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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.

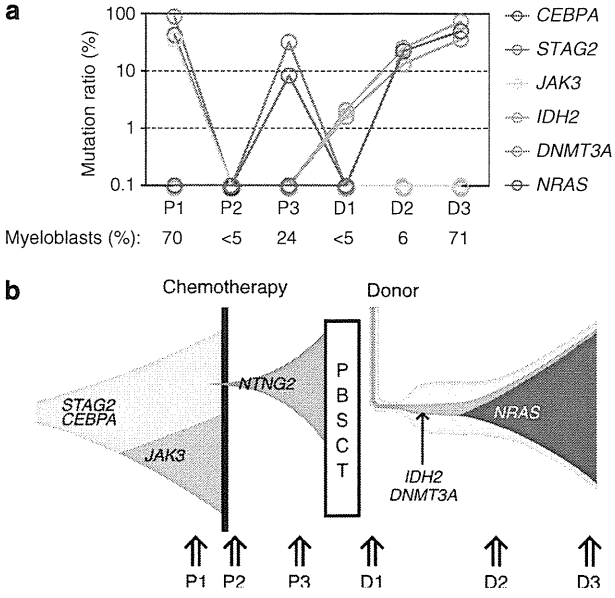

Figure 1. Genomic analysis of AML samples and donor PBSCs. (**a**) Genomic mutations corresponding to CEBPA(D107Tfs), STAG2(H73Rfs), JAK3(L857P), IDH2(R140Q), DNMT3A(V150Gfs) and NRAS(G13D) were examined by targeted deep sequencing in genomic DNA prepared from samples P1, P2, P3, D1, D2 and D3. The ratio 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

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.

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).

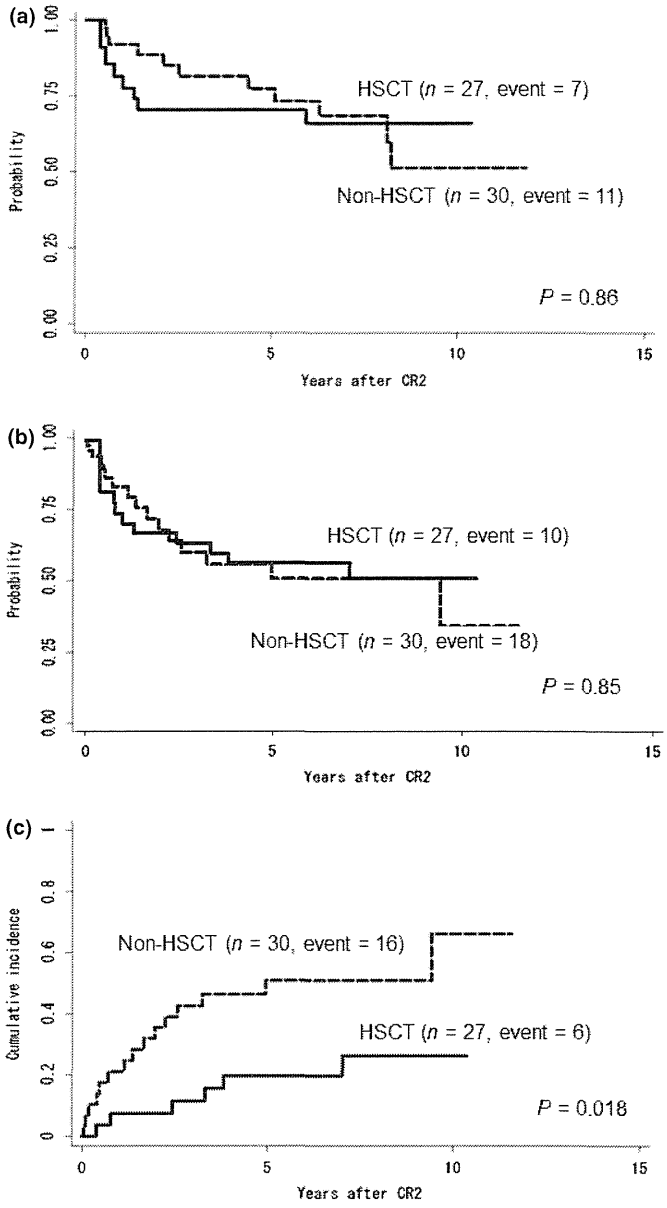

Fig. 1. Outcomes according to transplantation status. (a) Overall survival (OS). (b) Event-free survival (EFS). (c) Cumulative incidence of relapse. Probabilities of OS and EFS were assessed using a time-dependent covariate approach. CR2, second complete remission; HSCT, hematopoietic stem cell transplantation.

