Original Study

Comparison of Autologous Hematopoietic Cell Transplantation and Chemotherapy as Postremission Treatment in Non-M3 Acute Myeloid Leukemia in First Complete Remission

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Abstract

Randomized trials of acute myeloid leukemia (AML) in first complete remission (CR1) showed that autologous hematopoietic cell transplantation (auto-HCT) improves relapse-free survival (RFS) but not overall survival (OS), compared with chemotherapy. Using a database of 2518 adult patients with AML in CR1, we conducted a 5-month landmark analysis and found that auto-HCT improves 3-year RFS but not OS compared with chemotherapy. **Introduction:** A number of randomized trials in patients with AML in CR1 have been conducted and they showed that auto-HCT improves RFS but not OS, compared with chemotherapy. However, because these trials have had compliance problems, the value of auto-HCT still has not been clearly established. **Patients and Methods:** Using a database of 2518 adult patients with AML in CR1, we retrospectively analyzed the outcome of auto-HCT and compared it with intensive nonmyeloablative chemotherapy using landmark analyses. **Results:** In 103 auto-HCT recipients, OS and RFS at 3 years from treatment were 65% and 57%, respectively. Multivariate analysis showed that unfavorable risk cytogenetics and entry into CR1 after 2 courses of induction treatment predicted a poor outcome. Because the median time interval between CR1 and auto-HCT was 153 days, landmark analyses at 5 months after CR1 were performed to compare 1290 patients who received chemotherapy alone (median age, 52 years; range, 16-70) with 103 who received auto-HCT (median age, 48 years; range, 16-67). Auto-HCT improves 3-year RFS (58% vs. 37%; P < .001) but not OS compared with chemotherapy alone. Among patients with unfavorable risk cytogenetics or those who required 2 courses to reach CR1, there was no significant difference in RFS between the 2 groups. **Conclusion:** Auto-HCT can be considered as a postremission therapy for AML patients with favorable or intermediate risk cytogenetics who achieve CR1 after a single course of induction treatment.

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Introduction

Autologous hematopoietic cell transplantation (auto-HCT) has been investigated as a potential therapeutic option to improve the outcome in acute myeloid leukemia (AML) patients. However, its value in the treatment of adults in remission has not been clearly established. Compared with allogeneic hematopoietic cell transplantation (HCT), auto-HCT offers the possibility of performing the same myeloablative regimen without the risks associated with graft-versus-host disease. Though the toxic death rate in auto-HCT is much lower than that in allogeneic HCT, the relapse rate remains higher[1-6] because of either graft contamination by malignant cells[7] or the absence of a graft-versus-leukemia effect by donor lymphocytes. To date, randomized trials in patients with AML in first complete remission (CR1) have been conducted to compare the postremission strategies of intensive chemotherapy, allogeneic HCT, and auto-HCT.[8-19] All of these trials analyzed the outcome on an intention-to-treat basis, and only 66% of patients actually underwent the intended auto-HCT treatment.[2-4,20] This can clearly pose problems in interpretation when a significant proportion of patients do not actually undergo the intended treatment.[19] On the other hand, despite the limitations of biases that might be difficult or impossible to identify and/or adjust for, observational databases contain information on large numbers of diverse subjects who have received diverse therapies, and can be analyzed to potentially provide answers that are more useful to clinicians than those obtained from randomized controlled trials.[21]

In the present study, we used a database of 2518 adult AML patients who achieved CR1 to retrospectively compare auto-HCT with intensive nonmyeloablative chemotherapy in AML patients in CR1.

Patients and Methods

Data Source

We created a nation-wide database of AML patients in CR1.[22] The targeted patients were adults aged 16-70 years who had been diagnosed with AML between 1999 and 2006, and who had achieved CR1 after 1 or 2 courses of induction chemotherapy. The diagnosis of AML was determined according to the World Health Organization classification fourth edition.[23,24] The National Cancer Center Hospital's institutional review board approved the protocol. Clinical data for more than 2600 patients were collected from 70 institutions between June and December 2008. Among them, patients with acute biphenotypic leukemia who were treated with chemotherapy for acute lymphocytic leukemia and those who had extramedullary AML without marrow invasion, or extramedullary lesions that did not totally disappear after remission-induction chemotherapy were excluded. In this study, patients with acute promyelocytic leukemia and those who received allogeneic HCT in CR1 were also excluded. Information about the disease risk at diagnosis, clinical course, and conditioning regimen for auto-HCT were collected.

Statistical Analysis

Data were retrospectively reviewed and analyzed as of April 2010. The primary end point of the study was overall survival (OS) with respect to either auto-HCT or CR1. The unadjusted probabilities of OS, relapse-free survival (RFS), and relapse rate were estimated using the Kaplan–Meier product limit method. OS, RFS, and the incidence of relapse were estimated as probabilities at 3 years after either auto-HCT or CR1. The log-rank test was used to compare the probabilities among different subgroups. The Cox proportional hazards regression model was used to estimate relative hazard ratios for OS, RFS, and the incidence of relapse. As covariates, we considered age, sex, conditioning regimen, interval from CR1 to auto-HCT, cytogenetic risks according to the Southwest Oncology Group (SWOG)[25], French-American-British (FAB) classifications,[24,26-29] number of courses of chemotherapy required to achieve CR1, white blood cell (WBC) count, and antecedent hematological disorders or dysplasia at diagnosis. We judged 2-tailed P values < .05 to be statistically significant. Statistical analyses were performed with SPSS software version 11.0.1 (SPSS, Chicago, IL).

Results

Patient Characteristics

We excluded 494 patients who had received allogeneic HCT in CR1 and 386 acute promyelocytic leukemia patients from the total of 2518 patients. Table 1 summarizes the characteristics of the remaining 1638 patients. Auto-HCT was used to treat 103 patients (auto-HCT group), and the other 1535 were treated with chemotherapy alone (chemotherapy group). Median follow-up times for the total test population and auto-HCT group were 50 months (0.2-116 months) and 60 months (6-115 months), respectively.

The proportions of patients in the auto-HCT group with favorable, intermediate, unfavorable, and unknown risk cytogenetics according to the SWOG criteria were 26%, 49%, 17%, and 9%, respectively. These values were not significantly different from those in patients who were treated with chemotherapy alone. As a remission induction therapy, 95% or more of patients in both groups had received standard-dose cytarabine and anthracycline (daunorubicin or idarubicin) -based regimen. Consolidation therapy was continued with cytarabine-based regimens with or without maintenance therapy at the discretion of physicians.

There was no significant difference in FAB subtypes, the number of remission-induction therapies, or the WBC count at the time of diagnosis between the 2 groups. However, the proportion of patients who had antecedent hematological disorders or dysplasia at diagnosis was significantly lower in auto-HCT patients than in chemotherapy patients (P = .011). Auto-HCT patients were significantly younger than the chemotherapy patients (P = .006).

Among auto-HCT patients, 62 (70%) received granulocyte colony stimulating factor (G-CSF) combined with BEA (busulfan/etoposide/cytosine arabinoside)[30,31] as a conditioning regimen: busulfan (4 mg/kg per day, 1 mg/kg per dose, 4 times a day [days −9 to −6], for 16 doses), etoposide (20 mg/kg on days −5 to −4), cytarabine (100 mg/m² on days −10 to −4, 3 g/m² every 12 hours on days −3 to −2), and filgrastim (200 μg/m² on days −12 to −4). The median time interval between CR1 and transplantation was 153 days (21-749 days). Only 8 patients (8%) received transplants within 100 days after reaching CR1, and approximately half of the patients (n = 55; 53%) underwent transplantation between 101 and 180 days after CR1.

Table 1 Patient Characteristics According to Treatment in CR1

Characteristic	Chemotherapy, n = 1535 n (%)	Auto-HCT, n = 103 n (%)	P
Age			.006
Median, years	52	48	
Cytogenetic Risk (SWOG)			.895
Favorable	360 (24)	26 (26)	
Intermediate	777 (51)	49 (49)	
Unfavorable	246 (16)	17 (17)	
Unknown	129 (9)	9 (9)	
FAB			.125
M1, 2, 4, 5	1345 (88)	94 (91)	
M0, 6, 7	104 (7)	3 (3)	
RAEB-t	32 (2)	3 (3)	
Unknown	54 (4)	3 (3)	
Remission Induction			.725
1 course	1276 (83)	87 (84)	
Standard-dose cytarabine with anthracycline	1455 (95)	101 (98)	
Low-dose cytarabine-based	65 (4)	1 (1)	
Others/data not available	15 (1)	1 (1)	
2 courses	259 (17)	16 (16)	
Dysplasia[a]			.011
No	1264 (83)	95 (92)	
Yes	268 (17)	8 (8)	
WBC			.351
≤ 20,000/μL	887 (61)	55 (56)	
> 20,000/μL	570 (39)	43 (44)	

Abbreviations: auto-HCT = autologous hematopoietic cell transplantation; CR1 = first complete remission; FAB = French-American-British; RAEB-t = refractory anemia with excess of blasts in transformation; SWOG = Southwest Oncology Group; WBC = white blood cell.
[a] Dysplasia contains patients with RAEB-t.

Outcomes of Auto-HCT

The relapse rate for 103 patients who received auto-HCT was 42% at 3 years from HCT (Figure 1A). There was only 1 case of nonrelapse mortality: a 38-year-old male who died of pulmonary hemorrhage 2.9 months after auto-HCT. The RFS and OS at 3 years after auto-HCT were 57% and 65%, respectively (Figure 1B and C).

