Table 4. Pretransplant prognostic factors

Variables	No.	*OS at 2 years*				*Relapse at 2 years*				*Non-relapse mortality at 2 years*			
		Univariate		*Multivariate*		*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
		%	P-value	HR (95% CI)	P-value	%	P-value	HR (95% CI)	P-value	%	P-value	HR (95% CI)	P-value
Age (years)			0.147				0.729				0.069		0.180
50–59	71	59				27				21		1	
60–69	47	52				31				34		1.66 (0.79–3.46)	
Sex			0.280				0.919				0.086		0.310
Male	55	52				30				31		1	
Female	63	59				26				21		0.65 (0.29–1.48)	
WBC at diagnosis			0.012		0.007		0.729				0.017		0.064
$<30\times10^9$/L	77	63		1		30				19		1	
$\geqslant30\times10^9$/L	39	42		2.19 (1.24–3.89)		25				39		1.99 (0.96–4.12)	
Disease status at RIST			0.039		0.036		0.016		<0.001		0.733		
CR1	96	60		1		21		1		27			
CR2	22	36		2.02 (1.05–3.89)		56		3.83 (1.73–8.48)		23			
Donor			0.521				0.030		0.039		0.106		
Unrelated	80	53				22		1		31			
Related	38	60				39		2.34 (1.05–5.23)		16			
TBI			0.403				0.031		0.068		0.270		
No	43	54				39		1		23			
Yes	75	57				21		0.49 (0.23–1.06)		27			

Abbreviations: CI = confidence interval; HR = hazard ratio; RIST = reduced-intensity SCT.

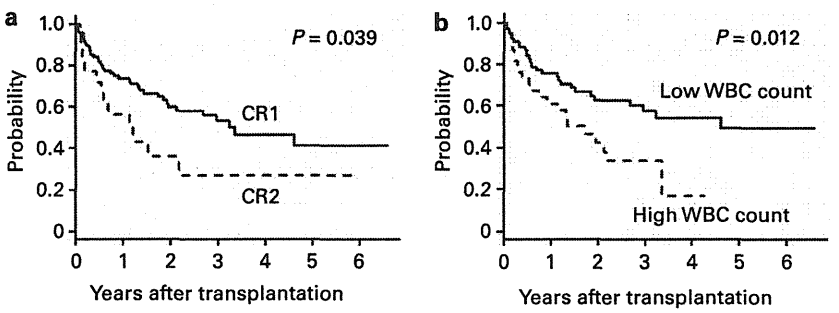

Figure 2. (**a**) OS of patients in their first (CR1) vs those in their second CR (CR2). (**b**) OS of patients with a low WBC count vs high WBC count at diagnosis.

transplantation after either full conditioning ($n = 1421$) or reduced-intensity conditioning ($n = 92$).[14] However, there has been no report about the clinical impact of WBC at diagnosis on survival among patients receiving RIST alone. Therefore, our results support the WBC count at diagnosis as one of the useful parameters for stratifying patients in studies of RIST.

Although there are limitations because this was a retrospective study based on registry data from multiple centers, our cohort consisted of a relatively large number of B-cell ALL patients aged 50 years or older and this study had the unique characteristic of investigating elderly B-cell ALL patients with 66% being Ph chromosome-positive. In contrast with the stem cell sources available in Western countries, only BM or CB was transplanted from unrelated donors in this study because transplantation with unrelated PB was not approved in Japan until 2009.

In conclusion, the results of this study support further investigation of fludarabine-containing RIST for ALL in the elderly, especially for patients in CR1, although longer follow-up is needed to confirm the durability of remission and the quality of life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank all of the staff from the participating institutions of the Japan Society for Hematopoietic Cell Transplantation for their assistance and cooperation.

AUTHOR CONTRIBUTIONS

HK (Kanamori) designed the study, analyzed the data and wrote the draft version of this manuscript. HN, MT, KI, TY, TF, KM and TE submitted and cleaned the data; T-NI, YM, RS and HS collected and reviewed the data; And SM, SK, HK (Kato), SN, KI, AS and JT interpreted the results and critically revised the manuscript.

REFERENCES

1 Kantarjian HM, O'Brien S, Smith TL, Cortes J, Giles FJ, Beran M *et al.* Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. *J Clin Oncol* 2000; **18**: 547–561.

2 Larson RA, Dodge RK, Burns CP, Lee EJ, Stone RM, Schulman P *et al.* A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: cancer and leukemia group B study 8811. *Blood* 1995; **85**: 2025–2037.

3 Rowe JM, Buck G, Burnett AK, Chopra R, Wiernik PH, Richards SM *et al.* Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALLXII/ECOG E2993. *Blood* 2005; **106**: 3760–3767.

4 Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK *et al.* In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood* 2008; **111**: 1827–1833.
5 Arnold R, Massenkeil G, Bornhäuser M, Ehninger G, Beelen DW, Fauser AA *et al.* Nonmyeloablative stem cell transplantation in adults with high-risk ALL may be effective in early but not in advanced disease. *Leukemia* 2002; **16**: 2423–2428.
6 Martino R, Giralt S, Caballero MD, Mackinnon S, Corradini P, Fernández-Avilés F *et al.* Allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning in acute lymphoblastic leukemia: a feasibility study. *Haematologica* 2003; **88**: 555–560.
7 Mohty M, Labopin M, Tabrizzi R, Theorin N, Fauser AA, Rambaldi A *et al.* Reduced intensity conditioning allogeneic stem cell transplantation for adult patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. *Haematologica* 2008; **93**: 303–306.
8 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
9 Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al.* Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**: 250–259.
10 Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M *et al.* Reduced intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant* 2009; **15**: 367–369.
11 Gray RJ. A class of *k*-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat* 1988; **16**: 1141–1154.
12 Fine JP, Gray RJ. A propotional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496–509.
13 Kanda Y. Investigation of the freely-available easy-to-use software 'EZR (Easy R) for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452–458.
14 Marks DI, Wang T, Pérez WS, Antin JH, Copelan E, Gale RP *et al.* The outcome of full-intensity and reduced-intensity conditioning matched sibling or unrelated donor transplantation in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia in first and second complete remission. *Blood* 2010; **116**: 366–374.
15 Mohty M, Labopin M, Volin L, Gratwohl A, Socié G, Esteve J *et al.* Reduced-intensity versus conventional myeloablative conditioning allogeneic stem cell transplantation for patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. *Blood* 2010; **116**: 4439–4443.
16 Cho BS, Lee S, Kim YJ, Chung NG, Eom KS, Kim HJ *et al.* Reduced-intensity conditioning allogeneic stem cell transplantation is a potential therapeutic approach for adults with high-risk acute lymphoblastic leukemia in remission: result of a prospective phase 2 study. *Leukemia* 2009; **23**: 1763–1770.
17 Rowe JM. Prognostic factors in adult acute lymphoblastic leukaemia. *Br J Haematol* 2010; **150**: 389–405.
18 Faderl S, Jeha S, Kantarjian HM. The biology and therapy of adult acute lymphoblastic leukemia. *Cancer* 2003; **98**: 1337–1354.
19 Yanada M, Takeuchi J, Sugiura I, Akiyama H, Usui N, Yagasaki F *et al.* High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol* 2006; **24**: 460–466.
20 Bachanova V, Verneris MR, DeFor T, Brunstein CG, Weisdorf DJ, Vermeris MR. Prolonged survival in adults with acute lymphoblastic leukemia after reduced-intensity conditioning with cord blood or sibling donor transplantation. *Blood* 2009; **113**: 2902–2905.

European Journal of Haematology

European Journal of Haematology 92 (137–146)

ORIGINAL ARTICLE

Positive impact of chronic graft-versus-host disease on the outcome of patients with *de novo* myelodysplastic syndrome after allogeneic hematopoietic cell transplantation: a single-center analysis of 115 patients

Nobuhiro Hiramoto, Saiko Kurosawa, Kinuko Tajima, Keiji Okinaka, Kohei Tada, Yujin Kobayashi, Akihito Shinohara, Yoshitaka Inoue, Ryosuke Ueda, Takashi Tanaka, Sung-Won Kim, Takuya Yamashita, Yuji Heike, Takahiro Fukuda

Hematopoietic Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan

Abstract

To evaluate the impact of graft-versus-host disease (GVHD) and prognostic factors for patients with myelodysplastic syndrome (MDS) after allogeneic hematopoietic cell transplantation (allo-HCT), we retrospectively reviewed 115 patients with MDS or acute myeloid leukemia with multilineage dysplasia (AML-MLD) after allo-HCT at our center. Eighty one patients received reduced-intensity conditioning (RIC) regimens, whereas 34 received myeloablative conditioning regimens. Although the RIC group was significantly older and included more patients with poor cytogenetic risk, no difference in 4-yr overall survival (OS) was seen between the two groups. In a multivariate analysis, covariates associated with a worse OS were the French-American-British stage of refractory anemia excess blasts in transformation/AML-MLD at peak, poor cytogenetic risk, bone marrow blasts of 20% or higher at HCT and the absence of chronic GVHD (cGVHD). By using semi-landmark analyses, we found that the presence of cGVHD significantly improved OS in high-risk patients or the RIC group. However, there was no difference in OS between those with and without cGVHD among low-risk MDS patients. These findings suggest that the graft-versus-leukemia effect may be more beneficial in high-risk patients who do not receive intensive preparative regimens.

Key words myelodysplastic syndrome; allogeneic hematopoietic cell transplantation; graft-versus-host disease; graft-versus-leukemia effect

Correspondence Saiko Kurosawa, MD, Hematopoietic Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Tel: +81 3 3542 2511; Fax: +81 3 3542 3815; e-mail: skurosaw@ncc.go.jp

Accepted for publication 9 October 2013

doi:10.1111/ejh.12214

Allogeneic hematopoietic cell transplantation (allo-HCT) has been assumed to be the only treatment modality with curative potential for patients with myelodysplastic syndrome (MDS). However, about 90% of MDS cases occur in elderly patients above the age of 60 yrs (1) and a substantial proportion of them are more likely to have a worse performance status and an increased comorbidity. As a result, myeloablative conditioning (MAC) regimens are less commonly used for patients with MDS because of an increased risk of non-relapse mortality (NRM). However, some studies have reported that the dose intensity of the conditioning regimen plays an important role in controlling the disease after allo-HCT for MDS or acute myeloid leukemia (AML) (2, 3). Reduced-intensity conditioning regimens (RIC) have been developed to decrease the risk of NRM with less-intensive conditioning for elderly or less-fit patients while preserving a graft-versus-leukemia (GVL) effect by an alloimmune reaction as an antitumor effect (4, 5). The European Group for Blood and Marrow Transplantation reported that, among patients with MDS who underwent allo-HCT from a sibling donor, the RIC group was associated with a lower incidence of NRM and a higher risk of relapse in comparison with the

MAC group, whereas overall survival (OS) was similar in both groups (6).

Although an alloimmune reaction by donor T-cells is important for disease control after allo-HCT, especially in the RIC setting, the significance of this effect has not been well documented in patients with MDS. Therefore, we retrospectively reviewed the medical records of 115 patients with *de novo* MDS or AML with multilineage dysplasia (AML-MLD) who underwent their first allo-HCT at our center, and evaluated the impact of graft-versus-host disease (GVHD) and prognostic factors for the outcome in patients with MDS after allo-HCT.

