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Supporting Information

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

Table S1. Actual treatment after CR2 in the non-HSCT group.

Original Article

The Demarcation Between Younger and Older Acute Myeloid Leukemia Patients

A Pooled Analysis of 3 Prospective Studies

Masamitsu Yanada, MD[1]; Shigeki Ohtake, MD[2]; Shuichi Miyawaki, MD[3]; Hisashi Sakamaki, MD[4]; Toru Sakura, MD[5]; Tomoya Maeda, MD[6]; Koichi Miyamura, MD[7]; Norio Asou, MD[8]; Iekuni Oh, MD[9]; Junichi Miyatake, MD[10]; Hiroyuki Kanbayashi, MD[11]; Jin Takeuchi, MD[12]; Masatomo Takahashi, MD[13]; Nobuaki Dobashi, MD[14]; Hitoshi Kiyoi, MD[15]; Yasushi Miyazaki, MD[16]; Nobuhiko Emi, MD[1]; Yukio Kobayashi, MD[17]; Ryuzo Ohno, MD[18]; and Tomoki Naoe, MD[15]; for the Japan Adult Leukemia Study Group

BACKGROUND: Contemporary treatment protocols for adult acute myeloid leukemia (AML) are age-specific, and older patients are generally treated less intensively than younger patients. However, it remains uncertain whether older but fit patients with AML really need to have their treatment attenuated. **METHODS:** To evaluate the contribution of age to outcome for patients with AML receiving intensive chemotherapy, data were analyzed for 2276 patients aged less than 65 years who were treated uniformly, regardless of age, in 3 consecutive prospective studies conducted by the Japan Adult Leukemia Study Group. **RESULTS:** A substantial drop in overall survival (OS) between patients aged 40 to 49 years and 50 to 64 years led to a focus on 2 comparisons: 1) age <50 versus ≥ 50 years; and 2) age 50 to 54 versus 55 to 59 versus 60 to 64 years. OS was significantly better for patients aged <50 years than that for those aged ≥ 50 years (49.6% and 37.0% at 5 years; $P<.001$); older patients were more susceptible to relapse, but not to early death or nonrelapse mortality. The significant differences in OS between these 2 age groups were equally seen for patients with favorable, intermediate, and adverse cytogenetics ($P<.001$ each). Outcomes for those aged 50 to 54, 55 to 59, and 60 to 64 years were similar, with 5-year OS rates of 38.2%, 35.1%, and 38.0%, respectively ($P=.934$), and no differences in early death or nonrelapse mortality were observed among these age groups. **CONCLUSIONS:** These findings justify the use of intensive chemotherapy without dose attenuation toward older but fit patients with AML, at least up to the age of 64 years. ***Cancer* 2013;119:3326-33.** © *2013 American Cancer Society.*

KEYWORDS: acute myeloid leukemia; age; overall survival; early death; relapse; nonrelapse mortality.

INTRODUCTION

Age is among the most important prognostic factors in acute myeloid leukemia (AML).[1-5] Increasing age in AML is associated with a higher frequency of unfavorable biological characteristics such as adverse cytogenetics, preceding myelodysplastic syndrome (MDS), and expression of the multidrug resistance phenotype, all of which are involved in intrinsic resistance to chemotherapy.[6-9] In addition to the disease biology, patient-related factors such as poor general condition and significant comorbidities also contribute to inferior outcomes for older patients.[8,10,11] Because of such distinct biological and clinical features, contemporary treatment protocols for adult AML are age-specific and are typically divided into those for younger and older patients, with older patients treated less intensively than younger patients. For this purpose, age 55 or 60 years is generally used as the demarcation between these 2 groups[1,2]; however, this cutoff age is quite arbitrary, and it remains uncertain whether patients over such age limits really need to have their treatment attenuated.

For the recent prospective AML studies conducted by the Japan Adult Leukemia Study Group (JALSG), age less than 65 years was used as the eligibility criterion, with dose modifications not having been adopted according to age. This

Corresponding author: Masamitsu Yanada, MD, Department of Hematology, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, 470-1192, Japan; Fax: (011) +81-562-95-0016; myanada@fujita-hu.ac.jp

[1]Fujita Health University School of Medicine, Toyoake, Japan; [2]Kanazawa University Graduate School of Medical Science, Kanazawa, Japan; [3]Tokyo Metropolitan Ohtsuka Hospital, Tokyo, Japan; [4]Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; [5]Saiseikai Maebashi Hospital, Maebashi, Japan; [6]Saitama Medical University International Medical Center, Saitama, Japan; [7]Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [8]Kumamoto University School of Medicine, Kumamoto, Japan; [9]Jichi Medical University, Tochigi, Japan; [10]Kinki University Faculty of Medicine, Osaka, Japan; [11]Ohta Nishinouchi Hospital, Kohriyama, Japan; [12]Nihon University School of Medicine, Tokyo, Japan; [13]St. Marianna University School of Medicine, Kawasaki, Japan; [14]Jikei University School of Medicine, Tokyo, Japan; [15]Nagoya University Graduate School of Medicine, Nagoya, Japan; [16]Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [17]National Cancer Center Hospital, Tokyo, Japan; [18]Aichi Cancer Center, Nagoya, Japan

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situation provides a welcome opportunity to evaluate the contribution of age to outcome for patients with AML treated with uniform intensive chemotherapy. For the study reported here, we integrated data for 2276 patients entered into 3 consecutive prospective studies between 1995 and 2005 for a comparison of patient characteristics and treatment outcomes among different age groups.

MATERIALS AND METHODS

Patients

All patients were subjects of one of the three phase 3 studies conducted by the JALSG, that is, the AML95 (from 1995-1997),[12] AML97 (from 1997-2001),[13,14] and AML201 (from 2001-2005) studies.[15,16] All of these studies adopted the same eligibility criteria: newly diagnosed AML (acute promyelocytic leukemia excluded), age 15 to 64, an Eastern Cooperative Oncology Group performance status 0 to 3, adequate functioning of the liver (serum bilirubin level < 2.0 mg/L), kidneys (serum creatinine level < 2.0 mg/dL), lungs ($PaO_2 \geq 60$ Torr or $SpO_2 \geq 93\%$), and heart (no significant abnormalities on electrocardiograms and echocardiograms). Patients with AML secondary to MDS or cytotoxic treatment were not eligible for enrollment. Written informed consent was obtained from all patients prior to registration. Each protocol was reviewed and approved by the institutional review boards of the participating centers, and was conducted in accordance with the Declaration of Helsinki.

Treatments

The treatment schedule for each study is described in detail elsewhere.[12-16] The AML95 study compared a fixed schedule (ie, "3+7") and an individualized schedule (up to "4+10" depending on the bone marrow findings on day 8) for induction therapy with idarubicin and cytarabine.[12] Postremission therapy consisted of 3 courses of consolidation therapy including behenoyl cytarabine and 12 months of maintenance therapy. The AML97 study adopted the 3+7 induction therapy with idarubicin and cytarabine for all patients.[13,14] After achieving complete remission (CR), patients were randomized to receive 3 or 4 consolidation courses that included standard-dose cytarabine, followed by 12 months of maintenance therapy only for the 3 courses. Those with a human leukocyte antigen (HLA)-identical sibling donor were assigned to allogeneic hematopoietic cell transplantation (HCT) if they were younger than 50 years and at intermediate or poor risk, as determined with a scoring system which took into account cytogenetics, white blood cell count, and other factors. The AML201 study compared idarubicin (12 mg/m^2 for 3 days) and daunorubicin (50 mg/m^2 for 5 days) both combined with cytarabine for induction therapy.[14,15] Patients in CR were randomly assigned to either 4 consolidation courses with standard-dose cytarabine or 3 courses with high-dose cytarabine. Allogeneic HCT was offered to patients aged 50 or younger if they presented with intermediate or adverse cytogenetics and had an HLA-identical sibling donor. In principle, doses were not modified according to age for any protocol. The single exception was for high-dose cytarabine in the AML201 study, in which reduction of the cytarabine dose from 2 g/m^2 to 1.5 g/m^2 was allowed for patients aged 60 years or older.

Definitions

Karyotypes were classified as favorable, intermediate, or adverse, in line with the revised UK Medical Research Council (MRC) criteria.[17] Monosomal karyotype was defined according to the criteria developed by Breems et al.[18]

CR was defined as the presence of all of the following: $< 5\%$ of blasts in bone marrow, no leukemic blasts in peripheral blood or extramedullary sites, and recovery of peripheral blood counts. Early death was defined as death from any cause occurring within 30 days after the start of induction therapy.[8] Overall survival (OS) was defined as the time from the start of treatment to death or last visit, and relapse-free survival as the time from CR to relapse, death or last visit. Patients undergoing allogeneic HCT were not censored at the time of transplantation unless indicated.

Statistical Analysis

Distributions of patient characteristics between and among groups were compared by using the chi-square test for categorical variables. Differences in continuous variables were compared by means of the Wilcoxon rank-sum test for distribution between 2 groups, and the Kruskal-Wallis test for distribution among 3 groups. The probabilities of OS and relapse-free survival were estimated by using the Kaplan-Meier method, with differences between groups qualified with the log-rank test. First, we examined OS by dividing patients into 4 age groups: 15-29, 30-39, 40-49, and 50-64 years. This provisional analysis disclosed a substantial drop in OS between patients aged 40-49 and 50-64 years (Fig. 1A). This finding led us to focus on 2 comparisons for subsequent analyses: 1) age < 50 versus ≥ 50 years; and 2) age 50 to 54 versus 55 to 59 versus 60 to 64 years. Relapse and nonrelapse mortality were considered as competing risk events for each other, and the probabilities of relapse

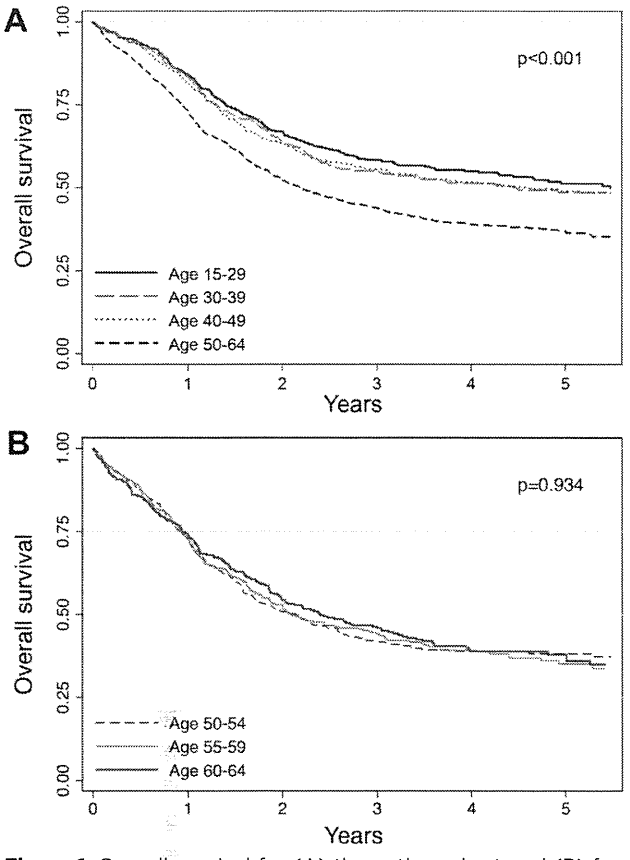

Figure 1. Overall survival for (A) the entire cohort and (B) for patients 50 to 64 years, is shown by age group. First, patients aged 15 to 29 (N = 438), 30 to 39 (N = 391), 40 to 49 (N = 510), and 50 to 64 (N = 937) are compared, and those aged 50 to 64 are then divided into 3 groups: 50 to 54 (N = 334), 55 to 59 (N = 322), and 60 to 64 (N = 281).

and nonrelapse mortality were estimated by using the cumulative incidence functions, with differences between groups qualified by the Gray test. The Cox proportional hazards regression model was used for multivariate analysis, and a hazard ratio (HR) was calculated in conjunction with a 95% confidence interval (CI). All statistical analyses were performed by using Stata version 12.0 software (StataCorp, College Station, Tex).

