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ORIGINAL ARTICLE

Comparative analysis of remission induction therapy for high-risk MDS and AML progressed from MDS in the MDS200 study of Japan Adult Leukemia Study Group

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Abstract A total of 120 patients with high-risk myelodysplastic syndrome (MDS) and AML progressed from MDS (MDS–AML) were registered in a randomized controlled study of the Japan Adult Leukemia Study Group (JALSG). Untreated adult patients with high-risk MDS and MDS–AML were randomly assigned to receive either idarubicin and cytosine arabinoside (IDR/Ara-C) (Group A) or low-dose cytosine arabinoside and aclarubicin (CA) (Group B). The remission rates were 64.7% for Group A (33 of 51 evaluable cases) and 43.9% for Group B (29 out of 66 evaluable cases). The 2-year overall survival rates and disease-free survival rates were 28.1 and 26.0% for Group A, and 32.1 and 24.8% for Group B, respectively. The duration of CR was 320.6 days for Group A and 378.7 days for Group B. There were 15 patients who lived longer than 1,000 days after diagnosis: 6 and 9 patients in Groups A and B, respectively. However, among patients enrolled in this trial, intensive chemotherapy did not produce better survival than low-dose chemotherapy. In conclusion, it is necessary to introduce the first line therapy excluding the chemotherapy that can prolong survival in patients with high-risk MDS and MDS–AML.

For the Japan Adult Leukemia Study Group.

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1 Introduction

Myelodysplastic syndrome (MDS) is a group of disorders in which abnormalities occur at the level of hematopoietic stem cells [1], leading to disturbance in the production of blood cells characterized by ineffective hematopoiesis [2], decrease in the number of peripheral blood cells and morphological/functional abnormalities in blood cells [3]. Allogeneic hematopoietic cell transplantation (allo-HCT) is the most effective curative therapy for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) [4]. However, for patients with high-risk MDS (those with refractory anemia with excess of blasts in transformation (RAEB)-t and some patients with RAEB) and patients with acute myeloid leukemia progressed from MDS (MDS–AML), chemotherapy aimed at remission is being used. The reasons for this are that MDS often affects elderly people [5], suitable donors are not always available at the time of disease onset, the necessity of pretransplant conditioning chemotherapy is controversial [6, 7] with a lack of sufficient evidence, and the optimal timing for transplantation varies widely depending on disease type [8].

On the other hand, reduced-intensity conditioning has extended the use of allo-HSCT to patients otherwise not eligible for this treatment due to older age or frailty [9]. However, allo-HSCT using traditional myeloablative preparative regimens is not easily tolerated by the elderly or frailer patient, and may lead to prohibitive treatment-related mortality rates. Most patients treated in the past were younger and devoid of comorbid clinical conditions. Novel reduced-intensity regimens have recently made allogeneic transplants applicable to the elderly, providing the benefit of the graft-versus-leukemia effect to a larger number of patients in need [10].

Low-dose chemotherapy, which has been used in clinical practice for 20 years, reduces the number of myeloblasts, improves pancytopenia and induces remission not only in MDS patients but also in some MDS–AML patients [11]. Common antineoplastic agents used in low-dose chemotherapy include cytosine arabinoside (Ara-C), aclarubicin (ACR), melphalan and etoposide. Nevertheless, despite improved Ara-C and regimens, the prognosis of AML in patients beyond 60 years of age remains dismal [4]. Low-dose antineoplastic drug therapy is still being used in some patients with MDS, which is common in elderly people, especially when the patient is at risk due to poor general condition or organ disorder [12].

The Japan Adult Leukemia Study Group (JALSG) previously conducted a pilot study for the treatment of high-risk MDS and MDS–AML to compare low-dose monotherapy with low-dose Ara-C plus granulocyte colony-stimulating factor (G-CSF) and multiple drug therapy with Ara-C plus Mitoxantrone plus VP-16. Later, JALSG conducted studies using a single protocol (JALSG MDS96) in 1996, in which remission induction and post-remission therapies using Ara-C and IDR in patients with high-risk MDS (RAEB-t) and in those with MDS–AML were performed, after which the efficacy and safety of these therapies were evaluated [13]. Furthermore, a randomized controlled study (JALSG MDS200) of intensive chemotherapy (IDR/Ara-C) or low-dose chemotherapy (CA) for high-risk MDS was also performed by JALSG.

Here, we present and analyze the results of the JALSG MDS200 study to assess and evaluate the validity of the MDS200 protocol for MDS treatment.

2 Patients and methods

2.1 Patient eligibility

A total of 120 patients were initially registered into the JALSG MDS200 study between June 2000 and March 2005. They were assigned into two groups, namely, Groups A and B (Table 1). Patients aged 15 years or more and diagnosed as having high-risk RAEB with high International Prognostic Scoring System score [14], RAEB-t or MDS-AML were eligible for this study. MDS–AML denotes secondary AML transformed from MDS.

Other eligibility criteria were as follows: patients with a performance status (PS) of 0–2 (ECOG); patients whose key organs other than the bone marrow retain intact function; patients who have not undergone any chemotherapy, except for pretreatment that does not affect the outcome of the main therapy; and patients who have given informed consent. Informed consent was obtained after carefully explaining the protocol and before registration.

2.2 Study protocol

The MDS200 protocol (Fig. 1) was designed based on the results of MDS96, and involved a dose-attenuation plan and allowed a wider range of chemotherapy. Patients were randomly assigned to either Group A or B.

In therapy A, the dose was adjusted according to a dose attenuation plan based on the presence of risk factors. The following 3 factors were regarded as risk factors: (1) Age ($\geq$60 years), (2) hypoplastic bone marrow and (3) PS $\geq$ 2. Patients with no risk factor received the standard dose, those with 1 risk factor received 80% of the dose and those with 2 or more risk factors received 60% of the dose (equivalent to the dose of MDS96). In therapy B, the use of

Table 1 Characteristics of patients

Group	A ($n = 53$)	B ($n = 67$)	P value (A vs. B)
Age (range)	63 (23–77)	61 (32–81)	0.505
Gender			
Male	37	52	0.332
Female	16	15	
Disease type			
HR-RAEB	4	11	0.269
RAEB-T	22	29	
MDS–AML	27	27	
Infection			
Presence	10	11	0.726
None	43	56	
Karyotype[a]			
Good	23 (44.2%) $n = 52$	21 (33.9%) $n = 62$	0.524
Int	11 (21.2%)	15 (24.2%)	
Poor	18 (34.6%)	26 (41.9%)	
PB (range)			
WBC (/μL)	2,500 (700–64,240)	2,720 (600–43,700)	0.665
Hb (g/dL)	8 (4.7–12.6)	7.9 (4.4–12.7) $n = 66$	0.562
Plt (/μL)	5.8 (0.2–31.4)	5.9 (0.5–36.7)	0.363
BM (range)			
Blast (%)	30 (4–95) $n = 51$	24.2 (1.9–96) $n = 66$	0.171
Biochemical data (range)			
LDH (IU/L)	296 (132–882)	303.5 (111–906) $n = 66$	0.998
CRP (mg/dL)	0.5 (0–20.2)	0.35 (0–11.7) $n = 66$	0.292

Patients who met all of the inclusion criteria and did not meet any of the stated exclusion criteria were included the study. The disease types were classified by FAB classification

Statistical analysis between Group A and Group B was done using χ^2 test or Mann–Whitney U-test

MDS myelodysplastic syndrome, *HR-RAEB* high risk-refractory anemia excess of blasts with high International Prognostic Scoring System Score, *RAEB-T* refractory anemia excess of blasts in transformation, *MDS–AML* MDS overt leukemia, *WBC* white blood cell, *Hb* hemoglobin, *Plt* platelet, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *PB* peripheral blood, *BM* bone marrow

