geneic HCT after relapse. In addition, genetic profile, including *KIT* mutation, in patients with CBF-AML may help to guide therapeutic decisions not only in first complete remission but also after first relapse.[21-23] The impact of cytogenetic profile at relapse on the benefit of allogeneic HCT needs to be evaluated in a study including a larger number of patients.

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 after relapse were chosen at the discretion of physicians, a lack of information regarding genetic profile, a lack of information regarding the presence of second relapse in those who did not undergo allogeneic HCT, and the relatively small number of patients analyzed. However, we showed that the prognosis differs significantly between groups of CBF-AML patients with different cytogenetic profiles at relapse. In addition, we found that optimal treatment strategies may vary between patients with t(8;21) and those with inv(16). These findings may help to guide therapeutic decisions including the indications for allogeneic HCT in patients with CBF-AML in first relapse. Further analyses using molecular profiling are warranted.

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Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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ORIGINAL ARTICLE

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

S Kurosawa[1], K Yakushijin[2], T Yamaguchi[3], Y Atsuta[4], T Nagamura-Inoue[5], H Akiyama[6], S Taniguchi[7], K Miyamura[8], S Takahashi[9], T Eto[10], H Ogawa[11], M Kurokawa[12], J Tanaka[13], K Kawa[14], K Kato[15], R Suzuki[4], Y Morishima[16], H Sakamaki[6] and T Fukuda[1]

The outcomes for allogeneic hematopoietic cell transplantation (allo-HCT) are heavily influenced by non-relapse mortality (NRM). We retrospectively assessed the changes in the incidence and causes of NRM after allo-HCT over the past 12 years. NRM, relapse rate and OS were analyzed using the Japan transplant outcome database of 6501 adult patients with acute leukemia or myelodysplastic syndrome who received their first allo-HCT in remission from 1997 through 2008. In multivariate analysis in patients aged 16–49 years, the adjusted hazard ratios (HRs) for NRM for 2001–2004 and 2005–2008 were 0.78 (95% confidence interval, 0.65–0.93) and 0.64 (0.54–0.78), respectively, compared with 1997–2000. The HR for overall mortality in 2005–2008 was 0.81 (0.70–0.93) compared with 1997–2000. In patients aged 50–70 years, the HRs for NRM and overall mortality in 2005–2008 were 0.56 (0.46–0.68) and 0.66 (0.47–0.93), respectively, compared with those in 2001–2004. We found that causes of death that contributed to the changes in NRM varied among subgroups. In conclusion, our study indicated that the incidence of NRM after allo-HCT has significantly decreased over the past 12 years, which has led to an improvement of OS, and also showed reductions in NRM in subgroups consisting of older patients and those who received unrelated cord blood transplantation.

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Keywords: leukemia; allogeneic hematopoietic cell transplantation; non-relapse mortality; GVHD; cord blood transplantation

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) has been recognized as a potent strategy for curing hematological malignancies. However, there have always been concerns about the risk of non-relapse mortality (NRM). As the risk of relapse is known to be significantly reduced after allo-HCT, the outcome of and indications for allo-HCT are heavily influenced by the risk for NRM.

Over the past few decades, many changes have been made to improve the outcome after allo-HCT, including improvements in the conditioning regimen, donor selection, and prophylaxis and treatment for organ complications, GVHD and infectious diseases, which have led to a reduction in NRM.[1–4]

Although an improvement in NRM has been reported in relatively younger patients who have received allo-HCT from a BM or peripheral blood (PB) donor, NRM has not been fully examined in other settings, such as in elderly patients, or in cord blood (CB) transplantation.

To evaluate the effects of these advances, we retrospectively assessed the changes in the incidence and causes of NRM over the past 12 years, using a nationwide registry database of more than 6000 patients who received various types of allo-HCT.

PATIENTS AND METHODS

Data source

The clinical data were extracted from a nationwide transplant outcome registry database provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP) and the Japan Cord Blood Bank Network (JCBBN). The JSHCT collect clinical data through the Transplant Registry Unified Management Program, as described previously.[5] This study was approved by the data management committees of JSHCT, JMDP and JCBBN, and by the Institutional Review Board at the National Cancer Center Hospital.

Patients and definitions

We evaluated the data on patients aged between 16 and 70 years who had AML, acute lymphocytic leukemia (ALL) or myelodysplastic syndrome (MDS), and who received their first allo-HCT between 1997 and 2008. We compared the incidence of NRM after allo-HCT in three consecutive 4-year

[1]Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan; [2]Division of Medical Oncology/Hematology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [3]Division of Biostatistics, Tohoku University Graduate School of Medicine, Sendai, Japan; [4]Department of HSCT Data Management/Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [5]Department of Cell Processing and Transfusion, The Institute of Medical Science, University of Tokyo, Tokyo, Japan; [6]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; [7]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [8]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [9]Department of Hematology and Oncology, The Institute of Medical Science, University of Tokyo, Tokyo, Japan; [10]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [11]Department of Internal Medicine, Division of Hematology, Hyogo College of Medicine, Hyogo, Japan; [12]Department of Haematology & Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [13]Stem Cell Transplantation Center, Hokkaido University Hospital, Sapporo, Japan; [14]Department of Hematology and Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; [15]Division of Haematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan and [16]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan. Correspondence: Dr T Fukuda, Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: tafukuda@ncc.go.jp
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periods (1997–2000, 2001–2004 and 2005–2008) for younger patients (16–49 years), and in the latter two periods for older patients (50–70 years). NRM was defined as death without recurrent disease after allo-HCT. Analyses were performed for patients with acute leukemia/MDS in remission or low-risk MDS (refractory anemia with or without ringed sideroblast: RA/RARS). Analyses were performed on the basis of patients' age (16–49 years and 50–70 years) and donor source (HLA-6/6-serum-matched or 1-Ag-mismatched related, unrelated BM and unrelated CB). In the era considered by this study, only BM from unrelated volunteer donors was used in Japan. In 2003, JMDP nationally recommended DNA typing of HLA-A and B, as well as HLA-DRB1. Since 2005, JMDP required all the candidates of unrelated allo-HCT to examine high-resolution typing of HLA-A, B and DRB1, and also recommended high-resolution typing of the C-locus. Conditioning regimens were classified as indicated by Giralt *et al.*[6] The incidences of mortality associated with GVHD, infection and organ failure were analyzed. In patients who had multiple causes among GVHD, infection and organ failure, information regarding the main cause of death was prioritized.

Statistical analysis

Data were retrospectively reviewed and analyzed as of June 2011. Among the three time periods, patient characteristics were compared using the χ^2 test. The primary end point of the study was NRM after allo-HCT. Probabilities of NRM and relapse were estimated with the use of cumulative incidence curves, with relapse viewed as a competing risk of NRM, and with NRM viewed as a competing risk of relapse. The Pepe and Mori test was used to evaluate the differences between groups. For the 151 patients (2%) who were known to have relapsed but whose date of relapse was unavailable, mid-point imputation was performed by substituting the midpoint from HCT to date of last contact as the date of relapse. The probability of OS was estimated using the Kaplan-Meier product limit method, and 95% confidence intervals (CIs) were calculated using the Greenwood formula. To compare the OS between groups, the log-rank test was used. Incidences of NRM, relapse and OS were estimated as probabilities at 3 years from allo-HCT. Multivariate analyses for NRM and relapse were performed using competing risk regression by the method of Fine and Gray, and for OS using a Cox proportional hazard regression model. The multivariate analyses were performed separately among patients aged 16–49 years and patients aged 50–70 years, where the year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008 among younger patients, 2001–2004 vs 2005–2008 among older patients or those who received unrelated CB transplantation (UCBT)), disease type (AML vs ALL or MDS), patient age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-6/6-Ag-matched sibling vs other family donors, HLA-6/6-Ag-matched unrelated BM, mismatched unrelated BM or unrelated CB) and conditioning regimens (myeloablative vs reduced-intensity) were considered as covariates. Multivariate analyses were also performed separately for those receiving related allo-HCT, where HLA-6/6-Ag-matched sibling vs other family donors were considered as covariates for the donor source, those receiving unrelated BM transplantation (UBMT), where HLA-6/6-Ag-matched unrelated BM vs mismatched BM were considered as covariates, and those who received UCBT, where the covariates above were examined other than the donor source. We considered two-sided *P*-values of <0.05 to be statistically significant. Statistical analyses were performed with SAS version 9.1.3 (SAS, Cary, NC, USA) and the SPSS software version 11.0.1 (SPSS, Chicago, IL, USA).

RESULTS

Patients

A total of 6501 patients registered from 266 institutions across the country[5] were analyzed, with a median age of 40 years and a median follow-up of 39 months. Characteristics of the patients and

Table 1. Patients' characteristics according to the time period of transplant

Characteristics	*1997–2000,* N *(%)*	*2001–2004,* N *(%)*	*2005–2008,* N *(%)*	P
Total number of patients	1354	2292	2855	
Age at transplant (years)				<0.001
16–34	740 (55)	892 (39)	862 (30)	
35–49	491 (36)	783 (34)	939 (33)	
50–59	116 (9)	489 (21)	743 (26)	
60–70	7 (1)	128 (6)	311 (11)	
Donor source				<0.001
Related BM	511 (38)	367 (16)	504 (18)	
Related peripheral blood	158 (12)	546 (24)	456 (16)	
Unrelated BM	588 (43)	998 (44)	1312 (46)	
Unrelated cord blood	14 (1)	321 (14)	534 (19)	
Others	83 (6)	60 (3)	49 (2)	
Disease type				0.991
AML	699 (52)	1226 (53)	1516 (53)	
ALL	505 (37)	744 (32)	949 (33)	
MDS	150 (11)	322 (14)	390 (14)	
Disease status				0.001
CR1	811 (60)	1288 (56)	1802 (63)	
CR2	311 (23)	552 (24)	654 (63)	
CR3 or beyond	76 (6)	96 (4)	77 (3)	
MDS RA/RARS	83 (6)	202 (9)	267 (9)	
Other remission state/no detailed data	73 (5)	154 (7)	55 (2)	
Conditioning				<0.001
Myeloablative	1131 (84)	1585 (69)	1788 (63)	
Reduced-intensity	21 (2)	394 (17)	689 (24)	
Not categorized	202 (15)	313 (14)	378 (13)	
GVHD prophylaxis				<0.001
CYA-based	1041 (77)	1367 (60)	1354 (47)	
Tacrolimus-based	270 (20)	825 (36)	1373 (48)	
No data available	43 (3)	100 (4)	128 (4)	

Abbreviations: MDS = myelodysplastic syndrome; RA/RARS = refractory anemia with or without ringed sideroblast.

transplantation procedures according to the time period are shown in Table 1. The overall proportions of AML, ALL and MDS were 53%, 34% and 13%, respectively. A total of 1354, 2292 and 2855 allo-HCTs were performed in 1997–2000, 2001–2004 and 2005–2008, respectively. The number and proportion of patients aged 50–70 years (1997–2000, $n=123$, 9%; 2001–2004, $n=617$, 27%; 2005–2008, $n=1054$, 37%), allo-HCT from an unrelated CB donor ($n=14$, 1%; $n=321$, 14%; $n=534$, 19%), and the use of a reduced-intensity conditioning regimen ($n=21$, 2%; $n=394$, 17%; $n=689$, 24%) increased over the three periods. Most of the myeloablative conditioning regimens (96%) consisted of high-dose CY with TBI or BU. Tacrolimus-based GVHD prophylaxis increased, especially in allo-HCT from an unrelated BM and CB donor (BM: $n=218$, 37%; $n=579$, 58%; $n=945$, 72%; CB: $n=3$, 21%; $n=99$, 31%; $n=229$, 43%).