RESULTS

Patient Characteristics

A total of 2276 patients (430 from AML95, 789 from AML97, and 1057 from AML201) were analyzed for this study, with a median follow-up of surviving patients of 4.2 years (range, 0.0-8.0 years). Table 1 shows baseline characteristics of the patients according to age groups (age < 50, 50-54, 55-59, and 60-64 years). There was no significant relationship between performance status and age. The distribution of cytogenetic risk differed modestly but significantly for patients aged < 50 and ≥ 50 ($P < .001$), but the difference was not significant for those aged 50 to 54, 54 to 59, and 60 to 64 years ($P = .577$). Comparison of patients aged < 50 and ≥ 50 years showed that t(8;21) and inv(16)/t(16;16) occurred more frequently in younger patients ($P < .001$ and $P = .043$, respectively), whereas the frequencies of add(5q)/del(5q)/-5, add(7q)/del(7q)/-7, complex karyotype, and monosomal karyotype were higher for older patients ($P = .002$, $P = .021$, $P < .001$ and $P < .001$, respectively). None of these cytogenetic aberrations, however, showed significant differences in distribution among those aged 50 to 54, 54 to 59, and 60 to 64 years.

Complete Remission and Early Death

Rates of CR and early death are summarized in Table 2. Patients younger than 50 years tended to show higher CR rates than those aged 50 or older, but the difference failed to reach statistical significance ($P = .078$), whereas there was no difference in the CR rates among those aged 50 to 54, 54 to 59, and 60 to 64 years ($P = .829$). Early death within 30 days after the start of induction therapy occurred in 1.8%, 1.5%, 2.5%, and 3.2% of patients aged < 50, 50 to 54, 55 to 59, and 60 to 64 years, respectively, with no significant difference between those aged < 50 and ≥ 50 ($P = .367$), or among those aged 50 to 54, 54 to 59, and 60 to 64 ($P = .356$). The rates of death within 60 days were 2.7%, 5.1%, 5.0%, and 6.1% for the respective age groups ($P = .003$ for age < 50 and ≥ 50, and $P = .813$ for age 50 to 54, 54 to 59, and 60 to 64 years).

Relapse and Nonrelapse Mortality

Cumulative incidences of relapse and nonrelapse mortality for the 1788 patients who attained CR are shown in Table 2. Patients 50 years of age or older were more likely to experience relapse than were those younger than 50 ($P = .008$), whereas there was no difference in relapse rates among those aged 50-54, 55-59, and 60-64 years ($P = .196$). The nonrelapse mortality rates did not differ significantly between patients aged < 50 and ≥ 50 years ($P = .695$), or among those aged 50 to 54, 54 to 59, and 60 to 64 years ($P = .388$).

Overall Survival

Figure 1A compares OS for patients divided into 4 age groups: 15 to 29, 30 to 39, 40 to 49, and 50 to 64 years. As mentioned above, this result prompted us to first postulate a distinction between patients younger and older than 50 years. OS was significantly better for patients aged < 50 years than that for those aged ≥ 50 years

TABLE 1. Patient Characteristics

Characteristic	Age <50 y N = 1339	Age 50-54 y N = 334	Age 55-59 y N = 322	Age 60-64 y N = 281
Protocol				
AML95	276 (21%)	53 (16%)	52 (16%)	49 (17%)
AML97	477 (36%)	112 (34%)	110 (34%)	90 (32%)
AML201	586 (44%)	169 (51%)	160 (50%)	142 (51%)
Sex				
Male	774 (58%)	220 (66%)	193 (60%)	161 (57%)
Female	565 (42%)	114 (34%)	129 (40%)	120 (43%)
Performance status				
0	668 (50%)	145 (43%)	162 (50%)	142 (51%)
1	504 (38%)	149 (45%)	107 (33%)	105 (37%)
2	100 (7%)	25 (7%)	32 (10%)	20 (7%)
3	55 (4%)	13 (4%)	16 (5%)	12 (4%)
Unknown	12 (1%)	2 (1%)	5 (2%)	2 (1%)
White blood cell count, $\times 10^9$/L				
Median	15.4	13.6	15.3	9.1
Range	0.1-450	0.3-247	0.5-367	0.5-709
Hemoglobin level, g/dL				
Median	8.5	8.4	8.4	8.6
Range	1.9-17.0	2.2-17.2	3.4-16.7	3.6-15.2
Platelet count, x10^9/L				
Median	48	46	50	54
Range	0-1150	1-736	0-468	0-999
Bone marrow blasts, %				
Median	71	68	66	66
Range	0-100	0-100	2-100	0-98
Peripheral blood blasts, %				
Median	59	49	45	44
Range	0-100	0-100	0-100	0-100
Cytogenetic risk				
Favorable	347 (26%)	69 (21%)	56 (17%)	43 (15%)
Intermediate	834 (62%)	220 (66%)	214 (66%)	193 (69%)
Adverse	124 (9%)	34 (10%)	36 (11%)	35 (12%)
Unevaluable	34 (3%)	11 (3%)	16 (5%)	10 (4%)
Specific cytogenetic aberrations				
t(8;21)	274 (20%)	54 (16%)	41 (13%)	39 (14%)
inv(16)/t(16;16)	73 (5%)	15 (4%)	15 (5%)	4 (1%)
add(5q)/del(5q)/-5	24 (2%)	8 (2%)	13 (4%)	16 (6%)
add(7q)/del(7q)/-7	44 (3%)	18 (5%)	16 (5%)	15 (5%)
t(11q23)	58 (4%)	12 (4%)	8 (2%)	6 (2%)
Complex karyotype	41 (3%)	18 (5%)	23 (7%)	20 (7%)
Monosomal karyotype	46 (3%)	21 (6%)	23 (7%)	19 (7%)

(49.6% and 37.0% at 5 years, $P < .001$). Among those aged ≥ 50, however, increasing age did not seem to affect OS, because survival curves for patients aged 50 to 54, 55 to 59, and 60 to 64 years were superimposed, with 5-year OS rates of 38.2%, 35.1%, and 38.0%, respectively ($P = .934$; Fig. 1B).

To evaluate whether the difference in OS between those aged < 50 and ≥ 50 years depends on cytogenetic risk, comparisons between these 2 groups were made within each cytogenetic risk group. This analysis showed that the intergroup difference was significant for favorable ($P < .001$; Fig. 2A), intermediate ($P < .001$; Fig. 2B), and adverse cytogenetic risk ($P < .001$, Fig. 2C). The effect of age on OS remained significant in a multivariate analysis adjusting for other covariates (Table 3). Allogeneic HCT was performed for 687 (51%) of the patients aged < 50 years, 101 (30%) of those aged 50 to 54 years, 58 (18%) of those aged 55 to 59 years, and 19 (7%) of those aged 60 to 64 years. Censoring the findings for these patients at the time of allogeneic HCT did not alter the main results; Kaplan-Meier survival curves with censoring of patients undergoing allogeneic HCT are shown for those aged < 50 and ≥ 50 years in Fig. 3.

Finally, we examined whether lack of significant interaction between age and OS in patients aged 50 to 64 years remains after adjusting for other potentially confounding factors. When a multivariate analysis was undertaken for these older patients by including the covariates listed in Table 3, age group had no impact on OS (HR = 0.93; 95% CI = 0.76-1.13, for patients aged

TABLE 2. Remission Induction Results and Outcomes at 5 Years by Age Group

Outcome	Age <50 y N = 1339 (1069)[a]	Age 50-54 y N = 334 (260)[a]	Age 55-59 y N = 322 (246)[a]	Age 60-64 y N = 281 (213)[a]	<50 vs ≥50 y	50-54 vs 55-59 vs 60-64 y
Complete remission	79.8%	77.8%	76.4%	75.8%	$P = .078$	$P = .829$
95% CI	77.6%-82.0%	73.0%-82.2%	71.4%-80.9%	70.1%-80.9%		
Early death[b]	1.8%	1.5%	2.5%	3.2%	$P = .367$	$P = .356$
95% CI	1.2%-2.7%	0.5%-3.5%	1.1%-4.8%	1.5%-6.0%		
Overall survival	49.6%	38.2%	35.1%	38.0%	$P < .001$	$P = .934$
95% CI	46.7%-52.5%	32.7%-43.7%	29.2%-41.1%	31.9%-44.1%		
Relapse-free survival	40.6%	29.2%	30.8%	37.3%	$P = .002$	$P = .126$
95% CI	37.5%-43.6%	23.6%-35.1%	24.7%-37.1%	30.6%-43.9%		
Relapse	53.4%	62.6%	63.6%	57.1%	$P = .008$	$P = .196$
95% CI	50.3%-56.5%	56.2%-68.3%	56.8%-69.7%	50.0%-63.6%		
Nonrelapse mortality	6.0%	8.2%	5.6%	5.6%	$P = .695$	$P = .388$
95% CI	4.6%-7.5%	5.2%-12.0%	3.1%-9.2%	3.1%-9.3%		

Abbreviation: CI, confidence interval.
[a] Figures in parentheses represent numbers of patients who achieved complete remission.
[b] Death within 30 days after the start of induction therapy.

55-59 years; HR = 0.93; 95% CI = 0.76-1.14, for patients aged 60-64 years; both with reference to those aged 50-54 years).