[a] Shows IPSS risk

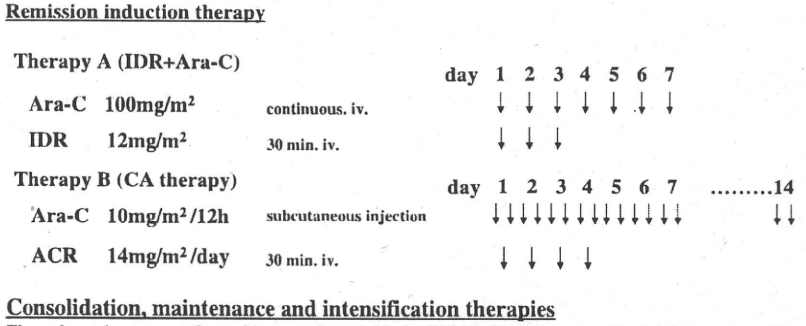

Fig. 1 Japan Adult Leukemia Study Group—myelodysplastic syndrome (JALSG MDS200 Protocol). In therapy A, the dose was adjusted according to a dose attenuation plan based on the presence of risk factors. The following 3 factors were regarded as risk factors: (1) Age (≥60 years), (2) hypoplastic bone marrow and (3) PS ≥ 2. Patients with no risk factor received the standard dose, those with 1 risk factor received 80% of the dose, and those with 2 or more risk factors received 60% of the dose (equivalent to the dose of MDS-96). In therapy B, the use of CAG therapy involving co-administration of G-CSF was allowed. *IDR* idarubicin, *Ara-C* cytosine arabinoside, *ACR* aclarubicin, *G-CSF* granulocyte colony-stimulating factor, *iv* intravenous injection, *min* minutes

CAG therapy involving the co-administration of granulocyte colony-stimulating factor (G-CSF) was allowed.

Untreated adult patients (≥15 years) with MDS (RAEB, RAEB-t or MDS–AML) were randomly assigned to receive either IDR/Ara-C (Group A) or CA (Group B) [15]. Complete remission (CR) rate, CR duration, overall survival (OS) rate and disease-/relapse-free survival (DFS/RFS) rate were compared between the two groups.

Consolidation therapy and maintenance therapy were performed in accordance with JALSG MDS96 [13].

2.3 Evaluation of response

Response to treatment was evaluated in accordance with JALSG criteria [13]. CR was considered achieved when the following conditions remained for at least 4 weeks. For the bone marrow: blasts accounting for ≤5% of all cells; absence of blasts with Auer body; and presence of normal erythroblasts, granulocytes and megakaryocytes. For peripheral blood: absence of blasts; neutrophils ≥ 1,000/ml; platelets ≥ 100,000/μL; and no evidence of extramedullary leukemia. CR duration was defined as the duration from the day when CR is achieved to the day of relapse or death, OS or DFS as the duration from the day of initiation of treatment to the day of death and DFS as the duration in which CR patients survived without relapse. Patients who were treated with HCST were not censored at the date of transplantation. All toxicity was graded using the World Health Organization criteria [16].

2.4 Statistical analysis

The primary endpoint of this study is DFS. Assuming a 1-year DFS rate of 60% in the Group A and 40% in the Group B, this design required the randomization of 200 patients. Eligible patients were randomized according to age, sex and disease type. Differences in background factors (e.g., age, gender and disease type) between Groups A and B were statistically analyzed using the χ^2 test or Mann–Whitney *U*-test. Probability of OS and DFS were estimated according to the method of Kaplan and Meier.

3 Results

3.1 Recruitment of patients and suspension of the study

The initially registered 120 patients were assigned into two groups, namely, Groups A and B. The clinical characteristics of the registered patients are shown in Table 1. The present protocol was originally planned to recruit 200 patients for Groups A and B within 3 years. However, the recruitment pace was slower than expected and thus the study period was extended from 3 years to 4.5 years. At the end of 2004, that is, after 4.5 years from the start of the study, the number of registered patients was only 113 in Groups A and B, which was 56.5% of the target number. At that point, the committee members discussed the progress of the MDS200 study and decided to suspend it at the end of March 2005. Since the final total number of patients did not reach the target number, we did not statistically compare DFS between Groups A and B, which was the primary endpoint of this study.

3.2 Characteristics of patients

There were no clear differences in the clinical characteristics of the patients between Groups A and B, such as FAB subtype, initial blood cell count, presence of infection, distribution in the karyotype group and biochemical data, as well as sex distribution (male/female ratio, 37/16 = 2.315 in Group A, and 52/15 = 3.467 in Group B).

3.3 Treatment outcome

The remission rates were 64.7% in Group A (33 out of 51 evaluable cases) and 43.9% in Group B (29 out of 66 evaluable cases). The 2-year overall survival (OS) rates were 28.1% in Group A and 32.1% in Group B, and the 2-year DFS rates were 26.0% in Group A and 24.8% in Group B. The mean duration of CR was 320.6 days (median: 213 days) in Group A and 378.7 days (median: 273 days) in Group B (Table 2). Reflecting the intensity of the remission induction chemotherapy, the period of WBC (<1,000/μL) after the therapy was longer in Group A than in Group B (19 days and 4 days, respectively). There were more grade 3 or 4 adverse events during the remission induction therapy in Group A (19 out of 53 evaluable patients) than in Group B (13 out of 67 evaluable patients). This difference was mostly attributable to infectious episodes (17 patients in Group A and 4 patients in Group B). In terms of bleeding episodes, 1 patient in Group A and 2 in Group B had grade 3/4 adverse events. The numbers of

Table 2 Treatment outcome (Group A vs. B)

	Group A (n = 53)	Group B (n = 67)
Remission rate (%)	64.7	43.9
Mean duration of remission (days)	320.6 (median: 213)	378.7 (median: 273)
2-Year survival rate (%)	28.1	32.1
2-Year disease-free survival rate (%)	26.0	24.8

The remission rates, 2-year overall survival (OS) rates and 2-year disease-free survival (DFS) rates are shown as percentages

early death in remission induction chemotherapy (death within 30 days) were 1 patient in Group A and 3 patients in Group B (Table 3). The cause of death in each group was infection or tumor progression. The completion rate of consolidation therapies were 37.3% in Group A (12 out of 33 evaluable cases), 37.9% in Group B (11 out of 29 evaluable cases). On the other hand, the maintenance therapies were completed 21.2% in Group A (7 out of 33 evaluable cases), and 15.2% in Group B (5 out of 33 evaluable cases). The numbers of dose attenuation in Group A were 30 patients of 100% dose, 21 patients of 80% or 60% dose and 2 patients of unknown.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was performed in 11 out of 50 patients (22%) in Group A and 19 out of 66 patients (28.8%) in Group B. Among those who received allo-HSCT, the transplantation was performed during the first remission in 40%, 21% of patients in Groups A, B, respectively.

There were 15 patients who lived longer than 1,000 days after diagnosis: 6, 9 patients in Groups A, B, respectively. Regarding the transplantation among long-term survivors, 3 out of 6 patients were transplanted in Group A, 6 out of 9 in Group B. Comparing the achievement of CR among these patients in Groups A and B, all 6 patients in Group A achieved CR, but only 4 out of 9 patients in Group B achieved CR.

Table 3 Toxicity of the induction therapy

	A ($n = 53$) (range)	B ($n = 67$) (range)	P value (A vs. B)
Period of WBC <1,000 (day)	19 (0–44) $n = 49$	4 (0–50) $n = 63$	<0.0001
Toxicity (grade 3/4)			
Presence	19	13	0.427
Bleeding	2	1	ND
Infection	17	11	0.04
Others	2	2	ND
Early death (<30 days)	1	3	ND

Statistical analysis between Groups A and B was performed using the χ^2 test or Mann–Whitney U-test

ND not done

4 Discussion

In this MDS200 study, patients with high-risk MDS and AML transformed from MDS (MDS–AML) were treated with either intensive or low-dose remission induction therapy, followed by intensive post-remission therapy that was the same as in the JALSG MDS96 study [13].

