



Comparison of Unrelated Cord Blood Transplantation and HLA-Mismatched Unrelated Bone Marrow Transplantation for Adults with Leukemia

Yoshiko Atsuta,¹ Yasuo Morishima,^{2,*} Ritsuro Suzuki,¹ Tokiko Nagamura-Inoue,³ Shuichi Taniguchi,⁴ Satoshi Takahashi,⁵ Shunro Kai,⁶ Hisashi Sakamaki,⁷ Yasushi Kouzai,⁸ Naoki Kobayashi,⁹ Takahiro Fukuda,¹⁰ Hiroshi Azuma,¹¹ Minoko Takanashi,¹² Takehiko Mori,¹³ Masahiro Tsuchida,¹⁴ Takakazu Kawase,¹⁵ Keisei Kawa,¹⁶ Yoshihisa Kodera,¹⁷ Shunichi Kato,^{18,*} for the Japan Marrow Donor Program and the Japan Cord Blood Bank Network

Recent advances in unrelated cord blood transplantation (UCBT) and high-resolution typing of human leukocyte antigen (HLA) from an unrelated donor have increased choices in alternative donor/stem cell source selection. We assessed HLA-mismatched locus-specific comparison of the outcomes of 351 single-unit UCB and 1,028 unrelated bone marrow (UBM) adult recipients 16 years old or older at the time of transplantation who received first stem cell transplantation with myeloablative conditioning for acute leukemia or myelodysplastic syndromes. With adjusted analyses, HLA 0 to 2 mismatched UCBT showed similar overall mortality (relative risk [RR] = 0.85, 95% confidence interval [CI], 0.68-1.06; $P = .149$) compared with that of single-HLA-DRB1-mismatched UBMT. UCBT showed inferior neutrophil recovery (RR = 0.50, 95% CI, 0.42-0.60; $P < .001$), lower risk of acute graft-versus-host disease (RR = 0.55, 95% CI, 0.42-0.72; $P < .001$), and lower risk of transplantation-related mortality (RR = 0.68, 95% CI, 0.50-0.92; $P = .011$) compared with single-HLA-DRB1-mismatched UBMT. No significant difference was observed for risk of relapse (RR = 1.28, 95% CI, 0.93-1.76; $P = .125$). HLA 0 to 2 antigen-mismatched UCBT is a reasonable second alternative donor/stem cell source with a survival outcome similar to that of single-HLA-DRB1-mismatched or other 7 of 8 UBMT.

Biol Blood Marrow Transplant 18: 780-787 (2012) © 2012 American Society for Blood and Marrow Transplantation

KEY WORDS: Unrelated cord blood transplantation, HLA-mismatched unrelated bone marrow transplantation

From the ¹Department of HSCT Data Management/Biostatistics Nagoya University Graduate School of Medicine, Nagoya, Japan; ²Department of Hematology and Cell Therapy Aichi Cancer Center Hospital, Nagoya, Japan; ³Department of Cell Processing & Transfusion, Research Hospital The Institute of Medical Science, The University of Tokyo, and Tokyo Cord Blood Bank Tokyo, Tokyo, Japan; ⁴Department of Hematology Toranomon Hospital, Tokyo, Japan; ⁵Department of Molecular Therapy The Institute of Medical Science The University of Tokyo, Tokyo, Japan; ⁶Department of Transfusion Medicine Hyogo College of Medicine, Nishinomiya, Japan; ⁷Division of Hematology Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; ⁸Department of Transfusion Medicine, Tokyo Metropolitan Tama Medical Center, Tokyo, Japan; ⁹Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan; ¹⁰Hematopoietic Stem Cell Transplantation Unit National Cancer Center Hospital, Tokyo, Japan; ¹¹Hokkaido Red Cross Blood Center, Sapporo, Japan; ¹²The Japanese Red Cross Tokyo Blood Center, Tokyo, Japan; ¹³Division of Hematology,

Department of Medicine, Keio University School of Medicine, Tokyo, Japan; ¹⁴Ibaraki Children's Hospital, Mito, Japan; ¹⁵Division of Epidemiology and Prevention, Aichi Cancer Center Hospital, Nagoya, Japan; ¹⁶Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; ¹⁷BMT Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; and ¹⁸Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan.

Financial disclosure: See Acknowledgments on page 786.

*Y.M. and S. Kato share senior authorship.

Correspondence and reprint requests: Yoshiko Atsuta, MD, PhD, Department of Hematopoietic Stem Cell Transplantation Data Management/Biostatistics, Nagoya University School of Medicine, 1-1-20 Daiko-Minami, Higashi-ku Nagoya 461-0047, Japan (e-mail: y-atsuta@med.nagoya-u.ac.jp).

Received July 20, 2011; accepted October 9, 2011

© 2012 American Society for Blood and Marrow Transplantation
1083-8791/\$36.00

doi:10.1016/j.bbmt.2011.10.008

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a widely used, curative treatment for hematologic malignancies. When available, a human leukocyte antigen (HLA)-identical sibling is the donor of choice. However, only about 30% of candidates eligible for allogeneic HSCT will have such a donor. In addition, older patients with older siblings have more difficulty finding such a donor capable of stem cell donation. High-resolution donor-recipient HLA matching has contributed to the success of unrelated donor marrow transplantation, and the current first recommended alternative donor after an HLA-matched sibling for HSCT is an HLA-A, -B, -C, and -DRB1 8 of 8-allele-matched unrelated donor [1-4]. However, there are still a significant number of patients for which finding an HLA 8 of 8-matched unrelated donor is difficult and for whom a second alternative donor/stem cell source should be found.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and single mismatched UBM donors are usually selected as a second alternative donor/stem cell source [1-4]. Lee et al. [3] showed that a single mismatch, antigen-level, or high-resolution, at HLA-A, -B, -C, or -DRB1 loci was associated with higher mortality and decreased survival. However, the reduction in survival may be acceptable in comparison with the survival rates for currently available alternative treatments. Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients; thus, single-DRB1-mismatched UBM donor is currently a second alternative in Japan [1,2,5].

