continuous variables. The probability of OS was estimated according to the Kaplan–Meier method, and the groups were compared using the log-rank test. The probabilities of the others were estimated on the basis of a cumulative incidence method to accommodate competing risks.[22] Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS, and a Fine and Gray proportional hazards model for the others.[23] The following variables were considered: age (<45 vs ⩾45 years), CMV serostatus (negative vs positive), disease status at CBT (standard risk vs high risk), cord blood $CD34^+$ cell count ($<1 \times 10^5$ vs $\geqslant 1 \times 10^5$/kg), cord blood nucleated cell count ($<2.5 \times 10^7$ vs $\geqslant 2.5 \times 10^7$/kg), HLA disparities (⩽2 vs ⩾3) and donor–recipient sex compatibility (F→F vs F→M vs M→F vs M→M). In this study, the F→F group was considered the reference group in the multivariate analyses, because this group is not influenced by H-Y Ags. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria).[24] $P<0.05$ was considered significant. Analysis of data was performed in August 2013.

RESULTS

Patients

The characteristics of patients and cord blood units are shown in Table 1. There were no significant differences among the four sex combination groups, except for cord blood nucleated cell counts (Table 1). The median period of follow-up for survivors after CBT was 92 months (range, 5–181 months).

GVHD

CBT in the F→M group had a theoretical increased risk of both aGVHD and cGVHD, because H-Y Ags can be targets for donor T cells and B cells. In univariate analysis, there was no significant difference in the cumulative incidence of grade II–IV aGVHD among the four groups (Figure 1a). In multivariate analysis, a lower cord blood $CD34^+$ cell count (hazard ratio (HR), 0.66; 95% confidence interval (CI), 0.46–0.94; $P=0.02$) and high risk of disease status at CBT (HR, 0.69; 95% CI, 0.48–0.98; $P=0.04$) were associated with a lower incidence of grade II–IV aGVHD. Nevertheless, the incidence of grade II–IV aGVHD was similar for the F→M group, M→F group and M→M group compared with the F→F group (Table 2). In addition, there was no significant difference in the cumulative incidence of grade III–IV aGVHD among the four groups ($P=0.34$) (Table 2). In univariate analysis, there was no significant difference in the cumulative incidence of extensive cGVHD among the four groups ($P=0.11$) (Figure 1b). Multivariate analysis indicated that the F→M group was a significant risk factor for extensive cGVHD compared with the F→F group (HR, 2.97; 95% CI, 1.14–7.69; $P=0.02$) (Table 2).

OS, relapse and TRM

The probability of OS at 7 years did not differ significantly among the four groups in univariate analysis ($P=0.32$) (Figure 1c) and

Table 1. Characteristics of patients, cord blood units and transplantation

	Total	*F→F*	*F→M*	*M→F*	*M→M*	P-*value*
Number (%)	191	37 (19)	59 (30)	39 (20)	56 (29)	
Age (years), median (range)	40 (16–55)	40 (18–53)	41 (16–52)	43 (16–55)	38 (16–55)	0.91
Disease type, number (%)						0.10
AML	101 (52)	17 (45)	28 (47)	28 (71)	28 (50)	
ALL	45 (23)	14 (37)	10 (16)	6 (15)	15 (26)	
MDS	25 (13)	4 (10)	11 (18)	1 (2)	9 (16)	
CML	11 (5)	1 (2)	6 (10)	2 (5)	2 (3)	
NHL	9 (4)	1 (2)	4 (6)	2 (5)	2 (3)	
Disease status at CBT, number (%)[a]						0.95
Standard risk	79 (41)	15 (40)	26 (44)	15 (38)	23 (41)	
High risk	112 (58)	22 (59)	33 (55)	24 (61)	33 (58)	
CMV serostatus, number (%)						0.60
Positive	166 (86)	34 (91)	52 (88)	34 (87)	46 (82)	
Negative	25 (13)	3 (8)	7 (11)	5 (12)	10 (17)	
Conditioning regimen, number (%)						0.07
TBI 12 Gy + Ara-C/G-CSF + CY	131 (68)	21 (56)	44 (74)	28 (71)	38 (67)	
TBI 12 Gy + Ara-C + CY	31 (16)	10 (27)	10 (6)	2 (5)	9 (16)	
TBI 12 Gy + CY	16 (8)	5 (13)	2 (3)	3 (7)	6 (10)	
TBI 12 Gy + others	13 (6)	1 (2)	3 (5)	6 (15)	3 (5)	
GVHD prophylaxis, number (%)						0.83
CsA + MTX	188 (98)	37 (100)	58 (98)	38 (97)	55 (98)	
CsA	3 (1)	0 (0)	1 (1)	1 (2)	1 (1)	
Number of nucleated cells, $\times 10^7$/kg, median (range)	2.43 (1.32–5.69)	2.66 (1.92–5.50)	2.38 (1.51–5.07)	2.58 (1.72–5.69)	2.28 (1.32–4.09)	<0.01
Number of $CD34^+$ cells, $\times 10^5$/kg, median (range)	0.92 (0.17–7.75)	0.89 (0.34–2.81)	0.93 (0.32–7.75)	1.07 (0.17–2.64)	0.92 (0.28–3.15)	0.77
HLA disparities, number (%)[b]						0.21
⩽2	129 (67)	30 (81)	37 (62)	27 (69)	35 (62)	
⩾3	62 (32)	7 (18)	22 (37)	12 (30)	21 (37)	

Abbreviations: Ara-C = cytosine arabinoside; MDS = myelodysplastic syndrome; NHL = non-Hodgkin's lymphoma. [a]Disease status at CBT was classified as standard risk or high risk; CR1 or CR2 without poor prognostic karyotype for AML and ALL, refractory anemia for MDS, chronic phase for CML, and CR1 or CR2 for NHL were classified as standard risk, whereas patients in all other situations were classified as high risk. [b]The number of HLA disparities defined as low resolution for HLA-A and -B and high resolution for HLA-DRB1.

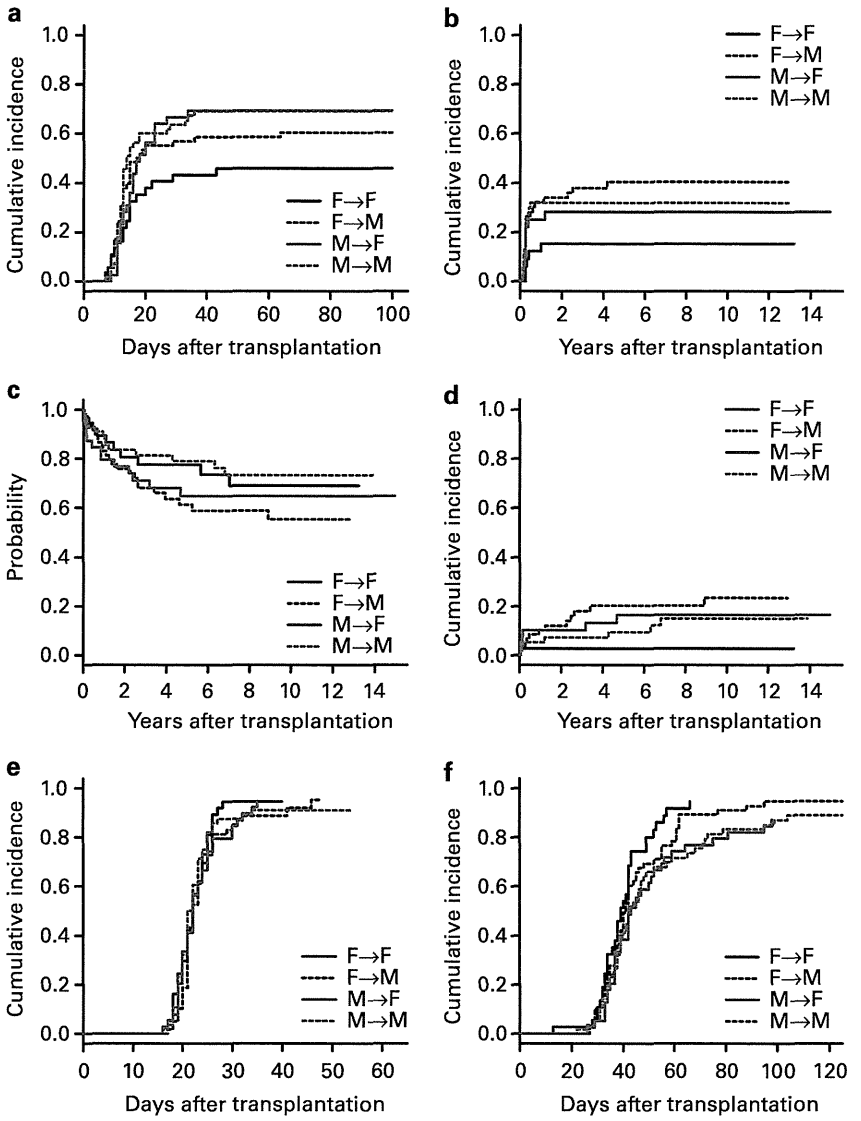

Figure 1. Cumulative incidence of grade II–IV aGVHD (**a**), extensive cGVHD (**b**), probability of OS (**c**), cumulative incidence of TRM (**d**), neutrophil engraftment (**e**) and platelet engraftment (**f**) according to donor–recipient sex combination after CBT.

multivariate analysis (Table 2). We also analyzed a subgroup of patients with standard risk or high risk of disease status at CBT. However, we were unable to find any impact of sex incompatibility on survival relating to disease risk at CBT (data not shown). Sex incompatibility was not associated with cumulative incidence of relapse (Table 2). A trend toward a higher incidence of TRM was observed in the F→M group compared with the F→F group, but this was not significant in univariate analysis ($P=0.09$) (Figure 1d) or multivariate analysis ($P=0.07$) (Table 2).

Engraftment

Female recipients with male donors have a theoretical increased risk of graft rejection, because the H-Y Ags can be a target for recipient T cells and B cells. In univariate analysis, there was no significant difference in the cumulative incidence of neutrophil engraftment among the four groups (Figure 1e). In multivariate analysis, a lower cord blood $CD34^+$ cell count (HR, 0.51; 95% CI, 0.37–0.70; $P<0.001$) and high risk of disease status at CBT (HR, 0.68; 95% CI, 0.51–0.93; $P=0.01$) were associated with a lower incidence of neutrophil engraftment, but sex incompatibility was not associated with neutrophil engraftment (Table 2). In univariate analysis, the cumulative incidence of platelet recovery was not significantly different among the four groups (Figure 1f). In multivariate analysis, the M→F group showed a significantly lower incidence of platelet engraftment when compared with the F→F group (HR, 0.56; 95% CI, 0.34–0.091; $P=0.02$) (Table 2). In addition, a lower cord blood $CD34^+$ cell count (HR, 0.58; 95% CI, 0.42–0.80; $P<0.01$), positive CMV serostatus (HR, 0.48; 95% CI, 0.32–0.74; $P<0.001$) and high risk of disease status at CBT (HR, 0.57; 95% CI, 0.41–0.78; $P<0.001$) were associated with a lower incidence of platelet engraftment.

DISCUSSION

We examined the effects of donor–recipient sex incompatibility on the outcome of CBT in our institute. The effect of sex incompatibility on transplant outcome might differ depending on the kinds of stem cell sources in allo-HSCT. Gallardo *et al.*[6] demonstrated the association of a sex-mismatch and grade III–IV aGVHD, higher TRM and lower OS in HLA-compatible BMT, but not HLA-compatible PBSC transplantation. In addition, Sterm *et al.*[25] reported the largest retrospective study for evaluating the effect of sex incompatibility in 53 988 allogeneic transplants using

Table 2. Univariate and multivariate analyses of sex compatibility for the outcomes of CBT

	Univariate analysis			*Multivariate analysis*		
	Number	*% (95% CI)*	*P-value*	*Hazard Ratio*	*95% CI*	*P-value*
Grade II–IV aGVHD at 100 days			0.17			
F→F	37	45.9 (29.2–61.2)		1.00		Reference
F→M	58	60.3 (46.4–71.7)		1.42	0.75–2.69	0.27
M→F	39	69.2 (51.7–81.5)		1.57	0.84–2.90	0.15
M→M	55	69.1 (54.8–79.7)		1.83	0.99–3.38	0.05
Grade III–IV acute GVHD at 100 days			0.34			
F→F	37	2.7 (0.2–12.3)		1.00		Reference
F→M	58	12.1 (5.3–21.9)		4.19	0.55–32.00	0.17
M→F	39	12.8 (4.6–25.4)		4.07	0.48–33.90	0.19
M→M	55	7.3 (2.3–16.2)		2.36	0.30–18.24	0.41
Extensive cGVHD at 3 years			0.11			
F→F	32	15.3 (5.4–29.7)		1.00		Reference
F→M	53	38.0 (24.9–51.0)		2.97	1.14–7.69	0.02
M→F	31	28.3 (13.9–44.6)		1.62	0.54–4.80	0.38
M→M	47	32.0 (19.2–45.5)		2.43	0.86–6.83	0.09
OS at 7 years[a]			0.32			
F→F	37	69.1 (49.3–82.4)		1.00		Reference
F→M	59	58.9 (44.2–71.0)		1.91	0.85–4.28	0.11
M→F	39	64.9 (47.2–78.0)		1.58	0.66–3.76	0.29
M→M	56	73.4 (58.2–83.9)		0.99	0.40–2.46	0.98
Relapse at 7 years			0.29			
F→F	37	31.5 (16.7–47.5)		1.00		Reference
F→M	59	21.6 (11.8–33.4)		0.80	0.32–1.97	0.63
M→F	39	23.4 (11.4–37.8)		0.89	0.34–2.32	0.82
M→M	56	13.1 (5.7–23.7)		0.52	0.17–1.55	0.25
TRM at 7 years			0.10			
F→F	37	2.7 (0.2–12.3)		1.00		Reference
F→M	59	20.1 (10.6–31.8)		7.96	0.82–77.19	0.07
M→F	39	16.4 (6.5–30.4)		5.62	0.58–53.67	0.13
M→M	56	15.0 (6.4–27.1)		3.95	0.38–40.64	0.25
Neutrophil engraftment at 60 days			0.76			
F→F	37	94.6 (75.8–98.9)		1.00		Reference
F→M	59	95.1 (75.9–99.1)		0.79	0.51–1.21	0.28
M→F	39	94.9 (73.5–99.1)		0.85	0.53–1.34	0.49
M→M	56	91.1 (78.8–96.4)		1.01	0.64–1.60	0.95
Platelet engraftment at 100 days			0.05			
F→F	37	94.6 (72.1–99.1)		1.00		Reference
F→M	59	94.7 (82.3–98.5)		0.80	0.51–1.24	0.33
M→F	39	87.2 (69.0–95.0)		0.56	0.34–0.91	0.02
M→M	56	87.0 (73.6–93.9)		0.63	0.38–1.03	0.06

Abbreviation: CI = confidence interval. [a]Hazards ratio for overall mortality.

various hematopoietic stem cell sources. As only 734 (1.3%) patients received CBT among the 53 988 patients, the details of CBT outcomes were not described. In the CBT setting, the sex of recipients has become a confounding factor for outcome.[26,27] Although sex incompatibility was considered as a factor influencing the outcome in several CBT studies,[14–16,28,29] there have been no reports detailing the effect of sex incompatibility on outcomes after CBT. Our results showed a higher risk of extensive cGVHD in the F→M group and a lower incidence of platelet engraftment in the M→F group compared with the F→F group in the multivariate analysis. These effects might be associated with allogeneic immune responses against H-Y Ags. However, there were no differences in OS, TRM and relapse among all four groups.

