who received transplantation using a single UCB or an RD/1AG-MM-GVH.

MATERIALS AND METHODS

Data collection

Data for patients (age: ⩾16 years) with acute myeloid leukemia, acute lymphoblastic leukemia, MDS and chronic myelogenous leukemia who received a first HCT using a single HLA 0–2 antigen-mismatched UCB unit or an RD/1AG-MM-GVH between 1 January 1998 and 31 December 2009 were obtained from the Transplant Registry Unified Management Program (TRUMP),[24] which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT). Our analysis included 2306 patients who received a single UCB graft (UCB group) and 541 patients who received a graft from an RD/1AG-MM-GVH (RD/1AG-MM-GVH group). As of January 2012, double UCB grafts for HCT are not available in Japan. The following patients were excluded: 26 patients who lacked data on survival status, survival date, sex of recipient, or GVHD prophylaxis and 8 patients who received stem cells that had been manipulated by *ex vivo* T-cell depletion or CD34 selection. Overall, 2288 patients who received a UCB unit and 525 who received a graft from an RD/1AG-MM-GVH fulfilled the criteria. The study was approved by the data management committees of TRUMP and by the institutional review boards of Japanese Red Cross Nagoya First Hospital and Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained from reports from the institution where the transplantation was performed or from cord blood banks. To reflect current practice in Japan, HLA matching in UCB or RD/1AG-MM-GVH transplantation was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci. An HLA mismatch in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, whereas a mismatch in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

End points

The primary end point of the study was to compare OS rates between the UCB and RD/1AG-MM-GVH groups. Other end points were the cumulative incidences of neutrophil and platelet engraftment, acute and chronic GVHD, relapse, and non-relapse mortality (NRM). Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded 0.5×10^9/l for 3 consecutive days following transplantation. Platelet recovery was considered to have occurred when the absolute platelet count exceeded 50×10^9/l without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to the traditional criteria.[25,26] The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Comparisons between groups were performed with the χ^2-test or extended Fisher's exact test as appropriate for categorical variables and the Mann–Whitney *U*-test for continuous variables. The probability of OS was estimated according to the Kaplan–Meier method, and the groups were compared with the log-rank test. The adjusted probability of OS was estimated according to the Cox proportional-hazards model, with other significant variables considered in the final multivariate model. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, NRM, and relapse were estimated on the basis of cumulative incidence methods, and the groups were compared with the Gray test;[27,28] competing events were death without engraftment for neutrophil and platelet engraftment, death or relapse without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. The Cox proportional-hazards model was used to evaluate variables that may affect OS, whereas the Fine and Gray proportional-hazards model was used to evaluate variables that may affect engraftment, GVHD, NRM and relapse.[29] We classified the conditioning regimen as myeloablative if either total body irradiation >8 Gy, oral busulfan ⩾9 mg/kg, intravenous busulfan ⩾7.2 mg/kg, or melphalan >140 mg/m^2 was used in the conditioning regimen, and otherwise classified it as reduced intensity, based on the report by the Center for International Blood and Marrow Transplant Research.[30] For patients for whom the doses of agents used in the conditioning regimen were not available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. Acute leukemia in the first or second remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts were defined as standard-risk diseases, and other conditions were defined as high-risk diseases. The following variables were considered when comparing the UCB and RD/1AG-MM-GVH groups: the recipient's age group (⩽50 years or >50 years at transplantation), sex of recipient, disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor and methotrexate, calcineurin inhibitor only, or other), year of transplantation (1998–2004, 2005–2009), and the time from diagnosis to transplantation (<6 months or ⩾6 months). In the analysis within the RD/1AG-MM-GVH group, the use of *in vivo* T cell depletion (no vs yes), stem cell source (peripheral blood (PB) stem cells vs bone marrow (BM)), and the number of HLA mismatches in the HVG direction (0–1 vs 2–3) were also considered. Factors without a variable of main interest were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. We then added a variable of main interest to the final model. All tests were two-sided, and $P<0.05$ was considered to indicate statistical significance. All statistical analyses were performed with Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[31] EZR is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria). 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

Characteristics of patients and transplants

Table 1 shows the patient and transplant characteristics. Recipients of an RD/1AG-MM-GVH were younger than recipients of a UCB unit. Approximately half of the recipients in the RD/1AG-MM-GVH group received PB. The number of HLA mismatches in the GVH direction between a UCB unit and recipient was 0 in 10%, 1 in 33% and 2 in 57%. In the RD/1AG-MM-GVH group, the number of antigen mismatches in the HVG direction was 0 in 12%, 1 in 68%, 2 in 18% and 3 in 3%. Most of the recipients of an RD/1AG-MM-GVH received a calcineurin inhibitor with methotrexate for GVHD prophylaxis, whereas 25% of UCB recipients received only calcineurin inhibitor. *In vivo* T-cell depletion including antithymocyte globulin (ATG) or alemtuzumab was used in 10% of the RD/1AG-MM-GVH group, but in only 1% of the UCB group. Alemtuzumab was used in only one patient, who received transplantation from an RD/1AG-MM-GVH. Information regarding the dose and type of ATG was missing in two-third of the patients who received ATG. Available data showed that the median dose of thymoglobulin was 2.5 (range 2.5–9.0, $n=9$) and 2.5 (range 1.25–5.0, $n=10$) mg/kg and the median dose of ATG-Fresenius was 8.0 (range 5.0–10.0, $n=3$) and 8.0 (range 5.0–10.0, $n=7$) mg/kg, in the UCB and RD/1AG-MM-GVH groups, respectively. Two-third of UCB transplantations were performed between 2005 and 2009. The median duration of follow-up for survivors was 2 and 4 years in the UCB and RD/1AG-MM-GVH groups, respectively.

Neutrophil and platelet engraftment

The incidence of neutrophil engraftment at day 50 in the RD/1AG-MM-GVH group was higher than that in the UCB group (UCB group, 73%, 95% confidence interval (CI), 71–75%; RD/1AG-MM-GVH group, 93%, 95% CI, 91–95%; Gray test, $P<0.001$; Figure 1a). The incidence of platelet engraftment at day 150 in the

Table 1. Patient characteristics

Variable	*UCB (n = 2288)*	*RD/1AG-MM-GVH (n = 525)*	P
Age at transplant, median (range)	49 (16–82)	43 (16–74)	<0.001
Recipient sex			
Female	1004 (44%)	239 (46%)	0.494
Male	1284 (56%)	286 (54%)	
Disease			
Acute myelogenous leukemia	1365 (60%)	269 (51%)	0.003
Acute lymphoblastic leukemia	498 (22%)	137 (26%)	
Chronic myelogenous leukemia	124 (5%)	42 (8%)	
Myelodysplastic syndrome	301 (13%)	77 (15%)	
Duration from diagnosis to transplant			
Median time (range), months	7.9 (0.2–768.5)	7.6 (0–251.7)	0.233
Disease risk			
Standard	959 (42%)	249 (47%)	0.050
High	1217 (53%)	257 (49%)	
Unknown	112 (5%)	19 (4%)	
Source of stem cells			
Bone marrow	—	251 (48%)	—
Peripheral blood	—	274 (52%)	
Cord blood	2288 (100%)	—	
HLA compatibility in the graft-versus-host direction			
Matched	225 (10%)	—	<0.001
One-antigen mismatch	753 (33%)	525 (100%)	
Two-antigen mismatch	1310 (57%)	—	
HLA compatibility in the host-versus-graft direction			
Matched	233 (10%)	62 (12%)	<0.001
One-antigen mismatch	716 (31%)	355 (68%)	
Two-antigen mismatch	1339 (59%)	94 (18%)	
Three-antigen mismatch	—	14 (3%)	
Conditioning regimen			
Myeloablative	1390 (61%)	253 (48%)	<0.001
CY + TBI ±	1062	164	
Other TBI regimen	130	20	
BU + CY ±	88	45	
Other non-TBI regimen	110	24	
Reduced intensity	894 (39%)	162 (31%)	
FLU ± TBI ±	840	138	
Other regimen	54	24	
Unclassifiable	4 (0.2%)	110 (21%)	
GVHD prophylaxis			
CSA/TAC + MTX	1410 (62%)	448 (85%)	<0.001
CSA/TAC + MMF	246 (11%)	12 (2%)	
CSA/TAC + Steroid	28 (1%)	13 (2%)	
CSA/TAC only	571 (25%)	45 (9%)	
Unknown	33 (1%)	7 (1%)	
Use of in vivo *T-cell depletion*			
No	2258 (99%)	472 (90%)	<0.001
Yes	30 (1%)	53 (10%)	
Year at transplant			
1998–2004	760 (33%)	260 (50%)	<0.001
2005–2009	1528 (67%)	265 (50%)	
Follow-up of survivors			
Median time (range), years	2.1 (0.0–10.0)	4.0 (0.1–12.2)	<0.001

Abbreviations: BU, busulfan; CSA, cyclosporine; CY, cyclophosphamide; FLU, fludarabine; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus; TBI, total body irradiation; UCB, unrelated cord blood.