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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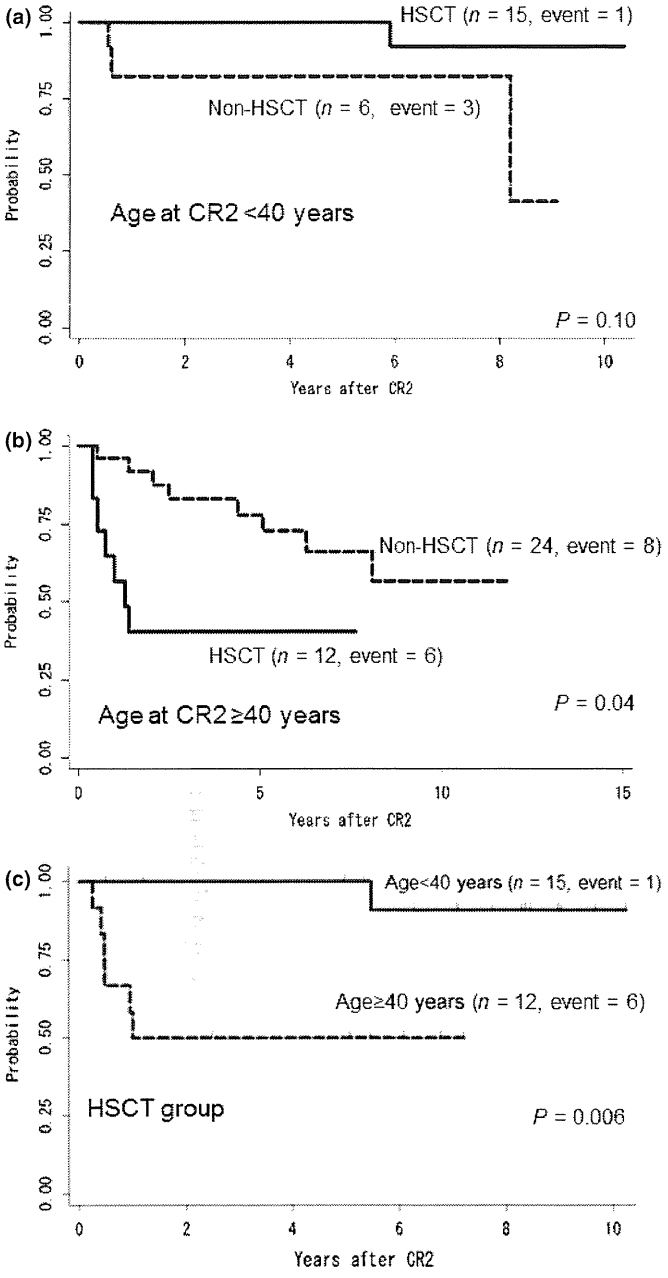

Fig. 2. Overall survival (OS) as a function of transplantation status in younger patients (a; age at second complete remission [CR2] <40 years) and older patients (b; age at CR2 ≥ 40 years) and as a function of age at CR2 in the hematopoietic stem cell transplantation (HSCT) group (c). A and B were assessed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