Recent advances in unrelated cord blood transplantation (UCBT) have provided patients with increased choices for a second alternative donor/stem cell source [6]. Clinical comparison studies of cord blood transplantation and HLA-A, -B, and -DRB1 6 of 6 allele-matched bone marrow transplantation for leukemia from unrelated donors in adult recipients showed comparable results [7-9]. More recently, promising outcomes of UCBT were shown compared with HLA-A, -B, -C, and -DRB1 8 of 8 allele-matched UBMT, the current first alternative donor/stem cell source [10-12].

The aim of this study was to determine the utility of UCBT as a second-alternative donor source in adult patients with acute leukemia or myelodysplastic syndromes. It is common today to perform high-resolution typing of HLA for donor selection of unrelated donors; thus, we performed mismatched-allele-specific analyses for comparison of HLA-mismatched UBMT and UCBT in terms of overall survival (OS) and other HSCT outcomes, setting single-DRB1-mismatched UBMT, the current second alternative, as the reference.

PATIENTS AND METHODS

Collection of Data and Data Source

The recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN) and the JMDP [13]. Peripheral blood stem cell donation from unrelated donors was not permitted in Japan during the study period. All 11 cord blood banks in Japan are affiliated with JCBBN. Both JCBBN and JMDP collect recipients' clinical information at 100 days posttransplantation. Patients' information on survival, disease status, and long-term complications including chronic graft-versus-host (cGVHD) disease and second malignancies is renewed annually using follow-up forms. This study was approved by the institutional review board of Nagoya University Graduate School of Medicine.

Patients

The subjects were adult patients of at least 16 years of age with acute myeloid leukemia, acute lymphoblastic leukemia, and myelodysplastic syndromes, who were recipients of first UBMT or UCBT with myeloablative conditioning. All patients in the UCBT cohort received a single-unit CB. Transplantation years were between 1996 and 2005 for UBMT and between 2000 and 2005 for UCBT to avoid the first 3 years of a pioneering period (1993-1995 for UBMT and 1997-1999 for UCBT). There were no statistically significant differences between UBMT in 1996-1999 and UBMT in 2000-2005 in probabilities of OS (41% versus 44%, at 3 years posttransplantation; $P = .86$) and in relapse-free survival (RFS) (40% versus 40%, at 3 years posttransplantation; $P = .93$).

Among 2,253 UBMT recipients with complete HLA high-resolution data, the following recipients with HLA -A, -B, -C, and -DRB1 8 of 8 allele match ($n = 1,079$) and more than three mismatches (5 of 8 allele match [$n = 117$], 4 of 8 allele match [$n = 24$], 3 of 8 allele match [$n = 4$], 2 of 8 allele match [$n = 1$]) were excluded. There were no statistically significant differences in risk of mortality or treatment failure (RFS) associated with single high-resolution (allele) versus single low-resolution (antigen) mismatches (data not shown), so in the analyses, allele and antigen mismatches were considered equivalent. HLA matching of cord blood was performed using low-resolution molecular typing methods for HLA-A and -B, and high-resolution molecular typing for HLA-DRB1. Of 557 recipients of CB with complete HLA data, 105 recipients with three mismatches and nine recipients with four mismatches were excluded. A total of 1,028 UBMT recipients (248 HLA class II locus mismatched, 424 HLA class I locus mismatched, and 356 HLA 2 loci mismatched) and 351 UCBT recipients (20 HLA-A, -B, low-resolution and -DRB1 matched, 87

locus mismatched, and 244 2 loci mismatched) were the subjects for analyses. Both host-versus-graft and graft-versus-host directions were accounted for in terms of HLA mismatch.

HLA Typing

Alleles at the HLA-A, -B, -C, and -DRB1 with unrelated bone marrow donor-recipient pairs and for HLA-DRB1 for unrelated cord blood donor-recipient pairs were identified by the methods described previously [1,5,14]. Serologic or antigen-level typing was performed with a standard two-stage complement-dependent test of microcytotoxicity or low-resolution DNA-based typing usually by collapsing the four-digit typing result back to its first two digits in part.

Definitions

The primary outcome of the analyses was OS, defined as time from transplantation to death from any cause. A number of secondary endpoints were also analyzed. Neutrophil recovery was defined by an absolute neutrophil count of at least 500 cells per cubic millimeter for three consecutive points; platelet recovery was defined by a count of at least 50,000 platelets per cubic millimeter without transfusion support. Diagnosis and clinical grading of acute GVHD (aGVHD) were performed according to the established criteria [15,16]. Relapse was defined as a recurrence of underlying hematologic malignant diseases. Transplantation-related death was defined as death during a continuous remission. RFS was defined as survival in a state of continuous remission.

