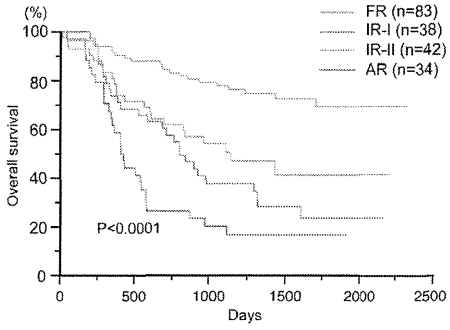
a

Genetic group	Subsets
Favorable	• CBF-AMLs • CN-AMLs with mutated *NPM1* without *FLT3*-ITD and mutated *DNMT3A* (normal karyotype) • CN-AMLs with mutated *CEBPA* without mutated *DNMT3A* (normal karyotype)
Intermediate-I	• CN-AMLs with mutated *DNMT3A* • CN-AMLs except for those included in the FR and AR
Intermediate-II	• AMLs included in the IR-II of the ELN stratification
Adverse	• CN-AMLs with *MLL*-PTD • AMLs included in the AR of the ELN stratification

b



c

Genetic group	Subsets
Favorable	• CBF-AMLs • CN-AMLs with mutated *NPM1* without *FLT3*-ITD and mutated *DNMT3A* (normal karyotype) • CN-AMLs with mutated *CEBPA* without mutated *DNMT3A* (normal karyotype)
Intermediate-I	• CN-AMLs with mutated *DNMT3A* • CN-AMLs except for those included in the FR and AR
Intermediate-II	• AMLs included in the IR-II of the ELN stratification except for those with mutated *TP53*
Adverse	• CN-AMLs with *MLL*-PTD • AMLs included in the AR of the ELN stratification except for those with mutated *TP53*
Very Adverse	• AMLs with mutated *TP53*

d

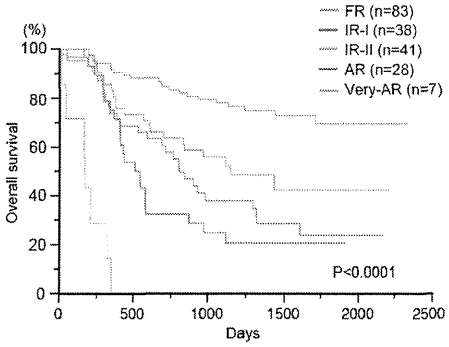


Figure 5. Risk stratification by modifying the ELN stratification system. When the CN-AML patients with *DNMT3A* mutations of the FR group and the patients with *MLL*-PTD of the IR-I group were included in the IR-I and the AR group, respectively (**a**), we could more clearly stratify the patients into four risk groups for OS than the original ELN system (**b**). When the patients with *TP53* mutations were classified as the very adverse-risk group (**c**), we could more clearly stratify the patients into five risk groups for OS (**d**).

CBFB-MYH11. Further study is required to clarify which combination is necessary for the clonal expansion, and whether different combinations cause clinical and phenotypical varieties.

After the completion of genetic alterations in AML, the most important issue is to clarify the prognostic impact of each mutation and/or co-occurring mutations.[7,42] The recently recommended ELN classification system is the first system that includes both cytogenetics and mutation status. Several groups reported that the ELN system clearly stratified the long-term prognosis of AML patients. However, the prognosis of FR groups except for the CBF-AML is still controversial. Paschka *et al.*[43] reported that the *IDH1/2* mutation was a poor prognostic factor in CN-AML with mutated *NPM1* without *FLT3*-ITD. In contrast, Patel *et al.*[5] reported that the *IDH1/2* mutation was a favorable prognostic factor in AML with mutated *NPM1* without *FLT3*-ITD. Furthermore, it has been reported that the *TET2* mutation was an adverse prognostic factor in AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD.[22] In the present cohort, we could not observe the statistically significant effects of *IDH1/2* and *TET2* mutations on the prognosis of CN-AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD, while we identified that *DNMT3A* mutation is an adverse prognostic factor in CN-AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD. In addition, we could not find the better prognostic impact of the *CEBPA* double mutations on the FR group recommended by the ELN in contrast to previous reports.[39,44]

Although different mutations might further stratify the prognosis of AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD, it was noteworthy that all mutations belonged to the class modifying methylation status.[13,38] These results collectively suggested that the epigenetic deregulation might contribute the pathogenesis of AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD. Prospective and large-scale study is necessary to clarify what genetic alterations influence the prognosis of AML with these genotypes.

In this study, we demonstrated that the prognosis of adult AML patients could be more clearly stratified by including the *DNMT3A* and *MLL*-PTD mutation status than the original ELN system, and that *TP53* mutations have a very adverse effect on the prognosis of AML patients. However, as most recurrently identified mutations were observed less than 5% of AML, it is highly expected to refine the genetic-based risk stratification system by much larger-scale studies. In addition, it is also important to evaluate the prognostic effects according to the functions of mutated genes rather than each sole mutation.

In the JALSG AML201 study, patients were randomized to the standard dose of IDR + Ara-C or HiDNR + Ara-C induction therapy, and the CR patients were again randomized to three courses of HiDAC or four courses of conventional standard-dose multiagent consolidation therapy. Therefore, we analyzed whether therapeutic regimens affect the CR rate and long-term survivals according to the mutation status and risk groups on the basis of the genetic status, while we could not observe any significant differences between therapeutic regimens and genetic status. Furthermore, we could not demonstrate that allo-SCT could improve the prognosis of the patients falling in the intermediate- and adverse-risk groups because of the small number of patients who underwent allo-SCT in the first CR in this cohort. It is, therefore, required to evaluate whether therapeutic regimens and allo-SCT affect the prognosis according to the genetic status.

In conclusion, we comprehensively analyzed 51 genes mutations in 197 *de novo* adult AML patients who were registered to a single prospective clinical study, and demonstrated that cooperative and exclusive mutation patterns and their prognostic impacts. Furthermore, we demonstrated that the prognosis of adult AML patients could be more clearly stratified by including the *DNMT3A*, *MLL*-PTD and *TP53* mutation status than the original ELN system. However, prognostic impacts of some mutation status are different from the previous reports. We must refine the risk

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stratification system by considering all known-risk factors in a large-scale and well-established cohort, although molecular genetic status has a strong impact on the prognosis of AML patients. We are now conducting a prospective large-scale study to confirm the present results.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

H Kiyoi, S Ogawa and TN designed the study, interpreted the data and wrote the manuscript; RK, YN, T Kato, EY, KS and FC performed molecular analysis and interpreted the data; YN, YS, KC, HT, SM and S Ogawa performed bioinformatics; NA, S Ohtake, SM, YM, TS, YO, N Usui, H Kanamori, T Kiguchi, KI, N Uike, FK, KK, CN, MO, AT, FI, HS, YK and HM collected samples and clinical data, contributed to the interpretation of the data and critically reviewed the draft; and all authors approved the final version submitted for the publication.

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Expression of CD56 is an unfavorable prognostic factor for acute promyelocytic leukemia with higher initial white blood cell counts

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Key words

Acute promyelocytic leukemia, all-trans retinoic acid, CD56 expression, chemotherapy, prognostic factor

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Expression of CD56 has recently been introduced as one of the adverse prognostic factors in acute promyelocytic leukemia (APL). However, the clinical significance of CD56 antigen in APL has not been well elucidated. We assessed the clinical significance of CD56 antigen in 239 APL patients prospectively treated with all-*trans* retinoic acid and chemotherapy according to the Japan Adult Leukemia Study Group APL97 protocol. All patients were prospectively treated by the Japan Adult Leukemia Study Group APL97 protocol. The median follow-up period was 8.5 years. Positive CD56 expression was found in 23 APL patients (9.6%). Expression of CD56 was significantly associated with lower platelet count ($P = 0.04$), severe disseminated intravascular coagulation ($P = 0.04$), and coexpression of CD2 ($P = 0.03$), CD7 ($P = 0.04$), CD34 ($P < 0.01$) and/or human leukocyte antigen-DR ($P < 0.01$). Complete remission rate and overall survival were not different between the two groups. However, cumulative incidence of relapse and event-free survival (EFS) showed an inferior trend in CD56$^+$ APL ($P = 0.08$ and $P = 0.08$, respectively). Among patients with initial white blood cell counts of 3.0×10^9/L or more, EFS and cumulative incidence of relapse in CD56$^+$ APL were significantly worse (30.8% *vs* 63.6%, $P = 0.008$, and 53.8% *vs* 28.9%, $P = 0.03$, respectively), and in multivariate analysis, CD56 expression was an unfavorable prognostic factor for EFS ($P = 0.04$). In conclusion, for APL with higher initial white blood cell counts, CD56 expression should be regarded as an unfavorable prognostic factor.

The clinical introduction of ATRA has dramatically improved the outcome of APL.[1–6] However, 13–33% of patients with APL still relapse after the first remission.[6] Therefore, various prognostic factors predicting outcome are being continuously analyzed, and initial high WBC count, low platelet count, and older age have been recognized as significant factors.[3,5–8] Recently, several investigators have suggested that the expression of CD56 antigen, a neural adhesion factor, is associated with higher incidence of relapse and poorer outcome in APL.[9–12] However, the number of reported cases and follow-up periods are still limited, and there has been no recommendation so far to modify standard treatment of APL on the basis of CD56 expression.[13,14] We analyzed the long-term outcome of 239 APL patients who were prospectively treated with ATRA combined with chemotherapies, including anthracycline and Ara-C, in the JALSG APL97 study, and assessed the clinical significance of CD56 expression in APL.