Male recipients with female donors had an increased incidence of GVHD, particularly cGVHD,[3–7,30] which led to a higher TRM and lower OS in the setting of HLA-compatible allo-HSCT. Recently, Newell *et al.*[31] demonstrated a shorter duration and a higher response of cGVHD to systemic immunosuppressive treatment in CBT recipients than in BMT or PBSC transplantation recipients, which might have contributed to extensive cGVHD not being shown to increase TRM in our study. It has been reported that a decreased risk of relapse is associated with aGVHD and cGVHD in leukemia patients after BMT.[32] In fact, several studies demonstrated that male recipients with female donors had a lower risk of relapse in leukemia and myeloma,[3–5] suggesting that sex incompatibility may contribute to the GVL effects. However, we were unable to find any impact of sex incompatibility on relapse in the entire cohort and in subgroup analysis on the basis of disease risk at CBT (data not shown). On the other hand, it has been reported that female recipients with male donors had a higher risk of graft failure in aplastic anemia following allo-HSCT.[8] Moreover, the role of H-Y Ags in graft rejection of solid organ transplantations has also been reported extensively, particularly in kidney transplantations.[33,34] In our study, female recipients with

male donors were significantly associated with a lower incidence of platelet engraftment, but not neutrophil engraftment. Our data showed that the most important factor in neutrophil and platelet engraftment was the cord blood $CD34^+$ cell count, indicating that this is more important than sex incompatibility in engraftment after CBT.

In HLA-compatible allo-HSCT, GVHD and GVL effects might be induced by allogeneic immune responses against mHA, which can be presented by HLA in normal or leukemia cells. mHA are HLA-restricted polymorphic peptides derived from intracellular proteins encoded by polymorphic genes. H-Y Ags can be recognized by female donor or recipient T cells in the setting of sex-mismatched allo-HSCT. Several H-Y Ags, such as SMCY, DFFRY, UTY, DBY, RPS4Y and TMSB4Y, have been identified and were recognized by either $CD4^+$ or $CD8^+$ T cells.[35,36] It has been reported that male recipients with female donors have a greater risk of cGVHD than aGVHD in allo-HSCT.[3–7,30] In addition, the detection of alloantibody and alloantibody-producing B cells against H-Y Ags was associated with cGVHD in sex-mismatched allo-HSCT.[37,38] In fact, our data also showed that male recipients with female donors were significantly associated with a higher incidence of extensive cGVHD, but not aGVHD. Although the existence of alloantibodies against H-Y Ags was not clarified in our study, specific alloantibodies against H-Y Ags should be investigated in future studies.

In conclusion, our data showed that donor–recipient sex combination affects the incidences of extensive cGVHD and platelet engraftment, but does not have a significant effect on the OS after CBT. However, these results should be interpreted with caution because this retrospective study included a relatively small number of Japanese patients who received single-unit CBT following 12 Gy TBI-based myeloablative conditioning regimens for hematological malignancies. Although these findings should be confirmed in prospective studies, donor–recipient sex combination does not appear to have a significant impact on survival after CBT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank all of the physicians and staff at the hospitals and the eight cord blood banks in Japan for their help in this study. This work was supported in part by The Kobayashi Foundation.

REFERENCES

1 Warren EH, Zhang XC, Li S, Fan W, Storer BE, Chien JW *et al.* Effect of MHC and non-MHC donor/recipient genetic disparity on the outcome of allogeneic HCT. *Blood* 2012; **120**: 2796–2806.
2 Gahrton G. Risk assessment in haematopoietic stem cell transplantation: impact of donor-recipient sex combination in allogeneic transplantation. *Best Pract Res Clin Haematol* 2007; **20**: 219–229.
3 Gratwohl A, Hermans J, Niederwieser D, van Biezen A, van Houwelingen HC, Apperley J. Female donors influence transplant-related mortality and relapse incidence in male recipients of sibling blood and marrow transplants. *Hematol J* 2001; **2**: 363–370.
4 Randolph SS, Gooley TA, Warren EH, Appelbaum FR, Riddell SR. Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. *Blood* 2004; **103**: 347–352.
5 Gahrton G, Iacobelli S, Apperley J, Bandini G, Björkstrand B, Bladé J *et al.* The impact of donor gender on outcome of allogeneic hematopoietic stem cell transplantation for multiple myeloma: reduced relapse risk in female to male transplants. *Bone Marrow Transplant* 2005; **35**: 609–617.
6 Gallardo D, Pérez-García A, de la Cámara R, Iriondo A, Jiménez-Velasco A, Torres A *et al.* Clinical outcome after sex-mismatched allogeneic stem cell transplantation from human lymphocyte antigen-identical sibling donors: influence of stem cell source. *Leukemia* 2006; **20**: 1461–1464.
7 Loren AW, Bunin GR, Boudreau C, Champlin RE, Cnaan A, Horowitz MM *et al.* Impact of donor and recipient sex and parity on outcomes of HLA-identical sibling allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006; **12**: 758–769.
8 Stern M, Passweg JR, Locasciulli A, Socié G, Schrezenmeier H, Békássy AN *et al.* Influence of donor/recipient sex matching on outcome of allogeneic hematopoietic stem cell transplantation for aplastic anemia. *Transplantation* 2006; **82**: 218–226.
9 Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood* 2013; **122**: 491–498.
10 Eapen M, Rocha V, Sanz G, Scaradavou A, Zhang MJ, Arcese W *et al.* Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. *Lancet Oncol* 2010; **11**: 653–660.
11 Atsuta Y, Suzuki R, Nagamura-Inoue T, Taniguchi S, Takahashi S, Kai S *et al.* Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. *Blood* 2009; **113**: 1631–1638.
12 Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M *et al.* Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood* 2007; **109**: 1322–1330.
13 Takahashi S, Iseki T, Ooi J, Tomonari A, Takasugi K, Shimohakamada Y *et al.* Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood* 2004; **104**: 3813–3820.
14 Atsuta Y, Kanda J, Takanashi M, Morishima Y, Taniguchi S, Takahashi S *et al.* Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia. *Haematologica* 2013; **98**: 814–822.
15 Kanda J, Atsuta Y, Wake A, Ichinohe T, Takanashi M, Morishima Y *et al.* Impact of the direction of HLA mismatch on transplantation outcomes in single unrelated cord blood transplantation. *Biol Blood Marrow Transplant* 2013; **19**: 247–254.
16 Eapen M, Klein JP, Sanz GF, Spellman S, Ruggeri A, Anasetti C *et al.* Effect of donor-recipient HLA matching at HLA A, B, C, and DRB1 on outcomes after umbilical-cord blood transplantation for leukaemia and myelodysplastic syndrome: a retrospective analysis. *Lancet Oncol* 2011; **12**: 1214–1221.
17 Stevens CE, Carrier C, Carpenter C, Sung D, Scaradavou A. HLA mismatch direction in cord blood transplantation: impact on outcome and implications for cord blood unit selection. *Blood* 2011; **118**: 3969–3978.
18 Barker JN, Scaradavou A, Stevens CE. Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood* 2010; **115**: 1843–1849.
19 Matsuno N, Wake A, Uchida N, Ishiwata K, Araoka H, Takagi S *et al.* Impact of HLA disparity in the graft-versus-host direction on engraftment in adult patients receiving reduced-intensity cord blood transplantation. *Blood* 2009; **114**: 1689–1695.
20 Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA *et al.* Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* 1974; **18**: 295–304.
21 Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE *et al.* Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; **69**: 204–217.
22 Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med* 1999; **18**: 695–706.
23 Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496–509.
24 Kanda Y. Investigation of the freely-available easy-to-use software 'EZR' (Easy R) for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452–458.
25 Stern M, Brand R, de Witte T, Sureda A, Rocha V, Passweg J *et al.* Female-versus-male alloreactivity as a model for minor histocompatibility antigens in hematopoietic stem cell transplantation. *Am J Transplant* 2008; **8**: 2149–2157.
26 Gluckman E, Rocha V, Arcese W, Michel G, Sanz G, Chan KW *et al.* Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. *Exp Hematol* 2004; **32**: 397–407.
27 Arcese W, Rocha V, Labopin M, Sanz G, Iori AP, de Lima M *et al.* Unrelated cord blood transplants in adults with hematologic malignancies. *Haematologica* 2006; **91**: 223–230.
28 Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R *et al.* Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. *N Engl J Med* 1997; **337**: 373–381.
29 Cohen YC, Scaradavou A, Stevens CE, Rubinstein P, Gluckman E, Rocha V *et al.* Factors affecting mortality following myeloablative cord blood transplantation in

adults: a pooled analysis of three international registries. *Bone Marrow Transplant* 2011; **46**: 70–76.
30 Flowers ME, Inamoto Y, Carpenter PA, Lee SJ, Kiem HP, Petersdorf EW *et al.* Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. *Blood* 2011; **117**: 3214–3219.
31 Newell LF, Flowers ME, Gooley TA, Milano F, Carpenter PA, Martin PJ *et al.* Characteristics of chronic GVHD after cord blood transplantation. *Bone Marrow Transplant* 2013; **48**: 1285–1290.
32 Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; **75**: 555–562.
33 Dierselhuis M, Goulmy E. The relevance of minor histocompatibility antigens in solid organ transplantation. *Curr Opin Organ Transplant* 2009; **14**: 419–425.
34 Gratwohl A, Döhler B, Stern M, Opelz G. H-Y as a minor histocompatibility antigen in kidney transplantation: a retrospective cohort study. *Lancet* 2008; **372**: 49–53.
35 Akatsuka Y, Morishima Y, Kuzushima K, Kodera Y, Takahashi T. Minor histocompatibility antigens as targets for immunotherapy using allogeneic immune reactions. *Cancer Sci* 2007; **98**: 1139–1146.
36 Feng X, Hui KM, Younes HM, Brickner AG. Targeting minor histocompatibility antigens in graft versus tumor or graft versus leukemia responses. *Trends Immunol* 2008; **29**: 624–632.
37 Sahaf B, Yang Y, Arai S, Herzenberg LA, Herzenberg LA, Miklos DB. H-Y antigen-binding B cells develop in male recipients of female hematopoietic cells and associate with chronic graft vs host disease. *Proc Natl Acad Sci USA* 2013; **110**: 3005–3010.
38 Miklos DB, Kim HT, Miller KH, Guo L, Zorn E, Lee SJ *et al.* Antibody responses to H-Y minor histocompatibility antigens correlate with chronic graft-versus-host disease and disease remission. *Blood* 2005; **105**: 2973–2978.

Biol Blood Marrow Transplant xxx (2014) 1–6

Comparable Long-Term Outcome of Unrelated Cord Blood Transplantation with Related Bone Marrow or Peripheral Blood Stem Cell Transplantation in Patients Aged 45 Years or Older with Hematologic Malignancies after Myeloablative Conditioning

Takaaki Konuma [1,*], Seiko Kato [1], Jun Ooi [2], Maki Oiwa-Monna [1], Toyotaka Kawamata [1], Arinobu Tojo [1], Satoshi Takahashi [1]

[1] *Department of Hematology/Oncology, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[2] *Department of Hematology/Oncology, Teikyo University School of Medicine, Tokyo, Japan*

Article history:
Received 25 February 2014
Accepted 4 April 2014

Key Words:
Cord blood transplantation
Bone marrow transplantation
Peripheral blood stem cell transplantation
Related donor
Donor age

A B S T R A C T

We investigated whether bone marrow or peripheral blood stem cells from older sibling donors or cord blood from unrelated donors provided a better outcome in allogeneic hematopoietic stem cell transplantation for relatively older patients who were candidates for myeloablative conditioning. Clinical outcomes of 97 patients aged 45 years or older with hematologic malignancies who received unrelated cord blood transplantation (CBT) (n = 66) or bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT) from related donors (n = 31) were compared. The cumulative incidences of grades III to IV acute and extensive chronic graft-versus-host diseases were similar between both groups. Although transplant-related mortality was significantly lower after CBT compared with BMT/PBSCT from related donors (hazard ratio [HR], .29, $P = .04$), overall mortality (HR, .72, $P = .47$) and relapse (HR, 2.02, $P = .23$) were not significantly different after CBT and BMT/PBSCT from related donors. These data suggest that CBT could be as safe and effective as BMT/PBSCT from older related donors for relatively older patients when it is used as a primary unrelated stem cell source.