The univariate analysis indicated that unfavorable risk cytogenetics according to the SWOG criteria and 2 courses of remission-induction treatment were associated with lower OS (P = .014 and P = .044, respectively) and RFS (P = .001 and P = .005, respectively), and a higher relapse rate (P = .001 and P = .004, respectively). The M0, 6, and 7 subgroups of the FAB classification, which are poor prognostic factors in the Japan Adult Leukemia Study Group scoring system,[32] were also shown to be significantly associ-

ated with lower RFS and a higher relapse rate (P = .018 and P = .018, respectively). Table 2 shows the results of multivariate analyses to determine independent prognostic factors. Although the M0, 6, and 7 subgroups of the FAB classification were associated with worse RFS and higher relapse rates in the univariate analysis, we excluded this factor from the multivariate analysis because of the small number of patients (n = 3). Unfavorable risk cytogenetics according to the SWOG criteria and 2 courses of remission-induction treatment were associated with lower OS and RFS, and a higher relapse rate.

The 3-year OS of patients with favorable, intermediate, and unfavorable risk cytogenetics were 84%, 60%, and 41%, respectively (P = .003, Figure 2A), and 3-year RFS were 77%, 63%, and 19%, respectively (P = .034, Figure 2B). Patients who required 2 courses of induction treatment to achieve complete remission (CR) had lower OS and RFS than those who required only 1 course of treatment (OS, 47% vs. 69%, P = .039; RFS, 34% vs. 62%, P = .002, Figure 2C and D).

In 89 patients for whom data regarding conditioning regimens were available, the 3-year OS did not differ between patients treated with G-CSF combined with BEA (74.5%, n = 62) and others (80%; n = 27; P = .834). Though the 3-year RFS was slightly higher in patients treated with G-CSF combined with BEA, the difference between the 2 groups was not statistically significant (69% vs. 59%) (P = .245).

When patients were divided into 4 groups according to the interval from CR1 to auto-HCT, there were no significant differences in the 3-year OS or RFS among the groups (Table 3). As shown in a multivariate Cox proportional hazard regression model (Table 2), there was no difference in survival rates when the groups were merged successively. Thus, we could not identify an appropriate cutoff point for the interval from CR1 to auto-HCT for OS and RFS. In the fourth group, the interval was rather broad, and ranged from 241 to 749 days. When we excluded this fourth group, the 3-year OS and RFS tended to be higher in subgroups with longer intervals.

Landmark Analysis Comparing Auto-HCT With Chemotherapy Alone

We next compared the outcomes for patients who received auto-HCT in CR1 (n = 103) with those for patients who did not receive either autologous or allogeneic HCT in CR1 (n = 1535). Because the median time interval between CR1 and auto-HCT was 153 days (21-749 days), landmark analyses at 5 months after CR1 were performed for all subgroups. We excluded 245 patients from the chemotherapy group who relapsed or died within 5 months after achieving CR1. The relapse rate in the auto-HCT group was significantly lower than that in the chemotherapy group (41% vs. 62% at 3 years from CR1, P < .001, Figure 3A). Nonrelapse mortality did not differ significantly between the auto-HCT and chemotherapy groups (1.1% vs. 1.4% at 3 years, P = .400). The 3-year RFS in the auto-HCT group was significantly higher than that in the chemotherapy group (58% vs. 37%, P < .001, Figure 3B). There was no significant difference between the auto-HCT and chemotherapy groups with regard to 3-year OS (68% and 64%, respectively, P = .169, Figure 3C).

By a subset analysis, patients with favorable and intermediate risk cytogenetics had the same trends in relapse rate, RFS, and OS

Figure 1 Outcomes of Autologous Hematopoietic Cell Transplantation (Auto-HCT) in Acute Myeloid Leukemia in First Complete Remission. Cumulative Incidence of Relapse (A), Relapse-Free Survival (B), and Overall Survival (C) After Auto-HCT are Shown
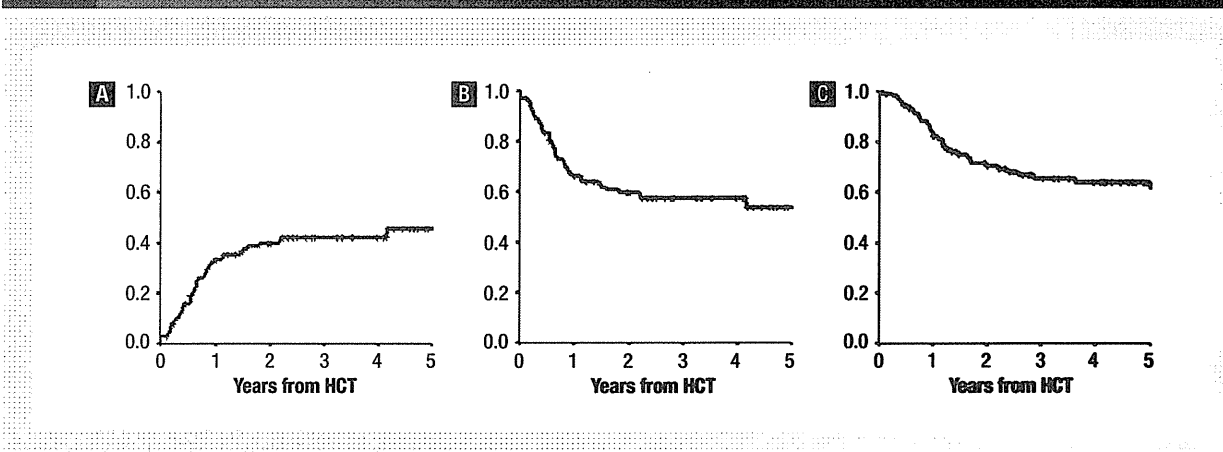

Table 2 Multivariate Analysis of the Auto-HCT Patients

Variables	OS		RFS		Relapse	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age (Vs. ≤ 49)						
≥ 50	1.25 (0.60-2.60)	.549	1.17 (0.57-2.39)	.671	1.29 (0.62-2.66)	.495
Cytogenetic Risk (SWOG: Vs. Favorable)						
Intermediate	1.64 (0.59-4.55)	.343	1.51 (0.54-4.22)	.436	1.37 (0.48-3.87)	.558
Unfavorable	3.43 (1.14-10.36)	.028	4.24 (1.46-12.31)	.008	4.27 (1.47-12.42)	.008
Remission Induction (Vs. 1 Course)						
2 Courses	3.28 (1.37-7.86)	.008	3.55 (1.56-8.06)	.003	3.89 (1.69-8.95)	.001
Conditioning (Vs. G-CSF with BEA)						
Others	1.05 (0.47-2.33)	.902	0.74 (0.35-1.55)	.418	0.72 (0.34-1.51)	.378
Interval from CR1 to Auto-HCT (Vs. > 150 Days)						
≤ 150 Days	1.37 (0.66-2.83)	.398	1.34 (0.65-2.75)	.432	1.23 (0.59-2.56)	.578

Abbreviations: auto-HCT = autologous hematopoietic cell transplantation; BEA = busulfan/etoposide/cytosine arabinoside; CR1 = first complete remission; G-CSF = granulocyte colony stimulating factor; HR = hazard ratio; OS = overall survival; RFS = relapse-free survival; SWOG = Southwest Oncology Group.

as in the total population (Figure 4). Among patients with favorable risk cytogenetics, the 3-year rate of relapse in the auto-HCT group (n = 26) was significantly lower than that in the chemotherapy group (n = 335, 23% vs. 56%, 3 years from CR1, *P* = .002). The 3-year RFS in the auto-HCT group was significantly higher than that in the chemotherapy group (77% vs. 42%, *P* = .002, Figure 4A). There was no significant difference between the auto-HCT and chemotherapy groups with regard to 3-year OS (85% vs. 72%, *P* = .234, Figure 4B).

Similarly, in patients with intermediate risk cytogenetics, the relapse rate in the auto-HCT group (n = 49) was significantly lower than that in the chemotherapy group (n = 658, 36% vs. 59% at 3 years from CR1, *P* = .002). The 3-year RFS in the auto-HCT group was significantly higher than that in the chemotherapy group (63%

vs. 40%, *P* = .002, Figure 4C). There was no significant difference between the auto-HCT and chemotherapy groups with regard to 3-year OS (65% vs. 66%, *P* = .484, Figure 4D). In contrast, in patients with unfavorable risk cytogenetics, there was no significant difference between the auto-HCT (n = 17) and chemotherapy (n = 178) groups with respect to relapse rate (81% vs. 69%, *P* = .778), RFS (19% vs. 31%, *P* = .735, Figure 4E), or OS (41% vs. 49%, *P* = .787, Figure 4F).