Materials and Methods

Patients. Adult patients with previously untreated APL were consecutively registered to the JALSG APL97 study between May 1997 and June 2002.[15] Eligibility criteria were: (i) diagnosis of APL with t(15,17) and/or the *PML-RARA* fusion gene amplified by RT-PCR; (ii) age between 15 and 70 years; (iii) ECOG PS 0 to 3; and (iv) sufficient functioning of the heart, lung, liver, and kidney. This study was approved by the

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Table 1. Clinical features of acute promyelocytic leukemia (APL) patients according to CD56 expression (n = 239)

Clinical features	CD56-positive		CD56-negtive		*P*-value
	No. of patients (%)	Median (range)	No. of patients (%)	Median (range)	
All patients					
No. of patients	23		216		
Age, years		48 (16–66)		47 (15–70)	0.84
15–59	20 (87)		181 (84)		0.69
60–65	3 (13)		35 (16)		
Sex					
Male	9 (39)		127 (59)		0.07
Female	14 (61)		89 (41)		
Initial WBC counts, $\times 10^9$/L		2.1 (0.04–98)		1.7 (0.01–257)	0.47
<3.0	12 (52)		129 (60)		0.78
3.0 to <10.0	6 (26)		46 (21)		
≥10.0	5 (22)		41 (19)		
Initial APL cell counts, $\times 10^9$/L		1.8 (0–92)		0.6 (0–253)	0.53
Initial platelet counts, $\times 10^9$/L		15 (6–120)		30 (2–238)	0.04
<10	5 (22)		28 (13)		0.30
10 to <40	13 (56)		111 (51)		
≥40	5 (22)		77 (36)		
ECOG performance status score					
0–2	19 (83)		202 (94)		0.05
3	4 (17)		13 (6)		
Albumin level, g/dL		4.2 (3.3–6.1)		4.2 (2.3–6.0)	0.51
<3.5	2 (9)		18 (9)		0.96
≥3.5	20 (91)		188 (91)		
Fibrinogen level, mg/dL		105 (55–389)		139 (20–513)	0.46
FDP ratio†		16.1 (4.0–322.4)		11.6 (0.3–524)	0.09
DIC score‡					
0–2	0 (0)		18 (9)		0.04
3–9	17 (77)		166 (82)		
≥10	5 (23)		18 (9)		
FAB subtype					
Typical	23 (100)		201 (93)		0.32
Variant	0 (0)		15 (7)		
ACAs	8 (42)		64 (35)		0.56
Patients with intial WBC counts ≥3.0 × 10^9/L					
No. of patients	11		87		
Age, years		41 (21–66)		45 (19–58)	0.87
15–59	10 (91)		73 (84)		0.54
60–65	1 (9)		14 (16)		
Sex					
Male	7 (64)		52 (60)		0.81
Female	4 (36)		35 (40)		
Initial WBC counts, $\times 10^9$/L		6.3 (3.2–98)		8.9 (3.0–257)	0.62
≥10.0	5 (45)		41 (47)		0.92
Initial APL cell counts, $\times 10^9$/L		4.8 (0–92)		7.0 (0.2–253)	0.37
Initial platelet counts, $\times 10^9$/L		14 (6–54)		23 (2–92)	0.38
<10	3 (30)		16 (18)		0.78
10 to <40	5 (40)		46 (53)		
≥40	3 (30)		25 (29)		
ECOG performance status score					
0–2	0 (0)		77 (90)		0.45
3	11 (100)		9 (10)		
Albumin level, g/dL		4.3 (3.5–4.7)		4.2 (2.6–5.8)	0.86
<3.5	2 (9)		8 (10)		0.29
≥3.5	20 (91)		76 (90)		
Fibrinogen level, mg/dL		104 (56–389)		104 (21–438)	0.84
FDP ratio†		26.7 (4.4–280)		14.1 (0.3–303)	0.24
DIC score‡					

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Table 1 (continued)

Clinical features	CD56-positive		CD56-negtive		*P*-value
	No. of patients (%)	Median (range)	No. of patients (%)	Median (range)	
0–2	0 (0)		3 (4)		0.02
3–9	7 (64)		75 (88)		
≥10	4 (36)		7 (8)		
FAB subtype					
Typical	11 (100)		74 (85)		0.17
Variant	0 (0)		13 (15)		
ACAs	2 (25)		22 (30)		0.76

†Fibrinogen degradation product (FDP) ratio calculated by dividing the FDP value by its upper normal limit. ‡Disseminated intravascular coagulation (DIC) score:[18] 0–2 indicates improbable DIC; score 3, suspected DIC; score 4–9, definitive DIC; ≥10, severe DIC. ACAs, additional chromosomal abnormalities; APL, Acute promyelocytic leukemia; ECOG, Eastern Cooperative Oncology Group; FAB, French–American–British; FDP, fibrin degradation product; WBC, white blood cell.

institutional review boards of each participating institution, and registered with the UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctrj/) under trial number C000000206. Informed consent was obtained from each patient before registration to the study in accordance with the Declaration of Helsinki.

Study design and treatments. The detail of treatment schedule was as described previously.[15] Remission induction therapy consisted of ATRA and chemotherapy with idarubicin and Ara-C, with dose and duration determined by initial WBC counts. After obtaining CR and receiving three courses of intensive consolidation chemotherapy including anthracyclines, Ara-C, and etoposide, patients negative for the *PML-RARA* fusion transcript were randomly allocated either to receive six courses of intensified maintenance chemotherapy or to observation. Patients who were positive for the *PML-RARA* fusion transcript received late ATRA therapy followed by maintenance therapy, and were scheduled to receive allogeneic hematopoietic stem cell transplantation, if they had a human leukocyte antigen-identical donor. Risk stratification according to initial WBC counts ($<3.0 \times 10^9$/L; 3.0×10^9/L to less than 10.0×10^9/L; $\geq 10.0 \times 10^9$/L) used in the current JALSG APL study are based on the results of the JALSG APL92 study.[3] In consideration of this background and the number of cases in each group, we adopted the value and divided the patients into two groups (i.e., initial WBC counts $<3.0 \times 10^9$ and $\geq 3.0 \times 10^9$) to analyze the prognostic impact of CD56 expression.

Immunophenotypic analysis. Immunophenotypic analysis was carried out using bone marrow samples taken at diagnosis and analyzed in the reference laboratory by standard immunofluorescence methods. Cells were stained with anti-CD45 (mAb), gated by CD45 expression and analyzed by flow cytometer. Cells were additionally stained with fluorescein-conjugated mAb against CD2, CD5, CD7, CD4, CD8, CD19, CD20, CD11b, CD13, CD14, CD15, CD33, CD34, CD56, and HLA-DR surface antigens. According to the criteria defined by the European Group for the Immunological Characterization of Leukemias,[16] surface markers were defined as positive if more than 20% of APL cells expressed a specific antigen.

Definition and evaluation of patients. Hematological response was evaluated by standard criteria.[17] Molecular relapse detected by RT-PCR analysis of *PML-RARA* was also considered as a relapse. Overall survival was calculated from the first day of therapy to death or last visit. Event-free survival was determined from the first day of therapy to relapse, death from any cause, or last visit. Cumulative incidence of relapse (extramedullary relapse) was measured from the date of CR to the first relapse, whereas non-relapse mortality was censored as a competing risk event.

Statistical analysis. Categorical data were compared using the χ^2-test or Fisher's exact test. Continuous data were compared using Wilcoxon's rank-sum test. The OS and EFS were estimated by Kaplan–Meier methods and compared by the log–rank test. The CIR was analyzed according to Kalbfleisch and Prentice, and differences were compared using Gray statistics. Cox's proportional hazards model was used for multivariate analysis of EFS. Factors significant at the 0.2 level in the univariate analysis were included in the multivariate analysis model. Statistical analyses were carried out using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA) and R 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org/). All hypothesis testing was two-tailed with a significance level of 0.05.

Results

Patient characteristics. Among 283 evaluable patients of 302 registered to the JALSG APL97 study,[15] 239 (85%) (median age, 48 years; range, 15–70 years) had satisfactory data for CD56 surface antigen expression, and were evaluated in this study. The median follow-up period was 8.5 years (0–12.2 years).

Of 239 patients, 23 (9.6%) were positive for CD56. The clinical and biological characteristics according to CD56 expression are shown in Tables 1 and 2. Expression of CD56 was significantly associated with lower platelet count ($<10 \times 10^9$/L) and severe DIC ($P = 0.04$ and $P = 0.04$, respectively); $CD56^+$ APL significantly coexpressed CD2, CD7, CD34, and/or HLA-DR antigen. ($P = 0.03$, $P = 0.04$, $P < 0.001$, and $P < 0.001$, respectively).

Treatment outcome. The CR rate and incidence of early death during induction therapy were not different between $CD56^+$ and $CD56^-$ APL (91% *vs* 95%, $P = 0.4$, and 9% *vs* 5%, $P = 0.54$, respectively; Table 3). Primary resistance to induction therapy was not observed in either group. The incidence of differentiation syndrome was not different between the two groups (22% *vs* 21%, $P = 0.9$; Table 3).

Overall survival was not different between the two groups (73.9% *vs* 79.2%, $P = 0.52$, at 9 years; Fig. 1a), whereas EFS

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Table 2. Immunophenotypic features of acute promyelocytic leukemia patients (n = 239) according to CD56 expression

Parameters	CD56-positive No. of patients (%)	CD56-negative No. of patients (%)	P-value
CD2			
Positive	5 (22)	16 (8)	0.03
Negative	18 (78)	191 (92)	
CD5			
Positive	1 (5)	3 (2)	0.25
Negative	18 (95)	195 (98)	
CD7			
Positive	2 (9)	4 (2)	0.04
Negative	20 (91)	208 (98)	
CD19			
Positive	1 (4)	5 (2)	0.56
Negative	22 (96)	210 (98)	
CD20			
Positive	0 (0)	1 (0.5)	0.75
Negative	19 (100)	191 (99.5)	
CD11b			
Positive	3 (19)	11 (7)	0.08
Negative	13 (81)	157 (93)	
CD15			
Positive	7 (54)	50 (33)	0.12
Negative	6 (46)	103 (67)	
CD41a			
Positive	1 (5)	19 (10)	0.46
Negative	20 (95)	177 (90)	
CD34			
Positive	9 (41)	27 (13)	$P < 0.01$
Negative	13 (59)	185 (87)	
HLA-DR			
Positive	7 (30)	16 (8)	$P < 0.01$
Negative	16 (70)	197 (92)	

HLA, human leukocyte antigen.

Table 3. Clinical outcomes of acute promyelocytic leukemia patients according to CD56 expression (n = 239)

Clinical features	CD56-positive No. of patients (%)	CD56-negative No. of patients (%)	P-value
No. of patients	23	216	
Induction outcome			
CR rate	21 (91)	206 (95)	0.40
Differentiation syndrome	5 (22)	44 (21)	0.90
Induction death	2 (9)	10 (5)	0.54
Hemorrhage	2 (100)	6 (60)	0.13
Infection	0 (0)	1 (10)	0.74
Differentiation syndrome	0 (0)	2 (20)	0.64
Others	0 (0)	1 (10)	0.74
Postremission outcome			
No. of patients	21	206	
Relapse			
All patients	9 (43)	49 (24)	0.06
Intial WBC counts <3.0	3 (14)	27 (13)	0.88
Initial WBC counts ≥3.0	6 (29)	22 (11)	0.02
Extramedullary relapse			
All patients	1 (5)	3 (1.5)	0.27
Intial WBC counts <3.0	0 (0)	2 (1.0)	0.65
Initial WBC counts ≥3.0	1 (5)	1 (0.5)	0.05
CIR (%)			
All patients	39.1	24.3	0.08
Intial WBC counts <3.0	20.0	20.1	0.98
Initial WBC counts ≥3.0	53.8	28.9	0.03
CIR (extramedullary relapse) (%)			
All patients	5.0	1.5	0.27
Intial WBC counts <3.0	0.0	1.8	0.69
Initial WBC counts ≥3.0	9.3	1.1	0.07

CIR, cumulative incidence of relapse; CR, complete remission; WBC, white blood cell.

and CIR tended to be inferior in $CD56^+$ APL (47.8% *vs* 64.8%, P = 0.08, and 39.1% *vs* 24.3%, P = 0.08, at 9 years, respectively; Figs 2a,3a). In patients with initial WBC counts $\geq 3.0 \times 10^9$/L, EFS and CIR for 11 $CD56^+$APL patients were significantly inferior to those for 87 $CD56^-$ APL patients (30.8% *vs* 63.6%, P = 0.008, and 53.8% *vs* 28.9%, P = 0.03, at 9 years, respectively; Figs 2b,3b). In patients with initial WBC counts $<3.0 \times 10^9$/L, EFS and CIR were not different between the two groups (P = 0.99 and P = 0.98, at 9 years, respectively). The OS in patients with initial WBC counts $\geq 3.0 \times 10^9$/L was similar between the two groups (61.5% *vs* 78.8%, P = 0.13, at 9 years; Fig. 1b). Although the number was small, EFS and CIR for five $CD56^+$ APL patients among those with initial WBC counts of $\geq 10 \times 10^9$/L were inferior to those for 41 $CD56^-$ APL patients (20.0% *vs* 60.9%, P = 0.03, and 60.0% *vs* 30.7%, P = 0.09, at 9 years, respectively). Cumulative incidence of extramedullary relapse tended to be more frequent in patients with $CD56^+$ APL whose initial WBC counts were $\geq 3.0 \times 10^9$/L (9.3% *vs* 1.1%, at 9 years, P = 0.07). We also analyzed the influence of CD56 expression on clinical outcomes according to Sanz's relapse risk score.[7] Both CIR and EFS in patients with $CD56^+$ APL were inferior in the high risk group (60.0% *vs* 31.4%, P = 0.09 and 20.0% *vs* 62.5%, P = 0.02, respectively), but not in low and intermediate risk groups (P = 0.17 and P = 0.55, respectively).