Outcomes of allo-HCT over the three periods

The incidence of NRM of the entire 6501 patients was 23% at 3 years after allo-HCT (Figure 1a). Overall, 265 patients died of acute or chronic GVHD (median OS: 143 days, range: 18–3360), 497 died of infection (median OS: 116 days, range: 0–3184) and 500 died of organ failure (median OS: 145 days, range: 0–4013).

Older patients had a significantly higher incidence of NRM than younger patients (31% vs 20%, $P<0.001$, Figure 1b). The donor source significantly affected the incidence of NRM, and unrelated CB had the highest risk of NRM (related, 17%; unrelated BM, 25%; unrelated CB, 31%, $P<0.001$, Figure 1c). In a comparison of the outcome after allo-HCT among the three time periods in the overall 6501 patients (Figure 2), there were no linear improvements in NRM and OS over the three periods (NRM: 23%, 25% and 21%; OS: 61%, 57% and 60% at 3 years after allo-HCT). By the multivariate analysis that adjusted for disease type, patient age, patient gender, donor source and conditioning regimens, in younger patients (Table 2), the hazard ratios (HRs) for NRM in 2001–2004 and 2005–2008 compared with 1997–2000 were 0.78 (95% CI 0.65–0.93, $P=0.005$) and 0.64 (95% CI 0.54–0.78, $P<0.001$), respectively. The HR for overall mortality in 2005–2008 was significantly lower than that in 1997–2000 (HR 0.81, 95% CI 0.70–0.93, $P=0.004$). The HRs for relapse did not differ significantly among the periods. In older patients, the HRs for NRM and overall mortality in 2005–2008 compared with 2001–2004 were 0.56 (95% CI 0.46–0.68, $P<0.001$) and 0.66 (95% CI 0.47–0.93, $P=0.017$), respectively. However, the HR for relapse in 2005–2008 significantly increased (HR 1.53, 95% CI 1.20–1.97, $P=0.001$).

Allo-HCT from an HLA-matched or 1-Ag-mismatched related donor

In younger patients who received allo-HCT from a related donor (Figure 3a), the incidence of NRM remained rather low throughout the 12 years. Although NRM and OS slightly improved in 2005–2008, the differences were not statistically significant.

In older patients who received allo-HCT from a related donor, NRM was significantly reduced in 2005–2008 compared with 2001–2004 (Figure 3b, HR 0.62, 95% CI 0.44–0.88, $P=0.007$, Table 2). The incidences of death associated with organ failure and GVHD were significantly reduced in 2005–2008 (organ failure, 11 and 6%, $P=0.007$; GVHD, 6 and 3%, $P=0.015$, Figure 4a). In contrast, a significant increase in relapse was observed in 2005–2008 compared with 2001–2004 (21 and 36%, $P<0.001$, data not shown), and the same result was also shown by a multivariate analysis (HR 1.97, 95% CI 1.38–2.81, $P<0.001$, Table 2). This result remained the same when the analyses were restricted to HCT using reduced-intensity regimens or myeloablative regimens. Consequently, the improvement in OS in 2005–2008 was not statistically significant (Figure 3b and Table 2).

Allo-HCT from an unrelated BM donor

A significant reduction in NRM was seen over the three periods among younger patients who received allo-HCT from an unrelated BM donor (Figure 3c), with the HRs of 0.69 (95% CI 0.55–0.88, $P=0.003$) and 0.61 (95% CI 0.47–0.78, $P<0.001$) in 2001–2004 and 2005–2008, respectively (Table 2). The incidences of death associated with GVHD and organ failure were significantly reduced over the three periods (GVHD, 7, 4 and 4%, $P=0.011$; organ failure, 12, 10 and 8%, $P=0.002$, Figure 4b). OS significantly improved in 2005–2008 (Figure 3c and Table 2).

In older patients who received allo-HCT from an unrelated BM donor, NRM and OS significantly improved in 2005–2008 compared with 2001–2004 (Figure 3d). The HR for NRM in 2005–2008 was 0.58 (95% CI 0.41–0.82, $P=0.002$). The incidences of death associated with infection and organ failure were reduced in 2005–2008 (infection, 14 and 10%, $P=0.054$; organ failure,

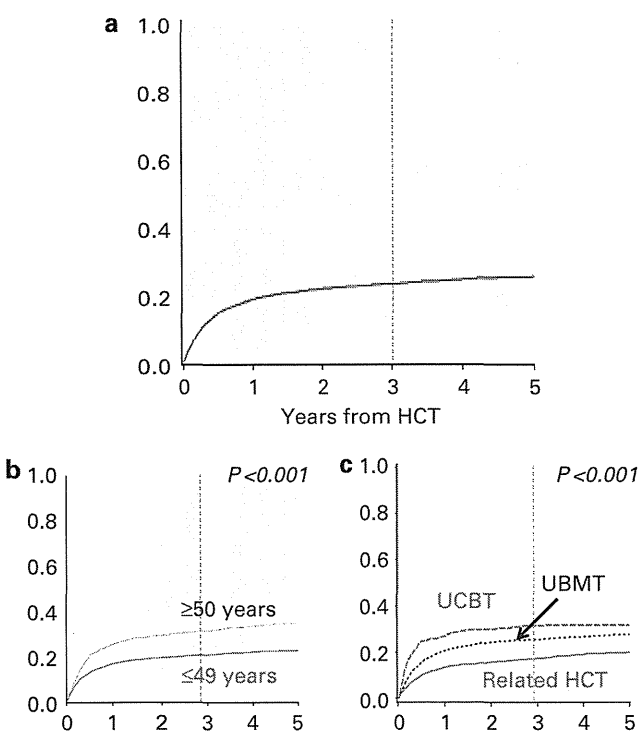

Figure 1. NRM over the past 12 years among 6501 patients who received allo-HCT in remission is shown in (**a**). NRM according to age (**b**) and donor source (**c**) are also shown.

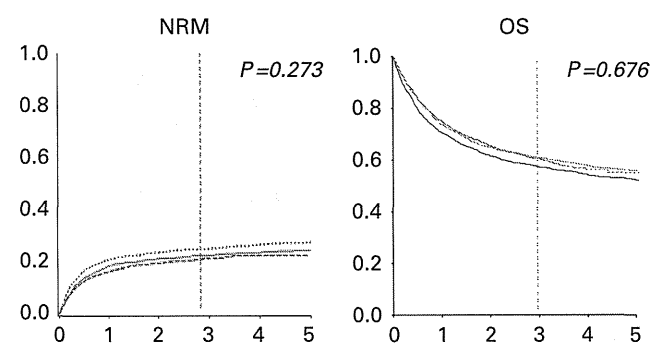

Figure 2. NRM and OS at 4-year periods (1997–2000, solid line; 2001–2004, dotted line; 2005–2008, dashed line) in the overall patients.

Table 2. Multivariate analyses for NRM, relapse and overall mortality after allo-HCT among the three periods

	All patients N = *6501*			*Related HCT* N = *2542*			*UBMT* N = *2898*			*UCBT* N = *869*		
	HR	*95% CI*	P *value*	*HR*	*95% CI*	P *value*	*HR*	*95% CI*	P *value*	*HR*	*95% CI*	P *value*
		N = 4707			*N* = 1846			*N* = 2202			*N* = 518	
Patient age at transplant, 16–49 years												
NRM												
1997–2000	1.00			1.00			1.00					
2001–2004	0.78	(0.65–0.93)	0.005	1.00	(0.75–1.33)	0.980	0.69	(0.55–0.88)	0.003	1.00		
2005–2008	0.64	(0.54–0.78)	<0.001	0.62	(0.44–0.88)	0.007	0.61	(0.47–0.78)	<0.001	1.04	(0.72–1.51)	0.830
Relapse												
1997–2000	1.00			1.00			1.00					
2001–2004	1.16	(0.98–1.37)	0.094	0.95	(0.74–1.21)	0.650	1.39	(1.39–1.06)	0.019	1.00		
2005–2008	1.12	(0.94–1.34)	0.220	1.20	(0.94–1.52)	0.150	1.20	(0.89–1.61)	0.240	0.66	(0.43–1.00)	0.049
Overall mortality												
1997–2000	1.00			1.00			1.00					
2001–2004	0.94	(0.82–1.06)	0.310	1.00	(0.82–1.22)	0.990	0.88	(0.73–1.06)	0.188	1.00		
2005–2008	0.81	(0.70–0.93)	0.004	0.89	(0.71–1.11)	0.285	0.77	(0.62–0.94)	0.010	0.84	(0.57–1.23)	0.373
		N = 1794			*N* = 696			*N* = 696			*N* = 351	
Patient age at transplant, 50–70 years												
NRM												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	0.56	(0.46–0.68)	<0.001	0.49	(0.33–0.71)	<0.001	0.58	(0.41–0.82)	0.002	0.57	(0.40–0.83)	0.003
Relapse												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	1.53	(1.20–1.97)	0.001	1.97	(1.38–2.81)	<0.001	1.46	(0.93–2.28)	0.100	0.96	(0.59–1.58)	0.880
Overall mortality												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	0.66	(0.47–0.93)	0.017	0.87	(0.67–1.15)	0.334	0.82	(0.61–1.09)	0.169	0.67	(0.49–0.91)	0.010

Abbreviations: CI = confidence interval; HCT = hematopoietic cell transplantation; HR = hazard ratio; NRM = non-relapse mortality; UBMT = unrelated BM transplantation; UCBT = unrelated cord blood transplantation. Year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008 among younger patients, 2001–2004 vs 2005–2008 among older patients or those who received UCBT), disease type (AML vs ALL or MDS), patient age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-matched sibling vs other family donors, HLA-matched unrelated BM, mismatched unrelated BM or unrelated CB), and conditioning regimens (myeloablative vs reduced-intensity) were considered as covariates. In the analysis for related HCT, donor source (HLA-matched sibling vs other family donors), year of allo-HCT, disease type, patient age, patient gender and conditioning regimens were considered as covariates. In the analysis for UBMT, donor source (HLA-matched BM vs mismatched unrelated BM), year of allo-HCT, disease type, patient age, patient gender and conditioning regimens were considered as covariates. In the analysis for UCBT, year of allo-HCT, disease type, patient age, patient gender and conditioning regimens were considered.

