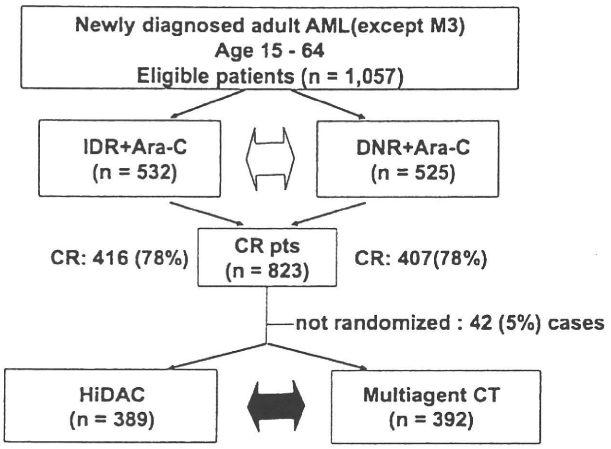

Figure 1. CONSORT diagram.

study to compare 4 courses of multiagent CT with 3 courses of HiDAC therapy after its approval in April 2001.

Methods

Patients

From December 2001 to December 2005, 1064 newly diagnosed adult patients 15 to 64 years of age with de novo AML were consecutively registered from 129 participating institutions. AML was first diagnosed by the French-American-British classification at each institution. Peripheral blood and bone marrow smears of registered patients were reevaluated by the central review committee. French-American-British M3 was not registered. Eligibility criteria included adequate function of liver (serum bilirubin < 2.0 mg/dL), kidney (serum creatinine < 2.0 mg/dL), heart and lung, and an Eastern Cooperative Oncology Group performance status between 0 and 3. Patients were not eligible if they had prediagnosed myelodysplastic syndrome or prior chemotherapy for other disorders. Cytogenetic abnormalities were grouped by standard criteria and classified according to the Medical Research Council classification.[8] The study was approved by institutional review boards at each participating institution. Written informed consent was obtained from all patients before registration in accordance with the Declaration of Helsinki.

Induction therapy consisted of Ara-C 100 mg/m^2 for 7 days and either IDR (12 mg/m^2 for 3 days) or DNR (50 mg/m^2 for 5 days). If patients did not achieve remission after the first course, the same therapy was administered once more. The outcome of induction therapy was reported to the JALSG Statistical Center before the consolidation therapy started. All CR patients were stratified according to induction regimen, number of courses of induction, age and karyotype, and randomized to receive either 4 courses of multiagent CT or 3 courses of HiDAC therapy. The first course of multiagent CT consisted of mitoxantrone (7 mg/m^2 by 30-minute infusion for 3 days) and Ara-C (200 mg/m^2 by 24-hour continuous infusion for 5 days). The second consisted of DNR (50 mg/m^2 by 30-minute infusion for 3 days) and Ara-C (200 mg/m^2 by 24-hour continuous infusion for 5 days). The third consisted of aclarubicin (20 mg/m^2 by 30-minute infusion for 5 days) and Ara-C (200 mg/m^2 by 24-hour continuous infusion for 5 days). The fourth consisted of Ara-C (200 mg/m^2 by 24-hour continuous infusion for 5 days), etoposide (100 mg/m^2 by 1-hour infusion for 5 days), vincristine (0.8 mg/m^2 by bolus injection on day 8), and vindesine (2 mg/m^2 by bolus injection on day 10). Each consolidation was started as soon as possible after neutrophils, white blood cells (WBCs), and platelets recovered to more than 1.5 × 10^9/L, 3.0 × 10^9/L, and 100.0 × 10^9/L, respectively. In the HiDAC group, 3 courses of Ara-C 2.0 g/m^2 by 3-hour infusion every 12 hours for 5 days were given. Each course was started 1 week after neutrophils, WBCs, and platelets recovered to the aforementioned counts.

Bone marrow examination was performed to confirm CR in both groups before each consolidation therapy and at the end of all consolidation therapy.

Best supportive care, including administration of antibiotics and platelet transfusions, was given if indicated. When patients had life-threatening documented infections during neutropenia, the use of granulocyte colony-stimulating factor was permitted.

After the completion of consolidation therapy, patients received no further chemotherapy. Allogeneic stem cell transplantation (allo-SCT) was offered during the first CR to patients of age 50 years or less with a histocompatible donor in the intermediate or adverse cytogenetic risk groups. Stem cell source was related donor or unrelated donor. Cord blood was not used. Conditioning before transplantation and prophylaxis for graft-versus-host disease were performed according to each institutional standard.