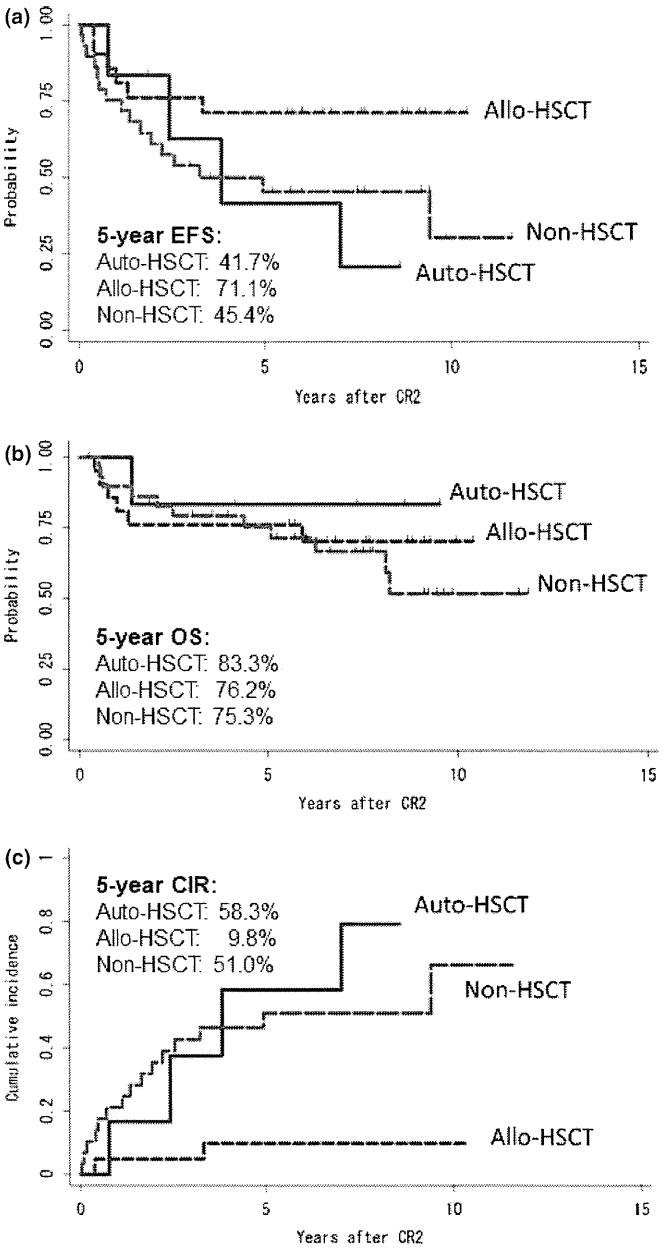

Fig. 3. Outcomes by type of hematopoietic stem cell transplantation (HSCT) (autologous [auto]-HSCT, allogeneic [allo]-HSCT or non-HSCT). (a) Event-free survival (EFS) rate. (b) Overall survival (OS) rate. (c) Cumulative incidence of relapse (CIR). CR2, second complete remission.

(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

(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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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

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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

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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

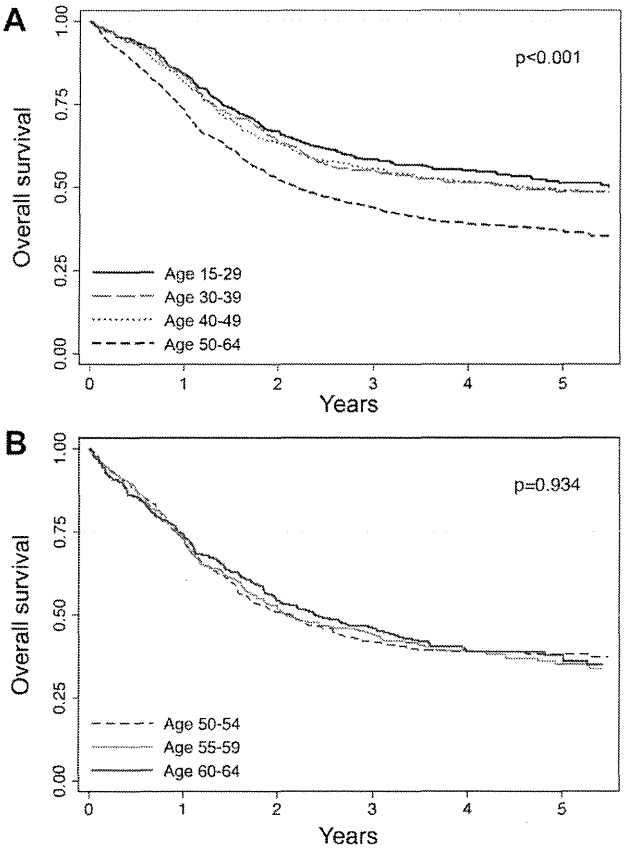

Figure 1. Overall survival for (A) the entire cohort and (B) for patients 50 to 64 years, is shown by age group. First, patients aged 15 to 29 (N = 438), 30 to 39 (N = 391), 40 to 49 (N = 510), and 50 to 64 (N = 937) are compared, and those aged 50 to 64 are then divided into 3 groups: 50 to 54 (N =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