INTRODUCTION

Donor age has been associated with transplant outcomes in allogeneic hematopoietic stem cell transplantation (allo-HSCT) after myeloablative conditioning or reduced-intensity conditioning (RIC) [1-5]. Older donor age resulted in an increased incidence of severe graft-versus-host disease (GVHD), which led to higher transplant-related mortality (TRM) or overall mortality after allo-HSCT from unrelated adult donors [1,2]. In contrast, it is difficult to determine the exact effect of the age of related donors, because increasing recipient age is frequently accompanied by increased donor age after allo-HSCT from related donors. However, older donor age of related donors may also be associated with adverse outcomes [3-5].

Several studies, including ours, comparing both cord blood transplantation (CBT) and bone marrow transplantation (BMT)/peripheral blood stem cell transplantation (PBSCT) from unrelated donors after myeloablative conditioning in adult patients demonstrated that the incidence of severe GVHD was significantly lower after CBT than after unrelated BMT/PBSCT. The survival rate and relapse incidence in CBT recipients were comparable with those in unrelated BMT/PBSCT recipients [6-9]. Moreover, we also demonstrated similar survival, relapse, and TRM between unrelated CBT and related BMT/PBSCT (rBMT/PBSCT) recipients [10]. The incidences of grades III to IV acute GVHD (aGVHD) and extensive chronic GVHD (cGVHD) among CBT recipients were also significantly lower than those among rBMT/PBSCT recipients. Because the lower risk of severe GVHD is one of the most attractive advantages of CBT, the use of cord blood instead of bone marrow or mobilized peripheral blood as a stem cell source might offer the possibility of decreasing severe GVHD in older patients. However, there has been no comparative study between CBT and BMT/PBSCT from older related donors after myeloablative conditioning in relatively older patients.

We previously reported that unrelated CBT after myeloablative conditioning is feasible in patients over the age of 45 years [11,12]. In this retrospective study, we report on a clinical comparison of CBT from unrelated donors and BMT/PBSCT from older related donors in patients older than 45 years of age with hematologic malignancies who were candidates for a myeloablative conditioning.

METHODS

Patients and Transplant Procedures

This retrospective study included 97 consecutive patients, 45 years of age or older, who received CBT (n = 66) from unrelated donors or BMT (n = 26) or PBSCT (n = 5) from related donors for acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and non-Hodgkin lymphoma (NHL) at the Institute of Medical Science, University of Tokyo between May 1992 and July 2013. Nineteen patients who received rBMT/PBSCT and 32 patients who received CBT were included from our previous study with extended

Financial disclosure: See Acknowledgments on page 5.

* Correspondence and reprint requests: Takaaki Konuma, Department of Hematology/Oncology, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail address: tkonuma@ims.u-tokyo.ac.jp (T. Konuma).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.04.005

Table 1
Characteristics of Patients, Grafts, and Transplantation

Characteristic	rBMT/PBSCT	CBT	*P*
Number of patients	31	66	
Recipient age, yr, median (range)	48 (45-58)	49 (45-55)	.60
Recipient sex, n (%)			.51
Male	20 (64)	37 (56)	
Female	11 (35)	29 (43)	
Recipient CMV serostatus, n (%)			.18
Positive	28 (90)	64 (96)	
Negative	0 (0)	2 (3)	
Unknown	3 (9)	0 (0)	
Disease type, n (%)			.08
AML	16 (51)	44 (66)	
MDS	2 (6)	8 (12)	
CML	6 (19)	3 (4)	
ALL	3 (9)	8 (12)	
NHL	4 (12)	3 (4)	
Disease status at transplantation,* n (%)			.48
Standard	8 (25)	23 (34)	
High	23 (74)	43 (65)	
Conditioning regimen, n (%)			<.01
TBI12Gy+Ara-C/G-CSF	21 (64)	0 (0)	
TBI12Gy+Ara-C/G-CSF+CY	2 (6)	52 (78)	
TBI12Gy+Ara-C/G-CSF+Flu	0 (0)	3 (4)	
TBI12Gy+CY	3 (9)	3 (4)	
TBI12Gy+Ara-C+CY	1 (3)	8 (12)	
TBI12Gy+VP16	4 (12)	0 (0)	
GVHD prophylaxis, n (%)			.23
Cyclosporine A+methotrexate	29 (93)	65 (98)	
Cyclosporine A	2 (6)	1 (1)	
Number of nucleated cells, $\times 10^7$/kg, median (range)	26.6 (3.13-50.0)‡	2.39 (1.72-5.07)	<.01
Number of $CD34^+$ cells, $\times 10^5$/kg, median (range)	40.5 (20.6-75.0)§	1.04 (.17-3.15)	<.01
Donor age, yr, median (range)	46.5 (38-58)	—	—
Sex compatibility, n (%)			.81
Female donor to male recipient	8 (25)	20 (30)	
Other	23 (74)	46 (69)	
HLA disparities,† n (%)			<.01
0	28 (90)	1 (1)	
1	2 (6)	13 (19)	
2	1 (3)	52 (78)	
ABO incompatibility, n (%)			.04
Match	19 (61)	20 (30)	
Major mismatch	4 (12)	17 (25)	
Minor mismatch	5 (16)	18 (27)	
Bidirectional mismatch	3 (9)	11 (16)	
Time from diagnosis to transplantation, days, median (range)	521 (59-2501)	390.5 (55-6783)	.84
<365 d, n (%)	12 (38)	31 (46)	.51
≥365 d, n (%)	19 (61)	35 (53)	
Year of transplantation, n (%)			<.01
1992-2002	27 (87)	17 (25)	
2003-2013	4 (12)	49 (74)	
Follow-up for survivors, mo, median (range)	185 (32-258)	87 (4-175)	<.01

CMV indicates cytomegalovirus; CY, cyclophosphamide; Flu, fludarabine; VP-16, etoposide.

* Disease status at transplantation was classified as standard risk or high risk; CR1 or CR2 without poor prognostic karyotype for AML and ALL, refractory anemia for MDS, chronic phase for CML, and CR1 or CR2 for NHL were classified as standard risk, whereas patients in all other situations were classified as high risk.

† Number of HLA disparities defined as low resolution for HLA-A, -B, and -DR.

‡ Number of nucleated cells was only for BMT recipients.

§ Number of $CD34^+$ cells was only for PBSCT recipients.

follow-up [10]. For disease status at transplantation, patients in first complete remission (CR1) or second complete remission (CR2) without poor prognostic karyotype for AML and ALL, refractory anemia for MDS, chronic phase for CML, and CR1 or CR2 for NHL were classified as standard risk, whereas patients in all other situations were classified as high risk.

Although bone marrow or mobilized peripheral blood from HLA-compatible related donors within immediate families is a frontline graft source, patients without a suitable closely HLA-compatible related donor were eligible for CBT as an alternative first treatment option, unless they had any type of anti-HLA antibody. Cord blood units were obtained from the Japan Cord Blood Bank Network and were selected as reported previously [9,10]. All patients received 12 Gy total body irradiation (TBI)-based myeloablative conditioning regimens, and cyclosporine-based GVHD prophylaxis regimens, as previously reported [9,10]. For myeloid disease, granulocyte colony-stimulating factor (G-CSF) was added to the conditioning regimen to increase the susceptibility to cytosine arabinoside (Ara-C) through induction of cell cycle entry of dormant leukemia cells, as previously reported [10]. Almost all patients received some supportive care, such as antibacterial, antifungal and antiviral agents, as previously reported [9,10]. The institutional review board of the Institute of Medical Science, University of Tokyo approved this study, which was conducted in accordance with the Declaration of Helsinki.

End Points and Definitions

The primary study end point was overall survival (OS), which was defined as the time from the date of transplantation to the date of death or last contact. Secondary end points were relapse, TRM, GVHD, and neutrophil and platelet recovery. Relapse was defined by morphologic evidence of disease in peripheral blood, bone marrow, or extramedullary sites. TRM was defined as death during a remission. Both aGVHD and cGVHD were graded according to previously published criteria [13,14]. The incidence of aGVHD

was evaluated in all engrafted patients, whereas the incidence of cGVHD was evaluated in engrafted patients surviving more than 100 days. Neutrophil engraftment was defined as the first of 3 consecutive days during which the absolute neutrophil count was at least .5 × 10^9/L. Platelet engraftment was defined as the first of 7 consecutive days with a platelet count of 20 × 10^9/L or higher without platelet transfusion.

Statistical Analysis

Baseline patient and transplant characteristics were compared using the chi-square test 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 groups were compared using Cox regression models or the log-rank test. The probabilities of relapse, TRM, aGVHD and cGVHD, and neutrophil and platelet engraftment were estimated based on a cumulative incidence method to accommodate competing risks. Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS and a Fine and Gray proportional hazards model for the others. In addition to the stem cell source (CBT versus rBMT/PBSCT), the following variables were considered: disease type (myeloid [AML, MDS, CML] versus lymphoid [ALL, NHL] disease), disease status at transplantation (standard risk versus high risk), time from diagnosis to transplantation (<365 days versus ≥365 days), sex compatibility between donor and recipient (female donor to male recipient versus other), ABO compatibility between donor and recipient (match versus mismatch), and year of transplantation (1992 to 2002 versus 2003 to 2013).

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphic user interface for R 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) [15]. $P < .05$ was considered significant. Analysis of data was performed in December 2013.

RESULTS

Characteristics of Patients and Grafts

The characteristics of patients, grafts, and transplant procedures are summarized in Table 1. Recipients' age, sex, cytomegalovirus serostatus, disease type, disease status at transplantation, GVHD prophylaxis, sex incompatibility between donors and recipients, and time from diagnosis to transplantation were almost the same between the CBT and rBMT/PBSCT recipients. On the other hand, there were significant differences in the following variables (Table 1). The conditioning regimen significantly differed between the CBT and rBMT/PBSCT recipients ($P < .01$). The most common conditioning regimen was TBI12Gy+Ara-C/G-CSF+cyclophosphamide (78%) for CBT and TBI12Gy+Ara-C/G-CSF (64%) for rBMT/PBSCT. The number of nucleated cells or $CD34^+$ cells for CBT recipients was 1 log lower than in rBMT or rPBSCT recipients, respectively. The proportion of HLA disparity and ABO incompatibility was higher among CBT recipients than rBMT/PBSCT recipients. CBT was more frequently performed in recent years, resulting in the significantly shorter follow-up period for CBT compared with that for rBMT/PBSCT. Median follow-up was 185 months (range, 32 to 258 months) for rBMT/PBSCT recipients and 87 months (range, 4 to 175 months) for CBT recipients ($P < .01$).

Table 2
Univariate and Multivariate Analysis of Transplant Outcomes after rBMT/PBSCT and CBT in Patients Aged 45 Years or Older

	Univariate Analysis		Multivariate Analysis*	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Neutrophil engraftment				
rBMT/PBSCT vs. CBT	.69 (.38-1.04)	.07	.46 (.26-.81)	<.01
Platelet engraftment				
rBMT/PBSCT vs. CBT	.30 (.14-.61)	<.01	.24 (.12-.50)	<.01
Grades III-IV aGVHD				
rBMT/PBSCT vs. CBT	.57 (.18-1.85)	.36	.53 (.15-1.90)	.34
Extensive cGVHD				
rBMT/PBSCT vs. CBT	1.01 (.53-1.91)	.97	1.08 (.49-2.35)	.84
Overall mortality				
rBMT/PBSCT vs. CBT	.69 (.36-1.32)	.26	.72 (.30-1.73)	.47
Relapse				
rBMT/PBSCT vs. CBT	1.42 (.52-3.87)	.49	2.02 (.63-6.42)	.23
TRM				
rBMT/PBSCT vs. CBT	.38 (.16-.93)	.03	.29 (.08-.99)	.04

* For neutrophil engraftment, lymphoid disease was also a significant variable (HR, 2.40; 95% CI, 1.52 to 3.79; $P < .01$). For platelet engraftment, lymphoid disease was also a significant variable (HR, 1.74; 95% CI, 1.17 to 2.59; $P < .01$). For grades III-IV aGVHD, ABO incompatibility was a significant variable (HR, 4.41; 95% CI, 1.06 to 18.24; $P = .04$). For extensive cGVHD, high risk of disease status at transplantation was a significant variable (HR, 3.14; 95% CI, 1.39 to 7.09; $P < .01$). For overall mortality, high risk of disease status at transplantation (HR, 3.33; 95% CI, 1.36 to 8.11; $P < .01$) and ABO incompatibility (HR, 3.14; 95% CI, 1.44 to 6.87; $P < .01$) were significant variables. For relapse, high risk of disease status at transplantation was a significant variable (HR, 4.55; 95% CI, 1.08 to 19.23; $P = .03$). For TRM, female donor to male recipient (HR, 2.89; 95% CI, 1.11 to 7.52; $P = .02$) and ABO incompatibility (HR, 5.20; 95% CI, 1.56 to 17.33; $P < .01$) were also significant variables.

