GVHD. Short tandem repeat (STR) analyses of the peripheral blood cells on days 29 and 92 demonstrated complete donor chimera. Bone marrow examinations on days 28 and 177 showed CR, and the cytogenetic analysis revealed a normal female karyotype, 46,XX.

Thirty-five months after the CBT, the patient complained of dyspnea. A CBC revealed a WBC of 2.8 $\times$ 10^9/L (blasts, 8%), a hemoglobin level of 5.6 g/dL, and a platelet count of 192 $\times$ 10^9/L. The bone marrow aspiration showed 77% blasts (Fig 1B) that were not distinguishable morphologically from the pretransplantation blasts. The immunophenotypes were similar but not identical to those of the pretransplantation blasts (CD10−, CD19+, CD13+, CD33+, CD34+, HLA-DR+). These results were compatible with the diagnosis of a relapse of the original leukemia. However, cytogenetic analysis showed a normal female karyotype, 46,XX, in each of the 20 metaphase cells examined (Fig 2B), and XY chromosome fluorescent in situ hybridization (FISH) showed an XX signal on 997 of 1,000 nuclei (99.7%). These results raised the concern that the recurrence was of donor-cell origin.

To determine the origin of the post-transplantation blasts, genomic DNA was extracted from the bone marrow smear, and a polymerase chain reaction of two informative autosomal STR loci, D3S3045 and D12S1064, was performed, as described by Ohashi et al.[1] STR analysis with the androgen receptor gene located on the X chromosome, and polymerase chain reaction analysis of the amelogenin gene with which both X-linked and Y-linked homologs could be amplified, were also performed. The results are shown in Figure 3. Red arrows, red triangles, gold arrows, and gold triangles indicate donor-specific, recipient-specific, X chromosome–specific, and Y chromosome–specific peaks, respectively. Additional stutter peaks (smaller in size) are visible to the left of the main peaks in STR analyses. The STR analysis showed that the percentage of recipient-derived DNA was 63.3% for D3S3045 and 75.0% for D12S1064 (Fig 3). In the STR analysis with the androgen receptor gene, two different peaks of donor origin and one peak of recipient origin were observed (Fig 3), thus demonstrating the presence of a recipient-derived X chromosome. In the amelogenin gene analysis, an X chromosome–specific peak was detected, but a Y chromosome–specific peak was not (Fig 3). This was consistent with the results of cytogenetic analysis and XY chromosome FISH.

The fact that the STR analysis of the peripheral blood cells on day 92 after CBT showed complete donor chimera (data not shown) strongly suggests that the residual normal hematopoiesis at the time of post-transplantation relapse was of donor origin. Accordingly, if the post-transplantation blasts had also been of donor origin, STR analyses of D3S3045 and D12S1064 should have demonstrated complete donor chimera. Therefore, the possibility of donor-cell leukemia (DCL) was ruled out. The pretransplantation and post-transplantation blasts were morphologically and immunophenotypically similar. In addition, the percentage of marrow blasts at the time of post-transplantation relapse, which was 77%, correlated well with the percentages of recipient-derived DNA detected in STR analyses of D3S3045 and D12S1064, which were 63.3% and 75.0%, respectively. These results strongly suggest that the post-transplantation blasts were of recipient origin. The simplest explanation for the normal female karyotype observed at the point of post-transplantation relapse is that it was a consequence of the loss of the Y chromosome, which occurred along with the duplication of an X chromosome. This hypothesis is supported by the fact that the recipient-derived X chromosome was detected in the specimen collected at the time of post-transplantation relapse by androgen receptor gene analysis (Fig 3). In light of these factors, we conclude that the post-transplantation blasts were of recipient origin.

Since the first report describing a patient with DCL in 1971,[2] several other patients with DCL have been described.[3-5] The simplest way to diagnose DCL is to demonstrate the opposite sex of the leukemic cells in sex-mismatched stem-cell transplantation (SCT), which is possible using cytogenetic analysis or XY chromosome FISH. However, our report clearly demonstrates that leukemic blasts from male patients sometimes lose their Y chromosome, and that doubling of an X chromosome can also occur. To the best of our knowledge, there is only one other report of this phenomenon. Spinelli et al[6] described a patient with relapsed leukemia after an allogeneic SCT, which was suggested to be of donor origin when examined by sex chromosome analysis, but later was demonstrated to be of recipient origin when examined using leukemia-specific molecular markers. Given that disease-specific molecular markers are not always available, more generally applicable techniques for demonstrating donor type are molecular analyses using polymorphic markers to differentiate donor and recipient cells. Our report describing this patient makes clear the need for adequate molecular analysis to determine the origin of leukemic relapse after allogeneic SCT.

It was reported recently that leukemic cells could escape from a donor's antileukemic T cells through the loss of a mismatched HLA

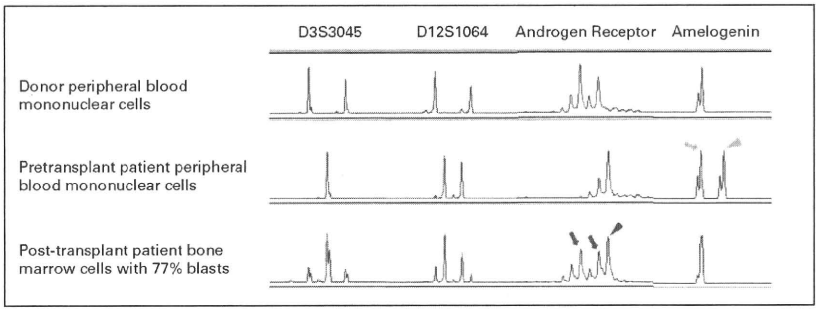

Fig 3.

haplotype after haploidentical SCT.[7] Because Y chromosome–encoded gene products serve as targets for female cytotoxic and helper T lymphocytes,[8] the loss of the Y chromosome observed in the current patient may have led to immune escape of the leukemic cells and relapse. The loss of the Y chromosome after female-to-male transplantations may be the consequence of selective pressure mediated by the donor's T cells and may not be a rare phenomenon.

The European Group for Blood and Marrow Transplantation reported that the incidence of DCL in allogeneic SCT recipients was about 0.1%.[3] Because molecular evaluation of the origin of relapsed leukemia cells is not routinely performed, the incidence of DCL may be higher or lower than that reported by the European Group for Blood and Marrow Transplantation survey. The routine use of molecular techniques to differentiate between donor and recipient cells enables us to determine an actual incidence of DCL, and this may lead to the clarification of the clinical features and pathogenesis of DCL. Moreover, a better understanding of DCL may provide new insights into the mechanism of leukemogenesis. The routine use of molecular techniques to evaluate the origin of relapsed leukemia after allogeneic SCT is warranted.

Nobuhiko Imahashi
Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

Haruhiko Ohashi and Kayo Arita
Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan

Kunio Kitamura
Ichinomiya Municipal Hospital, Ichinomiya, Japan

Taro Takahashi, Yukiyasu Ozawa, and Koichi Miyamura
Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
The author(s) indicated no potential conflicts of interest.

REFERENCES

1. Ohashi H, Kato C, Fukami S, et al: Leukemic relapse in the central nervous system after allogeneic stem cell transplantation with complete remission in the bone marrow and donor-type chimerism: Report of two cases. Am J Hematol 79:142-146, 2005
2. Fialkow PJ, Thomas ED, Bryant JI, et al: Leukaemic transformation of engrafted human marrow cells in vivo. Lancet 1:251-255, 1971
3. Hertenstein B, Hambach L, Bacigalupo A, et al: Development of leukemia in donor cells after allogeneic stem cell transplantation:A survey of the European Group for Blood and Marrow Transplantation (EBMT). Haematologica 90:969-975, 2005
4. Murata M, Ishikawa Y, Ohashi H, et al: Donor cell leukemia after allogeneic peripheral blood stem cell transplantation: A case report and literature review. Int J Hematol 88:111-115, 2008
5. Fraser CJ, Hirsch BA, Dayton V, et al: First report of donor cell–derived acute leukemia as a complication of umbilical cord blood transplantation. Blood 106:4377-4380, 2005
6. Spinelli O, Giussani U, Borleri G, et al: Need for an accurate molecular diagnosis to assess the donor origin of leukemia relapse after allogeneic stem cell transplantation. Haematologica 85:1153-1157, 2000
7. Vago L, Perna SK, Zanussi M, et al: Loss of mismatched HLA in leukemia after stem-cell transplantation. N Engl J Med 361:478-488, 2009
8. Spierings E, Vermeulen CJ, Vogt MH, et al: Identification of HLA class II-restricted H-Y-specific T-helper epitope evoking CD4+ T-helper cells in H-Y-mismatched transplantation. Lancet 362:610-615, 2003

DOI: 10.1200/JCO.2010.30.5813; published online ahead of print at www.jco.org on September 20, 2010

Acknowledgment

Supported by the Ministry of Health, Labor and Welfare of Japan Grant-in-Aid No. H20-meneki-ippan-017 (K.M.), the Research Committee for Idiopathic Hematopoietic Disorders of the Japanese Ministry of Health, Labor and Welfare, and the National Hospital Organization Research Fund (H.O.).