RD/1AG-MM-GVH group was also higher than that in the UCB group (UCB group, 53%, 95% CI, 51–55%; RD/1AG-MM-GVH group, 70%, 95% CI, 66–74%; Gray test, $P<0.001$; Figure 1b). The use of RD/1AG-MM-GVH was significantly associated with a higher incidence of neutrophil and platelet engraftment in the multivariate analysis (neutrophil engraftment, hazard ratio (HR), 3.46,

95% CI, 3.00–3.98, $P<0.001$; platelet engraftment, HR 2.20, 95% CI, 1.89–2.57, $P<0.001$; Supplementary Table 1). As our previous study revealed that an HLA-B mismatch had an adverse effect on OS in transplantation from an RD/1AG-MM-GVH, patients in the RD/1AG-MM-GVH group with an HLA-A, -B, or -DR mismatch were separately compared with the UCB group. We consistently observed superior neutrophil and platelet engraftment in each RD/1AG-MM-GVH group as compared with the UCB group (Supplementary Table 1).

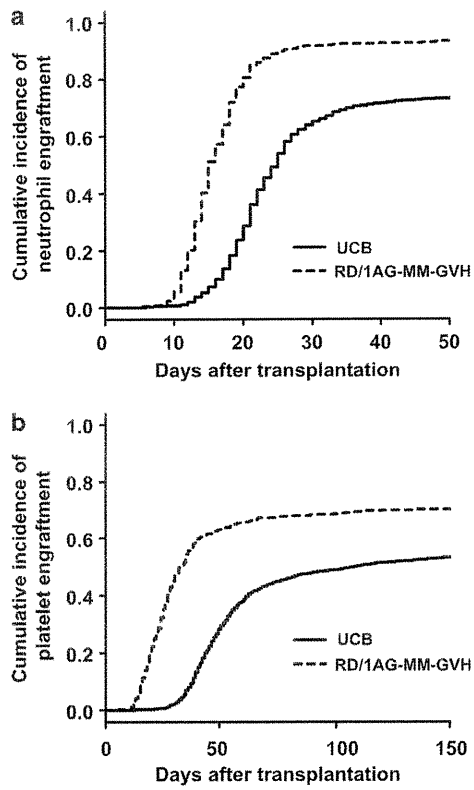

Figure 1. Neutrophil (**a**) and platelet engraftment (**b**).

Acute and chronic GVHD

The incidence of grade II–IV or grade III–IV acute GVHD in the RD/1AG-MM-GVH group was significantly higher than that in the UCB group (grade II–IV acute GVHD at day 100: UCB group, 34%, 95% CI, 32–36%; RD/1AG-MM-GVH group, 50%, 95% CI, 45–54%; Gray test, $P<0.001$; grade III–IV acute GVHD at day 100: UCB group, 11%, 95% CI, 10–13%; RD/1AG-MM-GVH group, 21%, 95% CI, 17–24%; Gray test, $P<0.001$; Figures 2a and b). The incidence of chronic GVHD or extensive type of chronic GVHD in the RD/1AG-MM-GVH group was also significantly higher than that in the UCB group (chronic GVHD at 3 years: UCB group, 25%, 95% CI, 23–27%; RD/1AG-MM-GVH group, 42%, 95% CI, 38–47%; Gray test, $P<0.001$; extensive chronic GVHD at 3 years: UCB group, 11%, 95% CI, 10–13%; RD/1AG-MM-GVH group, 29%, 95% CI, 25–34%; Gray test, $P<0.001$; Figures 2c and d). A multivariate analysis confirmed a higher risk of grade II–IV or grade III–IV acute GHVD, chronic or extensive chronic GVHD in the RD/1AG-MM-GVH group than in the UCB group (grade II–IV acute GVHD; HR 1.64, 95% CI, 1.43–1.90, grade III–IV acute GVHD; HR 2.28, 95% CI, 1.80–2.88, chronic GVHD; HR 1.47, 95% CI, 1.24–1.73, extensive chronic GVHD; HR 2.35, 95% CI, 1.90–2.91, Supplementary Table 2).

OS

The 3-year unadjusted OS rates in the UCB and RD/1AG-MM-GVH groups were 38% (36–41%) and 39% (34–43%), respectively ($P=0.115$). The use of either UCB or RD/1AG-MM-GVH was not associated with OS rates in the multivariate analysis (UCB vs RD/1AG-MM-GVH, HR, 0.99, 95% CI, 0.87–1.12, $P=0.833$) in all-risk patients, or either standard-risk ($P=0.588$) or high-risk patients ($P=0.639$; Table 2), after adjusting for the following significant risk factors: age >50 years, male recipient, acute myeloid leukemia vs MDS, high-risk disease, GVHD prophylaxis using only calcineurin inhibitor vs calcineurin inhibitor + methotrexate, and earlier year

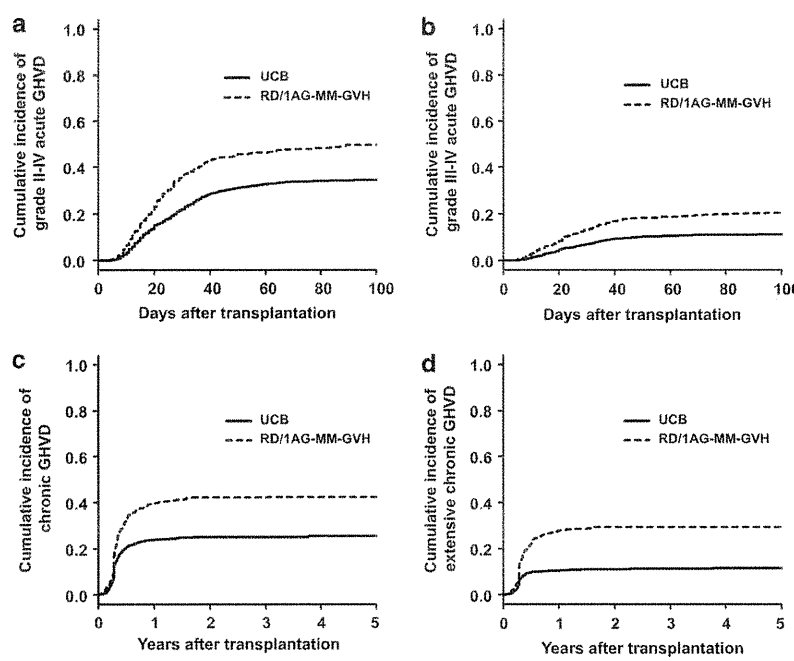

Figure 2. Acute and chronic GVHD. Cumulative incidences of grade II–IV (**a**) and grade III–IV acute GVHD (**b**) and chronic (**c**) and extensive chronic GVHD (**d**) are shown.

Table 2. Multivariate analysis of overall mortality

Variable	*Total*[a]		*Standard risk*[b]		*High risk*[c]	
	HR (95% CI)	P *value*	*HR (95% CI)*	P *value*	*HR (95% CI)*	P *value*
(A)						
UCB	1.00	reference	1.00	reference	1.00	reference
RD/1AG-MM-GVH	0.99 (0.87–1.12)	0.833	1.06 (0.86–1.31)	0.588	0.96 (0.81–1.13)	0.639
(B)						
UCB	1.00	reference	1.00	reference	1.00	reference
RD/HLA-A-MM-GVH	0.92 (0.72–1.18)	0.519	0.99 (0.66–1.48)	0.959	0.90 (0.64–1.26)	0.551
RD/HLA-B-MM-GVH	1.20 (1.01–1.44)	0.043	1.44 (1.05–1.96)	0.023	1.12 (0.89–1.41)	0.326
RD/HLA-DR-MM-GVH	0.85 (0.70–1.02)	0.084	0.88 (0.66–1.19)	0.411	0.84 (0.65–1.08)	0.170

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CI, confidence interval; CML, chronic myelogenous leukemia; CSA, cyclosporine; HR, hazard ratio; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus. [a]Other significant variables in model A were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.50, 95% CI, 1.35–1.66, $P<0.001$); sex of recipient, female (reference, 1.00), male (HR, 1.12; 95% CI, 1.02–1.24; $P=0.023$); diagnosis, AML (reference, 1.00),ALL (HR, 1.11, 95% CI, 0.98–1.26, $P=0.112$), CML (HR, 0.90, 95% CI, 0.72–1.13, $P=0.374$), MDS (HR, 0.81, 95% CI, 0.68–0.95, $P=0.001$); disease risk, standard risk (reference, 1.00), high risk (HR, 2.24; 95% CI, 2.00–2.50; $P<0.001$), status not known, (HR, 1.59; 95% CI, 1.21–2.09; $P=0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00),CSA/TAC only (HR, 1.23; 95% CI, 1.09–1.39; $P=0.001$), CSA/TAC + steroid/MMF (HR, 1.02; 95% CI, 0.86–1.21; $P=0.820$), other/missing (HR, 1.21; 95% CI, 0.82–1.78; $P=0.342$); year of transplantation, 1998–2004 (reference, 1.00), 2005–2009 (HR, 0.89; 95% CI, 0.80–0.99; $P=0.038$). [b]Other significant variables in model A were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.72, 95% CI, 1.42–2.07, $P<0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 1.43; 95% CI, 1.14–1.78; $P=0.002$), CSA/TAC + steroid/MMF (HR, 1.00; 95% CI, 0.73–1.37; $P=0.995$), other/missing (HR, 1.51; 95% CI, 0.67–3.39; $P=0.319$). [c]Other significant variables were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.41, 95% CI, 1.23–1.61, $P<0.001$); diagnosis, AML (reference, 1.00), ALL (HR, 1.13, 95% CI, 0.95–1.34, $P=0.183$), CML (HR, 0.94, 95% CI, 0.70–1.27, $P=0.704$), MDS (HR, 0.73, 95% CI, 0.60–0.89, $P=0.002$).