Although we did not perform statistical comparison of DFS or OS between these two treatment groups due to the insufficient number of patients enrolled, the results suggest that there was no significant difference, that is, survival curves were superimposable (Figs. 2, 3). Intensive chemotherapy similar to that for AML can produce a CR rate of 64.7% for high-risk MDS and MDS–AML patients, whereas low-dose induction therapy can result in a CR rate of 43.9%. However, among the patients enrolled in this trial, the difference in CR rate did not lead to better survival as described above. In terms of adverse events, patients who received intensive treatment had more grade 3 or 4 adverse events, particularly infectious events with a longer period of leukopenia. There was no increase in the number of patients succumbing to early death (death within 30 days after the

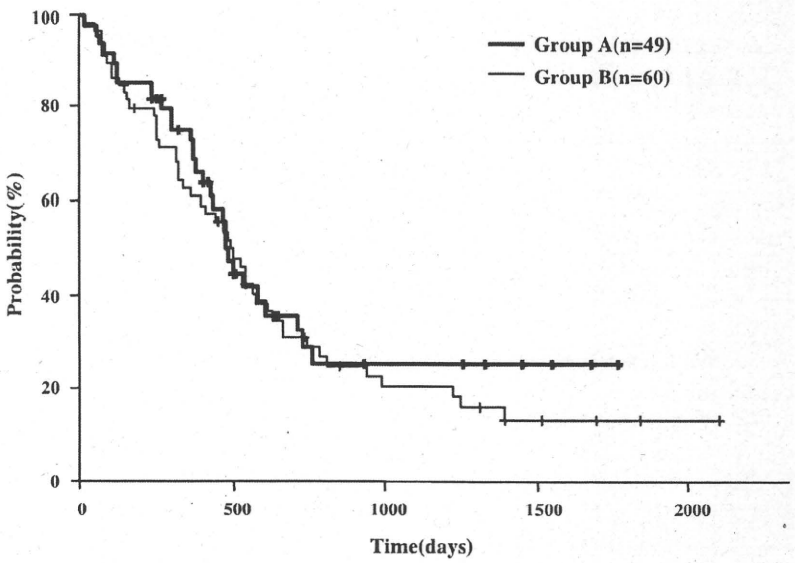

Fig. 2 Overall survival. Survival was calculated from the date of the start of treatment to the date of death due to any cause or to the date of the most recent follow-up. These data were not censored at the time of HSCT. All randomized patients were not included this data in each group. Due to this reason, some patients were not known to be CR or not, but known to be alive or not

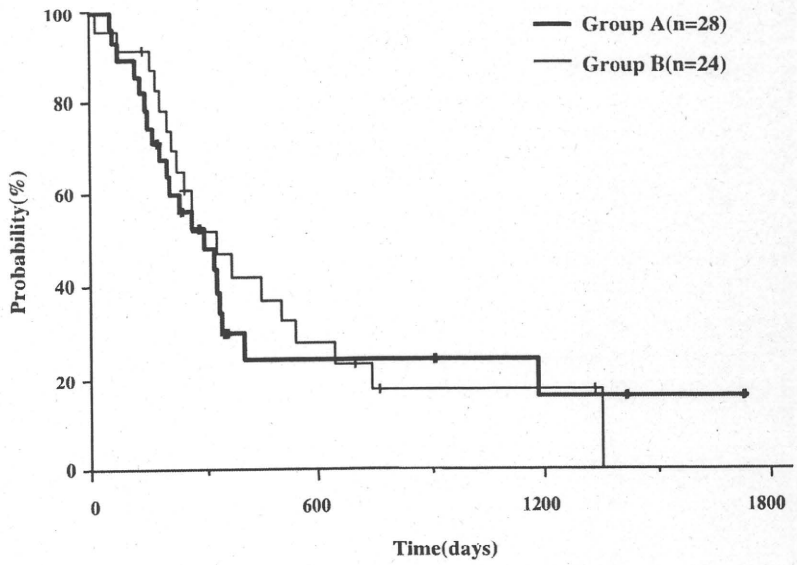

Fig. 3 Disease-/relapse-free survival. RFS was calculated from the date of achieving complete remission to the date of relapse, death or the most recent follow-up. These data were not censored at the time of HSCT. All randomized patients were not included this data in each group. Due to this reason, some patients were not known to be CR state or relapse, but known to be alive or not

start of treatment) in Group A, suggesting that intensive treatment produced higher CR rate, and higher toxicity resulted in a similar survival rate with low-dose induction therapy at least during the early phase of treatment.

There are several reasons that could explain why no difference in survival rate was observed regardless of the difference in CR rate. One could be the similar post-remission therapy between Groups A and B, as demonstrated by the almost similar DFS curves among the two groups. Another reason could be the disease status at the time of transplantation for patients in the two groups. In Group A, 60% of the transplantation was performed during the period other than that covering the first CR; this was 79% in Group B. Allo-HSCT has been shown to have the strongest antileukemia effect, and this was also found in the current study in which 6 out of 15 long-term survivors received allo-HSCT in Groups A and B. From the viewpoint of transplantation, intensive treatment merely selected cases that were suitable for transplantation, as observed in the case of transplantation for relapsed AML patients [17]. There are arguments against remission induction therapy for MDS patients in that it does not affect post-transplant prognosis [6, 18]. In the results of JSHCT, the chemotherapy before undergoing allo-SCT is not necessary in patients with MDS [6]. A group from the Institute of Medical Science of Tokyo University performed umbilical cord blood stem cell transplantation without remission induction therapy in high-risk MDS patients aged not more than 55 years and obtained favorable results with reduced time from diagnosis to transplantation [19]. It is important to perform clinical studies based on the concept that HCST should be performed immediately after diagnosis without remission induction, and determine the types of patients who would benefit from remission induction therapy prior to transplantation in terms of prognosis. In the present study, although suspended because of the insufficient number of patients enrolled, it appears that remission induction therapy with IDR and Ara-C did not produce better survival than that with low-dose chemotherapy despite higher CR rate. Therefore, it is suggested that CR rate is not a suitable surrogate marker for the evaluation of the outcome of chemotherapy for high-risk MDS and MDS–AML. In the latest reports, induction chemotherapy for patients with high-risk MDS and MDS–AML also provide no survival advantage [20, 21]. Considering the low survival rate of patients in this category, it is clearly necessary to introduce new strategies for the treatment of high-risk MDS and MDS–AML, such as molecular targeting agents and allo-HSCT with reduced-intensity conditioning regimens.

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Prognostic potential of detection of WT1 mRNA level in peripheral blood in adult acute myeloid leukemia

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Abstract

We retrospectively analyzed the potential of Wilms' tumor gene 1 (WT1) mRNA levels in peripheral blood for predicting the prognosis of 50 patients with AML. After achieving complete remission (CR), 34 patients (69.4%) were determined to be positive and 15 (30.6%) were negative for WT1. The relapse rate of the positive and negative patients was 73.5% and 40.0% ($p = 0.02$), respectively. After consolidation therapy, only 15 patients (32.6%) were positive and 31 (67.4%) were negative for WT1. Although the relapse rate of the positive and negative patients was 80.0% and 54.8% ($p = 0.10$), respectively, the rate of relapse within 1 year was 73.3% in positive patients and only 33.3% in negative patients ($p = 0.01$), respectively. The disease-free survival (DFS) rate at 3 years was 20.0% for positive patients and 50.0% for negative patients ($p = 0.01$). The overall survival (OS) rate at 3 years was 42.8% in positive patients and 69.8% in negative patients ($p = 0.04$), respectively. WT1 mRNA levels in the peripheral blood can predict relapse after CR, and its levels after consolidation therapy are closely correlated with DFS, OS, and early relapse.

Keywords: *AML, WT1, prognosis, real-time RT-PCR, minimal residual disease*

Introduction

Approximately 70–80% of newly diagnosed patients with adult acute myeloid leukemia (AML) achieve a complete remission (CR) when treated with anthracyclines such as daunorubicin (DNR) or idarubicin (IDR), and cytosine arabinoside (Ara-C); however, relapse is common, and only about one-third of these patients remain free of disease for more than 5 years [1–5]. If left untreated, almost all patients in CR will suffer relapse and die [6]. The leukemic clone may regrow on account of persisting leukemic cells and cause relapse after CR. Therefore, minimal residual disease (MRD) is the most reliable marker for prognosis. If a subset of patients with a high risk of relapse are identified, these patients can be treated with intensified chemotherapy or stem cell transplant (SCT). However, MRD cannot be detected by standard methods, and therefore more sensitive methods such as polymerase chain reaction (PCR) or multi-dimensional flow cytometry are needed. Since half of AML cases do not contain any leukemia-specific genetic alteration, samples from these patients cannot be subjected to PCR. Recent reports suggest that adult AML is characterized by high expression of Wilms' tumor gene 1 (WT1), and that the monitoring of WT1 expression might be an appropriate method to detect MRD [7].