Bone Marrow Transplantation (2010), 1–11

www.nature.com/bmt

ORIGINAL ARTICLE

Disease stage stratified effects of cell dose in unrelated BMT for hematological malignancies: a report from Japan marrow donor program

Y Inamoto[1], K Miyamura[2], S Okamoto[3], H Akiyama[4], H Iida[5], T Eto[6], Y Morishima[7], K Kawa[8], A Kikuchi[9], Y Nagatoshi[10], J Tanaka[11], T Ashida[12], M Hirokawa[13], M Tsuchida[14] and S Mori[15] for the Japan Marrow Donor Program

[1]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan;* [2]*Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan;* [3]*Department of Medicine, Keio University School of Medicine, Tokyo, Japan;* [4]*Hematology Division, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan;* [5]*Department of Hematology, Meitetsu Hospital, Nagoya, Japan;* [6]*Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan;* [7]*Department of Hematology and Cell Therapy, Aichi Cancer Center, Nagoya, Japan;* [8]*Department of Pediatrics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan;* [9]*Department of Pediatrics, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan;* [10]*Department of Pediatrics, National Kyushu Cancer Center, Fukuoka, Japan;* [11]*Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan;* [12]*Division of Hematology, Department of Internal Medicine, Kinki University School of Medicine, Osaka, Japan;* [13]*Akita University Hospital, Clinical Oncology Center, Akita, Japan;* [14]*Department of Pediatrics, Ibaragi Children's Hospital, Ibaragi, Japan and* [15]*Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo, Japan*

Cell dose is one of the major factors that can be manipulated in unrelated BMT. However, regarding disease-stage-stratified effects of cell dose, data are limited. We analyzed the registry data from 3559 patients with acute leukemia, CML and myelodysplastic syndrome who received T-cell replete unrelated BMT through the Japan Marrow Donor Program. Adjusted effects of cell dose were evaluated for various outcomes separately according to disease stages and children or adults. Acute GVHD and nonrelapse mortality were not affected by cell dose. Among children, a cell dose lower than 3.0×10^8/kg was associated with lower engraftment rates in advanced-stage diseases. Among adults, a cell dose of 3.4×10^8/kg or higher was associated with lower relapse rates and better survival rates only in early-stage diseases, whereas cell dose below 2.3×10^8/kg was associated with lower engraftment rates in advanced-stage diseases. In conclusion, effects of cell dose may differ among disease stages. A cell dose of 3.4×10^8/kg or higher is recommended only for adults with early-stage diseases. With the number of patients available for analysis in this study, we could not show any significant benefits associated with 4.6×10^8/kg or higher in children.

Bone Marrow Transplantation advance online publication, 8 November 2010; doi:10.1038/bmt.2010.281

Keywords: allogeneic; cell dose; disease stage; unrelated

Correspondence: Dr Y Inamoto, Clinical Research Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, D5-290, PO Box 19024, Seattle, WA 98109-1024, USA.
E-mail: yinamoto@js3.so-net.ne.jp
Received 29 March 2010; revised 21 June 2010; accepted 12 August 2010

Introduction

Allogeneic hematopoietic cell transplantation has been established as a curative therapy for hematological malignancies.[1,2] Because of the better understanding of the significance of HLA allele compatibility and the advances in supportive care, the results of BMT from unrelated donors are improving.[3–5]

Cell dose is one of the major factors that can be manipulated by physicians and affect transplant outcomes.[6–8] Historically, its importance for engraftment and hematological recovery has been documented in patients with aplastic anemia.[9,10] Several subsequent studies showed that cell dose was also associated with better survival due to decreased nonrelapse mortality (NRM) in hematological malignancies. However, other important factors, such as patient age, disease, conditioning, GVHD prophylaxis, ABO compatibility, donor characteristics and HLA matching, also affect the transplant outcome.[11,12] Therefore, the actual effect of cell dose should be confirmed after adjustment for all of these factors with a sufficient number of patients.

On the other hand, the GVL effect may work differently according to disease stages. Rocha *et al.*[13] showed that cell dose was associated with decreased relapse rates in AML in first CR, whereas no significant associations between cell dose and relapse rates were observed in other studies, including various diseases.[7,8,11] These conflicting results suggested that the cell dose effect is worth analyzing separately according to disease stages.

In this report, we examined adjusted effects of cell dose on various transplant outcomes according to disease stages and children or adults using the detailed registry data of 3559 patients who received T-cell replete unrelated BMT through the Japan Marrow Donor Program.

Patients and methods

Patients

The data set consisted of 5071 unrelated BMTs facilitated by the Japan Marrow Donor Program between 1993 and 2005. Of these 5071 patients, 3559 with AML, ALL, CML and myelodysplastic syndrome who received their first T-cell replete myeloablative transplantation with GVHD prophylaxis containing calcineurin inhibitor without antithymocyte globulin were selected for this study. The patients and donors were all Japanese. Informed consent for this registry study was obtained from patients and donors in accordance with the declaration of Helsinki. This study was approved by the data management committees of Japan Marrow Donor Program.

Transplantation procedure

Patients were conditioned with various regimens determined by each transplant center. The proportions of TBI regimen were assessed from the database. Red cells and/or plasma removal from the graft was performed for ABO-major and/or -minor mismatched transplantation. All grafts were BM because the donation of PBSCs from unrelated donors is not yet approved in Japan. GVHD prophylaxis was categorized into either a CsA-based or tacrolimus-based prophylaxis.

HLA matching

HLA-A, -B and -DRB1 alleles were identified by high-resolution DNA typing as described previously.[3,4] As our previous study showed that a single-allele mismatch at DRB1 locus had no impact on engraftment, acute and chronic GVHD, NRM, relapse and OS in the Japanese population,[4] it was considered as a HLA-matched transplantation in this study.

Definition of disease stage and outcomes

Early stage was defined as the status of the first and second CR of AML and ALL, the first chronic phase of CML and refractory anemia of myelodysplastic syndrome, whereas advanced stage was defined as other status. For cytogenetic categorization, patients were divided into three categories: good risk (AML with t(15;17), inv16 or t(8;21)), intermediate risk (other than good or poor risk) or poor risk (ALL with t(9;22) or t(4;11), CML with additional abnormalities other than t(9;21) or myelodysplastic syndrome with complex or chromosome 7 abnormalities).[14] Engraftment was defined as an ANC of more than 500/μl for 3 consecutive days in the peripheral blood, and analyzed among all patients. Acute GVHD was graded by established criteria.[15] Chronic GVHD was assessed in patients surviving beyond day +100, and was classified as limited or extensive according to the Seattle criteria.[16]

Statistical analysis

Cell dose was defined as harvested total nucleated cell dose. Analysis was performed separately for disease stages, and children or adults. Children were defined as patients who were aged 12 years or younger for two reasons. One reason was because cell dose per patient body wt had a stronger linear correlation with age at these ages. Another reason was because patients aged 12 years or younger were usually treated with children's protocols. To determine the impacts of low and high cell doses on the outcomes in the current practices, cut-off points were set at upper and lower 25% of the cell dose separately in children and adults. Patient characteristics and causes of NRM were tested for associations using the χ^2-test for discrete variables, and the Spearman rank correlation test for continuous variables. Cumulative incidences of NRM, relapse and GVHD were estimated by Gray's method. Relapse was considered as a competing risk in NRM, deaths without relapse as a competing risk in relapse, and deaths without GVHD as a competing risk in GVHD. OS was calculated using the Kaplan–Meier method and *P*-values were calculated using a Log-rank test. Multivariate analyses were performed using logistic regression model for engraftment, the Cox proportional hazard regression model for OS, and the multivariate proportional hazard modeling of subdistribution functions in competing risks for NRM, relapse and GVHD.[17] Variables considered in the analysis were cell dose, patient age (linear), ABO incompatibility (none, major or minor), disease stage (early or advanced), cytogenetics (good, intermediate or poor), the number of HLA-mismatched loci, patient sex, donor sex, female to male transplantation, conditioning (TBI regimen, antithymocyte globulin regimen, and reduced-intensity regimen), GVHD prophylaxis (CsA-based or tacrolimus-based), donor age (linear), year of transplant (categorical) and preceding grades II–IV acute GVHD (only for chronic GVHD analysis). Cell dose was kept in the final model even though it was not statistically significant. All statistical tests were two-sided, and *P*-values less than 0.05 were considered significant. Analysis was performed using STATA (Stata Statistical Software: Release 10.0., Stata Corporation, College Station, TX, USA) and R version 2.10.0 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

The number of patients with AML, ALL, CML and myelodysplastic syndrome were 1205 (34%), 1140 (32%), 755 (21%) and 459 (13%), respectively. The median volumes of harvested marrow for child and adult recipients were 426 mL (range, 83–1045) and 850 mL (range, 220–1500), respectively ($P<0.0001$). The median numbers of harvested cells for child and adult recipients were 3.63×10^8/kg (range, 0.58–13.7) and 2.92×10^8/kg (range, 0.16–12.1), respectively ($P<0.0001$). Cut-off points were set at 3.0 and 4.6×10^8/kg for children, and 2.3 and 3.4×10^8/kg for adults. Patient characteristics were summarized in Tables 1 and 2. Recipient age, recipient–donor gender compatibility, recipient body wt, GVHD prophylaxis and the year of transplantation showed statistically significant differences according to cell dose in children. Recipient age, recipient–donor gender compatibility, recipient body wt, ABO mismatch, disease type in early-stage malignancy, GVHD prophylaxis and the year of transplantation showed statistically significant differences according to cell dose in adults.

Table 1 Patient characteristics in children

Characteristic	*Cell dose*						P
	$<3.0\times10^8$/kg (n = 140)		*3.0–4.6 $\times10^8$/kg (n = 248)*		*$\geq4.6\times10^8$/kg (n = 128)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	
Recipient age, years							
Median	9		8		5		<0.001
Range	0–12		0–12		0–12		
Donor age, years							
Median	35		34		32		0.20
Range	21–50		20–50		20–50		
Sex (recipient/donor)							
Male/male	33	24	71	29	47	37	0.001
Female/female	41	29	65	26	23	18	
Male/female	50	36	58	23	25	20	
Female/male	16	11	54	22	33	26	
Recipient body wt, kg							
Median	27		25		17		<0.001
Range	5–72		5–49		4–44		
ABO mismatch							
Match	96	69	154	62	66	52	0.063
Major mismatch	29	21	55	22	37	29	
Minor mismatch	15	11	39	16	25	20	
Disease							
Early-stage malignancy							0.50
AML	18	20	53	30	23	26	
ALL	62	68	107	60	52	58	
CML	7	8	14	8	10	11	
MDS	4	4	4	2	4	4	
Advanced-stage malignancy							0.51
AML	10	20	18	26	9	23	
ALL	28	57	37	53	18	46	
CML	4	8	1	1	2	5	
MDS	7	14	14	20	10	26	
Cytogenetics							
Good risk	4	3	17	7	8	6	0.55
Intermediate risk	110	79	189	76	98	77	
Poor risk	18	13	25	10	17	13	
Not available	8	6	17	7	5	4	
Conditioning							
TBI regimen	122	87	209	84	102	80	0.25
Non-TBI regimen	18	13	39	16	26	20	
GVHD prophylaxis							
Cyclosporin-based	44	31	100	40	71	55	<0.001
Tacrolimus-based	96	69	148	60	57	45	
No. of HLA mismatch by DNA typing							
0	95	68	190	77	90	70	0.39
1 locus	40	29	52	21	33	26	
2 or more loci	5	4	6	2	5	4	
Year of transplantation							
1993–1996	18	13	44	18	31	24	0.009
1997–2000	39	28	67	27	50	39	
2001–2003	54	39	87	35	32	25	
2004–2005	29	21	50	20	15	12	

Abbreviation: MDS = myelodysplastic syndrome.