Among patients who achieved CR1 after a single induction treatment, 3-year RFS and relapse rate in the auto-HCT group (n = 87) were significantly better than those in the chemotherapy group (n = 1100, RFS, 63% vs. 40%, *P* < .001, Figure 4G; relapse, 37% vs. 60%, *P* < .001). There was no significant difference in 3-year OS between these 2 groups (72% vs. 67%, *P* = .193, Figure 4H). In

Figure 2 Overall Survival and Relapse-Free Survival After Autologous Hematopoietic Cell Transplantation (Auto-HCT) According to Cytogenetic Risks or the Number of Induction Treatment Courses Required to Reach First Complete Remission (CR1). Overall Survival (A and C) and Relapse-Free Survival (B and D) According to Cytogenetic Risks (A and B) or the Number of Induction Treatment Courses Required to Reach CR1 (C and D). *P* Values Were Calculated by the Log-Rank Test
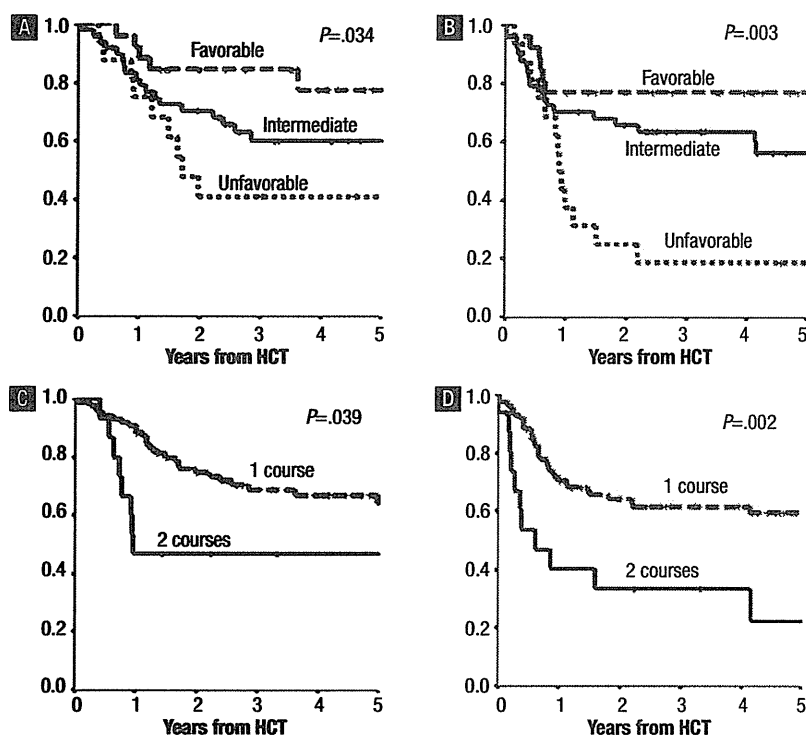
Table 3 Outcomes According to Interval from CR1 to Auto-HCT

Interval From CR1 (Days)	≤120, n = 21	121-150, n = 29	151-240, n = 30	≥241, n = 23	P
Relapse (%)	54.6	46.2	25.9	44.5	.37
RFS (%)	45.4	51.7	74.1	55.5	.32
OS (%)	59.1	61.5	73.9	63.2	.51

Outcomes are presented as the probability at 3 years from transplantation.
Abbreviations: auto-HCT = autologous hematopoietic cell transplantation; CR1 = first complete remission; OS = overall survival; RFS = relapse-free survival.

contrast, among those who received 2 courses of induction, there was no significant difference between the auto-HCT (n = 16) and chemotherapy (n = 190) groups with regard to relapse rate (66% vs. 75%, *P* = .414), RFS (34% vs. 24%, *P* = .367, Figure 4I), or OS (47% vs. 48%, *P* = .705, Figure 4J).

Among patients younger than 60 years of age, 3-year RFS and relapse rate in the auto-HCT group (n = 89) were significantly better than those in the chemotherapy group (n = 890, RFS, 60% vs. 38%, *P* < .001; relapse, 39% vs. 61%, *P* < .001). There was no difference

in 3-year OS between these 2 groups (68% vs. 67%, *P* = .545). In contrast, in patients aged 60 years or older, there was no difference between the auto-HCT (n = 14) and chemotherapy (n = 400) groups with respect to the relapse rate (52% vs. 63%, *P* = .294), RFS (47% vs. 36%, *P* = .237), or OS (77% vs. 59%, *P* = .224).

Discussion

In this study, we analyzed the outcomes in 103 AML patients who received auto-HCT in CR1. The 5-month landmark analyses indicated that auto-HCT improves RFS but not OS compared with chemotherapy alone. Our data are consistent with the findings of a meta-analysis of randomized studies of more than 1000 AML patients, which indicated that patients who received auto-HCT had a better RFS, albeit a similar OS, than those who received chemotherapy or no further treatment.[16-18] In addition, our findings were consistent with recently reported results of a randomized study in which more than 90% of randomized patients received their assigned treatment.[19]

We found that despite better RFS, auto-HCT did not improve OS in CR1 patients. Among the patients who relapsed after auto-HCT, the OS was only 21% at 3 years after relapse, although 46% of them received allogeneic HCT. We reported that, in patients who relapsed after receiving chemotherapy alone, the 3-year OS was 30% with

Figure 3 Comparison of Autologous Hematopoietic Cell Transplantation (Auto-HCT) and Chemotherapy Alone Using a Landmark Analysis at 5 Months After First Complete Remission (CR1). Cumulative Incidence of Relapse (A), Relapse-Free Survival (B), and Overall Survival (C), in the Auto-HCT and Chemotherapy Groups are Compared. *P* Values Were Calculated by the Log-Rank Test
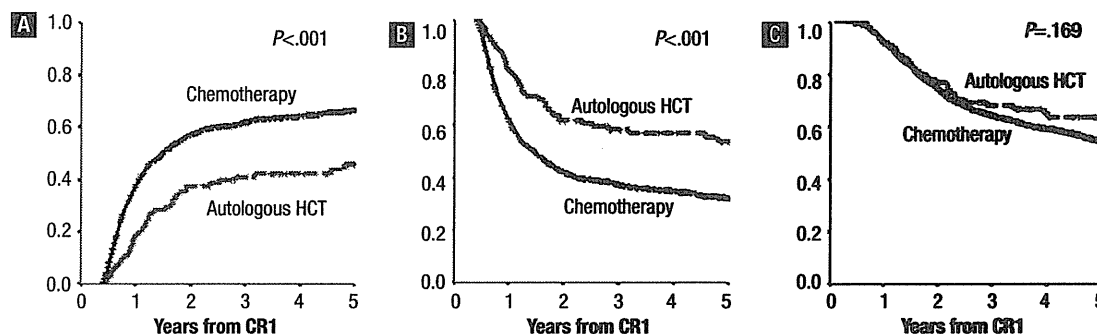
54% of them receiving salvage allogeneic HCT.[33] Thus, allogeneic HCT after relapse may have contributed to the improved OS in the chemotherapy group.

A European study[34] reported that a less favorable outcome was associated with a shorter interval from CR1 to auto-HCT. We found that, after excluding auto-HCT patients with an interval of > 240 days, a longer interval was associated with a better 3-year OS and RFS, although the differences were not statistically significant (Table 3). The better RFS with late transplants compared with early transplants might have resulted from the fact that patients who received transplantation later received more courses of chemotherapy, which also resulted in more intensive in vivo purging, as shown in previous studies.[35-37]

The WBC count at diagnosis has been reported to reflect the prognosis.[32,38] However, in the current study, a high WBC count did not have any prognostic significance. This suggests that myeloablative conditioning treatment might overcome the unfavorable nature of AML characterized by a high WBC count. In the current study, the influence of the source of stem cells was unclear, because the relevant data (peripheral blood [PB] or bone marrow [BM]) were not collected. However, our data were collected at a time when PB was commonly used for auto-HCT.

The number of induction treatments required to achieve CR1 has been reported to serve as a prognostic factor.[33,37] In this study, multivariate analysis for survival and relapse rates after auto-HCT showed that 2 courses of induction treatment to achieve CR1 were associated with a poor prognosis for auto-HCT patients. In patients who require a single induction, auto-HCT improved RFS compared with chemotherapy alone. In contrast, among those who required 2 courses of induction treatment, auto-HCT did not improve RFS compared with chemotherapy alone.

The present study showed that in favorable and intermediate risk cytogenetics patients, auto-HCT provides better RFS than chemotherapy alone. The multivariate analysis revealed that unfavorable risk cytogenetics was associated with poor outcomes. The results of a meta-analysis of 24 prospective trials with 6007 AML patients showed that, compared with nonallogeneic HCT therapies, alloge-

neic HCT improved RFS and OS for intermediate- or poor-risk AML patients in CR1.[39] Compared with nonallogeneic HCT therapies, allogeneic HCT was reported to offer no survival advantage for favorable risk AML in CR1.[39-42] It has been reported that, because of the low CR rate after reinduction therapy and an inferior survival duration especially after relapse with t(8;21),[40] HCT is the postremission therapy of choice in patients with additional adverse factors.

At present, the indications for allogeneic HCT for patients diagnosed with intermediate risk AML have not been fully defined when unrelated donors are used.[43,44] The present findings suggest that auto-HCT can serve as an alternative option for AML CR1 patients with intermediate risk AML who do not have an appropriate sibling donor.

Our study has several limitations, and thus the results must be interpreted with caution. These limitations include the retrospective nature of the study, leaving room for selection bias or chance effect. The auto-HCT group included significantly younger patients and fewer patients with myelodysplastic syndrome related AML than the chemotherapy group. This imbalance might have influenced the results. However, this large retrospective analysis using landmark methods should have important implications in determining the indication of auto-HCT.

Conclusion

On the basis of our results, we believe that auto-HCT might be recommended as a first-line postremission therapy for favorable or intermediate risk AML patients who have achieved CR1 after a single induction. It remains unclear whether auto-HCT is more beneficial than high-dose cytarabine for AML patients with favorable risk cytogenetics. Moreover, it also remains to be seen whether auto-HCT is a better option for those with intermediate risk AML in CR1 when they do not have a suitable related donor. Our observation needs to be confirmed in a prospective study.

