphamide, and etoposide (n = 47); or TBI, cyclophosphamide, and cytarabine (n = 40). Forty patients (17%) received a non–TBI-based myeloablative regimen, including a combination of busulfan and cyclophosphamide with or without other agents (n = 27); melphalan, thiotepa, and busulfan (n = 3); cytarabine, ranimustine, carboplatin, cyclophosphamide, and total lymphoid irradiation (n = 2); or cytarabine, etoposide, and busulfan (n = 2). The remaining 6 patients received individualized regimens. GVHD prophylaxis included a combination of cyclosporin and methotrexate in 204 (88%) or tacrolimus and methotrexate in 22 (9%). Two hundred twenty-six patients (97%) were treated with G-CSF, starting at days +1 to +6 after graft infusion until engraftment.

GVHD

Acute GVHD occurred in 155 (67%) of the 233 patients: grade I in 65 (28%), grade II to IV in 90 (39%), and grade III to IV in 37 (16%) patients. Of the 165 patients who survived the initial 100 days after allo-HSCT, chronic GVHD occurred in 79 (48%), with extensive type in 48 (29%). In allo-HSCT from related (n = 173) and unrelated (n = 60) donors, grade II to IV acute GVHD occurred, respectively, in 61 (35%) and 30 (50%), grade III to acute GVHD occurred in 25 (15%) and 12 (20%), chronic GVHD occurred in 54 (31%) and 25 (42%) patients, and chronic extensive

Table 1. Patient-, disease-, and transplantation-related characteristics

Variable	No. (%)*
Patient characteristics	
Younger than 40 y	158 (68)
40 y or older	75 (32)
Male sex	150 (64)
Disease characteristics at diagnosis	
Histology	
Indolent	38 (16)
Follicular	37 (16)
MALT	1 (0)
Aggressive	111 (48)
Diffuse large B cell	44 (19)
Peripheral T cell, unspecified	22 (9)
Extranodal NK/T cell, nasal type	19 (8)
Anaplastic large cell	7 (3)
Mantle cell	5 (2)
Others	14 (6)
Lymphoblastic	84 (36)
Precursor B cell	7 (3)
Precursor T cell	77 (33)
Stage I	9 (4)
Stage II	25 (11)
Stage III	30 (13)
Stage IV	150 (64)
No data	19 (8)
Disease characteristics at transplantation	
Response to chemotherapy†	
Sensitive	128 (55)
Complete remission‡	90 (39)
Partial remission	38 (16)
Resistant	104 (45)
Primary refractory disease	41 (18)
Refractory relapse	63 (27)
No. of prior chemotherapy regimens†	3 (0-11)
Fewer than 4 regimens	143 (61)
At least 4 regimens	90 (39)
Prior autograft	40 (17)
Prior radiotherapy	81 (35)
Transplantation characteristics	
Year of transplantation	
1990-1995	46 (20)
1996-2001	187 (80)
No. of patients receiving a transplant per hospital	
Fewer than 9 patients	146 (63)
At least 9 patients	87 (37)
Donor	
HLA-matched related	154 (66)
HLA-1 antigen-mismatched related	19 (8)
HLA-matched unrelated	43 (19)
HLA-1 antigen-mismatched unrelated	17 (7)
Donor-recipient sex match	
Male-male	80 (34)
Male-female	66 (28)
Female-male	33 (14)
Female-female	46 (20)
Donor-recipient CMV status§	
+/+	131 (57)
−/+	14 (6)
+/−	14 (6)
−/−	11 (5)
Source of stem cells	
Bone marrow	159 (68)
Peripheral blood cells	70 (30)
Bone marrow + peripheral blood cells	2 (1)
Cord blood	2 (1)
Conditioning regimen	
TBI-containing	193 (83)
Non-TBI	40 (17)
GVHD prophylaxis	
Cyclosporin + methotrexate	204 (88)
Tacrolimus + methotrexate	22 (9)
Others	7 (3)

The study included 233 patients. The median age was 31 years (range, 15-59 years). Age was a continuous variable.

MALT indicates extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue; NK, natural killer; HLA, human leukocyte antigen; CMV, cytomegalovirus; TBI, total body irradiation; GVHD, graft-versus-host disease.

*Categoric variable.

†One patient with mediastinal B-LBL did not receive prior chemotherapy for an unknown reason but did receive prior radiotherapy.