Engraftment

Engraftment was achieved in 500 of 516 (97%) child patients and 2882 of 3043 (95%) adult patients. Multivariate analysis showed that $<3.0\times10^8$/kg was associated with lower engraftment rates in children with advanced-stage diseases (odds ratio, 0.15; 95% confidence interval (CI), 0.03–0.74; $P=0.02$) and $<2.3\times10^8$/kg was associated with lower engraftment rates in adults with advanced-stage diseases (odds ratio, 0.60; 95% CI, 0.37–0.97; $P=0.039$).

Table 2 Patient characteristics in adults

Characteristic	*Cell dose*						P
	<2.3 × 10⁸/kg (n = 755)		*2.3–3.4 × 10⁸/kg (n = 1519)*		*≥3.4 × 10⁸/kg (n = 769)*		
	No.	*%*	*No.*	*%*	*No.*	*%*	
Recipient age, years							
Median	34		34		32		0.0076
Range	13–65		13–66		13–62		
Donor age, years							
Median	34		34		34		0.42
Range	20–51		20–68		20–51		
Sex (recipient/donor)							
Male/male	309	41	666	44	336	44	<0.001
Female/female	179	24	287	19	132	17	
Male/female	188	25	253	17	91	12	
Female/male	79	10	313	21	210	27	
Recipient body wt, kg							
Median	61		59		55		<0.001
Range	29–120		25–112		23–90		
ABO mismatch							
Match	401	53	800	53	355	46	<0.001
Major mismatch	191	25	417	27	271	35	
Minor mismatch	163	22	302	20	143	19	
Disease							
Early-stage malignancy							0.002
AML	187	40	347	37	149	32	
ALL	148	31	281	30	155	33	
CML	89	19	248	26	135	29	
MDS	48	10	62	7	34	7	
Advanced-stage malignancy							0.83
AML	104	37	189	33	98	33	
ALL	62	22	129	22	61	21	
CML	59	21	124	21	62	21	
MDS	58	20	139	24	75	25	
Cytogenetics							
Good risk	54	7	116	8	45	6	0.59
Intermediate risk	615	81	1215	80	622	81	
Poor risk	54	7	105	7	58	8	
Not available	32	4	83	5	44	6	
Conditioning							
TBI regimen	634	84	1245	82	621	81	0.25
Non-TBI regimen	121	16	274	18	148	19	
GVHD prophylaxis							
CsA-based	337	45	833	55	418	54	<0.001
Tacrolimus-based	418	55	686	45	351	46	
No of HLA mismatch by DNA typing							
0	584	77	1183	78	608	79	0.90
1 locus	158	21	306	20	146	19	
2 or more loci	13	2	30	2	15	2	
Year of transplantation							
1993–1996	70	9	227	15	113	15	<0.001
1997–2000	158	21	500	33	293	38	
2001–2003	329	44	509	34	230	30	
2004–2005	198	26	283	19	133	17	

Abbreviation: MDS = myelodysplastic syndrome.

Acute and chronic GVHD

The cumulative incidences of grades II–IV acute GVHD in children and adults were 50 and 43%, respectively. Multivariate analysis showed no statistically significant association of cell dose with incidences of grades II–IV acute GVHD in children and adults.

Table 3 Variables associated with relapse in (a) children and (b) adults

Variable	*Early-stage disease*						*Advance- stage disease*					
	Univariate			*Multivariate*			*Univariate*			*Multivariate*		
	HR	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P
(a)												
n − 358									*n − 158*			
Cell dose (× 10⁸/kg)												
3.0 4.6	1.00		1.00	1.00		1.00						
<3.0	1.06	(0.60 1.87)	0.84	0.99	(0.56 1.75)	0.98	1.18	(0.66 2.14)	0.57	1.03	(0.54 1.95)	0.93
⩾4.6	1.22	(0.70 2.14)	0.48	1.20	(0.69 2.09)	0.52	0.98	(0.54 1.81)	0.96	0.95	(0.53 1.72)	0.87
Recipient age												
Linear	0.95	(0.90 1.01)	0.14	0.99	(0.92 1.07)	0.83						
Donor age												
Linear	1.01	(0.99 1.04)	0.37	0.96	(0.92 0.99)	0.02	0.96	(0.92 0.99)				0.021
Cytogenetics												
Intermediate risk	1.00		1.00		1.00							
Good risk	Unevaluable[a]	<0.001	Unevaluable[a]	<0.001	1.71	(0.8 3.67)	0.16					
Poor risk	1.43	(0.76 2.69)	0.27	1.42	(0.76 2.65)	0.27	0.78	(0.27 2.24)	0.64			
ABO mismatch												
Match	1.00						1.00			1.00		
Major mismatch	1.11	(0.64 1.91)	0.72				0.48	(0.24 0.94)	0.031	0.48	(0.23 0.98)	0.043
Minor mismatch	0.80	(0.40 1.61)	0.54				0.66	(0.33 1.31)	0.23	0.25		
HLA mismatch												
Match	1.00						1.00					
Mismatch	0.95	(0.61 1.48)	0.81				0.63	(0.38 1.04)	0.072			
Recipient sex												
Male	1.00						1.00					
Female	0.97	(0.61 1.55)	0.90				0.92	(0.56 1.52)	0.76			
Donor sex												
Male	1.00						1.00					
Female	1.11	(0.70 1.76)	0.67				0.99	(0.61 1.63)	0.98			
Female donor to male recipient												
No	1.00						1.00					
Yes	1.20	(0.72 2.02)	0.48				1.17	(0.69 2)	0.56			
Conditioning												
Non-TBI regimen	1.00						1.00					
TBI regimen	0.62	(0.36 1.06)	0.08				0.67	(0.38 1.21)	0.18			
GVHD prophylaxis												
CsA-based	1.00						1.00					
Tacrolimus-based	0.91	(0.57 1.45)	0.68				1.02	(0.62 1.67)	0.93			
Year of transplantation												
1993 1996	1.00						1.00					
1997 2000	0.86	(0.44 1.70)	0.67				1.29	(0.64 2.6)	0.47			
2001 2003	1.02	(0.53 1.96)	0.95				1.20	(0.61 2.39)	0.60			
2004 2005	0.72	(0.32 1.61)	0.42				0.99	(0.4 2.44)	0.98			
(b)												
n − 1883									*n − 1160*			
Cell dose (× 10⁸/kg)												
2.3 3.4	1.00			1.00			1.00			1.00		
<2.3	1.13	(0.85 1.49)	0.41	1.09	(0.82 1.44)	0.56	1.20	(0.94 1.55)	0.14	1.21	(0.94 1.56)	0.13
⩾3.4	0.61	(0.43 0.85)	0.0042	0.60	(0.43 0.85)	0.004	0.91	(0.70 1.18)	0.48	0.90	(0.70 1.17)	0.44
Recipient age												
Linear	0.99	(0.98 1.00)	0.28				0.99	(0.98 1.00)	0.015	0.99	(0.98 1.00)	0.0088
Donor age												
Linear	0.99	(0.97 1.00)	0.088				0.99	(0.98 1.00)	0.20			
Cytogenetics												
Intermediate risk	1.00						1.00					
Good risk	0.97	(0.60 1.58)	0.91				1.33	(0.89 1.99)	0.16			
Poor risk	1.43	(0.91 2.24)	0.12				1.00	(0.66 1.51)	0.98			

Table 3 Continued

Variable	Early-stage disease						Advance- stage disease					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
ABO mismatch												
Match	1.00						1.00			1.00		
Major mismatch	1.10	(0.83 1.46)	0.52				0.70	(0.55 0.90)	0.0045	0.71	(0.56 0.92)	0.0081
Minor mismatch	0.97	(0.70 1.36)	0.88				0.77	(0.59 1.02)	0.07	0.76	(0.58 1.01)	0.055
HLA mismatch												
Match	1.00						1.00			1.00		
Mismatch	0.92	(0.70 1.22)	0.57				0.73	(0.57 0.92)	0.0093	0.73	(0.57 0.93)	0.01
Recipient sex												
Male	1.00						1.00					
Female	1.11	(0.87 1.43)	0.40				1.08	(0.87 1.33)	0.47			
Donor sex												
Male	1.00						1.00					
Female	1.05	(0.81 1.35)	0.72				0.90	(0.73 1.13)	0.37			
Female donor to male recipient												
No	1.00						1.00					
Yes	0.87	(0.62 1.22)	0.41				0.81	(0.61 1.09)	0.17			
Conditioning												
Non-TBI regimen	1.00						1.00					
TBI regimen	1.36	(0.95 1.95)	0.10				1.08	(0.82 1.42)	0.58			
GVHD prophylaxis												
CsA-based	1.00						1.00					
Tacrolimus-based	1.50	(1.17 1.92)	0.0014	1.49	(1.16 1.91)	0.0017	1.07	(0.87 1.31)	0.53			
Year of transplantation												
1993 1996	1.00						1.00					
1997 2000	1.20	(0.77 1.86)	0.42				1.06	(0.74 1.52)	0.74			
2001 2003	1.59	(1.05 2.43)	0.03				1.24	(0.87 1.76)	0.23			
2004 2005	2.02	(1.27 3.19)	0.0028				1.19	(0.81 1.76)	0.37			

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a]Hazard ratio was unevaluable because of no events.

The cumulative incidences of limited or extensive chronic GVHD in children and adults were 34 and 45%, respectively. Multivariate analysis in children showed a statistically significant association of $<3.0 \times 10^8$/kg with higher incidences of chronic GVHD in advanced-stage diseases (hazard ratio, 2.46; 95% CI, 1.17–5.17; $P=0.017$). Multivariate analysis in adults showed no statistically significant association of cell dose with incidences of chronic GVHD.