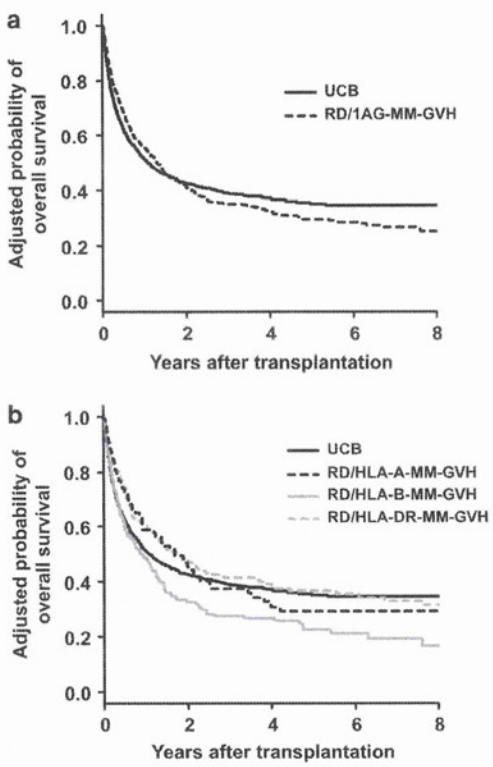

Figure 3. Overall survival. Overall survival rates in the transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (**b**) are shown.

of transplantation (1998–2004). Figure 3a shows the adjusted survival curves of the two groups. Next, the HLA-A, HLA-B and HLA-DR mismatched groups in transplantation from an RD/1AG-MM-GVH were compared with the UCB group. The OS rate of patients who received transplantation from an RD/1AG-MM-GVH involving an HLA-B mismatch was significantly lower than that in the UCB group ($P=0.043$; Figure 3b and Table 2), and a subgroup analysis revealed that the adverse effect of an HLA-B mismatch was significant only in standard-risk patients (standard-risk, $P=0.023$; high-risk, $P=0.326$; Table 2).

Relapse and NRM

The 3-year relapse rates in the UCB and RD/1AG-MM-GVH groups were 35% (95%CI, 33–37%) and 32% (95% CI, 28–36%), respectively (Gray test; $P=0.041$; Figure 4a), and a significant decrease in the incidence of relapse was found in the RD/1AG-MM-GVH group in the multivariate analysis (RD/1AG-MM-GVH vs UCB, HR, 0.78, 95%CI, 0.64–0.95, $P=0.012$; Table 3). The impact of reducing the incidence of relapse did not differ according to the HLA mismatch antigen in the RD/1AG-MM-GVH group (Table 3 and Figure 4b). The 3-year NRM rates in the UCB and RD/1AG-MM-GVH groups were 30% (95% CI, 28–32%) and 32% (95% CI, 28–36%), respectively (Gray test; $P=0.474$; Figure 4c), and a significant increase in the NRM rate was observed in the RD/1AG-MM-GVH group in the multivariate analysis (RD/1AG-MM-GVH vs UCB, HR, 1.24, 95% CI, 1.04–1.47, $P=0.016$; Table 3). In particular, the NRM rate of patients who received transplantation from an RD/1AG-MM-GVH with an HLA-B mismatch was significantly higher than that in the UCB group (RD/1AG-MM-GVH vs UCB, HR, 1.50, 95% CI, 1.17–1.92, $P=0.001$; Figure 4d and Table 3).

The causes of death in patients who died without relapse are shown in Supplementary Table 3. The rates of GVHD and organ failure in the RD/1AG-MM-GVH group were higher than those in the UCB group (GVHD, 18 vs 10%, organ failure, 28 vs 19%), whereas the rates of graft failure and infection were lower in the RD/1AG-MM-GVH group (graft failure, 1 vs 5%; infection, 26 vs 38%).

The impact of the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group

Based on the fact that the leading causes of death in the RD/1AG-MM-GVH group were GVHD and organ failure, we analyzed the risk factors for the development of acute GVHD in this group.

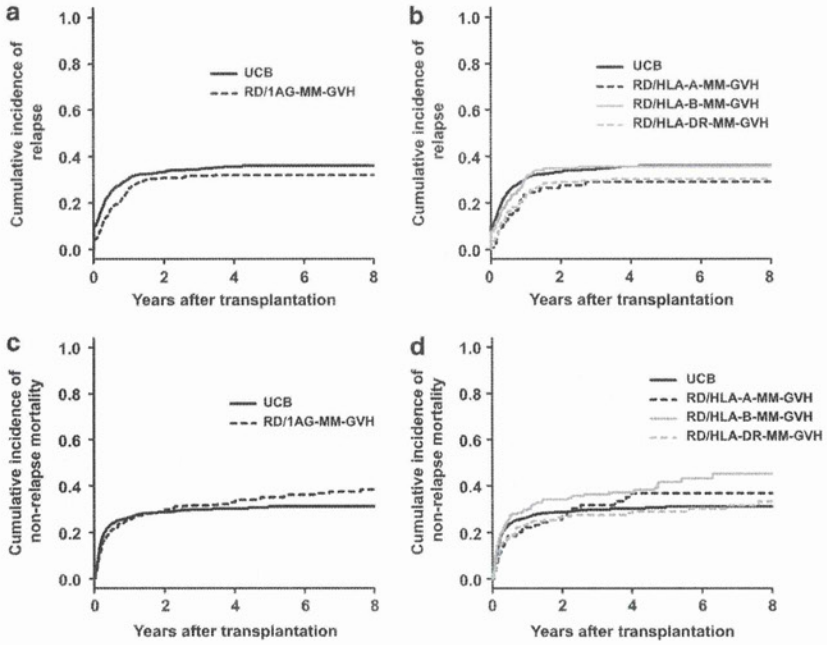

Figure 4. Relapse and non-relapse mortality. Cumulative incidence of relapse and non-relapse mortality after transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**, **c**) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (**b**, **d**) are shown.

Table 3. Multivariate analysis of relapse and non-relapse mortality

Variable	*Relapse*[a]		*Non-relapse mortality*[b]	
	HR (95% CI)	P *value*	*HR (95% CI)*	P *value*
(A)				
UCB	1.00	reference	1.00	reference
RD/1AG-MM-GVH	0.78 (0.64–0.95)	0.012	1.24 (1.04–1.47)	0.016
(B)				
UCB	1.00	reference	1.00	reference
RD/HLA-A-MM-GVH	0.70 (0.49–1.00)	0.050	1.28 (0.93–1.76)	0.130
RD/HLA-B-MM-GVH	0.81 (0.62–1.07)	0.134	1.50 (1.17–1.92)	0.001
RD/HLA-DR-MM-GVH	0.80 (0.61–1.04)	0.096	1.02 (0.78–1.32)	0.901

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CI, confidence interval; CML, chronic myelogenous leukemia; CSA, cyclosporine; HR, hazard ratio; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus. [a]Other significant variables in model A were; diagnosis, AML (reference, 1.00), ALL (HR, 1.09, 95% CI, 0.92–1.29, $P=0.336$), CML (HR, 1.39, 95% CI, 1.05–1.82, $P=0.019$), MDS (HR, 0.59, 95% CI, 0.46–0.76, $P<0.001$); time from diagnosis to transplantation, <6 months (reference, 1.00), ⩾6 months (HR, 0.80; 95% CI, 0.70–0.92; $P=0.002$); disease risk, standard risk (reference, 1.00), high risk (HR, 2.81; 95% CI, 2.41–3.27; $P<0.001$), status not known, (HR, 2.17; 95% CI, 1.45–3.23; $P<0.001$); conditioning intensity, myeloablative (reference, 1.00), reduced intensity (HR, 1.22; 95% CI, 1.04–1.44; $P=0.014$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 0.65; 95% CI, 0.53–0.78; $P<0.001$), CSA/TAC + steroid/MMF (HR, 0.75; 95% CI, 0.59–0.96; $P=0.024$), other/missing (HR, 0.94; 95% CI, 0.55–1.61; $P=0.825$). [b]Other significant variables in model A were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.70, 95% CI, 1.47–1.98, $P<0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00),CSA/TAC only (HR, 1.70; 95% CI, 1.44–2.01; $P<0.001$), CSA/TAC + steroid/MMF (HR, 1.18; 95% CI, 0.94–1.49; $P=0.158$), other/missing (HR, 1.47; 95% CI, 0.86–2.51; $P=0.154$); year of transplantation, 1998–2004 (reference, 1.00), 2005–2009 (HR, 0.76; 95% CI, 0.66–0.88; $P<0.001$).

In multivariate analysis, two factors were found to be significantly associated with the risk of developing grade II–IV acute GVHD in the RD/1AG-MM-GVH group: the use of *in vivo* T-cell depletion and source of stem cells (use of *in vivo* T-cell depletion, yes vs no, HR 0.40, $P=0.002$, PB vs BM, HR 1.61, $P<0.001$).