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The prognostic importance and the strong correlation of WT1 mRNA levels with the incidence of relapse and survival have been reported recently [8–12]. In most of these studies, WT1 mRNA level in the bone marrow, which contains normal CD34-positive cells expressing WT1 mRNA, was evaluated. Therefore, the background level of normal CD34-positive cells in remission limits the accurate assessment of WT1 mRNA levels in bone marrow cells. Therefore, we evaluated the WT1 mRNA levels in peripheral blood, which rarely contains normal CD34-positive cells and expresses low or undetectable levels of WT1.

In this study, we focused on the evaluation of WT1 expression in peripheral blood and its clinical significance for determining the relapse and survival of adult patients with AML by using a WT1 mRNA Assay Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), which produces uniform results across hospitals.

Patients and methods

Patients

From 1 June 2001 to 30 October 2003, the clinical utility of the WT1 mRNA Assay Kit was determined for the early detection of relapse in 191 patients with AML [13]. In this study, we selected subjects who achieved a CR, and also had their WT1 expression analyzed at presentation, after induction (before consolidation), and after the final consolidation therapy. Patients of M3 type or who had undergone SCT before relapse were excluded. Table I shows the characteristics of these patients.

All patients received induction, consolidation, and maintenance therapies according to each institutional standard. Induction therapy consisted of cytarabine or behenoyl cytarabine and anthracyclines (idarubicin or daunorubicin); however, two patients received a separate induction therapy consisting of mitoxantrone or aclarubicin. All patients received three or four courses of consolidation therapy. Consolidation therapy consisted of the Japan Adult Leukemia Study Group (JALSG) post-remission regimen for AML (27/50) or high-dose cytarabine therapy (23/50) [14]. The definition of relapse was established when more than 5% blasts were observed in the marrow, or when blasts were detected in peripheral blood. The study was approved by the ethics committees of the participating institutions. Informed consent was obtained from the patients or their families prior to initiation of the study.

Table I. Patient characteristics.

Selected patients	50
Male/female	28/22
Age, median (range)	56 (22–86 years)
FAB	
M0	0
M1	9
M2	21
M4	12
M5	6
M6	2
M7	0
Karyotype	
Favorable	14
Intermediate	28
Adverse	7
Unknown	1

FAB, French–American–British classification.

Quantification of WT1 mRNA levels

Total RNA was extracted from peripheral blood using the QIAamp RNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). WT1 mRNA levels were determined using the WT1 mRNA Assay Kit (Otsuka). An outline of the estimation method described in the kit is as follows. One microgram of total RNA was converted into cDNA containing random hexamer using reverse transcriptase, RNase inhibitor, and dNTPs. Quantitative PCR was performed by the TaqMan method according to the previously described procedure [15]. The TaqMan probe was labeled with 6-carboxy-fluorescein phosphoramide (FAM) as the reporter dye at the 5′-end and with carboxy-tetramethyl-rhodamine (TAMRA) as the quencher dye at the 3′-end. For PCR analysis of WT1, 8 μL of cDNA mixture (corresponding to 400 ng of RNA) was used, and for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression analysis, 1 μL of mixture (corresponding to 50 ng of RNA) was used. Known copy numbers of WT1 cDNA standard (5×10^3, 5×10^5, and 5×10^7 copies/mL) and GAPDH cDNA standard (5×10^5, 5×10^7, and 5×10^9 copies/mL) were simultaneously amplified by PCR, and standard curves for measuring WT1 and GAPDH mRNA levels in the samples were obtained. The WT1 mRNA level was adjusted according to that of GAPDH.

Procedures for assessing WT1 mRNA expression levels

The WT1 mRNA level was normalized with GAPDH mRNA level as follows. The ratio of WT1 transcripts to GAPDH transcripts was calculated, and the value of (WT1/GAPDH) $\times$ (2.7×10^7) (mean copy number/μg RNA of GAPDH in 94 normal human volunteers) was defined as copies/μg of WT1 RNA expression level in the samples. Next, 1 μg of RNA was dissolved in 20 μL of the reaction

solution and used for reverse transcriptase reactions, as described in the kit. Therefore, the WT1 mRNA expression level was 50 copies/μg RNA at the lower limit of detection (2500 copies/mL). This value was regarded as the 'cut-off' value for assessment of the presence or absence of WT1 mRNA expression. In other words, patients were negative for WT1 if the mRNA levels were less than 50 copies/μg RNA, and were positive if the mRNA levels were equal to or higher than 50 copies/μg RNA.

Statistical analysis

Overall survival (OS) was defined from the date of diagnosis to the date of death. Disease-free survival (DFS) for patients who had achieved CR was defined from the date of CR to the date of the first event (either relapse or death). The Kaplan–Meier method was used to estimate OS and DFS. The log-rank test was used for the comparison of OS and DFS. The χ^2 test was used for ordinal data, such as the detection of WT1 and relapse. For the comparison of time to relapse, the Wilcoxon/Kruskal–Wallis test was used. Statistical analyses were conducted using JMP software (SAS Institute, Inc., Cary, NC).

Results

WT1 mRNA levels according to FAB subtype

WT1 mRNA was detected in 107 of 114 patients (93.9%). The percentage of detection of WT1 mRNA was low in M5 (6/9) and M7 (0/1) types; however, the percentage in other types was high, and ranged from 85.7 to 100%. In 92 of the 94 healthy controls (97.9%), the expression of WT1 mRNA was undetectable.

WT1 mRNA levels during the course of chemotherapy

The median follow-up period was 39 months (range 34–46 months). The median WT1 mRNA level at diagnosis was 48 327 copies/μg RNA (range 137–329 185). After achieving CR, the WT1 mRNA level ranged from <50 copies/μg to 30 732 copies/μg RNA. Thirty-four (69%) patients were positive and 15 (31%) were negative for WT1. After consolidation therapy, the WT1 mRNA level ranged from <50 copies/μg RNA to 49 174 copies/μg RNA. Only 15 patients (33%) were WT1 positive and 31 (67%) were negative.

Prognosis and expression level of WT1 mRNA at diagnosis

The WT1 mRNA levels did not correlate with the relapse rate (Table II) or with DFS [Figure 1(A)] and OS [Figure 1(B)].

Prognostic relevance of detection of WT1 mRNA after CR

Six of the 15 negative patients relapsed after a median 7.6 months (range 3.7–11.7 months). In contrast, 25 of the 34 positive patients relapsed after a median 10.0 months (range 2.6–37.4 months). No significant differences were observed with regard to time to relapse between the negative and positive patients ($p=0.21$). The relapse rate of positive patients and that of negative patients was 74% and 40% ($p=0.02$; sensitivity = 81%; specificity = 50%), respectively (Table II). The DFS rate at 3 years was 30% in positive patients and 60% in negative patients ($p=0.09$) [Figure 2(A)]. The OS rate at 3 years was 53% in positive patients and 79% in negative patients ($p=0.12$) [Figure 2(B)].

Prognostic relevance of WT1 mRNA detection after consolidation

Seventeen of the 31 negative patients relapsed after a median 10.2 months (range 2.6–37.4 months). In contrast, 12 of the 15 positive patients relapsed after a median 6.3 months (range 3.7–27.0 months). There were significant differences in

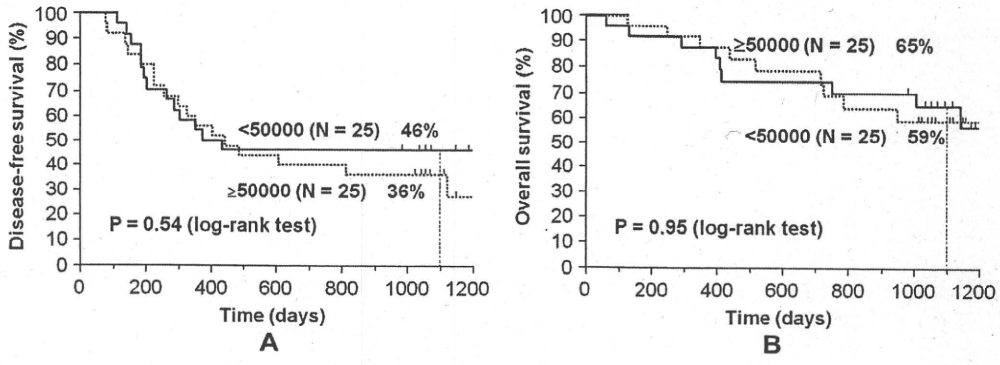

Figure 1. (A) Disease-free survival and (B) overall survival according to the expression level of WT1 mRNA at diagnosis.

time to relapse between the negative and positive patients ($p = 0.04$).