Clinical Practice Points

● A number of randomized trials in patients with AML in CR1 have been conducted and they showed that auto-HCT improves re-

Figure 4 Comparison of Autologous Hematopoietic Cell Transplantation (Auto-HCT) and Chemotherapy Alone Using a Landmark Analysis at 5 Months After First Complete Remission (CR1) According to Cytogenetic Risks or the Number of Induction Treatment Course Requiring CR1. Relapse-Free Survival (A, C, E, G, and I) and Overall Survival (B, D, F, H, and J). After Receiving Auto-HCT and Chemotherapy Alone are Shown in the Subgroups of the Southwest Oncology Group Criteria: (A and B) Favorable Risk Cytogenetics; (C and D) Intermediate Risk Cytogenetics; and (E and F) Unfavorable Risk Cytogenetics; or in the Subgroups of the Number of Induction Treatment Courses: (G and H) 1 Course; and (I and J) 2 Courses. P Values Were Calculated by the Log-Rank Test
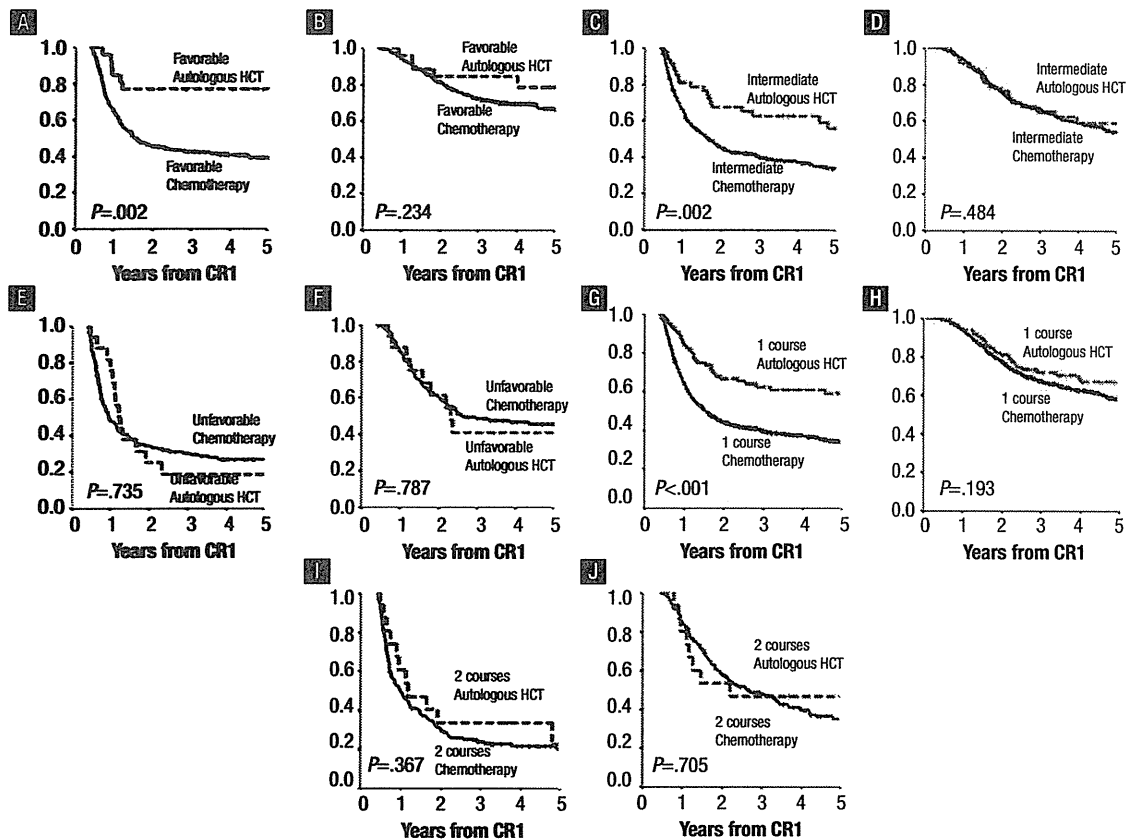
lapse-free survival but not overall survival, compared with chemotherapy.

● Because these trials have had compliance problems, the value of auto-HCT still has not been clearly established.

● To avoid this problem, we constructed a database of 2518 patients with non-M3 AML in CR1 collected from 70 institutions and conducted landmark analyses to compare the outcome of auto-HCT with intensive nonmyeloablative chemotherapy.

● In 103 auto-HCT recipients, multivariate analysis showed that unfavorable risk cytogenetics and entry into CR1 after 2 courses of induction treatment predicted a poor outcome.

● Because the median time interval between CR1 and auto-HCT was 153 days, landmark analyses at 5 months after CR1 were performed to compare 1290 patients who received chemotherapy alone with 103 who received auto-HCT. Auto-HCT improves 3-year RFS (58% vs. 37%; P < .001) but not OS compared with chemotherapy alone.

● Among patients with unfavorable risk cytogenetics or those who required 2 courses to reach CR1, there was no significant difference in RFS between the 2 groups.

● Auto-HCT can be considered as a postremission therapy for AML patients with favorable or intermediate risk cytogenetics who achieve CR1 after a single course of induction treatment.

Acknowledgments

Disclosure

All authors have no conflicts of interest.

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Expansion of CD8+/perforin+ T-cells predicts response to ciclosporin A therapy in patients with erythroid hypoplasia/aplasia

Erythroid hypoplasia or aplasia is a haematological condition observed in acquired pure red cell aplasia (PRCA) and aplastic anaemia. Myelodysplastic syndrome (MDS) with erythroid hypoplasia/aplasia is a rare form of MDS that is not included in existing classifications of MDS. Patients with erythroid hypoplasia/aplasia have common characteristics: transfusion dependence, immunological abnormalities, and successful immunosuppressive therapies, such as ciclosporin A (CsA) and/or antithymocyte globulin (ATG). Thus, we may regard erythroid hypoplasia/aplasia as an immunological disease entity. However, the pathogenic mechanisms of erythroid hypoplasia/aplasia have not been fully elucidated, although T-lymphocyte-mediated inhibition of erythropoiesis is considered the most likely mechanism of pathogenesis (Fisch *et al*, 2000). It has been reported that autoreactive CD8+ T cells in patients with thymoma-associated PRCA suppress erythropoiesis *in vitro* (Mangan *et al*, 1986). Recently, we reported that oligoclonal expansion of CD8+/perforin+ T cells in the bone marrow was observed in patients with thymoma-associated PRCA, and the oligoclonality was exclusively detected in CD8+ T cells, not in CD4+ T cells (Nitta *et al*, 2010). To clarify the pathogenic role of the T cells, we analysed the T-cell subsets and therapeutic responses in patients with erythroid hypoplasia/aplasia.

A total of 22 patients with erythroid hypoplasia/aplasia, including eight MDS with erythroid hypoplasia/aplasia, three idiopathic PRCA, three thymoma-associated PRCA, and eight aplastic anaemia, were enrolled in this study. Patients with erythroid hypoplasia/aplasia that were possibly due to viral infection were excluded from this study. All patients were treated with CsA alone, and improvement of anaemia was

evaluated in accordance with the International Working Group (IWG) 2006 criteria of MDS (Cheson *et al*, 2007) and guidelines of acquired aplastic anaemia and PRCA (Marsh *et al*, 2003; Sawada *et al*, 2007). Erythroid cellularity in all the patients was confirmed by performing bone marrow aspiration and/or biopsy. For T-cell subset analysis, mononuclear cells (MNCs) were purified from the bone marrow (BM) or peripheral blood (PB) of the patients. As controls, 30 patients without BM abnormalities and 30 patients with MDS without erythroid hypoplasia/aplasia were analysed. Patients gave written consent, in accordance with the Declaration of Helsinki. This study was approved by the Institutional Review Board of Hiroshima University.

For T-cell subset analysis, MNCs were stained with fluorescein isothiocyanate (FITC) [Becton Dickinson (BD), San Jose, CA, USA], phycoerythrin (PE) (BD), peridinin chlorophyll protein (PerCP) (BD), or allophycocyanin (APC) (BD)-conjugated antibodies for CD8 subpopulation analysis: FITC anti-CD8, PE anti-perforin or anti-CCR7 (CD197), PerCP anti-CD62L or anti-CD28, and APC anti-CD27 or anti-CD45RA or anti-CD45RO. In addition, to clarify the population of regulatory T cells (Tregs), CD4, CD25, and FoxP3 expressions were analysed in BM-MNCs by 3-colour flow cytometry using a PE-anti-human FoxP3 staining kit (BD); Th17 cells were identified by staining with anti-IL-17 antibody-PE and anti-CD4 antibody-PerCP. Intracellular analysis of perforin and FoxP3, and IL-17 staining were performed after fixation [BD Cytofix/Cytoperm fixatives (BD)]. The cells were washed with Perm/Wash buffer in accordance with the instruction manual. Data were analysed using a FACS Calibur flow cytometer (BD) with CellQuest software (BD).