Because the use of *in vivo* T-cell depletion significantly lowered the risk of acute GVHD, we re-compared the RD/1AG-MM-GVH group and the UCB group while focusing on the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group. The incidence of grade II–IV or grade III–IV acute GVHD or chronic or extensive chronic GVHD in the RD/1AG-MM-GVH group using *in vivo* T-cell depletion was comparable to that in the UCB group (Supplementary Figure 1 and Supplementary Table 4), whereas the incidences of neutrophil and platelet engraftment were significantly higher in the RD/1AG-MM-GVH group using *in vivo* T-cell depletion than in the UCB group (neutrophil engraftment, HR, 5.52, 95% CI, 3.36–9.05, $P<0.001$; platelet engraftment, HR 2.01, 95% CI, 1.26–3.21, $P<0.001$). Compared to the UCB group, the RD/1AG-MM-GVH group with T-cell depletion showed lower overall and NRM, albeit these differences were not significant, which suggests that the use of *in vivo* T-cell depletion may improve the outcome of transplantation from an RD/1AG-MM-GVH (Figure 5, Supplementary Table 5). It is interesting to note that the adverse impact of an HLA-B mismatch vs HLA-A or -DR

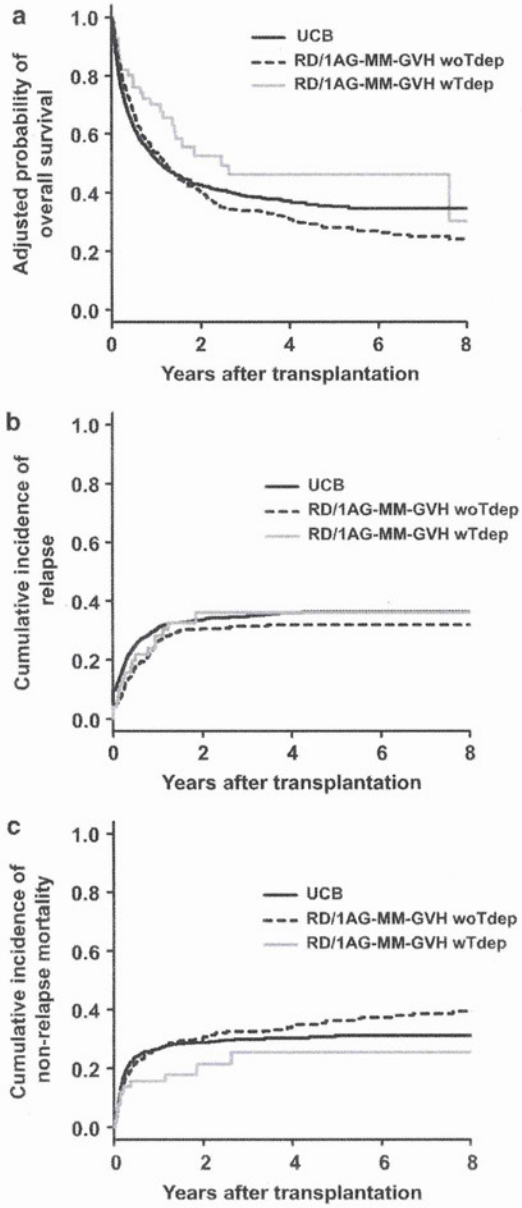

Figure 5. OS (**a**), relapse (**b**) and NRM (**c**) according to the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group.

mismatch in the RD/1AG-MM-GVH group disappeared with the use of *in vivo* T-cell depletion (with *in vivo* T-cell depletion; HLA-B vs HLA-A/DR mismatch; HR 1.08, 95% CI, 0.45–2.62, $P=0.864$, without *in vivo* T-cell depletion; HLA-B vs HLA-A/DR mismatch; HR 1.59, 95% CI, 1.25–2.01, $P<0.001$).

With regard to the effect of stem cell source, the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group using BM was lower than that with PB but higher than that with UCB (Supplementary Figure 2). The use of PB or BM did not affect OS, relapse, or NRM (Supplementary Table 5).

DISCUSSION

In this nationwide retrospective study, we found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group regardless of the disease risk. The RD/1AG-MM-GVH group with an HLA-B mismatch showed significantly higher overall and NRM, whereas the RD/1AG-MM-GVH group with an HLA-A or HLA-DR mismatch showed an OS comparable to that in the UCB group. Neutrophil and platelet engraftment in the RD/1AG-MM-GVH group were significantly faster than those in the UCB group, whereas the incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group was significantly higher. However, the incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a better, but not significantly better, OS than that in the UCB group.

In Japan, unrelated BM donor coordination (from donor search to transplantation) takes a median of 4 months, whereas much less time is required for UCB or RD/1AG-MM-GVH transplantation if there is a candidate. This was reflected in the longer duration from diagnosis to transplantation in unrelated BM transplantation.[32] In contrast, UCB and RD/1AG-MM-GVH transplantation show a similar and shorter duration (Table 1 ; 7.9 months vs 7.6 months). Therefore, in cases where both UCB and RD/1AG-MM-GVH are available, donors should be chosen based on their advantages and disadvantages. Compared with UCB, the use of RD/1AG-MM-GVH has a great advantage in neutrophil and platelet engraftment, which is not inconsistent with a previous finding that engraftment in the UCB group was significantly delayed comparing with that in MUD.[33] This translated into a lower rate of death from graft failure or infection in the RD/1AG-MM-GVH group. However, these advantages were offset by a substantial increase in the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group. The risk of grade III–IV acute GVHD and extensive chronic GVHD in the RD/1AG-MM-GVH group was twice that in the UCB group. If UCB units containing adequate total nucleated cell doses (ex. $>2.5\times10^7$/kg) are available,[34] the selection of UCB would be appropriate to avoid the risk of chronic GVHD. In contrast, RD/1AG-MM-GVH would be more appropriate when early neutrophil engraftment should be prioritized, such as for a patient with an active infectious disease at transplantation.

The high incidences of GVHD and GVHD-related death in the RD/1AG-MM-GVH group indicate the need for stronger immunosuppression to improve the clinical outcome. The use of T-cell depletion, mostly by ATG, was significantly associated with a lower incidence of grade III–IV acute GVHD and extensive chronic GVHD in the RD/1AG-MM-GVH group. Although this effect was not statistically significant, the RD/1AG-MM-GVH group with *in vivo* T-cell depletion showed lower overall and treatment-related mortality, which would outweigh a possible increased risk of relapse. These findings in our cohort suggest that ATG may be effective, and the addition of ATG in the RD/1AG-MM-GVH group should be assessed in a prospective study.

As shown in our previous study,[23] overall mortality in the RD/1AG-MM-GVH group involving an HLA-B mismatch was significantly higher than that in the RD/1AG-MM-GVH group with an HLA-A or -DR mismatch, probably because of an additional HLA-C antigen mismatch as expected from linkage disequilibrium between HLA-B and HLA-C and available data on HLA-C antigen.[23,35] The incidence of grade III–IV acute GVHD in the HLA-B mismatch group was higher than that in the HLA-DR mismatch group, but was comparable to that in the HLA-A mismatch group. In addition, the incidence of death from GVHD was similar in the HLA-B and HLA-A/DR mismatch groups (data not shown). Therefore, the reason for the lower overall morality in the RD/1AG-MM-GVH group with an HLA-B mismatch remains unclear. However, the adverse effect of an HLA-B mismatch disappeared when *in vivo* T-cell depletion was used, which suggests that an immunological effect is involved in this mechanism.

This study has several limitations. First, in clinical practice in Japan, matching of HLA-DR is counted at a low resolution, as with HLA-A and HLA-B, whereas it is counted at a high resolution in the

United States and Europe. To evaluate the impact of this difference, we divided patients in the UCB group with two antigen mismatches into two groups by using available HLA-DRB1 allele information: a group with two antigen mismatches with one additional HLA-DRB1 allele mismatch ($n = 609$) and another group with two antigen mismatches without an additional HLA-DRB1 mismatch ($n = 295$). We did not find a significant difference in OS between these two groups ($P = 0.758$), which suggests that HLA-matching using HLA-DR antigen or allele information will not affect OS in the present study. Second, the findings in the present study are based on Asian cohort who received a 'single' UCB or RD/1AG-MM-GVH transplantation. Lighter body weight in Asian population than Caucasian population may make it easy to find a suitable single UCB unit that contains adequate total nucleated cell doses. In addition, as suggested by Oh *et al.*,[36] limited heterogeneity of Japanese population may affect the outcomes of transplantation. Therefore, the findings should be externally validated in the non-Asian cohort or transplantation using double UCB units. Third, information on the dose and type of ATG was missing in two-third of the patients who received ATG. However, the available data showed that the median dose of thymoglobulin (2.5 mg/kg) or ATG-F (8 mg/kg) was equivalent to the dose that is widely used in our daily practice. Lastly, heterogeneous backgrounds may have resulted in a bias, although we tried to adjust for possible confounders by multivariate analyses. Lastly, the effect of multiple testing should be taken into account for the interpretation of secondary end points.

In conclusion, our findings suggest that both UCB and RD/1AG-MM-GVH are suitable as alternative donors for patients without an HLA-matched sibling or unrelated donor. However, the presence of an HLA-B-antigen mismatch in the GVH direction has an adverse effect on OS because of treatment-related complications. Neutrophil and platelet engraftment in the RD/1AG-MM-GVH group were significantly faster than those in the UCB group, whereas the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group was significantly higher, which translated into a high incidence of death from GVHD. Donor selection between UCB and RD/1AG-MM-GVH should be determined based on the presence of an HLA-B mismatch in RD/1AG-MM-GVH and from the risks and benefits derived from the risk of graft failure and infection in the UCB group and acute or chronic GVHD in the RD/1AG-MM-GVH group. Additional immune suppression using *in vivo* T-cell depletion may improve the clinical outcome in the RD/1AG-MM-GVH group by decreasing the incidences of GVHD and NRM and may also overcome the adverse effect of an HLA-B mismatch. This approach should be assessed in a prospective study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

JK and YK designed the research, organized the project and wrote the manuscript; JK, YA, and YK performed the statistical analysis and analyzed the data; KK and TN-I collected data from JCBBN; and all of the authors interpreted the data and reviewed and approved the final manuscript.