NRM

The cumulative incidences of NRM at 5 years in children and adults were 21 and 39%, respectively. Multivariate analysis showed no statistically significant association of cell dose with incidences of NRM in children (Supplementary Table S1a) and adults (Supplementary Table S1b). Causes of NRM according to cell dose were not statistically different in children. As a cause of NRM in adults, the proportions of idiopathic pneumonia syndrome were statistically different according to cell dose (13, 14 and 23% for <2.3, 2.3–3.4 and $>3.4 \times 10^8$/kg, respectively; $P=0.002$).

Relapse

The cumulative incidences of relapse at 5 years in children and adults were 27 and 25%, respectively. Multivariate analysis in children showed no statistically significant association of cell dose with incidences of relapse (Table 3a). Multivariate analysis in adults showed a statistically significant association of $>3.4 \times 10^8$/kg with lower incidences of relapse in early-stage diseases (hazard ratio, 0.60; 95% CI, 0.43–0.85; $P=0.004$) (Table 3b). Results were similar when CML in chronic phase was excluded from analysis in adults (data not shown).

OS

The median follow-up periods among survivors were 57 months (range, 9–140 months) in children and 55 months (range, 3–147 months) in adults. The OS rates at 5 years among children with early-stage diseases were 67, 75 and 68% for <3.0, 3.0–4.6 and $>4.6 \times 10^8$/kg, respectively ($P=0.74$; Figure 1a). The OS rates at 5 years among children with advanced-stage diseases were 31, 36 and 40% for <3.0, 3.0–4.6 and $>4.6 \times 10^8$/kg, respectively

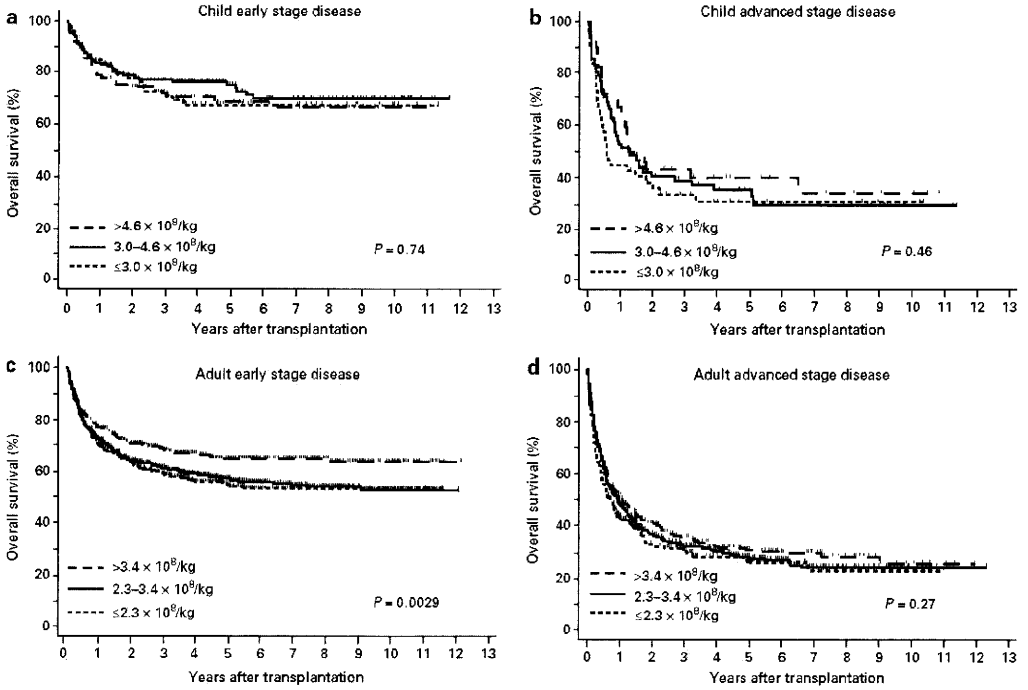

Figure 1 Kaplan–Meier estimates of OS according to cell dose: (**a**) among children with early-stage diseases; (**b**) among children with advanced-stage diseases; (**c**) among adults with early-stage diseases; and (**d**) among adults with advanced-stage diseases.

($P=0.46$; Figure 1b). The OS rates at 5 years among adults with early-stage diseases were 54, 57 and 65% for <2.3, 2.3–3.4 and $>3.4\times10^8$/kg, respectively ($P=0.0029$; Figure 1c). The OS rates at 5 years among adults with advanced-stage diseases were 26, 28 and 31% for <2.3, 2.3–3.4 and $>3.4\times10^8$/kg, respectively ($P=0.27$; Figure 1d).

Multivariate analysis in children showed no statistically significant association of cell dose with survival rates (Table 4a). Multivariate analysis in adults showed a statistically significant association of $>3.4\times10^8$/kg with better survival rates only in early-stage diseases (hazard ratio, 0.74; 95% CI, 0.62–0.90; $P=0.002$) (Table 4b).

Discussion

This study showed that effects of cell dose on transplant outcomes were different among disease stages. Among children, we could not show any statistically significant effects of cell dose except the lower engraftment rates and higher incidences of chronic GVHD associated with $<3.0\times10^8$/kg in advanced-stage diseases. Among adults, cell dose $>3.4\times10^8$/kg was associated with decreased relapse rates and better survival rates in early-stage diseases, whereas cell dose was not associated with outcomes except the lower engraftment rates with $<2.3\times10^8$/kg in advanced-stage diseases.

Although many studies reported that higher cell dose improved OS rates,[8,11,12,18,19] effects of cell dose on relapse and NRM rates were not consistent among studies probably because of the differences in diseases, stages and transplant procedures. Furthermore, it is not practical to analyze child and adult patients together because biology of disease, treatment protocols and harvested total nucleated cells per body wt are likely to differ between them. Therefore, we investigated cell dose effects separately according to disease stages and children or adults, and extended analysis to various outcomes.

Although several studies showed that engraftment rates were improved with higher cell dose,[6,11] our results did not show any statistically significant merits with high cell dose both in children and adults. Low cell dose was associated with worse engraftment rates in advanced-stage diseases in both children and adults. Effects of low cell dose would be particularly great in advanced-stage diseases considering that graft failure occurs more frequently in advanced-stage diseases.[7]

Effects of cell dose on relapse rates were controversial. Although several studies did not show any effects of cell dose on relapse rates,[7,8,11] the results of our study supported those by Rocha *et al.*[13] among patients with AML in the first CR, and those by Barrett *et al.*[20] after

Table 4 Variables associated with OS in (a) children and (b) adults

Variable	*Early stage disease (n = 358)*						*Advanced stage disease (n = 158)*					
	Univariate			*Multivariate*			*Univariate*			*Multivariate*		
	HR	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P
(a)												
n = 358							*n = 158*					
Cell dose (× 10^8/kg)												
3.0 4.6	1.00			1.00			1.00			1.00		
<3.0	1.15	(0.72 1.85)	0.56	1.09	(0.68 1.75)	0.73	1.59	(0.85 2.95)	0.14	1.39	(0.87 2.20)	0.17
⩾4.6	1.18	(0.74 1.89)	0.49	1.18	(0.74 1.89)	0.48	0.99	(0.63 1.56)	0.96	0.87	(0.53 1.43)	0.59
Recipient age												
Linear	1.01	(0.95 1.07)	0.86				1.04	(0.98 1.10)	0.20			
Donor age												
Linear	1.02	(1.00 1.05)	0.11				1.01	(0.98 1.04)	0.41			
Cytogenetics												
Intermediate risk	1.00						1.00					
Good risk	0.75	(0.27 2.06)	0.58				1.18	(0.55 2.56)	0.67			
Poor risk	1.09	(0.60 1.96)	0.79				1.20	(0.60 2.39)	0.61			
ABO mismatch												
Match	1.00						1.00					
Major mismatch	1.40	(0.88 2.22)	0.15				0.87	(0.54 1.39)	0.55			
Minor mismatch	1.49	(0.89 2.51)	0.13				0.71	(0.41 1.25)	0.24			
HLA mismatch												
Match	1.00			1.00			1.00					
Mismatch	1.72	(1.30 2.27)	<0.001	1.72	(1.30 2.27)	<0.001	1.11	(0.77 1.60)	0.58			
Recipient sex												
Male	1.00						1.00					
Female	1.04	(0.70 1.54)	0.86				1.25	(0.85 1.85)	0.25			
Donor sex												
Male	1.00						1.00					
Female	1.26	(0.85 1.87)	0.25				0.72	(0.49 1.07)	0.10			
Female donor to male recipient												
No	1.00						1.00			1.00		
Yes	1.10	(0.71 1.70)	0.68				0.63	(0.40 0.99)	0.05	0.57	(0.35 0.91)	0.02
Conditioning												
Non-TBI regimen	1.00						1.00					
BI regimen	1.01	(0.59 1.72)	0.98				1.26	(0.74 2.15)	0.40			
GVHD prophylaxis												
CsA-based	1.00						1.00					
Tacrolimus-based	1.07	(0.71 1.60)	0.75				0.83	(0.56 1.22)	0.34			
Year of transplantation												
1993 1996	1.00						1.00					
1997 2000	0.74	(0.44 1.25)	0.27				1.10	(0.65 1.87)	0.73			
2001 2003	0.59	(0.34 1.03)	0.06				0.87	(0.51 1.49)	0.61			
2004 2005	0.69	(0.35 1.36)	0.29				0.90	(0.46 1.76)	0.76			
(b)												
n = 1883									*n = 1160*			
Cell dose (× 10^8/kg)												
2.3 3.4	1.00			1.00			1.00			1.00		
<2.3	1.05	(0.88 1.25)	0.59	1.06	(0.89 1.26)	0.54	1.10	(0.93 1.31)	0.25	1.15	(0.97 1.37)	0.11
⩾3.4	0.75	(0.62 0.90)	0.002	0.74	(0.62 0.90)	0.002	0.94	(0.79 1.11)	0.47	0.94	(0.80 1.12)	0.52
Recipient age												
Linear	1.01	(1.01 1.02)	<0.001	1.01	(1.01 1.02)	<0.001	1.00	(1.00 1.01)	0.61			
Donor age												
Linear	1.01	(1.00 1.02)	0.01	1.01	(1.00 1.02)	0.02	1.00	(0.99 1.01)	0.42			
Cytogenetics												
Intermediate risk	1.00						1.00			1.00		
Good risk	0.79	(0.59 1.06)	0.12				1.05	(0.78 1.41)	0.75	1.04	(0.77 1.40)	0.80
Poor risk	1.09	(0.82 1.45)	0.56				1.59	(1.24 2.04)	<0.001	1.61	(1.26 2.07)	<0.001