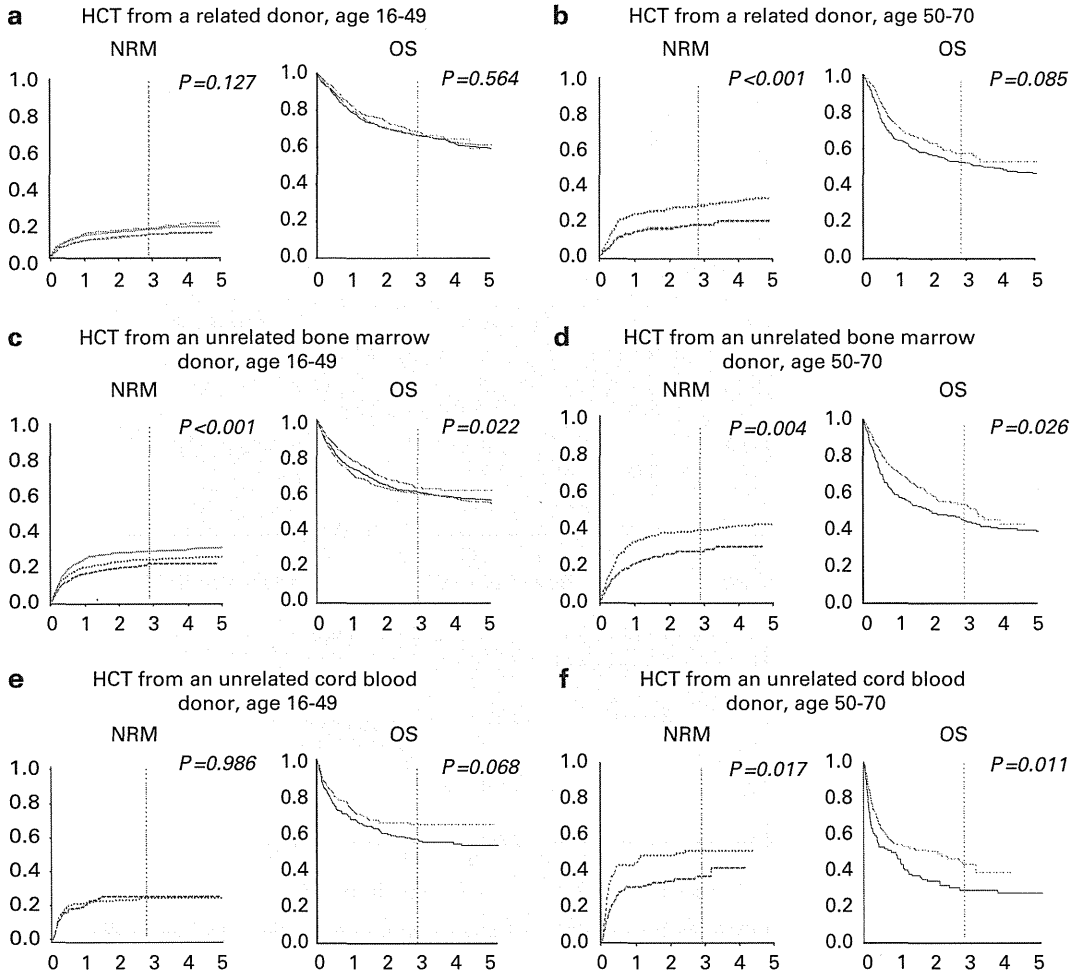

Figure 3. (**a**) NRM and OS at 3 years from HCT among younger patients (16–49 years) who received allo-HCT from a related donor were 15%, 16% and 12% ($P=0.127$), and 67%, 66% and 68% ($P=0.564$), respectively in the period of 1997–2000 ($n=587$, solid line), 2001–2004 ($n=620$, dotted line) and 2005-2008 ($n=639$, dashed line). (**b**) NRM and OS among older patients (50–70 years) who received related donor transplantation were 28% and 17% ($P<0.001$) and 52% and 57% ($P=0.085$), respectively in the period of 2001–2004 ($n=293$, dotted line) and 2005–2008 ($n=321$, dashed line). (**c**) NRM and OS among younger patients who received allo-HCT from an unrelated BM donor were 28%, 24% and 22% ($P<0.001$), and 60%, 60% and 63% ($P=0.022$), respectively in the period of 1997–2000 ($n=560$, solid line), 2001–2004 ($n=803$, dotted line) and 2005-2008 ($n=839$, dashed line). (**d**) NRM and OS among older patients who received allo-HCT from an unrelated BM donor were 39% and 27% ($P=0.004$) and 45% and 54% ($P=0.026$), respectively in the period of 2001–2004 ($n=195$, dotted line) and 2005–2008 ($n=473$, dashed line). (**e**) Non-relapse mortality and OS among younger patients who received allogeneic hematopoietic cell transplantation from an unrelated cord blood donor were 25% and 25% ($P=0.986$), and 55% and 65% ($P=0.068$), respectively in the period 2001–2004 ($n=214$, dotted line) and 2005–2008 ($n=292$, dashed line). (**f**) Non-relapse mortality and OS among older patients who received allogeneic hematopoietic cell transplantation from an unrelated cord blood donor were 51% and 37% ($P=0.017$), and 29% and 44% ($P=0.011$), respectively in the period of 2001–2004 ($n=107$, dotted line) and 2005–2008 ($n=242$, dashed line).

14 and 8%, $P=0.049$, Figure 4c). We found a significant reduction in mortality rates associated with bacterial and fungal infection.

Allo-HCT from an unrelated CB donor

In younger patients who received allo-HCT from an unrelated CB donor, there was no significant difference in the incidence of NRM between the two periods (Figure 3e). In this group, there was a marked reduction in the relapse rate (25 and 18%, $P=0.018$, data not shown; HR 0.66, 95% CI 0.43–1.00, $P=0.049$, Table 2). OS was better in 2005–2008; however, the difference was not statistically significant.

Significant improvements in NRM and OS were observed in 2005–2008 among older patients who received UCBT (Figure 3f). The HRs for NRM and overall mortality in 2005–2008 were 0.57 (95% CI 0.40–0.83, $P=0.003$) and 0.67 (95% CI 0.49–0.91, $P=0.010$), respectively. Reductions in the incidences of death associated with GVHD and infection seemed to contribute to the improvements in NRM (GVHD, 7 and 3%, $P=0.163$; infection, 23 and 13%, $P=0.136$). The mortality rate due to bacterial infection was significantly reduced.

Incidence of and mortality after severe acute GVHD

In subgroups that showed a significant reduction in the incidence of NRM, younger patients who received UBMT, older patients who received related HCT and older patients who received UCBT showed significant reductions in the incidence of GVHD-related mortality. In younger patients who received UBMT, the incidence of severe acute GVHD was significantly reduced over the three

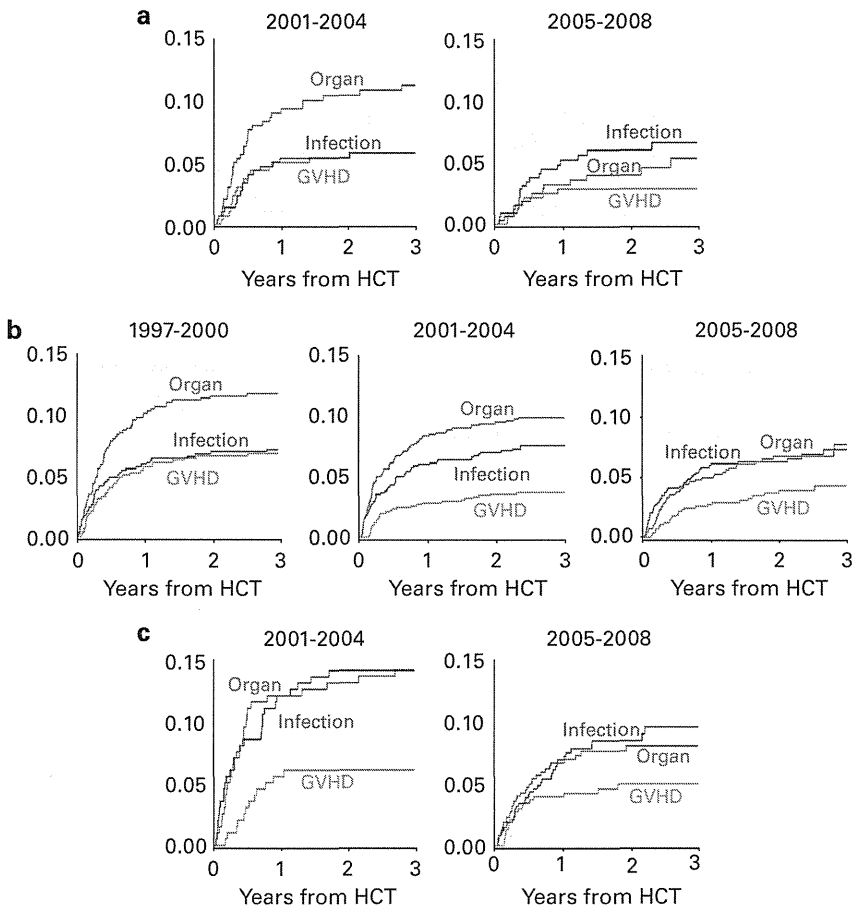

Figure 4. Change in the causes of NRM among different time periods is shown. Cumulative incidences of death due to GVHD, infection and organ failure are separately presented in each time period. (**a**) In older patients who received allo-HCT from a related donor, the incidences of death associated with organ failure and GVHD were significantly reduced in 2005–2008 (organ failure, 11 and 6%, $P=0.007$; GVHD, 6 and 3%, $P=0.015$). (**b**) In younger patients who received allo-HCT from an unrelated BM donor, the incidences of death associated with GVHD and organ failure were significantly reduced (GVHD, 7, 4 and 4%, $P=0.011$; organ failure, 12, 10 and 8%, $P=0.002$). (**c**) In older patients who received allo-HCT from an unrelated BM donor, the incidences of death associated with infection and organ failure were reduced in 2005–2008 (infection, 14 and 10%, $P=0.054$; organ failure, 14 and 8%, $P=0.049$).

periods (16, 15 and 12% at 100 days after allo-HCT, $P=0.021$). In older patients who received related HCT, the incidence of severe acute GVHD was reduced in 2005–2008 relative to 2001–2004, but this difference was not statistically significant (14 and 10%, $P=0.099$). In older patients who received UCBT, there was no remarkable reduction in the incidence of severe acute GVHD in the later period (18 and 16%, $P=0.542$). However, the mortality rate was significantly reduced among older patients who suffered severe acute GVHD after UCBT (92 and 67% at 3 years after allo-HCT, $P=0.022$).