Patients and methods

Patients

This study included patients with *de novo* MDS or AML-MLD who underwent their first allo-HCT at our center between January 2000 and December 2009. The study protocol was reviewed and approved by the institutional ethics committee. Therapy-related MDS and cord blood transplant recipients were excluded. Therapy-related MDS was defined as disease arising in patients who were treated with irradiation, chemotherapy, or both for hematologic malignancies or other cancers. Disease stages were categorized according to the French-American-British (FAB) classification (7). AML-MLD was defined as AML with more than 30% bone marrow (BM) myeloblasts and morphological features of myelodysplasia, or a prior history of MDS. Patients with MDS were classified into two diagnostic groups (Low/Intermediate-1 and Intermediate-2/High) at diagnosis and at peak according to the International Prognostic Scoring System (IPSS) (8). Cytogenetic risk groups were determined according to IPSS using the cytogenetic information at diagnosis. Matching between the donor and recipient was determined according to donor–recipient HLA-A, HLA-B, and HLA-DR compatibility.

Myeloablative conditioning regimens included cyclophosphamide (Cy, 60 mg/kg for 2 d) plus busulfan (Bu, orally 4 mg/kg for 4 d or i.v. 3.2 mg/kg for 4 d) (Bu/Cy) or total body irradiation (TBI, 12 Gy) (TBI/Cy). RIC regimens included Bu (orally 4 mg/kg for 2 d or i.v. 3.2 mg/kg for 2 d) plus fludarabine (Flu, 30 mg/m^2 for 6 d) (Flu/Bu) or cladribine (2-CdA, 0.11 mg/kg for 6 d) (2-CdA/Bu). In a subset of patients who received RIC, low-dose TBI (2 or 4 Gy) and/or low-dose antithymocyte globulin (ATG) (total dose 5–10 mg/kg Fresenius or 2.5–5 mg/kg Thymoglobulin) were added. GVHD prophylaxis included either cyclosporine or tacrolimus alone or a combination of either of the calcineurin inhibitors and methotrexate. The decision regarding the intensity of the conditioning regimen and GVHD prophylaxis for each patient was made at the discretion of the attending physicians based on a review of the patient's age, disease status, comorbidities, performance status and HLA compatibility.

Neutrophil and platelet engraftment dates were defined as the first of three consecutive days with an absolute neutrophil count of 0.5×10^9/L or higher and an untransfused platelet count of 2.0×10^9/L or higher. Acute and chronic GVHD (cGVHD) were diagnosed and graded according to standard criteria (9). Response and relapse of the disease were defined according to standard hematologic criteria.

Statistical analysis

We used the Chi-square analysis and Fisher's exact test to compare categorical covariates and the Mann–Whitney *U* test to compare continuous covariates. OS was estimated by the Kaplan–Meier method, and differences between groups were evaluated by the log-rank test. Relapse and NRM were considered as competing risk events for each other. The probabilities of relapse and NRM were estimated by the cumulative incidence functions, and differences between groups were evaluated by the Gray test (10, 11). OS and the incidences of relapse and NRM were estimated as probabilities at 4 yrs from allo-HCT. To evaluate the effect of cGVHD on OS, we performed semi-landmark analyses (12). For patients with cGVHD, OS was estimated as the probability from the onset of cGVHD by the Kaplan–Meier method. A landmark comparison group consisted of survivors without cGVHD at day 138 (landmark day), which was the median time of the onset of cGVHD with OS for this group estimated as the probability from the landmark day. The Cox proportional hazards regression model was used for univariate and multivariate analyses, and a hazard ratio was calculated in conjunction with a 95% confidence interval (CI). For the assumption of proportional hazards over time, acute GVHD (aGVHD) and cGVHD were treated as time-dependent covariates (13). For multivariate analyses, we decided to include covariates with a *P*-value of <0.1 in univariate analyses. In addition, we included conditioning regimens and GVHD in these models to evaluate their effects on the outcome. The statistical analysis was performed with R-Project (version 2.2.1; http://www.r-project.org/).

Results

Patient characteristics

The characteristics of a total of 115 patients are summarized in Table 1. The median age was 55 yrs (range: 19–68) and the median follow-up of surviving patients was 40 months (range: 4–130). Eighty one patients (70%) received RIC regimens, whereas 34 (30%) received MAC regimens. According to the FAB stage at peak, the proportions of patients with refractory anemia (RA)/refractory anemia with ringed sideroblasts (RARS), refractory anemia

Table 1 Patient characteristics

No. of patients	All $N = 115$	MAC $N = 34$	RIC $N = 81$
Period of HCT (%)			
2000–2004	71 (62)	18 (53)	53 (65)
2005–2009	44 (38)	16 (47)	28 (35)
Age at HCT, median (range)	55 (19–68)	46 (23–57)	57 (19–68)
Age at HCT, yrs			
≧50 yrs (%)	84 (73)	10 (29)	74 (91)
Patient sex, male (%)	82 (71)	24 (71)	58 (72)
FAB stage at diagnosis (%)			
RA/RARS	45 (39)	13 (38)	32 (40)
RAEB/CMMoL	44 (38)	12 (36)	32 (40)
RAEB-T/AML-MLD	26 (23)	9 (26)	17 (20)
IPSS at diagnosis (%)			
Low/Intermediate-1	37 (32)	13 (38)	24 (30)
Intermediate-2/High	64 (56)	16 (47)	48 (59)
Unknown	14 (12)	5 (15)	9 (11)
FAB stage at peak (%)			
RA/RARS	22 (19)	6 (18)	16 (20)
RAEB/CMMoL	38 (33)	10 (29)	28 (34)
RAEB-T/AML-MLD	55 (48)	18 (53)	37 (46)
IPSS at peak (%)			
Low/Intermediate-1	24 (21)	6 (18)	18 (22)
Intermediate-2/High	77 (67)	23 (68)	54 (67)
Unknown	14 (12)	5 (14)	9 (11)
Cytogenetic risk group (%)			
Good/Intermediate	75 (65)	27 (79)	48 (59)
Poor	40 (35)	7 (21)	33 (41)
BM blasts at HCT, median (range)	5 (0–78)	3 (0–46)	4 (0–78)
≦4%	60 (52)	18 (53)	42 (52)
5–19%	38 (33)	10 (29)	28 (35)
≧20%	10 (9)	3 (9)	7 (8)
Unknown	7 (6)	3 (9)	4 (5)
Disease duration, months, median (range)	9 (1–200)	8 (2–200)	10 (1–172)
Karnofsky score at HCT (%)			
90–100	96 (83)	29 (85)	67 (83)
Transfusion dependence (%)	89 (77)	27 (79)	62 (77)
Prior chemotherapy (%)	68 (59)	22 (65)	46 (57)
Donor (%)			
Related	55 (48)	12 (35)	43 (53)
Unrelated	60 (52)	22 (65)	38 (47)
HLA matching (%)			
HLA match (6/6)	101 (88)	31 (91)	70 (86)
HLA mismatch (5/6)	14 (12)	3 (9)	11 (14)
Source of stem cells (%)			
Peripheral blood	52 (45)	11 (32)	41 (51)
BM	63 (55)	23 (68)	40 (49)
Sex mismatch (%)			
Female donor/Male recipient	36 (31)	13 (38)	23 (28)
Other combination	79 (69)	21 (62)	58 (72)
Follow-up duration for survivors, months, median (range)	40 (4–130)	40 (4–130)	47 (4–125)
Conditioning regimen			
MAC (%)			
CY/TBI		15 (44)	
Bu/CY		19 (56)	
Reduced intensity conditioning			
Flu/Bu-based			65 (80)
2-CdA/Bu-based			16 (20)
TBI-containing			23 (28)
ATG-containing			26 (32)
GVHD prophylaxis (%)			
CSP			26 (32)
CSP+MTX		24 (71)	37 (46)
TAC			2 (2)
TAC+MTX		10 (29)	16 (20)

MAC, myeloablative conditioning; RIC, reduced intensity conditioning; HCT, allogeneic hematopoietic cell transplantation; FAB, French-American-British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; CMMoL, chronic myelomonocytic leukemia; RAEB-T, refractory anemia with excess blasts in transformation; AML-MLD, acute myeloid leukemia with multilineage dysplasia; BM, bone marrow; mons, months; CY, cyclophosphamide; TBI, total body irradiation; Bu, busulfan; ATG, antithymocyte globulin; Flu, fludarabine; 2-CdA, cladribine; CSP, cyclosporine; MTX, methotrexate; TAC, tacrolimus; GVHD, graft-versus-host disease; IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndrome.

with excess blasts (RAEB)/chronic myelomonocytic leukemia (CMMoL), and refractory anemia excess blasts in transformation (RAEB-T)/AML-MLD were 19%, 33%, and 48%, respectively. According to the cytogenetic risk at diagnosis, the proportions of patients with good/intermediate and poor risk were 65% and 35%, respectively. According to the IPSS risk at peak, the proportions of patients with Low/Intermediate-1 and Intermediate-2/High were 21% and 67%, respectively, and 12% of the patients did not have evaluable data. BM blast counts at allo-HCT were 4% or less in 52%, 5–19% in 33%, 20% or higher in 9%, and not evaluable in 6%. The RIC group was significantly older than the MAC group (median, 57 vs. 46 yrs, $P < 0.001$) and included more patients with poor cytogenetic risk (41% vs. 21%, $P = 0.03$).

Conditioning regimen and GVHD prophylaxis

The conditioning regimen and GVHD prophylaxis are shown in Table 1. The MAC group included either Bu/CY or TBI/CY, followed by a combination of methotrexate and tacrolimus or cyclosporine. The RIC group included Flu/Bu or 2-CdA/Bu, followed by either cyclosporine or tacrolimus alone or a combination of either of the calcineurin inhibitors and methotrexate.

Hematopoietic recovery

A total of 113 patients achieved primary engraftment with a median time to reach a neutrophil count of 0.5 × 10^9/L or higher and a platelet count of 2.0 × 10^9/L or higher of 14 d (range, 10–40 d) and 22 d (range, 8–105 d), respectively. The median times to reach these neutrophil and platelet counts were earlier in the RIC group than the MAC group (neutrophil: 14 vs. 19 d, $P < 0.001$; platelet: 21 vs. 29 d, $P = 0.005$), as shown in Table 2. None of the patients experienced primary graft failure. All but two patients, who died before day 30 after allo-HCT without evidence of engraftment, were assessed for hematopoietic recovery, and 6 (5%) experienced secondary graft failure.

Graft-versus-host disease

The 113 patients who achieved engraftment was evaluated for aGVHD. The incidence of grade II–IV aGVHD was 42% and that of grade III–IV aGVHD was 14%, as shown in Table 2. There was no significant difference between the RIC and MAC groups in the incidence of aGVHD. Among the 107 patients who survived more than 100 d after allo-HCT, 10 (9%) developed limited cGVHD and 48 (45%) developed extensive cGVHD. There was no significant difference between the RIC and MAC groups with regard to the incidence of cGVHD.