The relapse rate of positive patients and that of negative patients was 80% and 55% ($p = 0.10$), respectively (Table II).

The DFS rate at 3 years was 20% in positive patients and 50% in negative patients ($p = 0.01$) [Figure 3(A)]. The rate of relapse within 1 year was 73% in positive patients and only 33% in negative patients ($p = 0.01$). The OS rate at 3 years was 43% in positive patients and 70% in negative patients ($p = 0.04$) [Figure 3(B)].

Table II. Relapse and expression level of WT1 mRNA.

Status	WT1 level	Relapse rate	*p*-Value
At diagnosis	<50 000 copies/μg RNA	14/25 (56%)	0.38
	≥50 000 copies/μg RNA	17/25 (68%)	
After CR	<50 copies/μg RNA	6/15 (40%)	0.02
	≥50 copies/μg RNA	25/34 (74%)	
After consolidation	<50 copies/μg RNA	17/31 (55%)	0.10
	≥50 copies/μg RNA	12/15 (80%)	

CR, complete remission.

Discussion

MRD in AML is one of the most important prognostic factors, and the evaluation of MRD by reverse transcriptase (RT)-PCR amplification of chromosome translocations is the most sensitive and reliable method. However, more than 50% of patients with AML lack a known chromosomal abnormality or genetic lesions suitable for MRD determination. Thus, multi-dimensional flow cytometry has been used for MRD measurement. However, this method is not popular because of technical complexity, non-uniform values, and changes in expression of surface markers.

In the light of this situation, WT1 mRNA would be an efficient marker for MRD. In this study, we evaluated WT1 mRNA levels in peripheral blood using the WT1 mRNA Assay Kit (Otsuka). Previously, we reported that 107 of 114 untreated patients with AML were positive for WT1 mRNA in the peripheral blood; levels of WT1 mRNA were reduced to fewer than 50 copies/μg RNA ('negative')

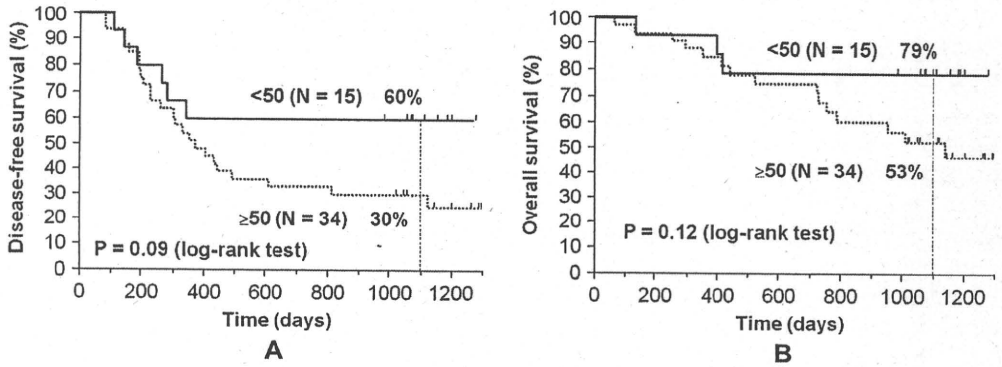

Figure 2. (A) Disease-free survival and (B) overall survival according to the detection of WT1 mRNA after CR.

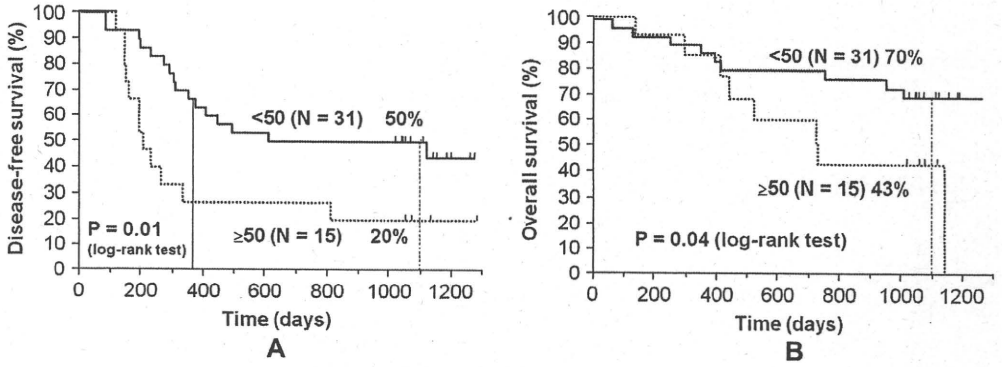

Figure 3. (A) Disease-free survival according to the detection of WT1 mRNA after consolidation. The solid line indicates the rate of relapse within 1 year after the date of CR: WT1 positive patients, 73%; WT1 negative patients, 33% ($p = 0.01$, χ^2 test). (B) Overall survival according to the detection of WT1 mRNA after consolidation.

after achieving remission and then increased again when the disease relapsed. However, some reports [16,17] indicate that WT1 mRNA is not a reliable marker for MRD. This discrepancy may have arisen mainly because normal hematopoietic progenitor cells also express WT1 mRNA. Weisser *et al.* [9] evaluated WT1 mRNA in bone marrow and reported that high levels of WT1 mRNA were associated with short OS and event-free survival (EFS) within the intervals of 61–120 and 120–180 days after the start of chemotherapy, but not at diagnosis and from days 16 to 60. They further insisted that future studies to investigate the applicability of peripheral blood as the test material were necessary. Therefore, we evaluated the WT1 mRNA level in peripheral blood, because peripheral blood does not contain normal progenitor cells. Until now, only Cilloni *et al.* [18] and Nowakowska-Kopera *et al.* [19] have analyzed WT1 mRNA levels in peripheral blood instead of bone marrow for MRD.

Our results showed that WT1 mRNA levels at diagnosis did not correlate with the relapse rate or with DFS and OS. Cilloni *et al.* [18] also reported that no significant differences were observed in WT1 mRNA levels at diagnosis between patients with CR and those without CR, and patients who persisted in CR and those who relapsed. Inoue *et al.* [7] reported that high expression of WT1 was an adverse prognostic factor for AML. Conversely, it was also reported that WT1 mRNA levels were remarkably high in patients with favorable cytogenetics [10]. Hence, WT1 mRNA level at diagnosis is not an adverse prognostic factor.

Our results indicated that patients with positive WT1 mRNA at achievement of CR tended to relapse, in comparison with WT1 mRNA negative patients. Cilloni *et al.* [18] also reported that all patients in CR whose WT1 mRNA levels were above normal relapsed. Moreover, Lapillonne *et al.* [10] demonstrated that an elevated WT1 mRNA level (2 SD greater than that of normal control bone marrow) after induction is an independent prognostic risk factor for relapse and death. Therefore, in order to prevent relapse, more intensified consolidation therapy such as high-dose Ara-C or SCT is recommended for WT1 mRNA positive patients upon achievement of CR.