Statistical Analysis

Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. Medians and ranges are provided for continuous variables and percentages for categorical variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of aGVHD and cGVHD, relapse, and transplantation-related mortality (TRM) [17]. Gray's test was used for group comparison of cumulative incidences [18]. Adjusted comparison of the groups on OS and RFS was performed with the use of the Cox proportional-hazards regression model [19]. For other outcomes with competing risks, Fine and Gray's proportional-hazards model for subdistribution of a competing risk was used [20]. For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event; for GVHD, death without GVHD and relapse were the competing events; for relapse, death without relapse was the competing

event; and, for TRM, relapse was the competing event [21]. Adjusted probabilities of OS and RFS were estimated using the Cox proportional-hazards regression model, with consideration of other significant clinical variables in the final multivariate models. The variables considered were the patient's age at transplantation, patient's sex, donor-patient sex mismatch, donor-patient ABO mismatch, diagnosis, disease status at conditioning, the conditioning regimen, and the type of prophylaxis against GVHD. Factors differing in distribution between CB and BM recipients and factors known to influence outcomes were included in the final models. Variables with more than two categories were dichotomized for the final multivariate model. Variables were dichotomized as follows: patient age >40 or <40 years at transplantation, recipient's sex, sex-mismatched donor-patient pair versus sex-matched pair, donor-recipient ABO major mismatch versus others for ABO matching, advanced versus standard (first and second complete remission of acute myeloid leukemia, first complete remission of acute lymphoblastic leukemia, or refractory anemia or refractory anemia with ring sideroblasts of myelodysplastic syndromes) risk of the disease, cyclophosphamide, and total-body irradiation (TBI) or busulfan and cyclophosphamide or others for conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against GVHD. No significant interactions were identified between each variable and HLA disparity/stem cell source groups. All *P* values were two-sided.

RESULTS

Patient Characteristics

Table 1 shows characteristics of patients, their disease, and transplantation regimens. Proportions of females, sex-mismatched donor-recipient pairs, and ABO mismatched donor recipient pairs were larger in cord blood recipients ($P < .001$, $P < .001$, and $P < .001$, respectively). UCB recipients were older than recipients of UBM (median age, 37 years versus 34 years; $P < .001$). A preparative regimen with TBI and cyclophosphamide was used in the majority of patients in all groups, and cytosine arabinoside was supplemented for CB recipients in addition to TBI and cyclophosphamide in about half the recipients with cyclophosphamide and TBI. For GVHD prophylaxis, tacrolimus and short-term methotrexate was used preferentially in BM recipients (61% of DRB1-one-mismatched BM recipients), while cyclosporine A and short-term methotrexate was used preferentially in CB recipients (61%). The median follow-up period for survivors was 2.1 years (range, 0.1-6.2) for CB recipients and 5.5 (range, 0.3-11.6) years for BM recipients.

Table 1. Patient, Disease, and Transplantation Characteristics According to Stem Cell Source and Number of Mismatched Loci

	Bone Marrow Transplant			
	Class II One Locus Mismatch	Class I One Locus Mismatch	Two Loci Mismatch	Cord Blood Transplantation
	N (%)	N (%)	N (%)	N (%)
Number of transplantations	248	424	356	351
Patient age at transplantation				
Median (range)	36 (16-60)	34 (16-67)	34 (16-59)	37 (16-58)
Patient sex				
Male	151 (61)	241 (57)	210 (59)	162 (46)
Female	97 (39)	183 (43)	146 (41)	189 (54)
Sex matching				
Matched	145 (58)	268 (63)	217 (61)	170 (48)
Male to female	52 (21)	82 (19)	73 (21)	97 (28)
Female to male	50 (20)	71 (17)	64 (18)	84 (24)
Unknown	1 (<1)	3 (1)	2 (1)	0 (0)
Diagnosis				
AML	135 (54)	204 (48)	172 (48)	193 (55)
ALL	78 (31)	149 (35)	135 (38)	113 (32)
MDS	35 (14)	71 (17)	49 (14)	45 (13)
Disease status				
Standard	124 (50)	214 (50)	168 (47)	147 (42)
Advanced	114 (46)	195 (46)	169 (47)	174 (50)
Unknown	10 (4)	15 (4)	19 (5)	30 (9)
ABO matching				
Matched	119 (48)	184 (43)	153 (43)	114 (32)
Minor mismatch	53 (21)	108 (25)	85 (24)	99 (28)
Major mismatch	67 (27)	116 (27)	97 (27)	73 (21)
Bidirectional	8 (3)	12 (3)	14 (4)	64 (18)
Unknown	1 (<1)	4 (1)	7 (2)	1 (<1)
HLA-mismatched number and direction				
Matched				20 (6)
One locus mismatched				87 (25)
HVG direction	16 (6)	38 (9)		8 (9)
GVH direction	17 (7)	30 (7)		8 (9)
Both directions	215 (87)	356 (84)		71 (82)
Two loci mismatched				244 (70)
Two HVG direction			4 (1)	2 (1)
One HVG direction and one GVH direction			6 (2)	4 (2)
Two GVH direction			4 (1)	3 (1)
One both directions and one HVG direction			42 (12)	40 (16)
One both directions and one GVH direction			29 (8)	28 (11)
Two both directions			271 (76)	167 (68)
No. of nucleated cells infused ($\times 10^7/kg$)				
Median	25.0	24.5	23	2.46
Range	2.40-59.8	2.10-97.5	1.5-66.0	1.41-6.01
Preparative regimen				
CY + TBI	94 (38)	168 (40)	151 (42)	109 (31)
CY + CA + TBI	46 (19)	78 (18)	74 (21)	124 (35)
CY + BU + TBI	20 (8)	39 (9)	27 (8)	15 (4)
Other TBI regimen	45 (18)	70 (17)	61 (17)	80 (23)
BU + CY	34 (14)	54 (13)	30 (8)	21 (6)
Other non-TBI regimen	9 (4)	15 (4)	13 (4)	2 (1)
GVHD prophylaxis				
Cyclosporine A + sMTX	87 (35)	221 (52)	150 (42)	213 (61)
Cyclosporine A \pm other	1 (<1)	5 (1)	5 (1)	24 (7)
Tacrolimus + sMTX	152 (61)	191 (45)	193 (54)	76 (22)
Tacrolimus \pm other	8 (3)	5 (1)	6 (2)	35 (10)
Others	0 (0)	2 (<1)	2 (<1)	3 (1)

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; BU, oral busulfan; CA, citarabine; CY, cyclophosphamide; GVH, graft-versus-host; HVG, host-versus-graft; MDS, myelodysplastic syndromes; sMTX, short-term methotrexate.