Correspondence

Table I. Characteristics of patients with erythroid hypoplasia/aplasia

Case no.	Age (years)/ sex	Disorder	Karyo type	Hb (g/l)	Ret (%)	Epo (iu/l)	Erythro blast% (BM)	NCC (x 10⁹/l)	CD4/8 ratio (BM)	CD8⁺/ Perforin⁺% (BM)	TRB@ rearrangement (BM)
CsA responders											
1	87/F	MDS (RA) with erythroid aplasia	46, XX	61	0·32	5220·0	2·4	15 8400	0·30	46·8	Rearranged
2	73/M	MDS (RA) with erythroid aplasia	46, XY	60	0·14	3360·0	0	48 050	0·32	64·8	Rearranged
3	70/M	MDS (RA) with erythroid hypoplasia	46, XY	72	0·70	25·9	11·0	25 000	0·42	50·2	Not rearranged
4	80/F	MDS (RA) with erythroid aplasia	46, XX	64	0·80	ND	3·0	24 000	0·78	41·9 (PB)	ND
5	67/F	Idiopathic PRCA	46, XX	47	0·19	3040·0	0·5	75 600	1·37 (PB)	45·2 (PB)	Rearranged
6	70/F	Thymoma-PRCA	46, XX	55	0·36	683·0	0	106 900	0·57	44·1	Rearranged
7	49/M	Thymoma-PRCA	46, XY	61	0·09	8590·0	0	329 200	0·36	34·8	Rearranged
8	58/F	Thymoma-PRCA	46, XX	43	0·13	ND	0	31 050	ND	51·8	Rearranged
9	81/F	Aplastic anaemia	46, XX	58	2·60	127·0	29·5	65 700	1·33	41·6 (PB)	Rearranged
10	57/M	Aplastic anaemia	46, XY	65	0·44	ND	6·5	57 150	0·88	36·1	ND
CsA non-responders											
11	76/F	MDS (RA) with erythroid aplasia	46, XX	68	0·32	3980.0	0.5	30 250	0·66	29·2	Rearranged
12	78/M	MDS (RA) with erythroid aplasia	46, XY	68	0.91	1810·0	1.0	150 200	1·15	25·3	Rearranged
13	73/M	MDS (RA) with erythroid aplasia	45, X, -Y [17/20]	57	0·19	346·0	3·0	102 750	1·03	11·9	Rearranged (PB)
14	56/F	MDS (RA) with erythroid hypoplasia	46, XX	74	0·72	ND	14·5	231 200	ND	13·6 (PB)	ND
15	33/F	Idiopathic PRCA	46, XX	53	0·38	ND	1·0	105 700	ND	20·8	Not rearranged
16	69/M	Idiopathic PRCA	46, XY	57	0·31	290·0	0·5	91 750	0·53	30·3	Not rearranged
17	52/M	Aplastic anaemia	46, XY	67	1·42	ND	26·0	21 100	0·24 (PB)	28·0	Rearranged (PB)
18	65/M	Aplastic anaemia	46, XY	53	1·47	ND	29·0	88 300	ND	19·0	ND
19	72/M	Aplastic anaemia	45, X, -Y [7/20]	59	1·33	ND	21·5	38 900	ND	6·3	ND
20	39/F	Aplastic anaemia	46, XX	85	2·18	ND	16·8	35 750	0·37	6·6	ND
21	71/M	Aplastic anaemia	46, XY	60	2·27	ND	37·5	11 900	ND	18·2	ND
22	66/M	Aplastic anaemia	46, XY	72	1·40	ND	16·4	40 400	ND	12·4	ND

CsA, ciclosporin A; Epo, erythropoietin; BM, bone marrow; PB, peripheral blood; NCC, nucleated cell count; MDS, myelodysplastic syndrome; RA, refractory anaemia; PRCA, pure red cell aplasia; M, male; F, female; ND, not done. [Correction added on 4 April 2012, after first online publication: Ages of patients in case numbers 6, 7, 8, 12 and 15 have been corrected.]

T-cell receptor beta gene (TRB@) rearrangements from BM-MNCs or PB-MNCs were assessed by polymerase chain reaction (PCR) assays using BIOMED-2 (Van Dongen et al, 2003) (InVivoScribe Technologies, San Diego, CA, USA).

Statistical significance of differences between independent groups was determined using Student's t-tests. P values <0·05 were considered statistically significant.

Among 22 patients with erythroid hypoplasia/aplasia, 10 patients (4 MDS with erythroid hypoplasia/aplasia, one idio-

Fig 1. (A) Comparison of CD8⁺/perforin⁺ T cells between ciclosporin A responders and non-responders. Patients in whom the proportion of CD8⁺/ perforin⁺ T cells expanded (45·7 ± 8·6%, n = 10) in the bone marrow or peripheral blood were ciclosporin A (CsA) responders. In contrast, patients in whom the proportion of CD8⁺/perforin⁺ T cells did not expand (18·5 ± 8·5%, n = 12, P < 0.0001) were CsA non-responders. (B) Immunophenotypic analysis of proliferative T cells in bone marrow mononuclear cells (BM-MNCs) of a patient with MDS with erythroid aplasia showing good response to CsA therapy. CD8⁺/perforin⁺ T cells in BM-MNCs of MDS with erythroid aplasia showing good response to CsA therapy were significantly increased compared with those in the normal controls (Case 1: 46·8% vs. Control: 7·1%). The T-cell subpopulation expressing CD8⁺ perforin⁺ CD62Lˡᵒʷ CD27⁺ CCR7ˡᵒʷ CD45RA⁺⁺ CD45RO⁺, which was consistent with the CD8⁺/perforin⁺ T_EM subset, increased in the bone marrow.

pathic PRCA, three thymoma-associated PRCA, and 2 aplastic anaemia) responded to CsA therapy within 2–8 weeks (Table I). The median blood haemoglobin concentration increased from 65 g/l at the baseline to 93 g/l with treatment, with a median haemoglobin increase of 28 g/l from the baseline. We attempted to compare the T-cell subsets between CsA responders and non-responders. Given that CD8$^+$/perforin$^+$ T cells expanded in the bone marrow of 3 patients with thymoma-associated PRCA in our previous study, we focused on a T-cell subpopulation expressing CD8$^+$/perforin$^+$. Intriguingly, the CD8$^+$/perforin$^+$ T cells were significantly increased in the CsA responders (45·7 ± 8·6%, $n = 10$) compared with those in the non-responders (18·5 ± 8·5%, $n = 12$, $P < 0.0001$, Fig 1A), normal BM controls (16·9 ± 7·0%, $n = 30$), and those with MDS without erythroid hypoplasia/aplasia (15·1 ± 7·0%, $n = 30$). Furthermore, we confirmed that the numbers and proportions of CD8$^+$/perforin$^+$ T cells in BM-MNCs or PB-MNCs decreased during remission following CsA therapy in 4 cases of CsA responders (data not shown). Among the CD8$^+$/perforin$^+$ T cells, the CD62L^{low} CD27$^+$ CCR7^{low} CD45RA^{++} CD45RO$^+$ population was prominent (Fig 1B), which is consistent with an effector memory T (T$_{EM}$) cell subset (Decrion et al, 2007). In contrast, Treg (CD4$^+$ CD25^{high} FoxP3$^+$) and Th17 (CD4$^+$ IL-17$^+$) cell populations were not associated with CsA responsiveness in patients with erythroid hypoplasia/aplasia (data not shown).

It has been reported that CsA therapy produced complete or partial remission in 19/20 (95%) thymoma-associated PRCA patients and all evaluable CsA responders became transfusion-independent within 2 weeks of the initiation of treatment (Hirokawa et al, 2008). However, most thymoma-associated PRCA patients, including our cases, required continuous CsA treatment (Hirokawa et al, 2008). These data suggest that CsA can predominantly suppress the cytotoxic function by CD8$^+$/perforin$^+$ T cells. Our study showed that the CD8$^+$/perforin$^+$ T-cell subset is a large population in patients with CsA-responsive erythroid hypoplasia/aplasia. It is suggested that the CD8$^+$/perforin$^+$ T-cell subset may have functions to reduce erythroid progenitors via immunological mechanisms. However, we could not confirm whether the CD8$^+$/perforin$^+$ T cells directly affect erythroid progenitors because the presence of CD8$^+$/perforin$^+$ T cells was identified by intracellular staining of perforin after fixation.

In conclusion, expansion of CD8$^+$/perforin$^+$ T cells predicts response to CsA therapy in patients with erythroid hypoplasia/aplasia. The disease entity of 'erythroid hypoplasia/aplasia with expansion of CD8$^+$/perforin$^+$ T-cell subset', including MDS, PRCA, and aplastic anaemia, may have common pathogenetic mechanisms. From our results, expansion of CD8$^+$/perforin$^+$ T cells in patients with erythroid hypoplasia/aplasia could be a new, useful, and simple marker in CsA therapy. We are convinced that CsA therapy should be actively applied for this disease entity.

Authorship and disclosures

H.N. performed research, analysed and interpreted data and wrote the manuscript; Y.H. interpreted data and revised the manuscript; H.H. provided patient samples; A.K. provided patient samples and revised the manuscript; and H.H designed the research and revised the manuscript.

Conflict of interest

The authors declare no competing financial interests.

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Serum ferritin and total units transfused for assessing iron overload in adults with sickle cell disease

Blood transfusion therapy usage in sickle cell disease (SCD) is increasing (Drasar *et al*, 2011) and with this comes the potential for morbidity and mortality associated with the consequent iron overload. Whilst patients treated with regular blood transfusions have their iron indices monitored regularly and are under regular clinical review, patients receiving sporadic blood transfusions can accrue a substantial iron load relatively unnoticed. Serum ferritin (SF) is a widely available and cost-effective screening test for iron overload but can be unreliable in SCD due to the inflammatory nature of the condition, even in the steady state (Adamkiewicz *et al*, 2009). R2 magnetic resonance imaging (R2MRI) is a recognized non-invasive method of estimating liver iron concentration (LIC) (St Pierre *et al*, 2005) but has limited availability. A simple method of assessing iron load in patients having regular and intermittent transfusions is therefore needed to enable appropriate targeting of resources.

