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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.

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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
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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
	$<3.0 \times 10^8$/kg (n = 140)		*3.0–4.6×10^8/kg (n = 248)*		*$\geqslant 4.6 \times 10^8$/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 $<3.0 \times 10^8$/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 $<2.3 \times 10^8$/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
	$<2.3 \times 10^8$/kg (n = 755)		*$2.3–3.4 \times 10^8$/kg (n = 1519)*		*$\geq 3.4 \times 10^8$/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 (× 10^8/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 (× 10^8/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

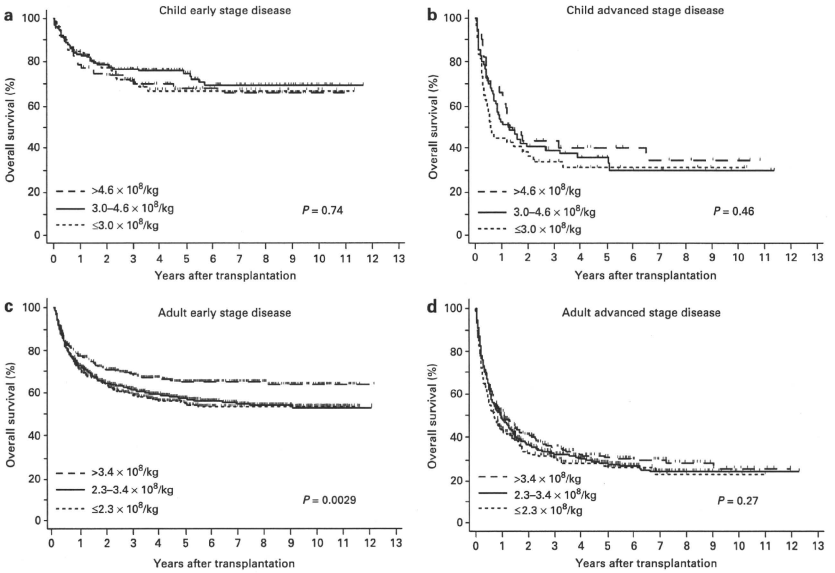

Figure 1 Kaplan–Meier estimates of OS according to cell dose: (**a**) among children with early-stage diseases; (**b**) among children with advanced-stage diseases; (**c**) among adults with early-stage diseases; and (**d**) among adults with advanced-stage diseases.

($P=0.46$; 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.

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Randomized study of induction therapy comparing standard-dose idarubicin with high-dose daunorubicin in adult patients with previously untreated acute myeloid leukemia: the JALSG AML201 Study

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Randomized study of induction therapy comparing standard-dose idarubicin with high-dose daunorubicin in adult patients with previously untreated acute myeloid leukemia: the JALSG AML201 Study

Shigeki Ohtake,[1] Shuichi Miyawaki,[2] Hiroyuki Fujita,[3] Hitoshi Kiyoi,[4] Katsuji Shinagawa,[5] Noriko Usui,[6] Hirokazu Okumura,[7] Koichi Miyamura,[8] Chiaki Nakaseko,[9] Yasushi Miyazaki,[10] Atsushi Fujieda,[11] Tadashi Nagai,[12] Takahisa Yamane,[13] Masafumi Taniwaki,[14] Masatomo Takahashi,[15] Fumiharu Yagasaki,[16] Yukihiko Kimura,[17] Norio Asou,[18] Hisashi Sakamaki,[19] Hiroshi Handa,[20] Sumihisa Honda,[21] Kazunori Ohnishi,[22] Tomoki Naoe,[4] and Ryuzo Ohno[23]

[1]Department of Clinical Laboratory Science, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan; [2]Leukemia Research Center, Saiseikai Maebashi Hospital, Maebashi, Japan; [3]Department of Internal Medicine and Clinical Immunology, Yokohama City University Graduate School of Medicine, Yokohama, Japan; [4]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [5]Hematology/Oncology Division, Okayama University Hospital, Okayama, Japan; [6]Division of Hematology and Oncology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan; [7]Department of Hematology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan; [8]Department of Internal Medicine, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [9]Department of Hematology, Chiba University Hospital, Chiba, Japan; [10]Department of Hematology and Molecular Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [11]Department of Hematology and Oncology, Mie University Graduate School of Medicine, Tsu, Japan; [12]Division of Hematology, Jichi Medical University, Shimotsuke, Japan; [13]Department of Hematology, Osaka City University, Osaka, Japan; [14]Department of Clinical Molecular Genetics and Laboratory Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan; [15]Division of Hematology and Oncology, Department of Internal Medicine, St Marianna University School of Medicine, Kawasaki, Japan; [16]Department of Hematology, Saitama Medical School, Hidaka, Japan; [17]Division of Hematology, First Department of Internal Medicine, Tokyo Medical University, Tokyo, Japan; [18]Department of Hematology, Kumamoto University School of Medicine, Kumamoto, Japan; [19]Department of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [20]Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Japan; [21]Department of Public Health, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [22]Oncology Center, Hamamatsu University School of Medicine, Hamamatsu, Japan; and [23]Aichi Cancer Center, Nagoya, Japan