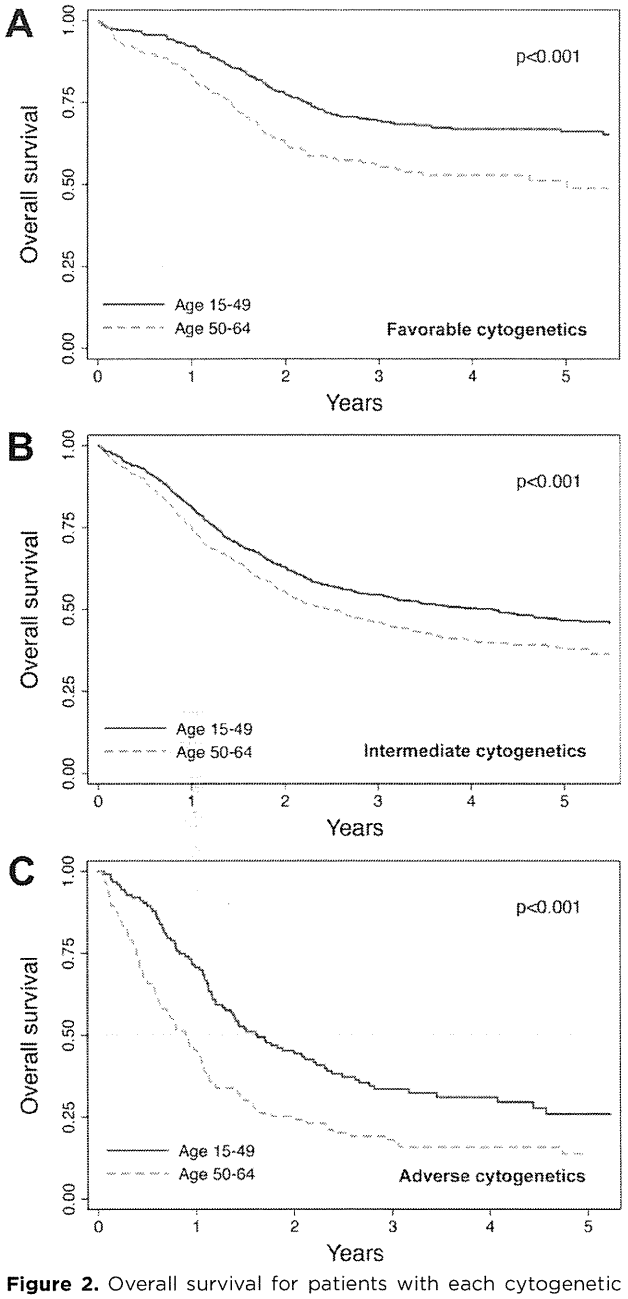

Figure 2. Overall survival for patients with each cytogenetic risk is shown by age group. Patients with (A) favorable (N = 515), (B) intermediate (N = 1461), and (C) adverse (N = 229) cytogenetics are shown separately.

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.

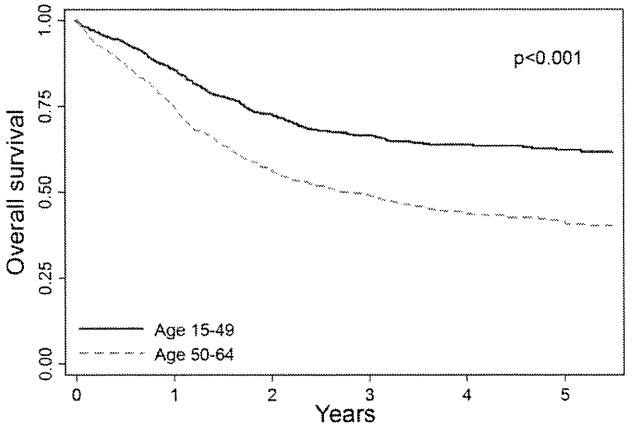

Figure 3. Overall survival is shown by age group with censoring of patients undergoing allogeneic hematopoietic cell transplantation. Patients aged 15 to 49 (N = 1339) and 50 to 64 years (N = 937) are compared, with those undergoing allogeneic hematopoietic cell transplantation censored at the time of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

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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 ≥60 mm Hg or SpO_2 ≥93%), and heart (no severe abnormalities detected on electrocardiograms). Patients who

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