DISCUSSION

In this study that used a large database of 6501 patients, we found that the incidence of NRM after allo-HCT for adult patients has significantly decreased over the past 12 years, which has led to an improvement of OS. As prior studies have primarily focused on the changes in NRM among younger patients who received allo-HCT with myeloablative conditioning,[2,4] this is the first study to show the changes in NRM in subgroups comprising older patients and UCBT.

We found that demographic, disease and transplantation characteristics have been changing, as previous studies reported.[1,2,4] The marked increase in the number of older patients, allo-HCT with reduced-intensity conditioning and UCBT might reflect an increase in allo-HCT for 'more vulnerable' patients. Gooley *et al.*[1] reported that the hematopoietic cell transplantation-specific comorbidity index (HCT-CI)[7] scores were higher in HCT recipients in more recent time periods. Unfortunately, we were not able to evaluate HCT-CI in the current study because of a lack of information.

Among patients who received related HCT, remarkable improvement in NRM was observed in older patients. Another distinguishing finding was an increase in relapse in overall older patients, especially among those who received related HCT in remission. There was no recent shift in the use of allo-HCT in a later remission state, and we obtained a similar result when the analyses were restricted to HCT using reduced-intensity regimens or myeloablative regimens. In addition, the proportional use of anti-thymocyte globulin has remained unchanged over the periods. Less use of PB donors and more aggressive selection of older patients as indicated for allo-HCT may have affected the result. Despite this increase in relapse, older patients who received HCT in remission showed, by multivariate analyses, a significant reduction in mortality with a remarkable reduction in HRs for NRM irrespective of donor sources.

In analyses based on the donor source, UBMT showed remarkable improvements in NRM and OS throughout the age subgroups. Along with high-resolution donor–recipient HLA

matching,[8,9] the lesser proportion of donor/patient pairs with allele mismatches may have reduced the incidence of GVHD-related mortality, and contributed to the improvement in outcomes after UBMT.

Among patients who received UCBT, we found a decreased risk of relapse in younger patients with no change in NRM. On the other hand, older patients had a decreased risk of NRM with no change in relapse. These outcomes may be explained by the changes in clinical practice in 2001–2004, 'learning phase' of UCBT, and that after 2005, including the indication of UCBT and the prophylaxis and treatment for GVHD/infection.

A recent reduction in the incidence of GVHD-related mortality was observed in younger patients receiving UBMT and older patients receiving related allo-HCT or UCBT. With the changes in prophylaxis and treatment against GVHD including high-resolution donor–recipient HLA matching,[8,9] the incidence of grade 3 to 4 severe acute GVHD has decreased in younger patients receiving UBMT and older patients receiving related HCT, which may have led to the reduction in GVHD-related mortality in these subgroups. Interestingly, in older patients receiving UCBT, there was no reduction in the incidence of severe acute GVHD; however, the mortality rate among those who developed severe acute GVHD was reduced. The prompt initiation of treatment after a more thorough examination to diagnose GVHD,[10] supportive care and nutritional management may have improved the prognosis of those who had severe GVHD. Alternatively, the unique HLA epidemiological genetics of Japanese patients may have affected the results.[11,12]

A recent reduction in the incidence of infection-related mortality was observed in older patients receiving UBMT or UCBT. New antifungal drugs, including mold-active azoles, micafungin or liposomal amphotericin B, are now more likely to be administered as empiric or preemptive strategies for patients who have a positive galactomannan Ag test or pulmonary nodules.[1,13,14] As GVHD and infection have been reported to be associated with each other's development and exacerbation,[13,15–18] an improved control of severe GVHD may have led to the reduction of the risk of infection-related mortality.[13,14]

We included all of the organ toxicities that were documented after allo-HCT as the cause of organ failure-related mortality, including conditioning regimen-related toxicity,[19,20] lung injury[15] and late effects on any organs.[21] We observed a reduction in the incidence of organ failure-related mortality in older patients receiving related HCT and those who received UBMT. In the future, more detailed analyses are warranted based on each specific organ toxicity.

As this analysis is based on a retrospectively collected multicenter database, our results may be susceptible to the disadvantages of any retrospective study, such as the heterogeneity in the treatment strategies chosen at the discretion of the physicians. Because of the nature of the multicenter registry, detailed data were not available regarding the incidences of infection and specific organ failure, and prophylactic treatment toward infection. Although we acknowledge this limitation, the results obtained from this large database that contains clinical data on over 6000 patients should provide valuable information. In addition, for the first time, we found reductions in NRM in subgroups consisting of older patients and those who received UCBT. We also showed the causes of death that contributed to the reduction of NRM in each donor/age subgroup. By further evaluating the risks of NRM and relapse in each demographic subgroup, we would be able to more clearly define the indications for allo-HCT, and tailor the strategy for individual patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contributions: SK designed the study, prepared the data file, performed the analysis, interpreted the data and wrote the manuscript; KY contributed to the study design, data file preparation, data analysis and interpretation of the data; TY was primarily responsible for the study design, data analysis and interpretation of the data; YA reviewed and cleaned the data, interpreted the data and helped to write the manuscript; TNI reviewed, cleaned and interpreted the data, HA, ST, KM, ST, TE, HO and MK obtained and interpreted the data; JT, KK, KK, RS, YM and HS reviewed, cleaned and interpreted the data; TF designed the study, interpreted the data and helped to write the manuscript.

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ORIGINAL ARTICLE

Recent decrease in non-relapse mortality due to GVHD and infection after allogeneic hematopoietic cell transplantation in non-remission acute leukemia

S Kurosawa[1], K Yakushijin[2], T Yamaguchi[3], Y Atsuta[4], T Nagamura-Inoue[5], H Akiyama[6], S Taniguchi[7], K Miyamura[8], S Takahashi[9], T Eto[10], H Ogawa[11], M Kurokawa[12], J Tanaka[13], K Kawa[14], K Kato[15], R Suzuki[4], Y Morishima[16], H Sakamaki[6] and T Fukuda[1]

Although recent improvements have been indicated in the outcome after allogeneic hematopoietic cell transplantation (allo-HCT), little information is available on how changes in transplant modalities have affected the outcomes after allo-HCT in non-remission, based on patient age, donor source and disease type. We compared the incidence and causes of non-relapse mortality (NRM) after allo-HCT in non-remission among three consecutive four-year periods using a nationwide transplant outcome registry database. A total of 3308 patients with acute leukemia in non-remission were analyzed. The risk of NRM decreased over the three periods, and the hazard ratios (HRs) in 2001–2004 and 2005–2008 compared with 1997–2000 were 0.86 (95% CI, 0.70–1.06; $P=0.16$) and 0.65 (95% CI, 0.53–0.80; $P<0.01$), respectively. A significant decrease in the HR for overall mortality was also observed in 2005–2008 (HR 0.85; 95% CI, 0.75–0.97; $P=0.02$). We found that a decrease in the incidences of death due to GVHD and infection contributed to the reduction in NRM, to which high-resolution donor-recipient HLA matching and other improvements may have contributed. As none of the subgroups showed improved survival without a reduction in NRM, the effective prevention of transplant-related complications appears to be necessary for improving outcomes after allo-HCT in non-remission.

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Keywords: allogeneic hematopoietic cell transplantation; acute leukemia; non-remission; non-relapse mortality; GVHD

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is recognized as a potentially curative therapy for patients with high-risk hematologic malignancies, which can lower the risk of relapse. However, treatment-related mortality, which may offset the benefit of a reduced risk of relapse, has long been a major problem. Several changes have been made in modalities of allo-HCT, including patient-donor HLA matching, conditioning regimens, immunosuppressive therapy, and the prophylaxis, diagnosis and treatment of GVHD and infection. As a result, the risk of non-relapse mortality (NRM) after allo-HCT has decreased over the past few decades.[1–6]

AML and ALL account for the largest proportion of diseases indicated for allo-HCT. Furthermore, a substantial number of patients with AML or ALL receive allo-HCT in non-remission. Despite the fact that high-risk acute leukemia is definitely indicated for allo-HCT, patients with non-remission leukemia carry various factors that lead to a higher risk of treatment-related toxicity, including comorbidities due to prior chemotherapy and intensified conditioning regimens in need of an antitumor effect,[7–11] and a deteriorated general condition due to refractory disease. Although prior studies have shown improvements in the outcome after allo-HCT,[1–5] little information is available on how changes in transplant modalities have affected the outcomes after allo-HCT in non-remission, based on patient age, donor source and disease type. We recently reported changes in the incidence and causes of NRM after allo-HCT in remission in Japan.[6] Using the same nationwide transplant outcome registry database, we compared the incidence and causes of NRM in patients with AML or ALL in non-remission in three consecutive four-year periods.

SUBJECTS AND METHODS

Data source

Clinical data were extracted from a nationwide transplant outcome registry database provided by the Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program and the Japan Cord Blood Bank Network, to which 267 institutions/departments contributed. The clinical data were consecutively collected through Transplant Registry Unified Management Program as described previously.[12] This study was

[1]Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan; [2]Division of Medical Oncology/Hematology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [3]Division of Biostatistics, Tohoku University Graduate School of Medicine, Sendai, Japan; [4]Department of HSCT Data Management/Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [5]Department of Cell Processing and Transfusion, The Institute of Medical Science, University of Tokyo, Tokyo, Japan; [6]Division of Hematology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; [7]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [8]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [9]Department of Hematology and Oncology, The Institute of Medical Science, University of Tokyo, Tokyo, Japan; [10]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [11]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan; [12]Department of Haematology & Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [13]Stem Cell Transplantation Center, Hokkaido University Hospital, Sapporo, Japan; [14]Department of Hematology Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; [15]Division of Haematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan and [16]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan. Correspondence: Dr T Fukuda, Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.
E-mail: tafukuda@ncc.go.jp
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approved by the data management committees of the Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program and the Japan Cord Blood Bank Network, and by the Institutional Review Board at the National Cancer Center Hospital.