DISCUSSION

To investigate how increasing age affects outcomes for patients with newly diagnosed AML, we analyzed data for 2276 patients 15 to 64 years of age who were treated uniformly, regardless of age, in 3 consecutive prospective AML studies by JALSG. This large-scale retrospective analysis yielded several relevant findings: 1) age 50 was a significant dividing point for outcomes; 2) patients aged 50 to 64 years were more susceptible to relapse, but not to early death or nonrelapse mortality than those younger than 50 years; and 3) outcomes did not differ among patients aged 50 to 54, 55 to 59, and 60 to 64 years.

Why were the survival rates for patients 50 years of age or older in our study significantly inferior to those of patients younger than 50? Our data indicate that worse outcomes for older patients resulted from higher relapse rates. In AML, it has been well established that cytogenetic findings at diagnosis are associated with the risk of relapse.[17-20] Comparison of the frequencies of distinct cytogenetic aberrations showed that younger patients were more likely to exhibit favorable cytogenetics such as t(8;21) and inv(16)/t(16;16), whereas older patients were more likely to show adverse cytogenetics such as abnormalities of chromosome 5 or 7, complex karyotype, and monosomal karyotype. However, such a difference in the distribution of cytogenetics alone could not have accounted for the difference in outcomes between patients aged < 50 and ≥ 50 years observed in this study, because the significant differences in OS between these 2 age groups were seen for all cytogenetic risk groups.

Moreover, it could be expected that allogeneic HCT would result in more favorable outcomes for younger patients. However, although the proportion of patients who had undergone allogeneic HCT was indeed higher among younger than older patients, censoring the findings obtained at the time of allogeneic HCT produced no major changes in the study results. Therefore, it seems that neither cytogenetics nor allogeneic HCT can explain why older patients suffered relapse more frequently than younger patients. Secondary AML could not have been the reason, either, because our study cohort consisted of only patients with de novo AML. Other mechanisms that had not been studied here, such as molecular profiles, may play a significant role in differences in outcomes for younger and older patients.[21-24]

The analytic results for data of patients 50 years of age or older in our study also provide insights into the treatment of older patients with AML. Patients aged 50 to 54, 55 to 59, and 60 to 64 years had similar long-term survival, and no differences in early death or nonrelapse mortality were observed among them. This finding calls into question whether older patients really need to be treated differently. Recently, Lowenberg et al compared the effect of a doubled dose of daunorubicin of 90 mg/m^2 with that of a conventional dose of 45 mg/m^2 in the context of the 3+7 regimen for patients aged 60 years or older.[25] Although no difference in outcome was observed overall, patients between 60 and 65 years of age significantly benefited from the doubled dose of daunorubicin. Taking these results into account, older and fit patients, especially those under the age of 65 years, may still benefit from intensified chemotherapy.

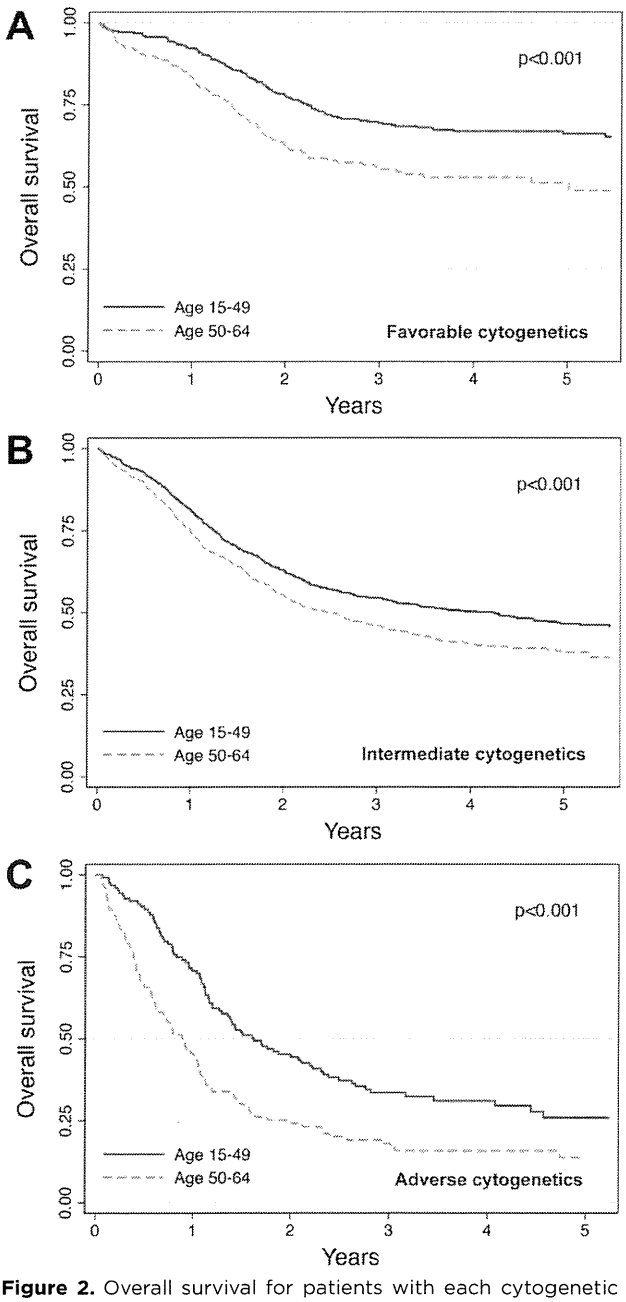

Figure 2. Overall survival for patients with each cytogenetic risk is shown by age group. Patients with (A) favorable (N = 515), (B) intermediate (N = 1461), and (C) adverse (N = 229) cytogenetics are shown separately.

TABLE 3. Multivariate Analysis of Risk Factors for Overall Survival

	HR	(95% CI)	*P*
Age			
<50	1.00		-
≥50	1.48	(1.32-1.66)	<.001
Sex			
Male	1.20	(1.07-1.36)	.002
Female	1.00		-
Performance status			
0-1	1.00		-
2-3	1.32	(1.12-1.56)	.001
White blood cell count			
Per 10 $\times 10^9$/L increase	1.02	(1.01-1.03)	<.001
Protocol			
AML95	1.25	(1.07-1.45)	.004
AML97	1.07	(0.94-1.22)	.318
AML201	1.00		-
Cytogenetics			
Favorable	0.65	(0.55-0.77)	<.001
Intermediate	1.00		-
Adverse	2.07	(1.75-2.45)	<.001
Unevaluable	1.41	(1.05-1.90)	.024

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

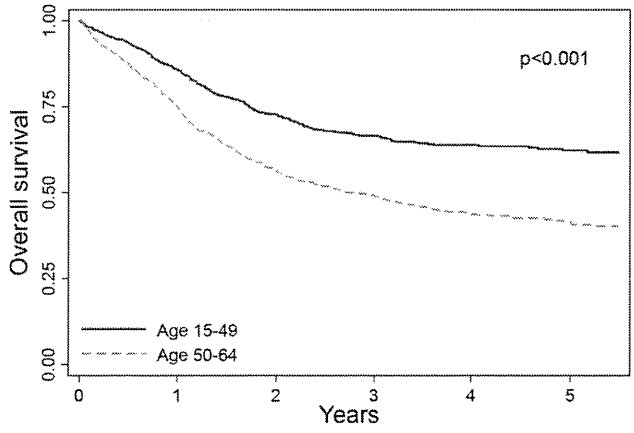

Figure 3. Overall survival is shown by age group with censoring of patients undergoing allogeneic hematopoietic cell transplantation. Patients aged 15 to 49 (N = 1339) and 50 to 64 years (N = 937) are compared, with those undergoing allogeneic hematopoietic cell transplantation censored at the time of transplantation.

When interpreting our data, we should bear in mind that our study cohort consisted exclusively of newly diagnosed AML patients under the age of 65 years who were entered into phase 3 studies. In addition, our cohort did not include patients with AML secondary to MDS or cytotoxic treatment. Secondary AML accounts for approximately 35% of the whole AML population,[26,27] and the frequency is even higher among older patients.[3,5] Our results therefore might not be applicable to the general AML population. This limitation may well be partly complemented by the findings of several previous studies. Buchner et al analyzed data of 2776 patients with de novo AML with no upper age enrolled onto 2 prospective studies by the German AML Cooperative Group.[9] In that study, OS for patients older than 60 years was only half that of younger patients, and this difference was attributable to less frequent CR and more frequent relapse in older patients. It seems likely that inclusion of elderly

patients might have contributed to a larger prognostic difference between younger and older patients. By using the combined data of 5 AML studies conducted by the Southwest Oncology Group, Appelbaum et al evaluated effect of age on outcomes.[8] Their study included not only patients with de novo AML but also those with secondary AML, with no upper age limit employed in trials for older patients. CR rates and OS were shown to worsen with advanced age, and this held true even if patients aged 56 to 65, 66 to 75, and older than 75 years were compared. It is conceivable that discrepant results among these studies, including ours, could be a reflection of differences in analyzed patient population. Through an entirely different approach, Juliusson et al evaluated the effect of age on outcomes for AML by using data for 2767 unselected patients with AML who were consecutively enrolled in the Swedish Acute Leukemia Registry.[11] They showed that intensive chemotherapy was associated with improved survival even for elderly patients, although it should be remembered that patients in that study were treated heterogeneously, and the choice of treatment must have been dependent on known and unknown confounding factors. Our study, in contrast, is advantageous in that the study population consisted of patients who were treated homogeneously regardless of age.

To summarize, we analyzed data for a large number of patients with AML aged 15 to 64 years who were treated uniformly in the context of clinical studies, and could not determine a specific age limit over which attenuation of treatment intensity is advisable. Our results justify the use of intensive chemotherapy without dose attenuation toward older but fit AML patients at least up to the age of 64.

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CONFLICT OF INTEREST DISCLOSURE

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Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Phase 2 study of arsenic trioxide followed by autologous hematopoietic cell transplantation for relapsed acute promyelocytic leukemia

Masamitsu Yanada,[1] Motohiro Tsuzuki,[1] Hiroyuki Fujita,[2] Katsumichi Fujimaki,[3] Shin Fujisawa,[4] Kazutaka Sunami,[5] Masafumi Taniwaki,[6] Akira Ohwada,[7] Kosuke Tsuboi,[8] Akio Maeda,[9] Akihiro Takeshita,[10] Shigeki Ohtake,[11] Yasushi Miyazaki,[12] Yoshiko Atsuta,[13] Yukio Kobayashi,[14] Tomoki Naoe,[13] and Nobuhiko Emi,[1] on behalf of the Japan Adult Leukemia Study Group

[1]Fujita Health University School of Medicine, Toyoake, Japan; [2]Yokohama City University Hospital, Yokohama, Japan; [3]Fujisawa City Hospital, Fujisawa, Japan; [4]Yokohama City University Medical Center, Yokohama, Japan; [5]National Hospital Organization Okayama Medical Center, Okayama, Japan; [6]Kyoto Prefectural University of Medicine, Kyoto, Japan; [7]Tokyo Metropolitan Bokutoh Hospital, Tokyo, Japan; [8]Tokai University School of Medicine, Isehara, Japan; [9]Hyogo Cancer Center, Akashi, Japan; [10]Hamamatsu University School of Medicine, Hamamatsu, Japan; [11]Kanazawa University Graduate School of Medical Science, Kanazawa, Japan; [12]Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [13]Nagoya University Graduate School of Medicine, Nagoya, Japan; and [14]National Cancer Center Hospital, Tokyo, Japan

Key Points

- We conducted a phase 2 study of ATO followed by autologous HCT for relapsed APL.
- This sequential treatment is effective and feasible.