Non-relapse mortality

The 4-yr incidence of NRM was 29% in the MAC group and 33% in the RIC group ($P = 0.89$) (Fig. 1A). In a univariate analysis, covariates associated with a higher incidence of NRM were recipient sex [female, hazard ratio (HR) 2.9, 95% CI 1.1–7.5, $P = 0.03$], IPSS risk at diagnosis (Int-2/High, HR 2.2, 95% CI 1.1–4.7, $P = 0.04$), the FAB stage at peak (RAEB/CMMoL, HR 2.8, 95% CI 1.0–7.7, $P = 0.05$), cytogenetic risk at diagnosis (poor, HR 2.0, 95% CI 1.1–4.0, $P = 0.03$), BM blasts at HCT (20% or higher, HR 4.1, 95% CI 1.7–10.2, $P = 0.002$), and the presence of aGVHD (grade III–IV, HR 4.4, 95% CI 2.2–9.0, $P < 0.001$), as shown in Table S1. In a multivariate analysis (Table 3), the covariates associated with a higher incidence of NRM were the presence of aGVHD (grade III–IV, HR 6.9, 95% CI 2.7–17.4, $P < 0.001$) and BM blasts at HCT (20% or higher, HR 3.6, 95% CI 1.3–9.9, $P = 0.01$). cGVHD in this model was not an independent factor for NRM when substituted for grade III–IV aGVHD (data not shown).

Relapse

The 4-yr incidence of relapse was 26% in the MAC group and 25% in the RIC group ($P = 0.97$) (Fig. 1B). In a univariate analysis, the only covariate associated with a higher relapse rate was prior chemotherapy (HR 2.5, 95% CI 1.1–5.8, $P = 0.04$), as shown in Table S1. In a multivariate analysis (Table 3), covariates associated with a higher relapse rate were prior chemotherapy (HR 4.3, 95% CI 1.2–15.9, $P = 0.03$), BM blasts at HCT (5–19%, HR 4.3, 95% CI 1.5–12.8, $P = 0.008$) and the absence of cGVHD (HR 12.7, 95% CI 3.1–52.6, $P < 0.001$). Grade II–IV or III–IV aGVHD in this model was not an independent factor for relapse when substituted for cGVHD (data not shown).

Table 2 Transplantation outcome

No. of patients	All $N = 115$	MAC $N = 34$	RIC $N = 81$
Graft failure (%)			
Primary	0 (0)	0 (0)	0 (0)
Secondary	6 (5)	1 (3)	5 (6)
Engraftment			
Neutrophils ≧ 0.5 × 10^9/L Median days (range)	14 (10–40)	19 (10–40)	14 (10–27)
Platelets ≧ 20 × 10^9/L Median days (range)	22 (8–105)	29 (13–90)	21 (8–105)
Acute GVHD (%)			
II–IV	48 (42)	12 (35)	36 (44)
III–IV	16 (14)	4 (11)	12 (15)
Onset, median days (range)	30 (5–98)	34 (9–66)	31 (9–68)
Chronic GVHD (%)			
Limited	10 (10)	4 (14)	6 (8)
Extensive	48 (47)	11 (39)	37 (50)
Onset, median days (range)	138 (100–1090)	124 (100–245)	134 (100–1090)

MAC, myeloablative conditioning; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease.

Overall survival

In the overall population, the 4-yr OS was 44%. Although patients in the RIC group were older and had a worse cytogenetic risk, no difference in OS was seen between the two groups (47% in the MAC group vs. 42% in the RIC group, $P = 0.84$) (Fig. 1C). Fifty two patients (45%) were alive and 63 (55%) had died. Disease relapse or progression (40%) was the most common cause of death, followed by non-relapse causes complicated by organ failure (23%), infection (19%), GVHD (6%), and others (12%) (Table 4). In a univariate analysis, covariates associated with a worse OS were older age (60 yrs or older, HR 1.7, 95% CI 1.0–2.9, $P = 0.04$), the FAB stage at diagnosis (RAEB/CMMoL, HR 1.8, 95% CI 1.0–3.2, $P = 0.04$), IPSS risk at diagnosis (Int-2/High, HR 2.4, 95% CI 1.3–4.4, $P < 0.001$), the FAB stage at peak (RAEB/CMMoL, HR 2.3, 95% CI 1.0–5.2, $P = 0.04$ RAEB-T/AML-MLD, HR 2.6, 95% CI 1.2–5.7,

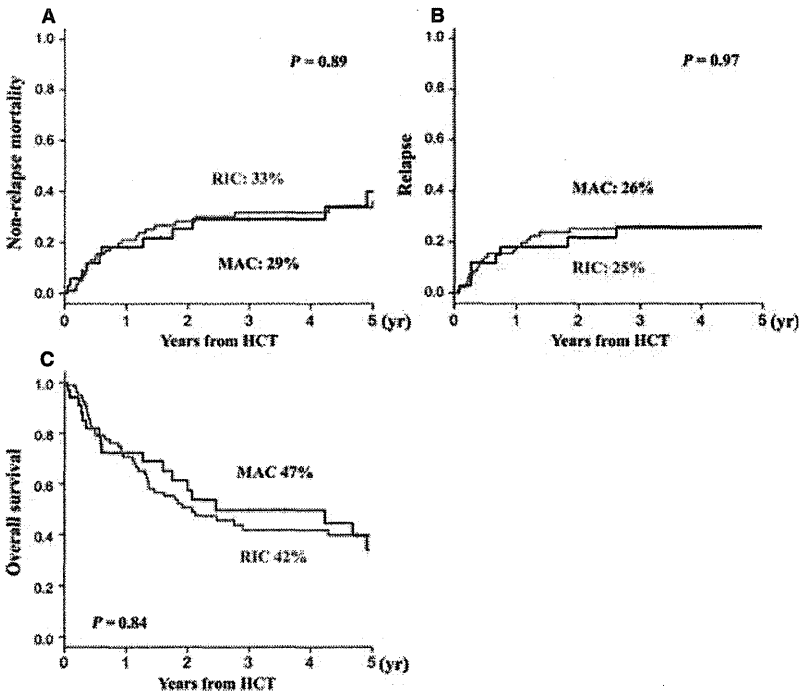

Figure 1 Outcomes stratified according to the intensity of the conditioning regimens. non-relapse mortality (A), Relapse (B) and overall survival (C) of patients with myelodysplastic syndrome receiving allo-hematopoietic cell transplantation after myeloablative conditioning or reduced-intensity conditioning regimens.

$P = 0.01$), IPSS risk at peak (Int-2/High, HR 2.3, 95% CI 1.1–5.0, $P = 0.02$), cytogenetic risk at diagnosis (poor, HR 2.2, 95% CI 1.3–3.7, $P < 0.001$), BM blasts at HCT (20% or higher, HR 3.4, 95% CI 1.6–7.2, $P < 0.001$), and the presence of aGVHD (Grade III–IV, HR 2.8, 95% CI 1.5–5.4, $P = 0.001$), as shown in Table S1. In a multivariate analysis (Table 3), covariates associated with a worse OS were the FAB stage at peak (RAEB-T/AML-MLD, HR 3.3, 95% CI 1.2–8.6, $P = 0.02$), cytogenetic risk at diagnosis (poor, HR 2.1, 95% CI 1.1–6.9, $P = 0.01$), BM blasts at HCT (20% or higher, HR 3.0, 95% CI 1.3–6.9, $P = 0.01$) and the absence of cGVHD (HR 2.0, 95% CI 1.1–4.0, $P = 0.04$). The presence of grade III–IV aGVHD was significantly associated with a worse OS (HR 5.4, 95% CI 2.5–11.4, $P < 0.001$) when this was substituted for cGVHD in this model.

In semi-landmark analyses for the entire population, the OS of patients with cGVHD tended to be better than that of patients without cGVHD ($P = 0.11$) (Fig. 2A). When the analysis was limited to the RIC group, the OS of patients with cGVHD was significantly better than that of patients without cGVHD ($P = 0.005$) (Fig. 2B). We also found that, in patients with poor cytogenetic risk, the OS of patients with cGVHD was significantly better than that of patients without cGVHD ($P = 0.003$) (Fig. 2C), whereas in patients with good/intermediate cytogenetic risk, there was no significant difference in OS between the two groups ($P = 0.76$) (Fig. 2D). In patients with BM blasts 5% or higher at HCT, the OS of patients with cGVHD was significantly better than that of patients without cGVHD ($P = 0.02$) (Fig. S1A), whereas in patients with BM blasts <5% at HCT, there was no significant difference in OS between the two groups ($P = 0.59$) (Fig. S1B).

Impact of extensive cGVHD in the RIC group

The median age in the RIC group was 57 (19–68) yrs. Among the 81 patients in the RIC group, 46 patients (58%) had cGVHD. The majority (86%) of patients with cGVHD developed extensive cGVHD. We also conducted a multivariate analysis limited to the patients pre-treated with RIC (Table S2) and found that the absence of extensive cGVHD was significantly associated with a worse OS (HR 2.4, 95% CI 1.2–5.5, $P = 0.001$) and a higher relapse rate (HR 13.1, 95% CI 4.0–43.9, $P < 0.001$). The presence of extensive cGVHD in this model was not an independent factor for NRM (HR 0.9, 95% CI 0.3–2.7, $P = 0.85$) when substituted for Grade III–IV aGVHD.

Discussion

We performed retrospective analyses of 115 patients with *de novo* MDS or AML-MLD who received their first allo-HCT at our center. By multivariate analyses, we found that the presence of cGVHD significantly reduced relapse and improved OS. To evaluate these results, we considered GVHD to be a time-dependent covariate and analyzed data from all patients to avoid bias from not considering patients

Table 3 Multivariate analysis for NRM, relapse, and OS

Variable	NRM		Relapse		OS	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age						
<60 yrs			1	0.72	1	0.33
≧60 yrs			1.2 (0.5–3.2)		1.4 (0.7–2.6)	
Prior chemotherapy						
No			1	0.03		
Yes			4.3 (1.2–15.9)			
Conditioning regimens						
MAC	1	0.33	1	0.77	1	0.63
RIC	0.7 (0.3–1.5)		0.9 (0.3–2.6)		1.2 (0.6–2.5)	
FAB stage at peak						
RA/RARS	1		1		1	
RAEB/CMMoL	1.2 (0.5–2.7)	0.68	0.6 (0.1–4.8)	0.57	1.9 (0.6–5.9)	0.28
RAEB-T/AML-MLD	2.3 (0.7–7.3)	0.14	0.7 (0.1–4.8)	0.73	3.3 (1.2–8.6)	0.02
Cytogenetic risk group						
Good/Intermediate	1	0.68	1	0.04	1	0.01
Poor	1.2 (0.5–2.7)		2.7 (1.1–6.9)		2.1 (1.1–6.9)	
BM blasts at HCT						
≦4%	1		1		1	
5–19%	1.2 (0.5–2.9)	0.75	4.3 (1.5–12.8)	0.008	1.6 (0.7–3.4)	0.28
≧20%	3.6 (1.3–9.9)	0.01	4.6 (0.9–23.4)	0.07	3.0 (1.3–6.9)	0.01
GVHD						
Grade III–IV aGVHD						
No	1	<0.001				
Yes	6.9 (2.7–17.4)					
cGVHD						
Yes			1	<0.001	1	0.04
No			12.7 (3.1–52.6)		2.0 (1.1–4.0)	

NRM, non-relapse mortality; OS, overall survival; HCT, allogeneic hematopoietic cell transplantation; HR, hazard ratio; CI, confidence interval; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; FAB, French-American-British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; CMMoL, chronic myelomonocytic leukemia; RAEB-T, refractory anemia with excess blasts in transformation; AML-MLD, acute myeloid leukemia with multilineage dysplasia; BM, bone marrow; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

Covariates examined for NRM; Period of HCT, Patient sex, Conditioning regimens, FAB stage at peak, Cytogenetic risk group, BM blast at HCT, The presence of Grade III–IV aGVHD. Covariates examined for Relapse rate; Period of HCT, Age, Patient sex, Prior chemotherapy, Conditioning regimens, FAB stage at peak, Cytogenetic risk group, BM blast at HCT, The presence of cGVHD. Covariates examined for OS; Period of HCT, Conditioning regimens, FAB stage at peak, Cytogenetic risk group, BM blast at HCT, The presence of cGVHD.

who died or relapsed too early to develop acute or chronic GVHD. Some studies that used the same statistical method reported that cGVHD had beneficial effects on relapse in patients receiving allo-HCT after MAC (14, 15). In addition, others showed that the presence of cGVHD was an independent factor in reducing relapse and improving progression-free survival (PFS) in the setting of non-MAC regimens (12) or RIC regimens (16). Similar to our study, Valcárcel *et al.* (16) demonstrated that the development of cGVHD was the strongest factor in reducing relapse and improving survival in patients with high-risk MDS and AML receiving allo-HCT after RIC.