Outcome

OS and RFS

OS and RFS for CB recipients were similar when compared with that of single-HLA-DRB1-mismatched BM recipients (relative risk [RR] = 0.85, 95% confidence interval [CI], 0.68-1.06; *P* = .149 for OS and RR = 0.97, 95% CI, 0.92-1.35; *P* = .747) (Table 2).

The adjusted probabilities of survival at 3 years posttransplantation of CB recipients (47%) were not

different from those of single HLA-DRB1 mismatched BM recipients (41%; *P* = .19) or single HLA class I-mismatched BM recipients (47%; *P* = .96), but superior to those of 6 of 8 BM recipients (38%; *P* = .014) (Figure 1A). Figure 1B shows adjusted RFS curves (42% for CB recipients, 36% for single HLA-DRB1-mismatched BM, 44% for single HLA class I-mismatched BM, and 36% for 6 of 8 BM recipients, at 3 years posttransplant) (*P* values of comparison between CB and single HLA-DRB1-mismatched BM, CB, and single HLA

Table 2. Multivariate Analyses of Overall Survival, Relapse-Free Survival, Relapse, and Transplant-Related Mortality

Degree of HLA Mismatch	N	Overall Survival			Relapse-Free Survival			Relapse			Transplant-Related Mortality		
		RR	(95% CI)	P value	RR	(95% CI)	P value	RR	(95% CI)	P value	RR	(95% CI)	P value
Bone marrow transplant	248	1.00			1.00			1.00			1.00		
Single DRB1 (7/8)	137	0.84	(0.64-1.11)	.216	0.82	(0.63-1.08)	.158	0.65	(0.41-1.01)	.056	1.07	(0.77-1.49)	.698
Single A or B (7/8)	287	0.89	(0.72-1.12)	.324	0.86	(0.69-1.07)	.170	0.60	(0.41-0.87)	.007	1.13	(0.86-1.48)	.391
Single C (7/8)	144	0.97	(0.74-1.27)	.831	0.95	(0.73-1.24)	.726	0.76	(0.49-1.17)	.208	1.10	(0.78-1.55)	.600
C + DRB1 (6/8)	122	1.22	(0.94-1.59)	.143	1.15	(0.88-1.49)	.300	0.70	(0.44-1.10)	.12	1.42	(1.03-1.96)	.032
A/B + C (6/8)	90	1.25	(0.92-1.68)	.146	1.13	(0.84-1.53)	.409	0.60	(0.35-1.02)	.061	1.48	(1.03-2.13)	.035
Other two loci (6/8)	351	0.85	(0.68-1.06)	.149	0.97	(0.92-1.35)	.747	1.28	(0.93-1.76)	.125	0.68	(0.50-0.92)	.011
Cord blood transplant													

RR indicates relative risk; CI, confidence interval. Adjusted by patient age at transplantation >40 versus ≤40, patient sex, donor-patient sex mismatch versus matched, ABO major mismatch versus others, advanced versus standard disease status at transplantation, cyclophosphamide and total-body irradiation or busulfan and cyclophosphamide for conditioning versus other conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against graft-versus-host disease.

class I-mismatched BM, and CB and 6 of 8 BM recipients were 0.80, 0.12, and 0.43, respectively).

Relapse and TRM

There was no significant increase of relapse rates among CB recipients when compared with DRB1 single-mismatched BM recipients (RR = 1.28, 95% CI, 0.93-1.76; *P* = .125). The risk of TRM was lower in CB recipients compared with that of single HLA-DRB1-mismatched BM recipients (RR = 0.68, 95% CI, 0.50-0.92; *P* = .011) (Table 2). The risk of TRM was also lower in CB recipients when compared with 6 of 8 BM recipients (RR = 0.52, 95% CI, 0.39-0.68; *P* < .001).

Hematologic recovery

Neutrophil and platelet recovery was inferior in CB recipients, as shown in Table 3 (RR = 0.50, 95% CI, 0.42-0.60; *P* < .001 for neutrophil recovery, RR = 0.52, 95% CI, 0.42-0.63; *P* < .001 for platelet recovery).

Acute GVHD and chronic GVHD

The risk of grade 2 to 4 or severe (grades 3-4) aGVHD was lower in CB recipients than that of single HLA-DRB1-mismatched BM recipients (RR = 0.55, 95% CI, 0.42-0.72; *P* < .001 for grade 2 to 4 aGVHD and RR = 0.43, 95% CI, 0.27-0.58; *P* < .001 for severe aGVHD) (Table 4). Unadjusted cumulative incidence of severe aGVHD was 9% for CB, 19% for single HLA-DRB1-mismatched BM, 18% for single HLA

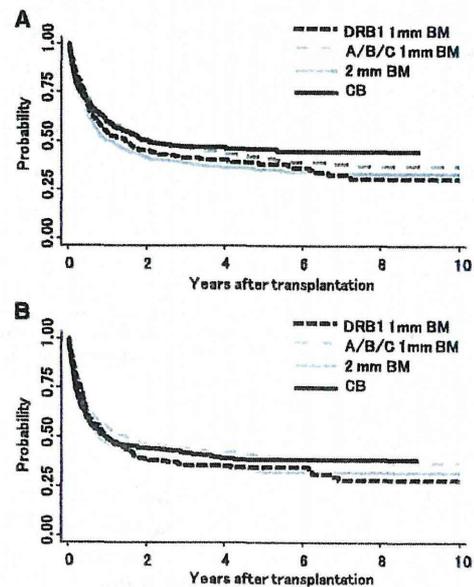


Figure 1. Adjusted probabilities of OS (A) and RFS (B). The adjusted 3-year probabilities of OS for unrelated cord blood recipients, single-HLA-DRB1-mismatched unrelated bone marrow (UBM) recipients, single-HLA-class-I-mismatched UBM, and 6 of 8 UBM recipients were 47%, 41%, 47%, and 38%, respectively (A). The adjusted 3-year probabilities of RFS were 42%, 36%, 44%, and 36%, respectively (B).