We present a retrospective review of the patients entered in our sickle cell database at King's College Hospital [KCH], London, covering data from a 20-year period, from 1st January 1990 to 31st January 2011. Six hundred and sixty adult SCD patients ranging from 16 to 80 years of age (mean 35 years, standard deviation [SD] ± 11·08) had steady-state SF levels and an accurate transfusion history. Fifty-seven percent of patients were female and patient genotypes consisted of HbSS 62·0%, HbSC 31·7%, HbSβ⁰ 1·8% and HbSβ⁺ 4·5%. LIC was assessed non-invasively by R2MRI (Ferriscan®) in 52 patients and cardiac T2* was performed concurrently in 18 cases. R2MRI is a well-validated method of assessing liver iron load and therefore these patients formed the final study group. Clinical characteristics obtained were age, frequency

of transfusion (regular *versus* sporadic), total top-up units transfused (TUT) and transfusion rate (TUT/top-up years). Exchange transfused units were not included in the analysis. Patient consent was formally obtained for the R2MRI scans under ethics number 08/H1101/123. Data were not normally distributed and therefore Spearman's rank test was used to compare the data ($P < 0.05$ was used to define statistical significance).

Of the 660 patients, 317 (48%) had received at least 1 unit of blood, 238 (75%) of which were HbSS. The study group of 52 patients (35 female) consisted of 48 HbSS, two HbSβ⁰ and two HbSC with age ranging from 19 to 63 years (mean 38 years).

We initially assessed which parameters most effectively predicted iron loading in the liver, as there is currently no consensus on this (Adamkiewicz *et al*, 2009; Inati *et al*, 2010). We found (in contrast to Inati *et al*, 2010) that TUT correlated more strongly with LIC rather than transfusion rate ($R = 0.71$ $P < 0.0001$ for TUT *versus* $R = 0.62$ $P < 0.0001$ for TUT/top-up years). A positive correlation was found between SF and TUT/top-up years, however this was weaker than other published data ($R = 0.48$ $P < 0.0001$). SF correlated significantly with LIC ($R = 0.91$ $P < 0.0001$) but in a non-linear manner. We subdivided our patients according to SF < or ≥ 1000 μg/l (as per National Institutes of Health guidelines (Adams *et al*, 2004)) and TUT < or ≥ 20 units TUT (see Fig 1).

Twenty-seven of the 52 patients had SF ≥ 1000 μg/l, with a mean of 3995 μg/l (range 1004–16 000 μg/l) and all 27 patients had LIC ≥ 2 mg/g dry-weight [g DW] (range 2·1–>43 mg/g DW, mean 22·6 mg/g DW). The normal range of LIC as estimated by R2MRI (Ferriscan®) was 0·7–

Clinical characteristics and outcome of refractory/relapsed myeloid leukemia in children with Down syndrome

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Myeloid leukemia in Down syndrome (ML-DS) is associated with good response to chemotherapy and favorable prognosis. Because little research has been focused on refractory/relapsed (R/R) cases, we conducted a retrospective analysis for R/R ML-DS. Among ML-DS patients diagnosed between 2000 and 2010 in Japan, 26 relapsed (25 in the BM and 1 in the skin), and 3 refractory patients were enrolled. The male/female ratio was 18/11. The median age at initial diagnosis of ML-DS was 2 years, and the median time to relapse was 8.6 months. Each patient initially had been treated with ML-DS–specific protocols. Thirteen of the 26 patients achieved complete remission with various kinds of reinduction chemotherapies; 2 of 8 survived without further recurrence after receiving allogeneic hematopoietic stem cell transplantation, and 4 of 5 maintained complete remissions with chemotherapy alone. Treatment failures mostly were associated with disease progression rather than treatment-related toxicities. The 3-year OS rate was 25.9% ± 8.5%. A longer duration from initial diagnosis to relapse was a significant favorable prognostic factor ($P < .0001$). We conclude that clinical outcome for patients with R/R ML-DS generally are unfavorable, even in those receiving hematopoietic stem cell transplantation. Novel methods to identify poor prognostic factors for ML-DS are necessary. (*Blood*. 2012; 120(9):1810-1815)

Introduction

Down syndrome (DS) is one of the most common congenital disorders and is associated with an increased risk of acute leukemia.[1] Acute myeloid leukemia (AML) in patients with DS is categorized as myeloid leukemia associated with DS (ML-DS) in the 4th edition of the World Health Organization classification. Clinical and biologic features of ML-DS in children are quite different from those of AML in children without DS and include: younger age at onset, lower white blood cell (WBC) count at diagnosis, and greater incidence of acute megakaryoblastic leukemia.[2,3] ML-DS is known to exhibit good sensitivity against cytotoxic agents, especially cytarabine (Ara-C), and outcomes in recent clinical trials are favorable: long-term event-free survival has been reported in approximately 80% of patients.[4-11] However, little attention has focused on refractory or relapsed (R/R) cases because most treatment failures are the result of toxicities rather than to resistant or recurrent leukemia.

We present is a nationwide retrospective analysis of patients with R/R ML-DS in Japan.

Methods

The present retrospective study was conducted on 120 institutions that belong to the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG), a Japanese nationwide collaborative study group for childhood hematologic malignancies, and data on 29 patients with R/R ML-DS treated in 26 hospitals were collected. Patients were either enrolled on one of the AML clinical trials or registered on patient database of the collaborative study group at initial diagnosis of ML-DS, of which the protocols and registrations were approved by the institutional review boards of each participating center with informed consent obtained in accordance with the Declaration of Helsinki. The present retrospective study was approved by the JPLSG steering committee and institutional review boards of Shiga University of Medical Science for all aspects of this investigation.

Inclusion criteria on current analyses were ML-DS patients initially treated with curative intent between January 1, 2000, and December 31, 2010, and age younger than 18 years at the onset of ML-DS. Patients with myelodysplastic syndrome with DS also were included because it is currently recognized that there are no biologic and clinical differences between myelodysplastic syndrome and overt AML in patients with DS. Clinical data at initial diagnosis of ML-DS, including sex, age, WBC count, extramedullary disease, French-American-British (FAB) morphology, therapy protocol given, and duration from initial diagnosis to relapse, were collected. In addition, treatment data, including achievement of further remission, HSCT, secondary cancer, outcome, and cause of death after diagnosis of R/R ML-DS, also were collected.

Statistical analyses

Descriptive statistical analyses to assess baseline characteristics and the clinical course of patients diagnosed with R/R ML-DS were performed by use of the χ^2 tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. Overall survival (OS) was defined as the length of time from the diagnosis of R/R ML-DS to death from any cause. OS percentages and standard errors were calculated with the Kaplan-Meier method, and log-rank tests were used for group comparisons. A Cox proportional hazards regression model was used to investigate risk factors that were associated with survival after diagnosis of R/R ML-DS. Variables including sex (female vs male), age at initial diagnosis (≤ 2 years vs > 2 years), WBC at initial diagnosis ($\geq 10\,000/\mu L$ vs $< 10\,000/\mu L$), FAB morphology at initial diagnosis (M7 vs others), disease status of R/R ML-DS (induction failure or relapse ≤ 6 months vs relapse > 6 months), achievement of further remission (yes vs no), and treatment of HSCT (yes vs no) that were significantly associated with survival in the univariate analyses were considered for inclusion in the model. Significant variables associated with survival were then identified. No statistical adjustment was made for performing multiple tests, but 2-sided P values greater than .05 were interpreted with caution. All data analyses were performed by the use of SAS Version 9.1.3 statistical software (SAS Institute). Follow-up data were actualized as of December 31, 2011.

Results

Patient characteristics and treatment at initial diagnosis

Relevant clinical data of the 29 patients at initial diagnosis are shown in Table 1; a slight predominance of male patients existed (male/female ratio was 18/11), median age at initial diagnosis for ML-DS was 2 years (range, 7 months to 16 years) at which 23 of 29 patients were younger than 4 years of age, median WBC count was 5600/μL (range, 900-143 600/μL), and only 1 patient had an extramedullary disease (at skin). Morphologically, 22 (75.8%) patients showed FAB M7 blasts. Karyotype analysis at initial diagnosis showed monosomy 7 in 2 patients, monosomy 7 associated with a ring or marker chromosome in 5 patients,[12] t(8;21)(q22;q22) with FAB M2 morphology in 1 patient, other various cytogenetic abnormalities in 13 patients, and 6 patients with normal karyotype and sole constitutional trisomy 21. Full karyotypes are listed in supplemental Table 1 (available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

All patients initially were treated with one of the protocols specifically designed for ML-DS. Twenty patients were treated according to the AML99 Down protocol of the Japanese Childhood AML Cooperative Study[6]; 7 patients were officially enrolled in the study, and the rest were treated according to the institutional choice. Among the remaining 9 patients, 8 patients were treated with the JPLSG AML-D05 protocol (registered at http://www.umin.ac.jp/ctr/ as UMIN000000989) and 1 patient with the Japanese Children's Cancer and Leukemia Study Group AML9805 Down protocol.[7] The AML99 and AML-D05 protocol, with which 28 of 29 patients were treated, consists of 5 courses of pirarubicin

Table 1. Clinical characteristics and treatment of the 29 ML-DS patients at initial diagnosis

	No.	%
Age, y		
Median (range)	2 (0.6-16)	
0- <1	2	6.9
1 ≤ - <2	9	31.0
2 ≤ - <3	6	20.7
3 ≤ - <4	6	20.7
4 ≤	6	20.7
Sex		
Male	18	62.1
Female	11	37.9
WBC, × 10⁹/L		
Median (range)	5.6 (0.9-143.6)	
FAB classification		
M1	1	3.5
M2	1	3.5
M5	1	3.5
M7	22	75.8
MDS/unclassified	4	13.7
Karyotype		
Constitutional trisomy 21	6	20.7
Monosomy 7	2	6.9
−7+ring/marker	5	17.2
t(8;21)(q22;q22)	1	3.5
Other abnormalities	13	44.8
Not available	2	6.9
Initial treatment for ML-DS		
AML99 Down protocol	20	69.0
JCCLSG AML9805 Down protocol	1	3.5
JPLSG AML-D05	8	27.5

FAB indicates French-American-British; JCCLSG, Japanese Children's Cancer and Leukemia Study Group; JPLSG, Japanese Pediatric Leukemia/Lymphoma Study Group; MDS, myelodysplastic syndrome; ML-DS, myeloid leukemia in Down syndrome; and WBC, white blood cell count.