Responses were evaluated by the recommendations of the International Working Group.[9] CR was defined as the presence of all of the following: less than 5% of blasts in bone marrow, no leukemic blasts in peripheral blood, recovery of peripheral neutrophil counts more than 1.0 × 10^9/L and platelet counts more than 100.0 × 10^9/L, and no evidence of extramedullary leukemia. Relapse was defined as the presence of at least one of the

Table 1. Clinical characteristics of randomized patients

Characteristic	HiDAC (n = 389)	Multiagent CT (n = 392)	P
Age, y, median (range)	46 (15-64)	47 (15-64)	.697
WBC, ×10^9/L, median (range)	15.6 (0.1-382)	14.9 (0.2-260)	.323
Karyotype, n			.210
Favorable	108	110	
Intermediate	242	256	
Adverse	27	14	
Unknown	12	12	
Induction, n			.914
IDR	196	196	
DNR	193	196	
Induction 1 cycle, %	81.0	81.4	.886

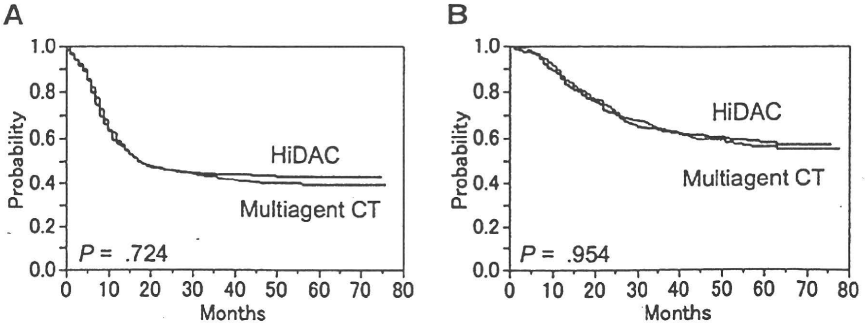

Figure 2. DFS and OS according to treatment arm. (A) DFS of CR patients. Predicted 5-year DFS was 43% for the HiDAC group (n = 389; red line) and 39% for the multiagent CT group (n = 392; blue line; P = .724). (B) OS of CR patients. Predicted 5-year OS was 58% for the HiDAC group (n = 389; red line) and 56% for the multiagent CT group (n = 392; blue line; P = .954).

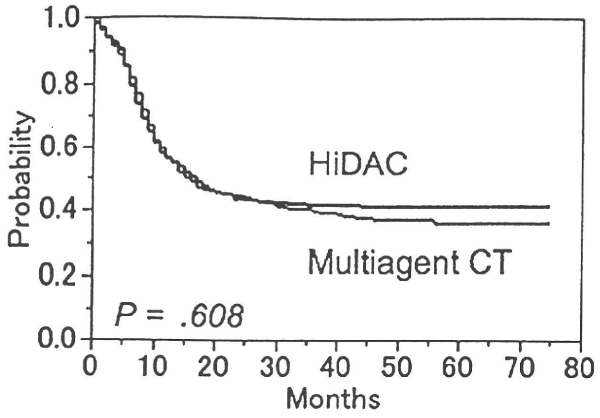

Figure 3. DFS according to treatment arm, after censoring the observation in transplanted patients. Predicted 5-year DFS was 41% for the HiDAC group (n = 389; red line) and 36% for the multiagent CT group (n = 392; blue line; $P = .608$).

following: reappearance of leukemic blasts in peripheral blood, recurrence of more than 5% blasts in bone marrow, and appearance of extramedullary leukemia.