Table 3. Multivariate Analyses of Neutrophil and Platelet Recovery

Degree of HLA Mismatch	N	Neutrophil Recovery			Platelet Recovery			
		RR	(95% CI)	P value	RR	(95% CI)	P value	
Bone marrow transplantation	Single DRB1 (7/8)	248	1.00			1.00		
	Single A or B (7/8)	137	1.31	(1.04-1.65)	.021	1.31	(1.01-1.70)	.039
	Single C (7/8)	287	1.19	(0.98-1.43)	.069	0.98	(0.79-1.21)	.840
	C + DRB1 (6/8)	144	0.96	(0.77-1.20)	.735	0.79	(0.62-1.02)	.065
	A/B + C (6/8)	122	1.14	(0.89-1.45)	.307	0.84	(0.63-1.13)	.255
	Other two loci (6/8)	90	0.89	(0.68-1.14)	.346	0.80	(0.58-1.10)	.174
Cord blood transplantation	351	0.50	(0.42-0.60)	<.001	0.52	(0.42-0.63)	<.001	

RR indicates relative risk; CI, confidence interval.

Adjusted by patient age at transplantation >40 versus <40, patient sex, donor-patient sex mismatch versus matched, ABO major mismatch versus others, advanced versus standard disease status at transplant, cyclophosphamide, and total-body irradiation or busulfan and cyclophosphamide for conditioning versus other conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against graft-versus-host disease.

class I-mismatched BM, and 22% for 6 of 8 BM at 100 days posttransplantation (*P* < .001 between CB and single HLA-DRB1-mismatched BM) (Figure 2A).

Among recipients who survived at least 100 days posttransplantation, the risk of developing cGVHD and extensive-type cGVHD was not significantly increased in all HLA disparity groups of CB recipients when compared with that of HLA-DRB1-allele/antigen-mismatched BM recipients (RR = 1.36, 95% CI, 0.99-1.88; *P* = .057 for cGVHD, and RR = 0.86, 95% CI, 0.55-1.34; *P* = .500 for extensive-type cGVHD). The unadjusted cumulative incidence of extensive-type cGVHD was 17% for CB recipients, 20% for single HLA-DRB1-mismatched BM, 25% for single HLA class I-mismatched BM, and 30% for 6 of 8 BM recipients at year posttransplantation (*P* = .34 between CB and single HLA-DRB1-mismatched BM) (Figure 2B).

DISCUSSION

Our main objective was to compare OS after transplantation of UCBT and single-HLA-mismatched UBM and to provide useful data for selection of an appropriate donor and graft source in second stem cell source/donor selection for adults with hematologic malignancy. To the best of our knowledge, this is the first study to involve mismatched allele/antigen-specific analyses including CB for the process of donor selection. Our results suggest that 0 to 2 HLA-mismatched UCB is a reasonable second alternative of choice for adult patients with leukemia, with similar survival to that of single DRB1-mismatched or other 7 of 8 UBM recipients, the current first choice for second alternative donor/stem cells.

Neutrophil and platelet recovery was slower in CB recipients than BM recipients, consistent with the results of previous reports [7-10,12]. This is the major limitation of the use of UCB, and several strategies have been studied to reduce the neutropenic period, such as screening for patients' pretransplantation anti-HLA antibodies and their specificity, transplantation of 2 UCB units if a single UCB unit with an ade-

quate cell dose is not available, or direct infusion of UCB into bone marrow [22-26].

Despite higher HLA disparity at the antigen level (69% 2 antigen mismatch, 25% antigen mismatch, and 6% matched), UCB recipients showed lower incidence of severe aGVHD than single DRB1-mismatched UBM recipients, consistent with other reports that compared UCB with single-mismatched UBM (7 of 8) [8,11,12]. In our study, tacrolimus and short-term methotrexate were used preferentially in BM recipients, whereas cyclosporine A was used in 68% of CB recipients. Prior studies have shown reduced severe aGVHD with tacrolimus, and this difference may have underscored the improved aGVHD control of UCB over mismatched BM in unadjusted analyses [27,28]. It is likely that decreased risk of grade 2 to 4 aGVHD in UCB recipients contributed to decreased risk of TRM among UCB recipients.

Increasing the number of HLA mismatches from 7 of 8 to 6 of 8 was associated with an approximately 10% reduction in survival in UBM recipients, which was quite similar to the results from the National Marrow Donor Program [3]. Because we eliminated data from the first 3 pioneering years of unrelated BMT, most of the bone marrow recipients and donors were allele-typed for at least HLA-A, -B, and -DRB1 before transplantation. Survival outcomes of single class I mismatch were not significantly different from those of single class II mismatch in the current analyses. We believe that allele typing of HLA-A, -B, and -DRB1 before transplantation led to better selection of the donor compared with that in the first several years of UBM. This study includes a large number of fully typed BM and CB recipients, but there are limitations. The choice of stem cell source is influenced by many unmeasured factors that can affect outcome. It is also influenced by the availability of acceptable HLA disparity for unrelated donors and mainly cell dose for cord blood units. Although we have adjusted for known risk factors and disparities between groups, we cannot rule out the influence of potential selection bias, which can only be excluded in a randomized controlled trial. Transplantation years

Table 4. Multivariate Analyses of Acute (Grades 2 to 4 and Grades 3 to 4), Chronic, and Extensive-Type Chronic Graft-versus-Host Disease