Table 4 Continued

Variable	*Early stage disease (n = 358)*						*Advanced stage disease (n = 158)*					
	Univariate			*Multivariate*			*Univariate*			*Multivariate*		
	HR	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P	*HR*	*95% CI*	P
ABO mismatch												
Match	1.00			1.00			1.00					
Major mismatch	1.16	(0.98 1.37)	0.08	1.18	(1.00 1.40)	0.05	1.10	(0.94 1.30)	0.23			
Minor mismatch	1.08	(0.89 1.31)	0.42	1.12	(0.92 1.36)	0.26	1.11	(0.93 1.33)	0.26			
HLA mismatch												
Match	1.00			1.00			1.00			1.00		
Mismatch	1.41	(1.22 1.63)	<0.001	1.38	(1.19 1.60)	<0.001	1.34	(1.18 1.53)	<0.001	1.31	(1.15 1.50)	<0.001
Recipient sex												
Male	1.00						1.00					
Female	0.88	(0.75 1.02)	0.08				0.96	(0.83 1.10)	0.55			
Donor sex												
Male	1.00						1.00					
Female	1.00	(0.86 1.16)	0.97				0.96	(0.83 1.11)	0.56			
Female donor to male recipient												
No	1.00						1.00					
Yes	1.11	(0.93 1.34)	0.25				1.06	(0.89 1.27)	0.50			
Conditioning												
Non-TBI regimen	1.00						1.00					
TBI regimen	0.90	(0.74 1.08)	0.26				1.00	(0.83 1.19)	0.97			
GVHD prophylaxis												
CsA-based	1.00						1.00					
Tacrolimus-based	1.04	(0.90 1.20)	0.60				0.85	(0.74 0.97)	0.02			
Year of transplantation												
1993 1996	1.00			1.00			1.00			1.00		
1997 2000	0.75	(0.60 0.93)	0.009	0.79	(0.63 0.99)	0.04	0.77	(0.62 0.95)	0.014	0.79	(0.63 0.98)	0.032
2001 2003	0.82	(0.66 1.02)	0.072	0.80	(0.64 1.00)	0.053	0.70	(0.56 0.87)	0.001	0.72	(0.58 0.90)	0.005
2004 2005	0.92	(0.72 1.19)	0.54	0.85	(0.65 1.11)	0.23	0.66	(0.51 0.85)	0.001	0.68	(0.53 0.88)	0.003

Abbreviations: CI = confidence interval; HR = hazard ratio.

identical twin BMT. Interestingly, our results showed lower relapse rates not associated with higher incidences of acute GVHD, which was also observed in the studies by Rocha *et al.*[13] and by Barrett *et al.*[20] GVL effect is influenced by disease types and stages possibly because of the differences in expression of tumor Ags, co-stimulatory molecules, resistance to killing and growth patterns.[21,22] It has been demonstrated that the GVL effect works more efficiently for minimal residual disease than for active disease.[23,24] Therefore, it is reasonable that decreased relapse rates with $\geqslant 3.4 \times 10^8$/kg was limited to early-stage diseases. Although it may be argued that patients with CML in chronic phase greatly influence the outcomes,[25] the results were similar even if these patients were excluded from analysis.

What are effector cells of cell dose effect? Calculated with the published data,[26] 1×10^8/kg nucleated BM cells include 8×10^6/kg T cells, 3×10^6/kg B cells and 2×10^6/kg nature killer cells. Considering the cell dose used in adaptive immunotherapies with these cells,[27–29] this number of T cells can alter the outcome but that of nature killer cells will not. Therefore, we speculated that T cells would be the most likely population affecting relapse rates. As the registry did not have data as to graft composition during the study period, we could not confirm this hypothesis in our data. Using total nucleated cells as the surrogate for cell dose may have limitations because some studies showed that more specific fractions, such as $CD34^+$ cell dose also predicted transplant outcomes.[30,31] Future studies analyzing the effect of subpopulations in grafts are warranted.

Many previous studies reported that higher cell dose decreased NRM, particularly related to infection.[7,8,12,32] However, no significant effects of cell dose on NRM rates were observed in our study. To address this discrepancy, we performed a further analysis on causes of NRM according to cell dose, which showed no significant differences in the proportions of deaths from infection both in children and adults. This would partly account for the discrepancy.

In light of the study which reported that 7×10^7/kg nucleated cells are enough to induce GVHD after donor leukocyte infusion,[33] higher cell dose may result in increased incidences of GVHD. However, most of the previous studies showed that cell dose had no effect on acute GVHD or that higher cell dose decreased acute GVHD.[7,8,18] They speculated a possible effect of accessory cells, such as MSCs, and a possibility that higher cell dose decreased early post transplant infections that might

amplify GVHD. Our results were compatible with these reports. We could not explain why $< 3.0 \times 10^8$/kg resulted in increased incidences of chronic GVHD among children with advanced-stage diseases.

There are two possible explanations for the discrepancy observed with regard to the effect of cell dose on OS in children and adults. First, a much greater volume of harvested marrow for adults as compared with children (almost twice the volume) might bring about higher contamination of peripheral blood and increase the dose of graft T cells to produce the different effects.[34] Second, cell dose effect might be already saturated in children because most children received much more cell dose than adults (7×10^7/kg more at median). Different analytical power between children and adults would not account for the discrepancy as the point estimate of hazard ratio in children with early-stage diseases was more than 1.0 with $> 4.6 \times 10^8$/kg (Table 4a).

In summary, our results suggested a strategy to determine an optimal cell dose of BMT according to disease stages to maximize the efficacy of BMT and minimize the risk of donors, although these results should be interpreted with caution because of their retrospective nature. In terms of overall benefits, cell dose of 3.4×10^8/kg or higher is recommended only for adults with early-stage diseases. With the number of patients available for analysis in our study, we could not show any significant benefits associated with 4.6×10^8/kg or higher in children.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported in part by a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan.

References

1 Thomas E, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE *et al.* Bone-marrow transplantation (first of two parts). *N Engl J Med* 1975; **292**: 832 843.
2 Kernan NA, Bartsch G, Ash RC, Beatty PG, Champlin R, Filipovich A *et al.* Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. *N Engl J Med* 1993; **328**: 593 602.
3 Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H *et al.* Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. *N Engl J Med* 1998; **339**: 1177 1185.
4 Morishima Y, Sasazuki T, Inoko H, Juji T, Akaza T, Yamamoto K *et al.* The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. *Blood* 2002; **99**: 4200 4206.
5 Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M *et al.* High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* 2007; **110**: 4576 4583.
6 Davies SM, Kollman C, Anasetti C, Antin JH, Gajewski J, Casper JT *et al.* Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the national marrow donor program. *Blood* 2000; **96**: 4096 4102.
7 Sierra J, Storer B, Hansen JA, Bjerke JW, Martin PJ, Petersdorf EW *et al.* Transplantation of marrow cells from unrelated donors for treatment of high-risk acute leukemia: the effect of leukemic burden, donor HLA-matching, and marrow cell dose. *Blood* 1997; **89**: 4226 4235.
8 Dominietto A, Lamparelli T, Raiola AM, Van Lint MT, Gualandi F, Berisso G *et al.* Transplant-related mortality and long-term graft function are significantly influenced by cell dose in patients undergoing allogeneic marrow transplantation. *Blood* 2002; **100**: 3930 3934.
9 Storb R, Prentice RL, Thomas ED. Marrow transplantation for treatment of aplastic anemia. An analysis of factors associated with graft rejection. *N Engl J Med* 1977; **296**: 61 66.
10 Deeg HJ, Self S, Storb R, Doney K, Appelbaum FR, Witherspoon RP *et al.* Decreased incidence of marrow graft rejection in patients with severe aplastic anemia: changing impact of risk factors. *Blood* 1986; **68**: 1363 1368.
11 Kollman C, Howe CW, Anasetti C, Antin JH, Davies SM, Filipovich AH *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.
12 Kimura F, Sato K, Kobayashi S, Ikeda T, Sao H, Okamoto S *et al.* Impact of AB0-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.
13 Rocha V, Labopin M, Gluckman E, Powles R, Arcese W, Bacigalupo A *et al.* Relevance of bone marrow cell dose on allogeneic transplantation outcomes for patients with acute myeloid leukemia in first complete remission: results of a European survey. *J Clin Oncol* 2002; **20**: 4324 4330.
14 Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H *et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (eds). IARC: Lyon France, 2008.
15 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825 828.
16 Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al.* Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**: 250 259.
17 Fine J, Gray R. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496 497.
18 Paulin T. Importance of bone marrow cell dose in bone marrow transplantation. *Clin Transplant* 1992; 6: 48 54.
19 Byrne JL, Stainer C, Cull G, Haynes AP, Bessell EM, Hale G *et al.* The effect of the serotherapy regimen used and the marrow cell dose received on rejection, graft-versus-host disease and outcome following unrelated donor bone marrow transplantation for leukaemia. *Bone Marrow Transplant* 2000; **25**: 411 417.
20 Barrett AJ, Ringden O, Zhang MJ, Bashey A, Cahn JY, Cairo MS *et al.* Effect of nucleated marrow cell dose on relapse and survival in identical twin bone marrow transplants for leukemia. *Blood* 2000; **95**: 3323 3327.
21 Han P, Story C, McDonald T, Mrozik K, Snell L. Immune escape mechanisms of childhood ALL and a potential countering role for DC-like leukemia cells. *Cytotherapy* 2002; **4**: 165 175.