‡Includes 2 patients in complete remission, unconfirmed.

§Sixty-three pairs were not evaluated for CMV status.

GVHD occurred in 33 (19%) and 16 (27%). In allo-HSCT from HLA-matched (n = 197) and mismatched (n = 36) donors, grade II to IV acute GVHD occurred, respectively, in 76 (39%) and 15 (42%), grade III to IV acute GVHD occurred in 30 (15%) and 7 (19%), chronic GVHD occurred in 65 (33%) and 14 (39%), and chronic extensive GVHD occurred in 41 (21%) and 7 (19%). The distribution pattern of the incidences of acute and chronic GVHD by background factors was analyzed by using a chi-square test. Although none of the factors correlated with acute GVHD, the incidence of chronic GVHD was higher in patients who had GVHD prophylaxis with tacrolimus plus methotrexate than in those with cyclosporin plus methotrexate ($P = .015$, chi-square test; $P = 0.023$, Fisher exact test).

Disease response

Of the 143 patients who had measurable disease at allo-HSCT, 89 (62%) achieved CR, 7 (5%) PR, 6 (4%) stable disease (SD), and 12 (8%) PD, whereas 29 (20%) were not evaluable because of early death. Of the 90 patients who were in CR at transplantation, 80 (89%) maintained CR, 4 (4%) showed PD, and 6 (7%) were not evaluable because of early death. Thirty-five patients died before the first response evaluation, with a median survival of 29 days (range, 0-72 days) after allo-HSCT. In the 27 patients with indolent lymphoma who had measurable disease at allo-HSCT, 22 (81%) achieved CR or PR. In the 72 patients with aggressive lymphoma who had measurable disease at allo-HSCT, 49 (68%) achieved CR or PR. In the 41 patients with lymphoblastic lymphoma who had measurable disease at allo-HSCT, 26 (63%) achieved CR.

TRM, disease relapse, and progression

Ninety-eight patients (42%) died of TRM, and its cumulative incidence is shown in Figure 1. Of the 98 patients who died of therapy-related complications, 60 (61%) died within day 100 of transplantation and 38 (39%) died thereafter. The major causes of TRM included GVHD (n = 11), infection (n = 29), interstitial pneumonitis (n = 16), venoocclusive disease of the liver (n = 11), thrombotic microangiopathy (n = 8), heart failure (n = 7), hemorrhage (n = 4), renal failure (n = 3), and others (n = 9), as shown in Table 2. The causes of infection-related mortality (n = 29) were bacterial (n = 13), fungal (n = 11), or viral (n = 5). Seventeen (59%) of 29 patients died of infections within 100 days of allo-HSCT, 7 (24%) from 101 days to 1 year and 5 (17%) thereafter. Fourteen patients died of TRM before engraftment. Of the 98 patients who died of TRM, 67 (68%) had GVHD, and 11 of

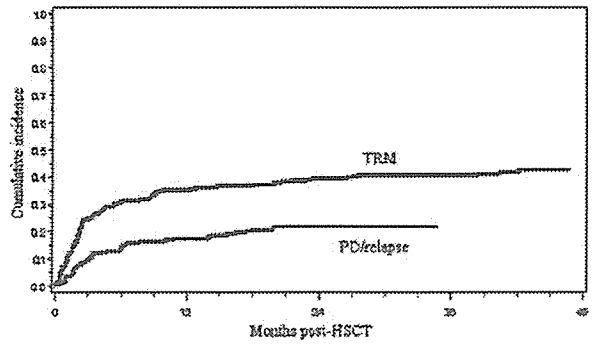

Figure 1. Cumulative incidences of treatment-related mortality (TRM) and disease relapse/progression (PD/relapse).

these died of GVHD (6 acute, 5 chronic) itself. The 14 factors shown in Table 3 were assessed with regard to their relation to TRM. A univariate analysis revealed that 6 factors, including older patient age, chemoresistant disease, prior autograft, prior radiotherapy, aggressive lymphoma other than PTCL, and chronic GVHD, were associated with a significantly increased risk of TRM. In a multivariate analysis using a logistic model, chemoresistant disease, prior autograft, and chronic GVHD remained significant.