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Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study

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Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study

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Allogeneic hematopoietic cell transplantation (HCT) is an effective treatment for adult T-cell leukemia (ATL), raising the question about the role of graft-versus-leukemia effect against ATL. In this study, we retrospectively analyzed the effects of acute and chronic graft-versus-host disease (GVHD) on overall survival, disease-associated mortality, and treatment-related mortality among 294 ATL patients who received allogeneic HCT and survived at least 30 days posttransplant with sustained engraftment. Multivariate analyses treating the occurrence of GVHD as a time-varying covariate demonstrated that the development of grade 1-2 acute GVHD was significantly associated with higher overall survival (hazard ratio [HR] for death, 0.65; $P = .018$) compared with the absence of acute GVHD. Occurrence of either grade 1-2 or grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD, whereas grade 3-4 acute GVHD was associated with a higher risk for treatment-related mortality (HR, 3.50; $P < .001$). The development of extensive chronic GVHD was associated with higher treatment-related mortality (HR, 2.75; $P = .006$) compared with the absence of chronic GVHD. Collectively, these results indicate that the development of mild-to-moderate acute GVHD confers a lower risk of disease progression and a beneficial influence on survival of allografted patients with ATL. (*Blood*. 2012;119(9):2141-2148)

Introduction

Adult T-cell leukemia (ATL) is a mature T-cell neoplasm that is causally associated with a retrovirus designated human T-cell leukemia virus type I (HTLV-I).[1-4] HTLV-I is endemic in southwestern Japan, sub-Saharan Africa, the Caribbean Basin, and South America.[3,4] In Japan, more than 1 million people were estimated to be infected with HTLV-I. Although the majority of HTLV-I–infected individuals remain asymptomatic throughout their lives, ~ 5% develop ATL at a median age of 40 to 60 years.[4,5]

ATL is categorized into 4 clinical variants according to its clinical features: smoldering, chronic, acute, and lymphoma types.[6] The acute and lymphoma variants of ATL have an extremely poor prognosis, mainly because of resistance to a variety of cytotoxic agents and susceptibility to opportunistic infections; the median survival time is ~ 13 months with conventional chemotherapy,[7,8] although encouraging results have been recently reported with the use of novel agents such as mogamulizumab.[9-11]

Over the past decade, allogeneic hematopoietic cell transplantation (HCT) has been increasingly performed with the aim of improving dismal prognosis of patients who developed ATL.[12-18] Notably, some patients with ATL who relapsed after allogeneic HCT were shown to achieve remission only with the cessation of immunosuppressive agents, raising the question of whether the graft-versus-leukemia effect against ATL can be induced as part of graft-versus-host reaction.[19,20] In 1 study, among 10 patients who experienced relapse of ATL after transplantation and were withdrawn from immunosuppressive therapy, 8 developed graft-versus-host disease (GVHD), and 6 of them subsequently achieved

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complete remission of ATL.[19] Similar observations have been rarely reported in other aggressive mature lymphoid neoplasms,[21] suggesting the unique susceptibility of ATL to graft-versus-host reactions. Recently, a combined analysis of 2 prospective studies including 29 ATL patients in total undergoing allogeneic HCT suggested that development of mild acute GVHD favorably affected overall survival and progression-free survival.[22] However, the impact of GVHD on the outcome of allogeneic HCT in ATL needs to be verified in a much larger cohort. We previously conducted a nationwide retrospective study to evaluate the current results of allogeneic HCT for ATL, and we confirmed that a substantial proportion of patients with ATL can enjoy long-term, disease-free survival after transplantation: the overall survival rate at 3 years among patients who received transplants in complete remission and not in complete remission was 51% and 26%, respectively.[23] Using the same cohort, we further evaluated the effects of acute and chronic GVHD on long-term outcomes of allografted patients with ATL.

Methods

Collection of data

Data on 417 patients with acute or lymphoma type ATL who had undergone allogeneic bone marrow, peripheral blood, or cord blood transplantation between January 1, 1996, and December 31, 2005, were collected through the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP), and the Japan Cord Blood Bank Network (JCBBN), the 3 largest HCT registries in our country; their roles were detailed previously.[23] The patients were included from 102 transplant centers; the data were updated as of December 2008. The study was approved by the data management committees of JSHCT, JMDP, and JCBBN, as well as by the institutional review boards of Kyoto University Graduate School of Medicine, where this study was organized.

Inclusion and exclusion criteria

Patients were included in the analysis if the following data were available: age at transplantation, sex of the recipient, donor type, stem cell source, agents used in the conditioning regimen and GVHD prophylaxis, the maximum grade and day of occurrence of acute GVHD, and the day of neutrophil recovery. Acute GVHD was reported according to the traditional criteria,[24] except that 1 patient was considered to have late-onset acute GVHD at day 133; neutrophil recovery was considered to have occurred when an absolute neutrophil count exceeded 0.5×10^9/L for 3 consecutive days after transplantation. Patients who missed any of these data (n = 37), who had a history of prior autologous or allogeneic HCT (n = 8), who had received an ex vivo T cell–depleted graft (n = 1), who experienced primary or secondary graft failure (n = 24) were excluded from the analysis. Because the association between the occurrence of acute GVHD and disease-associated mortality was difficult to evaluate in the event of early toxic death, patients who died within 30 days of transplantation (n = 53) also were excluded from the study. Among these 53 patients, 22 were evaluable for acute GVHD: grade 0 in 17 patients, grade 1-2 in 3 patients, and grade 3-4 in 2 patients. Two physicians (J.K. and T.I.) independently reviewed the quality of collected data, and 294 patients in total (158 males and 136 females), with a median age of 51 years (range, 18-79 years), were found to meet these criteria and included in the study: 163 patients from JSHCT, 82 patients from JMDP, and 49 patients from JCBBN. No overlapping cases were identified. Of these 294 patients, the effects of chronic GVHD, reported and graded according to using traditional criteria,[25] were considered evaluable for the 183 patients who survived at least 100 days after transplantation with complete information on the type and the day of occurrence of chronic GVHD.

End points

The primary end point of the study was the effect of acute GVHD on overall survival, defined as the period from the date of transplantation until the date of death from any cause or the last follow-up. The secondary end points of the study included the impact of acute GVHD on disease-associated and treatment-related mortality, and the impact of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality. Reported causes of death were reviewed and categorized into disease-associated or treatment-associated deaths. Disease-associated deaths were defined as deaths from relapse or progression of ATL, whereas treatment-related deaths were defined as any death other than disease-associated deaths.

Statistical analysis

The probability of overall survival was estimated by the Kaplan-Meier method. Treatment-related and disease-associated mortality were estimated with the use of cumulative incidence curves to accommodate the following competing events[26]: disease-associated death for treatment-related mortality and treatment-related deaths for disease-associated mortality. Data on patients who were alive at the time of last follow-up were censored. Semi-landmark plots were used to illustrate the effects of GVHD on overall survival and cumulative incidence of disease-associated and treatment-related deaths. For patients with acute or chronic GVHD, the probability of overall survival and the cumulative incidences of disease-associated and treatment-related deaths were plotted as a function of time from the onset of acute or chronic GVHD. Day 24.5, the median day of onset for acute GVHD, was termed as the landmark day in patients without acute GVHD. In the case of patients without chronic GVHD, day 116, the median day of onset for chronic GVHD, was termed as the landmark day.

Univariate and multivariate Cox proportional hazards regression models were used to evaluate variables potentially affecting overall survival, whereas the Fine and Gray proportional subdistribution hazards models were used to evaluate variables potentially affecting disease-associated and treatment-related mortality.[27] In these regression models, the occurrence of acute and chronic GVHD was treated as a time-varying covariate.[28] In the analysis of acute GVHD, patients were assigned to the "no acute GVHD group" at the time of transplantation and then transferred to the "grade 1-2 acute GVHD group" or to the "grade 3-4 acute GVHD group" at the onset of the maximum grade of acute GVHD. In the analysis of chronic GVHD, patients were assigned to the "no chronic GVHD group" at the time of transplantation and then transferred to the "limited chronic GVHD group" or to the "extensive chronic GVHD group" at the onset of the maximum grade of chronic GVHD. The variables considered were the age group of the recipient ($\leq$ 50 years or $>$ 50 years at transplantation), sex of the recipient (female or male), disease status before transplantation (complete remission, disease status other than complete remission, or unknown), intensity of conditioning regimen (myeloablative, reduced intensity, or unclassifiable), type of GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other), type of donor (HLA-matched related donor, HLA-mismatched related donor, unrelated donor for bone marrow, or unrelated cord blood), time from diagnosis to transplantation (within 6 months, $>$ 6 months, or unknown), and year of transplantation (1995-2002 or 2003-2005). We classified the intensity of conditioning regimen as myeloablative or reduced intensity based on the working definition by Center for International Blood and Marrow Transplant Research if data on dosage of agents and total-body irradiation (TBI) used in the conditioning regimen were available.[29] For 110 patients for whom such information was not fully available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by treating clinicians. The cutoff points for year of transplantation were chosen such that we could make optimal use of the data with a proviso that the smaller group contained at least 30% of patients. In the analysis of the effect of chronic GVHD, the prior history of grade 2-4 acute GVHD also was added to the multivariate models. We also assessed the interaction between acute GVHD and the intensity of conditioning regimen in the multivariate models. Only factors with a P value of less than .10 in univariate analysis were included in the multivariate models. In addition, the heterogeneities of the effects of grade 1-2 or grade 3-4 acute GVHD on overall survival according to background transplant characteristics were evaluated by the forest plots stratified by variables included in the regression analyses. Furthermore, landmark analysis treating the development of acute GVHD as a time-fixed covariate was performed to confirm