Statistical analysis

This was a multi-institutional randomized phase 3 study with a 2 × 2 factorial design. The primary endpoint of the first randomization was CR rate, and a sample size of 420 patients per group was estimated to have a power of 90% at a 1% level of significance to demonstrate noninferiority (assuming 80% CR rate for both groups). For the second randomization (ie, this study), the primary endpoint was DFS, and the secondary end points were OS and adverse events of grade 3 or more by National Cancer Institute Common Toxicity Criteria. A sample size of 280 patients per group was estimated to have a power of 80% at a 5% level of significance to demonstrate 10% superiority in 5-year DFS for the HiDAC arm (40% vs 30%). OS was defined as the time interval from the date of diagnosis to the date of death. DFS for patients who had achieved CR was defined as the time interval from the date of CR to the date of the first event (either relapse or death). Patients who underwent allo-SCT were not censored. The Kaplan-Meier method was used to estimate probabilities of DFS and OS. For comparison of DFS and OS, the log-rank test was used for univariate analysis and the proportional hazard model of Cox for multivariate analysis. Cumulative incidence of relapse and treatment-related mortality were estimated according to the competing risk method and were evaluated with Gray test. The Wilcoxon rank-sum test was used for continuous data, such as age and WBC count, whereas the χ^2 test was used for ordinal data, such as risk group and frequency of allo-SCT. Statistical analyses were conducted using the JMP program (SAS Institute) and R software Version 2.9.1 (www.r-project.org).

Results

Response to induction therapy

Of 1064 patients registered, 1057 patients were evaluable. Seven patients (1 misdiagnosis, 1 infectious complication, 1 without therapy, and 4 withdrawal of consent) were excluded. Median age was 47 years (range, 15-64 years). Cytogenetic studies were performed in 99.2% of registered patients and the results were available in 97%. Of 1057 evaluable patients, 823 (78%) achieved CR (662 of them after the first induction course). CR rate in the IDR and DNR arms was similar (78.2% vs 77.5%). Percentage of patients who reached CR after the first induction course was also similar (64.1% vs 61.1%, $P = .321$). Day to achieve CR was longer in the IDR arm than the DNR arm (33.8 vs 32.4 days, $P = .038$). The detailed result of induction phase of this study is reported in a separate paper.[10]

Postremission randomization

Of 823 patients who achieved CR, 42 did not undergo the second randomization for a variety of reasons, which included residual toxicity from induction therapy (12), allo-SCT (8), death (1), refusal (1), and unknown (20). The remaining 781 patients were randomly assigned to receive either the HiDAC regimen (389) or the multiagent CT regimen (392; Figure 1). Clinical characteristics of 2 treatment groups were well balanced in age, initial WBC count, cytogenetic risk, induction arm, and induction cycle (Table 1).

DFS and OS

The median follow-up period of living patients was 48 months (range, 5-78 months). Five-year DFS was 43% for the HiDAC group and 39% for the multiagent CT group ($P = .724$; Figure 2A). Five-year OS was 58% for the HiDAC group and 56% for the multiagent CT group ($P = .954$; Figure 2B). After censoring the observation on the date of SCT in transplanted patients, 5-year DFS was 41% for the HiDAC group and 36% for the multiagent CT group ($P = .608$; Figure 3).

The cumulative incidences of relapse and treatment-related mortality during CR, respectively, were 49% and 8% for the HiDAC group and 56% and 5% for the multiagent CT group ($P = .294$, $P = .172$; Figure 4A). After censoring the observation in transplanted patients, those were 55% and 4% for the HiDAC group and 61% and 3% for the multiagent CT group ($P = .402$, $P = .409$), respectively (Figure 4B).

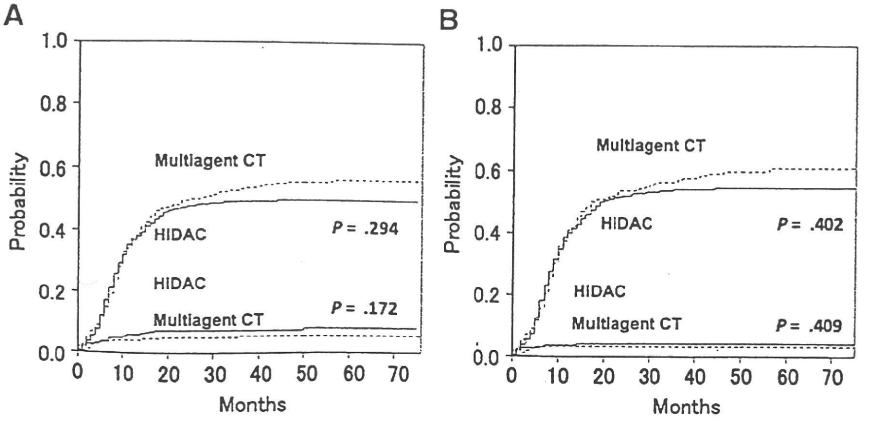

Figure 4. Cumulative incidence of relapse and treatment-related mortality in CR by treatment arm. (A) The incidences of relapse and mortality, respectively, were 49% and 8% for the HiDAC group (solid line) and 56% and 5% for the multiagent CT group (dotted line; $P = .324$, $P = .172$). (B) After censoring the observation in transplanted patients, the incidences of relapse and mortality, respectively, were 55% and 4% for the HiDAC group (solid line) and 61% and 3% for the multiagent CT group (dotted line; $P = .402$, $P = .409$).