In the multivariate analysis, CD56 expression was an independent adverse prognostic factor for EFS in patients whose initial WBC counts were $\geq 3.0 \times 10^9$/L (hazard ratio = 2.54; 95% confidence interval, 1.07–6.06, P = 0.04) (Table 4).

Discussion

Expression of CD56 has been reported as one of the adverse prognostic factors in AML with t(8;21), associated with a short remission duration and survival as well as higher incidence of extramedullary relapse.[19,20] Recently, several investigators have suggested that CD56 expression is also associated with short remission duration in APL, higher CIR, and extramedullary relapse (Table 5).[9–12] However, large-scale studies with long-term follow-up are limited,[12] and the prognostic significance of CD56 expression has not been fully elucidated.

Our study, analyzing 239 APL patients, showed a significant correlation between CD56 expression with lower platelet counts and severe DIC. In contrast to previous reports,[9,10,12] CD56 expression was not associated with higher WBC counts, lower albumin levels, or higher frequency of M3 variant. Severity of DIC was related to platelet counts in $CD56^+$ APL,

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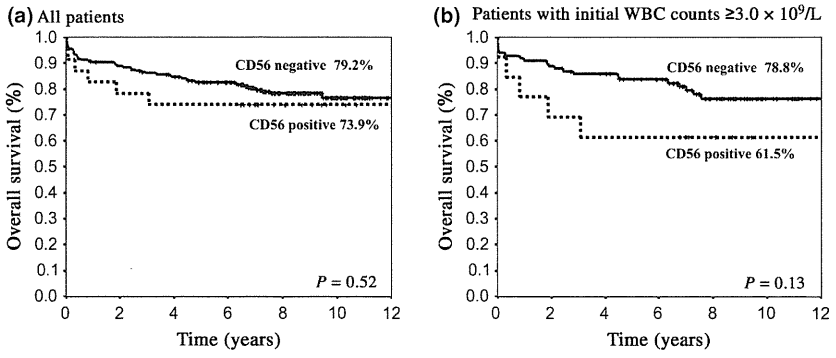

Fig. 1. Overall survival (OS) of patients with acute promylocytic leukemia according to CD56 expression. (a) OS was not different between the two groups for all patients (73.9% *vs* 79.2% at 9 years, $P = 0.52$). (b) In patients whose white blood cell (WBC) count was $\geq 3.0 \times 10^9$/L, OS did not differ between the two groups (61.5% *vs* 78.8%, $P = 0.13$).

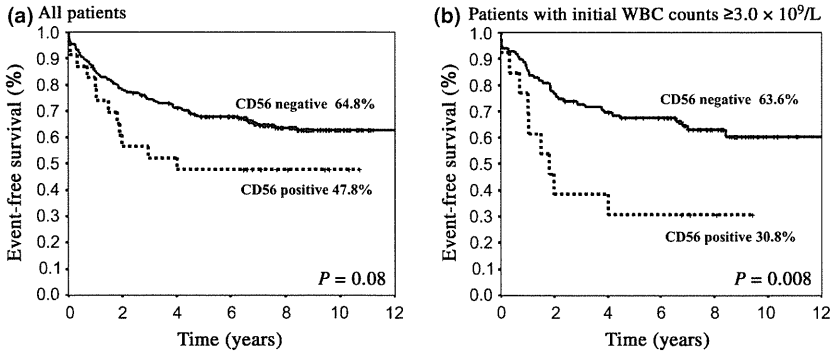

Fig. 2. Event-free survival (EFS) of patients with acute promylocytic leukemia (APL) according to CD56 expression. (a) EFS for all patients showed an inferior trend in $CD56^+$ APL (47.8% *vs* 64.8% at 9 years, $P = 0.08$). (b) In patients whose white blood cell (WBC) count was $\geq 3.0 \times 10^9$/L, EFS for $CD56^+$ APL was significantly inferior to that for $CD56^-$ APL (30.8% *vs* 63.8%, $P = 0.008$).

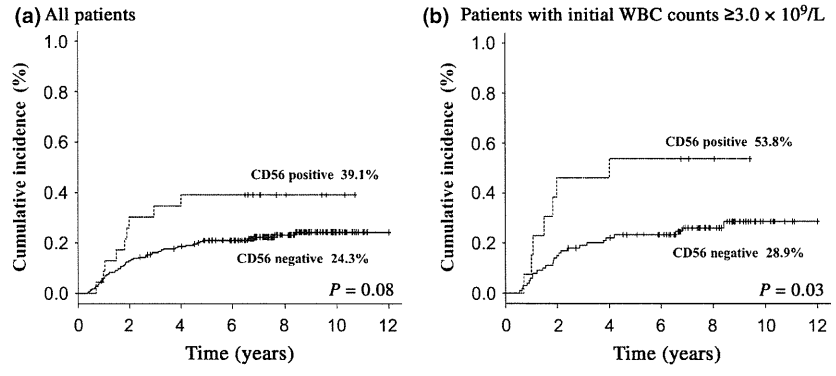

Fig. 3. Cumulative incidence of relapse (CIR) of patients with acute promylocytic leukemia (APL) according to CD56 expression. (a) CIR for all patients showed an inferior trend in the $CD56^+$ APL group (39.1% *vs* 24.3% at 9 years, $P = 0.08$). (b) In patients whose white blood cell (WBC) count was $\geq 3.0 \times 10^9$/L, CIR for the $CD56^+$ group was significantly higher compared to that for the $CD56^-$ APL group (53.8% *vs* 28.9%, $P = 0.03$).

although fibrinogen levels and fibrinogen degradation product ratios (fibrinogen degradation product value/its upper limit of normal value) were not different (Table 1). The relationship between CD56 expression and DIC in AML, including APL, has not been elucidated. As statistically significant findings associated with $CD56^+$ APL in previous reports were not the same as our present study, further studies with sufficient numbers of patients will be needed to clarify the characteristic features of $CD56^+$ APL.

Consistent with the report from the PETHEMA/HOVON group,[12] $CD56^+$ APL cells frequently coexpressed CD2, CD7, CD34, and/or HLA-DR antigen in our study. Although the mechanism leading to aberrant expression of lymphoid markers, such as CD2 and CD7 in $CD56^+$ APL cells, remains unclear, the expression of these antigens, as well as CD34 and HLA-DR, may indicate that $CD56^+$ APL cells arise in more immature, undifferentiated, and progenitor cells, as previously suggested in acute leukemia.[21]

The PETHEMA/HOVON group have reported lower CR rates in their patients with $CD56^+$ APL.[12] However, our study showed no difference in CR and induction mortality rates. Their patients with $CD56^+$ APL showed poorer ECOG PS scores and lower albumin levels compared with our patients. Higher ECOG PS scores and lower albumin levels were reportedly associated with induction mortality.[22] Therefore, the difference may be explained by the characteristics of patients enrolled in both studies.

Our study indicated that CD56 expression was correlated with higher CIR and inferior EFS, and was an independent adverse prognostic factor for EFS by multivariate analysis among APL patients whose initial WBC counts were $\geq 3.0 \times 10^9$/L. These results verified that CD56 expression was one of the adverse prognostic factors in APL patients. However, the direct molecular mechanism why CD56 expression in APL is associated with poor prognosis still remains unclear. CD56 expression is reportedly associated with higher

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expression of P-glycoprotein in AML,[23,24] but their adverse prognostic roles seem independent.[24] Unfortunately, neither ours nor other studies focusing on $CD56^+$ APL have tested the association between CD56 and P-glycoprotein. However, APL expressing CD34 was reportedly less sensitive to ATRA therapy.[25,26] Therefore, coexpression of CD34 antigen might explain the higher CIR in $CD56^+$ APL, although the RT-PCR negativity after the consolidation chemotherapy was not different between $CD56^+$ and $CD56^-$ APL.

In this study, CD56 expression was not determined as one of the prognostic factors in APL patients whose initial WBC counts were $<3.0 \times 10^9$/L. One explanation might be that it has become difficult to determine significant risk factors in patients with APL, whose prognosis has considerably improved.[1–5] In particular, in patients with lower initial WBC counts, the outcome has been dramatically improved in the ATRA era.[3,27] Another considerable reason is that there might be synergistic action between CD56 expression and some undetermined proliferation molecular factors. Additionally, extramedullary relapse, observed frequently in patients with $CD56^+$ APL whose initial WBC counts are $\geq 3.0 \times 10^9$/L, might also be a reason. The molecular mechanism behind why $CD56^+$ APL patients with higher initial WBC counts show poor prognosis should be clarified in a future study.

Recently, arsenic trioxide, gemtuzumab ozogamicin, and tamibarotene have been shown to be effective for APL,[28–33] and, in fact, most of our relapsed patients received these drugs as well as stem cell transplantation. This may be a plausible reason why EFS and CIR tended to be worse in $CD56^+$ APL, but not OS, because these drugs and transplantation salvaged the relapsed patients.