The optimal treatments for relapsed acute promyelocytic leukemia (APL) remain equivocal. We conducted a phase 2 study to evaluate the efficacy and feasibility of a sequential treatment consisting of induction and consolidation with arsenic trioxide (ATO), peripheral blood stem cell (PBSC) harvest after high-dose cytarabine chemotherapy, and autologous hematopoietic cell transplantation (HCT). Between 2005 and 2009, 35 patients (26 with hematologic and 9 with molecular relapse) were enrolled. Induction therapy resulted in complete remission in 81% of those with hematologic relapse, and most patients became negative for *PML-RARα* after the first ATO consolidation course, but 4 remained positive. Administration of the second ATO consolidation course further decreased the transcript levels in 3 patients. In total, 25 patients proceeded to PBSC harvest, all of whom successfully achieved the target CD34+ cell doses, and 23 underwent autologous HCT with *PML-RARα*–negative PBSC graft. Posttransplant relapse occurred in 3 patients, and there was no transplant-related mortality. With a median follow-up of 4.9 years, the 5-year event-free and overall survival rates were 65% and 77%, respectively. These findings demonstrate the outstanding efficacy and feasibility of the sequential treatment featuring ATO and autologous HCT for relapsed APL. This study was registered at http://www.umin.ac.jp/ctr/ as #C000000302. (*Blood.* 2013;121(16):3095-3102)

Introduction

Outcomes for acute promyelocytic leukemia (APL) have improved significantly since the advent of all-*trans* retinoic acid (ATRA), and the recently introduced frontline therapy that combines ATRA and chemotherapy can provide long-term complete remission (CR) for a majority of patients with newly diagnosed APL.[1-6] Nevertheless, relapse still occurs in ~20% of cases, for which arsenic trioxide (ATO) has been shown to provide high CR rates exceeding 80%,[7-9] thus making it a current recommendation for reinduction therapy.[10,11] After returning to CR, autologous or allogeneic hematopoietic cell transplantation (HCT) for consolidating the CR status is generally considered if the patient is eligible for the procedure.[10-12] However, because there have been few prospective studies for this very small patient population, the therapeutic approach after achievement of second or subsequent CR is mostly based on findings from retrospective studies.

In 2005, the Japan Adult Leukemia Study Group (JALSG) initiated a phase 2 study entitled APL205R for patients with relapsed APL. The main purpose of this study was to evaluate the efficacy and feasibility of a sequential treatment consisting of induction and consolidation with ATO, peripheral blood stem cell (PBSC) harvest after chemotherapy using high-dose cytarabine (AraC), and autologous HCT. This report presents and discusses the results of this study.

Methods

Patients

This study enrolled patients with relapsed APL between December 2005 and June 2009. At least a single documentation of cytogenetic and/or molecular evidence of t(15;17)/*PML-RARα* was required at the time of entry. Eligibility criteria consisted of age between 18 and 65 years; an Eastern Cooperative Oncology Group performance status between 0 and 3; and adequate functioning of the liver (serum bilirubin level <2.0 mg/L), kidneys (serum creatinine level <2.0 mg/dL), lungs ($Pao_2 \geq 60$ mm Hg or $SpO_2 \geq 93\%$), and heart (no severe abnormalities detected on electrocardiograms). Patients who

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Table 1. Treatment schedule

Drug	Dose	Route	Days
Induction			
ATO	0.15 mg/kg	IV (2 h)	1-*
IDA	12 mg/m^2	IV (30 min)	†
MTX, AraC, PSL	15 mg, 40 mg, 10 mg	IT	‡
Consolidation #1			
ATO	0.15 mg/kg	IV (2 h)	1-25
MTX, AraC, PSL	15 mg, 40 mg, 10 mg	IT	‡
Consolidation #2			
ATO	0.15 mg/kg	IV (2 h)	1-25
MTX, AraC, PSL	15 mg, 40 mg, 10 mg	IT	‡
Consolidation #3			
AraC	2 g/m^2, every 12 h	IV (3 h)	1-4
PBSCH			§
Autologous HCT			
Busulfan	1 mg/kg, every 6 h	po	−6, −5, −4
Melphalan	70 mg/m^2	IV (bolus)	−3, −2
PBSCT			0

IT, intrathecally; IV, intravenously; MTX, methotrexate; PBSCH, peripheral blood stem cell harvest; PBSCT, peripheral blood stem cell transplantation; po, by mouth; PSL, prednisolone.

*For induction, ATO was administered until complete remission or for 60 d, whichever was shorter.

†IDA was added for 2 d if the WBC count exceeded 20.0 × 10^9/L before or during the induction therapy, if the combined total count of myeloblasts and promyelocytes in the peripheral blood exceeded 5.0 × 10^9/L before or during the induction therapy, or if an extramedullary myeloid tumor was detected before the induction therapy.

‡Intrathecal injection was given when the platelet count recovered after the end of the courses. PSL could be replaced with 4 mg of dexamethasone.

§PBSCH was performed when the WBC count had recovered.

had previously undergone autologous or allogeneic HCT were not eligible for inclusion. Written informed consent was obtained from all patients prior to registration. The protocol was reviewed and approved by the institutional review board of each of the participating centers and was conducted in accordance with the Declaration of Helsinki. This study is registered at http://www.umin.ac.jp/ctr/ as #C000000302.

Treatments

The treatments used during the study are summarized in Table 1. For remission induction, ATO was administered by a 2-hour infusion at a daily dose of 0.15 mg/kg until CR or a maximum of 60 days. In addition, patients received 12 mg/m^2 of idarubicin (IDA) on days 1 and 2 if 1 or more of the following criteria were met when the treatment was started: (1) the white blood cell (WBC) count exceeded 20.0 × 10^9/L; (2) the combined total count of myeloblasts and promyelocytes in the peripheral blood exceeded 5.0 × 10^9/L; and (3) there was the presence of an extramedullary myeloid tumor. Patients who showed evidence of criteria 1 and/or 2 after the start of induction therapy were given 2 extra doses of 12 mg/m^2 of IDA at that point. Those who achieved CR were scheduled to receive an additional 2 courses of ATO (0.15 mg/kg for 25 days) for consolidation. During ATO administration, a 12-lead electrocardiogram, complete blood cell counts, and chemistry parameters including the electrolytes were monitored at least twice a week, and the serum potassium and magnesium levels were maintained above the lower limits of normal. After the end of each ATO course, central nervous system (CNS) prophylaxis was attained by means of intrathecal injection of methotrexate, AraC, and corticosteroids (3 times in total). Patients with cytological evidence of CNS leukemia received intrathecal injections twice a week simultaneously with ATO, until complete clearance of leukemic cells in the cerebrospinal fluid (CSF) had been achieved. Following the third course of ATO, patients proceeded to PBSC harvest. For this purpose, high-dose AraC was administered at 2 g/m^2 for 3 hours twice daily for 4 days, and granulocyte–colony-stimulating factor was initiated from day 6. Upon recovery, autologous PBSCs were harvested by means of apheresis. Patients who attained a target CD34+ cell dose of 2.0 × 10^6/kg or higher were allocated to undergo autologous HCT unless *PML-RARα* transcripts were detected in PBSCs. The conditioning regimen consisted of busulfan (1 mg/kg orally every 6 hours on days −6 to −4) and melphalan (70 mg/m^2 intravenously on days −3 to −2),[13] whereas unpurged autologous PBSCs were infused on day 0. The study flow is shown in Figure 1.

Assessments and definitions

Hematologic CR was defined as the presence of all of the following: <5% of blasts in the bone marrow, no leukemic blasts in the peripheral blood or extramedullary sites, and recovery of peripheral blood counts. Hematologic relapse was defined as the presence of at least 1 of the following: recurrence of >10% leukemic cells in the bone marrow, recurrence of any leukemic cells in the peripheral blood, or development of extramedullary disease.[3] Molecular relapse was defined as the reappearance of polymerase chain reaction (PCR) positivity for *PML-RARα* in a single bone marrow or peripheral blood sample for this study. Prospective molecular monitoring was performed with the real-time quantitative reverse-transcription PCR (qRT-PCR) assay in a single independent laboratory. The *PML-RARα* levels in bone marrow samples were assessed at enrollment and after each course of therapy. Harvested PBSCs were also subjected to the qRT-PCR assay. The number of transcript copies was normalized by means of glyceraldehyde-3-phosphate dehydrogenase, and then converted into molecules per μg RNA. The threshold for quantification was 50 copies per μg RNA, which corresponds to a sensitivity of 10^{-4}, whereas levels below the threshold were differentiated into "not detected" and "detected but not quantifiable," and PCR negativity was categorized as "not detected."

For posttransplant engraftment, neutrophil engraftment was defined as achievement of a neutrophil count of at least 0.5 × 10^9/L for 2 consecutive days, and platelet engraftment as achievement of a platelet count of at least 30 × 10^9/L independent of transfusions for 2 consecutive days.

Statistical analysis

The primary end point was event-free survival (EFS) at 1 year after registration, which was defined as the time from registration to failure to achieve CR, relapse, death, or last visit, whichever came first. The expected and threshold EFS rates at 1 year were estimated to be 50% and 20%, respectively. The threshold EFS rate of 20% was determined based on historical control data of Japanese patients with relapsed APL who were

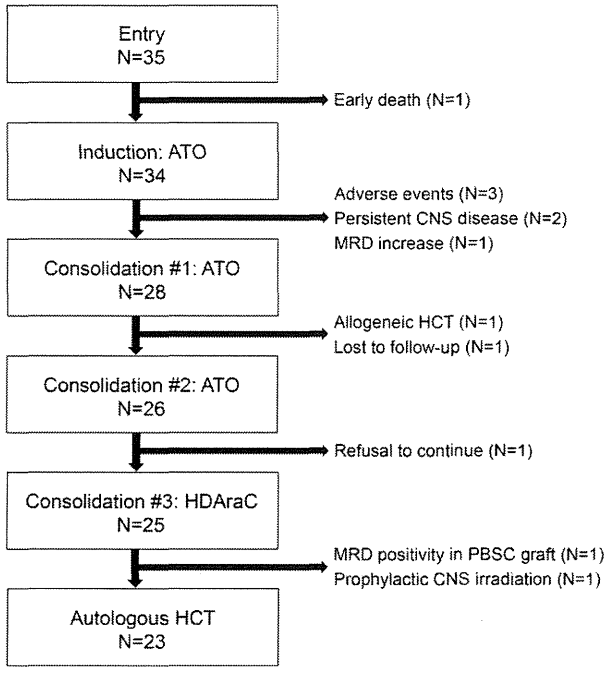

Figure 1. Patient flow diagram. HDAraC, high-dose cytarabine; MRD, minimal residual.