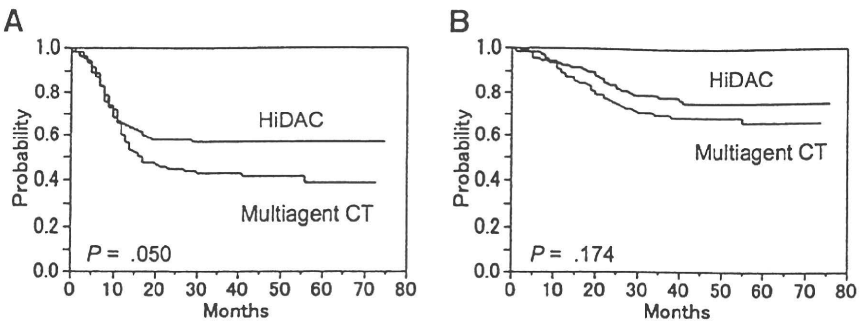

Figure 5. DFS and OS by treatment arm for the favorable cytogenetic risk group. (A) Predicted 5-year DFS was 57% for the HiDAC group (n = 108; red line) and 39% for the multiagent CT group (n = 110; blue line; $P = .050$). (B) Predicted 5-year OS was 75% for the HiDAC group (n = 108; red line) and 66% for the multiagent CT group (n = 110; blue line; $P = .174$).

In patients with the favorable cytogenetics, core-binding factor (CBF) leukemia with t(8;21) or inv(16), 5-year DFS was 57% in the HiDAC group and 39% in the multiagent CT group ($P = .050$; Figure 5A), and 5-year OS was 75% and 66%, respectively ($P = .174$; Figure 5B).

In patients with the intermediate cytogenetics, 5-year DFS was 38% in the HiDAC group and 39% in the multiagent CT group ($P = .403$; Figure 6A), and 5-year OS was 53% and 54%, respectively ($P = .482$; Figure 6B). In patients with the adverse cytogenetics, 5-year DFS was 33% in the HiDAC group and 14% in the multiagent CT group ($P = .364$; Figure 7A), and 5-year OS was 39% and 21%, respectively ($P = .379$; Figure 7B). Among younger patients (≤ 50 years), 5-year DFS was 45% in the HiDAC group and 46% in the multiagent CT group ($P = .590$), and 5-year OS was 62% and 66%, respectively ($P = .228$). Among the older patients (> 50 years), 5-year DFS was 40% in the HiDAC group and 28% in the multiagent CT group ($P = .230$), and 5-year OS was 51% and 40%, respectively ($P = .159$). In patients treated with the IDR regimen at induction, 5-year DFS was 42% in the HiDAC group and 41% in the multiagent CT group ($P = .641$), and 5-year OS was 58% and 57%, respectively ($P = .790$). In patients treated with the DNR regimen at induction, 5-year DFS was 44% in the HiDAC group and 37% in the multiagent CT group ($P = .339$), and 5-year OS was 58% and 56%, respectively ($P = .713$). There was no relationship between the duration of myelosuppression and DFS or OS.

Significant unfavorable prognostic features for DFS by the Cox proportional hazard model were WBC more than 20×10^9/L, the number of induction therapies, and age more than 50 years, and for OS, age more than 50 years, the number of induction therapies, WBC more than 20×10^9/L, and myeloperoxidase-positive blast less than 50%. Induction therapy, consolidation therapy, and cytogenetic risk group were not independent prognostic factors for DFS or OS by this multivariate analysis (Table 2).