Neutrophil and Platelet Engraftment

One patient in the CBT group died on day 21 due to encephalitis, and 1 patient in the rBMT/PBSCT group died on day 7 due to organ failure. Primary graft failure occurred in 3 of the surviving 65 patients in the CBT group, but there was no primary graft failure in the rBMT/PBSCT group. As expected, neutrophil recovery was significantly delayed after CBT as compared with rBMT/PBSCT. Median times to neutrophil recovery were 22 days (range, 18 to 34 days) after CBT, as compared with 18 days (range, 11 to 40 days) after rBMT/PBSCT ($P < .01$). The cumulative incidence of neutrophil recovery on day 60 was slightly lower after CBT (93.9%; 95% confidence interval [CI], 83.5% to 97.9%) compared with rBMT/PBSCT (96.8%; 95% CI, 57.8% to 99.8%) ($P = .07$). In the multivariate analysis, the hazard risk of neutrophil engraftment was significantly lower after CBT as compared with rBMT/PBSCT (hazard ratio [HR], .46; 95% CI, .26 to .81; $P < .01$, Table 2).

Platelet recovery was also significantly delayed after CBT as compared with rBMT/PBSCT. Median times to platelet recovery were 42 days (range, 13 to 104 days) after CBT, as compared with 24 days (range, 15 to 300 days) after rBMT/PBSCT ($P < .01$). The cumulative incidence of platelet recovery on day 100 was significantly lower after CBT (90.8%; 95% CI, 80.0% to 95.9%) compared with rBMT/PBSCT (93.5%; 95% CI, 71.5% to 98.7%) in the univariate analysis ($P < .01$); the difference was also significant in multivariate analyses (HR, .24; 95% CI, .12 to .50; $P < .01$, Table 2).

Acute and Chronic GVHD

The cumulative incidences of grades II to IV (HR, .90; 95% CI, .49 to 1.64; $P = .76$) and grades III to IV aGVHD (HR, .53; 95% CI, .15 to 1.90; $P = .34$) were similar between CBT and rBMT/PBSCT recipients in multivariate analyses (Table 2). The unadjusted cumulative incidence of grades III to IV aGVHD at 100 days was 9.2% (95% CI, 3.7% to 17.8%) in CBT recipients and 16.1% (95% CI, 5.7% to 31.2%) in rBMT/PBSCT recipients ($P = .35$). Extensive cGVHD developed in 27 of 58 CBT recipients and in 13 of 27 rBMT/PBSCT recipients surviving more than 100 days. In a multivariate analysis, the cumulative incidences of cGVHD (HR, .94; 95% CI, .55 to 1.62; $P = .84$) and extensive cGVHD (HR, 1.08; 95% CI, .49 to 2.35; $P = .84$) were similar between CBT and rBMT/PBSCT recipients in multivariate analysis (Table 2).

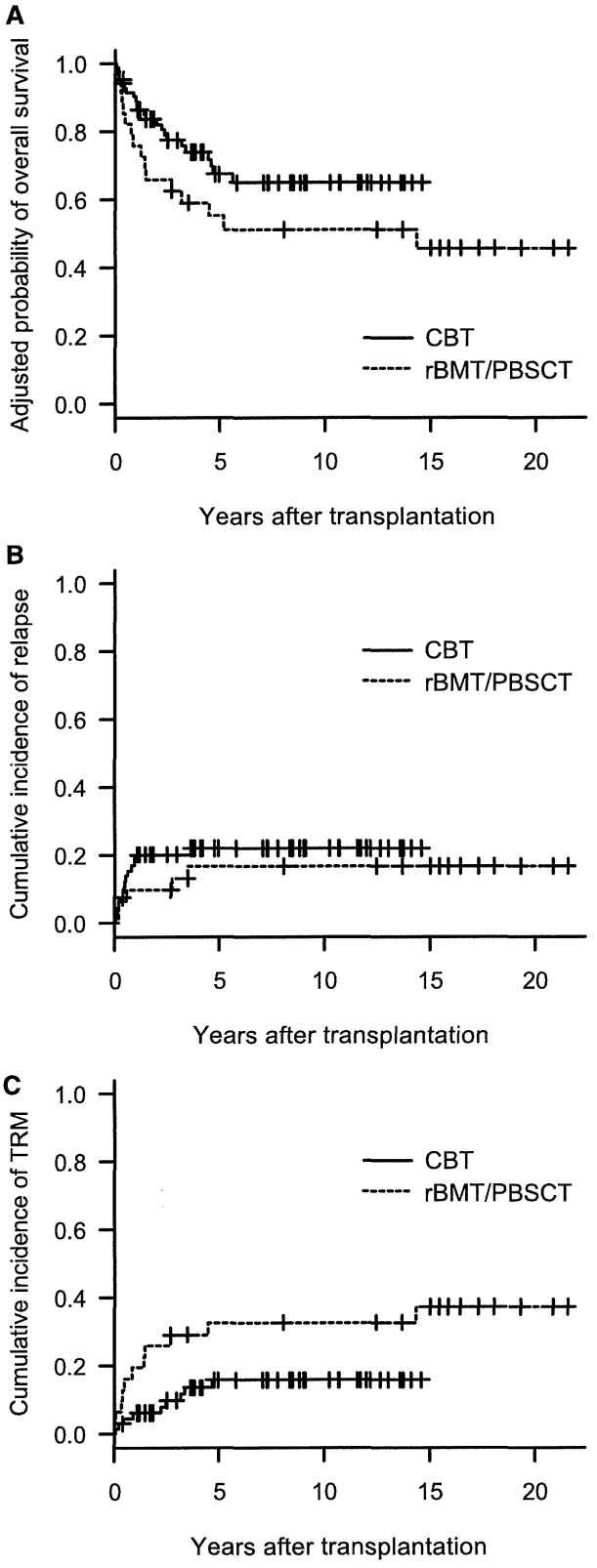

Figure 1. Outcomes after CBT or BMT/PBSCT from related donors in patients aged 45 years or older with hematologic malignancies after a myeloablative conditioning regimen. Adjusted probability of OS (A), unadjusted cumulative incidence of relapse (B), and TRM (C).

Table 3
Cause of Death

	Death before 100 Days		Death after 100 Days	
	rBMT/PBSCT (n = 2)	CBT (n = 4)	rBMT/PBSCT (n = 14)	CBT (n = 19)
Primary disease	0	2	5	12
GVHD	0	0	5	5
Infection	1	2	4	2
Organ failure	1	0	0	0

Survival, Relapse, and TRM

The adjusted probabilities of OS at 5 years were 67.4% (95% CI, 55.7% to 81.6%) for recipients of CBT and 55.2% (95% CI, 39.4% to 77.4%) for recipients of rBMT/PBSCT (Figure 1A). In multivariate analysis, the hazard risk of overall mortality was similar between CBT and rBMT/PBSCT recipients (HR, .72; 95% CI, .30 to 1.73; $P = .47$; Table 2). We also compared OS of both groups for each disease risk. However, OS of both recipient groups was also equivalent in standard-risk patients (n = 31) and high-risk patients (n = 66) (data not shown). The unadjusted cumulative incidence of relapse at 5 years was 22.0% (95% CI, 12.7% to 33.0%) in CBT recipients and 16.7% (95% CI, 5.9% to 32.3%) in rBMT/PBSCT recipients ($P = .48$) (Figure 1B). In multivariate analysis, the hazard risk for relapse was similar between CBT and rBMT/PBSCT recipients (HR, 2.02; 95% CI, .63 to 6.42; $P = .23$; Table 2). The unadjusted cumulative incidence of TRM was significantly lower after CBT at 100 days (3.0%; 95% CI, .6% to 9.4%) and 5 years (15.8%; 95% CI, 7.6% to 26.6%) compared with rBMT/PBSCT at 100 days (6.5%; 95% CI, 1.1% to 18.9%) and 5 years (32.7%; 95% CI, 16.8% to 49.6%) ($P = .04$) (Figure 1C). In multivariate analysis, the hazard risk of TRM was significantly lower after CBT as compared with rBMT/PBSCT (HR, .29; 95% CI, .08 to .99; $P = .04$; Table 2).

We also analyzed a subgroup of patients aged 50 years or older after CBT (n = 29) and rBMT/PBSCT (n = 11). In multivariate analysis, the hazard risk of overall mortality (HR, .36, $P = .10$) and relapse (HR, 2.73, $P = .41$) after CBT was comparable with that after rBMT/PBSCT, respectively. However, the hazard risk of TRM was lower after CBT than after rBMT/PBSCT (HR, .16; 95% CI, .04 to .56; $P < .01$).

The causes of death before and after 100 days after transplantation by donor type are summarized in Table 3. The major cause of death in both recipient groups was primary disease. However, GVHD and infection as a primary cause of late mortality were more common after rBMT/PBSCT compared with CBT.

DISCUSSION

The objective of this study was to compare the transplant outcomes after CBT and rBMT/PBSCT in relatively older patients who were candidates for myeloablative conditioning. Unexpectedly, there were no significant differences in aGVHD and cGVHD between CBT and rBMT/PBSCT recipients. However, TRM was higher after rBMT/PBSCT compared with CBT. The reduced TRM in CBT might be in part due to improved supportive care, because CBT was more frequently performed in recent years. However, year of transplantation did not affect any clinical results in our multivariate analysis. On the other hand, we used almost the same 12-Gy TBI-based myeloablative conditioning and cyclosporine-based GVHD prophylaxis regimens during the period for both recipients of CBT and rBMT/PBSCT. Among relatively older

patients who were candidates for such myeloablative conditioning, survival and relapse were not significantly different after CBT and rBMT/PBSCT.

Whether an older sibling donor or unrelated donor should be chosen as an optimal donor is an important question in allo-HSCT for older patients. There have been some clinical comparisons of allo-HSCT from older sibling donors and unrelated donors in older patients [16-18]. A European Group for Blood and Marrow Transplantation analysis by Kröger et al. [16] reported on comparisons of allo-HSCT from older sibling donors and young unrelated donors in 719 patients older than 50 years with MDS. They showed that recipients from young unrelated donors had improved survival compared with those from older sibling donors among older patients with MDS. A single-institute analysis by Ayuk et al. [17] showed similar outcomes from older sibling donors compared with young unrelated donors among older patients with AML in CR. On the other hand, Alousi et al. [18] of the Center for International Blood and Marrow Transplantation also performed a similar study in 2172 patients older than 50 years with leukemia or lymphoma. In contrast, their data showed that the risks of overall mortality, relapse, and TRM were lower after allo-HSCT from older sibling donors compared with those from young unrelated donors. However, comparative clinical outcomes of CBT and BMT/PBSCT from older related donors after myeloablative conditioning have yet to be clarified. Our data showed comparable outcomes for CBT and BMT/PBSCT from older related donors after myeloablative conditioning in relatively older patients when cord blood was selected as a primary unrelated donor source.

In comparison with other sources of allo-HSCT, the lower risk of GVHD without compromised graft-versus-leukemia effects is one of the most important advantages of CBT. In our study, the incidences of severe aGVHD and cGVHD were not significantly different after CBT and rBMT/PBSCT. Relapse was also similar between CBT and rBMT/PBSCT recipients. However, TRM was significantly lower after CBT compared with that after rBMT/PBSCT. GVHD-associated mortality was a common cause of late death after rBMT/PBSCT compared with CBT. Newell et al. [19] reported a shorter duration and a higher response of cGVHD to systemic immunosuppressive treatment in CBT recipients than in BMT/PBSCT recipients, suggesting that a longer duration of systemic immunosuppressive treatment for cGVHD might have contributed to higher infection-related late mortality after rBMT/PBSCT compared with CBT. In fact, we previously reported that the termination of immunosuppressive treatment for rBMT/PBSCT recipients was slower than those for CBT recipients [10]. These effects might have contributed to higher TRM after rBMT/PBSCT compared with CBT in our study. In addition, the absence of risk for donors may also be one of the most attractive advantages of CBT for older patients. Older patients generally have older donors as well when they have an HLA-compatible sibling. Because older donors are more likely to have organ dysfunction or comorbidity, older patients hardly ever find healthy sibling donors. These problems could be overcome with the advantages of CBT, especially in older patients.

Myeloablative conditioning regimens for allo-HSCT have been restricted to younger patients and those without comorbidities, because TRM occurs more frequently among older patients and those with serious comorbidities. RIC regimens have recently been expanded for use with graft sources not only from bone marrow or mobilized peripheral blood but also from cord blood. Although the risk of graft failure after CBT has been reported to be higher after RIC compared with myeloablative conditioning [20], several reports showed similar survival with acceptable engraftment between CBT and other graft sources from related and unrelated adult donors after RIC [21,22]. Further studies are warranted to establish optimal RIC regimens for CBT.

In conclusion, our data showed that CBT had almost equivalent results compared with rBMT/PBSCT after myeloablative conditioning for relatively older patients. However, these results should be interpreted with caution because this study was a retrospective single-institute analysis that included a heterogeneous population and a relatively small number of patients. In addition, although our study was performed in patients older than 45 years of age, it should be noted that most patients were younger than 55 years of age. This is because the patients in our cohort received myeloablative conditioning, which often excludes even older patients. As such, our results cannot be extended to patients older than 60 years of age until another similar study is performed using RIC in those older than 55 years. Although these findings should be confirmed in larger prospective studies, CBT could be as safe and effective as BMT/PBSCT from older related donors after myeloablative conditioning for relatively older patients when it is used as a primary unrelated stem cell source.

ACKNOWLEDGMENTS

The authors thank all the physicians and staff at the hospitals and the 8 cord blood banks in Japan for their help in this study.

Financial disclosure: Supported in part by The Kobayashi Foundation.

Conflict of interest statement: There are no conflicts of interest to report.