Table 2. Patient characteristics at enrollment

Characteristics	Values
Age in years, median (range)	46 (20-64)
Gender, male/female	23/12
WBC count, x 10^9/L	
Median (range)	2.6 (0.5-18.1)
≤10/>10	34/1
Platelet count, x 10^9/L	
Median (range)	79 (8-260)
≤40/>40	9/26
Performance status, 0/1/2/3	27/6/0/2
Number of prior relapses, 1/2	32/3
Type of relapse, hematologic/molecular	26/9
Interval between primary diagnosis and enrollment in years, median (range)	2.5 (0.8-11.0)

treated with ATRA-based therapy.[14] With a statistical power of 80% and a 1-sided, type I error of 5%, the minimum number of 17 eligible patients required for this study was calculated by means of binomial analysis. Allowing for a premature dropout rate of 15%, we aimed for inclusion of at least 20 patients. Primary end point analysis was performed with the Kaplan-Meier method for the calculation of probability of EFS. The treatment was considered to be effective if the lower limit of the 90% confidence interval (CI) exceeded the threshold EFS (ie, 20%). Overall survival (OS) was defined as the time from registration to death or last visit, and failure-free survival as the time from registration to failure to achieve CR, withdrawal from study, relapse, death, or last visit. Survival estimates and CIs were calculated with the Kaplan-Meier method and Greenwood's formula. The log-rank test was used for group comparison.

Results

Patient characteristics

A total of 35 patients with relapsed APL were enrolled in this study. Patient enrollment was allowed to exceed the originally planned minimum requirement after having ensured it ethical to expand the number of patients. Table 2 summarizes baseline characteristics of the patients. There were 23 males and 12 females, with a median age at enrollment of 46 years (range, 20-64 years). The median interval between primary diagnosis and enrollment was 2.5 years (range, 0.8-11.0 years).

All of the patients had been initially treated with ATRA-based therapy, and most of them in accordance with the protocols of JALSG or modifications thereof.[3,15] Thirty-two patients were in first relapse, and 3 in second relapse, with hematologic relapse accounting for 26, and molecular for 9. None of the patients had received ATO before.

Induction with ATO

ATO was administered to all patients except for 1 who developed intracranial hemorrhage immediately after enrollment and succumbed to early death (unique patient number [UPN] 26). Of the remaining 34 patients who underwent induction therapy, IDA was added for 2 patients on days 1 and 2, and during the induction course for 8 patients as per protocol. None of the patients developed differentiation syndrome. Three patients discontinued the study due to adverse events (grade 3 skin rash [UPN 10], grade 3 QT prolongation [UPN 19], and grade 4 QT prolongation accompanied by frequent ventricular premature contraction [UPN 23]). CSF examination performed at the end of the induction therapy revealed cytological evidence of CNS involvement in 4 patients, 2 of whom discontinued due to persistent CNS disease despite repeated intrathecal injections (UPN 13 and UPN 33). Of the 26 patients with hematologic relapse, 5 were taken off the study as mentioned previously, whereas the other 21 (81%) achieved CR. Of the 9 patients presenting with molecular relapse, 7 proceeded to consolidation therapy, and 2 were withdrawn from the study because of persistent CNS disease (UPN 13) or at the physician's discretion because the *PML-RARα* levels increased significantly after induction therapy (UPN 29).

Consolidation with ATO

During the 2 consolidation courses with ATO, 3 patients were taken off the study: 1 discontinued the protocol after the first consolidation course to receive umbilical cord blood transplantation (UPN 1), 1 was lost to follow-up after completing the first consolidation course (UPN 14), and the other refused to continue for unknown reasons after the second consolidation course (UPN 30). None of the patients discontinued the study because of relapse or adverse events during this phase of the treatment.

High-dose AraC and PBSC harvest

For PBSC harvest, 25 patients were given high-dose AraC as the third consolidation therapy, and all of them attained the target CD34+ cell doses of 2.0×10^6/kg. The median value of the CD34+ cell doses was 6.5×10^6/kg (range, 2.0-42.2 $\times$ 10^6/kg). One patient (UPN 18) whose PBSC sample was positive for *PML-RARα* was taken off the study because of ineligibility for autologous HCT as per protocol. One other patient (UPN 3), who had documented CNS leukemia at the end of induction therapy, but whose leukemic cells in the CSF were completely cleared with intrathecal injections, was withdrawn from the protocol at the physician's discretion to undergo prophylactic CNS irradiation. This patient received autologous HCT, but not as part of this study, and subsequently suffered posttransplant relapse in the CNS with fatal outcome. All of the other patients proceeded to autologous HCT. No dropouts due to relapse or adverse events were reported during this phase of the treatment.

Autologous HCT

The remaining 23 patients underwent autologous HCT as per protocol. The median time until engraftment was 12 days (range, 11-39 days) for neutrophils and 15 days (range, 12-136 days) for platelets. Posttransplant relapse occurred in 3 patients after a median duration of 5 months (range, 3-6 months). There was no transplant-related mortality.

Kinetics of the *PML-RARα* transcript levels

The results of the serial qRT-PCR tests during the treatment are summarized in Table 3. Most patients achieved PCR negativity after the first consolidation, but 4 were still positive for *PML-RARα* at this time. The PCR results turned negative after the second and third consolidation in 1 patient each (UPN 25 and 17, respectively). Of the 2 patients who remained positive for *PML-RARα* after the third consolidation, 1 (UPN 18) showed positive and the other (UPN 5) negative PCR test results for PBSCs. The latter underwent autologous HCT with a *PML-RARα*–negative graft but relapsed 5 months after transplantation.

Overall outcome

The probability of EFS was 77% at 1 year, with the 90% CIs ranging from 63% to 86%, thus demonstrating that this study has met its

Table 3. Kinetics of *PML-RARα* transcript levels

UPN	At entry	After induction	After consolidation #1	After consolidation #2	After consolidation #3
1	3000	N	N	Off study	Off study
2	460	N	N	N	N
3	60 000	<50	N	N	N
4	4200	<50	N	N	NA
5	69 000	28 000	760	140	<50
6	32 000	6000	N	N	N
7	15 000	290	N	N	N
8	360 000	<50	N	N	N
9	NA	1000	N	NA	N
10	NA	Off study	Off study	Off study	Off study
11	950	N	NA	N	N
12	64 000	50	N	NA	N
13	10 000	7100	Off study	Off study	Off study
14	120 000	400	NA	Off study	Off study
15	510 000	150	N	N	NA
16	190 000	<50	N	N	NA
17	95 000	1800	110	110	N
18	67 000	1500	480	390	280
19	130 000	Off study	Off study	Off study	Off study
20	450 000	280 000	N	N	N
21	140 000	170	N	N	N
22	26 000	61	N	N	N
23	24 000	Off study	Off study	Off study	Off study
24	730 000	<50	N	N	N
25	1900	2500	<50	N	N
26	440 000	Off study	Off study	Off study	Off study
27	NA	7800	N	N	N
28	NA	2600	N	N	N
29	510	6300	Off study	Off study	Off study
30	45 000	65	N	N	Off study
31	NA	300 000	NA	N	N
32	NA	50	N	N	N
33	180 000	NA	Off study	Off study	Off study
34	20 000	N	N	N	N
35	150 000	10 000	N	N	N

"Off study" indicates that the patient discontinued the study for reasons detailed in the text.

The threshold for quantification was 50 copies per μg RNA, which corresponds to a sensitivity of 10^{-4}. The levels below the threshold were differentiated into "not detected (N)" and "detected but not quantifiable (<50)."

N, not detected; NA, not assessed.

primary end point. Figure 2 shows Kaplan-Meier estimates for EFS and OS. With a median follow-up for surviving patients of 4.9 years (range, 0.3-6.3 years), the 5-year EFS and OS rates were 65% and 77%, respectively. The probability of failure-free survival was estimated to be 59% at 5 years.

Discussion

Current comprehensive practice guidelines have provided recommendations on the management of APL,[10,11] but what the optimal treatments for relapsed APL are remains equivocal. This is primarily because of the lack of prospective studies due to the rarity of relapses in APL, so that we initiated a phase 2 study for patients with relapsed APL in 2005 to evaluate the efficacy and feasibility of a sequential treatment featuring ATO and autologous HCT and enrolled 35 patients from 25 institutions nationwide. The treatment immediately induced molecular remission in a majority of patients, and only 3 patients were taken off the protocol because of adverse events throughout the entire study period, so that 23 patients could receive autologous HCT with a *PML-RARα*–negative PBSC graft. The 5-year probabilities of EFS and OS for the entire cohort were 65% and 75%, respectively. Of note, the EFS curve reached a stable plateau after 2 years from registration. These results have led us to conclude that this sequential treatment is effective and feasible.

ATO is currently the most active agent available for APL. Accumulated evidence has shown that >80% of patients with relapsed APL can achieve CR with ATO monotherapy.[7-9] In addition to high CR rates, the capability of this agent to induce molecular remission is another significant advantage because molecular remission is a prerequisite for long-term disease control in APL and is thus considered an important therapeutic milestone.[10,16] By contrast, ATRA alone is less likely to induce molecular remission, which results in this agent being used generally in combination with intensive chemotherapy rather than as monotherapy.[17] Although high CR rates can be expected for such combined use, this approach is limited by unsustained CR, especially for patients with hematologic relapse, and, more importantly, by quite high toxicity.[18,19] A retrospective study by Thomas et al[18] reported better survival for relapsed APL patients treated with ATO-based therapy than for historical control patients treated with ATRA-based therapy. The favorable safety profile of ATO is also an important advantage, as was seen in our study, where only 3 patients (8%) had to discontinue the protocol because of adverse events during induction therapy. This ratio seems to be only slightly higher than that observed in a US Intergroup study (5%).[8] It is further worth noting that none of our patients developed differentiation syndrome. This contrasts with a high incidence of this complication (25%) in the American study.[8] It can be assumed that the additional use of IDA for cases with high WBC counts may have contributed to reducing the risk of differentiation syndrome in our cohort.