Tolerance and toxicity of postremission therapy

All courses of consolidation were administered to 72.5% of patients in the HiDAC group and 70.2% in the multiagent CT group (Table 3). In the HiDAC group, 110 patients (28%) did not receive all 3 courses. The reasons included relapse (18), death in CR (10), allo-SCT (34), adverse events (27), patient's refusal (11), and unknown (10). In the multiagent CT group, 118 patients (30%) did not receive all 4 courses. The reasons included relapse (31), death in CR (8), allo-SCT (42), adverse events (13), patient's refusal (5), and unknown (19). The most common reason was allo-SCT in both groups. Of 125 patients received SCT in first CR, 49 (25 in HiDAC and 24 in multiagent CT) received SCT after completion of full courses of consolidation therapy. The second common reason was adverse events in the HiDAC group and relapse in the multiagent CT group. The patients older than 50 years could tolerate both regimens. Table 4 shows a comparison of both groups regarding the nadir of WBC count and the number of days of WBC less than 1.0×10^9/L. After each course of consolidation, the nadir of WBC count was significantly lower ($P < .0001$) and the day of WBC less than 1.0×10^9/L was significantly longer in the HiDAC group ($P < .001$). During each course of consolidation, the frequency and the number of days of granulocyte colony-stimulating factor administration were significantly higher in the HiDAC group. Table 5 shows toxic adverse events, excluding hematologic side effects. The frequency of documented infections was significantly higher in the HiDAC group ($P < .001$). The subset analysis showed the high incidence of documented infection in HiDAC regimen only in intermediate cytogenetic risk group ($P < .001$).

Discussion

To determine the best postremission therapy, there have been several prospective randomized studies comparing chemotherapy

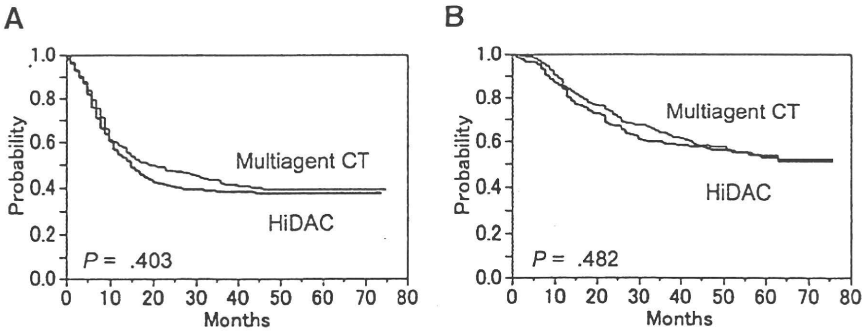

Figure 6. DFS and OS by treatment arm for the intermediate cytogenetic risk group. (A) Predicted 5-year DFS was 38% for the HiDAC group (n = 242; red line) and 39% for the multiagent CT group (n = 256; blue line; $P = .403$). (B) Predicted 5-year OS was 53% for the HiDAC group (n = 242; red line) and 54% for the multiagent CT group (n = 256; blue line; $P = .482$).

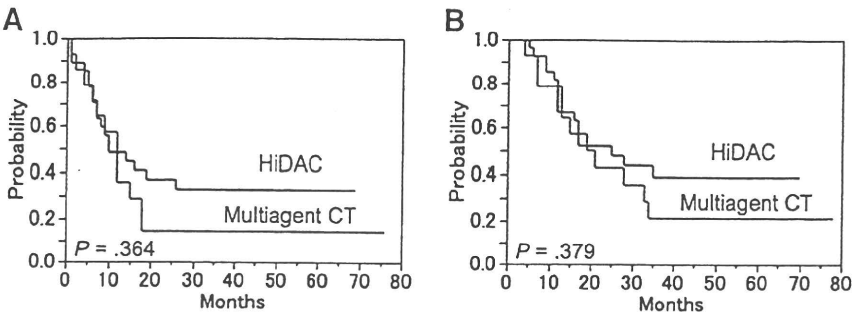

Figure 7. DFS and OS by treatment arm for the adverse cytogenetic risk group. (A) Predicted 5-year DFS was 33% for the HiDAC group (n = 27; red line) and 14% for the multiagent CT group (n = 14; blue line; $P = .364$). (B) Predicted 5-year OS was 39% for the HiDAC group (n = 27; red line) and 21% for the multiagent CT group (n = 14; blue line; $P = .379$).

with SCT. Although there is some limitation in SCT, such as patient age and availability of human leukocyte antigen–identical donors, most randomized studies demonstrate that SCT, the most intensive postremission modality, provides superior or at least noninferior prognosis in high- or intermediate-risk adult AML.[11-13]

As for postremission chemotherapy, HiDAC therapy is generally used in the United States and other countries after the landmark Cancer and Leukemia Group B-8525 (CALGB-8525) study.[14] In Japan, however, because HiDAC therapy was not approved by our national medical insurance system until 2001, combination chemotherapy using non–cross-resistant agents was commonly used in previous studies for adult AML. Therefore, in the current study, we compared conventional multiagent CT with HiDAC therapy.