Patients and definitions

We evaluated data on patients aged between 16 and 70 years who had AML or ALL and who received their first allo-HCT in non-remission between 1997 and 2008. Non-remission status was defined as any percentage of blasts in the peripheral blood, or a BM aspirate containing >5% blasts at the time of transplant. We compared the incidence of NRM after allo-HCT in three consecutive four-year periods (1997–2000, 2001–2004 and 2005–2008) for younger patients (16–49 years), and in the latter two periods for older patients (50–70 years). NRM was defined as death without the detection of recurrent disease after allo-HCT. In 154 patients who died without a confirmed hematological remission within 30 days from allo-HCT, the cause of death was defined as NRM. In 293 patients who died without a confirmed hematological remission after 31 days or later after allo-HCT, the cause of death was defined as refractory disease. A separate analysis that excluded these 447 patients who died without a confirmed remission was performed. We also changed the cutoffs from 30 days to 60 days or 90 days. Analyses were performed on the basis of patient's age (16–49 years and 50–70 years), disease (AML and ALL) and donor source (HLA-matched/1-Ag-mismatched related, unrelated BM and unrelated cord blood (CB)). In this study, matching of unrelated BM between recipient and donor were determined based on serum typing. In 2003, Japan Marrow Donor Program nationally recommended DNA typing of HLA-A and B, as well as HLA-DRB1. Since 2005, Japan Marrow Donor Program required all the candidates of unrelated allo-HCT to examine high-resolution typing of HLA-A, B and DRB1 and also recommended high-resolution typing of C-locus. In the era considered by this study, only BM was used from unrelated volunteer donors in Japan. Conditioning regimens were classified as indicated by Giralt *et al.*[13] The causes of death other than recurrent disease were obtained from the database and the incidences of mortality associated with GVHD, infection or organ failure were compared over the three time periods. In patients who had multiple causes among GVHD, infection and organ failure, information regarding the main cause of death was prioritized. The 447 patients who died without a confirmed hematological remission were excluded from the analyses regarding the causes of death.

Statistical analysis

Data were retrospectively reviewed and analyzed as of March 2012. Among the three time periods, patient characteristics were compared using the χ^2-test. The primary endpoint of the study was NRM after allo-HCT. Probabilities of NRM were estimated with the use of cumulative incidence curves, with relapse viewed as a competing risk of NRM. The Pepe and Mori test was used to evaluate the differences between groups. For the 337 patients (10%) who were known to have relapsed but whose date of relapse was unavailable, midpoint imputation was performed by substituting the midpoint from HCT to date of last contact as the date of relapse. The incidence of NRM was estimated as the probability at 2 years from allo-HCT. Multivariate analyses were performed for NRM and relapse using competing risk regression by the method of Fine and Gray, and for survival using a Cox proportional hazard regression model. The analyses were performed separately among younger patients aged 16–49 years and older patients aged 50–70 years. In the multivariate analyses, we considered the following factors as covariates: the year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008; because of the small number of HCT performed in 1997–2000, we considered 2001–2004 as reference vs 2005–2008 in subgroup analyses among older patients or those who received unrelated CB transplantation (UCBT)), disease type (AML vs ALL), patient age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-matched sibling vs other family donors, HLA-matched-unrelated BM, mismatched-unrelated BM or unrelated CB), and conditioning regimens (myeloablative vs reduce-intensity conditioning (RIC)). Multivariate analyses were also performed separately for patients who received related allo-HCT, patients who received unrelated BMT (UBMT), and patients who received UCBT. We considered two-sided *P*-values of <0.05 to be statistically significant. Statistical analyses were performed with SAS version 9.1.3 (SAS, Cary, NC, USA) and SPSS software version 11.0.1 (SPSS, Chicago, IL, USA).

Table 1. Patient characteristics according to the time period of transplant

Characteristics	*1997–2000* N(%)	*2001–2004* N(%)	*2005–2008* N(%)	P-*value*
Total number of patients	637	1165	1506	
Gender				*0.064*
Male	355(56)	674(58)	793(53)	
Female	281(44)	489(42)	505(34)	
Age (years)				*<0.001*
16–29	249(39)	277(24)	265(18)	
30–39	139(22)	240(21)	278(18)	
40–49	157(25)	246(21)	304(20)	
50–59	83(13)	296(25)	430(29)	
60–70	9(1)	106(9)	229(15)	
Donor source				*<0.001*
HLA-matched sibling	248(39)	380(33)	365(24)	
Related others	94(15)	165(14)	196(13)	
Matched-unrelated BM	213(33)	288(25)	461(31)	
Mismatched-unrelated BM	34(5)	83(7)	85(6)	
Unrelated CB	23(4)	176(15)	286(19)	
Others	25(4)	73(6)	113(8)	
Disease type				*<0.001*
AML	388(61)	840(72)	1209(80)	
ALL	249(39)	325(28)	297(20)	
Ph-positive ALL	66(10)	75(6)	48(3)	
Conditioning				*<0.001*
Myeloablative	504(79)	668(57)	837(56)	
Reduced-intensity	14(2)	290(25)	426(28)	
Not categorized	119(19)	207(18)	243(16)	
GVHD prophylaxis				*<0.001*
Cyclosporin-based	472(74)	679(58)	618(41)	
Tacrolimus-based	150(24)	423(36)	806(54)	
Disease status at HCT				*<0.001*
No treatment	20(3)	43(4)	115(8)	
Primary induction failure	148(23)	292(25)	576(38)	
First relapse	154(24)	372(32)	485(32)	
⩾Second relapse	55(9)	159(14)	157(10)	
Non-remission/no detailed data	260(41)	299(26)	173(11)	

Abbreviation: CB = cord blood.

RESULTS

Patients

A total of 3308 patients with a median age of 42 years and a median follow-up of 27 months (range, 0–150) was analyzed. The characteristics of the patients and transplantation procedures according to the time period are shown in Table 1. The number of allo-HCT procedures increased over time. The number and proportion of patients aged 50–70 years, allo-HCT from an unrelated CB donor and the use of a RIC regimen increased over the three periods. Most of the myeloablative regimens (96%) consisted of high-dose CY with TBI or BU. Tacrolimus-based GVHD prophylaxis increased, especially in allo-HCT from an unrelated BM and CB donor (BM: 1997–2000, $n=109$, 44%; 2001–2004, $n=231$, 62%; 2005–2008, $n=426$, 78%, CB: $n=5$, 22%; $n=50$, 28%; $n=174$, 61%). The proportion of allo-HCT given for ALL in non-remission decreased over the three periods with decreasing proportions of both Ph-positive ALL and Ph-negative

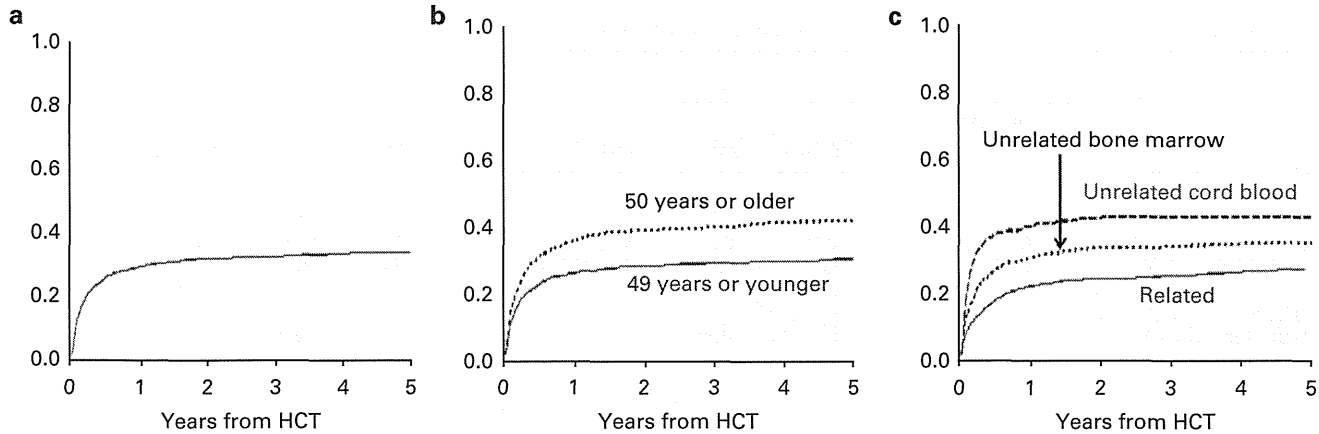

Figure 1. Cumulative incidence curves of NRM over the past 12 years among patients who received allo-HCT in non-remission are shown for the entire population (**a**), and subgroups based on age (**b**) and donor (**c**).

ALL. We categorized patients by detailed disease status; however, about 40% of allo-HCT performed in the earliest time period lacked the necessary information.

Transplant outcomes

Overall, the incidence of NRM was 31% at 2 years after allo-HCT (Figure 1a). Patients who were 50 years or older had a significantly higher incidence of NRM than patients who were 49 years or younger (39% vs 28%, $P<0.001$, Figure 1b). The donor source significantly affected the incidence of NRM, and unrelated CB had the highest risk of NRM (related, 23%; unrelated BM, 33%; unrelated CB, 42%, $P<0.001$, Figure 1c).

Hazard ratios (HRs) for NRM, relapse and overall mortality in 2001–2004 and 2005–2008 compared with 1997–2000, after adjusting for disease type, patient age, patient gender, donor source and conditioning regimens, are shown in Table 2. In the overall 3308 patients, HRs for NRM in 2001–2004 and 2005–2008 were reduced, with a significant decrease in 2005–2008. A significant decrease in the HR for overall mortality was also observed in 2005–2008. The HR for relapse did not change among the three periods. Other factors that were significantly associated with increased NRM were older age (HR 1.43; 95% CI, 1.19–1.71; $P<0.01$), male gender (HR 1.20; 95% CI, 1.04–1.41; $P=0.01$) and donor other than HLA-matched sibling (other family donors, HR 1.55; 95% CI, 1.22–1.97; $P<0.01$; HLA-matched-unrelated BM, HR 1.57; 95% CI, 1.30–1.90; $P<0.01$; HLA-mismatched-unrelated BM, HR 1.82; 95% CI, 1.35–2.47; $P<0.01$; unrelated CB, HR 2.45; 95% CI, 1.96–3.08; $P<0.01$). Younger age and HLA-matched sibling donor were also significantly associated with reduced overall mortality. Although the HR for NRM in the RIC group tended to be higher than that in the myeloablative group (HR 1.20; 95% CI, 0.99–1.47; $P=0.07$), this difference was NS. An analysis according to disease type showed that the HRs for NRM and overall mortality were reduced in AML patients, but not in ALL patients (Table 2). The incidences of NRM and OS are presented as Supplementary Figures 1a–c.