Until now, there have been only two reports on WT1 mRNA levels after consolidation therapy [12,19]. In this study, we found that the detection of WT1 mRNA after consolidation therapy is a poor prognostic factor for DFS or OS. Interestingly, WT1 positive patients suffered relapse after consolidation therapy earlier than negative patients, and the majority of the former suffered relapse within 1 year. The median CR duration of relapsed patients among the WT1 mRNA positive patients was 6.3 months. It is well known that the most important prognostic factor for relapsed AML is the duration of first CR, and unlike late relapsed patients, early relapsed patients fail to achieve a CR [20]. On the other hand, the results of SCT for patients in first CR did not differ from those of patients in second CR, and the results of SCT for patients in first or second CR differed considerably from those of patients without CR. On the basis of these data, WT1 positive patients should be recommended SCT after consolidation therapy in the first CR. In other words, we could define suitable patients and appropriate timing for SCT according to evaluation of the WT1 mRNA level in the peripheral blood after consolidation therapy.

In conclusion, we have shown that the detection of WT1 mRNA in the peripheral blood at achieving CR is closely correlated with the relapse rate, and the detection of WT1 mRNA after consolidation therapy indicates an early relapse and short survival. Although our results need to be confirmed with a large-scale prospective study, we consider that the detection of WT1 mRNA in the peripheral blood is essential for the prevention of relapse by therapeutic interventions such as intensified consolidation chemotherapy or SCT in CR. In a large-scale prospective study, the detection of WT1 mRNA in peripheral blood using the WT1 mRNA Assay Kit (Otsuka) can provide uniform results across hospitals, and this method is less stressful for patients.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Appendix. Names of leaders of the institutions who contributed to this study but are not included among the authors of this article (based on information at time of study)

No.	Name of institution and department	Investigator		Contract period (trial period*)
		Title	Name	
1	Department of Cell Therapy and Transplantation Medicine, Faculty of Medicine, The University of Tokyo Hospital	Lecturer	Shigeru Chiba	2001/06/21–2003/06/30 (2002/05/02–2003/05/19)
2	Department of Hematology and Cell Therapy, Aichi Cancer Center	Director	Yasuo Morishima	2001/08/01–2004/03/31 (2002/02/13–2003/07/30)
3	Internal Medicine, Nissay Hospital	Deputy Director	Masashi Nakagawa	2002/01/31–2003/09/30 (2002/04/22–2003/06/19)
4	Division of Hematology, Yaizu City Hospital	Director	Tadashi Tobita	2002/02/01–2003/09/30 (2002/02/21–2003/04/22)
5	Internal Medicine III, Hamamatsu University School of Medicine	Associate Professor	Kazunori Onishi	2001/08/20–2003/09/30 (2001/10/23–2003/05/21)
6	Department of Hematology and Oncology, Osaka University	Associate Professor	Hiroyasu Ogawa	2001/09/13–2003/12/31 (2001/12/12–2003/08/14)
7	Internal Medicine, Ogaki Municipal Hospital	Head	Hirofumi Kosugi	2002/02/20–2003/09/30 (2002/03/15–2003/08/12)
8	Internal Medicine, Nagoya National Hospital	Head	Motohiro Hamaguchi	2002/04/01–2003/03/31 (2002/04/25–2003/01/14)
9	Internal Medicine I, Tokyo Medical College	Professor	Kazuma Oyashiki	2001/11/15–2003/09/30 (2002/01/15–2003/08/19)

*Date of collection of samples from first patient–date of collection of samples from last patient.

Prognostic factors and outcomes of adult patients with acute myeloid leukemia after first relapse

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background
Patients with acute myeloid leukemia who are treated with conventional chemotherapy still have a substantial risk of relapse; the prognostic factors and optimal treatments after relapse have not been fully established. We, therefore, retrospectively analyzed data from patients with acute myeloid leukemia who had achieved first complete remission to assess their prognosis after first relapse.

Design and Methods
Clinical data were collected from 70 institutions across the country on adult patients who were diagnosed with acute myeloid leukemia and who had achieved a first complete remission after one or two courses of induction chemotherapy.

Results
Among the 1,535 patients who were treated with chemotherapy alone, 1,015 relapsed. Half of them subsequently achieved a second complete remission. The overall survival was 30% at 3 years after relapse. Multivariate analysis showed that achievement of second complete remission, salvage allogeneic hematopoietic cell transplantation, and a relapse-free interval of 1 year or longer were independent prognostic factors. The outcome after allogeneic transplantation in second complete remission was comparable to that after transplantation in first complete remission. Patients with acute myeloid leukemia and cytogenetic risk factors other than inv(16) or t(8;21) had a significantly worse outcome when they did not undergo salvage transplantation even when they achieved second complete remission.

Conclusions
We found that both the achievement of second complete remission and the application of salvage transplantation were crucial for improving the prognosis of patients with acute myeloid leukemia in first relapse. Our results indicate that the optimal treatment strategy after first relapse may differ according to the cytogenetic risk.

Key words: acute myeloid leukemia, allogeneic hematopoietic cell transplantation, first relapse, second remission, cytogenetic risk.

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Introduction

Although up to 80% of patients with acute myeloid leukemia achieve first hematologic complete remission (CR1) with current induction chemotherapy, a substantial number of patients have an individualized risk of relapse.[1] Several risk factors have been defined in CR1 and these are used to stratify the treatment strategy in CR1.[2-4] However, once patients relapse, the probability of achieving a second complete remission (CR2) becomes lower and the duration of the second disease-free interval is generally reported to be shorter, meaning that the prognosis of patients who relapse is still challenging.[5-10]

Several retrospective studies have tried to identify the prognostic factors and optimal treatment strategies after first relapse.[7-12] Breems *et al.* evaluated the prognosis of patients with acute myeloid leukemia in first relapse including those after allogeneic hematopoietic cell transplantation (HCT) and showed that age, relapse-free interval, cytogenetic risks and previous allogeneic HCT were independent prognostic factors.[12] With regard to the treatment strategy, salvage allogeneic HCT has been shown to improve the outcome after relapse.[11] However, clinically important facts, such as the impact of the disease status at salvage allogeneic HCT and what treatment strategy should be used after relapse according to the disease risk have not yet been fully clarified. In addition, these issues have been difficult to analyze in a randomized study setting. We, therefore, performed a retrospective analysis of patients with non-M3 acute myeloid leukemia who relapsed after being treated with conventional chemotherapy in CR1.

Design and Methods

Patients

The study protocol was approved by the Institutional Review Board at the National Cancer Center Hospital. We constructed a new database of adult patients, aged 16 to 70 years, who were diagnosed with acute myeloid leukemia according to the World Health Organization classification between 1999 and 2006, and who had achieved CR1 after one or two courses of induction chemotherapy. Clinical information on over 2,500 patients was collected from 70 institutions across the country. Data from patients with biphenotypic leukemia who were treated with chemotherapy for acute lymphocytic leukemia and those who had extramedullary acute myeloid leukemia without marrow invasion, an extramedullary lesion that did not totally disappear after remission induction chemotherapy or acute promyelocytic leukemia were excluded from the analysis. As patients who relapsed after treatment with conventional chemotherapy alone were analyzed in this study, those who received autologous HCT in CR1 were also excluded.

Statistical analysis

Data were retrospectively reviewed and analyzed as of February 2010. Background differences between two groups were examined with the χ^2 test for categorical variables and the t-test for continuous variables. The primary end-point of the study was overall survival after first relapse. Overall survival from CR1, overall survival and cumulative incidences of relapse and non-relapse mortality from the date of allogeneic HCT were also estimated. The unadjusted probabilities of overall survival were estimated using the Kaplan-Meier product limit method, and 95% confidence intervals were calculated using the Greenwood formula. The log-rank test was used to compare overall survival among different subgroups. The Pepe-Mori test was used to evaluate differences in the cumulative incidence among groups. Overall survival and incidences of relapse and non-relapse mortality were estimated as probabilities at 3 years from the time of the first relapse, allogeneic HCT or CR1. A Cox proportional hazard regression model was used to estimate relative hazard ratios for overall survival, and a risk ratio regression model was used to estimate risk ratios for the achievement of CR2. The following factors were considered as covariates: age, relapse-free interval from CR1, achievement of CR2, application of salvage allogeneic HCT, number of courses of chemotherapy required to achieve CR1, cytogenetic risk according to Southwest Oncology Group,[4] French-American-British cytological classification, white blood cell count, and dysplasia at diagnosis. We considered two-sided *P* values less than 0.05 to be statistically significant. Statistical analyses were performed with the SPSS software package and SAS version 9.1.3 (SAS, Cary, NC, USA).