Although our study confirmed CD56 expression as an independent adverse prognostic factor in APL patients with higher initial WBC counts who were treated with ATRA and chemotherapy (Table 4), the clinical significance of CD56 expression might change with the introduction of more potent agents as front-line therapy. Expression of CD56 has not been included so far in standard treatments recommended by the European LeukemiaNet.[14] However, some recent

Table 4. Prognostic factors affecting event-free survival of acute promyelocytic leukemia patients (initial white blood cell counts $\geq 3.0 \times 10^9$/L) (n = 239)

Factors for event-free survival	Univariate analysis		Multivariate analysis	
	P-value	Hazard ratio	95% CI	*P*-value
DIC score† >10 (*vs* DIC score ≤10)	0.17	1.06	0.90–1.24	0.48
Age >60 years (*vs* age ≤60 years)	0.04	2.00	0.86–4.65	0.11
HLA-DR antigen positive (*vs* negative)	0.02	1.46	0.49–4.33	0.49
CD56 antigen positive (*vs* negative)	0.008	2.54	1.07–6.06	0.04

†Disseminated intravascular coagulation (DIC) score:[18] 0–2 indicates improbable DIC; score 3, suspected DIC; score 4–9, definitive DIC; ≥10, severe DIC. Factors with *P*-value <0.20 in univariate analysis were included in the multivariate analysis. CI, confidence interval; HLA, human leukocyte antigen; HR, hazard ratio.

Table 5. Clinical features and outcomes in acute promyelocytic leukemia (APL) patients with CD56 expression, as reported in published works

Authors	No. of patients	Treatment	$CD56^+$ APL (%)	Clinical features in patients with $CD56^+$ APL*	CR rate		CIR		CIR (extramedullary)		DFS†		OS	
					$CD56^+$	$CD56^-$	$CD56^+$	$CD56^-$	$CD56^+$	$CD56^-$	$CD56^+$	$CD56^-$	$CD56^+$	$CD56^-$
Murray *et al.*[9]	50	CT alone / ATRA alone / ATRA + CT	24%	S-isoform↑, Fibrinogen↓	50%*	84%	NA	NA	NA	NA	NA	NA	5 weeks*	232 weeks
Ferrara *et al.*[10]	100	ATRA + CT	15%	No effect	87%	94%	NA	NA	13%	8%	22 months	NR	62%*	86%
Ito *et al.*[11]	28	ATRA + CT	14%	Coexpression of CD34	100%	87%	NA	NA	75%*	0%	4 months*	NR	26 months	NR
Montesinos *et al.*[12]	651	CT alone / ATRA + CT	11%	Initial WBC counts↑, Albumin↓, S-isoform↑, Coexpression of CD2, CD7, CD15, CD34, CD117, and HLA-DR	85%	92%	22%*	10%	7%*	1%	73%*	85%	78%	84%
Present study (all patients)	225	ATRA + CT	10%	Initial platelet counts↓, Severe DIC↑, Coexpression of CD2, CD7, CD34, and HLA-DR	91%	95%	39%	24%	5%	1.5%	48%	65%	74%	79%
Present study (initial WBC counts ≥3.0 × 10^9/L)	112	ATRA + CT	12%		92%	94%	54%*	29%	9.3%	1.1%	31%*	64%	62%	79%

*Significant difference. †Event-free survival in present study. APL, acute promyelocytic leukemia; ATRA, all-*trans* retinoic acid; CIR, cumulative incidence of relapse; CR, complete remission; CT, chemotherapy; DFS, disease-free survival; DIC, disseminated intravascular coagulation; HLA, human leukocyte antigen; NA, not available; NR, not reached; OS, overall survival; WBC, white blood cell.

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published research, including ours (summarized in Table 5), will promote the modification of treatment for $CD56^+$ APL. In fact, it is proposed in some recently published studies. We should not only continue to monitor CD56 expression in APL patients, but use more effective therapeutic strategies for patients with $CD56^+$ APL, especially those with higher initial WBC counts.

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Disclosure Statement

The authors have no conflict of interest.

Abbreviations

AML	acute myeloid leukemia
APL	acute promyelocytic leukemia
Ara-C	cytosine arabinoside
ATRA	all-*trans* retinoic acid
CIR	cumulative incidence of relapse
CR	complete remission
DIC	disseminated intravascular coagulation
ECOG	Eastern Cooperative Oncology Group
EFS	event-free survival
HLA	human leukocyte antigen
HOVON	Hemato-Oncologie voor Volwassenen Nederland
JALSG	Japan Adult Leukemia Study Group
OS	overall survival
PETHEMA	Programa de Estudio y Tratamiento de las Hemopatı'as Malignas
PS	performance status
WBC	white blood cell

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Leukemic evolution of donor-derived cells harboring *IDH2* and *DNMT3A* mutations after allogeneic stem cell transplantation

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Although allogeneic stem cell transplantation is effective for the treatment of leukemia with poor prognosis, some such treated individuals experience disease relapse at various times after transplantation. Chimerism analysis of the relapsed disease has revealed infrequent cases in which the malignant cells originate from the donor and not from the initial leukemic clones.[1,2] Such donor cell leukemia (DCL) is often refractory to further treatment, with a mean overall survival for the affected patients of only 32.8 months.[2]

We recently described a 47-year-old Japanese man with acute myeloid leukemia (AML) who underwent a transplantation of peripheral blood stem cells (PBSCs) from his HLA-matched brother.[3] Although the allogeneic transplantation was successful, AML again became apparent in the patient 27 months later and chimerism analysis revealed that the leukemia was DCL. Genomic DNA was isolated and subjected to whole-exome sequencing from specimens of the initial AML (containing 70% myeloblasts, referred to as sample P1), the first complete remission after chemotherapy (sample P2), the first relapse (containing 24% myeloblasts; sample P3), donor PBSCs (sample D1), DCL at 27 months after allogeneic transplantation (containing 6% myeloblasts, sample D2) and DCL at 36 months after transplantation (containing 71% myeloblasts, sample D3).

Exome sequencing yielded a total of ~84.7 million, ~31.6 million, ~73.5 million, ~44.3 million and ~53.2 million unique, high-quality, paired-end reads for samples P1, P2, P3, D1 and D3, respectively (Supplementary Information). Although chimerism analysis for short tandem repeats had indicated that D3 was derived from D1 clones,[3] we further examined this possibility in a genome-wide manner. As demonstrated in Supplementary Figure 1a, the allele frequency of single-nucleotide polymorphisms (SNPs) detected in our data sets was highly concordant between P1 and P2 (Pearson's correlation coefficient (r) of 0.978) as well as between P1 and P3 ($r = 0.986$), suggesting that these three samples originate from a single individual. However, as expected, the concordance dropped substantially for the P1 and D3 pair ($r = 0.628$). In contrast, the concordance between D1 and D3 was high ($r = 0.983$), suggesting that the relapsed leukemia after transplantation was indeed derived from the donor cell. Of note, the allele frequency of SNPs showed only a low level of concordance ($r = 0.285$) between P1 and a cell line (KCL22)[4] derived from an unrelated Japanese patient with chronic myeloid leukemia (Supplementary Figure 1b). The correlation coefficient of 0.628 for P1 and D3 thus indicated that the patient and donor siblings share a substantial number of SNPs.

We next searched for somatic nonsynonymous mutations among the leukemic samples. For P1 and P3, we used P2 as a paired normal control. Given that D3 was shown to be derived from D1, we used the latter as the germline control for the former. Through our computational pipeline (Supplementary Information), nine missense mutations and two out-of-frame insertions/deletions (indels) were detected for P1, two missense mutations for P3 and nine missense mutations and one out-of-frame indel for D3 (Table 1). As described previously,[3] a 4-bp deletion of *CEBPA* was present in the initial AML but absent from the DCL. Similarly,

Table 1. Confirmed somatic mutations in the specimens analyzed

Specimen	*Gene symbol*	*GenBank accession no.*	*Nucleotide change*	*Amino-acid change*	*Mutation ratio (%)*				
					P1	*P2*	*P3*	*D1*	*D3*
P1	*ACSL5*	NM_016234	c.280G>A	p.V94I	40.6	0.0	30.6	0.0	0.0
	ANO4	NM_178826	c.2441C>T	p.S814L	42.3	0.0	16.7	0.0	0.0
	APOB	NM_000384	c.9175C>T	p.R3059C	32.8	0.0	7.4	0.0	0.0
	BANK1	NM_017935	c.222C>G	p.N74K	36.4	0.0	9.2	0.0	0.0
	CCDC88C	NM_001080414	c.3748G>A	p.E1250K	36.4	0.0	0.0	0.0	0.0
	FAM178B	NM_001122646	c.81G>A	p.M27I	41.2	0.0	25.0	0.0	0.0
	GABRB2	NM_021911	c.1009C>T	p.R337C	44.8	0.0	14.5	0.0	0.0
	JAK3	NM_000215	c.2570T>C	p.L857P	40.8	0.0	0.0	0.0	0.0
	SPATA31D1	NM_001001670	c.3793C>T	p.R1265C	36.6	0.0	6.7	0.0	0.0
	CEBPA	NM_004364	c.319_322delGACT	p.D107Tfs	63.6	0.0	10.0	0.0	0.0
	STAG2	NM_001042750	c.219_220insCG	p.H73Rfs	100.0	0.0	27.6	0.0	0.0
P3	*ACSL5*	NM_016234	c.280G>A	p.V94I	40.6	0.0	30.6	0.0	0.0
	NTNG2	NM_032536	c.1348G>T	p.G450C	0.0	0.0	37.5	0.0	0.0
D3	*CCDC168*	NM_001146197	c.11761G>C	p.D3921H	0.0	0.0	0.0	0.0	55.6
	GAL3ST1	NM_004861	c.1086G>T	p.M362I	0.0	0.0	0.0	0.0	32.6
	IDH2	NM_002168	c.419G>A	p.R140Q	0.0	0.0	0.0	7.1	50.0
	MYO7B	NM_001080527	c.635G>A	p.R212H	0.0	0.0	0.0	0.0	45.8
	NFATC1	NM_172390	c.736G>A	p.V246I	0.0	0.0	0.0	0.0	48.6
	PSMB8	NM_004159	c.637C>T	p.P213S	0.0	0.0	0.0	0.0	40.9
	TCAIM	NM_173826	c.668C>G	p.S223C	0.0	0.0	0.0	0.0	70.0
	TMEM132D	NM_133448	c.481G>A	p.A161T	0.0	0.0	0.0	0.0	35.3
	UBA2	NM_005499	c.419G>A	p.G140E	0.0	0.0	0.0	0.0	47.4
	DNMT3A	NM_153759	c.449delT	p.V150Gfs	0.0	0.0	0.0	8.7	61.1
	NRAS[a]	NM_002524	c.38G>A	p.G13D	0.0	0.0	0.0	0.0	18.4

[a]Below the threshold in the initial screening.

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none of the identified somatic mutations were shared between the initial AML and DCL, providing further support for the distinct nature of the two leukemias.

Given that P3 contains only 24% myeloblasts, our computational pipeline could not accurately detect all of the associated somatic mutations. Indeed, most of the somatic mutations found in P1 (such as those in *ANO4*, *APOB*, *BANK1*, *STAG2* and *CEBPA*) were still present in P3 at lower frequencies (Table 1) but were not isolated in our pipeline analysis for P3. Lowering the threshold for somatic calls, however, increased the number of pseudopositive mutations in all specimens. We therefore applied the 30% threshold for mutation calls to all analyses. Of note, our data still indicate that P3 is not completely identical to P1. Nonsynonymous mutations of *CCDC88C* and *JAK3* detected in P1 were thus absent in P3, whereas a mutation of *NTNG2* was newly apparent in P3, suggestive of a clonal evolution in P3 divergent from the original P1 clones.