There has been no previous study on the effect of cGVHD on OS according to the conditioning regimen and disease status at allo-HCT. To clarify these questions, we used semi-landmark analyses to evaluate the effect of cGVHD on OS in various subgroups. In the current study, the presence of cGVHD predominantly improved OS in the setting of RIC, but did not affect OS in the MAC group (data not shown). In addition, the presence of cGVHD was significantly associated with the improvement in OS in high-risk patients with BM blasts of 5% or higher at allo-HCT or poor cytogenetic risk, whereas it did not affect OS in low-risk patients. These findings suggest that the benefit of the GVL effect appeared to be more prominent in patients with high-risk MDS who did not receive intensive preparative regimens.

Our findings may suggest that extensive cGVHD is beneficial for patients pre-treated with RIC because of elderly age or less-fit conditions. Valcárcel *et al.* reported that cGVHD was significantly associated with reducing relapse and improving OS without increasing NRM in high-risk AML and MDS patients pre-treated with RIC. In their study,

Table 4 Cause of death

No. of patients	All $N = 115$	MAC $N = 34$	RIC $N = 81$
Cause of death			
All Causes (% of all patients)	63 (55)	18 (53)	45 (56)
Progression (% of all death)	25 (40)	7 (39)	18 (40)
Organ failure (%)	14 (23)	5 (28)	9 (20)
Multiple organ failure	3	1	2
Veno-occlusive disease	3	1	0
Renal failure	1	0	1
Cardiac failure	1	1	0
Diffuse alveolar hemorrhage	7	2	5
Infection (%)	12 (19)	3 (17)	9 (20)
Bacterium	7	2	5
Fungus	3	0	3
Virus	2	1	1
Bleeding (%)	2 (3)	0 (0)	2 (4)
Secondary cancer (%)	4 (6)	0 (0)	4 (10)
GVHD (%)	4 (6)	2 (11)	2 (4)
Unknown (%)	2 (3)	1 (5)	1 (2)

MAC, myeloablative conditioning; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease.

the cumulative incidence of cGVHD was 53% and extensive cGVHD accounted for the majority (94%) of that (16). Baron *et al.* (12) showed a comparable incidence of extensive cGVHD and reported the same results in AML and MDS patients with extensive cGVHD pre-treated with non-MAC regimens.

It is difficult to induce cGVHD 'moderately' on purpose, and the induction of cGVHD may lead to an increased risk of NRM. When we wish for the presence of cGVHD without a devastating outcome, there are two possible choices. First, G-CSF-mobilized peripheral blood mononuclear cells (G-PBMC) may be a preferable stem cell source when compared with BM. Some studies have shown that the use of G-PBMC as a stem cell source increased the frequency of cGVHD with comparable survival as compared with BM (17–19). Second, GVHD prophylaxis without ATG may be another beneficial option, as ATG has been shown to significantly decrease the incidence of cGVHD (20–22).

As the major causes of treatment failure were disease relapse and progression, treatment strategies before or after allo-HCT to reduce the risk of relapse remain a significant consideration for patients with high-risk MDS. The use of some additional treatment might be effective, especially for patients with high-risk MDS without cGVHD. Azacitidine is a DNA hypomethylating agent to show a significantly prolonged OS compared with conventional care regimens in patients with intermediate-2 and high-risk MDS (23, 24). The use of low-dose azacitidine as pre-emptive and maintenance treatment may prolong survival in patients with higher-risk MDS or AML after allo-HCT (25–27). Azacitidine also appears to induce leukemic cell differentiation and increase the expression of human leukemic antigen DR-1 (HLA-DR) and several tumor-associated antigens that could potentially enhance the GVL effect (28–30). We were not

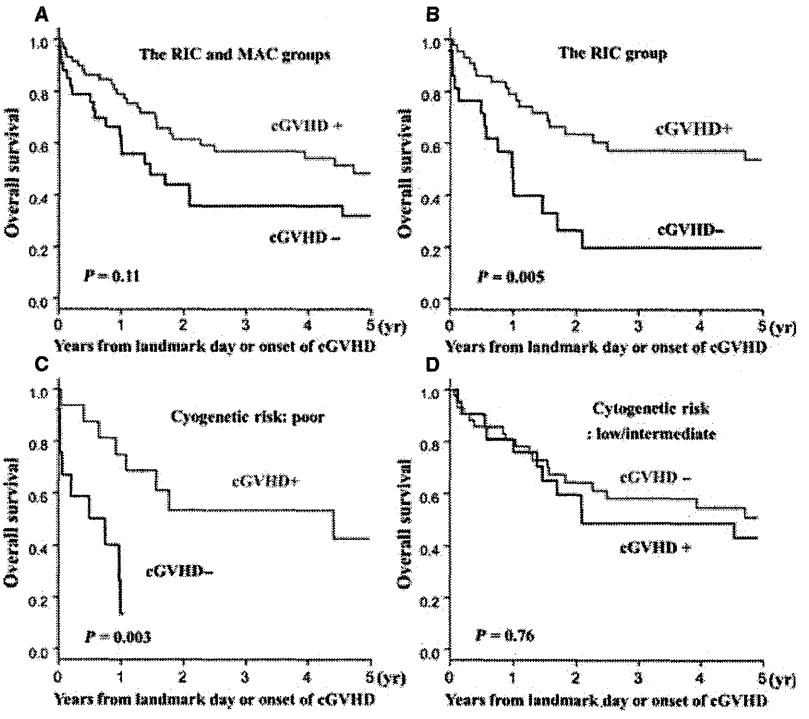

Figure 2 Semilandmark plots illustrating the impact of chronic graft-versus-host disease (GVHD) on overall survival (OS) of patients with myelodysplastic syndrome receiving allo-hematopoietic cell transplantation. OS curves of patients with or without chronic GVHD are shown for the entire population (A), the reduced-intensity conditioning group (B), patients with poor cytogenetic risk (C), and patients with low/intermediate cytogenetic risk (D).

able to assess the effect of Azacitidine before or after allo-HCT in patients with MDS, because patients who received Azacitidine were not included in our study. These issues need to be addressed in a prospective study.

We also analyzed the impact of aGVHD on outcomes after allo-HCT. The presence of grade II–IV aGVHD did not significantly influence the outcome. On the other hand, the presence of grade III–IV aGVHD was significantly associated with a worse OS and a higher incidence of NRM. Several studies have analyzed the effect of aGVHD on the prognosis after allo-HCT, but only a few have shown that aGVHD has a positive impact (12, 15, 16, 31). Kanda *et al.* (31) reported that grade I aGVHD had a beneficial effect on PFS in high-risk patients. However, we were not able to evaluate the effect of grade I aGVHD because of the small number of patients.

In the present study, OS, relapse and NRM did not differ significantly between the MAC and RIC groups, although the RIC group had significantly higher proportions of elderly patients and those with poor cytogenetic risk. Several previous studies have analyzed MDS and AML patients who received allo-HCT after MAC or RIC regimens (2, 6, 32, 33). In some studies, OS and PFS tended to be similar between the MAC and RIC groups, with a decreased incidence of NRM offset by an increased incidence of relapse in the RIC group. In other studies, there were no differences in relapse or NRM between the MAC and RIC groups, with a comparable OS (34, 35), and our results were consistent with the latter results.

The other major covariates that influenced OS in the present study were poor cytogenetic risk at diagnosis and the disease status at allo-HCT. Poor cytogenetic risk was also a significant factor for the increased risk of relapse, which was consistent with previous reports (32, 33, 36, 37). Although some studies have reported that a low pre-transplant tumor burden was essential for the success of allo-HCT in patients with MDS (35, 38, 39), it remains to be determined whether induction chemotherapy should be given to reduce the tumor burden before allo-HCT. Previous studies have shown that chemotherapy prior to allo-HCT did not improve OS because of the possibility of an increased incidence of NRM (38–40). In the present study, prior chemotherapy was significantly associated with an increased risk of relapse, but did not affect OS or NRM. This result may be explained by the fact that patients who need chemotherapy prior to HCT are probably those with high-risk disease.

Our study has several limitations, and thus the results must be interpreted with caution. These limitations include the retrospective nature of the study including the fact that therapeutic strategies were chosen at the discretion of physicians, the small number of patients analyzed, the heterogeneity of the groups of patients, and a short follow-up period. Nevertheless, the present data from more than 100 patients treated in a single center allowed us to identify factors that were associated with the prognosis in patients with MDS after allo-HCT.

In summary, the presence of cGVHD significantly reduced the risk of relapse and improved OS without increasing the incidence of NRM in patients with MDS. We also found that the presence of cGVHD significantly improved OS in high-risk patients or the RIC group, which suggests that the GVL effect may be beneficial in high-risk patients who do not receive intensive preparative regimens. For elderly or unfit patients with MDS, allo-HCT with RIC regimens was a potentially curative therapeutic option comparable with MAC regimens. As the major causes of treatment failure were disease relapse and progression, the treatment strategies to reduce the risk of relapse before and after allo-HCT are still a significant consideration for patients with high-risk MDS.

Acknowledgements

This work was supported by grants from the Japanese Ministry of Health, Labour and Welfare and the Advanced Clinical Research Organization. The results were presented at the 53rd annual meeting of the American Society of Hematology, San Diego, CA, on December 10, 2011.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

N.H. designed the study, prepared the data file, performed the analysis, interpreted data, and wrote the manuscript; S.K was primarily responsible for the study design, data analysis, and interpretation of the data; K.O., T.K., Y.K., A.S., Y.I., R.U. and T.T. provided the patients' data; S-W.K., Y.T., and Y.H. interpreted data and reviewed the manuscript; K.T. supported the statistical analysis; T.F. provided the patients' data, interpreted data, and helped to write the manuscript.

References

1. Rollison DE, Howlader N, Smith MT, Strom SS, Merritt WD, Ries LA, Edwards BK, List AF. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001–2004, using data from the NAACCR and SEER programs. *Blood* 2008;**112**:45–52.
2. de Lima M, Anagnostopoulos A, Munsell M, *et al.* Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. *Blood* 2004;**104**:865–72.