22 Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**: 2097 2100.
23 Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD *et al.* Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979; **300**: 1068 1073.
24 Levine JE, Braun T, Penza SL, Beatty P, Cornetta K, Martino R *et al.* Prospective trial of chemotherapy and donor leukocyte infusions for relapse of advanced myeloid malignancies after allogeneic stem-cell transplantation. *J Clin Oncol* 2002; **20**: 405 412.
25 Porter DL, Roth MS, McGarigle C, Ferrara JL, Antin JH. Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. *N Engl J Med* 1994; **330**: 100 106.
26 Theilgaard-Monch K, Raaschou-Jensen K, Palm H, Schjodt K, Heilmann C, Vindelov L *et al.* Flow cytometric assessment of lymphocyte subsets, lymphoid progenitors, and hematopoietic stem cells in allogeneic stem cell grafts. *Bone Marrow Transplant* 2001; **28**: 1073 1082.
27 Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W *et al.* Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* 1995; **86**: 2041 2050.
28 Collins Jr RH, Shpilberg O, Drobyski WR, Porter DL, Giralt S, Champlin R *et al.* Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol* 1997; **15**: 433 444.
29 Passweg JR, Stern M, Koehl U, Uharek L, Tichelli A. Use of natural killer cells in hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2005; **35**: 637 643.
30 Mavroudis D, Read E, Cottler-Fox M, Couriel D, Molldrem J, Carter C *et al.* CD34+ cell dose predicts survival, posttransplant morbidity, and rate of hematologic recovery after allogeneic marrow transplants for hematologic malignancies. *Blood* 1996; **88**: 3223 3229.
31 Bittencourt H, Rocha V, Chevret S, Socie G, Esperou H, Devergie A *et al.* Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 2002; **99**: 2726 2733.
32 Dominietto A, Raiola AM, van Lint MT, Lamparelli T, Gualandi F, Berisso G *et al.* Factors influencing haematological recovery after allogeneic haemopoietic stem cell transplants: graft-versus-host disease, donor type, cytomegalovirus infections and cell dose. *Br J Haematol* 2001; **112**: 219 227.
33 Shiobara S, Nakao S, Ueda M, Yamazaki H, Takahashi S, Asano S *et al.* Donor leukocyte infusion for Japanese patients with relapsed leukemia after allogeneic bone marrow transplantation: indications and dose escalation. *Ther Apher* 2001; **5**: 40 45.
34 Batinic D, Marusic M, Pavletic Z, Bogdanic V, Uzarevic B, Nemet D *et al.* Relationship between differing volumes of bone marrow aspirates and their cellular composition. *Bone Marrow Transplant* 1990; **6**: 103 107.

Supplementary Information accompanies the paper on Bone Marrow Transplantation website (http://www.nature.com/bmt)

blood

2010 116: 4368-4375
Prepublished online July 27, 2010;
doi:10.1182/blood-2010-02-269571

Allogeneic stem cell transplantation for adult Philadelphia chromosome –negative acute lymphocytic leukemia: comparable survival rates but different risk factors between related and unrelated transplantation in first complete remission

Satoshi Nishiwaki, Yoshihiro Inamoto, Hisashi Sakamaki, Mineo Kurokawa, Hiroatsu Iida, Hiroyasu Ogawa, Takahiro Fukuda, Yukiyasu Ozawa, Naoki Kobayashi, Masanobu Kasai, Takehiko Mori, Koji Iwato, Takashi Yoshida, Makoto Onizuka, Keisei Kawa, Yasuo Morishima, Ritsuro Suzuki, Yoshiko Atsuta and Koichi Miyamura

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

Allogeneic stem cell transplantation for adult Philadelphia chromosome–negative acute lymphocytic leukemia: comparable survival rates but different risk factors between related and unrelated transplantation in first complete remission

Satoshi Nishiwaki,[1] Yoshihiro Inamoto,[2] Hisashi Sakamaki,[3] Mineo Kurokawa,[4] Hiroatsu Iida,[5] Hiroyasu Ogawa,[6] Takahiro Fukuda,[7] Yukiyasu Ozawa,[1] Naoki Kobayashi,[8] Masanobu Kasai,[9] Takehiko Mori,[10] Koji Iwato,[11] Takashi Yoshida,[12] Makoto Onizuka,[13] Keisei Kawa,[14] Yasuo Morishima,[14] Ritsuro Suzuki,[15] Yoshiko Atsuta,[15] and Koichi Miyamura[1]

[1]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [2]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Department of Hematology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; [4]Department of Cell Therapy and Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan; [5]Department of Hematology, Meitetsu Hospital, Nagoya, Japan; [6]Divison of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [7]Department of Stem Cell Transplantation, National Cancer Center, Tokyo, Japan; [8]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan; [9]Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital, Nagoya, Japan; [10]Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan; [11]Fourth Department of Internal Medicine, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Hiroshima, Japan; [12]Department of Internal Medicine, Toyama Prefectural Central Hospital, Toyama, Japan; [13]Department of Hematology and Oncology, Tokai University School of Medicine, Isehara, Japan; [14]Japan Marrow Donor Program, Tokyo, Japan; and [15]Japan Society for Hematopoietic Cell Transplantation, Nagoya, Japan

To identify factors to improve the outcomes of related and unrelated allogeneic stem cell transplantations (allo-SCT) for Philadelphia chromosome–negative acute lymphocytic leukemia (Ph⁻ ALL) in the first complete remission (CR1), we retrospectively analyzed 1139 Ph⁻ ALL patients using the registry data, particularly the details of 641 patients transplanted in CR1. Overall survival was significantly superior among patients transplanted in CR1, but no significant difference was observed between related and unrelated allo-SCTs (related vs unrelated: 65% vs 62% at 4 years, respectively; $P = .19$). Among patients transplanted in CR1, relapse rates were significantly higher in related allo-SCT compared with unrelated allo-SCT, and multivariate analysis demonstrated that less than 6 months from diagnosis to allo-SCT alone was associated with relapse. On the other hand, nonrelapse mortality (NRM) was significantly higher in unrelated allo-SCT compared with related allo-SCT, and multivariate analysis demonstrated that 10 months or longer from diagnosis to allo-SCT, human leukocyte antigen mismatch, and abnormal karyotype were associated with NRM. In conclusion, our study showed comparable survival rates but different relapse rates, NRM rates, and risk factors between related and unrelated allo-SCTs. After a close consideration of these factors, the outcome of allo-SCT for adult Ph⁻ ALL in CR1 could be improved. (*Blood.* 2010;116(20):4368-4375)

Introduction

The indication of allogeneic stem cell transplantation (allo-SCT) for Philadelphia chromosome–negative acute lymphocytic leukemia (Ph⁻ ALL) is still controversial.[1,2] As for related allo-SCT, one prospective study suggested that related allo-SCT for Ph⁻ ALL in first complete remission (CR1) could provide the most potent antileukemic therapy and considerable survival benefits.[3] As for unrelated allo-SCT, the largest retrospective study of Ph⁻ ALL patients in CR1 showed worse overall survival (OS) rates because of higher incidences of nonrelapse morality (NRM) than those in related allo-SCT,[4] whereas another reported that there were no differences in OS rates and NRM rates between related and unrelated allo-SCTs for adult ALL in CR1.[5] These data indicated that unrelated allo-SCT could also be a treatment option for adult Ph⁻ ALL patients in CR1 if NRM rates were low enough, although it is not yet routinely performed.

Although the analyses of the outcome of allo-SCT alone have some biases, such as excluding death during chemotherapy, and there may be potential differences in the baseline characteristics of patients between related and unrelated allo-SCTs, the comparison of transplantation outcomes and risk factors between related and unrelated allo-SCTs for adult Ph⁻ ALL could indicate strategies to improve transplantation outcomes for this disease. We particularly focused on allo-SCT in CR1 because this is the area of controversy.

Methods

Collection of data and data sources

The recipients' clinical data were provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP). Both JSHCT and JMDP collect recipients' clinical data at 100 days after allo-SCT. The patient's data on survival, disease status, and long-term complications, including chronic graft-versus-host disease (GVHD) and second malignancies, are renewed annually by follow-up forms. More than 99% of unrelated allo-SCT in Japan was captured in the JMDP database, and approximately 75% of related allo-SCT was captured in the JSHCT database. This study was approved by the data management committees of JSHCT and JMDP. Informed consent was obtained from both recipients and donors in accordance with the Declaration of Helsinki.

Submitted February 11, 2010; accepted July 19, 2010. Prepublished online as *Blood* First Edition paper, July 27, 2010; DOI 10.1182/blood-2010-02-269571.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Patients

Data of 1976 patients who underwent their first allo-SCT for Ph⁻ ALL between 1993 and 2007 were available in the registration database of JSHCT and JMDP. Excluding 662 patients whose age was 15 years or younger, 67 patients without data of GVHD prophylaxis and the interval from diagnosis to allo-SCT, 22 patients who underwent 2 or more human leukocyte antigen (HLA) loci mismatched related allo-SCT, and 86 patients who received reduced-intensity conditioning regimens, we analyzed 1139 adult Ph⁻ ALL patients (499 related and 640 unrelated). We particularly analyzed details of 641 patients transplanted in CR1, according to donor types (310 related and 331 unrelated). All but 4 patients were donated from Japanese donors harvested in Japanese harvest centers. Only bone marrow grafts were used in unrelated allo-SCT because peripheral blood stem cell donation from unrelated donors is not yet approved in Japan. HLA high-resolution molecular typing methods were performed for HLA-A, -B, -Cw, and -DRB1 for all patients in JMDP. Donor and recipient pairs were considered matched when HLA was matched at -A, -B, and -DRB1 loci in related allo-SCT and at -A, -B, -Cw, and -DRB1 loci in unrelated allo-SCT. Mismatches were defined as at least one disparity of these loci.

Definition

Neutrophil recovery was defined by an absolute neutrophil count of at least 0.5×10^9/L for 3 consecutive days; platelet recovery was defined by a count of at least 50×10^9/L without transfusion support. Acute and chronic GVHD was diagnosed and graded according to consensus criteria.[6,7] Relapse was defined as hematologic leukemia recurrence. NRM was defined as death during continuous remission. For analyses of OS, failure was death from any cause, and surviving patients were censored at the date of last contact. The date of allo-SCT was the starting time point for calculating all outcomes. Patients were classified at diagnosis by the Japan Adult leukemia Study Group (JALSG) risk stratification: low risk was defined as less than 30 years at diagnosis and white blood cell count less than 30 000/μL at diagnosis, high risk as 30 years or more at diagnosis and white blood cell count 30 000/μL or more at diagnosis, and intermediate risk as other.[8] To determine the cut-off for the upper limit of tolerability by age, we analyzed the cumulative incidence of NRM by categorizing the patients' age every 5 years. Because NRM rates of 45- to 49-year-old and 50-year-old or older categories showed higher incidences compared with other categories, we determined the best cut-off point as 45 years old.