Surprisingly, whereas most somatic mutations detected in D3 were not present in D1, our results suggested that IDH2(R140Q) and DNMT3A(V150Gfs) were already present in the healthy donor at a low frequency (Table 1). Polymerase chain reaction (PCR)-based cloning of the genomic fragments and Sanger sequencing for *IDH2* and *DNMT3A* from D1 indeed confirmed the presence of the corresponding mutations in 2 (2.3%) out of 87 DNA clones and 1 (1.1%) out of 93 clones, respectively (Supplementary Figure 2). Furthermore, although the mutation rate (18.4%) was below the threshold of the present study, the oncogenic mutation NRAS(G13D)[5] in D3 (Table 1) was confirmed by Sanger sequencing of the corresponding genomic DNA (Supplementary Figure 2).

We then verified these infrequent mutations by sequencing the corresponding DNA fragments at extra-high coverage (hundreds of thousand times) with the use of a next-generation sequencer. The D2 sample, which contains only 6% myeloblasts, was also examined in this analysis. We confirmed that 1.6% (5.96×10^3 mutant reads out of 3.67×10^5 total reads at the corresponding nucleotide position) and 2.1% (1.24×10^4 out of 6.01×10^5 reads) of D1 cells already harbored the IDH2(R140Q) and DNMT3A(V150Gfs) mutations, respectively (Figure 1a). These mutations were not detected in the primary AML (P1 to P3). Whereas the *NRAS* mutation was not detected in D1, it became apparent in D2 and D3 at a frequency similar to that of the *IDH2* mutation. In addition, the *JAK3* mutation present in P1 was no longer evident at the relapsed stage P3.

On the basis of the genetic mutation profiles identified in the present case, we propose the following scheme for disease progression (Figure 1b). Given the high frequency of *STAG2* and *CEBPA* mutations in the primary AML, the 2-bp insertion in *STAG2* on the X chromosome (with there being only one copy of *STAG2* per cell in the male patient) as well as the heterozygous 4-bp deletion in *CEBPA* may characterize the founding clone of the original leukemia, with subsets of this clone subsequently acquiring additional oncogenic hits such as JAK3(L857P). The disappearance of *JAK3* and *CCDC88C* mutations in P3 suggests that the leukemic subclones harboring these mutations were sensitive to the initial chemotherapy.

The molecular pathogenesis of DCL has been unclear and may differ among cases. For instance, germline predisposition to cancer, such as the Li-Fraumeni syndrome or Bloom syndrome, may be shared between recipients and related donors.[6] However, in the present case, mutations in *IDH2* and *DNMT3A* were detected only in the donor, not in the primary AML, rendering this scenario unlikely. Alternatively, occult leukemia may already be present in the donor blood system and is inadvertently transmitted to the recipient.[7] In such cases, however, leukemia usually emerges in the donor soon after transplantation. Our donor, in contrast, has not developed any hematologic malignancy at 10 years after the donation of his PBSCs.

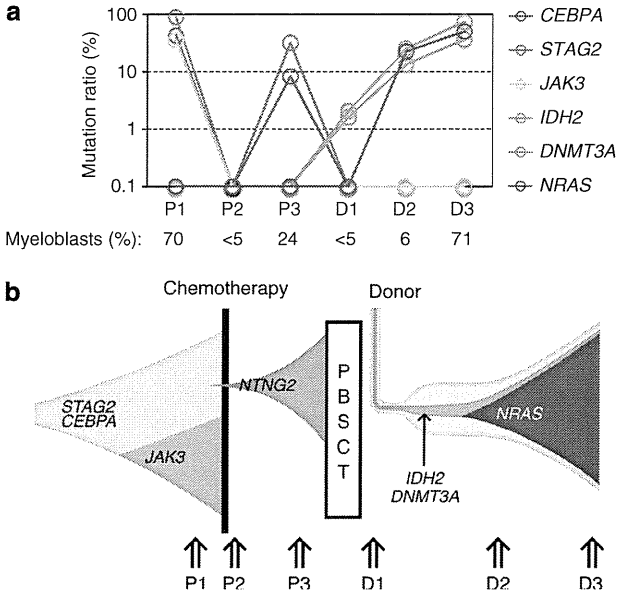

Figure 1. Genomic analysis of AML samples and donor PBSCs. (**a**) Genomic mutations corresponding to CEBPA(D107Tfs), STAG2(H73Rfs), JAK3(L857P), IDH2(R140Q), DNMT3A(V150Gfs) and NRAS(G13D) were examined by targeted deep sequencing in genomic DNA prepared from samples P1, P2, P3, D1, D2 and D3. The ratio of mutant reads to all reads at the corresponding position is shown as a percentage, with mutation frequencies of $<0.1\%$ being considered as 0.1% in the graph. The percentage of myeloblasts in each sample is indicated below the graph. (**b**) Founding clones of the primary AML harbored nonsynonymous mutations of *STAG2* and *CEBPA* and gave rise to subclones harboring a *JAK3* mutation. Whereas the latter cell population was sensitive to the initial chemotherapy, a subclone positive for an *NTNG2* mutation emerged from the former population and gave rise to relapse. All of these leukemic clones were successfully eradicated by peripheral blood stem cell transplantation (PBSCT). PBSCs of the donor, however, contained a small clonal population of cells positive for *IDH2* and *DNMT3A* mutations that eventually gave rise to AML on acquisition of additional mutations including NRAS(G13D).

Our present data therefore strongly suggest that apparently healthy individuals may harbor preleukemic subclones in their blood system (Figure 1b). Indeed, somatic mutations of *TET2* and *DNMT3A* were recently identified in clonal blood cells from one healthy elderly individual.[8] Furthermore, the *IDH2* and *DNMT3A* mutations identified in the present study may have had a specific role in the initiation of leukemia, given that mutations in the epigenetic modifiers including TET1/2, IDH1/2 and DNMT3A have been identified as early genetic events in AML progression.[9,10] Such mutations are indeed among the most frequently detected somatic alterations in AML.[11] These observations raise an important concern as to how 'appropriate' donors should be chosen, especially given that the incidence of DCL is increasing with the prevalence of molecular analysis for donor/recipient chimerism.[2] Prospective studies of whether and how examination of preleukemic subclones should be incorporated into the donor selection process for stem cell transplantation are thus warranted.

Furthermore, in our case, the oncogenic mutation NRAS(G13D) was likely a driver for leukemia progression, given that the frequency of this mutation was almost identical to that of the *IDH2* mutation in the D2 and D3 specimens. In contrast to the absence of leukemia in the donor, DCL rapidly developed in the recipient after transplantation in association with the accumulation of additional genetic hits, possibly as a result of a growth-promoting condition of the bone marrow after transplantation and due to a

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defective immune surveillance resulting from the immunosuppressive treatment to control graft-versus-host disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Cytogenetics and outcome of infants with acute lymphoblastic leukemia and absence of *MLL* rearrangements

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Acute lymphoblastic leukemia (ALL) in infants less than 1 year of age is rare and the biological features are different from ALL in older children.[1] Infant ALL is characterized by a high frequency of rearrangements of the *MLL* gene (*MLL*-R) and heterogeneous outcome. However overall, their event-free survival (EFS) is much worse than older children with ALL.[1–5] A large collaborative trial, Interfant-99, demonstrated improved outcome, while characterizing definitively the independent prognostic variables in infant ALL.[6] While cytogenetic data are reported within individual infant ALL clinical trials, the numbers are typically small and many reports are less detailed for those patients without *MLL* gene rearrangements (*MLL*-G). However, it was previously suggested that *MLL*-G had an important predictive influence on outcome.[7,8] These observations were later confirmed in Interfant-99,[6] in which *MLL*-G patients showed a threefold reduced risk of an event compared with *MLL*-R patients, although all *MLL*-G patients were grouped together into a single category. To better understand the association of different chromosomal abnormalities and outcome among *MLL*-G infants, here we have carried out detailed cytogenetic investigation of two infant ALL trials: Interfant-99 and Children's Oncology Group (COG)-P9407.

Patients were 365 days old or less with newly diagnosed ALL without a rearrangement of the *MLL* gene enrolled to Interfant-99 (May 1999–December 2005; $n = 110$) and COG-P9407 (June 1996–October 2006; $n = 52$).[6,9] Individual study groups obtained ethical approval, and treating physicians obtained informed consent from parents or guardians. The presence of *MLL* gene rearrangements was excluded using fluorescence *in situ* hybridization (FISH), reverse transcription (RT)-PCR and/or Southern blotting, as previously reported.[6] Each national study group provided patient data, including cytogenetics, FISH and molecular results. EFS and overall survival (OS) were calculated from the date of trial enrolement to the date of the first event (induction failure, relapse, second malignancy or death) or last follow-up. Median follow-up time was 7 years.

Among 162 *MLL*-G patients, no cytogenetic data were available for 34 (21%), resulting in a success rate of 79%. An abnormal karyotype was detected in 90/128 (70%) patients with a successful cytogenetic result (Supplementary Table 1) with the remainder classified as normal based on the presence of at least 10 (but usually 20) normal metaphases. They were categorized according to cytogenetic risk group as previously defined for childhood ALL.[10] Compared with childhood ALL (1–18 years) using data from the UKALL97/99 treatment trial,[10] the frequency of good risk cytogenetic abnormalities among *MLL*-G infants was significantly lower (12 vs 60%, $P < 0.01$), whereas the frequency of poor risk abnormalities (excluding *MLL* translocations) was similar (8 vs 10%). Although *ETV6–RUNX1* fusion is present in 25% of childhood ALL, we found no *ETV6–RUNX1* cases among the 75 patients tested by FISH or RT-PCR. High hyperdiploidy (HeH) was the most

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Role of hematopoietic stem cell transplantation for relapsed acute promyelocytic leukemia: A retrospective analysis of JALSG-APL97

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For patients with relapsed acute promyelocytic leukemia (APL), all-*trans* retinoic acid-based salvage regimens can achieve second complete remission (CR2), but the optimal post-remission strategy for APL patients after CR2 remains unclear. Hematopoietic stem cell transplantation (HSCT) during CR2 might be effective, but data on the role of HSCT for APL patients after CR2 are limited in Japan. We retrospectively analyzed outcomes for 57 relapsed APL patients who achieved CR2 in the JALSG APL97 study. Of those, six received autologous (auto)-HSCT, 21 received allogeneic (allo)-HSCT, and 30 received various regimens other than HSCT. The 5-year event-free survival (EFS) rate, overall survival (OS) rate and cumulative incidence of relapse (CIR) were 50.7%, 77.4% and 51.0% in the non-HSCT group, 41.7%, 83.3% and 58.3% in the auto-HSCT group and 71.1%, 76.2% and 9.8% in the allo-HSCT group, respectively. Both the EFS rate and CIR were significantly better in the allo-HSCT group than in other groups. Allo-HSCT appears effective in APL patients in CR2, with a low relapse rate beyond a relatively early transplantation-related mortality (19%). Among older patients (age ≥40 years), the 5-year OS was significantly better in the non-HSCT group than in the HSCT group (78.0% *vs* 40.5%; $P = 0.04$). Further prospective studies with larger patient numbers are required to confirm the impact of HSCT alone and in combination with arsenic trioxide on outcomes for patients with APL in CR2. (*Cancer Sci* 2013; 104: 1339–1345)