3. Alyea EP, Kim HT, Ho V, Cutler C, DeAngelo DJ, Stone R, Ritz J, Antin JH, Soiffer RJ. Impact of conditioning regimen intensity on outcome of allogeneic hematopoietic cell transplantation for advanced acute myelogenous leukemia and myelodysplastic syndrome. *Biol Blood Marrow Transplant* 2006;**12**:1047–55.
4. Giralt S, Estey E, Albitar M, *et al.* Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997;**89**:4531–6.
5. Slavin S, Nagler A, Naparstek E, *et al.* Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998;**91**:756–63.
6. Martino R, Iacobelli S, Brand R, *et al.* Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. *Blood* 2006;**108**:836–46.
7. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol* 1982;**51**:189–99.
8. Greenberg P, Cox C, LeBeau MM, *et al.* International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood* 1997;**89**:2079–88.
9. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995;**15**:825–8.
10. Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part I: unadjusted analysis. *Bone Marrow Transplant* 2001;**28**:909–15.
11. Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part 2: regression modeling. *Bone Marrow Transplant* 2001;**28**:1001–11.
12. Baron F, Maris MB, Sandmaier BM, *et al.* Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol* 2005;**23**:1993–2003.
13. Prentice RL, Kalbfleisch JD, Peterson AV Jr, Flournoy N, Farewell VT, Breslow NE. The analysis of failure times in the presence of competing risks. *Biometrics* 1978;**34**:541–54.
14. Horowitz MM, Gale RP, Sondel PM, *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990;**75**:555–62.
15. Sullivan KM, Weiden PL, Storb R, *et al.* Influence of acute and chronic graft-versus-host disease on relapse and survival after bone marrow transplantation from HLA-identical siblings as treatment of acute and chronic leukemia. *Blood* 1989;**73**:1720–8.
16. Valcarcel D, Martino R, Caballero D, *et al.* Sustained remissions of high-risk acute myeloid leukemia and myelodysplastic syndrome after reduced-intensity conditioning allogeneic hematopoietic transplantation: chronic graft-versus-host disease is the strongest factor improving survival. *J Clin Oncol* 2008;**26**:577–84.
17. Blaise D, Kuentz M, Fortanier C, *et al.* Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Societe Francaise de Greffe de Moelle. *J Clin Oncol* 2000;**18**:537–46.
18. Bensinger WI, Martin PJ, Storer B, *et al.* Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *N Engl J Med* 2001;**344**:175–81.
19. Couban S, Simpson DR, Barnett MJ, *et al.* A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. *Blood* 2002;**100**:1525–31.
20. Finke J, Bethge WA, Schmoor C, *et al.* Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol* 2009;**10**:855–64.
21. Bacigalupo A, Lamparelli T, Bruzzi P, *et al.* Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood* 2001;**98**:2942–7.
22. Socie G, Schmoor C, Bethge WA, *et al.* Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. *Blood* 2011;**117**:6375–82.
23. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, *et al.* Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol* 2009;**10**:223–32.
24. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, *et al.* Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J Clin Oncol* 2010;**28**:562–9.
25. de Lima M, Giralt S, Thall PF, de Padua Silva L, Jones RB, Komanduri K, Braun TM, Nguyen HQ, Champlin R, Garcia-Manero G. Maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation for recurrent acute myelogenous leukemia or myelodysplastic syndrome: a dose and schedule finding study. *Cancer* 2010;**116**:5420–31.
26. Platzbecker U, Wermke M, Radke J, *et al.* Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial. *Leukemia* 2012;**26**:381–9.
27. Jabbour E, Giralt S, Kantarjian H, Garcia-Manero G, Jagasia M, Kebriaei P, de Padua L, Shpall EJ, Champlin R, de Lima M. Low-dose azacitidine after allogeneic stem cell transplantation for acute leukemia. *Cancer* 2009;**115**:1899–905.

28. Pinto A, Attadia V, Fusco A, Ferrara F, Spada OA, Di Fiore PP. 5-Aza-2′-deoxycytidine induces terminal differentiation of leukemic blasts from patients with acute myeloid leukemias. *Blood* 1984;**64**:922–9.
29. Pinto A, Maio M, Attadia V, Zappacosta S, Cimino R. Modulation of HLA-DR antigens expression in human myeloid leukaemia cells by cytarabine and 5-aza-2′-deoxycytidine. *Lancet* 1984;**2**:867–8.
30. Momparler RL, Bouchard J, Samson J. Induction of differentiation and inhibition of DNA methylation in HL-60 myeloid leukemic cells by 5-AZA-2′-deoxycytidine. *Leuk Res* 1985;**9**:1361–6.
31. Kanda Y, Izutsu K, Hirai H, *et al.* Effect of graft-versus-host disease on the outcome of bone marrow transplantation from an HLA-identical sibling donor using GVHD prophylaxis with cyclosporin A and methotrexate. *Leukemia* 2004;**18**:1013–9.
32. Shimoni A, Hardan I, Shem-Tov N, Yeshurun M, Yerushalmi R, Avigdor A, Ben-Bassat I, Nagler A. Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. *Leukemia* 2006;**20**:322–8.
33. Lim Z, Brand R, Martino R, *et al.* Allogeneic hematopoietic stem-cell transplantation for patients 50 years or older with myelodysplastic syndromes or secondary acute myeloid leukemia. *J Clin Oncol* 2010;**28**:405–11.
34. Khabori MA, El-Emary M, Xu W, Guyatt G, Galal A, Kuruvilla J, Lipton J, Messner H, Gupta V. Impact of intensity of conditioning therapy in patients aged 40–60 years with AML/myelodysplastic syndrome undergoing allogeneic transplantation. *Bone Marrow Transplant* 2011;**46**:516–22.
35. Scott BL, Sandmaier BM, Storer B, Maris MB, Sorror ML, Maloney DG, Chauncey TR, Storb R, Deeg HJ. Myeloablative vs nonmyeloablative allogeneic transplantation for patients with myelodysplastic syndrome or acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. *Leukemia* 2006;**20**:128–35.
36. Kuendgen A, Strupp C, Aivado M, Hildebrandt B, Haas R, Gattermann N, Germing U. Myelodysplastic syndromes in patients younger than age 50. *J Clin Oncol* 2006;**24**:5358–65.
37. Sorror ML, Sandmaier BM, Storer BE, Maris MB, Baron F, Maloney DG, Scott BL, Deeg HJ, Appelbaum FR, Storb R. Comorbidity and disease status based risk stratification of outcomes among patients with acute myeloid leukemia or myelodysplasia receiving allogeneic hematopoietic cell transplantation. *J Clin Oncol* 2007;**25**:4246–54.
38. Scott BL, Storer B, Loken MR, Storb R, Appelbaum FR, Deeg HJ. Pretransplantation induction chemotherapy and posttransplantation relapse in patients with advanced myelodysplastic syndrome. *Biol Blood Marrow Transplant* 2005;**11**:65–73.
39. Warlick ED, Cioc A, Defor T, Dolan M, Weisdorf D. Allogeneic stem cell transplantation for adults with myelodysplastic syndromes: importance of pretransplant disease burden. *Biol Blood Marrow Transplant* 2009;**15**:30–8.
40. Nakai K, Kanda Y, Fukuhara S, *et al.* Value of chemotherapy before allogeneic hematopoietic stem cell transplantation from an HLA-identical sibling donor for myelodysplastic syndrome. *Leukemia* 2005;**19**:396–401.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Semilandmark plots illustrating impact of chronic GVHD on OS of patients with MDS receiving allo-HCT.

Table S1. Univariate analysis for NRM, relapse, and OS.

Table S2. Multivariate analysis for NRM, relapse and OS in the RIC group (patients pretreated with RIC).

Int J Hematol (2013) 98:608–614
DOI 10.1007/s12185-013-1449-y

ORIGINAL ARTICLE

Reduced-intensity conditioning regimen with low-dose ATG-F for unrelated bone marrow transplant is associated with lower non-relapse mortality than a regimen with low-dose TBI: a single-center retrospective analysis of 103 cases

Shigeo Fuji · Niina Ueno · Nobuhiro Hiramoto · Yoshitaka Asakura · Kimikazu Yakushijin · Yutaro Kamiyama · Saiko Kurosawa · Sung-Won Kim · Yuji Heike · Takuya Yamashita · Takahiro Fukuda

Received: 31 March 2013 / Revised: 17 September 2013 / Accepted: 17 September 2013 / Published online: 4 October 2013

Abstract Although anti-T lymphocyte globulin-Fresenius (ATG-F) is commonly used as prophylaxis for graft-versus-host disease (GVHD), the appropriate dosage of ATG-F in the setting of a reduced-intensity conditioning (RIC) regimen has not been determined. In the present study, we retrospectively analyzed the clinical outcomes of 103 patients after unrelated bone marrow transplant (uBMT) with RIC regimens. RIC regimens consisted of purine analogue plus busulfan with low-dose TBI or ATG-F (5–10 mg/kg in total). Median age was 57 years (range 20–68). The incidence of grade II–IV acute GVHD and chronic GVHD with ATG-F was significantly lower than that with TBI 2 Gy (15 vs. 61 %, $P < 0.05$; 33 vs. 57 %, $P < 0.05$). The incidence of 2-year NRM with ATG-F was significantly lower than that with TBI 2 Gy (6 vs. 28 %, $P < 0.05$). There was no statistically significant difference in the cumulative incidence of 2-year relapse between the ATG-F and TBI 2 Gy groups (37 vs. 20 %, $P = 0.13$). In conclusion, the addition of low-dose ATG-F to GVHD prophylaxis in patients who received uBMT resulted in decreased incidence of acute and chronic GVHD, which led to a significantly reduced risk of NRM without compromising overall survival. The beneficial effect of low-dose ATG-F should be assessed in a prospective clinical trial.

S. Fuji · N. Ueno · N. Hiramoto · Y. Asakura · K. Yakushijin · Y. Kamiyama · S. Kurosawa · S.-W. Kim · Y. Heike · T. Yamashita · T. Fukuda (✉)
Hematopoietic Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-Ku, Tokyo 104-0045, Japan
e-mail: tafukuda@ncc.go.jp

Keywords ATG · Unrelated bone marrow transplant · GVHD

Introduction

After Slavin and co-workers [1–3] introduced a reduced-intensity conditioning (RIC) regimen using fludarabine (Flu)/busulfan (Bu), similar regimens have been widely used worldwide. Bornhäuser et al. [2] reported that a RIC regimen using Flu/Bu/Anti-T lymphocyte globulin (ATG) was associated with a high risk of graft failure (GF). The incidence of GF was higher in patients who received bone marrow (BM, 31 %) as compared to unmanipulated peripheral blood stem cells (PBSC, 10 %), although this difference was not statistically significant [2]. In contrast, Nagler et al. [3] reported that a RIC regimen using Flu/Bu/ATG followed by an unrelated BMT (uBMT) was not associated with GF. In general, PBSC is preferred in the setting of RIC, due to the risk of GF [4]. However, in Japan, only BM was able to be harvested from an unrelated volunteer donor. Therefore, we incorporated TBI 4 Gy in addition to purine analogue plus Bu to ensure engraftment [5]. With this conditioning regimen, while all patients ($n = 17$) achieved engraftment, 5 had grade IV acute graft-versus-host disease (GVHD) and the incidence of non-relapse mortality (NRM) was unacceptably high (1-year NRM 46 %). Therefore, we thereafter reduced the dose of TBI from 4 to 2 Gy.