Although the beneficial effect of ATO for induction has been well documented in relapsed APL, it is far less clear what the best consolidation strategy is after achieving CR. Previous studies showed that patients who achieved second or subsequent CR with ATO but did not receive transplantation thereafter had poor outcome; the proportion of those remaining alive and relapse-free ranged from 22% to 37%.[7,17,20] Although some patients may remain in CR without transplantation, overall prognosis is far from satisfactory, and the outcome seems much better for those who receive autologous or allogeneic HCT.[17,20] Owing to its posttransplant graft-versus-leukemia effect, allogeneic HCT is generally considered the most effective treatment of preventing relapse in acute myeloid leukemia.[21] In APL, however, the relapse rate after autologous HCT may be quite low provided the patient is in molecular remission at the time of transplantation.[22-25] Given the lower risk of transplant-related mortality with autologous HCT, the balance of benefits and risks may well favor autologous HCT over allogeneic HCT. For autologous HCT to be successful, it is imperative to reduce the tumor burden substantially at the molecular level before transplantation. For this reason, what constitutes an adequate number of cycles of ATO therapy is a subject of clinical interest. Similar to the observation by the US Intergroup,[8] our study found that 2 courses of ATO therapy induced most patients into molecular remission, although 4 patients remained positive for *PML-RARα* after the second course (ie, consolidation #1). Administration of the third ATO course reduced the transcript levels in 3 of the patients, whereas the level stayed unchanged in the remaining patient. It was possible to administer the third course of ATO because none of the 26 patients who had received this course had to withdraw from the study due to relapse or adverse events. These findings lead us to consider that

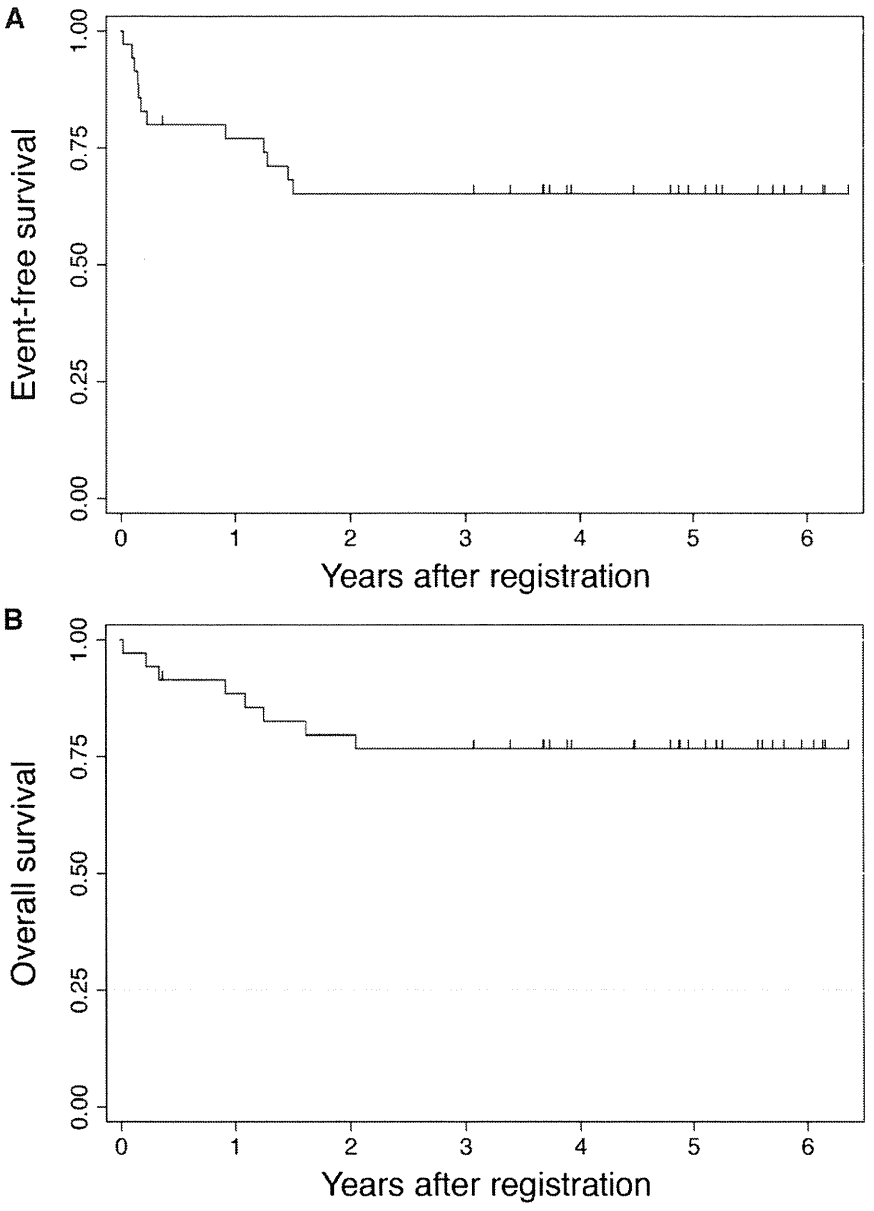

Figure 2. Kaplan-Meier curves for EFS (A) and OS (B). The probabilities of EFS and OS for the entire cohort (N = 35) were 65% and 77% at 5 years, respectively.

administration of a total of 3 courses of ATO before PBSC collection is feasible.

For the PBSC-mobilizing regimen, we chose high-dose AraC, hoping it would produce highly efficient mobilization as well as exert a systemic antileukemic effect. The fact that all the 25 patients undergoing this procedure successfully achieved the target CD34+ cell doses has convinced us of the usefulness of this regimen. In addition, high-dose AraC is known to provide good coverage of the CNS, the most common site of extramedullary involvement in APL.[26,27] Above and beyond our expectations, routine CSF examination at the end of the induction therapy identified 4 patients with cytological evidence of CNS involvement, although they did not show any CNS-related symptoms. This suggests that high-dose AraC may also play a part in protecting against the potential risk of subsequent CNS relapse for these patients.

Except for 1 patient whose PBSC sample was positive for *PML-RARα* and another who was withdrawn from the study to receive off-protocol prophylactic CNS irradiation, all the remaining patients who had undergone PBSC harvest proceeded to autologous HCT without any subsequent transplant-related mortality. This contrasts with a previous prospective study conducted before the advent of ATO, in which a combination of ATRA and intensive chemotherapy was used.[28] In that study, severe toxicity of induction therapy precluded the subsequent conduct of PBSC harvest or autologous HCT for some patients, and nearly 10% of the autografted patients suffered transplant-related mortality. These results highlight the need for active and less toxic therapies that give patients a better chance to proceed to and receive autologous HCT safely. For this reason, ATO can be considered to be an ideal treatment because of its strong antileukemic effect and favorable safety profile.

Although relatively few patients were analyzed in our study, to our knowledge this is the first prospective study to evaluate the use of ATO in conjunction with autologous HCT for relapsed APL. The results presented here provide evidence of the outstanding efficacy and feasibility of the sequential treatment consisting of induction and consolidation with ATO, PBSC harvest after high-dose AraC chemotherapy, and autologous HCT. For patients who are not eligible for this strategy, such as those for whom autologous HCT is

not suitable or whose *PML-RARα* levels do not decrease sufficiently during treatment, other treatment approaches need to be investigated that incorporate, for example, allogeneic HCT,[24,29] gemtuzumab ozogamicin,[30,31] tamibarotene,[32] or novel agents. It is desirable that such studies can be conducted prospectively. Finally, we should remember that the incorporation of ATO into initial therapy is expected to further improve the outcome for newly diagnosed APL,[33,34] which will hopefully lead to reduction in the number of patients who require salvage therapy.

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Authorship

Contribution: M.Y. collected and analyzed data, interpreted results, and drafted the manuscript; M.T., H.F., and A.T. designed the study, collected data, interpreted results, and reviewed the manuscript; K.F., S.F., K.S., M.T., A.O., K.T., and A.M. collected data, interpreted results, and reviewed the manuscript; S.O. contributed to data management, designed the study, collected data, interpreted results, and reviewed the manuscript; Y.M. contributed to data management, interpreted results, and reviewed the manuscript; Y.A. designed the study, analyzed data, interpreted results, and drafted the manuscript; Y.K. designed the study, provided administrative support, interpreted results, and reviewed the manuscript; T.N. provided administrative support, interpreted results, and reviewed the manuscript; and N.E. served as the principal investigator, designed the study, collected and analyzed data, interpreted results, and drafted the manuscript.

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A complete list of the members of the JALSG appears in "Appendix: study group members."

Correspondence: Masamitsu Yanada, Department of Hematology, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, 470-1192, Japan; e-mail: myanada@fujita-hu.ac.jp.

Appendix: study group members

The members of the JALSG are Nihon University School of Medicine, Kasukabe Municipal Hospital, Tokyo Metropolitan Komagome Hospital, Tokyo Metropolitan Ohtsuka Hospital, Nagoya University Graduate School of Medicine, Nagoya Ekisaikai Hospital, JA Aichi Showa Hospital, Okazaki City Hospital, Daido Hospital, Yokkaichi Municipal Hospital, Ichinomiya Municipal Hospital, Komaki City Hospital, Toyohashi Municipal Hospital, Ogaki Municipal Hospital, Tosei General Hospital, National Center for Geriatrics and Gerontology, Aichi Cancer Center, Toyota Kosei Hospital, Japanese Red Cross Nagoya First Hospital, Fujita Health University School of Medicine, Mie University Graduate School of Medicine, Suzuka Kaisei Hospital, Takeuchi Hospital, Yamada Red Cross Hospital, JA Suzuka General Hospital, Matsusaka Chuo General Hospital, Kinki University School of Medicine, Osaka Minami Medical Center, Sakai Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Hiroshima Red Cross Hospital & Atomic-Bomb Survivors Hospital, Shikoku Cancer Center, Nagasaki University Graduate School of Biomedical Sciences, Sasebo City General Hospital, Nagasaki Medical Center, Kumamoto University School of Medicine, Kumamoto City Hospital, Kumamoto Shinto General Hospital, Jichi Medical School, Okayama University Hospital, Minami-Okayama Medical Center, Okayama City Hospital, Chugoku Central Hospital, Okayama Medical Center, Okayama Rosai Hospital, Kagawa Rosai Hospital, Gunma University Graduate School of Medicine, Nishi-Gunma National Hospital, Fujioka General Hospital, Fukaya Red Cross Hospital, University of Fukui, Kurashiki Central Hospital, Kanazawa Medical Center, Fukui Red Cross Hospital, Fukui Prefectural Hospital, National Cancer Center Hospital, Saitama Medical School, Hyogo College of Medicine, Osaka National Hospital, Takarazuka Municipal Hospital, Uegahara Hospital, Amagasaki Central Hospital, Kawasaki Medical School, Kochi Health Sciences Center, Chiba University Hospital, Chiba Aoba Municipal Hospital, Funabashi Central Hospital, Saiseikai Narashino Hospital, Oami Hospital, Nara Medical University, Jikei University School of Medicine, Dokkyo University School of Medicine, Nagoya Medical Center, Ohta Nishinouchi Hospital, Kochi Medical School, Shiga University of Medical Science, National Cancer Center East, Anjo Kosei Hospital, St. Marianna University School of Medicine, Yokohama Seibu Hospital, Shinshu University School of Medicine, Nagano Red Cross Hospital, Matsumoto Medical Center Matsumoto Hospital, Showa Inan General Hospital, Tokyo Women's Medical University, Tama-Hokubu Medical Center, Hamamatsu University School of Medicine, Hamamatsu Medical Center, Kagoshima University Hospital, Tochigi Cancer Center, Kanazawa University Graduate School of Medical Science, Keijyu Medical Center, NTT West Kanazawa Hospital, Toyama City Hospital, Ishikawa Central Hospital, JA Takaoka Hospital, Tokyo Medical University, Tokyo Medical University Hachioji Medical Center, Kyorin University School of Medicine, Hokkaido University Graduate School of Medicine, Sapporo Kousei Hospital, Sapporo Aiiku Hospital, Asahikawa City Hospital, Hakodate City Hospital, Hokkaido Cancer Center Hospital, Saiseikai Maebashi Hospital, Nagoya City University Graduate School of Medical Sciences, Enshu General Hospital, Shizuoka Saiseikai General Hospital, Tokai University School of Medicine, Ebina General Hospital, Yamaguchi University School of Medicine, Yamaguchi Prefecture Central Hospital, The University of Tokyo, Osaka City University, Saiseikai Nakatsu Hospital, Osaka University Graduate School of Medicine, University of Tokyo, Niigata University Medical and Dental Hospital, Oita University Faculty of Medicine, Oita Prefectural Hospital, Almeida Memorial Hospital, Kouseiren Tsurumi Hospital, National Kyushu Cancer Center, Kyushu Medical Center, Fukuoka Postal Services Agency Hospital, Aso Iizuka Hospital, Teikyo University School of Medicine, Teikyo University Mizonokuchi Hospital, Sapporo Hokuyu Hospital, Aichi Medical University, Kitasato University Hospital, Yamagata University Faculty of Medicine, Keio University, Aomori Prefectural Central Hospital, Hyogo Cancer Center, Kyoto Prefectural University of Medicine, Kyoto Hospital, Kobe Central Hospital, Matsushita Memorial Hospital, Osaka City General Hospital, National Defense Medical College, Akita University School