The introduction of all-*trans* retinoic acid (ATRA) has brought about marked progress in the treatment of acute promyelocytic leukemia (APL), but relapse still occurs in approximately 15–25% of patients.[1–3] Most of the relapsed patients were able to achieve second complete remission (CR2) using ATRA-based salvage regimens[4–6] or recent arsenic trioxide (ATO)-based salvage regimens.[7,8] After achieving CR2, most patients need to receive post-remission treatments to reduce minimal residual disease (MRD). A variety of post-remission strategies have been used, including further consolidation chemotherapy,[3] hematopoietic stem cell transplantation (HSCT),[6,9–11] continued treatment with ATO[7,8,12] or a combination of such therapies; however, the optimal post-remission therapy remains controversial. Previous studies have reported that ATO-based post-remission therapy for patients with APL in CR2 resulted in superior survival compared with chemotherapy alone or HSCT alone.[13] Likewise, HSCT strategies for patients with APL in CR2 resulted in better outcomes than chemotherapy alone, despite being associated with high transplantation-related mortality (TRM).[9–11] Moreover, autologous HSCT (auto-HSCT) was much better than allogeneic HSCT (allo-HSCT) for patients in CR2 who achieved molecular remission.[6,9]

Recently, in a phase 2 prospective study, our Japan Adult Leukemia Study Group (JALSG) reported the efficacy of sequential treatment using ATO followed by auto-HSCT for 25 patients with relapsed APL.[14] However, evidence has been lacking in terms of the role of auto-HSCT alone on the cumulative relapse rate or efficacy for patients with APL in CR2 who were ineligible for the phase 2 study regimens. Moreover, in situations where no guidelines regarding the optimal choice of auto- or allo-HSCT in CR2 have been determined, the role of HSCT alone in post-remission therapies for patients with APL in CR2 is yet to be evaluated. Therefore, the present study aimed to evaluate in detail the efficacies of HSCT alone for APL patients in CR2 by comparing outcomes, including cumulative relapse rate, both for APL patients who underwent auto-HSCT or allo-HSCT during CR2 and for those who did not receive HSCT during long-term follow up.

Materials and Methods

Data source. Information on patients with APL in CR2 and the salvage treatment applied were obtained from the JALSG APL97 study.[15] Between May 1997 and June 2002, a total of 302 adult patients with previously untreated de novo APL were registered in this study. The main eligibility criteria included diagnosis of APL with t(15;17) and/or the *PML-RARA* fusion gene and age between 15 and 70 years. For remission induction therapy, patients received ATRA either

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alone or with chemotherapy, followed by three courses of consolidation therapy consisting of cytarabine and anthracyclines. After completing consolidation therapy, patients negative for the *PML-RARA* fusion gene were randomly allocated to undergo either six courses of intensified maintenance chemotherapy or observation alone. More detailed eligibility criteria and the treatment schedule have been described previously.[15] Of the 283 assessable patients with t(15;17) and/or *PML-RARA*, 267 (94.3%) achieved complete remission (CR). Of the 267 patients who achieved CR, 67 (26.1%) experienced a first relapse during the median follow-up duration of 100 months (range, 11–155 months) from first achieving CR.

Salvage treatment in first relapse. All 67 relapses occurred between 1998 and 2005, during which time ATRA was mainly used as the salvage treatment for relapsed patients because ATO was not commercially available in Japan. Among the relapsed patients, two were unable to complete the follow-up survey and 65 received salvage treatment with ATRA alone (n = 17), ATRA plus chemotherapy (n = 33), tamibarotene (Am80) alone (n = 7), chemotherapy alone (n = 6), allo-HSCT alone (n = 1) or unknown (n = 1). Of those patients who received salvage treatments, 58 (89%) achieved CR2.

Of the 58 patients who achieved CR2, 27 had received HSCT (auto-HSCT, n = 6; allo-HSCT, n = 21) during CR2, 30 had not and one was unassessable. Therefore, the present study included 57 patients. We defined 27 patients in CR2 who received HSCT (six auto-HSCT and 21 allo-HSCT) as the HSCT group and 30 patients in CR2 who received regimens other than HSCT as the non-HSCT group. Clinical characteristics of the 57 APL patients in CR2 are summarized in Table 1.

Hematopoietic stem cell transplantation group. Stem cells for auto-HSCT were harvested in CR2 from peripheral blood in all six patients. Peripheral blood stem cell (PBSC) collection was made after mobilization using granulocyte colony-stimulating factor (G-CSF) following chemotherapy. All patients who underwent auto-HSCT achieved molecular CR of *PML-RARA* in bone marrow according to nested reverse transcriptase–polymerase chain reaction (RT-PCR) (n = 3), real-time quantitative PCR (RQ-PCR) (n = 2) or RT-PCR (n = 1) just before PBSC collection. For allo-HSCT, bone marrow cells were used in 15 patients, G-CSF-mobilized PBSC in four patients and cord blood cells in two patients. Donors were unrelated in 13 patients (bone marrow, 11 patients; cord blood, two patients). Seven of 15 patients who were examined for *PML-RARA* in the marrow before allo-HSCT were positive for MRD.

Patients were administered various conditioning regimens for HSCT. All six autografted patients received a myeloablative regimen using total body irradiation (TBI)/cyclophosphamide

Table 1. Clinical characteristics of the 57 APL patients in CR2 according to treatment after CR2

	Auto-HSCT (n = 6) No. (%) or median (range)	Allo-HSCT (n = 21) No. (%) or median (range)	Non-HSCT (n = 30) No. (%) or median (range)	All (n = 57) No. (%) or median (range)
At diagnosis	6	21	30	57
Sex				
Male	3 (50)	16 (76)	18 (60)	37 (64)
Female	3 (50)	5 (24)	12 (40)	20 (36)
Age (years)	40 (24–59)	33 (21–55)	50 (15–70)	45 (15–70)
15–29	2 (33)	8 (38)	5 (17)	15 (26)
30–49	2 (33)	11 (52)	9 (30)	22 (39)
50–70	2 (33)	2 (10)	16 (53)	20 (35)
WBC counts (x10^9/L)	6.5 (2.1–33.7)	3.2 (0.4–46.1)	1.9 (0.1–63.7)	2.7 (0.1–63.7)
<3.0	1 (17)	9 (43)	19 (63)	29 (51)
3.0–10.0	4 (66)	6 (29)	6 (20)	16 (28)
10.0 or higher	1 (17)	6 (29)	5 (17)	12 (21)
At first relapse				
Age (years)	44 (27–60)	36 (22–59)	53 (16–72)	47 (16–72)
First CR duration (months)	22 (10–81)	22 (6–63)	18 (6–90)	21 (6–90)
Salvage treatment				
ATRA alone	1 (17)	3 (14)	12 (40)	16 (28)
ATRA plus chemotherapy	5 (83)	9 (43)	12 (40)	26 (46)
Tamibarotene alone	0	3 (14)	4 (13)	7 (12)
Chemotherapy alone	0	5 (24)	2 (7)	7 (12)
Unknown	0	1 (5)	0	1 (2)
In CR2 achievement				
Age at CR2 (years)	44 (27–60)	36 (22–59)	53 (16–72)	47 (16–72)
Time to HSCT after CR2 (months)	7 (4–20)	5 (1–13)	–	–
Stem-cell source				
Peripheral blood	6	4	–	–
Bone marrow	0	15	–	–
Cord blood	0	2		
Donor	–		–	
HLA-identical sibling	–	8	–	–
Unrelated donor	–	13	–	–

Allo-HSCT, allogeneic HSCT; APL, acute promyelocytic leukemia; ATRA, all-*trans* retinoic acid; Auto-HSCT, autologous HSCT; CR, complete remission; CR2, second complete remission; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; Non-HSCT, patients who received regimens other than HSCT; WBC, white blood cell.

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(CY) (n = 1), busulfan (BU)/CY (n = 3), BU/melphalan (n = 1) or BU/etoposide/cytarabine (n = 1). Allografted patients received a myeloablative regimen using TBI/CY (n = 11), TBI /BU/CY (n = 2), BU/CY (n = 6) or a non-myeloablative fludarabine-based regimen (n = 2).

Non-HSCT group. Patients in this group received various consolidation and/or maintenance regimens with chemotherapy and/or ATRA (Table 1). Reasons for not undergoing HSCT in CR2 included age >60 years (n = 6), relatively poor condition (n = 4), patient refusal (n = 5), lack of an appropriate donor (n = 1), medical decision (n = 13) and unknown reasons (n = 1). Among patients in the non-HSCT group, 10 received allo-HSCT (n = 8) or auto-HSCT (n = 2) during the third CR (CR3) or more. After achievement of CR2, patients were treated with a variety of consolidation regimens, including chemotherapy, ATO, gemtuzumab ozogamycin and observation alone (Table S1).

Definitions. Hematological CR was defined as the presence of all of the following: <5% blasts in bone marrow; no leukemic blasts in peripheral blood or extramedullary sites; and recovery of peripheral blood counts. Relapse was defined as the presence of at least one of the following: two consecutive positive RT-PCR obtained 1 month apart after achieving molecular remission; recurrence of >10% leukemic cells in bone marrow; recurrence of any leukemic cells in peripheral blood; or development of extramedullary disease.[15]

Statistical analysis. Overall survival (OS) was calculated from the date of CR2 to the date of death or last follow up. Event-free survival (EFS) was calculated from the date of CR2 to an event (relapse or death) or to the date of last follow up. Cumulative incidence of relapse (CIR) was calculated from the date of CR2 to the date of second relapse or last follow up for patients alive in CR2. Results were analyzed as of 31 March 2010, allowing for median follow ups of 84 months (range, 16–120 months) and 87 months (range, 2–136 months) from the date of CR2 for the HSCT and non-HSCT groups, respectively. Differences in categorical factors between the HSCT and non-HSCT groups were compared using the χ^2 test. Age at CR2 was dichotomized using a cut-off point of 40 years to create a younger group (<40 years) and an older group (≥40 years) by taking the transplantation risk of age in the risk score of the European Group for Blood and Marrow Transplantation into consideration.[16] Continuous data were compared using the Mann–Whitney test. The OS and EFS were estimated using the Kaplan–Meier method and compared using the log-rank test. To adjust for effects of the timing of HSCT in the survival analysis, HSCT was treated as a time-dependent covariate in the Kaplan–Meier estimates of OS and EFS. The CIR was estimated using the cumulative incidence method, where death in CR2 was considered as a competing risk and compared using Gray's test. All tests were two tailed and a value of $P < 0.05$ was considered statistically significant. All analyses were performed using STATISTICA version 6.0 software (Statsoft Inc., Tulsa, OK, USA) and STATA 11 software (STATA Corp LP, College Station, TX, USA).