We conducted a multi-institutional randomized study to determine whether high-dose daunorubicin would be as effective as standard-dose idarubicin in remission-induction therapy for newly diagnosed adult patients younger than 65 years of age with acute myeloid leukemia. Of 1064 patients registered, 1057 were evaluable. They were randomly assigned to receive either daunorubicin (50 mg/m^2 daily for 5 days) or idarubicin (12 mg/m^2 daily for 3 days) in combination with 100 mg/m^2 of cytarabine by continuous infusion daily for 7 days as induction therapy. Complete remission was achieved in 407 (77.5%) of 525 patients in the daunorubicin group and 416 (78.2%) of 532 in the idarubicin group ($P = .79$). Patients achieving complete remission received intensive postremission therapy that consisted of either 3 courses of high-dose cytarabine or 4 courses of standard-dose therapy. Overall survival rates at 5 years were 48% for the daunorubicin group and 48% for the idarubicin group ($P = .54$), and relapse-free survival rates at 5 years were 41% and 41% ($P = .97$), respectively. Thus, high-dose daunorubicin and standard-dose idarubicin were equally effective for the treatment of adult acute myeloid leukemia, achieving a high rate of complete remission and good long-term efficacy. This study is registered at http://www.umin.ac.jp/ctrj/ as C000000157. (*Blood*. 2011;117(8): 2358-2365)

Introduction

The combination of anthracycline and cytarabine (Ara-C) with or without other antileukemia drugs is a standard induction therapy for acute myeloid leukemia (AML),[1-3] and a combination of daunorubicin at a dose of 45 to 50 mg/m^2 given daily for 3 days and Ara-C at a dose of 100 to 200 mg/m^2 given daily for 7 days generally has been used. In the late 1980s, however, idarubicin was introduced into clinics, and 3 randomized studies comparing idarubicin with daunorubicin reported significantly higher complete remission (CR) rates in favor of idarubicin.[4-6] A meta-analysis also confirmed a superior effect of idarubicin at a dose of 10 to 12 mg/m^2 for 3 days versus daunorubicin at a dose of 45 to 60 mg/m^2 for 3 days in the achievement of CR.[7] Nevertheless, the long-term follow-up of the above-mentioned 3 randomized studies comparing idarubicin with daunorubicin revealed that the idarubicin group had better overall survival (OS) than the daunorubicin group in only 1 study.[8]

The Japan Adult Leukemia Study Group (JALSG) used idarubicin and Ara-C as induction therapy in the AML95 and AML97 studies,[9-11] after idarubicin was registered and approved for the national health insurance system in 1995. Both studies resulted in satisfactorily high CR rates (80% and 79%, respectively); however, these CR rates were not superior to those of our earlier AML87, AML89, and AML92 studies, which used daunorubicin in combination with other antileukemia drugs.[12-14] In these 3 previous studies,

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daunorubicin and other drugs were administered in a response-oriented individualized manner; that is, additional drugs were given for a few days when the bone marrow at day 8 was not hypoplastic, containing a substantial number of blasts. Therefore, the total doses of daunorubicin administered during the first course of induction therapy were 240 to 280 mg/m^2 given for more than 5 to 7 days, which was more than the conventional dose of 40 to 60 mg/m^2 given for 3 days. Usui et al also reported that the optimal dose of daunorubicin in their induction therapy for newly diagnosed adult AML was approximately 280 mg/m^2 (40 mg/m^2 for 7 days).[15]

Because there had been no prospective randomized study comparing a higher dose of daunorubicin with the standard dose of idarubicin (12 mg/m^2) in adult AML, in the present multi-institutional randomized study, we prospectively compared idarubicin (12 mg/m^2 for 3 days) with daunorubicin (50 mg/m^2 for 5 days), in combination with Ara-C (100 mg/m^2 for 7 days), as induction therapy for previously untreated adult AML. High-dose daunorubicin resulted in the same CR rate and predicted 5-year OS compared with standard-dose idarubicin.

Methods

Patients

From December 2001 to December 2005, 1064 newly diagnosed adult patients 15 to 64 years of age with de novo AML were consecutively registered from 129 participating institutions. AML was first diagnosed by the French-American-British (FAB) classification at each institution. Peripheral blood and bone marrow smears from all registered patients were sent to Nagasaki University and examined by May-Giemsa, peroxidase, and esterase staining. Next, diagnosis was reevaluated by the central review committee. Patients with the FAB M3 subtype were not registered in the present study. Eligibility criteria included adequate function of liver (serum bilirubin level < 2.0 mg/dL), kidney (serum creatinine < 2.0 mg/dL), heart, and lung and an Eastern Cooperative Oncology Group performance status between 0 and 3. Patients were not eligible if they had prediagnosed myelodysplastic syndrome, but they were eligible if they had no definite diagnosis of myelodysplastic syndrome confirmed by bone marrow histologic analysis even when they had a previous history of hematologic abnormality. Cytogenetic abnormalities were grouped by standard criteria and classified according to the Medical Research Council classification.[16] The study was approved by the institutional review boards at each participating institution. Written informed consent was obtained from all patients before registration in accordance with the Declaration of Helsinki. The study was registered at http://www.umin.ac.jp/ctr/ as C000000157.