Transplant outcomes based on patient age

As the transplantation modality may vary according to the patient's age, HRs in comparison to those in the reference era were investigated separately for patients aged 49 years or younger (reference era: 1997–2000) and those aged 50 years or older (reference era: 2001–2004). As shown in Table 3, in patients aged 16–49, HRs for NRM and overall mortality in 2005–2008 were significantly reduced. In contrast, in patients aged 50–70, there were no remarkable changes in the HRs for NRM and overall mortality between 2001–2004 and 2005–2008. The incidences of NRM and OS are presented as Supplementary Figures 1d and e. RIC was used in 47% of patients aged 50 years or older (50–59: 36%, 60–70: 72%). There was no remarkable difference in the HR for NRM between the myeloablative and RIC groups (RIC: HR 0.97; 95% CI, 0.74–1.28; $P=0.85$).

Transplant outcomes based on donor

We also performed analyses based on the donor source separately among younger and older patients (Table 3). In related donor transplantation, there were no differences in the HRs for NRM, relapse and OS among the time periods in both younger and older patients. In younger patients who received UBMT, there were significant reductions in the HRs for NRM in 2001–2004 and 2005–2008. The HR for overall mortality was also significantly reduced in 2005–2008. In younger patients who received UCBT, there were significant reductions in the HRs for NRM and overall mortality in 2005–2008. The incidences of NRM and OS are presented as Supplemental Figures 1f–k. The HRs for relapse among younger patients who received UBMT were significantly higher in recent periods. In patients aged 50 years or older, no significant changes in HRs for NRM, relapse or overall mortality were observed among the different time periods in either of the donor subgroups.

Causes of death that accounted for changes in NRM

The causes of death were obtained in 98% of patients who died without recurrent disease. In 17% of patients for whom multiple causes of death were provided, GVHD, infection, or organ failure given as a main cause of death was prioritized. Overall, 151 patients died of acute or chronic GVHD (median OS: 101 days, range: 12–1979), 337 died of infection (median OS: 63 days, range: 1–2700), and 251 died of organ failure (median OS: 88 days, range: 0–2283). In the overall population, no remarkable decrease in the incidences of mortality due to these three causes was observed although the HRs for NRM and overall mortality decreased (Table 2). Meanwhile, significant reductions in the incidences of GVHD-related and infection-related mortality were observed among younger patients who received UBMT (Figure 2a) and UCBT (Figure 2b). In older patients or allo-HCT from a related donor, no remarkable differences were observed in the incidences of mortality due to GVHD, infection or organ failure among the time periods. The incidence of organ failure-related mortality did not decrease in any of the subgroups.

DISCUSSION

We evaluated the changes in NRM after allo-HCT for acute leukemia in non-remission over the last 12 years. Overall, we found higher NRM rates compared with those after allo-HCT in

Table 2. Multivariate analyses for NRM, relapse and overall mortality after allogeneic HCT

	All patients			*AML*			*ALL*		
	HR	*N = 3308 95% CI*	*P value*	*HR*	*N = 2437 95% CI*	*P value*	*HR*	*N = 871 95% CI*	*P value*
NRM									
1997–2000	1.00			1.00			1.00		
2001–2004	0.86	(0.70–1.06)	0.16	0.82	(0.64–1.05)	0.12	0.96	(0.67–1.38)	0.83
2005–2008	0.65	(0.52–0.80)	<0.01	0.59	(0.46–0.75)	<0.01	0.85	(0.58–1.25)	0.42
Relapse									
1997–2000	1.00			1.00			1.00		
2001–2004	1.01	(0.87–1.18)	0.88	1.05	(0.86–1.27)	0.64	0.92	(0.70–1.20)	0.53
2005–2008	1.07	(0.92–1.25)	0.38	1.08	(0.89–1.30)	0.43	1.07	(0.80–1.43)	0.65
Overall mortality									
1997–2000	1.00			1.00			1.00		
2001–2004	0.94	(0.82–1.07)	0.32	0.91	(0.78–1.07)	0.26	0.97	(0.77–1.21)	0.76
2005–2008	0.85	(0.75–0.97)	0.02	0.79	(0.67–0.92)	<0.01	1.07	(0.85–1.36)	0.56

Abbreviations: CI = confidence interval; HCT = hematopoietic cell transplantation; HR = hazard ratio; NRM = non-relapse mortality
Year of allo-HCT (1997–2000 versus 2001–2004 or 2005–2008), disease type (AML versus ALL), patient age (16–29 years versus 30–39, 40–49, 50–59 or 60–70), patient gender (male versus female), donor source (HLA-matched sibling versus other family donors, HLA-matched unrelated bone marrow, mismatched unrelated bone marrow or unrelated cord blood), and conditioning regimens (myeloablative versus reduced-intensity) were considered as covariates. In the analysis for AML and ALL, the 5 covariates were considered other than disease type.

remission (31 vs 22% at 2 years after HCT).[6] The HRs for NRM and overall mortality were lower in more recent time periods. Although several studies have shown changes in outcomes after allo-HCT,[1–6] this is the first analysis restricted to allo-HCT in non-remission, based on the patient age, donor source and disease type. The reduction in the HR for NRM was reflected in the reduced HR for overall mortality, and none of the subgroups showed a reduced risk for overall mortality without an improvement in NRM. This may indicate that lowering the risk of treatment-related mortality is, so far, an absolute requirement for improving outcomes after allo-HCT in non-remission, where a high-risk of relapse has always been an obstacle.

The reductions in the HRs for NRM and overall mortality in the overall population were accounted for by the reductions in HRs in patients with AML, and there was no improvement in NRM or overall mortality in those with ALL in non-remission over the three time periods. We also found that the number and proportion of patients who received allo-HCT for ALL in non-remission decreased over the three time periods despite an increase in the total number of allo-HCT. The proportions of both Ph-positive ALL and Ph-negative ALL decreased and, interestingly, more patients with Ph-positive ALL have received allo-HCT in remission after 2000. The introduction of imatinib may have helped more patients with Ph-positive ALL to receive allo-HCT in a controlled disease status.[14–16] In addition, a lowered expectation for the effect of allo-HCT in ALL in non-remission may have also impacted the indication. In patients who receive allo-HCT in non-remission, strategies that can provide intensified preparative regimens and a GVL effect without increasing toxicity need to be pursued.

No improvement in the HRs for NRM and overall mortality was observed in patients aged 50–70 who received allo-HCT in non-remission. Older patients with acute leukemia have been reported to have a worse prognosis because of more unfavorable disease profiles, deteriorated general conditions and an increased risk of comorbidities.[17] As the eradication of residual disease by provoking GVHD may increase toxicity and become unbearable for elderly patients, it may be necessary to reduce the tumor burden before transplantation. We previously demonstrated a significant reduction in NRM in patients aged 50 years or older who received allo-HCT in remission.[6] The safety and efficacy of modified induction chemotherapy or preparative regimen for elderly patients need to be validated.[18–25]

We found that decreases in GVHD-related and infection-related mortality contributed to the reduced risk of NRM. These findings are consistent with prior reports.[2,3] Based on an analysis of 14 403 patients with leukemia in the first CR who received allo-HCT from a matched sibling donor, Gratwohl *et al.*[3] showed that the rate of mortality due to infection decreased. In a detailed analysis in a single-center study, Gooley *et al.*[2] showed that the rates of severe GVHD and infection were recently reduced. There have been substantial improvements in HLA typing over the period of 1997–2008, with more accuracy in defining HLA haplotypes at high-resolution.[26,27] In addition to high-resolution donor-recipient HLA matching, the more frequent use of tacrolimus,[28–30] the prompt initiation of treatment after a more thorough examination to diagnose GVHD,[31] and supportive care and nutritional management[32] may have contributed to the reduced risk of GVHD-related mortality as did in allo-HCT in remission. Alternatively, the unique HLA epidemiological genetics of Japanese patients may have affected the results.[33,34] As GVHD and infection have been reported to be associated with each other's development and exacerbation,[35–37] an improved control of severe GVHD, along with the introduction of new antifungal drugs, may have led to the reduction of the risk of infection-related mortality. We did not find a reduction in the risk of organ failure-related mortality in any of the subgroups. Although intensified antitumor treatment may be required in allo-HCT in non-remission, continuous effort is needed for monitoring, prevention and intervention with regard to regimen-related toxicity, including late effects.[38–40]

As this analysis is based on a retrospectively collected multicenter database, our results may be susceptible to the disadvantages of any retrospective study using a multicenter registry database. In patients who died without a confirmed hematological remission, we assumed the disease status from the survival time. The impact on transplant outcome of detailed disease status in non-remission patients[41] was not assessed because of the lack of information. In addition, detailed data regarding the incidences of infection or other complications were not available. While we acknowledge these limitations, our data showed that the risks of NRM have decreased after allo-HCT for patients with acute

Table 3. Multivariate analyses for NRM, relapse and overall mortality after allogeneic HCT based on age and donor source

	All patients			*Related HCT*			*UBMT*			*UCBT*		
	N = *3308*			N = *1448*			N = *1164*			N = *485*		
	HR	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*
Patient's age at transplant: 16–49 years												
NRM												
1997–2000	1.00			1.00			1.00					
2001–2004	0.80	(0.63 –1.01)	0.06	0.93	(0.62 –1.40)	0.74	0.64	(0.44–0.92)	0.02	1.00		
2005–2008	0.52	(0.41 –0.68)	<0.01	0.79	(0.51 –1.24)	0.31	0.44	(0.30–0.62)	<0.01	0.60	(0.37 –0.97)	0.04
Relapse												
1997–2000	1.00			1.00			1.00					
2001–2004	1.02	(0.85 –1.21)	0.86	0.83	(0.64 –1.06)	0.14	1.42	(1.07–1.88)	0.02	1.00		
2005–2008	1.13	(0.94 –1.35)	0.19	0.91	(0.70 –1.18)	0.47	1.45	(1.10–1.92)	<0.01	1.28	(0.87 –1.89)	0.22
Overall mortality												
1997–2000	1.00			1.00			1.00					
2001–2004	0.90	(0.77 –1.04)	0.14	0.88	(0.71 –1.08)	0.22	0.92	(0.73–1.15)	0.45	1.00		
2005–2008	0.79	(0.68 –0.92)	<0.01	0.88	(0.71 –1.10)	0.26	0.79	(0.62–0.99)	0.05	0.68	((0.52 –0.90)	<0.0l
Patient's age at transplant: 50–70 years												
NRM												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	0.87	(0.68 –1.11)	0.27	0.91	(0.59 –1.40)	0.67	0.75	(0.49–1.13)	0.17	0.83	(0.54 –1.29)	0.41
Relapse												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	1.00	(0.80 –1.25)	0.99	0.97	(0.72 –1.30)	0.83	1.04	(0.66–1.64)	0.87	1.39	(0.82 –2.35)	0.22
Overall mortality												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	0.95	(0.80 –1.13)	0.53	0.96	((0.75 –1.22)	0.72	0.81	(0.60–1.11)	0.18	1.13	(0.75 –1.72)	0.56

Abbreviations: *CI* = confidence interval; *HCT* = hematopoietic cell transplantation; *HR* = hazard ratio; *NRM* = non-relapse mortality; *UBMT* = unrelated BMT; UCBT = unrelated CB transplantation.
Year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008 among younger patients; because of the small number of HCT performed in 1997–2000, we considered 2001–2004 as reference vs 2005–2008 in subgroup analyses among older patients or those who received unrelated CB transplantation), disease type (AML vs ALL), patient's age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-matched sibling vs other family donors, HLA-matched-unrelated BM, mismatched-unrelated BM or unrelated CB), and conditioning regimens (myeloablative vs reduced-intensity) were considered as covariates. In the analysis for related HCT, HLA-matched sibling vs other family donors were considered as covariates in donor source in addition to the other five factors. In the analysis for unrelated BMT, HLA-matched BM vs mismatched-unrelated BM were considered as covariates in donor source in addition to the other five factors. In the analysis for unrelated CB transplantation, the five covariates were considered other than the donor source.