REFERENCES

1. Kollman C, Howe CW, Anasetti C, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood.* 2001;98:2043-2051.
2. Jagasia M, Arora M, Flowers ME, et al. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. *Blood.* 2012;119:296-307.
3. Mehta J, Gordon LI, Tallman MS, et al. Does younger donor age affect the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies beneficially? *Bone Marrow Transplant.* 2006;38:95-100.
4. Yakoub-Agha I, Mesnil F, Kuentz M, et al. Allogeneic marrow stem-cell transplantation from human leukocyte antigen-identical siblings versus human leukocyte antigen-allelic-matched unrelated donors (10/10) in patients with standard-risk hematologic malignancy: a prospective study from the French Society of Bone Marrow Transplantation and Cell Therapy. *J Clin Oncol.* 2006;24:5695-5702.
5. Flowers ME, Inamoto Y, Carpenter PA, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. *Blood.* 2011;117:3214-3219.
6. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med.* 2004;351:2265-2275.
7. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med.* 2004;351:2276-2285.
8. Eapen M, Rocha V, Sanz G, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. *Lancet Oncol.* 2010;11:653-660.
9. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood.* 2004;104:3813-3820.
10. Takahashi S, Ooi J, Tomonari A, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related

donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood*. 2007;109:1322-1330.
11. Ooi J, Iseki T, Takahashi S, et al. Unrelated cord blood transplantation after myeloablative conditioning in patients over the age of 45 years. *Br J Haematol*. 2004;126:711-714.
12. Konuma T, Ooi J, Takahashi S, et al. Unrelated cord blood transplantation after myeloablative conditioning in patients with acute leukemia aged between 50 and 55 years. *Bone Marrow Transplant*. 2006;37:803-804.
13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
14. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.
15. Kanda Y. Investigation of the freely-available easy-to-use software "EZR" (Easy R) for medical statistics. *Bone Marrow Transplant*. 2013;48: 452-458.
16. Kröger N, Zabelina T, de Wreede L, et al. Allogeneic stem cell transplantation for older advanced MDS patients: improved survival with young unrelated donor in comparison with HLA-identical siblings. *Leukemia*. 2013;27:604-609.
17. Ayuk F, Zabelina T, Wortmann F, et al. Donor choice according to age for allo-SCT for AML in complete remission. *Bone Marrow Transplant*. 2013;48:1028-1032.
18. Alousi AM, Le-Rademacher J, Saliba RM, et al. Who is the better donor for older hematopoietic transplant recipients: an older-aged sibling or a young, matched unrelated volunteer? *Blood*. 2013;121: 2567-2573.
19. Newell LF, Flowers ME, Gooley TA, et al. Characteristics of chronic GVHD after cord blood transplantation. *Bone Marrow Transplant*. 2013; 48:1285-1290.
20. Cutler C, Ballen K. Reduced-intensity conditioning and umbilical cord blood transplantation in adults. *Bone Marrow Transplant*. 2009;44: 667-671.
21. Majhail NS, Brunstein CG, Tomblyn M, et al. Reduced-intensity allogeneic transplant in patients older than 55 years: unrelated umbilical cord blood is safe and effective for patients without a matched related donor. *Biol Blood Marrow Transplant*. 2008;14:282-289.
22. Peffault de Latour R, Brunstein CG, Porcher R, et al. Similar overall survival using sibling, unrelated donor, and cord blood grafts after reduced-intensity conditioning for older patients with acute myelogenous leukemia. *Biol Blood Marrow Transplant*. 2013;19:1355-1360.

Biol Blood Marrow Transplant 20 (2014) 577–592

Brief Articles

Effect of ABO Blood Group Incompatibility on the Outcome of Single-Unit Cord Blood Transplantation after Myeloablative Conditioning

Takaaki Konuma [1,*], Seiko Kato [1], Jun Ooi [2], Maki Oiwa-Monna [1], Yasuhiro Ebihara [1], Shinji Mochizuki [1], Koichiro Yuji [1], Nobuhiro Ohno [1], Toyotaka Kawamata [1], Norihide Jo [1], Kazuaki Yokoyama [1], Kaoru Uchimaru [1], Arinobu Tojo [1], Satoshi Takahashi [1]

[1] *Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan*
[2] *Department of Hematology/Oncology, Teikyo University School of Medicine, Tokyo, Japan*

Article history:
Received 1 November 2013
Accepted 18 December 2013

Key Words:
Cord blood transplantation
ABO incompatibility
Graft-versus-host disease
Myeloablative conditioning

A B S T R A C T

ABO blood group incompatibility between donor and recipient has been associated with poor transplant outcomes in allogeneic hematopoietic stem cell transplantation. However, its effect on the outcome of cord blood transplantation (CBT) has yet to be clarified. We retrospectively analyzed 191 adult patients who received single-unit CBT after myeloablative conditioning for malignant disease in our institute. Major mismatch showed a significantly lower incidence of platelet engraftment compared with ABO match as a reference (hazard ratio, .57; $P = .01$). Nevertheless, there was no increase in graft-versus-host disease, transplant-related mortality, and overall mortality after ABO-incompatible CBT. These data suggested that donor–recipient ABO incompatibility does not have a significant impact on outcome after myeloablative CBT for hematological malignancies.

INTRODUCTION

In contrast to solid organ transplantation, ABO blood group incompatibility between donor and recipient is reportedly a more common situation after allogeneic hematopoietic stem cell transplantation (allo-HSCT). It is well known that ABO incompatibility of allo-HSCT can cause an increased risk of delayed erythroid reconstitution, pure red cell aplasia, and acute and delayed hemolysis [1,2]. However, the association between ABO incompatibility and transplantation outcomes, such as neutrophil and platelet recovery, graft-versus-host disease (GVHD), and survival, is controversial [1,2]. Moreover, most of these studies analyzed patients receiving allo-HSCT using bone marrow or mobilized peripheral blood as a stem cell source from related and unrelated donors [1-5].

Cord blood transplantation (CBT) from an unrelated donor is increasingly used as an alternative transplant method for adult patients without HLA-compatible related or unrelated donors. Although most patients receive an HLA-mismatched cord blood unit, the lower risk of GVHD without compromising graft-versus-leukemia effects is one of the most attractive advantages of CBT. We previously reported that ABO incompatibility influenced platelet engraftment and transfusion requirement of RBCs and platelets in CBT [6]. However, the effects of ABO incompatibility on GVHD and survival after myeloablative CBT are limited. In the present study, we analyzed the neutrophil and platelet recovery, GVHD, transplant-related mortality (TRM), relapse, and survival in myeloablative CBT in adult patients with malignant disease in our institute.

METHODS

This retrospective study included data from 191 adult patients who underwent unrelated first allogeneic transplantation using single-unit CBT at our institute between August 1998 and February 2013. Donor–recipient ABO compatibility was categorized as follows: ABO match in 55 patients, major mismatch in 47, minor mismatch in 58, and bidirectional mismatch in 31. All patients received 12 Gy total body irradiation (TBI)-based myeloablative conditioning regimens and cyclosporine with or without short-term methotrexate as a GVHD prophylaxis, and cord blood units were selected as reported previously [7,8]. The institutional review board of the Institute of Medical Science, The University of Tokyo approved this study. This study was conducted in accordance with the Declaration of Helsinki.

The primary study endpoint was overall survival (OS), defined as the time from the date of transplantation to the date of death or last contact. Secondary endpoints were relapse, TRM, GVHD, and neutrophil and platelet recovery. Relapse was defined by morphologic evidence of disease in peripheral blood, bone marrow, or extramedullary sites. TRM was defined as death during a remission. Both acute GVHD (aGVHD) and chronic GVHD (cGVHD) were graded according to previously published criteria [9,10]. The incidence of aGVHD was evaluated in all engrafted patients, whereas the incidence of cGVHD was evaluated in engrafted patients surviving more than 100 days. Neutrophil engraftment was defined as being achieved on the first of 3 consecutive days during which the absolute neutrophil count was at least 0.5×10^9/L. Platelet engraftment was defined as being achieved on the first of 3 days when the platelet count was higher than 50×10^9/L without transfusion support.

Baseline patient and transplant characteristics were compared using the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables. The probability of OS was estimated according to the

Financial disclosure: See Acknowledgments on page 580.

* Correspondence and reprint requests: Takaaki Konuma, Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail address: tkonuma@ims.u-tokyo.ac.jp (T. Konuma).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.12.563

Kaplan-Meier method, and groups were compared using the log-rank test. The probabilities of the others were estimated based on a cumulative incidence method to accommodate competing risks. Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS, and a Fine and Gray proportional hazards model for the others.

The following variables for multivariate analysis were considered: age (<45 versus ≥45 years), disease status at CBT (standard risk versus high risk), cord blood nucleated cell count (<2.5 × 10^7 versus ≥2.5 × 10^7/kg), cord blood CD34 + cell count (<1 × 10^5 versus ≥1 × 10^5/kg), HLA disparities based on antigen level HLA-A and -B and allele level HLA-DRB1 (1 versus 2 versus ≥3), sex compatibility between donor and recipient (female donor to male recipient versus other), year of CBT (1998 to 2005 versus 2006 to 2013), and ABO compatibility between donor and recipient (match versus major mismatch versus minor mismatch versus bidirectional mismatch). The ABO match was considered the reference group in the multivariate analyses.

All statistical analyses were performed with EZR, a graphic user interface for R 2.13.0 [11]. $P < .05$ was considered significant. Analysis of data was performed in August 2013. The median follow-up of surviving patients was 92 months (range, 5 to 181) after CBT in the entire cohort.

RESULTS

The characteristics of patients and cord blood units are shown in Table 1. There were no significant differences among the 4 groups, except for HLA disparities. The major mismatch group contained a slightly higher number of HLA disparities as compared with the minor mismatch group ($P = .07$) or the bidirectional mismatch group ($P = .08$), although these were not statistically significant.

The probability of OS at 5 years significantly differed among the 4 groups in univariate analysis ($P = .03$) (Figure 1A). However, multivariate analysis of mortality adjusting for other variables showed no significant difference between ABO match and major (hazard ratio [HR], 1.20; $P = .62$), minor (HR, .72; $P = .41$), or bidirectional (HR, 1.76; $P = .14$) mismatch (Table 2). In univariate analysis, ABO incompatibility was not associated with cumulative incidence of TRM (Figure 1B) or relapse (Table 2). In multivariate analysis, a trend toward a higher incidence of TRM was observed in the major mismatch compared with the match group, but this was not significant ($P = .05$).

In univariate analysis, there was no significant difference in the cumulative incidence of grades II to IV aGVHD among the 4 groups ($P = .91$) (Figure 1C). In multivariate analysis, a higher number (≥3) of HLA disparities (HR, 1.56; 95% confidence interval [CI], 1.05 to 2.32; $P = .02$), a higher cord blood CD34 + cell count (HR, 1.51; 95% CI, 1.05 to 2.18; $P = .02$), and older year of CBT (HR, 1.85; 95% CI, 1.30 to 2.65; $P < .01$) were associated with a higher incidence of grades II to IV aGVHD, but ABO incompatibility was not associated with the incidence of grades II to IV aGVHD (Table 2). The cumulative incidence of grades III to IV aGVHD significantly differed among the 4 groups in univariate analysis ($P = .02$). However, multivariate analysis adjusting for other variables showed no significant difference in the cumulative incidence of grades III to IV aGVHD between ABO match and major (HR, 2.56; $P = .19$), minor (HR, .59; $P = .56$), or bidirectional (HR, 1.46; $P = .67$) mismatch (Table 2). In univariate analysis, there was no significant difference in the cumulative incidence of extensive cGVHD among the 4 groups ($P = .86$) (Figure 1D). In multivariate analysis, older age (HR, 1.85; 95% CI, 1.06 to 3.23;

Table 1
Characteristics of Patients, Cord Blood Units, and Transplantation

	Total	Match	Major Mismatch	Minor Mismatch	Bidirectional Mismatch	P
Number (%)	191	55 (28)	47 (24)	58 (30)	31 (16)	
Age, yr, median (range)	40 (16-55)	40 (16-55)	40 (16-53)	40 (16-53)	41 (18-52)	.94
Disease type, n (%)						.61
AML	101 (52)	30 (54)	24 (51)	30 (51)	17 (54)	
ALL	45 (23)	17 (30)	10 (21)	11 (18)	7 (22)	
MDS	25 (13)	5 (9)	5 (10)	10 (17)	5 (16)	
CML	11 (5)	1 (1)	4 (8)	4 (6)	2 (6)	
NHL	9 (4)	2 (3)	4 (8)	3 (5)	0 (0)	
Disease status at CBT,* n (%)						.09
Standard risk	79 (41)	24 (44)	17 (36)	30 (51)	8 (25)	
High risk	112 (58)	31 (54)	30 (64)	28 (48)	23 (74)	
Conditioning regimen, n (%)						.36
TBI12Gy+Ara-C/G-CSF+CY	131 (68)	34 (61)	33 (70)	40 (68)	24 (77)	
TBI12Gy+Ara-C+CY	31 (16)	9 (16)	11 (23)	9 (15)	2 (6)	
TBI12Gy+CY	16 (8)	6 (10)	1 (2)	5 (8)	4 (12)	
TBI12Gy+others	13 (6)	6 (10)	2 (4)	4 (6)	1 (3)	
GVHD prophylaxis, n (%)						.10
Cyclosporine A + methotrexate	188 (98)	55 (100)	47 (100)	57 (98)	29 (93)	
Cyclosporine A	3 (1)	0 (0)	0 (0)	1 (2)	2 (6)	
Number of nucleated cells, ×10^7/kg, median (range)	2.43 (1.32-5.69)	2.52 (1.32-5.50)	2.47 (1.65-4.92)	2.38 (1.51-5.69)	2.58 (1.65-5.07)	.79
Number of CD34$^+$cells, ×10^5/kg, median (range)	.92 (.17-7.75)	.88 (.28-3.15)	.93 (.17-1.99)	.91 (.28-7.75)	1.14 (.44-2.84)	.20
HLA disparities,† n (%)						.05
1	23 (12)	4 (7)	7 (14)	8 (13)	4 (12)	
2	106 (55)	32 (58)	16 (34)	37 (63)	21 (67)	
3	57 (29)	17 (30)	23 (48)	12 (20)	5 (16)	
4	5 (2)	2 (3)	1 (2)	1 (1)	1 (3)	
Sex compatibility, n (%)						
Female donor to male recipient	58 (30)	19 (34)	13 (27)	17 (29)	9 (29)	.88
Other	133 (69)	36 (65)	34 (72)	41 (70)	22 (70)	
Year of CBT, n (%)						.58
1998-2005	102 (53)	28 (50)	22 (46)	33 (56)	19 (61)	
2006-2013	89 (46)	27 (49)	25 (53)	25 (43)	12 (38)	

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia, MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; NHL, non-Hodgkin lymphoma, Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide.