(25 mg/m², 1-hour intravenous infusion on days 1 and 2), intermediate-dose Ara-C (100 mg/m², 1-hour intravenous infusion on days 1-7), with or without etoposide (150 mg/m², 2-hour intravenous infusion on days 3-5). Because of few incidences of CNS leukemia and CNS relapse among patients with ML-DS, cerebrospinal fluid was not routinely examined, and no CNS prophylaxis was delivered throughout the treatment on these patients. None of the patients received HSCT before the diagnosis of R/R ML-DS.

Patient characteristics at induction failure or relapse

Relevant clinical data of the patients at induction failure or at first relapse are shown in Table 2. There were 3 induction failures and 26 relapsed cases. Among the 26 relapsed cases, duration from initial diagnosis to relapse was 2.4 -71.8 months (median, 8.6 months). All patients who relapsed within 6 months (n = 8) were on chemotherapy for ML-DS. Twenty-four patients (92%) relapsed within 2 years after the initial chemotherapy for ML-DS. Twenty-five patients relapsed in the BM, and 1 relapsed with an isolated extramedullary mass in a skin lesion. The WBC count at relapse was between 1700 and 25 700/μL (median, 4100/μL). Twenty-three (88.5%) showed FAB M7 morphology. Ten patients had chromosomal abnormalities that were the same as at the initial diagnosis, and 12 patients had additional abnormalities.

GATA1 mutation status

Nine patients were examined for GATA1 mutation of the leukemic blasts either at initial diagnosis of ML-DS or at relapse; 8 of them were confirmed to have the mutation (Table 3).

Table 2. Clinical characteristics of the 29 ML-DS patients at induction failure or at relapse

	No	%
Disease status		
Refractory AML	3	10.3
Relapsed AML	26	89.7
Duration from initial diagnosis to relapse, mo (n = 26)		
Median (range)	8.6 (2.4-71.8)	
< 6	8	30.8
≤ 6 to < 12	13	50.0
≤ 12	5	19.2
Site of relapse (n = 26)		
Bone marrow	25	96.2
Extramedullary (skin)	1	3.8
FAB classification at relapse (n = 26)		
M1	1*	3.8
M7	23†	88.5
Not available	2‡	7.7
Karyotype at relapse (n = 26)		
Same as before	10	38.5
Additional abnormalities	12	46.1
Not available	4	15.4

AML indicates acute myeloid leukemia; FAB, French-American-British; and MDS, myelodysplastic syndrome.

*This patient was M7 at initial diagnosis.

†A total of 18 patients were M7, 1 was M1, 3 were MDS, and 1 unknown at initial diagnosis.

‡One patient was M5a, and 1 was M7 at initial diagnosis.

Treatment outcome for R/R ML-DS

The clinical data and outcome of all the 29 patients in this study are described in Table 3. Twenty-six of the 29 patients received various salvage chemotherapies with curative intent. Six patients were treated with an ML-DS–oriented induction regimen as previously reported[6,7]; 12 patients were treated with etoposide, mitoxantrone, and intermediate dose of Ara-C with continuous intravenous infusion[13]; and the other 8 patients were treated with various chemotherapy regimen, such as FLAG (fludarabine, high-dose Ara-C, and G-CSF), AVC (pirarubicin, vincristine, and Ara-C with continuous intravenous infusion), low-dose Ara-C + etoposide, and vincristine + asparaginase. No deaths because of toxicities were observed during reinduction therapy. Two patients only received palliative therapy and eventually died of disease progression (Table 3, no. 19 and 28). The details of the postrelapse clinical course could not be identified in one patient (no. 26), and this patient died of unknown cause after undergoing HSCT.

Among the 26 patients who were treated with curative intent, 13 patients (50%) achieved complete remission (CR). Eight of the 13 patients who achieved CR subsequently received allogeneic HSCT, and 2 survived without leukemia. The remaining 5 patients who achieved subsequent CR were treated with chemotherapy alone, and 4 were alive without any evidence of leukemia (no. 15, 16, 17, and 23). The 3-year OS rate of the patients who achieved CR was 57.7% ± 14.7%. All 13 patients who did not achieve CR eventually died because of disease progression. Six of those patients received allogeneic HSCT without attaining CR: 4 died because of disease progression, and 2 died because of transplantation-related toxicities. No secondary cancer was observed.

Preconditioning regimen varied among the 14 patients who received HSCT. We therefore categorized the conditioning regimen into 4 groups[14]: busulfan (BU)–based myeloablative conditioning (MAC) regimen (BU-MAC), when > 8 mg/kg of BU combination

was used; BU-based reduced intensity conditioning (RIC) regimen (BU-RIC), when a lower dose of BU combination was used; total body irradiation (TBI)–based MAC (TBI-MAC), when ≥ 8 Gy of fractionated TBI combination was used; and TBI-based RIC (TBI-RIC), when a lower dose of the TBI combination was used. A total of 5 patients received BU-MAC, 3 received BU-RIC, 4 received TBI-MAC, and 2 received TBI-RIC. We did not find any difference in survival and toxic death among these 4 conditioning regimen (data not shown).

Finally, the 3-year OS rate of all patients was 25.9% ± 8.5% (Figure 1). The median follow-up period for all 29 patients was 0.9 years (range, 0.2-7.0 years). Among the 8 patients with GATA1 mutation, 7 patients, including 1 patient with palliative therapy (no. 19), did not achieve subsequent remission and died of disease progression. In contrast, 11 of the 20 patients whose GATA1 status was not examined attained subsequent remission, and 6 of 11 patients are alive without disease. The patient with wild-type GATA1 (no. 10) died of transplantation-related toxicity although receiving HSCT in second CR.

Prognostic factors

Several predictive factors for OS were evaluated by univariate and multivariate analyses (Table 4). The unadjusted 3-year OS was found to be better for patients with a longer duration from initial diagnosis to relapse (patients with the duration from diagnosis to relapse > 12 months, 66.7% ± 27.2%; ≤ 12 to > 6 months, 23.1% ± 11.7%; and ≤ 6 months, 0%). In Cox regression analysis, the adjusted hazard ratios of patients who relapsed more than 6 months after the initial diagnosis were significantly better compared with those who did not achieve CR or who relapsed within 6 months after the diagnosis (hazard ratio 3.14; 95% confidence interval 1.28-7.67; $P = .012$), and this finding was still detected after we controlled for reinduction regimens (etoposide, mitoxantrone, and intermediate dose of Ara-C with continuous intravenous infusion vs others), HSCT (yes vs no), and biologic factors, including chromosomal abnormalities and GATA1 status (hazard ratio 4.56; 95% confidence interval 1.07-19.41; $P = .04$). Two clinical factors were found to be associated with the duration from initial diagnosis to relapse. First, patients older than 2 years of age at initial diagnosis were more likely to relapse in a short period from initial diagnosis (≤ 6 months) compared with younger patients (trend test, $P = .02$). Second, patients who relapsed earlier were less likely to achieve CR when they received second-line chemotherapy ($P = .001$). Other factors, including sex, WBC at initial diagnosis, FAB morphologies (M7 vs others), chromosomal abnormalities, and GATA1 status, were not significantly associated with survival. HSCT did not influence the prognosis even if performed after achieving further remission.