Table 1. Characteristics of patients and transplants

Variable	No. of patients, n = 294 (%)
Age group at transplant, y	
≤ 30	7 (2)
> 30-40	30 (10)
> 40-50	109 (37)
> 50-60	123 (42)
> 60	25 (9)
Sex	
Male	158 (54)
Female	136 (46)
Disease status	
Complete remission	99 (34)
Not in complete remission	178 (61)
Unknown	17 (6)
Conditioning regimen	
Myeloablative	102 (34)
Reduced intensity	128 (44)
Unclassifiable	64 (22)
GVHD prophylaxis*	
Cyclosporine-based	195 (66)
Tacrolimus-based	94 (32)
Other	5 (2)
Source of stem cells	
Bone marrow	132 (45)
Peripheral blood	111 (38)
Bone marrow + peripheral blood	2 (1)
Cord blood	49 (17)
Type of donor†	
HLA-matched related	132 (45)
HLA-mismatched related	31 (11)
Unrelated, bone marrow	82 (28)
Unrelated, cord blood	49 (17)
Time from diagnosis to transplant	
≤ 6 mo	141 (48)
> 6 mo	141 (48)
Uncertain/missing	12 (4)
Year of transplant	
1995-1999	22 (7)
2000-2002	91 (31)
2003-2005	181 (62)
Follow-up of survivors	
Median time, mo (range)	42.8 (1.5-102.3)

Data are numbers (%) unless specified otherwise.

*Cyclosporine-based indicates cyclosporine with or without other agents; tacrolimus-based indicates tacrolimus with or without other agents.

†HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, B, and DR antigens.

the results of analyses treating the occurrence of acute GVHD as a time-varying covariate; the landmark day was set at day 68 after transplantation, the date until when more than 95% of patients developed acute GVHD.

Results are expressed as hazard ratios (HRs) and their 95% confidence intervals (CI). All tests were 2-sided, and a P value of less than .05 was considered to indicate statistical significance. All statistical analyses were performed with STATA Version 11 software (StataCorp).

Results

Characteristics of patients and transplants

Characteristics of the patients and transplants are shown in Table 1. Most of the patients received transplants at the age of 41 to 60 years (median, 51 years). The disease status at transplantation was mainly defined as other than complete remission. The intensity of conditioning regimen was classified as myeloablative in 102 (35%) patients and reduced intensity in 128 (44%) patients; the remaining 64 (22%) patients were reported to receive cyclophosphamide plus TBI in 16 patients; busulfan plus cyclophosphamide in 15 patients; busulfan plus melphalan in 1 patient; purine analog-containing regimen in 6 patients; and other TBI-based regimens in 26 patients, although the intensity of these regimens was considered unclassifiable because of lack of dosage information. Cyclosporine-based prophylaxis against GVHD was used in more than half of patients. Patients underwent transplantation using HLA-matched related donor in 132 patients (45%), HLA-mismatched related donor in 31 patients (11%), unrelated bone marrow donor in 82 patients (28%), and unrelated cord blood unit in 49 patients (17%). Half of the patients received transplants within 6 months of diagnosis. The median time of follow-up among the survivors was 42.8 months (range, 1.5-102.3 months).

Effects of acute GVHD on overall survival

The median onset day of acute GVHD of any grade after transplantation was 24.5 (range, 5-133). Acute GVHD of grades 1-4, 2-4, and 3-4 occurred in 202 patients (69%), 150 patients (51%), and 65 patients (22%), respectively. The effect of acute GVHD on overall survival was evaluated using semi-landmark plots with reference to the following 3 categories: no acute GVHD, grade 1-2 acute GVHD, and grade 3-4 acute GVHD (Figure 1A). The impact of grade 1-2 or grade 3-4 acute GVHD on overall survival also was evaluated by forest plots stratified by background characteristics of patients and transplants (Figure 2). These analyses revealed that development of grade 1-2 acute GVHD was consistently associated with higher overall survival compared with the absence of acute GVHD, whereas occurrence of grade 3-4 acute GVHD was consistently associated with lower overall survival, except that adverse impact of grade 3-4 acute GVHD was not observed in the subgroups of patients who received transplants from an HLA-matched related or HLA-mismatched related donor. Multivariate analysis treating an occurrence of acute GVHD as a time-dependent covariate also confirmed the positive impact of grade 1-2 acute GVHD (HR, 0.65; 95% CI, 0.45-0.93; $P = .018$) and the adverse impact of grade 3-4 acute GVHD on overall survival (HR, 1.64; 95% CI, 1.10-2.42; $P = .014$; Table 2). Patients who received reduced intensity conditioning and myeloablative conditioning had similar rates of overall survival by both univariate (HR of reduced intensity vs myeloablative transplant, 1.19; 95% CI, 0.85-1.68; $P = .318$) and multivariate analysis (HR, 0.95; 95% CI, 0.61-1.47; $P = .814$). There was no interaction effect between conditioning intensity and grade 1-2 ($P = .704$) or grade 3-4 acute GVHD ($P = .891$) on overall survival. The effect of each grade of acute GVHD on overall survival was additionally evaluated. It showed that only grade 2 acute GVHD was associated with superior overall survival, whereas only grade 4 acute GVHD was associated with inferior survival (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). In the landmark analysis treating an occurrence of acute GVHD as a time-fix covariate, consistent results were obtained for patients who survived at least 68 days (landmark day), although the adverse impact of grade 3-4 acute GVHD on overall survival became no longer significant (supplemental Table 2).

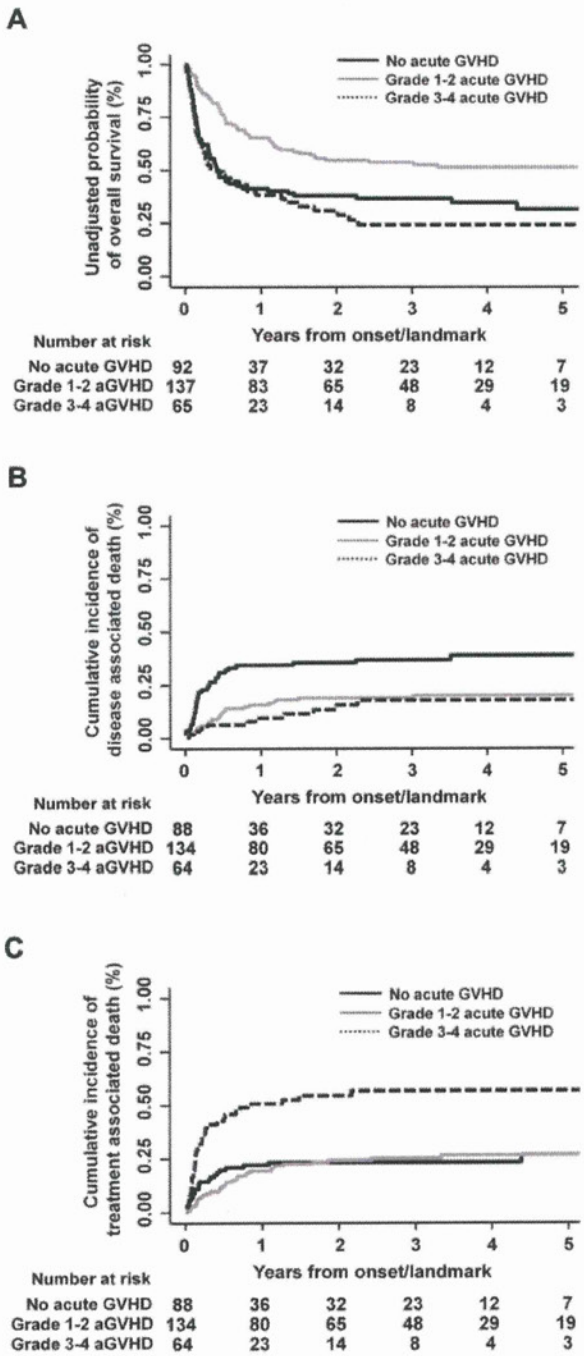

Figure 1. Semi-landmark plots for effects of acute GVHD. Semi-landmark plots illustrating the effects of acute GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