* For disease status at CBT, patients in complete remission (CR) 1 or CR2 without poor prognostic karyotype for AML and ALL, refractory anemia for MDS, chronic phase for CML, and CR1 or CR2 for NHL were classified as standard risk, whereas patients in all other situations were classified as high risk.

† The number of HLA disparities defined as low resolution for HLA-A and -B and high resolution for HLA-DRB1.

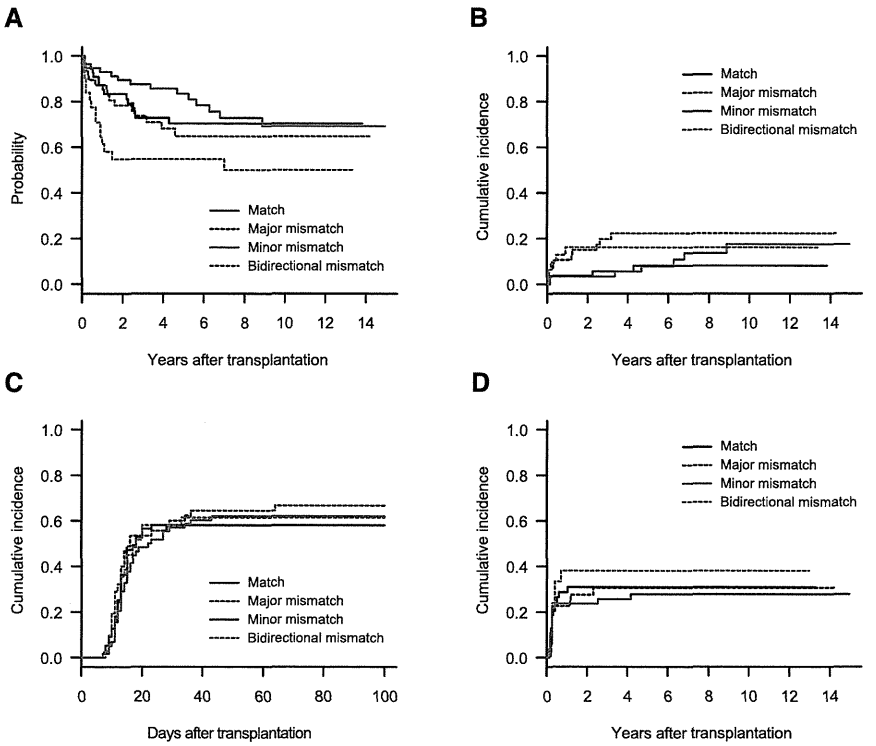

Figure 1. Probability of OS (A), cumulative incidence of TRM (B), grades II to IV aGVHD (C), and extensive cGVHD (D) according to donor–recipient ABO incompatibility after myeloablative CBT.

$P = .03$) and female donor to male recipient (HR, 1.79; 95% CI, 1.02 to 3.15; $P = .04$) were associated with a higher incidence of extensive cGVHD, but ABO incompatibility was not associated with the incidence of extensive cGVHD (Table 2).

ABO incompatibility was not associated with cumulative incidence of neutrophil engraftment among the 4 groups in univariate analysis ($P = .73$). In multivariate analysis, a lower cord blood CD34 + cell count (HR, .51; 95% CI, .37 to .70; $P < .001$), high risk of disease status at CBT (HR, .68; 95% CI, .50 to .93; $P = .01$), and older year of CBT (HR, .71; 95% CI, .53 to .96; $P = .02$) were associated with a lower incidence of neutrophil engraftment, but ABO incompatibility was not associated with neutrophil engraftment (Table 2). The cumulative incidence of platelet recovery was not significantly different among the 4 groups in univariate analysis ($P = .30$). In multivariate analysis, major mismatch (HR, .57; $P = .01$) showed a significantly lower incidence of platelet engraftment when compared with ABO match (Table 2). In addition, a lower cord blood CD34 + cell count (HR, .63; 95% CI, .45 to .88; $P < .01$), lower cord blood nucleated cell count (HR, .70; 95% CI, .52 to .94; $P = .01$), and high risk of disease status at CBT (HR, .65; 95% CI, .45 to .94; $P = .02$) were associated with a lower incidence of platelet engraftment.

We also analyzed the effect of major/bidirectional mismatch group defined as combined group of major and bidirectional mismatch. However, we were unable to find any impact of major/bidirectional mismatch on outcomes in multivariate analysis, except for platelet engraftment (Supplemental Table 1).

DISCUSSION

The ABO blood group antigens consist of oligosaccharide glycoproteins and are expressed not only in erythrocytes but also in neutrophils, platelets, and, vascular endothelial and epithelial cells. The ABO antigens could be immunological targets for ABO-incompatible donor or recipient lymphocytes, affecting GVHD and engraftment. Many previous studies have reported an increased risk of aGVHD after ABO-incompatible allogeneic bone marrow transplantation from related and unrelated donors, particularly in minor and bidirectional mismatch [3-5]. Igarashi et al. [12] reported an association between the anti-host isohemagglutinin produced by donor-derived B lymphocytes and the development of aGVHD after minor and bidirectional mismatched allogeneic bone marrow transplantation and peripheral blood stem cell transplantation from related and unrelated donors. These effects might be associated with ABO-incompatible immune responses against ABO antigens in vascular endothelial and epithelial cells of recipients. However, it has been reported that donor-derived isohemagglutinin was not identified in patients after minor and bidirectional mismatched CBT [12,13]. The higher proportion of naïve B lymphocytes in cord blood grafts might contribute to defective isohemagglutinin production after ABO-incompatible CBT, which might have contributed to the low incidence of severe GVHD even after ABO-incompatible CBT. Therefore, the effect of ABO incompatibility on transplant outcome might differ depending on the kinds of stem cell sources in allo-HSCT.

Several studies have reports on associations between ABO incompatibility and outcomes after CBT [14-19]. Romee et al. [14] reported no impact of ABO incompatibility on aGVHD and cGVHD in 503 CBT recipients. Berglund et al. [15] reported an increased incidence of grades II to IV aGVHD in major mismatch recipients (n = 23) of CBT. Moreover, previous studies demonstrated lower survival for major

Table 2
Univariate and Multivariate Analysis of ABO Compatibility for the Outcomes of CBT

	Univariate Analysis			Multivariate Analysis		
	Number	Percent (95% CI)	*P*	HR	95% CI	*P*
OS*		At 5 yr	.03			
Match	55	70.2 (55.3-81.0)		1.00		Reference
Major mismatch	47	64.8 (48.0-77.3)		1.20	.57-2.50	.62
Minor mismatch	58	83.2 (70.1-90.9)		.72	.33-1.57	.41
Bidirectional mismatch	31	54.6 (35.7-70.1)		1.76	.82-3.77	.14
Relapse†		At 5 yr	.09			
Match	55	26.9 (15.6-39.6)		1.00		Reference
Major mismatch	47	15.8 (6.8-28.2)		.54	.20-1.42	.21
Minor mismatch	58	14.4 (6.6-24.9)		.54	.22-1.32	.18
Bidirectional mismatch	31	32.5 (16.7-49.3)		1.08	.43-2.71	.86
TRM‡		At 5 yr	.19			
Match	55	8.1 (2.5-18.1)		1.00		Reference
Major mismatch	47	22.2 (11.3-35.4)		3.19	.97-10.46	.05
Minor mismatch	58	7.9 (2.5-17.6)		1.34	.34-5.33	.67
Bidirectional mismatch	31	16.1 (5.7-31.2)		1.99	.49-8.03	.33
Grades II-IV aGVHD		At 100 d	.91			
Match	55	58.2 (43.9-70.1)		1.00		Reference
Major mismatch	45	66.7 (50.5-78.6)		1.06	.64-1.73	.81
Minor mismatch	58	62.1 (48.1-73.3)		1.11	.68-1.80	.66
Bidirectional mismatch	31	61.3 (41.4-76.2)		1.28	.73-2.24	.37
Grades III-IV aGVHD§		At 100 d	.02			
Match	55	5.5 (1.4-13.7)		1.00		Reference
Major mismatch	45	20.0 (9.8-32.8)		2.56	.63-10.37	.19
Minor mismatch	58	3.4 (.6-10.7)		.59	.10-3.46	.56
Bidirectional mismatch	31	9.7 (2.4-23.2)		1.46	.25-8.44	.67
Extensive cGVHD		At 5 yr	.86			
Match	49	28.6 (16.7-41.6)		1.00		Reference
Major mismatch	40	30.5 (16.9-45.3)		1.18	.56-2.47	.65
Minor mismatch	55	27.9 (16.5-40.4)		1.24	.57-2.72	.58
Bidirectional mismatch	21	38.1 (17.8-58.3)		1.56	.67-3.63	.30
Neutrophil engraftment		At 60 d	.73			
Match	55	96.4 (83.6-99.2)		1.00		Reference
Major mismatch	47	92.6 (75.2-98.0)		.82	.56-1.20	.33
Minor mismatch	58	94.8 (83.3-98.5)		1.09	.78-1.53	.59
Bidirectional mismatch	31	88.7 (64.1-96.8)		1.06	.66-1.68	.80
Platelet engraftment		At 100 d	.30			
Match	55	88.9 (76.0-95.0)		1.00		Reference
Major mismatch	47	70.0 (53.6-81.6)		.57	.36-.90	.01
Minor mismatch	58	93.1 (81.2-97.6)		.92	.66-1.28	.64
Bidirectional mismatch	31	73.3 (51.5-86.4)		.78	.45-1.34	.37

* HR for overall mortality. In multivariate analysis, there were no significant variables, but there was a trend toward a higher mortality among those with a high risk of disease status at CBT (HR, 1.60; 95% CI, .88-2.89; $P = .11$) and female donor to male recipient (HR, 1.64; 95% CI, .94-2.85; $P = .07$).

† In multivariate analysis, there were no significant variables, but there was a trend toward a higher relapse among those with a high risk of disease status at CBT (HR, 1.71; 95% CI, .85-3.44; $P = .13$).

‡ In multivariate analysis, there were no significant variables, but there was a trend toward a higher TRM among those with female donor to male recipient (HR, 2.05; 95% CI, .87-4.81; $P = .09$).

§ In multivariate analysis, there were no significant variables, but there was a trend toward a higher incidence of grades III-IV aGVHD among those with a lower cord blood CD34 + cell count (HR, 2.75; 95% CI, .84-9.00; $P = .09$) and a high risk of disease status at CBT (HR, 3.98; 95% CI, .80-19.65; $P = .08$).

mismatch recipients of single-unit CBT [16,17], whereas other studies did not [14,18,19]. However, these studies included a relatively heterogeneous group of patients receiving single or double CBT after reduced-intensity or myeloablative conditioning regimen. In most of these studies, 3 groups of ABO mismatch, namely, major, minor, and bidirectional mismatch, were not evaluated separately. Of note, the advantage of our study is the relatively homogeneous adult patient population with hematological malignancies treated with single-unit CBT after 12 Gy TBI-based myeloablative conditioning regimens and a cyclosporine-based GVHD prophylaxis. Moreover, 3 groups of ABO mismatch were evaluated separately. Therefore, we were able to determine the potential effect of ABO incompatibility in CBT.

In conclusion, our data showed that ABO incompatibility affected the incidences of platelet engraftment but did not have a significant effect on the incidence of GVHD, relapse, TRM, and OS after CBT. These results should be interpreted with caution because this retrospective study included a relatively small number of Japanese patients who received single-unit CBT after 12 Gy TBI-based myeloablative conditioning regimens for hematological malignancies. Although these findings should be confirmed in large prospective studies, ABO incompatibility does not appear to have had a significant impact on the outcome after CBT in our study.

ACKNOWLEDGMENTS

The authors thank all physicians and staff at the hospitals and the 8 cord blood banks in Japan for their help in this study.

Financial disclosure: This work was supported in part by The Kobayashi Foundation.

Conflict of interest statement: There are no conflicts of interest to report.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.12.563.