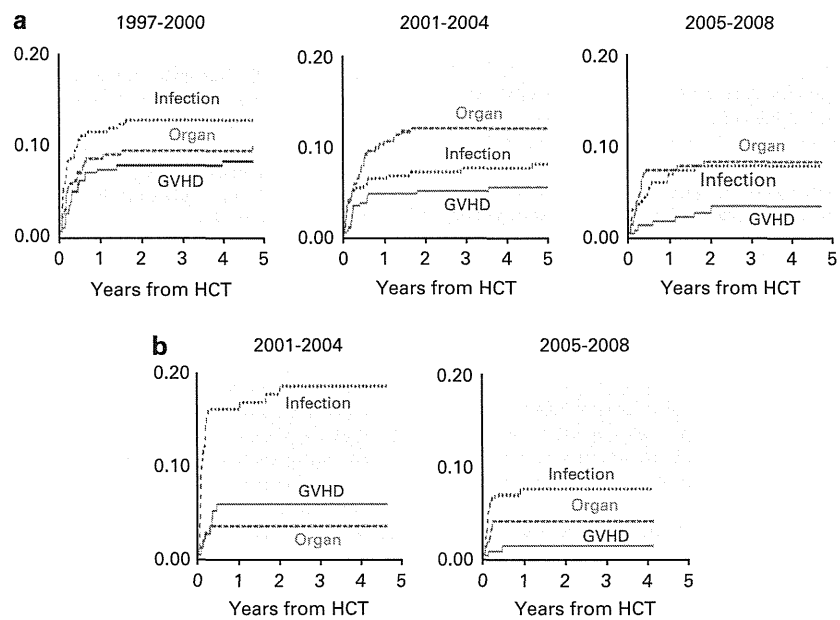

Figure 2. Change in causes of NRM among different time periods in younger patients who received allo-HCT from an unrelated BM donor (**a**), and younger patients who received allo-HCT from an unrelated CB donor (**b**). Because of the small number of transplantation performed in 1997–2000, we considered 2001–2004 as reference in patients who received CB transplantation (Figure 2b). Cumulative incidences of death associated with GVHD (solid line), infection (dotted line) and organ failure (dashed line) are shown in each time period. Significant reductions in the incidences of GVHD-related and infection-related mortality were observed among younger patients who received UBMT (**a**, GVHD: 1997–2000, 8%; 2001–2004, 5%; 2005–2008, 3%; $P = 0.01$, infection: 13%, 7%, 7%; $P = 0.04$, organ failure: 9%, 12%, 8%; $P = 0.58$) and UCBT (**b**, GVHD: 2001–2004, 6%; 2005–2008, 1%; $P = 0.04$, infection: 18%, 7%; $P = 0.02$, organ failure: 3%, 4%; $P = 0.77$).

leukemia in non-remission, using a large nationwide consecutive data. In subgroups that showed a reduced risk of NRM, significant reductions in the incidences of GVHD- and infection-related mortality were observed. We also indicated that there has been no decrease of NRM in older patients or in related donor transplant. In addition, our study showed that non-remission ALL continues to remain a major challenge. As none of the subgroups showed improved survival without a reduction in the HR for NRM, not only the control of refractory disease but also effective prevention, monitoring and treatment of transplant-related complications may be necessary to improve outcomes after allo-HCT in non-remission. Our findings may provide a foundation for future studies to improve outcomes of allo-HCT for acute leukemia in non-remission.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Rapid T-cell chimerism switch and memory T-cell expansion are associated with pre-engraftment immune reaction early after cord blood transplantation

Cord blood (CB) contains immature immune cells and is thought to be less active in inducing allogeneic immune reaction than other sources of stem cells. However, a high incidence of immune-mediated complications has been reported, such as pre-engraftment immune reaction (PIR) and haemophagocytic syndrome (HPS) early after cord blood transplantation (CBT) (Kishi *et al*, 2005; Narimatsu *et al*, 2007; Frangoul *et al*, 2009; Takagi *et al*, 2009; Patel *et al*, 2010). In addition, we reported that human leucocyte antigen (HLA) disparity in the graft-*versus*-host (GVH) direction adversely affected engraftment kinetics when single calcineurin inhibitors were used for GVH disease (GVHD) prophylaxis (Matsuno *et al*, 2009). These observations suggested that the GVH reaction plays a critical role in engraftment. Here, we report the engraftment kinetics of donor-derived T cells using a multicolour flow cytometry-based method (HLA-Flow method) (Watanabe *et al*, 2008) and also describe the results of naïve/memory T-cell phenotype analyses early after CBT.

Between November 2009 and September 2010, 73 adult patients underwent single-unit CBT at Toranomon hospital. This study reports 41 patients who were eligible for chimerism analysis using the HLA-Flow method and survived more than 14 d after CBT. Characteristics of the patients and CB are summarized in Table SI. All patients provided written informed consent, and the study was conducted in accordance with institutional review board requirements. Peripheral blood was collected at 1, 2, 3, 4, and 8 weeks after CBT. Anti-HLA monoclonal antibodies in combination with lineage-specific antibodies were used to analyse the lineage-specific chimerism as previously reported (Watanabe *et al*, 2008). Anti-HLA antibodies specific for donor and recipient HLA in all patients are summarized in Table SII. At 2, 4, and 8 weeks after CBT, T-cell subsets were analysed using the following monoclonal antibodies: peridinin-chlorophyll-protein – cyanin 5·5 (PerCP-Cy5·5)-CD8, phycoerythrin – cyanin 7 (PE-Cy7)-CCR7, allophycocyanin (APC)-CD4, APC-Cy7-CD3 (BD Pharmingen, San Jose, CA, USA), and Pacific Blue-CD45RA (CALTAG, Carlsbad, CA, USA). Absolute numbers of $CD4^+$ T cells ($CD3^+CD4^+$), $CD8^+$ T cells ($CD3^+CD8^+$), and naïve ($CD45RA^+CCR7^+$) and memory ($CD45RA^-CCR7^{+/-}$) T cells were calculated by multiplying the peripheral lymphocyte counts by the percentage of positive cells. PIR was characterized by non-infectious high-grade fever (>38·5°C) coexisting with skin eruption, diarrhoea, jaundice and/or body weight gain greater than 5% of baseline, developing 6 or more days before engraftment (Kishi *et al*, 2005; Uchida *et al*, 2011). Cumulative incidence of neutrophil engraftment, PIR, and GVHD were calculated using Gray's method. Intergroup comparisons were performed using the Mann–Whitney *U*-test.

We analysed lineage-specific chimerism for 32, 40, 40, 34, and 34 patients at a median of 8 (range, 7–11; week 1), 15 (14–20; week 2), 22 (21–25; week 3), 29 (28–36; week 4), and 57 (56–62; week 8) days post-transplant, respectively. Fig 1A shows representative results for $CD4^+$ T-cell chimerism. $CD4^+$ and $CD8^+$ T-cell chimerism results in all patients are shown in Fig 1B. Of 41 enrolled patients, 37 achieved neutrophil engraftment at a median of 19 d (range, 13–38 d). Thirty-nine patients achieved donor-dominant T-cell chimerism (>90%) by 3 weeks after CBT, whereas the remaining two patients, with recipient-dominant T-cell chimerism (>90%) at every point tested, developed graft failure because of early relapse (day 14 post-transplant) and rejection, respectively. Among the 39 patients who achieved donor-dominant T-cell chimerism, two died before engraftment due to non-relapse causes on day 28 (infection) and day 25 (diffuse alveolar haemorrhage), respectively. Among those with donor-dominant chimerism, 24 (63%) of 38 evaluable patients developed PIR at a median of 8 (6–11) days after CBT. Patients who achieved donor-dominant T-cell chimerism (>90%) at 1 week had a higher incidence of PIR compared to those who did not ($P = 0{\cdot}017$, Fig 1C). In a representative patient at 2 weeks after CBT, rapid conversion from naïve to memory phenotype was observed in both $CD4^+$ and $CD8^+$ T cells (Fig 2A). Fig 2B shows the relative proportion of naïve $CD4^+$ and $CD8^+$ T cells at 2, 4, and 8 weeks after CBT in 37 evaluable patients who achieved donor-dominant T-cell chimerism. Patients who developed PIR had significantly more lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+$ memory T cells, and $CD8^+$ memory T cells at 2 weeks after CBT compared with those without PIR (Fig 2C and data not shown).