Degree of HLA Mismatch	Grade 2-4 acute GVHD			Grade 3-4 acute GVHD			Chronic GVHD			Extensive cGVHD				
	N	RR	(95% CI)	P-value	RR	(95% CI)	P-value	N	RR	(95% CI)	P-value	RR	(95% CI)	P-value
Bone marrow transplantation														
Single DRB1 (7/8)	248	1.00			1.00		.698	199	1.00			1.00		.651
Single A or B (7/8)	137	0.76	(0.55-1.06)	.103	0.91	(0.56-1.47)	.635	111	0.91	(0.61-1.36)	.646	0.89	(0.57-1.50)	.003
Single C (7/8)	287	0.93	(0.72-1.20)	.584	0.91	(0.61-1.35)	.610	227	1.56	(1.15-2.10)	.004	1.79	(1.22-2.63)	.097
C + DRB1 (6/8)	144	0.85	(0.60-1.18)	.320	0.88	(0.54-1.44)	.003	109	1.44	(1.01-2.05)	.007	2.26	(1.46-3.50)	<.001
A/B + C (6/8)	122	1.40	(1.04-1.90)	.028	1.90	(1.25-2.87)	.183	87	1.64	(1.14-2.34)	.007	1.15	(0.82-2.13)	.652
Other two loci (6/8)	90	0.88	(0.60-1.28)	.501	0.65	(0.34-1.22)	<.001	60	1.35	(0.86-2.12)	.191	0.86	(0.55-1.34)	.500
Cord blood transplantation	351	0.55	(0.42-0.72)	<.001	0.43	(0.27-0.58)		252	1.36	(0.99-1.88)	.057			

GVHD indicates graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

Adjusted by patient age at transplantation >40 versus <40, patient sex, donor-patient sex mismatch versus matched, ABO major mismatch versus others, advanced versus standard disease status at transplantation, cyclophosphamide, and total-body irradiation or busulfan and cyclophosphamide for conditioning versus other conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against graft-versus-host disease.

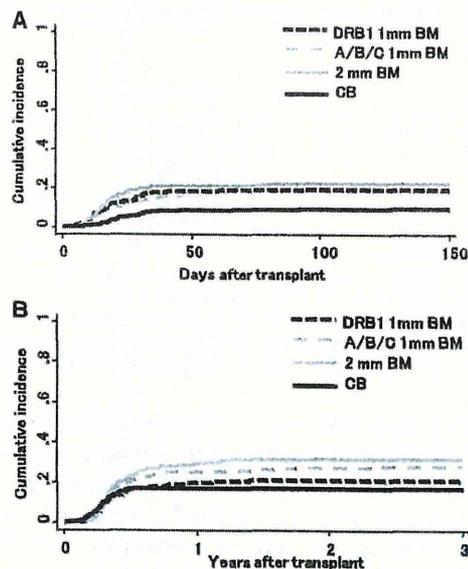


Figure 2. Cumulative incidence of grade 3 to 4 aGVHD (A) and extensive-type cGVHD (B). The cumulative incidences of grade 3 to 4 aGVHD at 100 days posttransplantation for unrelated cord blood recipients, single HLA-DRB1-mismatched unrelated bone marrow (UBM) recipients, and single HLA class I-mismatched UBM were 9%, 19%, 18%, and 22% (A). The cumulative incidences of extensive-type cGVHD at 1-year posttransplantation were 17%, 20%, 25%, and 30% (B).

of UBM recipients included from 1996 and 1999, for which there were no significant outcome differences between UBM performed in 1996 to 1999 and after 2000. In these periods, there were advances including in supportive care and nutritional management, introduction of new antifungal agents, and more frequent use of tacrolimus, which may have affected transplantation outcomes [27-32].

In conclusion, we suggest that 0 or 2 HLA-mismatched UCB is a comparable second alternative for adult patients with leukemia in the absence of the first alternative, an 8 of 8 UBM donor, with survival similar to that of single DRB1-mismatched or other 7 of 8 UBM recipients. UCB may be preferred over single mismatched UBM when a transplantation is needed urgently, considering the short time needed for UCBT.

ACKNOWLEDGMENTS

The authors are grateful for the assistance and cooperation of all the staff members of the collaborating institutes of the Japan Cord Blood Bank Network and Japan Marrow Donor Program. This work was supported by a Research Grant for Tissue Engineering (H17-014), a Research Grant for Allergic Disease and Immunology (H20-015), a Research Grant for Cancer (H19-1), and a Research Grant for Allergic Disease and Immunology (H23-010) from the Japanese Ministry of Health, Labor, and Welfare.

Financial disclosure: The authors have nothing to disclose.

AUTHORSHIP STATEMENT

Contributions: Y.A., Y.M., R.S., and S. Kato designed the study, and wrote the article; Y.A. analyzed results and created the figures; T.N.I., H.A., and M. Takashi reviewed and cleaned the Japan Cord Blood Bank Network data, and reviewed the results; S. Taniguchi, S. Takahashi, S. Kai., H.S., Y. Kouzai., N.K., T.M., T.F., and Y. Koderu submitted and cleaned the data; M. Tsuchida, K.K., T.K., and Y.M. reviewed and cleaned the Japan Marrow Donor Program data, and reviewed the results.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2011.10.008.