Discussion

Treatment strategy for ML-DS is based on reducing the intensity of chemotherapy protocol designed for non-DS AML patients, considering both the potential risk of treatment-related toxicities and greater sensitivity to cytotoxic agents. With this strategy, there have been successful reports on treating patients with ML-DS from several collaborative groups.[4-11] However, it is assumed that salvage of patients with R/R ML-DS is quite difficult because the OS and event-free survival rate are almost the same in these reports. Because little attention has been focused on these cases so far, a nationwide retrospective study was conducted. The present

Table 3. Clinical data on 29 Down syndrome patients with refractory/relapsed myeloid leukemia

Clinical characteristics at initial diagnosis of ML-DS

No.	Sex	Age, y	WBC, /μL	FAB	Karyotype	GATA1 status	IF/RL	Time to IF/RL, mo*	Subsequent CR	HSCT	Survival, mo†	Cause of death
1	F	3	3000	M7	Constitutional	NE	RL	4	No	No	5	Leukemia
2	M	3	51 700	M7	Constitutional	NE	IF	1	No	UCBT	8	Leukemia
3	M	2	10 000	M7	Other	NE	RL	2	No	No	3	Leukemia
4	F	2	5800	M7	Other	NE	RL	8	No	No	9	Leukemia
5	F	1	18 000	M7	−7+ring/marker	NE	RL	12	Yes	UCBT	16	Leukemia
6	M	0.7	19 200	M7	Other	NE	RL	8	Yes	RBMT	28	TRM
7	M	0.7	6500	M7	Other	mutated	RL	3	No	UCBT	7	TRM (non-CR)
8	F	5	25 800	M2	t(8;21)(q22;q22)	NE	IF	0	Yes	UCBT	>54‡	
9	F	1	4800	M7	Monosomy 7	mutated	RL	3	No	RPBSCT	10	Leukemia
10	M	14	1900	M7	Na	WT	RL	17	Yes	RBMT	60	TRM
11§	M	1	11 600	M7	Monosomy 7	NE	RL	18	Yes	No	33	Leukemia
12	M	3	2600	M7	Constitutional	NE	RL	3	No	RBMT	19	Leukemia
13	M	4	2800	MDS	−7+ring/marker	NE	RL	12	Yes	UBMT	11	Leukemia
14	M	15	1800	RAEBt	Other	NE	IF	2	No	UCBT	11	Leukemia
15	F	10	900	M1	Other	NE	RL	39	Yes	No	>33	
16	M	3	6700	M7	Constitutional	mutated	RL	72	Yes	No	>28	
17	M	1	5100	M7	Other	NE	RL	10	Yes	No	>46	
18	M	2	5600	MDS	−7+ring/marker	mutated	RL	9	No	No	10	Leukemia
19	M	1	7400	M7	Constitutional	mutated	RL	7	No	No	3	Leukemia
20	M	1	143 600	M7	Constitutional	NE	RL	11	Yes	RBMT	>84	
21	M	2	8600	M7	Other	mutated	RL	5	No	No	4	Leukemia
22	F	3	5900	M7	Other	NE	RL	11	Yes	UBMT	>32	
23	M	16	38 800	M5a	Other	NE	RL	20	Yes	No	>23	
24	M	1	3480	M7	Other	NE	RL	9	Yes	UCBT	14	Leukemia
25	F	3	2100	MDS	Other	mutated	RL	7	No	No	3	Leukemia
26	F	2	2900	M7	−7+ring/marker	NE	RL	11	NA	Yes	11	unknown
27	F	2	2700	M7	Other	mutated	RL	4	No	No	4	Leukemia
28	M	1	2470	M7	−7+ring/marker	NE	RL	8	No	No	4	Leukemia
29	F	1	3680	M7	Na	NE	RL	4	No	UCBT	5	Leukemia

CR indicates complete response; F, female; IF, induction failure; M, male; NA, data not available; NE, not evaluated; other, other cytogenetic abnormalities; RBMT, related bone marrow transplantation; RPBSCT, related peripheral blood stem cell transplantation; RL, relapse; TRM, transplantation-related mortality; UBMT, unrelated bone marrow transplantation; and UCBT, unrelated cord blood transplantation

*Months from initial diagnosis to either induction failure or first relapse.
†Duration of survival from induction failure or first relapse.
‡Patient no. 8 is alive with disease.
§Patient no. 11 underwent HSCT in third CR on day 981 after relapse and died of transplantation-related toxicity on day 981.

study clearly showed that patients with R/R ML-DS are resistant to second-line chemotherapy and that the disease course rarely is salvaged by allogeneic HSCT.

It is well recognized that the outcome of ML-DS is much better than that of AML in non-DS patients; however, the OS rate of R/R ML-DS cases in the present study (26%) was no better than that of the reported survival rate of non-DS AML patients, which is 23%-33%.[15,16] Usually, non-DS AML patients are heavily pre-

Figure 1. OS curve of R/R ML-DS. The 3-year OS rate was 25.9% ± 8.5%.

treated before relapse (eg, HSCT), and this explains one aspect of therapeutic resistance to second-line treatment that could lead to the low salvage rate in these patients. It is notable that the salvage rate of patients with ML-DS in the R/R setting also was very poor, even though these patients had initially received the low-intensive ML-DS–oriented chemotherapy. The initial therapies given for the present cases are even less intensive than the other ML-DS protocols used in developed countries and, of course, than that of the non-DS AML protocols.

Only 50% of patients with R/R ML-DS in the present study had achieved further remission with attempts of various reinduction therapies. It has been reported that the reinduction rate for non-DS patients with AML is 65%-77%, and achievement of subsequent CR was uniformly a good prognostic factor.[15,16] When we consider the results of the present study, which indicate that the achievement of further remission is a good prognostic factor, improvement of the reinduction rate for R/R ML-DS would be mandatory for a better prognosis.

The reported 5-year OS rate of patients with non-DS AML who attained second CR and subsequently received HSCT were 58%-62%.[17-19] In the present study, 8 of the 13 patients subsequently received allogeneic HSCT, but only 2 of those 8 patients (25%) survived. It is well known that the transplantation-related mortality
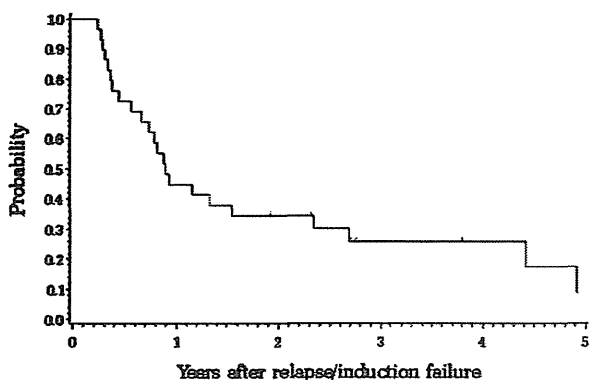
Table 4. Univariate and multivariate analyses of prognostic factors for refractory/relapsed ML-DS patients

| | Univariate analysis | | Multivariate analyses | | | |
| | | | Model 1 (baseline) | | Model 2 (relapsed phase) | |
Variables	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Sex: male vs female	0.78 (0.33-1.83)	.56	0.71 (0.30-1.71)	.45	0.85 (0.36-2.05)	.72
Age at initial diagnosis: ≤ 2 y vs > 2 y	1.98 (0.83-4.72)	.12	2.73 (0.89-8.37)	.08	*	
WBC at initial diagnosis: ≥ 10 000/µL vs < 10 000/µL	0.57 (0.22-1.47)	.24	0.43 (0.15-1.19)	.10	0.60 (0.22-1.60)	.30
FAB classification: M7 vs others	1.63 (0.60-4.44)	.34	0.92 (0.27-3.09)	.89	1.88 (0.66-5.37)	.24
Disease status: IF or RL ≤ 6 mo vs RL > 6 mo	2.92 (1.24-6.88)	.01	−		3.14 (1.28-7.67)	.012
Response to r-induction therapy: non-CR vs CR	16.54 (4.44-61.59)	< .0001	−		*	

Reference groups: female > 2 years, < 10 000/µL, others (FAB), RL > 6 months, and CR.

CI indicates confidence interval; CR, complete response, HR, hazard ratio; IF, induction failure; mo, month(s); and RL, relapse.

*A variable of "patient age at initial diagnosis" was excluded from model 2 because it was a predictive factor for the variable of "type of relapse/induction failure." Patients who relapsed in the later phase (after 6 months) were more likely to achieve in CR, and therefore, a variable of "response to reinduction therapy" was also excluded from the model 2 in the multivariate analysis.

of patients with ML-DS is greater than in patients with non-DS AML[20]; however, the main cause of transplantation failure was disease progression, not transplantation-related complications, where only one death in remission was documented. Our result was consistent with the report by Meissner et al in which they described that relapse was the major cause of treatment failure in children with DS treated by HSCT for acute leukemia.[21]

However, 5 of the 13 R/R ML-DS patients with further remission subsequently were treated with chemotherapy only, and 4 of the 5 patients survived (duration after relapse, 22-45 months). Three of those 4 patients had relapsed more than 12 months from the initial diagnosis, which was found to be good prognostic factor in this study. Moreover, most of these patients were treated with continuous and/or high-dose Ara-C, which might be a key component of a salvage regimen for R/R ML-DS. Allogeneic HSCT might not be essential for these patients, especially for "late" relapsed patients, as for the non-DS cases[15,16]

The duration from initial diagnosis to R/R was shown as the strongest prognostic factor, but biologic factors (including chromosomal abnormalities and GATA1 status) were not relevant in this R/R ML-DS study. Although more than 80% of patients with ML-DS could be cured with low-intensive chemotherapy, methods that can be used to identify the remaining poor subgroups with a poor prognosis and a treatment strategy distinct from "usual" ML-DS are urgently needed. This necessity is because of the fact that they are rarely salvageable once they experience morphologic induction failure or relapse, as was indicated in this retrospective study. Future treatment protocols in this patient population could include adherence to a very low-intensity chemotherapy for the majority of patients with ML-DS, identification of the subgroup with a poor prognosis using minimal residual disease, and stratification of these patients to receive a more intensive chemotherapy containing high-dose and/or continuous infusion of intermediate-dose Ara-C.

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Authorship

Contribution: T. Taga and D.T. designed, organized, and performed research, analyzed data, and wrote the paper; A.M.S. designed research and collected and analyzed clinical data; K. Kudo, H.M., A.K., S.I., H.N., H.T., A.T., and A.S. designed, organized, and performed research and analyzed data; K.T. performed mutation screening and designed, organized, performed research and analyzed data; T. Taki designed, organized, and performed research and analyzed chromosomal data; K. Koh and H.K. provided clinical samples and data; and S.A. provided clinical data, designed and organized research, analyzed data, and wrote the paper.

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