Our data confirmed that a majority of patients achieved donor-dominant T-cell chimerism around 2 weeks after CBT. We also found that early recipient-type T-cell chimerism was closely associated with graft rejection. A remarkable finding was that a rapid recipient-to donor-dominant switch

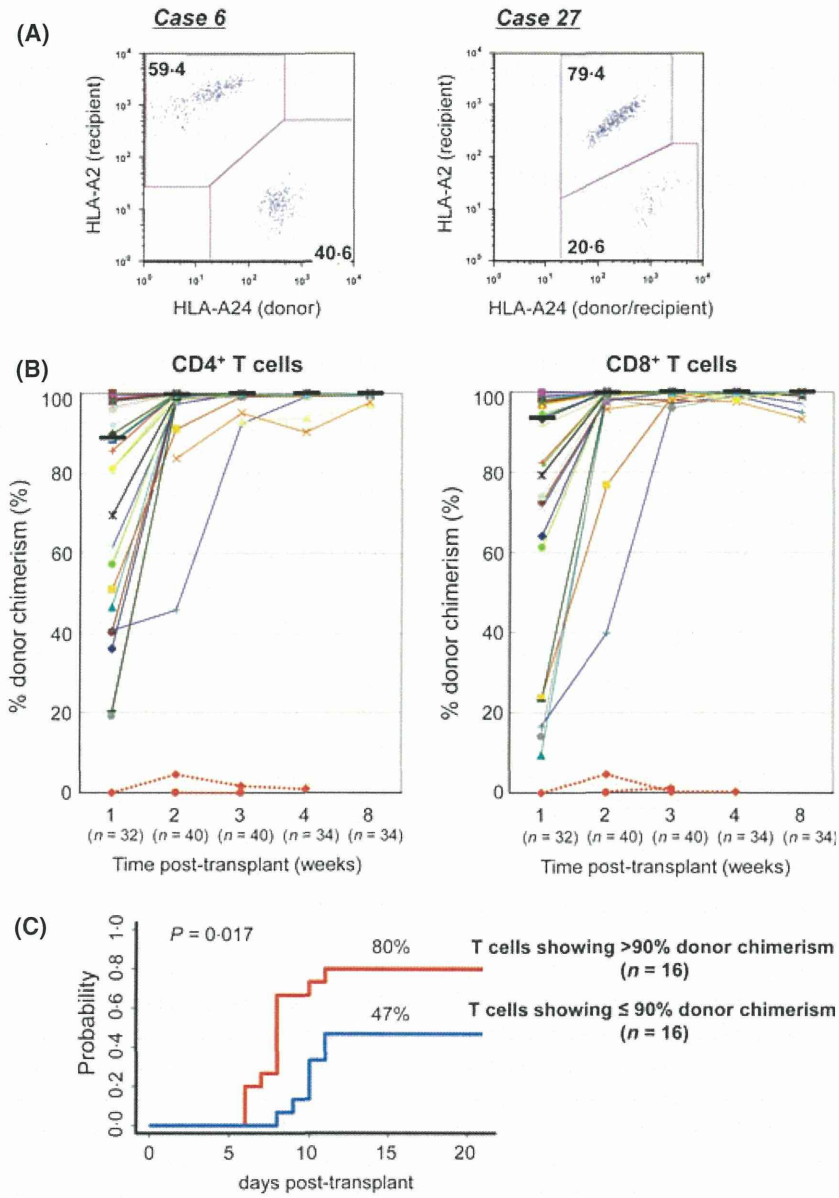

Fig 1. T-cell chimerism analysed by HLA-Flow method. (A) Chimerism analysis by the HLA-Flow method separated donor- vs. recipient-derived cells among $CD4^+$ T cells at 1 week after cord blood transplant (CBT). In Case 6, human leucocyte antigen (HLA)-A2 was recipient-specific and HLA-A24 was donor-specific. In Case 27, HLA-A2 was recipient-specific, whereas HLA-A24 was shared by both donor and recipient, indicating that HLA-A2-negative and HLA-A24-positive cells were donor-derived. (B) The median percentages of donor-derived $CD4^+$ T cells and $CD8^+$ T cells at 1 week after CBT were 88·9%, and 93·5%, respectively. Red dotted lines indicate recipient-dominant chimerism in two patients who developed graft failure. (C) Cumulative incidence of pre-engraftment immune reaction (PIR) according to chimerism status of T cells at 1 week after CBT

of T-cell chimerism at 1 week post-transplant was associated with a higher incidence of PIR, supporting a hypothesis that PIR could be an early variant form of GVH reaction caused by donor-derived T cells. CB T cells are naïve and do not include pathogen-specific effector T cells. Grindebacke *et al* (2009) demonstrated that about 80% of $CD4^+$ T cells kept the naïve phenotype during the first 18 months after birth. In contrast, we found a rapid conversion from naïve to memory phenotype at 2 weeks after CBT. In addition, PIR could be associated with peripheral expansion of donor-derived memory T cells. Recently, Gutman *et al* (2010) reported that $CD8^+$ T cells predominately expressed effector memory or effector phenotype early after double-unit CBT, reflecting an immune response of the dominant unit against the non-engrafting unit. These findings suggest that donor-derived naïve T cells will be activated by alloantigens and differentiate into mature cells early after CBT. Most of the present patients with PIR responded promptly after a

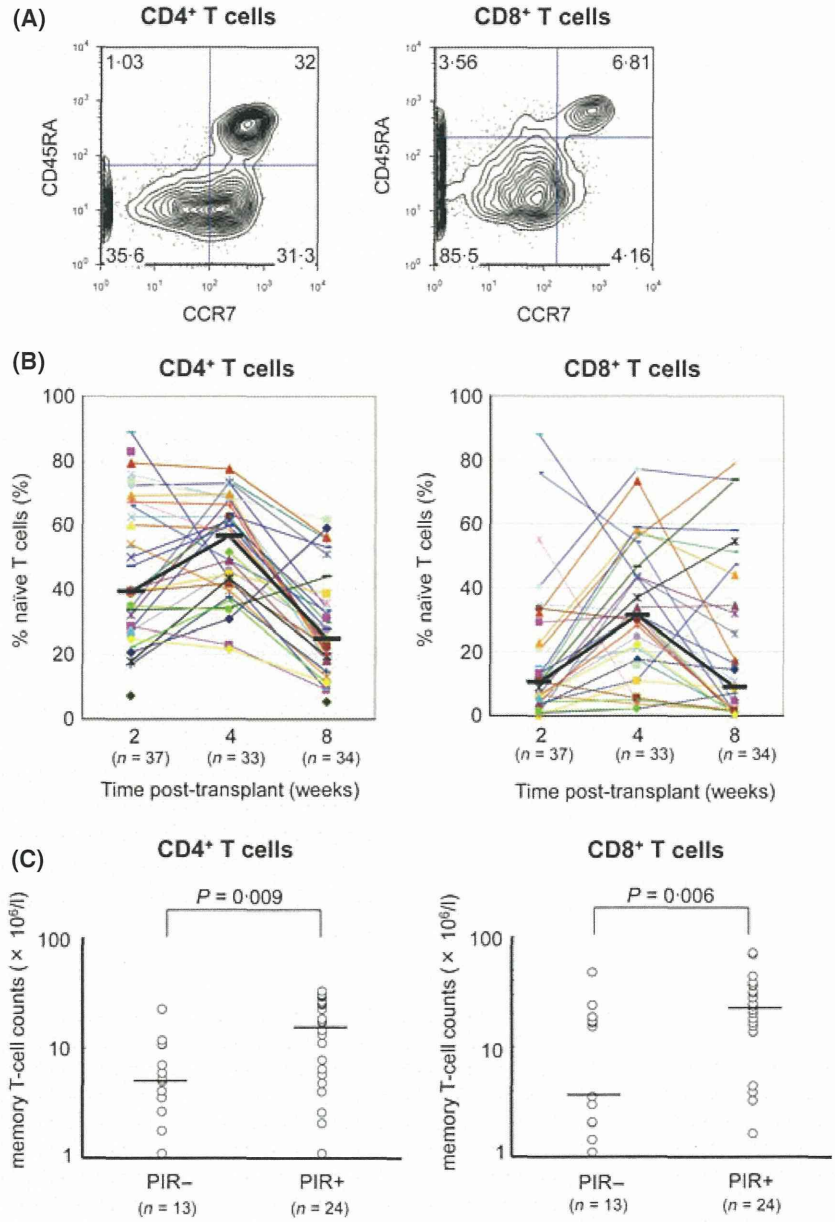

Fig 2. Conversion from naïve to memory T-cell phenotype. (A) A rapid conversion from naïve phenotype ($CD45RA^{+}CCR7^{+}$) to memory phenotype ($CD45RA^{-}CCR7^{+/-}$) in a representative sample at 2 weeks after cord blood transplant (CBT) (Case 5). (B) Relative proportion of naïve $CD4^{+}$ and $CD8^{+}$ T cells at 2, 4, and 8 weeks after CBT. Bold horizontal lines denote median values. (C) Memory T-cell counts at 2 weeks after CBT in patients with or without pre-engraftment immune reaction (PIR).

short course of steroid treatment, and none experienced graft failure due to HPS. This observation could be attributed to more intensive immunosuppression from adding mycophenolate mofetil to tacrolimus in the majority of patients (Uchida *et al*, 2011). Although neither the T-cell chimerism nor the memory T-cell counts affected the incidence of acute GVHD, steroid treatment for PIR could suppress the onset of acute GVHD. In conclusion, rapid T-cell chimerism switch and donor-derived memory T-cell expansion were associated with PIR, supporting a significant role of donor-derived T cells in the pathogenesis of the early immune reaction after CBT.

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Author contributions

NM, HY, NW, HN, and ST designed the study; NM, HY, and NW performed the research; NM and HY analysed data; HY, NU, HO, AN, TI, K Ishiwata, NN, MT, Y A-M, K Izutsu, KM, AW, and ST performed transplantation; AY reviewed histopathological findings; and NM, HY, NW, NU, HN, and ST contributed to writing the paper.

Competing interests

The authors have no competing interests.

Naofumi Matsuno[1,2]*
Hisashi Yamamoto[2]*
Nobukazu Watanabe[1]
Naoyuki Uchida[2]
Hikari Ota[2]
Aya Nishida[2]
Taichi Ikebe[2]
Kazuya Ishiwata[2]
Nobuaki Nakano[2]
Masanori Tsuji[2]
Yuki Asano-Mori[2]
Koji Izutsu[2]
Kazuhiro Masuoka[2]
Atsushi Wake[2]
Akiko Yoneyama[3]
Hiromitsu Nakauchi[4]
Shuichi Taniguchi[2,5]

[1]*Laboratory of Diagnostic Medicine, Division of Stem Cell Therapy, Centre for Stem Cell Biology and Regenerative Medicine, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan,* [2]*Department of Haematology, Toranomon Hospital, Tokyo, Japan,* [3]*Department of Infectious Diseases, Toranomon Hospital, Tokyo, Japan,* [4]*Division of Stem Cell Therapy, Centre for Stem Cell Biology and Regenerative Medicine, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan and* [5]*Okinaka Memorial Institute for Medical Research, Tokyo, Japan*
E-mail: nwatanab@ims.u-tokyo.ac.jp

*The first two authors contributed equally to this work.

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Supporting Information

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

Table SI. Patient and cord blood characteristics.

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