REFERENCES

1. Morishima Y, Sasazuki T, Inoko H, 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.
2. Morishima Y, Yabe T, Matsuo K, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. *Biol Blood Marrow Transplant*. 2007;13:315-328.
3. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110:4576-4583.
4. Bray RA, Hurley CK, Kamani NR, et al. National marrow donor program HLA matching guidelines for unrelated adult donor hematopoietic cell transplants. *Biol Blood Marrow Transplant*. 2008;14:45-53.
5. Sasazuki T, Fuji T, Morishima Y, 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.
6. Gluckman E. Ten years of cord blood transplantation: from bench to bedside. *Br J Haematol*. 2009;147:192-199.
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. 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.
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. Atsuta Y, Suzuki R, Nagamura-Inoue T, 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.
11. Eapen M, Rubinstein P, Zhang MJ, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet*. 2007;369:1947-1954.
12. 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.
13. Koderu Y, Morishima Y, Kato S, et al. Analysis of 500 bone marrow transplants from unrelated donors (UR-BMT) facili-

- tated by the Japan Marrow Donor Program: confirmation of UR-BMT as a standard therapy for patients with leukemia and aplastic anemia. *Bone Marrow Transplant*. 1999;24:995-1003.
14. Kawase T, Morishima Y, Matsuo K, et al. High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. *Blood*. 2007;110:2235-2241.
15. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15:825-828.
16. Flowers ME, Kansu E, Sullivan KM. Pathophysiology and treatment of graft-versus-host disease. *Hematol Oncol Clin North Am*. 1999;13:1091-1112. viii-ix.
17. 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.
18. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141-1154.
19. Cox DR. Regression model and life tables. *J R Stat Soc B*. 1972;34:187-200.
20. Fine JP, Gray RJ. A proportional hazards model for subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:456-509.
21. Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part I: unadjusted analysis. *Bone Marrow Transplant*. 2001;28:909-915.
22. Rocha V, Gluckman E. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. *Br J Haematol*. 2009;147:262-274.
23. Takashi M, Atsuta Y, Fujiwara K, Kodo H, Kai S, Sato H, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. *Blood*. 2010;116:2839-2846.
24. Spellman S, Bray R, Rosen-Bronson S, et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. *Blood*. 2010;115:2704-2708.
25. Brunstein CG, Gutman JA, Weisdorf DJ, et al. Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. *Blood*. 2010;116:4693-4699.
26. Frassoni F, Gualandi F, Podesta M, et al. Direct intrabone transplant of unrelated cord-blood cells in acute leukaemia: a phase I/II study. *Lancet Oncol*. 2008;9:831-839.
27. Nash RA, Etzioni R, Storb R, et al. Tacrolimus (FK506) alone or in combination with methotrexate or methylprednisolone for the prevention of acute graft-versus-host disease after marrow transplantation from HLA-matched siblings: a single-center study. *Blood*. 1995;85:3746-3753.
28. Yanada M, Emi N, Naoe T, et al. Tacrolimus instead of cyclosporine used for prophylaxis against graft-versus-host disease improves outcome after hematopoietic stem cell transplantation from unrelated donors, but not from HLA-identical sibling donors: a nationwide survey conducted in Japan. *Bone Marrow Transplant*. 2004;34:331-337.
29. Fuji S, Kim SW, Fukuda T, Kamiya S, Kuwahara S, Takaue Y. Positive impact of maintaining minimal caloric intake above 1.0 x basal energy expenditure on the nutritional status of patients undergoing allogeneic hematopoietic stem cell transplantation. *Am J Hematol*. 2009;84:63-64.
30. Fuji S, Kim SW, Mori S, et al. Hyperglycemia during the neutropenic period is associated with a poor outcome in patients undergoing myeloablative allogeneic hematopoietic stem cell transplantation. *Transplantation*. 2007;84:814-820.
31. Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. *Clin Infect Dis*. 2007;44:531-540.
32. Yokoe D, Casper C, Dubberke E, et al. Infection prevention and control in health-care facilities in which hematopoietic cell transplant recipients are treated. *Bone Marrow Transplant*. 2009;44:495-507.

Low incidences of acute and chronic graft-versus-host disease after unrelated bone marrow transplantation with low-dose anti-T lymphocyte globulin

Kazuo Hatanaka · Shigeo Fuji · Kazuhiro Ikegame · Ruri Kato · Atsushi Wake · Michihiro Hidaka · Toshiro Ito · Masami Inoue · Yoshihisa Nagatoshi · Akiyoshi Takami · Naokuni Uike · Hisashi Sakamaki · Hiromasa Yabe · Yasuo Morishima · Ritsuro Suzuki · Yoshiko Atsuta · Takahiro Fukuda

Received: 25 August 2012/Revised: 12 October 2012/Accepted: 16 October 2012/Published online: 7 November 2012
© The Japanese Society of Hematology 2012

Abstract Anti-T lymphocyte globulin (ATG) is commonly used as prophylaxis for graft-versus-host disease (GVHD), especially in patients who are at high risk of GVHD. The appropriate dosage of ATG in Japan has not yet been assessed. We therefore conducted a nationwide survey of patients who received ATG-Fresenius as GVHD prophylaxis for unrelated bone marrow transplantation (uBMT). A total of 86 patients were identified (median age 31 years, range 1–68). The median total dose of ATG was

10 mg/kg. The cumulative incidence of neutrophil engraftment was 90 %. The probability of 2-year overall survival (OS) was 67 %. The cumulative incidence of 2-year non-relapse mortality was 25 %. The incidences of grade II–IV and grade III–IV acute GVHD were 20 and 8 %, respectively. The incidences of chronic and extensive chronic GVHD were 19 and 8 %, respectively. In adult patients, there was a reduction of acute GVHD with high-dose ATG (>10 mg/kg), which did not reach statistical significance. In conclusion, the addition of low-dose ATG to GVHD prophylaxis in Japanese patients who received uBMT resulted in decreased incidences of both acute and chronic GVHD without compromising OS. The effects of low-dose ATG should be assessed in a prospective clinical trial.

K. Hatanaka and S. Fuji contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s12185-012-1209-4) contains supplementary material, which is available to authorized users.