The cumulative incidence of relapse and PD is shown in Figure 1. Relapse or progression of lymphoma after allo-HSCT was observed in 49 patients (21%; 5 indolent, 19 aggressive, 25 LBL), and 32 (14%; 3 indolent, 13 aggressive, and 16 LBL) died of PD. Of the 105 patients with chemoresistant disease before allo-HSCT, 61 (58%) died of treatment-related complications, 19 (18%) died of PD, and 25 (24%) are alive with a median follow-up of 20.9 months (range, 1.8-136.0 months). Of the 128 patients with chemosensitive disease before allo-HSCT, 37 (29%) died of treatment-related complications, 12 (9%) died of PD, and 79 (62%) are alive with a median follow-up of 35.2 months (range, 4.4-140.2 months). Eight (16%) of the 49 patients who showed PD died of treatment-related complications such as infection (n = 4), interstitial pneumonitis (n = 3), and GVHD (n = 1). Only 6 of the 70 patients who had passed 2 years after transplantation developed relapse thereafter.

Donor lymphocyte infusion

Donor lymphocyte infusions (DLIs) were given after the withdrawal of immunosuppressive therapy to those who relapsed or showed evidence of disease progression or persistent disease without any sign of GVHD. A total of 7 patients, including 5 with T-LBL, received DLI after allo-HSCT from an HLA-matched related donor (n = 6) or a -matched unrelated donor (n = 1). Two patients who received DLI from an HLA-matched related donor developed grade II acute GVHD, which subsequently extended to extensive chronic GVHD; one of them with T-LBL died without a response, whereas the other with T-cell lymphoma is still alive without disease progression 3.8 years after allo-HSCT. Five patients did not develop GVHD following DLI; 3 patients subsequently died of disease progression, but 2 patients with T-LBL are still alive without disease progression at 361 and 783 days after allo-HSCT.

OS and PFS

One hundred four (45%) of the 233 patients are currently alive with a median follow-up of 31 months (range, 1.8-138 months). The OS and PFS are, respectively, 45% and 40% at 2 years, and 39% and 36% at 5 years after allo-HSCT (Figure 2). Median OS and PFS are, respectively, 15.6 months (95% confidence interval, 9.6-27.6 months) and 9.6 months (6-18 months). The 2-year OS of those with indolent, aggressive, and lymphoblastic lymphoma was, respectively, 57%, 42%, and 41%. Patients with indolent lymphoma tended to have a better survival ($P = .131$, log rank test; $P = .064$, G. Wilcoxon test) (Figure 3). Kaplan-Meier estimates of OS of patients with 4 histologic subtypes of aggressive lymphoma, including diffuse large B-cell lymphoma (n = 44), PTCL (n = 22), extranodal NK/T-cell lymphoma, nasal type (n = 19), and others (n = 26), are shown in Figure 4.

The 14 clinical factors shown in Table 4 were assessed with regard to their relation to OS. A univariate analysis revealed that 5 factors, including chemoresistant disease, prior autograft, prior radiotherapy, aggressive lymphoma other than PTCL, and clinical subtype (aggressive versus indolent), were associated with a significantly worse OS. In a multivariate analysis using Cox proportional hazard models, chemoresistant disease, prior autograft, and prior radiotherapy were associated with a worse OS (Table 4). Acute GVHD, which was treated as a time-dependent variable, was not a significant factor for OS in both univariate and multivariate models. The relation between OS and response to chemotherapy is shown in Figure 5.

Discussion

This report describes the general outcome of patients with NHL who underwent modern allo-HSCT with a myeloablative regimen

Table 2. Causes of treatment-related mortality

Causes of TRM	Patients, no. (%)	No. of patients with GVHD	No. of patients without GVHD	Early death, no.*
GVHD	11 (11)			
Infection	29 (30)	15	8	6
Interstitial pneumonitis	16 (17)	15	0	1
Venoocclusive disease	11 (11)	5	4	2
Thrombotic microangiopathy	8 (8)	7	1	0
Heart failure	7 (7)	3	1	3
Hemorrhage	4 (4)	3	1	0
Renal failure	3 (3)	2	1	0
Others†	9 (9)	6	1	2
Total	98 (100)	56	17	14

GVHD indicates graft-versus-host disease.

*Early death was defined as treatment-related death before engraftment.

†Others (n = 9) were acute respiratory distress syndrome (n = 2), hepatic failure (n = 2), leukoencephalopathy (n = 1), secondary solid cancer (n = 1), suicide (n = 1), and unknown cause (n = 2).