Treatments

Patients were randomly assigned by use of a centralized computer system to receive either idarubicin or daunorubicin. Randomization was stratified by age (younger or older than 50 years) and type of AML (FAB classification). All patients received 100 mg/m^2/d Ara-C by 24-hour continuous infusion from days 1 to 7. In the idarubicin group, patients received 12 mg/m^2/d idarubicin for 3 days, and in the daunorubicin group, they received 50 mg/m^2/d daunorubicin for 5 days. If patients did not achieve CR by the first course, the same induction therapy was repeated after an approximately 3- to 4-week interval. If patients did not achieve CR with 2 courses, they were judged as failure cases.

All patients who achieved CR were again randomized to receive either 4 courses of conventional consolidation therapy or 3 courses of high-dose Ara-C therapy. In the conventional consolidation-therapy group, the first course consisted of mitoxantrone (7 mg/m^2 by 30-minute infusion on days 1 to 3) and Ara-C (200 mg/m^2 by 24-hour continuous infusion on days 1 to 5). The second course consisted of daunorubicin (50 mg/m^2 by 30-minute infusion on days 1 to 3) and Ara-C (200 mg/m^2 by 24-hour continuous infusion on days 1 to 5). The third course consisted of aclarubicin (20 mg/m^2 by 30-minute infusion on days 1 to 5) and Ara-C (200 mg/m^2 by 24-hour continuous infusion on days 1 to 5). The fourth course consisted of Ara-C (200 mg/m^2 by 24-hour continuous infusion on days 1 to 5), etoposide (100 mg/m^2 by 1-hour infusion on days 1 to 5), vincristine (0.8 mg/m^2 by bolus injection on day 8), and vindesine (2 mg/m^2 by bolus injection on day 10). Each consolidation was administered as soon as possible after the neutrophils, white blood cells (WBCs), and platelets recovered to more than 1.5×10^9/L, 3.0×10^9/L, and 100×10^9/L, respectively. In the high-dose Ara-C group, 3 courses of 2.0 g/m^2 Ara-C were given by 3-hour infusion every 12 hours on days 1 to 5. Each course was administered 1 week after the neutrophils, WBCs, and platelets recovered to the above counts.

The best supportive care, including administration of antibiotics and platelet transfusions, was given as indicated. When patients had life-threatening documented infections during neutropenia, the use of granulocyte colony-stimulating factor was permitted.

After completion of consolidation therapy, no patients received further chemotherapy. Allogeneic stem cell transplantation (SCT) was offered during the first CR to patients 50 years of age or younger and with a histocompatible donor in the intermediate or adverse cytogenetic risk groups.

Definitions and study end points

Responses were evaluated according to the recommendations of the International Working Group.[17] CR was defined as the presence of all of the following: fewer than 5% blasts in bone marrow, no leukemic blasts in peripheral blood, recovery of peripheral neutrophil counts to more than 1.0×10^9/L and platelet counts to more than 100×10^9/L, and no evidence of extramedullary leukemia. Relapse after CR was defined as the presence of at least 1 of the following: reappearance of leukemic blasts in the peripheral blood, recurrence of more than 5% blasts in the bone marrow not attributable to any other cause (eg, bone marrow regeneration after consolidation therapy), and appearance of extramedullary leukemia.

This was a multi-institutional, randomized, phase 3 study with a 2×2 factorial design. The primary end point of the first randomization was CR rate. The result of the second randomization is reported here in part but will be presented fully in a separate paper. OS was calculated from the date of entry into the study until death due to any cause and was censored at the last follow-up. Relapse-free survival (RFS) for patients who achieved CR was measured from the date of CR until the date of AML relapse or death of any cause and was censored at the last follow-up. Patients who underwent allogeneic SCT were not censored at the date of SCT.

Statistical analysis

This study was prospectively powered to demonstrate noninferiority of daunorubicin compared with idarubicin. With a sample size of 420 patients per group (840 in total), the study had a power of 90% at a 1% level of significance to demonstrate noninferiority (assuming an 80% CR rate for both groups). Statistical testing for the noninferiority trial was performed according to the method of Blackwelder.[18] The Kaplan-Meier method was used to estimate probabilities of OS and RFS.[19] To test factors that predict CR, the χ^2 test and Wilcoxon rank sum test were used for univariate analysis, and the multiple logistic regression model was used for multivariate analysis. For comparison of OS and RFS, the log-rank test was used for univariate analysis and the proportional hazard model of Cox for multivariate analysis.[20,21] Cumulative rates of CR, neutrophil recovery, and platelet recovery were estimated according to the Kaplan-Meier method and were evaluated with the log-rank test. The JMP program (SAS Institute Inc) was used for these analyses. All analyses were performed according to the intention-to-treat principle. All statistical tests except the method of Blackwelder were 2-sided, and the significance level was set at .05.