K. Hatanaka
Department of Hematology/Oncology, Wakayama Medical University Hospital, Wakayama, Japan

K. Hatanaka
Department of Internal Medicine,
Rinku General Medical Center, Izumisano, Japan

S. Fuji · T. Fukuda (✉)
Hematopoietic Stem Cell Transplantation Division,
National Cancer Center Hospital, 5-1-1, Tsukiji,
Chuo-Ku, Tokyo 104-0045, Japan
e-mail: tafukuda@ncc.go.jp

K. Ikegame · R. Kato
Division of Hematology, Department of Internal Medicine,
Hyogo College of Medicine, Nishinomiya, Japan

K. Ikegame · R. Kato
Department of Medicine III,
Osaka University Hospital, Suita, Japan

A. Wake
Department of Hematology,
Toranomon Hospital, Tokyo, Japan

A. Wake
Department of Hematology,
Toranomon Hospital Kajigaya, Kawasaki, Japan

M. Hidaka
Department of Internal Medicine, National Hospital
Organization, Kumamoto Medical Center, Kumamoto, Japan

T. Ito
Division of Hematology, Department of Internal Medicine,
Shinshu University School of Medicine, Nagano, Japan

M. Inoue
Department of Hematology/Oncology, Osaka Medical Center
and Research Institute for Maternal and Child Health,
Iaumi, Japan

Y. Nagatoshi
Section of Pediatrics,
National Kyushu Cancer Center, Fukuoka, Japan

Y. Nagatoshi
Department of Pediatrics,
Fukuoka National Hospital, Fukuoka, Japan

Keywords ATG · Unrelated bone marrow transplant · GVHD

Introduction

Over the past several decades, significant advances have been made in the field of allogeneic hematopoietic stem cell transplantation (HSCT), and allogeneic HSCT has become an integral part of treatment for a variety of hematological malignancies and some non-malignant diseases [1, 2]. However, acute and chronic graft-versus-host diseases (GVHD) are still major morbidities after allogeneic HSCT, especially in patients who receive stem cells from an unrelated donor. Finke et al. [3] conducted a large randomized-control trial which showed that anti-T lymphocyte globulin Fresenius (ATG-F) has a beneficial effect as GVHD prophylaxis in patients who have undergone unrelated HSCT. Furthermore, a long-term follow-up of this study demonstrated that the use of ATG-F significantly reduced the incidence of chronic GVHD, which should reduce late non-relapse mortality (NRM) and improve the quality of life (QOL) after unrelated HSCT [4].

Different preparations of ATG including Thymoglobulin and ATG-F have been tested as part of conditioning regimens to achieve *in vivo* T cell depletion. These two ATG preparations can differ substantially in their potency due to differences in production methods, i.e., in the cells used for immunization [5]. In addition, the intensity of GVHD prophylaxis may depend on the patient's ethnicity, since the risk of GVHD might differ among races [6, 7]. For example, regarding Thymoglobulin, 6.0–7.5 mg/kg is the

usual dose for preventing GVHD, and 4 mg/kg was shown to be insufficient in Caucasian patients [5]. In contrast, in Korea, Kim et al. reported that the use of a very low dose of Thymoglobulin (total 2.5 mg/kg) was still associated with lower incidences of acute GVHD and NRM [8, 9]. Furthermore, ATG has multiple immunomodulatory effects, including the expansion of regulatory T cells [10]. Therefore, the effectiveness of ATG as GVHD prophylaxis cannot be assessed simply in terms of the degree of lymphodepletion. Profound immunosuppression could lead to an inferior outcome due to a high rate of infections or possibly to an increase in relapse caused by a loss of graft-versus-leukemia (GVL) effects.

Therefore, we assessed the clinical outcomes of patients who underwent unrelated HSCT using ATG-F as GVHD prophylaxis to identify a candidate dose of ATG-F for testing in a prospective clinical trial.

Patients and methods

Study design

This was a retrospective study that surveyed the use of ATG-F as GVHD prophylaxis in Japan. Patients who received ATG-F as GVHD prophylaxis for bone marrow transplant from an unrelated donor (uBMT) from April 2001 to December 2006 were included. In the era of this study, only BMT could be collected as a stem cell source from an unrelated volunteer donor. We performed a nationwide questionnaire survey at institutions in Japan that performed allogeneic HSCT. For patients who received ATG-F as GVHD prophylaxis, we also collected data regarding the dose and duration of ATG-F along with data from the national registry system of the Japan Society of Hematopoietic Cell Transplantation [11]. We identified 581 cases who received ATG for related or unrelated HSCT, and questionnaires were obtained for 509 (87.6 %). Among these 509 cases, 182 (35.8 %) received ATG-F. We only included patients who received ATG-F for an unrelated BMT, because most of the patients who received ATG for a related HSCT underwent a haploidentical HSCT. In terms of HLA typing, allele typing of 6 loci including HLA A, B and DRB1 was available, while information regarding HLA-C was unavailable. This study was approved by the Institutional Review Board of the Japan Society of Hematopoietic Cell Transplantation and Rinku General Medical Center, Osaka, Japan.

Clinical outcomes

Endpoints included neutrophil recovery, overall survival (OS), NRM, acute GVHD and chronic GVHD. Neutrophil

A. Takami
Department of Hematology and Oncology,
Kanazawa University Hospital, Kanazawa, Japan

N. Uike
Department of Hematology,
National Kyushu Cancer Center, Fukuoka, Japan

H. Sakamaki
Hematology Division, Tokyo Metropolitan Cancer and
Infectious Diseases Center-Komagome Hospital, Tokyo, Japan

H. Yabe
Department of Cell Transplantation and Regenerative Medicine,
Tokai University School of Medicine, Tokyo, Japan

Y. Morishima
Division of Epidemiology and Prevention,
Aichi Cancer Center Research Institute, Nagoya, Japan

R. Suzuki · Y. Atsuta
Department of HSCT Data Management/Biostatistics,
Nagoya University, Nagoya, Japan