of Medicine, NTT Kanto Medical Center, Yokohama City University Hospital, Yokohama City University Medical Center, Kanagawa Cancer Center, Yokosuka City Hospital, Fujisawa City Hospital, Shizuoka Red Cross Hospital, Yamato Municipal Hospital, Saiseikai Yokohama Nanbu Hospital, Tohoku University School of Medicine, Osaki Citizen Hospital, Hiroshima University, Hiroshima-Nishi Medical Center, Kagawa University, Kagawa Prefectural Central Hospital, Sakaide City Hospital, Juntendo University School of Medicine, Kanazawa Medical University, Kobe University Graduate School of Medicine, Imamura Bun-In Hospital, Ehime University School of Medicine, Bokutoh Hospital, Ohtsu Red Cross Hospital, Matsue Red Cross Hospital, Tokyo Medical and Dental University, Yokohama City Minato Red Cross Hospital, Jichi Medical School Omiya Medical Center, Shizuoka Cancer Center Hospital, Ehime Prefectural Central Hospital, International Medical Center of Japan, Kure Medical Center, Nagoya Daini Red Cross Hospital, University of Yamanashi, Heart Life Hospital, Musashino Red Cross Hospital, Saitama Medical School, PL General Hospital, Toyama Prefectural Central Hospital, Shimane Prefectural Central Hospital, Tottori Prefectural Central Hospital, National Disaster Medical Center, Shimane University Hospital, Otemae Hospital, Nakagami Hospital, Tsukuba University Hospital, Mito Medical Center, National Hospital Organization, Tsuchiura Kyodo General Hospital, Hitachi General Hospital, JA Toride Medical Center, Fuchu Hospital, Ibaraki Prefectural Central Hospital, Saga University, Yamanashi Prefectural Central Hospital, Hiroshima City Asa Hospital, Sendai Medical Center, Kurume University of Medicine, Kitakyushu Municipal Medical Center, Kyushu Kosei-Nenkin Hospital, University of Miyazaki Hospital, Hamanomachi Hospital, Kyushu University Graduate School of Medical Sciences, Harasanshin Hospital, Toranomon Hospital, Matsuyama Red Cross Hospital, Fukushima Medical University, Fukuoka University Hospital, Yokohama Municipal Citizen's Hospital, Nagoya City West Medical Center, Takanohara Central Hospital, The University of the Ryukyus, Osaka Red Cross Hospital, and Sapporo Medical University School of Medicine.

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Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia

Yoshiko Atsuta,[1] Junya Kanda,[2] Minoko Takanashi,[3] Yasuo Morishima,[4] Shuichi Taniguchi,[5] Satoshi Takahashi,[6] Hiroyasu Ogawa,[7] Kazuteru Ohashi,[8] Yuju Ohno,[9] Yasushi Onishi,[10] Nobuyuki Aotsuka,[11] Tokiko Nagamura-Inoue,[12] Koji Kato,[13] and Yoshinobu Kanda,[2] on behalf of the HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation

[1]Department of Hematopoietic Stem Cell Transplantation Data Management / Biostatistics, Nagoya University Graduate School of Medicine, Nagoya; [2]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama; [3]The Japanese Red Cross Tokyo Blood Center, Tokyo; [4]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya; [5]Department of Hematology, Toranomon Hospital, Tokyo; [6]Department of Molecular Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo; [7]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo; [8]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo; [9]Department of Internal Medicine, Kitakyushu Municipal Medical Center, Kitakyushu; [10]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai; [11]Department of Hematology and Oncology, Japanese Red Cross Narita Hospital, Narita; [12]Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, and Tokyo Cord Blood Bank, Tokyo; and [13]Department of Pediatrics, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

ABSTRACT

Recent advances in unrelated cord blood transplantation have increased chances and options available in allogeneic stem cell transplantation. The effect of HLA disparity on outcomes after cord blood transplantation was studied recently in mainly pediatric populations. Results showed that HLA matching in combination with total nucleated cell dose positively affects survival. The effect of HLA disparity after single-unit cord blood transplantation may be different in adults because their total nucleated cell dose is much lower compared to pediatric patients. We investigated the effect of HLA disparity on the outcome of single-unit unrelated cord blood transplantation separately in 498 children aged 15 years or under (HLA-A, HLA-B low-resolution, and HLA-DRB1 high-resolution matched [6/6], n=82, and one locus- [5/6], n=222, two loci- [4/6], n=158, three loci- [3/6] mismatched, n=36) and 1,880 adults (6/6, n=71; 5/6, n=309; 4/6, n=1,025; 3/6, n=475) with leukemia. With adjusted analyses, in children, 4/6 showed significantly increased risks of overall mortality (relative risk [RR]=1.61, P=0.042) and transplant-related mortality (RR=3.55, P=0.005) compared to 6/6. The risk of grade 2 to 4 acute GVHD was increased in 5/6 (RR=2.13, P=0.004) and 4/6 (RR=2.65, P<0.001). In adults, the risk of mortality did not increase with the number of mismatched loci (RR=0.99, P=0.944 for 5/6; RR=0.88, P=0.436 for 4/6). The risk of relapse was significantly decreased in 4/6 (RR=0.67, P=0.034). The risk of transplant-related mortality (TRM) or acute GVHD was not increased in 5/6 or 4/6. The effect of HLA disparity on transplant outcome differed between children and adults. In children, an increased number of mismatched HLA loci correlated with an increased risk of mortality. In adults, there was no increase in mortality with an increase in the number of mismatched HLA loci.

Introduction

Recent advances in unrelated cord blood transplantation (UCBT) have provided increased opportunities for patients with hematologic malignancies to receive hematopoietic stem cell transplantation (HSCT). This has led to an increased number of UCBT procedures over the past decade.[1,2] Clinical comparison studies of cord blood and bone marrow from unrelated donors have shown comparable results, which indicates that cord blood is a reasonable alternative donor / stem cell source.[3-12] These studies support the use of HLA-A, HLA-B, low-resolution and HLA-DRB1 zero- to two-loci-mismatched UCB for patients with leukemia in the absence of an HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched unrelated adult donor, and the use of UCB as a first-line option when a transplant is urgently required.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched bone marrow is currently the first alternative for HLA-identical sibling donors.[13-16] An increase in the number of HLA mismatches, antigen-level, or high-resolution, at HLA-A, HLA-B, HLA-C, or HLA-DRB1 loci from 8/8 to 7/8, or 7/8 to 6/8 was associated with higher mortality with an approximately 10% reduction in survival in UBM recipients.[12,13,15] Since HLA mismatches are better tolerated after UCB with a lower incidence of severe graft-*versus*-host disease (GVHD), up to two HLA antigen mismatches of HLA-A, HLA-B, low resolution and HLA-DRB1 high resolution are considered in the current CB selection algorithm. Several reports have recently described the effect of HLA disparity on the transplant outcomes after UCBT.[9,17,18] Eapen *et al.* reported the pos-

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Correspondence: y-atsuta@med.nagoya-u.ac.jp

sibility of a better outcome in HLA 6/6 matched UCB in 35 recipients, and Barker *et al.* confirmed these results with a larger number of UCB recipients.[9,18] However, these studies, which assessed the effect of HLA disparity on the outcome of single-unit CBT, were mainly conducted in pediatric populations in which the infused cell dose is much greater than that in adult recipients.

The aim of this study was to assess the effect of HLA disparity on the transplant outcomes after single-unit UCBT in pediatric and adult recipients. The accumulation of single-unit CBT in adult recipients has enabled us to assess separately the effect of HLA disparity on CBT outcomes in children and adults.

Design and Methods

Study design and data source

For this retrospective observational study, recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN). All 11 cord blood banks in Japan are affiliated with the JCBBN. JCBBN collected the recipients' clinical information at 100 days post-transplant through the Transplant Registry Unified Management Program (TRUMP) of the Japan Society of Hematopoietic Cell Transplantation (JSHCT).[19] Information on survival, disease status, and long-term complications including chronic graft-*versus*-host disease and second malignancies is renewed annually. Patient consent is not required for TRUMP registration of the JSHCT for the registry data consists of anonymized clinical information. This study was approved by the data management committees of the JSHCT and the JCBBN, and by the institutional review boards of Saitama Medical Center, Jichi Medical University and Nagoya University Graduate School of Medicine, Japan.