Statistical analysis

The 2-sided χ^2 test was used for categorical variables. OS rates were estimated by the Kaplan-Meier method, and P values were calculated using a log-rank test.[9,10] Cumulative incidences of relapse, NRM, and GVHD were calculated by the Gray method.[11,12] Death without relapse was considered as a competing event for relapse, and relapse as a competing event for NRM. Univariate and multivariate analyses were performed using Cox proportional hazard regression model.[13] A significance level of P less than .05 was used for all analyses.

Results

Patient characteristics

Of 1139 patients, 641 received allo-SCT in CR1 (310 related and 331 unrelated), 199 in subsequent remission (56 related and 143 unrelated), and 299 in nonremission (133 related and 166 unrelated). The characteristics of the patients transplanted in CR1 are shown in Table 1. The frequencies of HLA mismatched donor and tacrolimus-based GVHD prophylaxis were higher, and the interval from diagnosis to allo-SCT was longer among patients who underwent an unrelated allo-SCT than among those who underwent a related allo-SCT. There was no significant difference in the age at allo-SCT, the white blood cell counts at diagnosis, JALSG risk stratification, and year of allo-SCT between related and unrelated allo-SCTs.

Survival

Median follow-up periods among survivors were 47.7 months (range, 1.3-162 months). OS rates at 4 years were 64% in CR1, 39% in subsequent CR, and 16% in non-remission ($P < .0001$). Although OS rates were significantly different among disease stages at allo-SCT, there were no significant differences in OS rates at 4 years between related and unrelated allo-SCTs in any disease stage (related vs unrelated: 65% vs 62% in CR1, $P = .19$; 44% vs 38% in subsequent CR, $P = .66$; and 17% vs 16% in non-remission, $P = .59$; respectively; Figure 1). There was no statistical difference in OS rates and NRM rates over transplantation years (data not shown). Among 641 patients transplanted in CR1, JALSG risk stratification did not have a significant impact on the OS after allo-SCT (68% in low risk, 62% in intermediate risk, and 58% in high risk, at 4 years, respectively; $P = .31$). To address our main issue, we performed the following analyses among patients transplanted in CR1 according to donor types.

Among 310 patients who underwent a related allo-SCT in CR1, multivariate analysis showed that age at allo-SCT and less than 6 months from diagnosis to allo-SCT were significant risk factors for OS. Among 331 patients who underwent an unrelated allo-SCT in CR1, multivariate analysis showed that abnormal karyotype [except for t(4;11) and t(1;19)], HLA mismatch, and 10 months or longer from diagnosis to allo-SCT were significant risk factors for OS (Table 2).

Relapse and NRM among patients transplanted in CR1

The cumulative incidence of relapse was significantly higher in patients who underwent a related allo-SCT compared with those who underwent an unrelated allo-SCT (related vs unrelated: 32% vs 22% at 4 years, $P = .03$; Figure 2A). Multivariate analyses according to donor type showed that less than 6 months from diagnosis to allo-SCT alone was associated with relapse among 310 patients who underwent a related allo-SCT in CR1, whereas only abnormal karyotype [except for t(4;11) and t(1;19)] was associated with relapse among 331 patients who underwent an unrelated allo-SCT in CR1 (Table 3).

The cumulative incidence of NRM was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 14% vs 27% at 4 years, $P = .0002$; Figure 2B). Multivariate analyses according to donor type showed that age only 45 years or older at allo-SCT was associated with NRM among 310 patients who underwent a related allo-SCT in CR1, whereas abnormal karyotype [except for t(4;11) and t(1;19)], HLA mismatch, and 10 months or longer from diagnosis to allo-SCT were associated with NRM among 331 patients who underwent an unrelated allo-SCT in CR1 (Table 4).

Acute and chronic GVHD among patients transplanted in CR1

The cumulative incidence of grade II-IV acute GVHD was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 30% vs 42% at day 100; $P = .0003$). The cumulative incidence of grade III-IV acute GVHD was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 7% vs 16% at day 100; $P = .0006$).

Table 1. Characteristics of patients transplanted in CR1, according to donor type

	Related (n = 310)		Unrelated (n = 331)		
No. of patients	**No.**	**%**	**No.**	**%**	***P***
Median WBC count at diagnosis/μL (range)	10 250 (109-328 000)		11 000 (700-892 000)		.43
Median patient age at allo-SCT, y (range)	30 (16-66)		31 (16-59)		.95
16-20	66	21.3	77	23.3	
21-30	93	30.0	82	24.8	
31-40	71	22.9	86	26.0	
41-50	58	18.7	68	20.5	
51 or older	22	7.1	18	5.4	
Sex					.09
Male	157	50.6	190	57.4	
Female	153	49.4	141	42.6	
Source					< .0001
BM	212	68.4	331	100.0	
PB	98	31.6	0	0.0	
Lineage					.01
T	50	16.1	54	16.3	
B	218	70.3	203	61.3	
Other	42	13.5	74	22.4	
Cytogenetics					.07
Normal	193	62.3	208	62.8	
t(4;11)	11	3.5	5	1.5	
Other MLL/11q23 translocations	1	0.3	3	0.9	
t(1;19)	10	3.2	6	1.8	
t(8;14)	3	1.0	3	0.9	
14q32 translocations	1	0.3	0	0.0	
del(6q)	3	1.0	1	0.3	
del(7p)	2	0.6	1	0.3	
−7*	5	1.6	2	0.6	
+8*	2	0.6	0	0.0	
+X*	0	0.0	1	0.3	
del(9p)	3	1.0	9	2.7	
abnormality of 11q	0	0.0	3	0.9	
del(12p)	2	0.6	1	0.3	
del(13q)/−13	1	0.3	2	0.6	
del(17p)	0	0.0	1	0.3	
Complex	10	3.2	15	4.5	
Low hypodiploidy/near triploidy	2	0.6	0	0.0	
High hyperdiploidy	16	5.2	12	3.6	
Other abnormality (no t(9;22))†	45	14.5	58	17.5	
JALSG risk stratification					.21
Low	39	12.6	45	13.6	
Intermediate	163	52.6	192	58.0	
High	108	34.8	94	28.4	
HLA matching					< .0001
Match	285	91.9	192	58.0	
Class I 1 locus-mismatch	18	5.8	53	16.0	
Class II 1 locus-mismatch	7	2.3	32	9.7	
2 or more loci mismatch	0	0.0	54	16.3	
Time from diagnosis to transplantation, mo (range)	5.7 (1.9-36.6)		10.0 (4.0-43.0)		< .0001
< 6	169	54.5	23	6.9	
6-9	109	35.2	143	43.2	
10 or longer	32	10.3	165	49.8	
Preparative regimen					.004
CY + TBI	140	45.2	156	47.1	
CA + CY + TBI	66	21.3	84	25.4	
BU + CY + TBI	17	5.5	15	4.5	
VP + CY + TBI	23	7.4	32	9.7	
Other TBI myeloablative regimens	39	12.6	32	9.7	
BU + CY	17	5.5	12	3.6	
Other non-TBI myeloablative regimens	8	2.6	0	0.0	
GVHD prophylaxis					< .0001
Cyclosporine A with or without other	283	91.3	171	51.7	
Tacrolimus with or without other	27	8.7	160	48.3	

Table 1. Characteristics of patients transplanted in CR1, according to donor type (continued)

No. of patients	Related (n = 310) No.	Related (n = 310) %	Unrelated (n = 331) No.	Unrelated (n = 331) %	P
Years of allo-SCT					.26
1993-1997	48	15.5	55	16.6	
1998-2002	132	42.6	120	36.3	
2003-2007	130	41.9	156	47.1	

WBC indicates white blood cell; BM, bone marrow; PB, peripheral blood; related HLA match, identical HLA-A, -B, and -DRB1 loci; unrelated HLA match, HLA-A, -B, -Cw, and -DRB1 loci; HLA mismatch, at least one disparity at one of these loci; CY, cyclophosphamide; TBI, total body irradiation; CA, cytarabine; BU, busulfan; and VP, etoposide.

*These groups exclude cases with low hypodiploidy and high hyperdiploidy.

†Abnormal karyotypes excluding those with any of the aforementioned abnormalities.

Among evaluable patients who survived at least 100 days after allo-SCT, no significant difference was observed between related and unrelated allo-SCTs in the incidence of chronic GVHD (related vs unrelated: 41% vs 41% at 2 years; $P = .76$). Extensive disease was observed in 60 (55%) of 109 with chronic GVHD after related allo-SCT and in 80 (74%) of 118 after unrelated allo-SCT ($P = .048$).

Causes of death among patients transplanted in CR1

Although relapse was the leading cause of death in both related and unrelated allo-SCTs, the proportion of relapse was significantly lower in those transplanted from unrelated donors ($P = .01$). Infection, GVHD, and organ failure were the major causes of NRM, and the incidence of interstitial pneumonia was higher in patients transplanted from unrelated donors ($P = .06$; Table 5).

Discussion

This study reports the largest series of adult Ph⁻ ALL patients who underwent allo-SCT. There was no significant survival difference between related and unrelated allo-SCTs in any disease stage. Among patients who underwent a related allo-SCT in CR1, a shorter interval from diagnosis to allo-SCT was associated with relapse, and age at allo-SCT was associated with NRM. On the other hand, among patients who underwent an unrelated allo-SCT, abnormal karyotype was associated with both relapse and NRM, and a longer interval from diagnosis to allo-SCT and HLA mismatch were associated with NRM. These results indicated that factors affecting transplantation outcomes were different according to donor type.

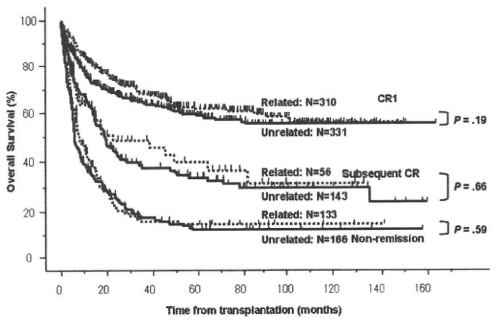

Figure 1. OS according to disease status and donor type. OS rates were significantly superior among patients transplanted in CR1. There was no significant difference between related and unrelated allo-SCTs (related vs unrelated: 65% vs 62% in CR1, $P = .19$; 44% vs 38% in subsequent CR, $P = .66$; and 17% vs 16% in nonremission, $P = .59$; respectively).