Results

The characteristics and prognosis of patients with APL who achieved CR2 by salvage treatment with HSCT (n = 27) or non-HSCT (n = 30) are summarized in Table 2.

Clinical consequences for the HSCT group. In the 27 patients (six auto-HSCT and 21 allo-HSCT) with a median duration of first CR at 22 months (range, 6–81 months), six patients relapsed and seven patients died, including four patients with TRM (Table 2).

Among the six patients who received auto-HSCT, the median duration of first CR was 22 months (range, 10–81 months), the median time from achievement of CR2 to HSCT was 6 months (range, 4–20 months), no TRM was seen and four patients (67%) relapsed at 9, 29, 46 and 84 months after auto-HSCT. Among those who relapsed, one died from APL progression 12 months after auto-HSCT. Another three patients achieved CR3 through treatment with ATO, Am80 or high-dose cytarabine and remained in CR3 at 7, 22, and 54 months after CR3, respectively. Of those who received auto-HSCT in CR2, four relapsed and one died, and the remaining two patients were alive in CR2.

In the 21 patients who received allo-HSCT in CR2, the median duration of first CR was 22 months (range, 6–63 months) and the median time from achievement of CR2 to HSCT was 6 months (range, 1–13 months). Of the 21 patients, four patients (19%) died of TRM (two patients died due to graft-versus-host disease [GVHD] and two patients died due to multiple organ failure) and two patients (9.5%) relapsed at 4 and 34 months after salvage HSCT and died. No significant difference in 5-year OS, EFS rates and CIR in seven patients with MRD before allo-HSCT was observed compared with eight patients negative for MRD (data not shown). Among those who received allo-HSCT in CR2, four died of TRM, two relapsed and died and the remaining 15 patients were alive in CR2.

Clinical consequences for the non-HSCT group. In the 30 patients in CR2 who did not receive any HSCT as post-remission therapy, the median duration of first CR was 18 months (range, 6–91 months) (Table 2). In CR2, these patients received consolidation treatment with various chemotherapy regimens, sometimes followed by maintenance treatment with ATRA. Of the 30 patients, 14 (47%) remained in CR2 after a median of 69 months (range, 2–133 months), but 16 (53%) experienced a second relapse after a median of 14 months (range, 1–113 months). One of the 14 patients who remained in CR2 died from secondary acute lymphoblastic leukemia.[17] Among the 16 patients who experienced a second relapse, eight received allo-HSCT (three in CR3, one in CR4, two in the second relapse and two in the third relapse) and two received auto-HSCT in CR3. Of these eight patients who

Table 2. Clinical consequences of APL patients in CR2 according to treatment with HSCT or non-HSCT after CR2

	HSCT group (n = 27)	Non-HSCT group (n = 30)	P-value
Male sex, n (%)	19 (70)	18 (60)	0.579
WBC counts at diagnosis, median (range) (x10^9/L)	3.9 (0.4–46.1)	1.9 (0.1–63.7)	0.129
Duration of first CR, median (range) (months)	22 (6–81)	18 (6–91)	0.415
Age at CR2, median (range) (years)	36 (22–59)	53 (16–72)	0.006
Relapses after CR2, n (%)	6 (22)	16 (53)	0.016
TRM, n (%)	4 (15)	N/A	N/A
Total deaths, n (%)	7 (26)	11 (37)	0.384
5-year EFS,% (95% CI)†	56.4 (32.1–74.9)	50.8 (30.4–68.0)	0.852
5-year OS,% (95% CI)†	70.4 (44.6–85.8)	77.4 (57.8–88.7)	0.860
5-year CIR,% (95% CI)	19.7 (7.1–36.8)	51.0 (31.0–67.9)	0.018

†Analyses were performed using a time-dependent covariate approach. APL, acute promyelocytic leukemia; CI, confidence interval; CIR, cumulative incidence of relapse; CR, complete remission; CR2, second complete remission; EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; N/A, not available; OS, overall survival; TRM, transplantation-related mortality; WBC, white blood cell.

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received allo-HSCT, four died from TRM (GVHD in two patients, pneumonia in one patient and multiple organ failure in one patient), two died from APL progression with further relapse after HSCT and two survived in a disease-free state. Of the two patients who received auto-HSCT, both remained in CR3. Of the six patients who experienced a second relapse and did not receive HSCT, one failed to obtain CR and died from APL progression and five patients achieved CR3 (two died of APL progression after the third relapse, one died of myocardial infarction and two remained in CR3 as of 22 and 23 months). Of those who received no HSCT in CR2, 13 patients were alive in CR2 and one patient died in CR2. Of the remaining 16 patients who relapsed, 10 patients died and six were alive in CR3 or more.

Comparisons between the HSCT and non-HSCT groups. Median age at CR2 was significantly younger in the HSCT group than in the non-HSCT group (P = 0.006) (Table 2). No significant differences were observed between these two groups in the frequency of male sex, white blood cell count at diagnosis or duration of first CR. The frequency of relapse after CR2 was significantly higher in the non-HSCT group (22% *vs* 53%; P = 0.016) (Table 2). However, the frequency of death did not differ between the two groups.

Although no significant differences in the 5-year OS rate (Table 2, Fig. 1a) or 5-year EFS rate (Table 2, Fig. 1b) were evident between the two groups, the CIR was significantly lower in the HSCT group than in the non-HSCT group (5-year CIR, 19.7% *vs* 51.0%; P = 0.018) (Table 2, Fig. 1c).

When we analyzed the data by dividing each group into two age subgroups of younger patients (age <40 years) and older patients (age ≥40 years), younger patients showed no significant difference in 5-year OS rate between the HSCT group (100%) and non-HSCT group (82.5%; P = 0.10), but did show a tendency in favor of allo-HSCT (Fig. 2a). Conversely, among the older patients, the OS rate was significantly higher in the non-HSCT group than in the HSCT group (5-year OS, 78.0% *vs* 40.5%; P = 0.04) (Fig. 2b). In the HSCT group, OS rate was significantly better in younger patients (age <40 years, n = 15; 5-year OS, 100%) than in older patients (age ≥40 years, n = 12; 5-year OS, 50.0%; P = 0.006) (Fig. 2c).

Comparisons among auto-HSCT, allo-HSCT and non-HSCT groups. We compared several outcomes among auto-HSCT, allo-HSCT and non-HSCT groups. No significant differences were seen in the 5-year EFS rate (auto-HSCT, 41.7%; allo-HSCT, 71.1%; non-HSCT, 45.4%) (Fig. 3a) or 5-year OS rate (auto-HSCT, 83.3%; allo-HSCT, 76.2%; non-HSCT, 75.3%) (Fig. 3b). However, 5-year CIR differed significantly between patients who underwent auto-HSCT (58.3%) and allo-HSCT (9.8%; P = 0.007) and between patients who underwent non-HSCT (51.0%) and allo-HSCT (9.8%; P = 0.009), while no significant difference was evident between the auto-HSCT and non-HSCT groups (P = 0.603) (Fig. 3c).

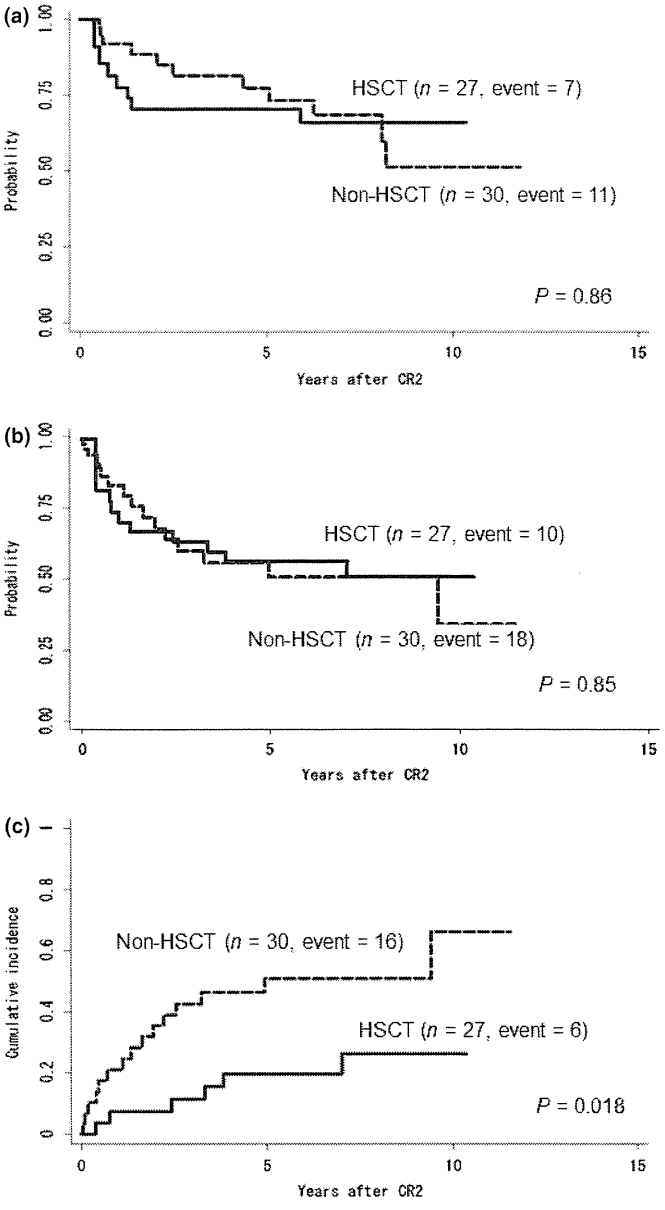

Fig. 1. Outcomes according to transplantation status. (a) Overall survival (OS). (b) Event-free survival (EFS). (c) Cumulative incidence of relapse. Probabilities of OS and EFS were assessed using a time-dependent covariate approach. CR2, second complete remission; HSCT, hematopoietic stem cell transplantation.

Discussion

The main results of the present study indicate that the 5-year CIR was significantly better in patients who underwent allo-HSCT than in those who did not and the 5-year OS rate was significantly better in the non-HSCT group than in the HSCT group among older patients (age ≥40 years).