Patients

The subjects were patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), or myelodysplastic syndrome (MDS), who were recipients of their first UCBT between January 2000 and December 2009. Among 2,461 recipients of single-unit UCB with complete HLA-A, HLA-B, low-resolution and HLA-DRB1 high-resolution data, 51 recipients with 4 HLA mismatches were excluded. Thirty recipients who did not receive GVHD prophylaxis and 2 recipients for whom information regarding the conditioning regimen was missing were excluded. A total of 2378 single-unit UCB recipients (498 children aged 15 years or under at transplant, and 1880 adults aged 16 years or over at transplant) were subjects for analysis.

HLA typing

Histocompatibility data for low-resolution typing for the HLA-A, HLA-B, and HLA-DR loci and high-resolution typing for HLA-DRB1 were obtained from the TRUMP database which includes HLA information provided by cord blood banks or transplant centers. The level of HLA typing in the present study was HLA-A, HLA-B, low-resolution, and HLA-DRB1 high-resolution, as in other studies in Europe and North America. However, according to current practice in Japan, mismatches in HLA-DR loci were counted at the low-resolution level at UCB unit selection. Therefore, results regarding the effect of HLA mismatches in HLA-A, HLA-B, and HLA-DR low-resolution are also provided (*Online Supplementary Table S1*). Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients compared to HLA class I mismatched recipients. Thus, in Japan, a single-DRB1-mismatched UBM donor is preferred over a single-A-mismatched UBM or single-B-mismatched UBM donor.[15,20] This background affected HLA typing strategy of HLA-DR low-resolution typing instead of high-resolution typing for selection of cord blood units in Japan. This observation may explain the fact that the frequency of 4/6 grafts is higher in this cohort than in cohorts in Europe and the USA.

Definitions

The primary outcome of the analyses was overall survival, defined as time from transplant to death from any cause. Several secondary end points were also analyzed. Neutrophil recovery was defined as an absolute neutrophil count of at least $0.5x10^9$/L cells per cubic millimeter for three consecutive points; platelet recovery was defined as a count of at least $50x10^9$ platelets per cubic millimeter without transfusion support. The recipients of reduced-intensity conditioning were also defined with the criteria above, according to the previous report that confirmed complete donor chimeras of all engrafted patients after CBT with reduced-intensity conditioning.[21] Diagnosis and clinical grading of acute GVHD were performed according to the established criteria.[22,23] Relapse was defined as the recurrence of underlying hematologic malignant diseases. Transplant-related death was defined as death during a 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 are shown for categorical variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and transplant-related mortality (TRM).[24] Gray's test was used for group comparisons of cumulative incidences.[25] An adjusted comparison of the groups with regard to overall survival (OS) was performed with the use of the Cox's proportional-hazards regression model.[26] For other outcomes with competing risks, Fine and Gray's proportional-hazards model for the subdistribution of a competing risk was used.[27] For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event. For GVHD, death without GVHD and relapse were competing events. For relapse, death without relapse was the competing event, and for transplant-related mortality (TRM), relapse was the competing event.[28] For acute GVHD, subjects were limited to those who engrafted, and for chronic GVHD, subjects were limited to those who engrafted and survived at least 100 days after transplantation.

The variables considered were the patient's age at transplant (5 years or over *vs.* under 5 years for pediatric recipients, and 50 years or over *vs.* under 50 years for adult recipients; cut-off points were around the median in each group), patient's sex, donor-patient sex mismatch (matched *vs.* male to female *vs.* female to male), donor-patient ABO mismatch (major mismatch *vs.* matched or minor mismatch), diagnosis (AML, ALL, CML or MDS), disease status at conditioning (first or second complete remission (CR) of AML, 1CR of ALL, first chronic phase of CML, and refractory anemia or refractory anemia with ringed sideroblasts as standard-risk diseases *vs.* advanced for all others), the conditioning regimen (reduced-intensity conditioning *vs.* myeloablative conditioning), and the type of prophylaxis against GVHD (tacrolimus-based *vs.* cyclosporine-based). Conditioning regimens were classified as myeloablative if total-body irradiation >8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan ≥7.2 mg/kg, or melphalan >140 mg/m^2 was used based on the report from the Center for International Blood and Marrow Transplant Research.[29,30] We cat-

egorized patients for whom there was insufficient information regarding the doses of agents or radiation used for the conditioning regimen according to information on the conditioning intensity (i.e. whether or not the conditioning regimen was intended to be myeloablative) as reported by the treating clinicians. The cryopreserved total nucleated cell dose was categorized as >10.0x10^7/kg, 5.0-9.9 x 10^7/kg, 2.5-4.9x10^7/kg, or <2.5 x 10^7/kg for children, and >3.0x10^7/kg, 2.5-2.9x10^7/kg, 2.0-2.4x10^7/kg, or <2.0x10^7/kg for adults. HLA disparity and nucleated cell dose were maintained in the model. Since patient age was highly correlated with the total nucleated cell dose in children, age was excluded from multivariate analyses for pediatric recipients. Other variables were selected in a backward stepwise manner with a variable retention criterion of P<0.05. Interaction between HLA disparity and adult (patient age at transplant 16 years or over) or child (patient age at transplant 15 years or under) was tested for overall survival by using a Cox's proportional-hazards regression model adjusted by other significant covariates in the final model for adult and pediatric recipients except for patient age. All P values were two-sided.

Results

Patients' characteristics

Table 1 shows patients' characteristics, their disease, and transplant regimens. Median age at transplant was five years (range 0-15) in 498 pediatric and 49 years (range 16-82) in 1880 adult recipients of single-unit CBT. The proportion of females was 45% in both children and adults. Among children, the proportion of patients with ALL was greatest (58%) followed by that of patients with AML (34%). Among adults, the most frequent disease was AML (59%), followed by ALL (22%) and MDS (13%). The median number of cryopreserved total nucleated cells received in children was 5.30 x 10^7/kg, which was significantly greater (approximately double) than the number of nucleated cells received in adult patients (2.52 x 10^7/kg). In adults, only 33 patients (2%) received CB with a total nucleated cell dose greater than or equal to 5.0 x 10^7/kg. In children, 82 patients (16%) received HLA-matched (6/6) UCB, 222 (45%) received one-locus-mismatched (5/6), 158 (32%) received two-loci-mismatched (4/6), and 36 (7%) received three-loci-mismatched (3/6) UCB. For adults, the numbers and proportions of recipients were 71 (4%) for 6/6, 309 (16%) for 5/6, 1025 (55%) for 4/6, and 475 (25%) for 3/6. Among those who received 3/6 UCB, only 2 pediatric and 11 adult patients received three HLA-A, HLA-B, HLA-DR low-resolution mismatched UCB. Eighty-eight percent (TBI regimen 62%, non-TBI regimen 26%) and 62% (TBI regimen 56%, non-TBI regimen 6%) of children and adults, respectively, received myeloablative conditioning. Fludarabine-based reduced-intensity conditioning was given to 34% of adult recipients. T-cell depletion *in vivo* with antithymocyte globulin or antilymphocyte globulin was performed in only 6 (2%) child recipients and 26 (1%) adult recipients. The median follow-up period for survivors was 2.4 years (range 0.1-9.5) for pediatric recipients and 2.1 (range 0.1-9.0) years for adult recipients.

Outcome

Overall survival, relapse, and transplant-related mortality: among children, overall mortality in 4/6 UCB recipients was significantly higher than that in 6/6 UCB recipients (RR=1.61, 95% confidence interval [CI], 1.02-2.56, P=0.042) (Table 2). Overall mortality increased with the number of mismatched loci in children (P for trend 0.043). The increased mortality in 4/6 UCB recipients was mainly affected by increased transplant-related mortality (TRM) (RR=3.55, 95%CI: 1.47-8.58, P=0.005) (P for trend 0.002) but not by the risk of relapse (RR=0.77, 95%CI: 0.48-1.24, P=0.392) in children. Among children, there were no differences in the risks of mortality and relapse between 5/6 UCB recipients (RR=1.07, P=0.765 for overall mortality; RR=1.06, P=0.794 for relapse; and RR=1.29, P=0.58 for TRM) and 6/6 UCB recipients (Table 2).

In adults, the number of HLA mismatches was not significantly associated with increased mortality (for overall mortality: RR=0.99, P=0.944 for 5/6; RR=0.88, P=0.436 for 4/6; RR=0.95, P=0.751 for 3/6; for TRM, RR=1.41, P=0.205 for 5/6; RR=1.24, P=0.408 for 4/6; RR=1.29, P=0.339 for 3/6). A two-loci mismatch was associated with a decreased risk of relapse in adult recipients (RR=0.70, P=0.075 for 5/6; RR=0.67, P=0.034 for 4/6; RR=0.70, P=0.07 for 3/6) (Table 2). The risks of mortality were similar when subjects were limited to those with standard risk disease status or to those with advanced risk disease status at transplant, to those who received myeloablative conditioning or to those who received reduced-intensity conditioning (*Online Supplementary Table S2*). A decreased risk of relapse was more prominent in patients with acute myeloid leukemia, and those who received reduced-intensity conditioning (*Online Supplementary Table S2*).)

Figure 1 shows unadjusted overall survival curves in children and adults. In children, the unadjusted probabilities of survival at three years post-transplant were 66% for 6/6, 62% for 5/6, 45% for 4/6, and 62% for 3/6 (P=0.032) (Figure 1A). In adults, the survival probabilities in all of the HLA disparity groups were similar (38% for 6/6, 37% for 5/6, 39% for 4/6, and 40% for 3/6 at three years post-transplant, P=0.567) (Figure 1B). A similar trend was seen when subjects were limited to standard-risk disease status at transplant (81% for 6/6, 76% for 5/6, 57% for 4/6, and 81% for 3/6 at three years post-transplant, P=0.035, for children; 51% for 6/6, 57% for 5/6, 58% for 4/6, and 55% for 3/6 at three years post-transplant, P=0.375, for adults) (*Online Supplementary Figure S1*).

A test of the interaction between HLA disparity and age (adult *vs.* child) revealed that the effect of HLA disparity on overall survival differed significantly between the pediatric and adult patient groups (P=0.009 for HLA disparity of 0-1 mismatches *vs.* 2-3 mismatches).

Hematologic recovery

The cryopreserved total nucleated cell dose significantly affected neutrophil and platelet recovery in children and neutrophil recovery in adults (Table 3). HLA disparity did not significantly affect neutrophil or platelet recovery in adults or children for neutrophil recovery: RR=1.03, P=0.823 for 5/6; RR=0.96, P=0.799 for 4/6; RR=0.67, P=0.068 for 3/6 in children; RR=0.89, P=0.436 for 5/6; RR=0.92, P=0.576 for 4/6; RR=0.84, P=0.243 for 3/6 in adults; for platelet recovery: RR=0.89, P=0.438 for 5/6; RR=0.75, P=0.09 for 4/6; RR=0.71, P=0.164 for 3/6 in children; RR=1.05, P=0.775 for 5/6; RR=1.05, P=0.791 for 4/6; RR=0.99, P=0.951 in 3/6 in adults (Table 3).