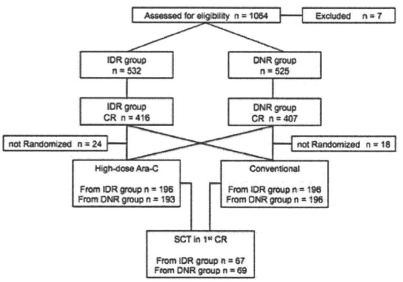

Figure 1. CONSORT flow diagram. IDR indicates idarubicin; DNR, daunorubicin; CR, complete remission; Ara-C, cytarabine; and SCT, stem cell transplantation.

Results

Patient characteristics

Among 1064 registered patients, 7 did not meet the inclusion criteria (misdiagnosis, 1; infectious complication, 1; without therapy, 1; and withdrawal of consent, 4). The study population thus comprised 1057 patients (Figure 1). Patient characteristics are presented in Table 1. Median age was 47 years (range, 15-64 years). Cytogenetics data were available for 1021 patients (96.6%). Among these, 247 (24.2%) were classified in the favorable-risk group, 681 (66.7%) in the intermediate-risk group, and 93 (9.1%) in the adverse group. Five hundred thirty-two patients were assigned to the idarubicin group and 525 to the daunorubicin group. The 2 groups were well balanced with regard to pretreatment characteristics such as age, initial WBC counts, FAB classification, and cytogenetic prognostic grouping.

Table 1. Patient characteristics

	IDR group (n = 532)	DNR group (n = 525)	*P*
Median age, y (range)	47 (15-64)	47 (15-64)	.781
≤ 50	310	306	
> 50	222	219	.996
Median WBC count, $\times 10^9$/L (range)	13.7 (0.1-382)	15.3 (0.1-334)	.769
≤ 20 × 10^9/L	304	297	
20= ≤ 50 × 10^9/L	95	104	
> 50 × 10^9/L	125	121	
Unknown	8	3	.427
FAB type			
M0	30	30	
M1	95	94	
M2	232	233	
M4	100	100	
M5	56	51	
M6	17	16	
M7	2	1	.997
Cytogenetic group			
Favorable	128	119	
Intermediate	335	346	
Adverse	49	44	
Unknown	20	16	.561
MPO-positive blasts, %			
< 50	169	187	
≥ 50	307	292	
Unknown	56	46	.330
Performance status			
0, 1, 2	512	509	
3	20	16	.524

Values are number of patients unless otherwise indicated.

IDR indicates idarubicin; DNR, daunorubicin; WBC, white blood cell count; FAB, French-American-British classification; and MPO, myeloperoxidase.

Table 2. Results of induction therapy

	IDR group, n (%)	DNR group, n (%)
Patients	532	525
CR	416 (78.2)	407 (77.5)
CR by 1 course	341 (64.1)	321 (61.1)
CR by 2 courses	75 (14.1)	86 (16.4)
95% CI	74.5-81.5	73.8-80.9

IDR indicates idarubicin; DNR, daunorubicin; and CR, complete remission.

Response to induction therapy

Overall, of 1057 evaluable patients, 823 (77.9%) achieved CR. Of 532 patients in the idarubicin group, 416 (78.2%) achieved CR, and of 525 in the daunorubicin group, 407 (77.5%) obtained CR ($P = .79$). Noninferiority for the primary end point was assessed by determining whether the lower bound of the 95% confidence interval (CI) of the difference between the CR rates for the daunorubicin and idarubicin groups was less than −10%. The CR rate of the daunorubicin group was noninferior to that of the idarubicin group (Table 2). In the idarubicin group, 341 patients (64.1%) achieved CR after the first course, and in the daunorubicin group, 321 (61.1%) did so ($P = .39$). The average period to achieve CR was 33.8 days (95% CI 32.9 to 34.6 days) in the idarubicin group and 32.4 days (95% CI 31.6 to 33.2 days) in the daunorubicin group ($P = .038$). CR rates related to FAB classification, age, and cytogenetics are shown in Table 3. Although they were few, patients with FAB M6 responded better to idarubicin: 78% of 17 patients in the idarubicin group and 38% of 16 in the daunorubicin group achieved CR ($P = .037$). There were no differences in CR rate between the 2 groups in other FAB subtypes, cytogenetic risk groups, age, myeloperoxidase positivity of blasts, initial WBC count, or performance status (Table 3). Overall, logistic regression analysis revealed that induction regimen was not an independent prognostic factor but that cytogenetic group and percentage of myeloperoxidase-positive blasts were significant independent factors for achieving CR (Table 4). A cutoff value of WBCs at 20 or 50 × 10^9/L did not change the result.