Finke et al. [6, 7] reported that ATG-F as GVHD prophylaxis reduced the incidences of acute and chronic GVHD without comprising survival in patients with an unrelated HSCT following a myeloablative conditioning regimen. Therefore, we also incorporated low-dose ATG-F instead of TBI to decrease GVHD-related deaths. Since

Asian populations have a lower risk of GVHD than Caucasian populations, we used low-dose ATG-F (5 or 10 mg/kg in total) [8–10]. Here, we retrospectively analyzed the clinical outcomes in patients who received uBMT with a RIC regimen in our institute. We focused on a comparison of currently used regimens, and particularly those that contain TBI 2 Gy and ATG-F.

Patients and methods

Study design

This was a single-center retrospective study that compared 3 RIC regimens (TBI 4 Gy-containing, $n = 30$; TBI 2 Gy-containing, $n = 40$; ATG-F-containing, $n = 33$) in patients who received uBMT from December 2001 to May 2009. In Japan in the era considered in this study, only BMT could be performed from an unrelated volunteer donor. This study was approved by the Institutional Review Board of National Cancer Center, Tokyo, Japan.

Clinical outcomes

Endpoints included neutrophil recovery, overall survival (OS), progression-free survival (PFS), NRM, acute GVHD, chronic GVHD and discontinuation of immunosuppressive drugs. Neutrophil recovery was defined as an absolute neutrophil count (ANC) of $\geq 0.5 \times 10^9$/L for 3 consecutive days. Incidences of grade II–IV or III–IV acute GVHD were based on standard criteria [11]. Chronic GVHD was defined according to Seattle's group criteria [12]. When typical chronic GVHD occurred before 100 days after uBMT, it was also defined as chronic GVHD in this study. Primary GF was defined in accordance with a previous report as an ANC that did not exceed 500/mm^3 or the absence of donor T cells (<5 %) before relapse, disease progression, second HSCT, or death [13]. Secondary GF was defined as a decrease in ANC of <100/mm^3 at 3 determinations or the absence of donor T cells (<5 %) after the initial engraftment without recovery before relapse, disease progression, second HSCT, or death.

Statistical analysis

The probabilities of OS and PFS were calculated by the Kaplan–Meier method. Cox proportional-hazards regression model was used to analyze OS and PFS. The cumulative incidences of engraftment, NRM, GVHD and discontinuation of immunosuppressive drugs were evaluated using a model by Fine and Grey for the univariate and multivariate analyses of cumulative incidence. In the competing risk models for engraftment, GVHD and discontinuation of immunosuppressive drugs, relapse and death before these events were defined as competing risks. In the competing risk models for NRM, relapse was defined as a competing risk. In the competing risk models for GF, relapse and NRM were defined as competing risks. Factors that were associated with a two-sided P value <0.10 in the univariate analysis were included in a multivariate analysis. We used a backward-stepwise selection algorithm and retained only the statistically significant variables in the final model. A two-sided P value <0.05 was considered statistically significant. The variables evaluated in these analyses were as follows: sex, patient's age at the time of uBMT (age $\geq$ 55 years vs. age < 55), disease risk (standard risk vs. high risk), conditioning regimen (TBI 4 Gy vs. TBI 2 Gy vs. ATG-F), and HLA disparity assessed by allele typing of HLA A, B, C and DRB1. Standard risk was defined as the first complete remission of acute leukemia or the first chronic phase of chronic myeloid leukemia. High risk was defined as other hematological malignancies. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) [14]. More precisely, it is a modified version of R commander (version 1.6-3) that was designed to add statistical functions that are frequently used in biostatistics.

Results

Patients' characteristics

The characteristics of the 103 patients are shown in Table 1. The median age was 57 years (range 20–68). Thirty-six (35 %) and 15 (15 %) patients received bone marrow from a donor with an HLA antigen and allele mismatch, respectively. All patients received a RIC regimen as defined by previous reports [15, 16]. The conditioning regimen included Flu ($n = 87$, 180 mg/kg) or cladribine ($n = 16$, 0.66 mg/kg) + Bu (8 mg/kg oral or 6.4 mg/kg i.v.). Targeting of Bu was not performed. The total dose of ATG-F was 10 mg/kg ($n = 13$) or 5 mg/kg ($n = 20$). As GVHD prophylaxis, tacrolimus (TAC) was mainly used in the TBI 2 Gy group and ATG-F group (90 and 90 %, respectively) and cyclosporine (CSP) was mainly used in the TBI 4 Gy group (93 %).

Posttransplant complications

Surviving patients were followed up for a median of 1,494 days after uBMT (range 524–3,466 days). The median follow-up of surviving patients in the TBI 4 Gy group was significantly longer than those in the other

Table 1 Patient characteristics

	TBI 4 Gy *N* (%)	TBI 2 Gy *N* (%)	ATG-F *N* (%)	*P* value
No. of patients	30	40	33	
Age, median (range), year	57 (27–67)	56 (20–68)	57 (24–66)	
Sex (Male/Female)	17/13	28/12	17/16	0.25
Diagnosis				
AML	15 (50)	17 (43)	15 (50)	0.02
MDS	6 (20)	3 (8)	3 (10)	
Lymphoma	5 (17)	20 (50)	14 (47)	
Others[a]	4 (13)	0 (0)	1 (3)	
Disease risk				
Standard	5 (17)	7 (18)	6 (18)	1.00
High	25 (83)	33 (83)	27 (82)	
HLA mismatch				
None	17 (57)	22 (55)	13 (39)	0.30
Mismatch	13 (43)	18 (45)	20 (61)	
GVHD prophylaxis				
CSP-based	28 (93)	4 (10)	5 (15)	<0.05
TAC-based	2 (7)	36 (90)	28 (85)	
Conditioning				
Fludarabine	19 (63)	35 (88)	33 (100)	<0.05
Cladribine	11 (37)	5 (13)	0 (0)	
Time period				
2001–2003	22 (73)	0 (0)	0 (0)	<0.05
2004–2006	8 (27)	16 (40)	11 (33)	
2007–2009	0 (0)	24 (60)	22 (66)	

Cladribine group included more patients with CSP as GVHD prophylaxis (10 patients, 63 %) and more patients transplanted from an HLA matched donor (14 patients, 88 %) as compared to fludarabine group. In patients who received ATG-F, 20 and 13 patients received 5 mg/kg and 10 mg/kg ATG-F, respectively. Whereas all patients with 5 mg/kg received TAC as GVHD prophylaxis, 8 patients (62 %) received TAC in patients who received 10 mg/kg

AML acute myeloid leukemia, *MDS* myelodysplastic syndrome, *CSP* cyclosporin, *TAC* tacrolimus

[a] Others included multiple myeloma, myeloproliferative disorder and acute lymphoblastic leukemia

groups (TBI 4 Gy 2,782 days, TBI 2 Gy 1,164 days, ATG-F 1,473 days), which reflects the change in our clinical practice, but there was no significant difference in the follow-up period between the TBI 2 Gy group and ATG-F group. Neutrophil engraftment was observed in 102 patients (median 18 days, range 11–32 days, Fig. 1a). Engraftment was achieved in 100, 93 and 97 % of the patients at 30 days after uBMT in the TBI 4 Gy, TBI 2 Gy and ATG-F groups, and there was no significant difference among the 3 groups. Primary GF occurred in one patient who received a conditioning regimen with ATG-F. Secondary GF occurred in 7 patients (TBI 4 Gy $n = 1$, ATG-F $n = 6$). Five patients who had GF after uBMT underwent salvage HSCT (cord blood transplant $n = 4$, haploidentical transplant $n = 1$), and 4 patients were successfully rescued. The other two patients had an autologous recovery and one patient had progressive disease before a planned salvage HSCT. The proportion of patients with GF with ATG-F was significantly higher than those in the other 2 groups (3, 0, 21 % in TBI 4 Gy, TBI 2 Gy and ATG-F, respectively; $P = 0.002$). The cumulative incidences of GF including both primary and secondary GF were 3.3, 0, 21.2 % in the TBI 4 Gy, TBI 2 Gy and ATG-F groups, respectively (Fig. 1b). In multivariate analysis, the use of ATG-F was associated with an increased risk of GF as compared to the use of low-dose TBI including both 2 and 4 Gy (HR 16.5, 95 % CI 2.1–130.9, $P = 0.008$).

The cumulative incidences of grade II-IV acute GVHD were 50, 61 and 15 % in the TBI 4 Gy, TBI 2 Gy and ATG-F groups, respectively (Fig. 1c). The use of ATG-F was associated with a significantly lower incidence of grade II–IV acute GVHD as compared to TBI 2 Gy ($P < 0.001$). Multivariate analysis showed that the ATG-F group was associated with a decreased risk of grade II–IV acute GVHD as compared to TBI 2 Gy (hazard ratio [HR] 0.17, 95 % CI 0.06–0.44, $P < 0.001$). There was no statistically significant difference between TBI 4 Gy and ATG-F groups. The cumulative incidences of grade III–IV acute GVHD were 30, 8 and 0 % with TBI 4 Gy, TBI 2 Gy and ATG-F, respectively (Fig. 1d).

The cumulative incidences of chronic GVHD were 50, 57 and 33 % with TBI 4 Gy, TBI 2 Gy and ATG-F, respectively (Fig. 1e). The ATG-F group had a significantly lower incidence of chronic GVHD as compared to the TBI 2 Gy group ($P = 0.038$). There was no statistically significant difference between TBI 4 Gy and ATG-F groups. Multivariate analysis showed that the use of ATG-F was associated with a decreased risk of chronic GVHD as compared to TBI 2 Gy (HR 0.45, 95 % CI 0.23–0.88, $P = 0.019$). The cumulative incidences of extensive chronic GVHD were 30, 45 and 21 % with TBI 4 Gy, TBI 2 Gy and ATG-F, respectively (Fig. 1f). There was no statistically significant difference between TBI 4 Gy and ATG-F groups. The ATG-F group had a significantly lower incidence of chronic GVHD than the TBI 2 Gy group ($P = 0.022$). Multivariate analysis showed that the use of ATG-F was associated with a lower incidence of extensive chronic GVHD as compared to TBI 2 Gy (HR 0.38, 95 % CI 0.17–0.89, $P = 0.025$). There was no statistically significant difference between TBI 4 Gy and ATG-F groups. In the ATG-F group, there was no statistically significant difference between 5 and 10 mg/kg ATG-F in terms of posttransplant complications, including acute and chronic GVHD (data not shown).