Our study demonstrated that there is no difference in DFS and OS between the multiagent CT regimen and the HiDAC regimen. The HiDAC regimen, however, was accompanied with more frequent infectious events resulting from more severe and longer-lasting neutropenia. In the CALGB-8525 study,[14] patients randomized to 4 cycles of HiDAC regimen were administered 3 g/m^2 of Ara-C by 3-hour infusion, twice daily on days 1, 3, and 5, and our patients randomized to 3 cycles of HiDAC regimen were given 2 g/m^2 of Ara-C by 3-hour infusion, twice daily for 5 days. Although there were some differences in schedule and dose administered, the total dose of Ara-C was almost the same (72 g/m^2 vs 60 g/m^2). The Acute Leukemia French Association Group compared a timed-sequential consolidation consisting of etoposide, mitoxantrone, and Ara-C with a postremission chemotherapy, including 4 cycles of HiDAC (3 g/m^2), and reported that there were no statistically significant differences between the 2 groups in the rates of event-free survival and OS at 3 years.[15] The British Medical Research Council also compared a conventional Medical Research Council schedule (MACE/MidAC) with 2 courses of HiDAC regimens (3 g/m^2 or 1.5 g/m^2) and reported that there were no significant differences in DFS and OS at 5 years.[16]

On the contrary, the CALGB-8525 study[14] revealed that their HiDAC regimen was superior to the intermediate dose of Ara-C (400 mg/m^2 for 5 days) or to the conventional dose of Ara-C (100 mg/m^2 for 5 days) regimens in DFS and OS; this plausibly comes from the lower dose intensity of the intermediate- or standard-dose Ara-C regimens. Indeed, the CALGB-9222 study[17] showed no difference in DFS and OS between the HiDAC group and the intensified sequential multiagent chemotherapy group.

Cytogenetics is considered one of the most valuable prognostic determinants in adult AML.[8,18] In the present study, although in the intermediate-risk group, the DFS and OS of both consolidation groups were almost identical; in the favorable risk group, the outcome of the HiDAC group (n = 108) tended to be superior to that of the multiagent CT group (n = 110) in DFS (57% vs 39%; $P = .050$) and OS (75% vs 66%; $P = .174$) but not at statistically significant level; and in the adverse risk group, the similar but statistically nonsignificant trend in DFS (33% vs 14%) and OS (39% vs 21%) was noted. Bloomfield et al[19] reported that the HiDAC regimen is the most effective to CBF leukemia. In their study, patients with CBF leukemia (n = 18) had a 78% chance of remaining CR at 5 years when treated with the HiDAC regimen. However, our study showed that DFS of CBF leukemia (n = 108) treated with the HiDAC regimen was only 57% at 5 years.

There are 2 possible explanations of difference between our results and those reported by Bloomfield et al.[19] One is that their superior results may come from a small number of patients (n = 18). Indeed, the CALGB-9222 study,[17] including 28 patients with CBF leukemia, demonstrated that the 5-year DFS and OS of CBF leukemia treated with HiDAC was 60% and 70%, respectively. These data are similar to our results. The other is that CBF leukemia reveals different sensitivity to HiDAC therapy. Some patients with CBF abnormality have KIT mutations, which confer

Table 2. Factors to predict unfavorable prognostic features for DFS and OS by multivariate analysis

Survival type/variable	Category	Hazard ratio	P
DFS			
Initial WBC count	≥ 20 × 10^9/L	1.49	< .0001
No. of induction therapies	2 courses	1.50	.0006
Age, y	> 50	1.33	.0028
Consolidation therapy	Multiagent CT	1.04	.7128
OS			
Age, y	> 50	2.00	< .0001
No. of induction therapies	2 courses	1.58	.0033
Initial WBC count	≥ 20 × 10^9/L	1.41	.0070
MPO-positive blast	< 50 %	1.42	.0149
Consolidation therapy	Multiagent CT	0.96	.7768

MPO indicates myeloperoxidase.

Table 3. Tolerance of consolidation

	% receiving the full courses	
	HiDAC	Multiagent CT
All patients	72.5	70.2
Patients ≤ 50 y	71.9	69.0
Patients > 50 y	73.4	71.9
Reason for not receiving the full courses (no. of patients)		
Relapse	18	31
Death	10	8
SCT in first CR	31	42
Adverse event*	27	13
Patient refusal	11	5
Unknown	10	19

*$P < .05$.