REFERENCES

1. Booth GS, Gehrie EA, Bolan CD, Savani BN. Clinical guide to ABO-incompatible allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2013;19:1152-1158.
2. Rowley SD, Donato ML, Bhattacharyya P. Red blood cell-incompatible allogeneic hematopoietic progenitor cell transplantation. *Bone Marrow Transplant.* 2011;46:1167-1185.
3. Seebach JD, Stussi G, Passweg JR, et al. ABO blood group barrier in allogeneic bone marrow transplantation revisited. *Biol Blood Marrow Transplant.* 2005;11:1006-1013.
4. Kimura F, Sato K, Kobayashi S, et al. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. *Haematologica.* 2008;93:1686-1693.
5. Bacigalupo A, Van Lint MT, Occhini D, et al. ABO compatibility and acute graft-versus-host disease following allogeneic bone marrow transplantation. *Transplantation.* 1988;45:1091-1094.
6. Tomonari A, Takahashi S, Ooi J, et al. Impact of ABO incompatibility on engraftment and transfusion requirement after unrelated cord blood transplantation: a single institute experience in Japan. *Bone Marrow Transplant.* 2007;40:523-528.
7. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood.* 2004;104:3813-3820.
8. Takahashi S, Ooi J, Tomonari A, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood.* 2007;109:1322-1330.
9. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995;15:825-828.
10. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med.* 1980;69:204-217.
11. Kanda Y. Investigation of the freely available easy-to-use software "EZR" for medical statistics. *Bone Marrow Transplant.* 2013;48:452-458.
12. Igarashi A, Kakihana K, Haraguchi K, et al. Anti-host isohemagglutinin production is associated with a higher risk of acute GVHD in ABO-incompatible transplantation. *Bone Marrow Transplant.* 2012;47:1356-1360.
13. Snell M, Chau C, Hendrix D, et al. Lack of isohemagglutinin production following minor ABO incompatible unrelated HLA mismatched umbilical cord blood transplantation. *Bone Marrow Transplant.* 2006;38:135-140.
14. Romee R, Weisdorf DJ, Brunstein C, et al. Impact of ABO-mismatch on risk of GVHD after umbilical cord blood transplantation. *Bone Marrow Transplant.* 2013;48:1046-1049.
15. Berglund S, Le Blanc K, Remberger M, et al. Factors with an impact on chimerism development and long-term survival after umbilical cord blood transplantation. *Transplantation.* 2012;94:1066-1074.
16. Arcese W, Rocha V, Labopin M, et al. Unrelated cord blood transplants in adults with hematologic malignancies. *Haematologica.* 2006;91:223-230.
17. Narimatsu H, Miyakoshi S, Yamaguchi T, et al. Chronic graft-versus-host disease following umbilical cord blood transplantation: retrospective survey involving 1072 patients in Japan. *Blood.* 2008;112:2579-2582.
18. Cohen YC, Scaradavou A, Stevens CE, et al. Factors affecting mortality following myeloablative cord blood transplantation in adults: a pooled analysis of three international registries. *Bone Marrow Transplant.* 2011;46:70-76.
19. Blin N, Traineau R, Houssin S, et al. Impact of donor-recipient major ABO mismatch on allogeneic transplantation outcome according to stem cell source. *Biol Blood Marrow Transplant.* 2010;16:1315-1323.

Unrelated Donor Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Hemoglobinopathies Using a Reduced-Intensity Conditioning Regimen and Third-Party Mesenchymal Stromal Cells

Sandhya Kharbanda [1,*], Angela R. Smith [2], Stephanie K. Hutchinson [1], David H. McKenna [3], James B. Ball [4], Lawrence S. Lamb Jr. [5], Rajni Agarwal [1], Kenneth I. Weinberg [1], John E. Wagner Jr. [2]

[1] *Division of Pediatric Hematology/Oncology/Stem Cell Transplantation, Stanford University, Palo Alto, California*
[2] *Division of Blood and Marrow Transplant Program, University of Minnesota, Minneapolis, Minnesota*
[3] *Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota*
[4] *Rocky Mountain Pediatric Hematology Oncology, Rocky Mountain Hospital for Children, Denver, Colorado*
[5] *Departments of Medicine and Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama*

Article history:
Received 3 November 2013
Accepted 19 December 2013

Key Words:
Hemoglobinopathies
Sickle cell disease
Thalassemia
Hematopoietic stem cell transplant
Reduced-intensity conditioning

ABSTRACT

Allogeneic hematopoietic stem cell transplantation for patients with a hemoglobinopathy can be curative but is limited by donor availability. Although positive results are frequently observed in those with an HLA-matched sibling donor, use of unrelated donors has been complicated by poor engraftment, excessive regimen-related toxicity, and graft-versus-host disease (GVHD). As a potential strategy to address these obstacles, a pilot study was designed that incorporated both a reduced-intensity conditioning and mesenchymal stromal cells (MSCs). Six patients were enrolled, including 4 with high-risk sickle cell disease (SCD) and 2 with transfusion-dependent thalassemia major. Conditioning consisted of fludarabine (150 mg/m^2), melphalan (140 mg/m^2), and alemtuzumab (60 mg for patients weighing > 30 kg and .9 mg/kg for patients weighing <30 kg). Two patients received HLA 7/8 allele matched bone marrow and 4 received 4-5/6 HLA

Financial disclosure: See Acknowledgments on page 585.

* Correspondence and reprint requests: Sandhya Kharbanda, Division of Hematology/Oncology/Stem Cell Transplantation, Department of Pediatrics, Stanford University, 1000 Welch Road, Suite 301, Palo Alto, CA 94304.
E-mail address: tkonuma@ims.u-tokyo.ac.jp (T. Konuma).

1083-8791/$ – see front matter

http://dx.doi.org/10.1016/j.bbmt.2013.12.564

Biol Blood Marrow Transplant 20 (2014) 396–401

Single-Unit Cord Blood Transplantation after Granulocyte Colony-Stimulating Factor–Combined Myeloablative Conditioning for Myeloid Malignancies Not in Remission

Takaaki Konuma [1,*], Seiko Kato [1], Jun Ooi [2], Maki Oiwa-Monna [1], Yasuhiro Ebihara [1], Shinji Mochizuki [1], Koichiro Yuji [1], Nobuhiro Ohno [1], Toyotaka Kawamata [1], Norihide Jo [1], Kazuaki Yokoyama [1], Kaoru Uchimaru [1], Shigetaka Asano [3], Arinobu Tojo [1], Satoshi Takahashi [1]

[1] *Department of Hematology/Oncology, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[2] *Department of Hematology/Oncology, Teikyo University School of Medicine, Tokyo, Japan*
[3] *System Medical Biology Laboratory, School of Advanced Science and Engineering, Waseda University, Tokyo, Japan*

Article history:
Received 9 November 2013
Accepted 4 December 2013

Key Words:
Cord blood transplantation
Granulocyte colony-stimulating factor
Acute myelogenous leukemia
Myelodysplastic syndrome
not in remission

A B S T R A C T

High disease burden in myeloablative allogeneic hematopoietic stem cell transplantation is associated with adverse outcomes in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS). Quiescent leukemia stem cells could be induced to enter cell cycle by granulocyte colony-stimulating factor (G-CSF) administration and become more susceptible to chemotherapy. We report on the outcome of unrelated cord blood transplantation (CBT) using a conditioning regimen of 12 Gy total body irradiation, G-CSF–combined high-dose cytarabine, and cyclophosphamide in 61 adult patients with AML or advanced MDS not in remission. With a median follow-up of 97 months, the probability of overall survival and cumulative incidence of relapse at 7 years were 61.4% and 30.5%, respectively. In multivariate analysis, poor-risk cytogenetics and high lactate dehydrogenase values at CBT were independently associated with inferior survival. These data demonstrate that CBT after G-CSF–combined myeloablative conditioning is a promising curative option for patients with myeloid malignancies not in remission.

INTRODUCTION

The prognoses of patients with acute myelogenous leukemia (AML) and advanced myelodysplastic syndrome (MDS) who have not achieved remission after chemotherapy have been poor. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only potentially curative therapy for such patients, high disease burden has been reported to be associated with increased relapse or poor survival rate after allo-HSCT [1-9]. Recently, cord blood (CB) has been considered an acceptable alternative as a source of hematopoietic stem cells in unrelated allo-HSCT for adult patients without HLA-identical related or unrelated donors [9-16]. In comparison with other sources of allo-HSCT, one of the main advantages of using CB for patients with a high disease burden who require urgent transplantation is its rapid and convenient availability. Because it was shown that administration of granulocyte colony-stimulating factor (G-CSF) increased the susceptibility of cell-cycle–specific agent cytarabine in leukemia cells in vitro [17], we administered G-CSF–combined high-dose cytarabine in myeloablative conditioning for allo-HSCT [18,19] and reported that a G-CSF–combined conditioning regimen provided better engraftment and survival results in cord blood transplantation (CBT) for myeloid malignancies [13-16]. The objective of this retrospective study was to confirm the effects of CBT after G-CSF–combined myeloablative conditioning in adult patients with myeloid malignancies not in remission and to identify variables influencing long-term outcomes.

PATIENTS AND METHODS

Patients and Transplantation Procedures

This retrospective study included 61 consecutive adult patients who underwent unrelated transplantation using single-unit CB for AML or advanced MDS not in remission at our institute between 1998 and 2013. Thirty-two patients were included in our previous study [15,16] and extended the follow-up. The diagnoses of AML and MDS were made according to the World Health Organization classification. Advanced MDS was defined as having refractory anemia with excess blasts type 1 or refractory anemia with excess blasts type 2 by World Health Organization classification. Myeloid malignancies not in remission were defined as more than 5% blasts in the bone marrow (BM), or circulating blasts in peripheral blood (PB) or central nervous system. The cytogenetic subgroups were defined according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML [20] and International Prognostic Scoring System criteria for MDS [21]. All patients received 12 Gy total body irradiation (TBI) in 4 divided fractions on days −8 and −7, cytarabine on days −5 and −4 (total dose 12 g/m2, and 3 g/m2 every 12 hours for 2 days) with 5 μg/kg G-CSF (lenograstim) from 12 hours before the first dose of cytarabine to the end of cytarabine dosing, and cyclophosphamide (total dose 120 mg/kg) on days −3 and −2 [15,16]. Fifty-eight patients received cyclosporine (CSP) (3 mg/kg/day) with a short course of methotrexate (15 mg/m2 on day +1 and 10 mg/m2 on days +3 and +6), and 3 patients received CSP only as graft-versus-host disease (GVHD) prophylaxis. CB units were obtained from the Japanese Cord Blood Bank Network. Donor-recipient HLA-matching status was based on antigen level HLA-A and -B and on allele level HLA-DRB1 typing. All patients received similar supportive care and CB units were

Financial disclosure: See Acknowledgments on page 400.

* Correspondence and reprint requests: Takaaki Konuma, Department of Hematology/Oncology, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail address: tkonuma@ims.u-tokyo.ac.jp (T. Konuma).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.12.555

Table 1
Characteristics of Patients, Cord Blood Units, and Transplantation

Characteristic	Value
No. of patients	61
Sex	
Male	36 (59)
Female	25 (41)
Age, median (range), yr	41 (18-55)
CMV serostatus	
Positive	54 (86)
Negative	7 (11)
Disease type	
De novo AML	24 (39)
AML secondary to MDS	24 (39)
Advanced MDS*	13 (21)
Cytogenetics†	
Good	1 (2)
Intermediate	27 (44)
Poor	30 (49)
Unknown	3 (5)
Bone marrow blasts at CBT, median (range), %	17.7 (1.4-86.0)¶
< 25%	39
≥ 25%	22
Peripheral blood blasts at CBT, median (range), %	6.5 (0-68.5)
Absent	12
Present	49
LDH at CBT	
≤ ULN	41 (67)
> ULN	20 (33)
Disease status at CBT‡	
Untreated	31 (51)
Primary refractory	14 (23)
Refractory relapse	16 (26)
Time from diagnosis to CBT, median (range), mo	7 (1-219)
Conditioning regimen	
TBI12Gy+Ara-C/G-CSF+CY	61
GVHD prophylaxis	
CyclosporineA+methotrexate	58 (95)
CyclosporineA	3 (5)
Number of nucleated cells, median (range), $\times 10^7$/kg	2.43 (1.32-5.50)
Number of CD34$^+$cells, median (range), $\times 10^5$/kg	1.03 (.21-2.27)
HLA disparities§	
1	13 (21)
2	32 (52)
3	14 (22)
4	2 (3)

CMV indicates cytomegalovirus; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; CBT, cord blood transplantation; LDH, lactate dehydrogenase; ULN, upper limit of normal; TBI, total body irradiation; Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide; GVHD, graft-versus-host disease; HLA, human leukocyte antigen.
Data presented are n (%) unless otherwise indicated.
* Advanced MDS are defined as having refractory anemia with excess blasts-1 (RAEB-1) or RAEB-2 by WHO criteria.
† The cytogenetic subgroups according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML and International Prognostic Scoring System criteria for MDS.
‡ Untreated was defined as no treatment before conditioning regimen, indicating that the majority of patients with AML secondary to MDS or advanced MDS received CBT as an up-front treatment. Primary refractory was defined as failure to achieve complete remission with induction chemotherapy. Refractory relapse was defined as failure to achieve complete remission with salvage chemotherapy after first or subsequent relapse.
§ The number of HLA disparities, defined as the low resolution for HLA-A and -B and the high resolution for HLA-DRB1.
¶ The 5 patients with less than 5% blasts in the bone marrow included circulating blasts in peripheral blood (n = 3) or central nervous system (n = 2).

selected, as previously reported [15,16]. The institutional review board of the Institute of Medical Science, University of Tokyo approved this study. This study was conducted in accordance with the Declaration of Helsinki.