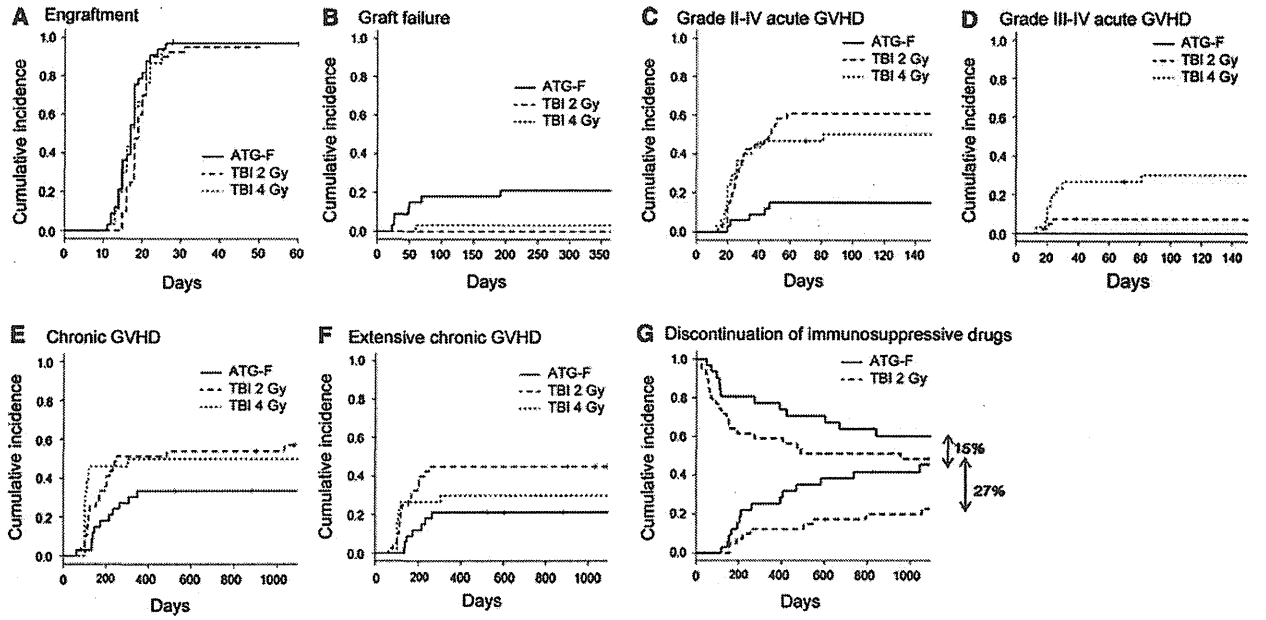

Fig. 1 **a** Engraftment, **b** graft failure, **c** grade II–IV acute GVHD, **d** grade III–IV acute GVHD, **e** chronic GVHD, **f** extensive chronic GVHD and **g** discontinuation of immunosuppressive drugs (*2 lower curves*). The competing risks of relapse and death are shown in the *2 upper curves*. *Bidirectional arrows* show the proportion of surviving patients who continued to receive immunosuppressive drugs at 3 years after uBMT (27 vs. 15 % with TBI 2 Gy and ATG-F, respectively; $P = 0.09$)

The cumulative incidences of the discontinuation of immunosuppressive drugs are shown in Fig. 1g. We excluded the TBI 4 Gy group from this analysis because the incidence of competing events (NRM and relapse) was high. At 3 years after uBMT, immunosuppressive drugs were discontinued in 46 % and 23 % of the patients with ATG-F and TBI 2 Gy, respectively. The probability that surviving patients would continue immunosuppressive drugs in the TBI 2 Gy group tended to be higher than that in the ATG-F group (27 vs. 15 %, $P = 0.09$).

There were no reported cases of PTLD in this case series.

Survival

The probabilities of 2-year OS were 40, 60 and 69 % with TBI 4 Gy, TBI 2 Gy and ATG-F, respectively (Fig. 2a). There was no statistically significant difference among three groups. One year after uBMT, the probability of OS with ATG-F was significantly better than that with TBI 2 Gy and TBI 4 Gy (ATG-F 91 % vs. TBI 2 Gy 68 %, $P = 0.01$; vs. TBI 4 Gy 47 %, $P < 0.01$). A multivariate analysis for OS showed that older age (age ≥55) was associated with an inferior outcome (HR 2.1, 95 % CI 1.1–4.2, $P = 0.03$). The probabilities of 2-year PFS were 40, 53 and 57 % with TBI 4 Gy, TBI 2 Gy and ATG-F, respectively (Fig. 2b). There was no statistically significant difference among three groups. The cumulative incidences of relapse at 2 years after uBMT were 10, 20 and 37 % with TBI 4 Gy, TBI 2 Gy and ATG-F, respectively (Fig. 2c). There was no statistically significant difference among three groups. No covariate including the use of ATG-F was associated with an increased risk for relapse. The cumulative incidences of 2-year NRM were 50, 28 and 6 % with TBI 4 Gy, TBI 2 Gy and ATG-F, respectively (Fig. 2d). A multivariate analysis showed that the ATG-F group had a lower NRM as compared to both TBI 2 Gy and TBI 4 Gy groups, respectively (HR 0.24, 95 % CI 0.07–0.80, $P = 0.020$; HR 0.12, 95 % CI 0.04–0.38, $P < 0.01$). In comparison between TBI 2 Gy and TBI 4 Gy, there was a trend toward an increased risk of NRM with TBI 4 Gy as compared to TBI 2 Gy ($P = 0.074$). In the ATG-F group, there was no statistically significant difference in the clinical outcomes between 5 and 10 mg/kg ATG-F, including OS, PFS and NRM (data not shown). In terms of the type of purine analogue, there was no statistically significant difference in the clinical outcomes between Flu and cladribine, including OS, PFS and NRM (data not shown).

Discussion

We assessed the impact of low-dose ATG-F and low-dose TBI on the clinical outcomes in patients who received an uBMT with a purine analogue plus busulfan-based RIC

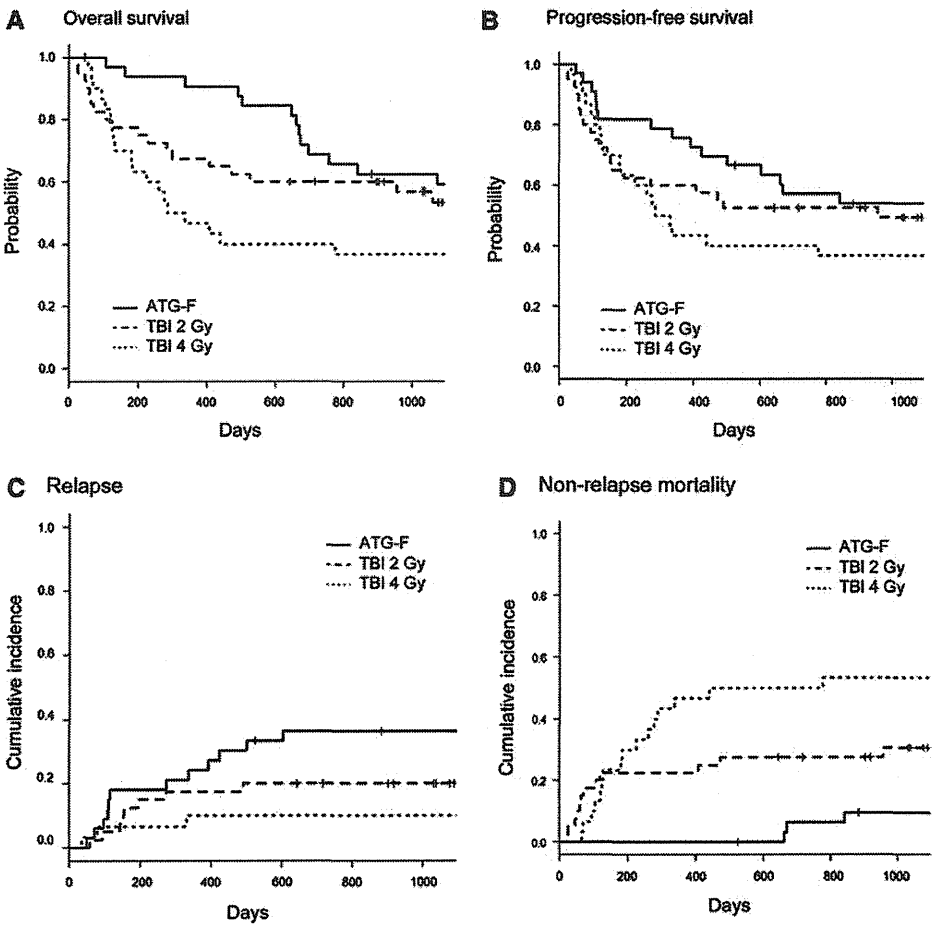

Fig. 2 **a** Overall survival, **b** progression-free survival, **c** relapse and **d** non-relapse mortality

regimen. The incidence of acute and chronic GVHD in patients with ATG-F was low, considering that all patients received BMT from an unrelated donor and about half of the patients received BMT from an unrelated donor with HLA mismatch. The promisingly low incidence of NRM in the ATG-F group is also an important finding in this study, considering the patients' characteristics such as old age and HLA mismatch. Although this study was not a prospective randomized-controlled trial, the incidence of NRM in the ATG-F group was significantly lower than that in the TBI 4 Gy and TBI 2 Gy groups.

In Western countries, the total dose of ATG-F for GVHD prophylaxis is usually 30–60 mg/kg [6]. In Asian countries, a smaller dose of ATG is commonly used, since the incidence of GVHD itself in Asian patients has been shown to be lower than that in Caucasian patients [8, 9]. Kim et al. [17] reported that the use of low-dose ATG (Thymoglobulin, 2.5 mg/kg) was associated with a low incidence of acute GVHD in patients who received an HLA-mismatched unrelated HSCT. In our study, we used ATG-F at a dose of 5 or 10 mg/kg. Use of the lower dose of ATG (5 mg/kg) did not increase the incidence of GVHD and was associated with similar clinical outcomes as compared to the higher dose (10 mg/kg), albeit the size of the study was limited. Soiffer et al. [18] reported that the adverse impact of ATG, which increased the incidence of infectious diseases and relapse, outweighed the lowered risk of GVHD in patients who received a RIC regimen. Therefore, the optimal dosage of ATG-F could differ depending on the intensity of the conditioning regimen. As shown in this study, the regimen with low-dose ATG-F significantly reduced the incidence of GVHD as compared to the TBI-containing regimen without compromising OS, possibly because low-dose ATG-F did not intensively suppress the recovery of lymphocytes that recognize infectious organisms or hematological malignancies. To confirm our finding, prospective studies are needed to assess the impact of low-dose ATG-F with a uniform conditioning regimen and GVHD prophylaxis.

Finke et al. [6] conducted a large randomized-control trial which demonstrated that the incidence of acute GVHD was reduced with ATG-F as GVHD prophylaxis without compromising OS in patients who underwent unrelated HSCT following a myeloablative conditioning regimen.

Furthermore, long-term follow-up revealed that the use of ATG-F significantly reduced the incidence of chronic GVHD [7]. Another randomized trial using ATG in unrelated HSCT also showed that chronic GVHD, especially chronic lung dysfunction, was reduced in patients who received ATG [19]. Consistent with their results, the incidences of acute and chronic GVHD with low-dose ATG-F were significantly lower than those with low-dose TBI in our study. The probability of OS at 1 year after uBMT was significantly better with low-dose ATG-F than with low-dose TBI, which reflects the decrease in GVHD-related deaths in the early phase. In addition to the decreased risk of death in the earlier time period after uBMT, more surviving patients with ATG-F discontinued immunosuppressive drugs as compared to those with TBI 2 Gy. The reduction and better control of chronic GVHD should be associated with an improved quality of life. Such beneficial effects are important in long-term survivors after uBMT.

The major concern with ATG-F in combination with RIC in this study was the high incidence of GF ($n = 7$, 21 %). Even though 4 patients were rescued by salvage HSCT and 2 patients had autologous recovery, GF is a lethal complication after allogeneic HSCT. Therefore, it would be better to avoid a RIC regimen with ATG-F in patients with a high risk of GF, for example untreated MDS with a history of transfusion [20]. One option is to use PBSC instead of BM, since BM is a well-known risk factor for GF [21, 22]. Although another option to improve the rate of engraftment in such cases could be the combination of TBI 2 Gy and ATG-F, we have not yet tested this regimen.