OS and RFS

At a median follow-up of 48 months, 5-year predicted OS rates were 48% for the idarubicin group (95% CI 43% to 53%) and 48% for the daunorubicin group (95% CI 43% to 53%; $P = .54$; Figure 2A), and 5-year predicted RFS rates of CR patients were 41% (95% CI 36% to 46%) and 41% (95% CI 35% to 45%), respectively ($P = .97$; Figure 2B). Significant unfavorable prognostic features for OS by the Cox proportional hazard model were adverse cytogenetic risk group, age greater than 50 years, WBC count more than 20 × 10^9/L, myeloperoxidase-positive blasts less than 50%, and FAB classification of either M0, M6, or M7; for RFS, the significant unfavorable prognostic features were adverse cytogenetic risk group, WBC count more than 20 × 10^9/L, myeloperoxidase-positive blasts less than 50%, lactate dehydrogenase of 500 IU/L or more, and age greater than 50 years. Induction regimen was not an independent prognostic factor for either OS or RFS by this multivariate analysis.

Table 3. CR rates by induction therapy

	CR rate, %		
	IDR group (n = 532)	DNR group (n = 525)	*P*
FAB type			
M0	43	63	.195
M1	86	79	.236
M2	80	82	.718
M4	81	79	.86
M5	77	75	.96
M6	76	38	.037
M7	50	100	.999
Cytogenetic group			
Favorable	91	96	.134
Intermediate	79	76	.359
Adverse	51	43	.534
Unknown	50	69	.257
Age, y			
≤ 50	83	77	.108
> 50	73	78	.225
Myeloperoxidase-positive blasts, %			
< 50	68	66	.709
≥ 50	87	88	.699
WBC at diagnosis, $\times 10^9$/L			
≤ 20	79	76	.767
20 = ≤ 50	82	82	.993
> 50	74	77	.824
Performance status			
0, 1, 2	79	78	.762
3	80	75	.999

CR indicates complete remission; IDR, idarubicin; DNR, daunorubicin; FAB, French-American-British classification; and WBC, white blood cell count.

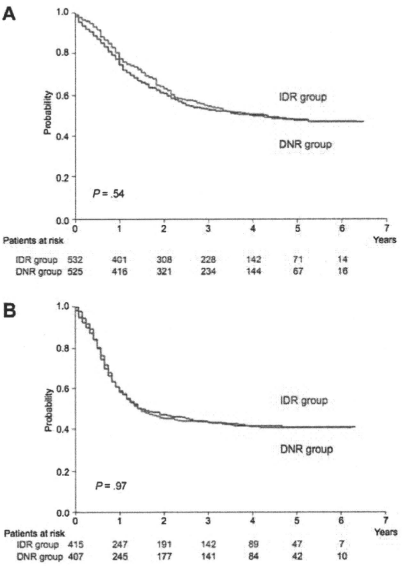

Figure 2. OS and RFS. (A) Predicted 5-year overall survival (OS) was 48% for the idarubicin group (IDR; n = 532; red line) and 48% for the daunorubicin group (DNR; n = 525; blue line; *P* = .54). (B) Predicted 5-year relapse-free survival (RFS) was 41% for the idarubicin group (IDR; n = 416; red line) and 41% for the daunorubicin group (DNR; n = 407; blue line; *P* = .97).

Adverse events

Patients receiving idarubicin required a slightly but significantly longer time to recover from neutropenia and thrombocytopenia. Median duration with a neutrophil count less than 1.0×10^9/L was 28 days for the idarubicin group and 27 days for the daunorubicin group (*P* = .0011; Figure 3A). Median duration with a platelet count less than 100×10^9/L was 25 days for the idarubicin group and 24 days for the daunorubicin group (*P* = .0034; Figure 3B). Sepsis occurred more frequently in the idarubicin group than in the daunorubicin group (8.7% and 4.9%, respectively; *P* = .02). Early death within 60 days occurred more frequently in the idarubicin group than in the daunorubicin group (4.7% and 2.1%, respectively; *P* = .03; Table 5).

Postremission therapy

Of the 823 CR patients, 781 were randomly assigned to receive either 4 courses of conventional standard-dose consolidation therapy (392 patients) or 3 courses of high-dose Ara-C therapy (389 patients), and 136 patients (16% of CR patients) underwent allogeneic SCT in the first CR. There was no significant difference in OS or RFS by postremission therapy between the idarubicin and daunorubicin groups (Table 6). In the idarubicin group, predicted 5-year OS rates were 57% for the conventional standard-dose consolidation arm (95% CI 49% to 65%) and 58% for the high-dose Ara-C arm (95% CI 51% to 66%; *P* = .79; Figure 4A). In the daunorubicin group, predicted 5-year OS rates were 56% (95% CI 48% to 63%) and 58% (95% CI 50% to 65%; *P* = .71; Figure 4B), respectively. If 2 groups were evaluated together, predicted 5-year OS rates were 56% (95% CI 51% to 62%) and 58% (95% CI 53% to 62%; *P* = .95), and predicted 5-year RFS rates were 39% (95% CI 34% to 44%) and 43% (95% CI 38% to 48%), respectively (*P* = .72). The detailed results of this consolidation phase will be reported in a separate paper.[22]

Table 4. Factors that predicted CR in all evaluable patients by multivariate analysis

Variables	Odds ratio	*P*
Cytogenetic group		
Favorable	10.39	< .0001
Intermediate	4.67	< .0001
Myeloperoxidase-positive blast ≥ 50%	2.64	< .0001
Induction therapy: IDR arm	0.97	.854

CR indicates complete remission; and IDR, idarubicin.