Table 4. Intensity of consolidation

	HiDAC	Multiagent CT	P
After first consolidation			
Lowest WBC, $\times 10^9$/L	0.17	0.40	< .0001
Days WBC < 1.0 × 10^9/L	13 (0-40)	12 (0-36)	.0005
After second consolidation			
Lowest WBC, $\times 10^9$/L	0.10	0.40	< .0001
Days WBC < 1.0 × 10^9/L	14 (0-34)	13 (0-241)	.0007
After third consolidation			
Lowest WBC, $\times 10^9$/L	0.10	0.40	< .0001
Days WBC < 1.0 × 10^9/L	14 (0-38)	11.5 (0-28)	< .0001
After fourth consolidation			
Lowest WBC, $\times 10^9$/L		0.40	
Days WBC < 1.0 × 10^9/L		12 (0-34)	

Values are median (range).

higher relapse risk on CBF AML.[20,21] CALGB reported that 29.5% of patients with inv(16) and 22% of patients with t(8;21) had KIT mutations, and the cumulative incidence of relapse was higher for patients with mutated KIT than for those with wild-type KIT.[20] The difference of mutation rates of KIT might result in the difference in DFS. Unfortunately, in our present study, KIT mutations were not prospectively evaluated. However, a high mutation rate of KIT is reported among Asian patients with t(8;21) from Japan (37.8%)[22] and China (48.1%).[23] Consequently, JALSG is prospectively evaluating KIT mutation and its impact on the outcome in patients with CBF leukemia treated with repetitive HiDAC therapy. In the adverse cytogenetic risk group, the outcome of the HiDAC group also tends to be better than that of the multiagent CT group, but the difference is not statistically significant. The small number of this cohort may explain the statistical insignificance. Nevertheless, HiDAC therapy may be recommended to this group if patients have no human leukocyte antigen–matched donor.

Recently, IDR is frequently included into induction regimen for AML because of its better effectiveness compared with DNR.[24-26] A meta-analysis of randomized trials showed that the use of IDR instead of DNR results in a high CR rate.[27] However, a German group reported that the advantage of IDR in response rate may be lost during HiDAC consolidation therapy because of increased toxicity in the IDR group.[28] However, our current study demonstrated that, among the HiDAC group, there is no difference in DFS and OS between patients receiving IDR or DNR in induction phase. In our study, although one or 2 courses of the IDR regimen were given before the HiDAC consolidation, only 19% of patients required 2 courses to obtain CR. In contrast, the German group gave 2 courses of IDR induction regimen before the HiDAC consolidation. Thus, severe adverse events during HiDAC therapy probably depend on the total dose of prior IDR. Nevertheless, the HiDAC regimen could be given safely in our patients who had received IDR as induction therapy.

Table 5. Adverse events (CTC grades 3 and 4) during consolidation therapy

	HiDAC, %	Multiagent CT, %	P
Documented infection	20.9	14.5	< .001
Febrile neutropenia	66.5	66.4	.311
Bleeding	0.8	0.7	.601
Early death*	0.9	0.6	.389

*Death within 30 days after consolidation chemotherapy.

In conclusion, postremission consolidation regimen should be selected on the basis of prognostic factors, such as cytogenetics. Although several types of HiDAC regimen have been widely adopted as the optimal postremission therapy, the conventional multiagent CT may be recommendable for the intermediate or adverse cytogenetic risk groups. However, our HiDAC regimen should be recommended to the favorable cytogenetic risk group.

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Authorship

Contribution: S.M. designed and performed research, interpreted data, and wrote the manuscript; S.O. designed and performed research, collected and analyzed data, and participated in writing the manuscript; S.F., H.K., K.S., N.U., T.S., K.M., C.N., Y.M., M. Taniwaki, T. Nagai, T.Y., A.F., M. Takahashi, F.Y., Y.K., N.A., H.S., H.H., S.H., K.O., and T. Naoe performed research; and R.O. interpreted data and participated in writing manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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ORIGINAL ARTICLE

Pre-transplant imatinib-based therapy improves the outcome of allogeneic hematopoietic stem cell transplantation for *BCR–ABL*-positive acute lymphoblastic leukemia