Several studies have demonstrated that auto-HSCT for APL in CR2 yields favorable results with a relatively low relapse rate.[6,9] In an Italian study, it was reported that of 15 patients receiving auto-HSCT for APL in CR2 only two of eight patients who were negative for *PML-RARA* transcript by RT-PCR in bone marrow before auto-HSCT relapsed, whereas all seven patients with positive findings from the RT-PCR relapsed.[6] In a study from the European Acute Promyelocytic Leukemia Group reported, among 28 auto-grafted patients who were in molecular remission at the time of stem cell harvest, only three relapsed (7-year EFS rate, 76.5%).[9] A recent prospective study of our JALSG also observed a relatively low relapse rate, in which there were only three relapses among 23 auto-grafted patients with molecular remission at the time of stem cell harvest (5-year EFS rate, 65%).[14] These studies show a prognostic importance of MRD negativity using molecular analysis before HSCT on the outcome. However, the results of the present study differ from previous report in that the MRD negativity is well associated with the low relapse rates in auto-HSCT. Contrary to our expectation, both the 5-year EFS rate (41.7%) and the 5-year CIR (58.3%) were worse for the auto-HSCT group than for the allo-HSCT group

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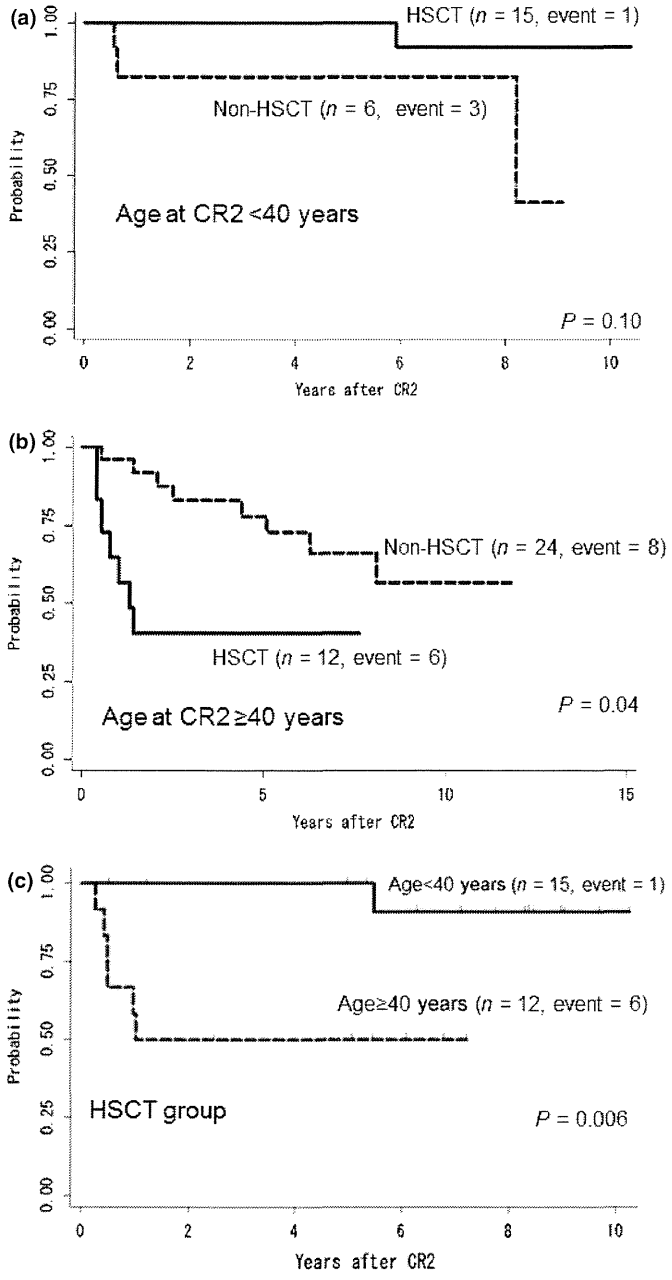

Fig. 2. Overall survival (OS) as a function of transplantation status in younger patients (a; age at second complete remission [CR2] <40 years) and older patients (b; age at CR2 ≥ 40 years) and as a function of age at CR2 in the hematopoietic stem cell transplantation (HSCT) group (c). A and B were assessed using a time-dependent covariate approach.

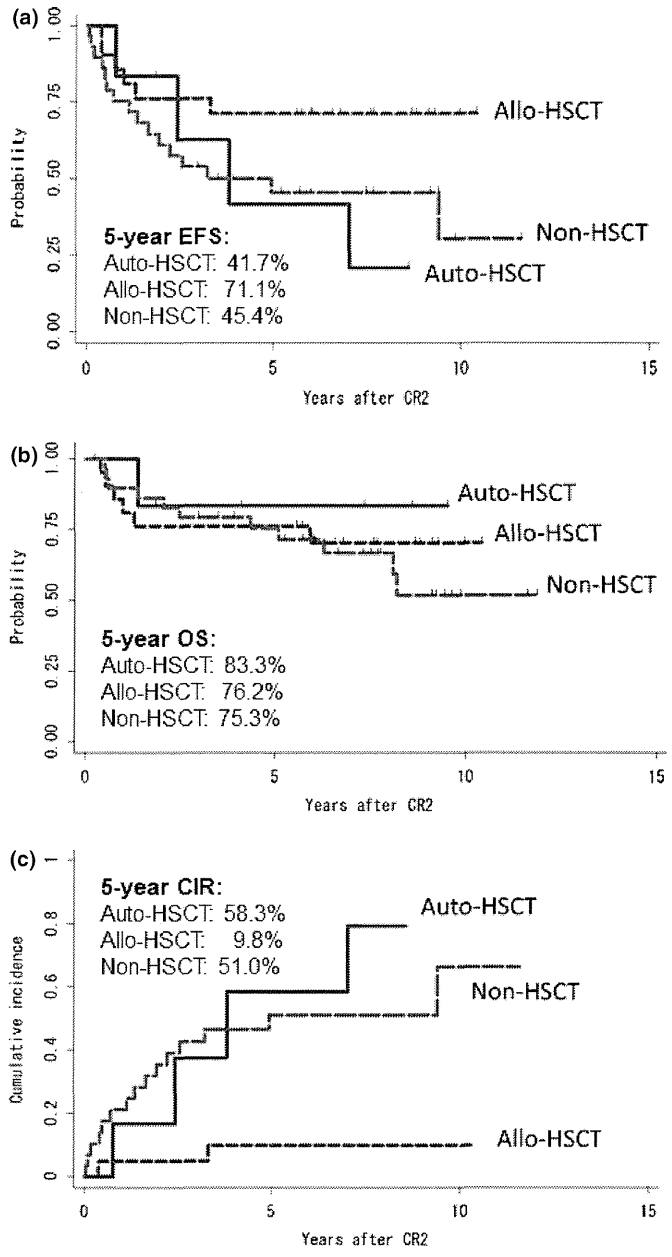

Fig. 3. Outcomes by type of hematopoietic stem cell transplantation (HSCT) (autologous [auto]-HSCT, allogeneic [allo]-HSCT or non-HSCT). (a) Event-free survival (EFS) rate. (b) Overall survival (OS) rate. (c) Cumulative incidence of relapse (CIR). CR2, second complete remission.

(Fig. 3a,c), even though all six patients were confirmed to have achieved molecular CR in bone marrow by nested RT-PCR or RT-PCR just before peripheral hematopoietic stem cell collection. Therefore, auto-HSCT was less effective for relapse in APL in CR2 and pre-transplant MRD had no predictive significance with respect to relapse in the present study. This might be due primarily to the small number of patients (n = 6) who received auto-HSCT in our analyses, which was the major limitation in the present study. Another possible explanation is the difference in sensitivity for the detection of MRD. In the APL97 study,[15] although all patients who received auto-HSCT were MRD negative before transplantation, the detection limit of the *PML-RARA* fusion transcript was 10^{-4}, whereas in the report by de Botton *et al.*[9], nested RT-PCR for *PML-RARA* amplification was used with a sensitivity of 10^{-5} to 10^{-6}. Although the 5-year EFS and 5-year CIR were worse in the auto-HSCT group in the present study, the 5-year OS rate (83.3%) was not inferior to that in the allo-HSCT and non-HSCT groups. No TRM was seen in the six patients who underwent auto-HSCT and all but one patient achieved CR3 by means of a range of post-relapse salvage treatments.

In the present study, none of the young patients (age <40 years) died within 5 years from the date of CR2. Taken together with our results that the 5-year OS rate tended to be better in the allo-HSCT group than in the non-HSCT group among younger patients (Fig. 2a), the 5-year EFS rate was better in allo-HSCT than in the auto-HSCT and non-HSCT groups

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(Fig. 3a) and the 5-year CIR was lower in allo-HSCT than in the auto-HSCT and non-HSCT groups (Fig. 3c), we suggest that conventional allo-HSCT represents an effective option for young APL patients who achieve CR2. The reason for this is that allo-HSCT is originally aimed at producing a graft-versus-leukemia effect in addition to direct antitumor effects of conditioning and is also regarded as an acceptable method of treatment in patients who are positive for pre-transplant MRD.

It is to be expected that patients in the non-HSCT group (those who did not undergo transplantation during CR2) would be older and those with complications, so the outcomes would be poorer than those of the transplant groups. However, counter to our expectations, survival outcomes in the non-HSCT group were relatively high (5-year EFS rate, 45.4%; 5-year OS rate, 75.3%) and not inferior to those in the HSCT groups. A similar result was reported by the European Acute Promyelocytic Leukemia Group, in which a consistent proportion of relapsed APL patients in CR2 who did not undergo transplantation were almost completely cured (EFS rate, 30.4%; OS rate, 39.5%).[9] Outcomes for the non-HSCT group were less favorable in the present study, but 13 of the 30 patients (43%) remained in CR2. The European APL study group also reported that 39% remained in CR2 in the non-HSCT group.[9] Such findings suggest that HSCT might not always be necessary for all patients in CR2 to prevent further relapse, given the potential for unnecessary TRM.

More recently, ATO has been used worldwide for the treatment of relapsed APL patients,[7,13,18] and has been included in the design of several front-line studies, with the aim of reducing therapy-related toxicities and obtaining more profound molecular remission. However, the efficacy of ATO alone in relapsed APL patients remains contentious. A study from France that treated relapsed APL reported that OS in patients with an ATO-based regimen was superior to that in patients with conventional combination chemotherapy or allo-HSCT,[13] but others have reported that an ATO-based regimen offered a high response rate but also a high relapse rate.[8,19] Moreover, a recent study from India that treated relapsed APL patients who had achieved molecular CR with ATO reported that the EFS rate was significantly inferior in patients who underwent continuous administration of ATO+ATRA without auto-HSCT (34%, $n = 19$) compared with that in patients treated with auto-HSCT after CR2 (83%, $n = 14$; $P = 0.001$).[20] The reason for such discrepancies in the effects of ATO-based regimen among different studies might be attributed to the small numbers of patients, selection bias and differences in economic constraints. Nevertheless, approximately 40% and 60% of patients receiving an ATO-based regimen relapsed in the French and Indian studies, respectively. In the present study, none of the relapsed patients were treated with ATO, because all relapsed before ATO gained approval for use in Japan. Only quite recently, our study group has reported better efficacy of a regimen of ATO followed by auto-HSCT for relapsed APL in the phase 2 study ($n = 23$; 5-year EFS, 65%).[14]

In conclusion, the present study suggests that allo-HSCT is favorably recommended for younger APL patients during CR2, but for older APL patients, safer and less toxic treatments such as non-myeloablative transplantation might be preferable. Nevertheless, given the small number of patients in the present study and the retrospective nature of the analysis, clear conclusions are difficult to reach. Further prospective studies with larger numbers of patients are required to confirm the role of HSCT both alone and in combination with ATO on the outcomes for patients with APL in CR2.

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Disclosure Statement

The authors have no conflict of interest.

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