End Points and Statistical Analysis

The primary study end point was overall survival (OS), defined as time from the date of transplantation to the date of death or last contact. Secondary end points were relapse, including disease progression before engraftment; transplantation-related mortality (TRM); neutrophil and platelet engraftment; acute graft-versus-host disease (aGVHD); and chronic GVHD (cGVHD). Relapse was defined as morphologic evidence of disease in PB, BM, or extramedullary sites. TRM was defined as death during remission. Neutrophil engraftment was defined as the first of 3 consecutive days during which the absolute neutrophil count was at least $.5 \times 10^9$/L. Platelet engraftment was achieved on the first of 3 days when the platelet count was higher than 50×10^9/L without transfusion support. Both aGVHD and cGVHD were graded according to the previously published criteria [22,23]. The incidence of aGVHD was evaluated in all engrafted patients, whereas the incidence of cGVHD was evaluated in engrafted patients surviving more than 100 days.

The probability of OS was estimated according to the Kaplan-Meier method, and the groups were compared using the log-rank test. The probabilities of relapse, TRM, neutrophil and platelet engraftment, and acute and chronic GVHD were estimated based on a cumulative incidence method to accommodate competing risks [24]. Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS and Fine and Gray proportional hazards model for relapse [25]. The following variables were considered: age (< 45 versus ≥ 45 years), disease type (de novo AML versus AML secondary to MDS versus advanced MDS), cytogenetic risk (other than poor versus poor), proportion of blasts in BM (< 25 versus ≥ 25%), the presence of blasts in PB (absent versus present), lactate dehydrogenase (LDH) at CBT (≤ upper limit of normal versus > upper limit of normal), disease status at CBT (untreated versus primary refractory versus refractory relapse), cord blood nucleated cell count (< 2.5 versus ≥ 2.5×10^7/kg), and HLA disparities based on antigen level HLA-A and -B and allele level

HLA-DRB1 (≤ 2 versus ≥ 3). All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria) [26]. $P < .05$ was considered significant. Analysis of data was performed in August 2013.

RESULTS

Patient and CB unit characteristics are shown in Table 1. The median age was 41 years (range, 18 to 55 years), the median number of nucleated cells was 2.43×10^7/kg (range, 1.32 to 5.50×10^7/kg), and the median number of CD34+ cells was 1.03×10^5/kg (range, .21 to 2.27×10^5/kg). Disease types were de novo AML in 24 patients, AML secondary to MDS in 24, and advanced MDS in 13. The majority of patients with de novo AML with multilineage dysplasia (n = 2), AML secondary to MDS (n = 19), or advanced MDS (n = 10) received CBT as an up-front treatment, which was classified as untreated group (n = 31). Among patients with primary refractory status (n = 14), 3 patients received CBT after the first cycle of induction chemotherapy. The median number of prior chemotherapy treatments before CBT for primary refractory status was 3 (range, 1 to 5). The median time from diagnosis to CBT was 7 months (range, 1 to 219 months), and the median period of follow-up for survivors after CBT was 97 months (range, 5 to 181 months).

The cumulative incidence of neutrophil recovery was 93.4% (95% confidence interval [CI], 81.0% to 97.8%) at 60 days after CBT with a median time to achieve greater than $.5 \times 10^9$/L neutrophils of 22 days (range, 18 to 41 days). Disease progression before engraftment occurred in 2 patients. The cumulative incidence of platelet recovery was 78.7% (95% CI, 65.7% to 87.2%) at 100 days after CBT with a median time to an untransfused platelet count greater than 50×10^9/L of 50 days (range, 30 to 179 days). The cumulative incidences of grade II to IV acute GVHD and extensive chronic GVHD were 62.3% (95% CI, 48.7% to 73.2%) at 100 days and 32.9% (95% CI, 21.4% to 44.9%) at 3 years after CBT, respectively. The probability of OS at 7 years was 61.4% (95% CI, 47.1% to 72.9%). The cumulative incidence of relapse at 7 years was 30.5% (95% CI, 19.2% to 42.6%). The cumulative incidence of TRM at 100 days and at 1 year was 6.6% (95% CI, 2.1% to 14.7%) and 8.2% (95% CI, 3.0% to 16.9%), respectively (Figure 1).

In multivariate analysis, poor-risk cytogenetics (hazard ratio [HR], 7.14; 95% CI, 2.33 to 21.80; $P < .001$) and high LDH value (HR, 4.00; 95% CI, 1.33 to 12.07; $P = .013$) were associated with inferior survival (Figure 2, Table 2). De novo AML (HR, 9.66; 95% CI, 1.06 to 87.75; $P = .044$), primary refractory status at CBT (HR, 6.47; 95% CI, 1.86 to 22.51; $P = .003$), and high LDH value (HR, 3.75; 95% CI, 1.11 to 12.57; $P = .032$) were associated with an increased relapse incidence (Table 3, Supplemental Figure 1). In contrast, the proportion of blasts in BM and the presence of blasts in PB did not show any impact on survival and relapse incidence.

DISCUSSION

Previous reports have suggested that the only potentially curative therapy for patients with myeloid malignancies not in remission is allo-HSCT. However, the incidence of relapse has been reported to be high, and several reports showed long-term survival rates of only 10% to 30% [1-6]. Several factors, including blasts in BM or PB, cytogenetics, and donor availability, have been associated with outcome. In this study, poor-risk cytogenetics and high LDH value were significantly associated with inferior OS. De novo AML, primary refractory status, and high LDH value were associated with increased relapse. However, we found no impact of disease burden on survival and relapse. In fact, several retrospective studies did not show any advantage of induction chemotherapy before allo-HSCT to reduce the disease burden for patients with advanced MDS or AML secondary to MDS [27-29]. Therefore, the majority of patients with advanced MDS or AML secondary to MDS received G-CSF–combined myeloablative conditioning followed by CBT without prior induction chemotherapy in our institute.

After physicians have decided that allo-HSCT is appropriate for patients with myeloid malignancy not in remission, the elective timing of the transplantation is the main advantage of CBT. In fact, CBT timing is decided depending on the patient's conditions, such as control of infection and disease burden. Such elective timing of CBT might have contributed to disease burden not being shown to influence outcome in our study. On the other hand, the use of CB as a source of hematopoietic stem cells could offer the opportunity for patients to receive allo-HSCT without related or unrelated donors. Moreover, the lower incidence of severe GVHD without compromising graft-versus-leukemia effects in CBT may also have contributed to long-term survival in our study.

Relapse is the most important cause of treatment failure after allo-HSCT, particularly in patients with myeloid malignancies not in remission. This is mainly due to the residual leukemic cells that have escaped the cytotoxic effect of conditioning before transplantation. To reduce disease relapse, the role of a more intense conditioning regimen has been analyzed extensively [30]. Since chemosensitization of leukemia cells with G-CSF enhances the cytotoxicity of the cell-cycle–specific agent cytarabine [17], we administered G-CSF–combined high-dose cytarabine in the standard conditioning regimen of TBI/cyclophosphamide. The clinical efficacy of concomitant use of G-CSF with chemotherapy has remained controversial in newly diagnosed or relapsed refractory AML and MDS [31,32]. Recently, Pabst et al. reported

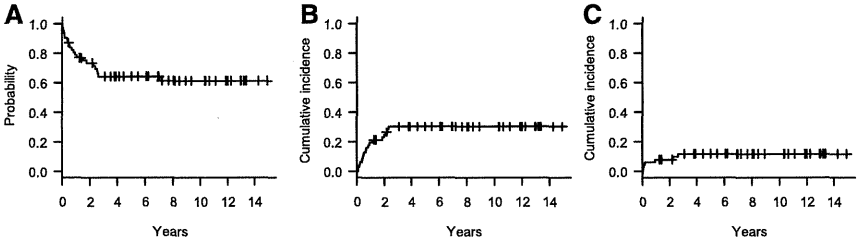

Figure 1. Probability of overall survival and cumulative incidences of relapse and transplant-related mortality after G-CSF–combined myeloablative CBT. Overall survival (A), relapse (B), and transplantation-related mortality (C) in 61 patients with AML or advanced MDS not in remission after G-CSF–combined myeloablative CBT.

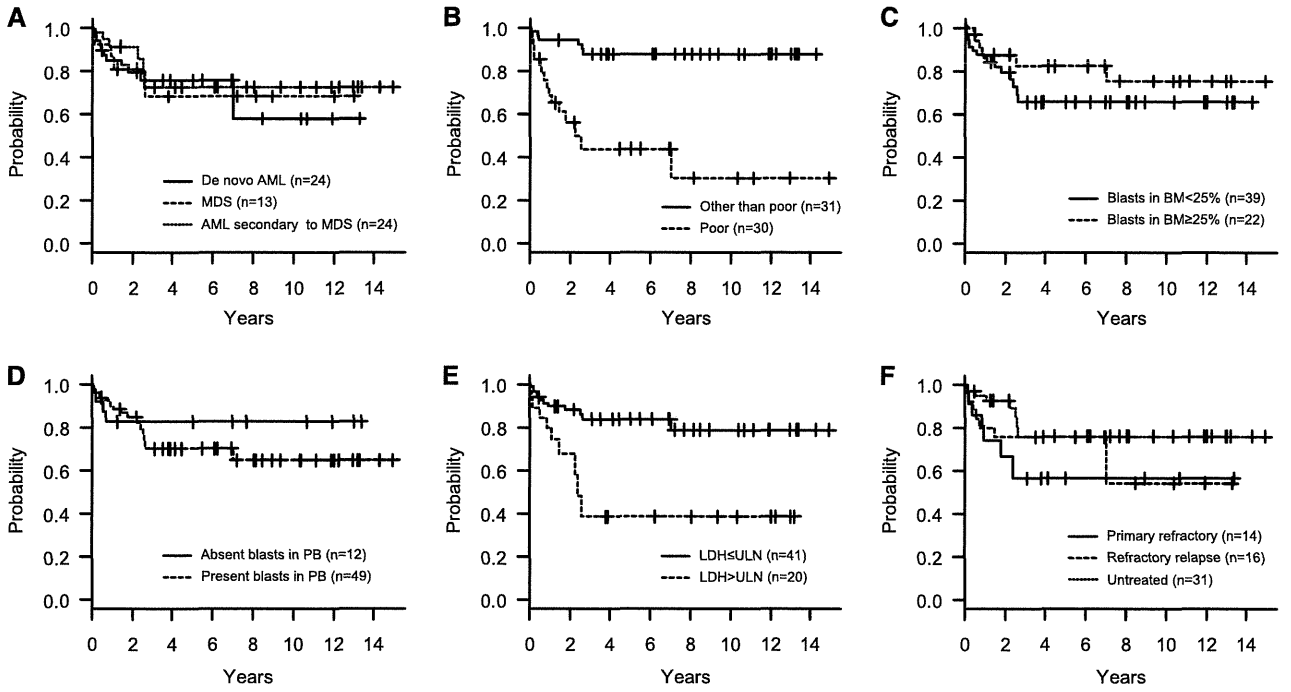

Figure 2. Adjusted probabilities of overall survival in 61 patients with AML and advanced MDS not in remission after G-CSF–combined myeloablative CBT. The adjusted probabilities of overall survival grouped according to the disease type (A), cytogenetic risk (B), the proportion of blasts in bone marrow (BM) (C), the presence of blasts in peripheral blood (PB) (D), the lactate dehydrogenase (LDH) value at cord blood transplantation (CBT) (E), and disease status at CBT (F). Multivariate analysis for overall survival is shown in Table 2.

Table 2
Univariate and Multivariate Analysis of Prognostic Factors for Survival

Variable	Univariate Analysis			Multivariate Analysis		
	Number	7-year OS (95% CI)	P	Hazard Ratio*	95% CI	P
Age						
< 45 years	36	63.5 (44.1-77.7)		1		
≥ 45	25	58.7 (36.7-75.4)	.555	.69	.25-1.86	.464
Disease type						
Advanced MDS	13	59.3 (27.5-81.0)		1		
AML secondary to MDS	24	74.4 (51.6-87.6)		.58	.13-2.54	.471
De novo AML	24	47.4 (23.0-68.4)	.234	.97	.18-5.16	.978
Cytogenetics†						
Other than poor	31	80.3 (61.3-90.6)		1		
Poor	30	38.9 (18.8-58.6)	.002	7.14	2.33-21.80	<.001
Bone marrow blasts at CBT, %						
< 25	39	58.0 (40.8-71.8)		1		
≥ 25	22	68.2 (41.2-84.7)	.297	.59	.16-2.09	.418
Peripheral blood blasts at CBT						
Absent	12	66.7 (33.7-86.0)		1		
Present	49	60.2 (44.0-73.1)	.983	1.18	.34-4.10	.787
LDH value at CBT						
≤ ULN	41	67.4 (48.9-80.4)		1		
> ULN	20	50.0 (27.1-69.2)	.147	4.00	1.33-12.07	.013
Disease status at CBT						
Untreated	31	71.1 (50.1-84.5)		1		
Primary refractory	14	50.0 (22.9-72.2)		2.76	.78-9.77	.114
Refractory relapse	16	50.0 (20.2-74.1)	.234	1.75	.30-10.22	.530
Number of nucleated cells, $\times 10^7$/kg						
≥ 2.5	29	59.2 (37.9-75.3)		1		
< 2.5	32	64.1 (44.3-78.4)	.989	.99	.38-2.58	.989
HLA disparities‡						
≤ 2	45	60.3 (43.7-73.4)		1		
≥ 3	16	65.0 (35.1-83.7)	.597	.98	.30-3.18	.975

MDS indicates myelodysplastic syndrome; AML, acute myelogenous leukemia; CBT, cord blood transplantation; LDH, lactate dehydrogenase; ULN, upper limit of normal; HLA, human leukocyte antigen; OS, overall survival; CI, confidence interval.

* Hazards ratio for overall mortality.

† The cytogenetic subgroups according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML and International Prognostic Scoring System criteria for MDS.

‡ The number of HLA disparities defined as the low resolution for HLA-A and -B and the high resolution for HLA–DRB1.