Effects of acute GVHD on disease-associated and treatment-related mortality

We next evaluated the effects of acute GVHD on disease-associated and treatment-related mortality (Figure 1B-C). Disease-associated mortality was defined as cumulative incidence of death directly attributable to relapse or progression of ATL, whereas treatment-related mortality was calculated as cumulative incidence of any death not included in disease-associated deaths. Multivariate analysis revealed that disease-associated mortality was lower in the presence of grade 1-2 and grade 3-4 acute GVHD compared with the absence of acute GVHD (grade 1-2 acute GVHD: HR, 0.54; 95% CI, 0.32-0.92; $P = .023$ and grade 3-4 acute GVHD: HR, 0.44; 95% CI, 0.22-0.90; $P = .024$; Table 2), and each grade of acute GVHD showed consistent inverse association with disease-associated mortality (supplemental Table 1). Although the risk of treatment-related mortality was not higher in the presence of grade 1-2 acute GVHD, development of grade 3-4 acute GVHD was significantly associated with higher treatment-related mortality compared with the absence of acute GVHD (HR, 3.50; 95% CI, 2.01-6.11; $P < .001$; Table 2). Patients undergoing reduced intensity transplantation and those undergoing myeloablative transplantation had similar risks of disease-associated death (HR, 0.99; 95% CI, 0.46-2.13; $P = .975$) and treatment-related death (HR, 0.98; 95% CI, 0.60-1.59; $P = .928$) by multivariate analysis. There was no interaction effect between conditioning intensity and grade 1-2 or grade 3-4 acute GVHD on disease-associated mortality and treatment-related mortality. Of 95 patients who experienced treatment-related deaths, 27 patients succumbed to infectious complications: bacterial in 13 patients, viral in 7 patients (including 3 cases of cytomegalovirus disease), viral and bacterial in 1 patient, fungal in 5 patients, and no specific organism reported in 1 patient. The proportions of patients who died of infectious complication among those without acute GVHD (n = 92), those with grade 1-2 (n = 137), and those with grade 3-4 acute GVHD (n = 65) were 4%, 9%, and 17%, respectively (supplemental Table 3). By multivariate analysis, development of grade 3-4 acute GVHD was significantly associated with higher risk of death related to infection (HR, 4.74; 95% CI, 1.51-14.8; $P = .008$), whereas the adverse influence on the infection-related deaths was less evident in the presence of grade 1-2 acute GVHD (HR, 2.17; 95% CI, 0.72-6.56; $P = .169$).

Effects of chronic GVHD on overall survival and mortality

Chronic GVHD was evaluated in 183 patients who survived at least 100 days after transplantation. The median day of chronic GVHD occurrence after transplantation was 116 (range, 100-146 days). Limited and extensive chronic GVHD occurred in 29 (16%) and 63 patients (34%), respectively. Semi-landmark plots were constructed to illustrate the effects of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality with reference to the following subgroups: no chronic GVHD, limited chronic GVHD, and extensive chronic GVHD (Figure 3). In multivariate analysis treating an occurrence of chronic GVHD as a time-dependent covariate, neither overall survival nor disease-associated mortality was significantly associated with severity of chronic GVHD, whereas treatment-related mortality was higher in the presence of extensive chronic GVHD (HR, 2.75; 95% CI, 1.34-5.63; $P = .006$) compared with the absence of chronic GVHD (Table 3). The proportions of patients who died of infectious complication among those without chronic GVHD (n = 91), those with limited chronic GVHD (n = 29), and those with extensive chronic GVHD (n = 63) were 7%, 10%, and 8%, respectively. In multivariate analysis, no statistically significant association was found between infection-related death and the occurrence of either limited ($P = .289$) or extensive GVHD ($P = .836$).

Discussion

To our knowledge, this is the largest retrospective study to analyze the impact of acute and chronic GVHD on clinical

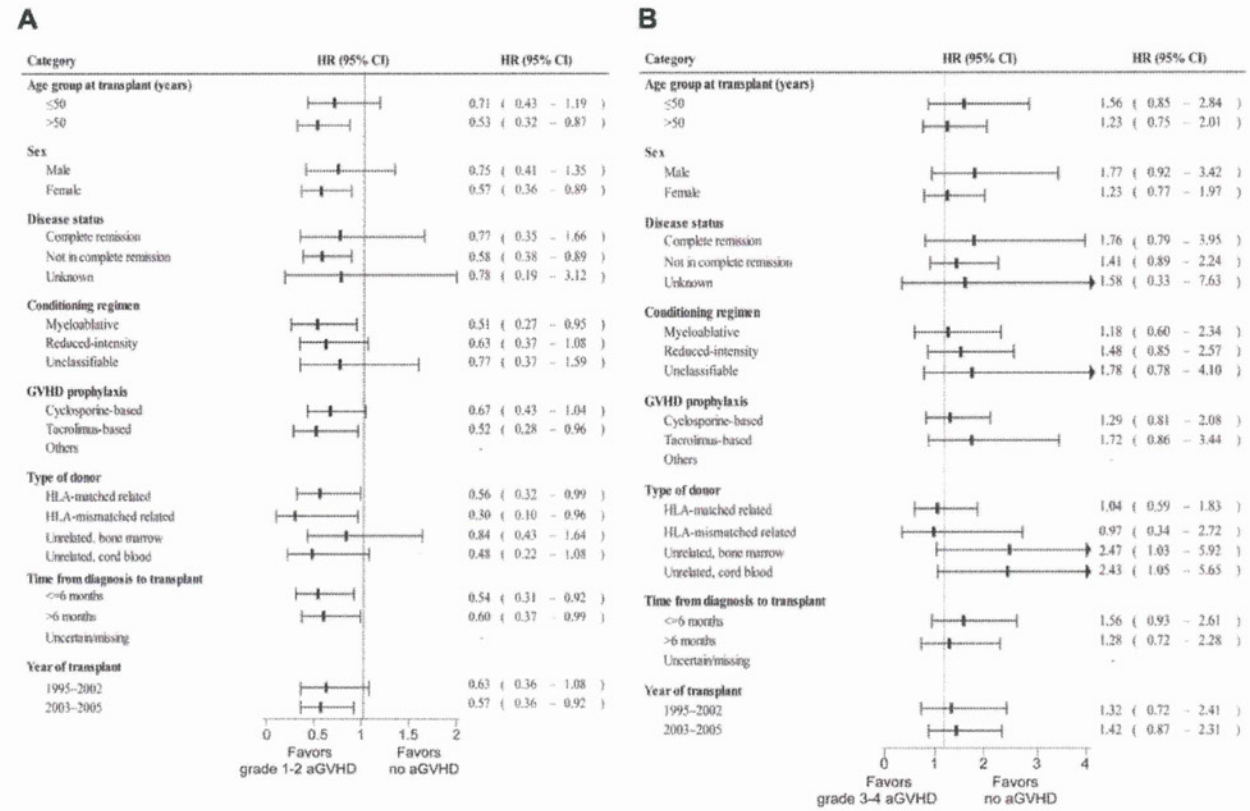

Figure 2. Impact of the grade of acute GVHD on overall survival in each stratified category. Effects of grade 1-2 (A) and grade 3-4 acute GVHD (B) on overall survival are shown as forest plots. Square boxes on lines indicate hazard ratios compared with "no acute GVHD group," and horizontal lines represent the corresponding 95% CI. Abbreviations used are the same as described in the footnotes to Tables 1 and 2.

outcomes including overall survival, disease-associated mortality, and treatment-related mortality after allogeneic HCT for ATL. In the present study, the occurrence of both grade 1-2 and grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD. However, positive effect of GVHD on reduced disease-associated mortality was counterbalanced by increased treatment-related mortality among patients who developed severe acute GVHD, and an overall beneficial effect on survival was observed only with the development of mild-to-moderate acute GVHD. In contrast to acute GVHD, no beneficial effect was observed in association with the development of chronic GVHD, although the point estimate of the HR comparing limited chronic GVHD versus the absence of chronic GVHD

Table 2. Effect of acute GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

	Univariate analysis		Multivariate analysis	
Outcome	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Overall survival*				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.60 (0.42-0.85)	.004	0.65 (0.45-0.93)	.018
Grade 3 or 4 acute GVHD vs no acute GVHD	1.38 (0.94-2.01)	.099	1.64 (1.10-2.42)	.014
Disease-associated mortality†				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.47 (0.28-0.79)	.005	0.54 (0.32-0.92)	.023
Grade 3 or 4 acute GVHD vs no acute GVHD	0.41 (0.21-0.81)	.010	0.44 (0.22-0.90)	.024
Treatment-related mortality‡				
Grade 1 or 2 acute GVHD vs no acute GVHD	1.13 (0.67-1.89)	.649	1.22 (0.72-2.07)	.461
Grade 3 or 4 acute GVHD vs no acute GVHD	3.34 (1.94-5.74)	< .001	3.50 (2.01-6.11)	< .001

*Other significant variables were sex of recipient, female (reference, 1.00) and male (HR, 1.70; 95% CI, 1.24-2.32; $P = .001$); achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.05; 95% CI, 1.44-2.92; $P < .001$), and status not known (HR, 2.21; 95% CI, 1.15-4.22; $P = .017$); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 1.71; 95% CI, 1.04-2.84; $P = .036$), unrelated donor of bone marrow (HR, 1.39; 95% CI, 0.94-2.06; $P = .096$), and unrelated cord blood (HR, 1.86; 95% CI, 1.22-2.83; $P = .004$).

†Other significant variables were achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.98; 95% CI, 1.62-5.47; $P < .001$), and status not known (HR, 0.96; 95% CI, 0.21-4.49; $P = .963$); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 2.14; 95% CI, 1.00-4.55; $P = .049$), unrelated donor of bone marrow (HR, 1.45; 95% CI, 0.81-2.61; $P = .214$), and unrelated cord blood (HR, 1.25; 95% CI, 0.63-2.49; $P = .517$).

‡Another significant variable was achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 1.17; 95% CI, 0.74-1.84; $P = .498$) and status not known (HR, 2.31; 95% CI, 1.04-5.15; $P = .040$).