Discussion

The present randomized study demonstrates that if the dose intensity is increased appropriately, daunorubicin is as effective as a standard dose of idarubicin for adults less than 65 years of age

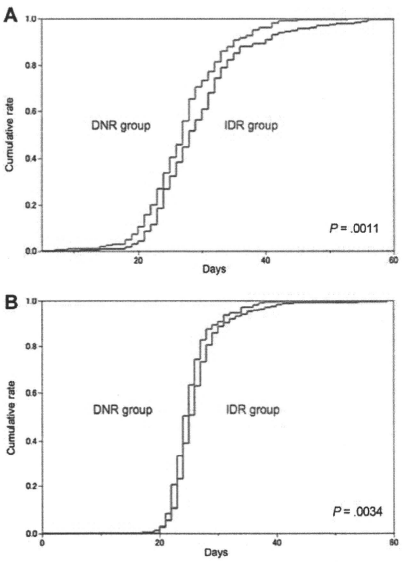

Figure 3. Hematologic recovery. (A) Day of recovery from neutropenia after the first induction course. Neutropenia was defined as neutrophil count $< 1.0 \times 10^9$/L. Median duration until recovery was 28 days for the idarubicin group (IDR; red line) and 27 days for the daunorubicin group (DNR; blue line; $P = .0011$). (B) Day of recovery from thrombocytopenia after the first induction course. Thrombocytopenia was defined as platelet count $< 100 \times 10^9$/L. Median duration until recovery was 25 days for the idarubicin group (IDR; red line) and 24 days for the daunorubicin group (DNR; blue line; $P = .0034$).

who have been newly diagnosed with AML. Remission-induction therapy with 50 mg/m^2 of daunorubicin for 5 days resulted in almost the same CR rate and long-term outcome as seen with 12 mg/m^2 of idarubicin for 3 days in combination with 100 mg/m^2 of Ara-C for 7 days. Generally, daunorubicin is used at a dose of 45 to 50 mg/m^2 for 3 days in combination with 100 to 200 mg/m^2 of Ara-C for 7 days, and 50% to 70% of newly diagnosed adult patients with AML achieve CR. As stated in the "Introduction," JALSG used a response-oriented individualized induction therapy in the AML87, AML89, and AML92 studies for AML, which permitted the additional daunorubicin and other antileukemia drugs

Table 5. Adverse events (World Health Organization grades 3 to 5) after the start of induction therapy

	IDR group, no. of patients (%)	DNR group, no. of patients (%)	P
Sepsis	46 (8.7)	26 (4.9)	.021
Early death*	25 (4.7)	11 (2.1)	.026
Bleeding	19 (3.6)	23 (4.4)	.532
Febrile neutropenia	416 (78.2)	406 (77.4)	.761
Acute cardiac toxicity	10 (1.9)	4 (0.8)	.112
Late-onset cardiac failure	2 (0.38)	2 (0.38)	.998

IDR indicates idarubicin; and DNR, daunorubicin.
*Death within 60 days after the start of induction therapy.

Table 6. Effect of induction therapy on outcome by postremission therapies

	5-year OS		5-year RFS	
Consolidation arm	IDR group	DNR group	IDR group	DNR group
Conventional standard-dose, %	57	56	41	37
P	.759		.332	
High-dose Ara-C, %	58	58	42	44
P	.725		.658	
Allogeneic SCT in first CR, %	59	59	58	64
P	.469		.394	

Number of patients in the conventional standard-dose arm was 196 in the IDR group and 196 in the DNR group; in the high-dose Ara-C arm, the numbers were 196 and 193, respectively; and in the SCT group, the numbers were 67 and 69, respectively, as shown in Figure 1.

OS indicates overall survival; RFS, relapse-free survival; IDR, idarubicin; DNR, daunorubicin; Ara-C, cytarabine; and CR, complete remission.

to be administered according to bone marrow status on day 8 or later.[12-14] The CR rates in these 3 studies ranged from 77% to 80%, and the median total dose of daunorubicin was 240 mg/m^2.