Results

Patients

Among the 2,029 patients with acute myeloid leukemia who achieved CR1, 494 patients underwent allogeneic HCT in CR1. The remaining 1,535 patients were treated with conventional chemotherapy alone, and 1,015 subsequently relapsed at a median interval of 8.8 months after having attained CR1 (range, 0.3-98.7 months, Figure 1). The median age of those who relapsed was 53 years (range, 16-70 years), and the median follow-up of patients who relapsed was 49 months (range, 5-116 months). As shown in Table 1, there were significant differences in clinical characteristics between patients who underwent allogeneic HCT in CR1 and those who did not, and between patients who relapsed after being treated with chemother-

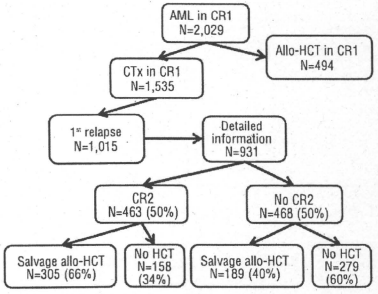

Figure 1. In first complete remission, 494 patients underwent allogeneic hematopoietic cell transplantation (allo-HCT). The remaining 1,535 patients were treated with conventional chemotherapy, and 1,015 of them subsequently relapsed. Of 931 patients for whom detailed information was available, 463 achieved second complete remission, and 305 of them underwent salvage allogeneic transplantation. Among 468 patients who did not achieve second complete remission, 189 underwent salvage allogeneic transplantation.

apy alone and those who did not. As remission induction therapy, 87% of 2,029 patients had received cytarabine- and anthracycline- (daunorubicin or idarubicin) based regimens. The remaining patients were treated with low dose cytarabine-based regimens (5%), BHAC-based regimens (5%), or others (3%). Consolidation therapy was also continued with cytarabine-based regimens with or without maintenance therapy. After first relapse, most patients received cytarabine plus anthracycline-based re-induction chemotherapy at the discretion of their physicians.

Outcome after first relapse

The overall survival of the 1,015 patients who relapsed was 30% at 3 years after first relapse (Figure 2A). Overall

Table 1. Patients' characteristics.

Characteristics	Allo-HCT in CR1	Chemotherapy in CR1			P values		
		1st relapse		No relapse			
	n=494 (%)	Allo-HCT n=527 (%)	No allo-HCT n=488 (%)	n=520 (%)	HCT vs. CTx in CR1*	Relapse vs. no relapse†	HCT in CR1 vs. HCT after relapse†
Age, years median (range)	43 (16-70)	43 (16-70)	60 (16-70)	52 (16-70)	*<0.001*	*0.356*	*0.048*
FAB classification					*<0.001*	*0.007*	*<0.001*
M1, 2, 4, 5	339 (69)	472 (90)	401 (82)	472 (91)			
M0, 6, 7	81 (16)	33 (6)	48 (10)	23 (4)			
Others	74 (15)	22 (4)	39 (8)	25 (5)			
Cytogenetic risk (SWOG)					*<0.001*	*<0.001*	*<0.001*
Favorable	29 (6)	138 (26)	69 (14)	153 (29)			
Intermediate	272 (55)	238 (45)	259 (53)	280 (54)			
Unfavorable	115 (23)	88 (17)	98 (20)	60 (12)			
Unknown	78 (16)	63 (12)	62 (13)	27 (5)			
Remission induction					*<0.001*	*<0.001*	*<0.001*
1 course	340 (69)	432 (82)	376 (77)	468 (90)			
2 courses	154 (31)	95 (18)	112 (23)	52 (10)			
White blood cell count (×10⁹/L)					*0.123*	*0.005*	*<0.001*
≤20	303 (61)	254 (48)	300 (61)	339 (65)			
>20	163 (33)	224 (43)	171 (35)	175 (34)			
Data not available	28 (6)	49 (9)	17 (3)	6 (1)			
Dysplasia					*<0.001*	*0.016*	*<0.001*
No	338 (68)	458 (87)	363 (74)	446 (86)			
Yes	156 (32)	69 (13)	125 (26)	74 (14)			

*CR1: first complete remission; allo-HCT: allogeneic hematopoietic cell transplantation; CTx: chemotherapy; FAB, French-American-British; others of FAB includes refractory anemia with excess blasts in transformation, and others which were not categorized in the FAB classification. *P value of "Allo-HCT in CR1" versus "Chemotherapy in CR1**"*. †P-value of "1st relapse**" versus "No relapse*". ‡P-value of "Allo-HCT in CR1" versus "Allo-HCT after relapse*".*

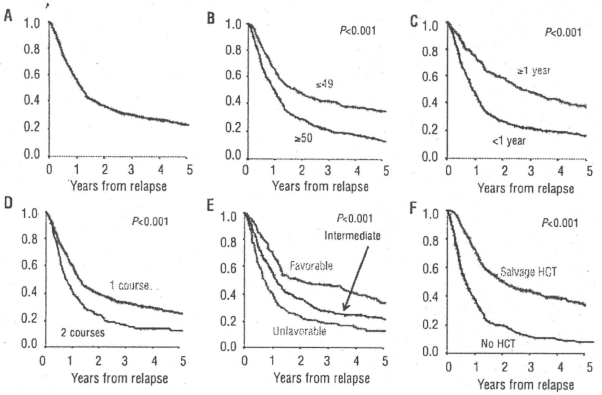

Figure 2. Overall survival after first relapse (A) for the total population, and according to (B) age, (C) relapse-free interval from first complete remission, (D) the number of courses of remission induction chemotherapy to achieve first complete remission, (E) cytogenetic risk according to the SWOG criteria, and (F) application of salvage allogeneic hematopoietic cell transplantation.

survival after relapse was significantly affected by age, relapse-free interval from CR1, the number of courses of chemotherapy required to achieve CR1 and cytogenetic classification (Figure 2B-E).

Salvage allogeneic hematopoietic cell transplantation after first relapse

Among 931 patients for whom detailed information after relapse was available, 463 achieved CR2 (50%, Figure 1) with different probabilities according to the cytogenetic risk [inv(16), 84%; t(8;21), 58%; intermediate, 48%; unfavorable, 31%]. After CR2 had been achieved, 305 patients (66%) underwent salvage allogeneic HCT, of whom 242 (80%) received the transplant while remaining in CR2. On the other hand, 189 (40%) of the 468 patients who did not achieve CR2 underwent salvage allogeneic HCT in non-remission status. Overall, half of the patients underwent salvage allogeneic HCT after their first relapse and had a better overall survival than that of patients who survived at least 2 months after relapse and did not undergo allogeneic HCT (44% *versus* 14% at 3 years from the first relapse, $P<0.001$, Figure 2F).

Comparison of disease status at allogeneic hematopoietic cell transplantation

We compared the outcome after salvage allogeneic HCT to that after allogeneic HCT in CR1. As shown in Table 1, 527 patients who underwent allogeneic HCT after relapse were less frequently associated with unfavorable factors compared to 494 patients who underwent allogeneic HCT in CR1. The source of cells for salvage HCT were HLA-matched related donors (31%), one-antigen mismatched related donors (6%), bone marrow from unrelated donors (40%), or cord blood from unrelated donors (24%). The conditioning regimens were myeloablative (65%, median age: 37 years) or reduced-intensity (35%, median age: 55 years) regimens (*Online Supplementary Table S1*). The source of stem cells was more frequently an unrelated donor, especially in the form of unrelated cord blood, in allogeneic HCT after relapse and there was a slight increase in the use of a reduced-intensity conditioning regimen for these transplants. Overall survival was significantly better after allogeneic HCT in CR1 than after relapse (67% *versus* 51% at 3 years from CR1, $P<0.001$, Figure 3A). This result did not change when patients who relapsed within 2 months of CR1 were excluded from among those who underwent allogeneic HCT after relapse. The statistical difference between the outcomes of the two groups also remained whether the donor was a matched relative or an unrelated donor.