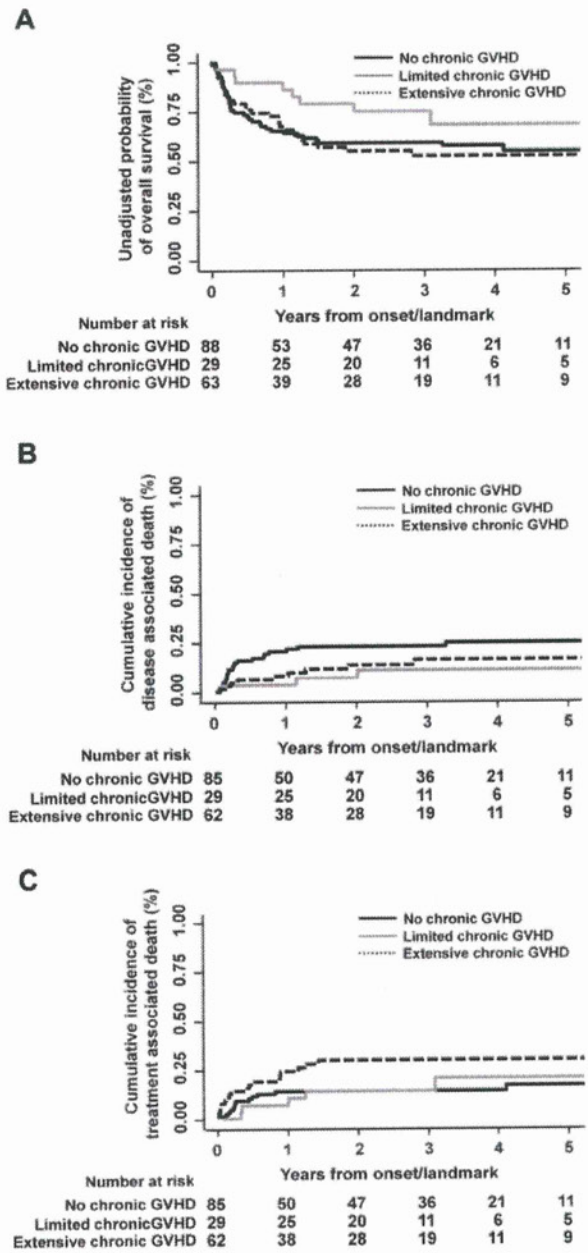

Figure 3. Semi-landmark plots for impact of chronic GVHD. Semi-landmark plots illustrating impact of chronic GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

suggested the trend toward a reduced risk of disease-associated deaths in the limited chronic GVHD group.

Our present findings are in contrast to the previous reports showing the beneficial effects of chronic GVHD rather than acute GVHD on the prevention of disease recurrence after allogeneic HCT. It is less likely that the particular characteristics of chronic GVHD in patients with ATL biased the results, because the incidence rate and median onset day of chronic GVHD in our cohort were similar to those reported in previous studies evaluating the incidence of chronic GVHD among Japanese patients, most of whom had received allogeneic HCT for myeloid neoplasms or acute lymphoblastic leukemia.[30-32] Conceivably, the rapid tempo of disease recurrence of ATL might be such that chronic GVHD is less potent in terms of harnessing clinically relevant graft-versus-leukemia responses compared with acute GVHD. However, the results of our analysis regarding the effect of chronic GVHD should be interpreted with caution because the number of patients evaluable for chronic GVHD was relatively small in our study for providing sufficient statistical power. The effect of chronic GVHD on outcomes after HCT for ATL should be further explored in a larger cohort.

The occurrence of GVHD has been shown to exert a potent graft-versus-leukemia effect in terms of reducing relapse incidence in acute leukemia or chronic myeloid leukemia.[33,34] In contrast, multiple studies have documented a correlation between GVHD in its acute or chronic form and treatment-related mortality. In a study of patients undergoing HLA-identical sibling HCT for chronic myeloid leukemia, the overall beneficial effect on long-term survival was demonstrated only in a group of patients who developed grade I acute GVHD or limited chronic GVHD.[33] In another study of HLA-identical sibling HCT for leukemia using cyclosporine and methotrexate as GVHD prophylaxis, a benefit of mild GVHD was only seen in high-risk patients but not in standard-risk patients. Therefore, the therapeutic window between decreased relapse incidence and increased transplant-related mortality in association with the development of GVHD has been considered to be very narrow.[34]

With regard to the effectiveness of allogeneic HCT for ATL, it is also of note here that posttransplant eradication of ATL cells can be achieved without the use of high-dose chemoradiotherapy: patients who received a transplant with reduced intensity conditioning had survival outcomes similar to those who received a transplant with myeloablative conditioning in our study. Intriguingly, several small cohort studies exhibited that abrupt discontinuation of immunosuppressive agents resulted in disappearance or reduction in the tumor burden in allografted patients with ATL. In some cases, remission of ATL was observed along with the development of GVHD.[19,20,22] Taken together with the findings of this study, it is suggested that ATL is particularly susceptible to immune modulation following allogeneic HCT. To clarify the presence of such "graft-versus-ATL" effect, further investigations are needed to assess the efficacy of donor lymphocyte infusion or withdrawal of immunosuppressive agents on relapse after transplantation.

Of the HTLV-I gene products, Tax is a dominant target of HTLV-I–specific cytotoxic T lymphocytes. The vigorous Tax-specific cytotoxic T-cell responses were demonstrated in recipients who obtained complete remission after allogeneic HCT for ATL, suggesting that "graft-versus-HTLV-I" responses might contribute to the eradication of ATL cells.[35,36] However, Tax is generally undetectable or present in very low levels in primary ATL cells.[37,38] In addition, small amounts of HTLV-I provirus can be detected in peripheral blood of recipients who attained long-term remission of ATL, even after HCT from HTLV-I–negative donors.[39,40] These findings suggest that "graft-versus-ATL" effect can be harnessed without complete elimination of HTLV-I. It is also important to note that allogeneic HCT is emerging as an effective treatment option for other mature T-cell neoplasms not related to HTLV-I, such as mycosis fungoides/Sézary syndrome and various types of aggressive peripheral T-cell lymphomas.[41,42] These observations raised the possibility that the common targets for alloimmune responses might exist across a spectrum of malignant T-cell neoplasms, including ATL. The minor histocompatibility antigens or tumor-specific antigens can be other targets of alloimmune anti-ATL effect.[43-45] Therefore, the elucidation of the mechanism underlying an immunologic eradication of primary ATL cells may

Table 3. Effect of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

	Univariate analysis		Multivariate analysis	
Outcome	HR (95% CI)	P	HR (95% CI)	P
Overall survival*				
Limited chronic GVHD vs no chronic GVHD	0.71 (0.34-1.47)	.353	0.72 (0.35-1.50)	.385
Extensive chronic GVHD vs no chronic GVHD	1.45 (0.90-2.35)	.131	1.40 (0.86-2.30)	.176
Disease-associated mortality†				
Limited chronic GVHD vs no chronic GVHD	0.45 (0.14-1.46)	.183	0.45 (0.14-1.44)	.178
Extensive chronic GVHD vs no chronic GVHD	0.81 (0.39-1.67)	.563	0.80 (0.39-1.64)	.536
Treatment-related mortality‡				
Limited chronic GVHD vs no chronic GVHD	1.59 (0.64-3.95)	.316	1.56 (0.63-3.87)	.342
Extensive chronic GVHD vs no chronic GVHD	2.85 (1.41-5.77)	.004	2.75 (1.34-5.63)	.006

*There was no significant variable.
†There was no significant variable.
‡There was no other significant variable.

lead to a new strategy for improving outcomes of allogeneic HCT not only for ATL but also for other intractable T-cell neoplasms.

This study has several limitations. First, acute GVHD might be intentionally induced for some patients considered at high risk of relapse by treating clinicians. Second, the information on the day when each grade of GVHD occurred was not available. Therefore, we treated the development of acute and chronic GVHD in their worst severity as a time-varying covariate. To validate the results, we also performed the landmark analysis and obtained consistent results. Third, the relatively small number of patients with chronic GVHD might mask or bias the effect of chronic GVHD on outcomes. Last, the effect of multiple testing should be taken into account for the interpretation of the secondary end points.

In conclusion, the development of acute GVHD was associated with lower disease-associated mortality after allogeneic HCT for ATL compared with the absence of acute GVHD. However, improved survival can be expected only among a group of patients who developed mild-to-moderate acute GVHD because those who developed severe acute GVHD were at high risk of treatment-related mortality. New strategies that enhance the allogeneic anti-ATL effect without exacerbating GVHD are required to improve the outcomes of patients undergoing allogeneic HCT for ATL.

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The views expressed in this report are those of authors and do not indicate the views of the JSHCT, JMDP, or JCBBN.

This work is in memory of T.U., who died during the preparation of this manuscript.

Authorship

Contribution: T.I. and T.U. designed the research and organized the project; M. Hishizawa, J.K., T.I., and T.U. reviewed and analyzed data and wrote the paper; J.K., T.I., and K.M. performed statistical analysis; Y.A., R.S., and H.S. collected data from JSHCT; T.K. and Y. Morishima collected data from JMDP; T.N.-I., and S. Kato collected data from JCBBN; and A.U., S.T., T.E., Y. Moriuchi, R.T., F.K., Y. Miyazaki, M.M., K.N., M. Hara, M.T., S. Kai, and J.O. interpreted data and reviewed and approved the final manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A list of other members who contributed data on allogeneic HSCT for ATL to JSHCT, JMDP, and JCBBN appears in the online supplemental Appendix.

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