On the basis of these experiences and also because of the regulation of our national medical insurance system, we used a dose and schedule of daunorubicin of 50 mg/m^2 for 5 days, that is, a total dose of 250 mg/m^2. In addition,we avoided higher daily doses, such as 80 mg/m^2 for 3 days, because higher plasma concentration might cause more cardiotoxicity in older patients.[23]

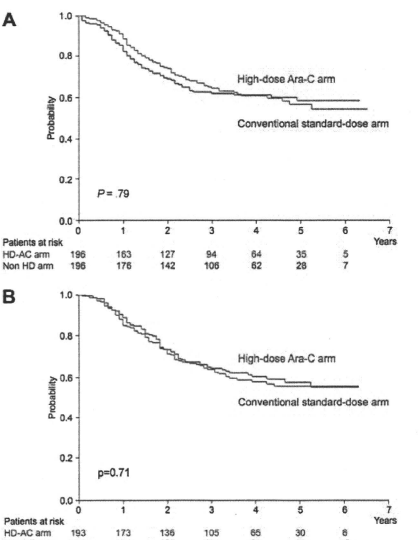

Figure 4. OS of CR patients randomized to receive consolidation therapy. (A) In the idarubicin group, predicted 5-year OS was 58% for the high-dose Ara-C arm (n = 196; red line) and 57% for the conventional standard-dose arm (n = 196; blue line; $P = .79$). (B) In the daunorubicin group, predicted 5-year OS was 58% for the high-dose Ara-C arm (n = 193; red line) and 56% for the conventional standard-dose arm (n = 196; blue line; $P = .71$). Ara-C indicates cytarabine; HD-AC arm, high-dose Ara-C arm; and Non HD arm, conventional standard-dose arm.

Three randomized studies in the early 1990s[4-6] and subsequent studies[24,25] and meta-analyses[7] reported a superior effect of idarubicin (12 to 13 mg/m^2 × 3 days) over that of daunorubicin (45 to 50 mg/m^2 × 3 days), in combination with Ara-C, and AML patients receiving idarubicin obtained 70% to 80% CR without a significant increase in toxic mortality, whereas those receiving daunorubicin achieved 58% to 65% CR.[4-6] However, because the duration of neutropenia and thrombocytopenia was longer in the idarubicin groups, it was questioned whether the doses used in these comparisons were equivalent in terms of levels of toxicity and whether any observed advantage represented an inherent biological advantage of idarubicin rather than biological dose equivalence.[1,2]

In these randomized studies, Wiernik et al reported that patients with initial WBC counts > 50 × 10^9 cells/L obtained only 32% CR by the daunorubicin regimen compared with 68% CR by the idarubicin regimen, whereas patients with WBC counts < 50 × 10^9/L obtained 65% and 69% CR, respectively.[5] Berman et al also reported that patients in the idarubicin group did well regardless of their initial WBC count, whereas patients in the daunorubicin group had a decreased response rate as the WBC count increased.[4] In the present study, however, a total of 250 mg/m^2 of daunorubicin resulted in almost the same CR rate as a total dosage of 36 mg/m^2 of idarubicin regardless of initial WBC counts and other prognostic factors such as cytogenetics, age, and FAB classification except M6. Although among patients with FAB M6, 16 patients in the daunorubicin group had a significantly lower CR rate than 17 patients in the idarubicin group, we have no clear explanation for this observation, because the small number of patients made further analysis difficult. Thus, the increased total dosage of daunorubicin administered in 5 days would be responsible for almost the same satisfactory CR rate and long-term outcome as idarubicin administered in 3 days in the present study. As for adverse events, the recovery from neutropenia and thrombocytopenia was slightly but significantly delayed in the idarubicin group, and sepsis and early mortality occurred more frequently in the idarubicin group, as shown in Figure 3 and Table 5.

Before we initiated the present AML201 study, there was no evidence that a higher dose of daunorubicin was more effective than its standard dose because of the lack of a prospective randomized study. In the sequential studies reported by Southwest Oncology Group, however, the CR rate with daunorubicin at a dose of 70 mg/m^2 was better than that with 45 mg/m^2.[26,27] Very recently, 2 groups reported that a higher dose of daunorubicin improved the CR rate and OS in prospective randomized studies.[28,29] A collaborative group composed of the Dutch-Belgian Cooperative Trial Group for Hemato-Oncology, the German AML Study Group, and the Swiss Group for Clinical Cancer Research compared 3-day daunorubicin at 90 mg/m^2 with 3-day daunorubicin at 45 mg/m^2, in combination with 7-day Ara-C, in elderly patients 60 to 83 years of age who had AML or high-risk refractory anemia and reported a higher CR rate for the escalated-treatment group (52% vs 35%, $P = .002$).[28] Although survival end points did not differ significantly overall, among patients 60 to 65 years of age, the CR rate (73% vs 51%) and OS rate (38% vs 23%) were significantly higher for the 90-mg/m^2 group. The Eastern Cooperative Oncology Group also compared 3-day daunorubicin at 90 mg/m^2 with 3-day daunorubicin at 45 mg/m^2, in combination with 7-day Ara-C, in patients 17 to 60 years of age with AML and reported a higher CR rate (70.6% vs 57.3%, $P < .001$) and longer OS (median 23.7 vs 15.7 months, $P = .003$) for the high-dose group.[29] Given these previous reports and the present report, the optimal total dose of daunorubicin is still to be explored but may rest somewhere between 250 and 270 mg/m^2. Because we used the FAB classification in the present study, we did not include either patients with 20% to 30% of blasts in the bone marrow or those with refractory anemia with excess blasts; therefore, it is unclear whether the present result is applicable to those patients.