In our study, unrelated allo-SCT resulted in OS rates similar to those from related allo-SCT, which was compatible with the result of one prospective study for standard-risk hematologic malignancies.[14] The rates of OS, relapse, and NRM among patients who underwent a related allo-SCT in CR1 were 65%, 32%, and 14%, respectively, which were compatible with those observed in the United Kingdom Medical Research Council UKALL XII/Eastern Cooperative Oncology Group E2993 trial (53%, 24%, and 18%, respectively).[3] Some patients were transplanted from a 1-locus mismatched related donor because it was reported that the outcome of allo-SCT from a 1 locus-mismatched related donor was similar to that of matched unrelated allo-SCT in the Japanese population.[15] On the other hand, the rates of OS, relapse, and NRM among patients who underwent an unrelated allo-SCT were 62%, 22%, and 27%, respectively, which were better than those reported from the Center for International Blood and Marrow Transplant Research (39%, 20%, and 42%, respectively).[4] These differences in NRM could be explained by the lower incidence of acute GVHD in our population, which possibly resulted from the genetic homogeneity in the Japanese population.[16,17] Interestingly, abnormal karyotype was associated with NRM. This could be explained by the possibility that patients with abnormal karyotype received intensive chemotherapy before allo-SCT because of persistent minimal residual disease, which might result in increased NRM rates. Another possibility is that rapid taper of immunosuppressive treatment might also cause GVHD leading to NRM.

In this study, NRM rates were higher in unrelated allo-SCT compared with related allo-SCT, whereas comparable NRM rates were reported in some recent reports,[18] suggesting that NRM rates after unrelated allo-SCT could be reduced with further efforts, such as better HLA matching. Because HLA-C was not routinely typed before 2003, most of the HLA-C data in this study were examined retrospectively, revealing that considerable numbers of patients had received class I allele-mismatched unrelated allo-SCT. Better HLA matching might reduce NRM after unrelated allo-SCT in the future. Although slower hematopoietic recovery after bone marrow transplantation compared with peripheral blood stem cell transplantation might affect the timing of deaths, there was no statistical difference in early mortality between the grafts (data not shown).

There was no statistical difference in the incidence of chronic GVHD between related and unrelated allo-SCTs, although acute GVHD was observed more frequently in unrelated allo-SCT. This was compatible with a past report in which the incidence of chronic GVHD was similar between related and unrelated allo-SCTs, whereas acute GVHD was observed more frequently in related allo-SCT.[14]

It was noteworthy that the interval from diagnosis to allo-SCT revealed a different effect on related and unrelated allo-SCTs. In Japanese clinical practice, the JALSG protocols have been common, where 1.5-month induction chemotherapy was followed by

Table 2. Univariate and multivariate analyses of factors influencing OS among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	N	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	1.19 (0.78-1.82)	.42	—		101	0.83 (0.56-1.25)	.38	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	0.73 (0.34-1.77)	.52	—		54	0.81 (0.44-1.48)	.35	—	
Other	42	0.94 (0.54-1.64)	.84	—		74	1.08 (0.70-1.67)	.72	—	
Karyotype										
Normal	199	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.51 (0.14-1.54)	.19	—		11	1.49 (0.54-4.09)	.44	1.59 (0.58-4.36)	.37
Other (not(9;22))	96	1.03 (0.67-7.14)	.89	—		112	1.49 (1.03-2.17)	.04	1.43 (1.13-2.40)	.01
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	1.36 (0.87-2.12)	.18	—		192	1.06 (0.71-1.59)	.77	—	
High	108	1.77 (0.95-3.31)	.07	—		94	1.02 (0.56-1.88)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	2.04 (1.30-3.13)	.002	2.13 (1.36-3.34)	.0009	50	1.05 (0.63-1.73)	.86		
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	0.95 (0.46-1.96)	.90	—		139	1.44 (1.01-2.06)	.04	1.45 (1.01-2.07)	.04
Stem cell source										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.43 (0.94-2.13)	.09	1.40 (0.93-2.11)	.11					
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.75 (1.16-2.63)	.007	1.80 (1.19-2.71)	.005	308	0.33 (0.10-1.04)	.06	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	0.56 (0.26-1.20)	.14	—		165	1.54 (1.07-2.21)	.02	1.62 (1.12-2.34)	.01
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.72 (0.38-1.35)	.30	—		319	0.59 (0.27-1.26)	.17	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	2.02 (1.15-3.56)	.01	—		160	1.38 (0.96-1.97)	.08	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.

6-month consolidation chemotherapy and 16-month maintenance chemotherapy.[8] Therefore, a shorter interval from diagnosis to allo-SCT, which was more common in related cases, might result in insufficient consolidation chemotherapy and worse survival because of increased relapse rates in related allo-SCT. Alternatively, effects from insufficient consolidation chemotherapy might be more prominent in related allo-SCT because graft-versus-leukemia effects might be weaker after related allo-SCT than unrelated allo-SCT.[19] On the other hand, a longer interval from diagnosis to allo-SCT, which was more common in unrelated cases, might result in the cumulative toxic sequelae of chemotherapy responsible for interstitial pneumonia indicated in the past reports.[20-25] Because the JALSG protocols do not define the timing of allo-SCT, it is possible that chemotherapy before allo-SCT might be prolonged because of persistent minimal residual disease. However, we could not confirm this because there were no data concerning minimal residual disease in the registry database.

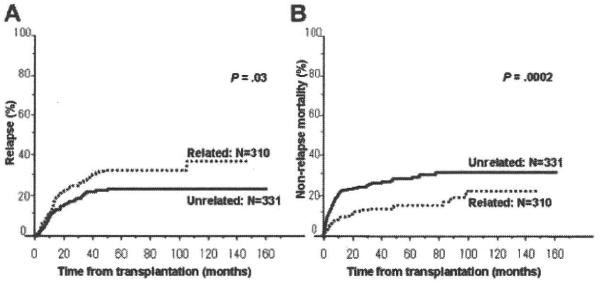

Figure 2. Cumulative incidence of relapse and NRM in patients transplanted in CR1 according to donor type. (A) Cumulative incidence of relapse among patients transplanted in CR1 was significantly higher in patients who underwent a related allo-SCT compared with those who underwent an unrelated allo-SCT (related vs unrelated: 32% vs 22% at 4 years, $P = .03$). (B) Cumulative incidence of NRM among patients transplanted in CR1 was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 14% vs 27% at 4 years, $P = .0002$).

Table 3. Univariate and multivariate analyses of factors influencing relapse among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	N	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	0.88 (0.52-1.47)	.62	—		101	1.11 (0.62-1.98)	.72	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	0.54 (0.22-1.37)	.09	—		54	1.31 (0.57-3.02)	.62	—	
Other	42	1.21 (0.66-2.21)	.54	—		74	1.06 (0.53-2.11)	.87	—	
Karyotype										
Normal	193	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.64 (0.19-2.12)	.36	—		11	1.97 (0.46-8.35)	.91	—	
Other (no t(9;22))	96	1.11 (0.68-1.82)	.67	—		112	2.15 (1.24-3.73)	.01	2.15 (1.24-3.73)	.01
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	0.96 (0.59-1.55)	.87	—		192	1.04 (0.57-1.91)	.90	—	
High	108	0.81 (0.35-1.84)	.61	—		94	1.04 (0.43-2.52)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	0.82 (0.41-1.64)	.57	—		50	0.74 (0.42-1.32)	.08	—	
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	0.82 (0.33-2.02)	.66	—		139	0.74 (0.42-1.32)	.31	—	
Stem cell source*										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.07 (0.65-1.76)	.79	—					—	
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.68 (1.05-2.69)	.03	1.68 (1.05-2.69)	.03	308	0.47 (0.11-1.92)	.29	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	0.49 (0.18-1.34)	.16	—		165	0.92 (0.54-1.58)	.76	—	
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.62 (0.31-1.25)	.18	—		319	0.47 (0.15-1.52)	.21	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	1.62 (0.81-3.26)	.18	—		160	1.39 (0.81-2.38)	.24	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.

*Stem cell source (peripheral blood) was not a significant risk factor for relapse in the multivariate analysis.

Although we mainly focused on patients in CR1, our results also indicated that some, but not all, patients with refractory disease could be rescued by allo-SCT. These patients could not have survived long with chemotherapy alone, and complete unresponsiveness, even to allo-SCT, was often assumed. These results were compatible with some reports showing that long-term survival could be achieved for patients receiving allo-SCT, even in refractory disease.[26-28]

Our study has several limitations. First, there might be some selection biases between related and unrelated allo-SCTs. It was possible that eligibility was more stringent in patients who received unrelated allo-SCT, and they might have had better pretransplantation conditions. Second, a time-censoring effect might impact the outcome. The longer interval from diagnosis to unrelated allo-SCT eliminates the effect of patients who die during that period. This bias might improve the outcome of unrelated allo-SCT. Third, we could not make the comparison between chemotherapy and allo-SCT in this study.

The time-censoring effect could be the major bias in this study, which resulted in lower relapse rates, especially in patients transplanted from unrelated donors. We tried to correct this bias by the previously described method.[29] In the JALSG ALL study, it was reported that approximately 80% and 75% of patients were alive 6 months and 10 months after enrollment, respectively.[8] Because 6 months and 10 months were the median interval from diagnosis to related and unrelated allo-SCTs, respectively, a crude way to apply a correction factor for the survival seen in our study is to lower the survival estimate at any given time point by 20% for related allo-SCT and 25% for unrelated allo-SCT, respectively. Thus, the corrected OS rates at 4 years were 52% ± 5% for related allo-SCT and 47% ± 4% for unrelated allo-SCT, which showed no statistical difference between related and unrelated allo-SCTs. Time-censoring effects would not change the results.

The change of transplantation indication for adolescents through the observation period might affect the outcome. In the JALSG protocol ALL202 (from September 2002), we treated patients less than 25 years old with a similar protocol performed for pediatric patients. Because allo-SCT was recommended only for high-risk patients, such as those with t(4;11) or MLL-rearrangement in the pediatric protocol, the outcome of young patients might be affected by the difference in the indication for allo-SCT between pediatric and adult protocols after 2002. However, the effect of this small population would not be so large.