Another concern with ATG-F is PTLD. The incidence of PTLD with in vivo T cell depletion using ATG varies. Soiffer et al. [18] reported that the incidence of PTLD was 2 % in patients who received a RIC regimen with ATG. In our study, there were no cases of PTLD. Although the size of our study was small, the risk of PTLD might be tolerable because T cell depletion was not intense.

The limitations of this study should be clarified. This was a retrospective study that assessed the impact of ATG-F or TBI on the clinical outcomes in patients who received a RIC regimen. At first, TBI 4 Gy group included patients who received uBMT during an earlier time period as compared to the other 2 groups (Table 1). Poor clinical outcome in TBI 4 Gy group was similar to our previous report ($n = 17$, NRM 46 % at 1 year) [4]. The high incidence of NRM in the TBI 4 Gy group in the current study ($n = 30$, NRM 50 % at 2 years) might be partly explained by the increased regimen-related toxicity. Considering the high incidence of severe acute GVHD in the TBI 4 Gy group, GVHD prophylaxis using CSP might be insufficient in uBMT with a RIC regimen. The improvement of supportive care might also affect the incidence of NRM in recent years in the TBI 2 Gy and ATG-F groups as reported [23, 24]. Second, the decision on whether a patient received ATG-F or TBI 2 Gy was based on the preference of each transplant physician and patient, which could lead to a significant selection bias. Furthermore, the characteristics of the patients were heterogeneous. Especially, the underlying disease varied significantly. The benefit of low-dose ATG-F should be re-evaluated.

In conclusion, the use of low-dose ATG-F in combination with a purine analogue plus Bu-based RIC regimen was associated with a promisingly low incidence of acute and chronic GVHD without compromising OS. The NRM rate in the ATG-F group was significantly lower than that in the TBI 4 Gy and TBI 2 Gy groups. The role of low-dose ATG-F as prophylaxis for GVHD should be further assessed with a uniform conditioning regimen in a prospective clinical trial.

Acknowledgments We thank the medical, nursing, data-processing, laboratory, and clinical staff at the participating centers for their important contributions to this study and their dedicated care of the patients. This work was supported by grants from the Japanese Ministry of Health, Labour and Welfare and the National Cancer Research and Development Fund (23-A-28).

Conflict of interest There is no conflict of interest to declare.

References

1. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;91:756–63.
2. Bornhäuser M, Thiede C, Platzbecker U, Jenke A, Helwig A, Plettig R, et al. Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. Clin Cancer Res. 2001;7:2254–62.
3. Nagler A, Aker M, Or R, Naparstek E, Varadi G, Brautbar C, et al. Low-intensity conditioning is sufficient to ensure engraftment in matched unrelated bone marrow transplantation. Exp Hematol. 2001;29:362–70.
4. Maris MB, Niederwieser D, Sandmaier BM, Storer B, Stuart M, Maloney D, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood. 2003;102:2021–30.
5. Onishi Y, Mori S, Kusumoto S, Sugimoto K, Akahane D, Morita-Hoshi Y, et al. Unrelated-donor bone marrow transplantation with a conditioning regimen including fludarabine, busulfan, and 4 Gy total body irradiation. Int J Hematol. 2007;85:256–63.
6. Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009;10:855–64.
7. Socié G, Schmoor C, Bethge WA, Ottinger HD, Stelljes M, Zander AR, et al. Chronic graft-versus-host disease: long-term

results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. Blood. 2011;117:6375–82.
8. Oh H, Loberiza FR Jr, Zhang MJ, Ringdén O, Akiyama H, Asai T, et al. Comparison of graft-versus-host-disease and survival after HLA-identical sibling bone marrow transplantation in ethnic populations. Blood. 2005;105:1408–16.
9. Hahn T, McCarthy PL Jr, Zhang MJ, Wang D, Arora M, Frangoul H, et al. Risk factors for acute graft-versus-host disease after human leukocyte antigen-identical sibling transplants for adults with leukemia. J Clin Oncol. 2008;26:5728–34.
10. Hatanaka K, Fuji S, Ikegame K, Kato R, Wake A, Hidaka M, et al. Low incidences of acute and chronic graft-versus-host disease after unrelated bone marrow transplantation with low-dose anti-T lymphocyte globulin. Int J Hematol. 2012;96:773–80.
11. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18:295–304.
12. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transpl. 1995;15:825–8.
13. Petersdorf EW, Hansen JA, Martin PJ, Woolfrey A, Malkki M, Gooley T, et al. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. N Engl J Med. 2001;345:1794–800.
14. Kanda Y. Free statistical software: EZR (Easy R) on R commander. Available from: http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html.
15. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transpl. 2009;15:1628–33.
16. Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. Biol Blood Marrow Transpl. 2009;15:367–9.
17. Kim HJ, Min WS, Cho BS, Eom KS, Kim YJ, Min CK, et al. Successful prevention of acute graft-versus-host disease using low-dose antithymocyte globulin after mismatched, unrelated, hematopoietic stem cell transplantation for acute myelogenous leukemia. Biol Blood Marrow Transpl. 2009;15:704–17.
18. Soiffer RJ, Lerademacher J, Ho V, Kan F, Artz A, Champlin RE, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. Blood. 2011;117:6963–70.
19. Bacigalupo A, Lamparelli T, Barisione G, Bruzzi P, Guidi S, Alessandrino PE, et al. Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. Biol Blood Marrow Transpl. 2006;12:560–5.
20. Mattsson J, Ringdén O, Storb R. Graft failure after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transpl. 2008;14:165–70.
21. Shaw BE, Russell NH, Devereux S, Das-Gupta E, Mackinnon S, Madrigal JA, et al. The impact of donor factors on primary non-engraftment in recipients of reduced intensity conditioned transplants from unrelated donors. Haematologica. 2005;90:1562–9.
22. Passweg JR, Zhang MJ, Rocha V, Kan F, Champlin RE, Isola LM, et al. Donor characteristics affecting graft failure, graft-versus-host disease, and survival after unrelated donor transplantation with reduced-intensity conditioning for hematologic malignancies. Biol Blood Marrow Transpl. 2011;17:1869–73.
23. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010;363:2091–101.
24. Kurosawa S, Yakushijin K, Yamaguchi T, Atsuta Y, Nagamura-Inoue T, Akiyama H, et al. Changes in incidence and causes of non-relapse mortality after allogeneic hematopoietic cell transplantation in patients with acute leukemia/myelodysplastic syndrome: an analysis of the Japan Transplant Outcome Registry. Bone Marrow Transpl. 2013;48:529–36.

Biol Blood Marrow Transplant 19 (2013) 1026–1032

Efficacy and Long-Term Outcome of Treatment for Pure Red Cell Aplasia after Allogeneic Stem Cell Transplantation from Major ABO-Incompatible Donors

Makoto Hirokawa [1,*], Takahiro Fukuda [2], Kazuteru Ohashi [3], Michihiro Hidaka [4], Tatsuo Ichinohe [5], Koji Iwato [6], Heiwa Kanamori [7], Makoto Murata [8], Toru Sakura [9], Masahiro Imamura [10], Soichi Adachi [10], Ritsuro Suzuki [10], Yasuo Morishima [10], Hisashi Sakamaki [10], for The PRCA Collaborative Study Group

[1] *Akita University, Akita, Japan*
[2] *National Cancer Center Hospital, Tokyo, Japan*
[3] *Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan*
[4] *National Hospital Organization, Kumamoto Medical Center, Kumamoto, Japan*
[5] *Kyoto University, Kyoto, Japan*
[6] *Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, Hiroshima, Japan*
[7] *Kanagawa Cancer Center, Yokohama, Japan*
[8] *Nagoya University Graduate School of Medicine, Nagoya, Japan*
[9] *Saiseikai Maebashi Hospital, Maebashi, Japan*
[10] *Japan Society of Hematopoietic Cell Transplantation, Nagoya, Japan*

Article history:
Received 12 November 2012
Accepted 5 April 2013

Key Words:
Major ABO-mismatched HSCT
PRCA
Long-term outcome

A B S T R A C T

No standard of care for pure red cell aplasia (PRCA) after major ABO-incompatible hematopoietic stem cell transplantation (HSCT) has been established. We conducted a retrospective cohort study to learn the efficacy and outcome of treatment for PRCA. One hundred forty-five recipients who showed delayed recovery of erythropoiesis and survived >100 days after transplantation without early disease progression were selected from 2846 records of major ABO-incompatible transplantation in the registry database in Japan, and detailed data of 46 recipients were collected. Treatment of PRCA, such as rapid tapering of calcineurin inhibitors, corticosteroids, or additional immunosuppressants, was given to 22 patients but not to the other 24 patients. The overall response rate of the treatment group was 54.5%. The number of days from diagnosis of PRCA to recovery of reticulocytes >1% and the cumulative number of red blood cell transfusions were not significantly different between the 2 groups. Infections accounted for the death of 7 of 11 patients in the treatment group. Univariate analysis identified 5 variables influencing survival, including graft-versus-host disease, disease progression, and treatment of PRCA; disease progression remained as the only factor negatively affecting survival by multivariate analysis. The present study could not provide supportive evidence for the beneficial effects of treatment for PRCA after major ABO-mismatched HSCT.

INTRODUCTION

Pure red cell aplasia (PRCA) is a syndrome characterized by anemia, reticulocytopenia, and an absence of erythroblasts from otherwise normal bone marrow. The causes and courses of this syndrome are highly variable, and the management of this anemia depends on its underlying mechanisms and diseases [1-7].

Incompatibility of ABO blood antigens is not supposed to be a barrier to allogeneic hematopoietic stem cell transplantation (HSCT). However, delayed recovery of erythropoiesis is one of the major complications in allogeneic HSCT from major ABO-incompatible donors [8]. Controversy still exists regarding the influence of ABO incompatibility on the outcome of allogeneic HSCT [9-14]. Incompatible hemagglutinins in recipients are attributed to the delayed recovery of reticulocyte counts and hypoplasia of erythroblasts in bone marrow [15,16]. Previous studies have demonstrated several risk factors for the development of PRCA after major ABO-incompatible HSCT, such as the presence of incompatible anti-A hemagglutinin in recipients [8,17], the use of a reduced-intensity preparatory regimen [18,19], the use of cyclosporine, use of sibling donors, or the absence of acute graft-versus-host-disease (GVHD) [20].

Few publications exist on the successful treatment of this PRCA, including rapid tapering of calcineurin inhibitors [21], corticosteroids [22,23], donor lymphocyte infusion [24], rituximab [25,26], erythropoiesis-stimulating agents [27,28], antilymphocyte globulin [29], immunoadsorption [30,31], plasma exchange [32], and mesenchymal stem cells [33,34]. However, no standard of care for PRCA after allogeneic HSCT from major ABO-incompatible HSCT has been established. To learn the efficacy and long-term outcome of treatment for PRCA after allogeneic HSCT from major ABO-incompatible donors, we conducted a retrospective cohort study and established a patient cohort of 46 patients with this PRCA.

Financial disclosure: See Acknowledgments on page 1031.

* Correspondence and reprint requests: Makoto Hirokawa, MD, PhD, Clinical Oncology Center, Akita University Hospital, 1-1-1 Hondo, Akita 010-8543, Japan.
E-mail address: mhirokawa@hos.akita-u.ac.jp (M. Hirokawa).

METHODS

Study Design

The present study was conducted as a retrospective observational study. It was approved by the Institutional Review Board of Akita University Graduate School of Medicine and the Data Management Committees of the

1083-8791/$ – see front matter © 2013 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.04.004