Idarubicin is a derivative of daunorubicin and differs from its parent compound by the deletion of a methoxy group at position 4 of the chromophore ring. In vitro and preclinical data have shown that idarubicin is more lipophilic, is faster in cellular uptake, exhibits increased cellular retention, is lower in susceptibility to P-glycoprotein–dependent resistance, and is less cardiotoxic than daunorubicin. Both idarubicin and daunorubicin undergo conversion to their respective alcohol metabolites, idarubicinol and daunorubicinol. Unlike the latter, idarubicinol has a prolonged plasma half-life and is thought to have a pharmacologic advantage.[30-33]

The pediatric Berlin-Frankfurt-Münster group previously compared idarubicin 12 mg/m^2 for 3 days with daunorubicin 30 mg/m^2 twice daily for 3 days, in combination with Ara-C and etoposide, and reported almost the same CR rates (85% vs 86%, respectively) and predicted 5-year event-free survival (55% vs 49%, respectively, $P = .29$) in newly diagnosed childhood AML.[34] Furthermore, daunorubicin at a dose of 60 mg/m^2 for 3 days and idarubicin at a dose of 12 mg/m^2 for 3 days achieved similar CR rates in the studies by Eastern Cooperative Oncology Group that consisted of a large number of adult patients.[35,36]

Recently, the French Acute Leukemia Association reported a randomized study comparing standard doses of idarubicin (12 mg/m^2 for 3 days) with high doses of daunorubicin (80 mg/m^2 for 3 days) or idarubicin (12 mg/m^2 for 4 days) for remission induction in newly diagnosed elderly patients 50 to 70 years of age (median 60 years old) with AML.[37] CR rates were significantly higher for the standard-dose idarubicin group (83%) than for the high-dose daunorubicin group (70%, $P = .007$) but not for the high-dose idarubicin group (78%, $P = .12$). Although OS, relapse incidence, and event-free survival were not different among the 3 arms of the study, daunorubicin (80 mg/m^2 for 3 days) did not improve the CR rate of elderly AML patients to the level of the standard-dose idarubicin regimen.

With regard to adverse events, recovery from myelosuppression was faster and sepsis was less frequent in the daunorubicin group. Both acute and late-onset cardiotoxicity were reported only in a small number of patients in both groups. Given that there was no increase in severe cardiac toxicities in patients receiving high-dose daunorubicin (90 mg/m^2 for 3 days) compared with standard-dose daunorubicin (45 mg/m^2 for 3 days) in the Eastern Cooperative Oncology Group study (7.9% and 7.2%, respectively),[29] daunorubicin may not necessarily be administered for 5 days as in the present study (50 mg/m^2 for 5 days), although further follow-up observation is needed for late-onset cardiotoxicity.

Since the landmark study of the Cancer and Leukemia Group B,[38] it has been believed that high-dose Ara-C is superior to consolidation therapy with intermediate (400 mg/m^2 for 5 days) or conventional (100 mg/m^2 for 5 days) doses of Ara-C. In the present study, we prospectively compared high-dose Ara-C with consolidation therapy that included a conventional dose of Ara-C and non–cross-resistant agents. Our results clearly demonstrate that there is no difference in RFS and OS between the 2 consolidation arms, regardless of whether idarubicin or daunorubicin is used as induction chemotherapy.

In conclusion, the intensified dose of daunorubicin in the present setting, that is, 50 mg/m^2 for 5 days, proved to be biologically equivalent in terms of efficacy and no more toxic in terms of myelosuppression than the standard dose and schedule of idarubicin, that is, 12 mg/m^2 for 3 days, for remission-induction therapy in newly diagnosed younger patients (15 to 64 years old, median 47 years) with AML.

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Authorship

Contribution: S.O. designed and performed research, collected and interpreted data, and wrote the manuscript; S.M. designed and performed research, analyzed data, and participated in writing the manuscript; H.F., H.K., K.S., N.U., H.O., K.M., C.N., Y.M., A.F., T. Nagai, T.Y., M. Taniwaki, M. Takahashi, F.Y., Y.K., N.A., H.S., and H.H. performed research; S.H. analyzed data; K.O. and T. Naoe conducted and performed research; and R.O. conducted research, interpreted data, and participated in writing the manuscript.

For a complete list of the members of the JALSG, see the supplemental Appendix (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

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Correspondence: Shigeki Ohtake, MD, PhD, Department of Clinical Laboratory Science, Kanazawa University Graduate School of Medical Science, 5-11-80 Kodatsuno, Kanazawa, 920-0942, Japan; e-mail: sohtake@med3.m.kanazawa-u.ac.jp.

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