When overall survival was compared in relation to disease status at allogeneic HCT after relapse, patients who underwent their transplant in CR2 had a significantly better overall survival than those who achieved CR2 but subsequently relapsed by the time of the transplant and those who never achieved CR2 (59%, 29%, and 21% at 3 years from HCT, $P<0.001$, Figure 3B). This result led us to compare the outcomes of allogeneic HCT in CR1 and CR2. There was no significant difference in terms of overall survival, non-relapse mortality or relapse after allogeneic HCT between the two groups (overall survival, 64% *versus* 59%, $P=0.090$; non-relapse mortality, 18% *versus* 20%, $P=0.316$; relapse, 22% *versus* 27%, $P=0.061$, Figure 3C, E, and F). The overall survival was also compared

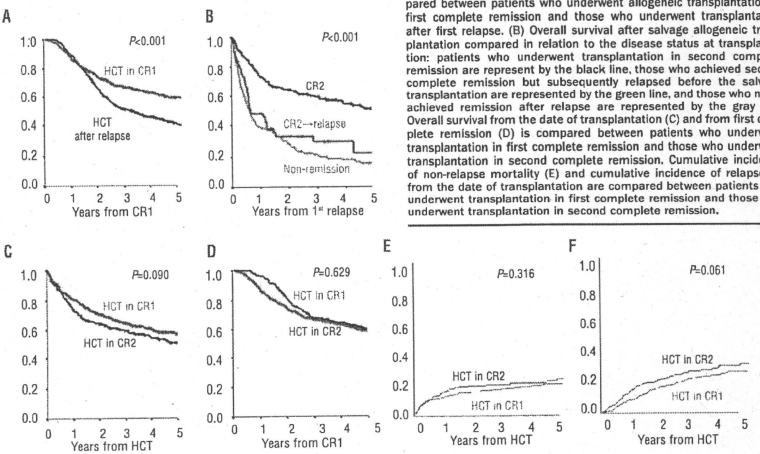

Figure 3. (A) Overall survival from first complete remission is compared between patients who underwent allogeneic transplantation in first complete remission and those who underwent transplantation after first relapse. (B) Overall survival after salvage allogeneic transplantation compared in relation to the disease status at transplantation: patients who underwent transplantation in second complete remission are represent by the black line, those who achieved second complete remission but subsequently relapsed before the salvage transplantation are represented by the green line, and those who never achieved remission after relapse are represented by the gray line. Overall survival from the date of transplantation (C) and from first complete remission (D) is compared between patients who underwent transplantation in first complete remission and those who underwent transplantation in second complete remission. Cumulative incidence of non-relapse mortality (E) and cumulative incidence of relapse (F) from the date of transplantation are compared between patients who underwent transplantation in first complete remission and those who underwent transplantation in second complete remission.

from CR1, and the survival curves were almost identical (67% *versus* 68%, *P*=0.629, Figure 3D).

Treatment strategy after first relapse

We also investigated the outcomes of patients who did or did not undergo subsequent allogeneic HCT after the achievement of CR2 and the effectiveness of allogeneic HCT when CR2 was not achieved or sustained (Figure 4).

We divided the 1,015 patients who relapsed into four subgroups according to their cytogenetic risk: a subgroup with inv(16) (n=61), another with t(8;21) (n=139), a subgroup with intermediate risk (n=469) and a subgroup with unfavorable risk (n=177) according to Southwest Oncology Group criteria (cytogenetic risk unknown, n=125; data not available on treatment after first relapse, n=44). Among patients with inv(16), overall survival after

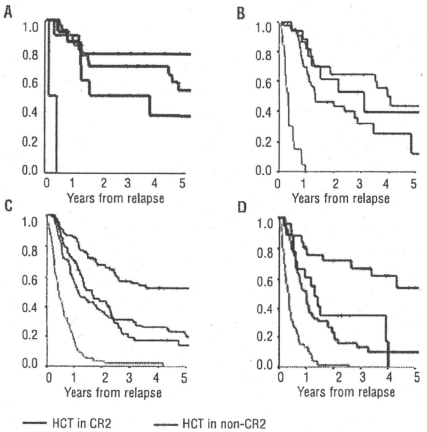

Figure 4. Overall survival after first relapse is shown according to treatment after relapse: allogeneic hematopoietic cell transplantation in second complete remission (HCT in CR2, indicated by red line), no allogeneic transplantation after achievement of second complete remission (CR2/no HCT, black line), allogeneic transplantation in a disease status other than second complete remission (HCT in non-CR2, green line), and no achievement of second complete remission without salvage allogeneic transplantation (NR/no HCT, gray line). *P* values among each of the cytogenetics groups are in the following order: (i) HCT in CR2 *vs.* CR2/no HCT, (ii) HCT in CR2 *vs.* HCT in non-CR2, (iii) HCT in CR2 *vs.* NR/no HCT, (iv) CR2/no HCT *vs.* HCT in non-CR2, (v) CR2/no HCT *vs.* NR/no HCT, (vi) HCT in non-CR2 vs. NR/no HCT. (A) inv(16): 3-year overall survival from relapse: HCT in CR2, n=31, 70%; CR2/no HCT, n=14, 78%; HCT in non-CR2, n=13, 50%; NR/no HCT, n=3, 0%; *P* values, (i) 0.415, (ii) 0.280, (iii)<0.001, (iv) 0.130, (v)<0.001, (vi) 0.003. (B) t(8;21): HCT in CR2, n=46, 64%; CR2/no HCT, n=18, 53%; HCT in non-CR2, n=50, 32%; NR/no HCT, n=25, 0%; *P* values, (i) 0.600, (ii) 0.012, (iii) <0.001, (iv) 0.163, (v) <0.001, (vi) <0.001. (C) intermediate-risk acute myeloid leukemia: HCT in CR2, n=109, 58%; CR2/no HCT, n=82, 19%; HCT in non-CR2, 31%, n=129; NR/no HCT, n=149, 2%; *P* values, (i) <0.001, (ii) <0.001, (iii) <0.001, (iv) 0.814, (v)<0.001, (vi)<0.001 (D) unfavorable-risk acute myeloid leukemia: HCT in CR2, n=27, 67%; CR2/no HCT, n=18, 35%; HCT in non-CR2, n=61, 13%; NR/no HCT, n=71, 0%; *P* values, (i) 0.005, (ii) <0.001, (iii)<0.001, (iv) 0.288, (v) <0.001, (vi) <0.001.

Table 2. Multivariate analysis.

Variables	Overall survival			Achievement of CR2		
	Hazard ratio	(95%CI)	*P*	Risk ratio	(95%CI)	*P*
Achievement of CR2 (*versus* Yes)						
No	3.23	(2.65-3.94)	<0.001	-	-	-
Salvage allo-HCT (*versus* Yes)						
No	2.61	(2.10-3.25)	<0.001	-	-	-
Interval from CR1 to relapse (*versus* ≥1 year)						
<1 year	1.80	(1.45-2.23)	<0.001	1.56	(1.37-1.78)	<0.001
Age (*versus* ≤49 years)						
≥50 years	1.15	(0.92-1.44)	0.21	1.04	(0.92-1.19)	0.530
Cytogenetic risk (SWOG, *versus* Favorable)						
Intermediate	1.11	(0.86-1.42)	0.421	1.14	(0.99-1.31)	0.074
Unfavorable	1.34	(1.00-1.78)	0.049	1.64	(1.24-2.17)	<0.001
Unknown	0.93	(0.66-1.32)	0.693	1.05	(0.86-1.27)	0.644
FAB (*vs.* M1, 2, 4, 5)						
M0, 6, 7	1.07	(0.80-1.43)	0.633	1.49	(1.02-2.17)	0.040
Remission induction (*versus* 1 course)						
2 courses	1.23	(1.01-1.52)	0.044	1.27	(1.02-1.59)	0.031
Dysplasia (*versus* No)						
Yes	1.24	(0.97-1.57)	0.084	1.43	(1.09-1.88)	0.011
WBC at diagnosis (*versus* ≤20×10⁹/L)						
>20×10⁹/L	1.08	(0.90-1.29)	0.414	0.88	(0.79-0.98)	0.025

CR2: second complete remission; allo-HCT: allogeneic hematopoietic cell transplantation; CR1: first complete remission; FAB: French-American-British classification.