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A high complete remission (CR) rate has been reported in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) following imatinib-based therapy. However, the overall effect of imatinib on the outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is undetermined. Between 2002 and 2005, 100 newly diagnosed adult patients with Ph + ALL were registered to a phase II study of imatinib-combined chemotherapy (Japan Adult Leukemia Study Group Ph + ALL202 study) and 97 patients achieved CR. We compared clinical outcomes of 51 patients who received allo-HSCT in their first CR (imatinib cohort) with those of 122 historical control patients in the pre-imatinib era (pre-imatinib cohort). The probability of overall survival at 3 years after allo-HSCT was 65% (95% confidence interval (CI), 49–78%) for the imatinib cohort and 44% (95% CI, 35–52%) for the pre-imatinib cohort. Multivariate analysis confirmed that this difference was statistically significant (adjusted hazard ratio, 0.44, $P=0.005$). Favorable outcomes of the imatinib cohort were also observed for disease-free survival ($P=0.007$) and relapse ($P=0.002$), but not for non-relapse mortality ($P=0.265$). Imatinib-based therapy is a potentially useful strategy for newly diagnosed patients with Ph + ALL, not only providing them more chance to receive allo-HSCT, but also improving the outcome of allo-HSCT.
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Introduction

The Philadelphia chromosome (Ph) presents in 20–25% of adult patients with acute lymphoblastic leukemia (ALL) and is an extremely unfavorable prognostic factor. The outcome of patients with Ph-positive ALL (Ph + ALL) following conventional chemotherapy is dismal, showing <20% long-term survival.[1–4] Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) has offered a curative option in Ph + ALL,[3–5] relatively high rates of relapse and non-relapse mortality (NRM) impair the treatment success even after allo-HSCT. The International Bone Marrow Transplant Registry reported a leukemia-free survival rate of 38% following human leukocyte antigen (HLA)-identical allo-HSCT for Ph + ALL patients transplanted in the first complete remission (CR).[6] Previously, we and others reported that imatinib-based chemotherapy produced very high CR rate, thus allowing a high proportion of patients to prepare for allo-HSCT.[7,8] However, because of the short observation period, the impact of imatinib-based therapy upon the survival outcomes after allo-HSCT remains unclear. To address whether allo-HSCT after imatinib-based therapy is a superior treatment approach to that after conventional chemotherapy, we conducted a retrospective analysis of Ph + ALL patients who underwent allo-HSCT before and after imatinib became available, using data from the Japan Adult Leukemia Study Group (JALSG) Ph + ALL202 study and from the nationwide database of the Japan Society of Hematopoietic Stem-cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP).

Patients and methods

Data source and patient selection criteria

We compared the transplantation outcome of patients treated by the JALSG Ph + ALL 202 study (imatinib cohort) with those in the historical control data in the pre-imatinib era from the JSHCT and JMDP (pre-imatinib cohort), in which information on patient survival, disease status and long-term complications, including chronic graft-versus-host disease (cGVHD) and second malignancies, is renewed annually using follow-up forms.[9,10] To

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attain an adequate level of comparability in terms of allo-HSCT, patients were selected according to the following criteria: (1) patients with *de novo* Ph + ALL; (2) age range of 15–65 years and (3) allo-HSCT during their first CR. A total of 122 patients who received allo-HSCT between January 1995 and December 2001 (before the approval of imatinib by the Japanese government) were selected. This study period of the pre-imatinib cohort included the pioneering period of cord blood transplantation (CBT) when the relevance of cell dose and HLA matching had not yet been recognized. Thus, the subjects were limited to those who received bone marrow (BM) or peripheral blood (PB) as a treatment graft.

Patients

Between September 2002 and May 2005, 100 newly diagnosed patients with Ph + ALL were registered to the JALSG Ph + ALL202 study, and received a phase 2 imatinib-combined chemotherapy as described previously.[7] Ph + ALL was diagnosed by the presence of Ph through chromosome and/or FISH analysis, and positivity for *BCR–ABL* fusion transcripts detection by real-time quantitative polymerase chain reaction (RQ–PCR) analysis.

Of 97 patients who achieved CR, 60 patients received allo-HSCT in their first CR. Of these 60 patients, 9 patients who received unrelated CBT were excluded in this analysis because of the reason as described at the selection criteria for control patients in the pre-imatinib era. Thus, 51 patients transplanted between February 2003 and December 2005 were analyzed. In the JALSG Ph + ALL202 study, allo-HSCT was recommended after achieving CR if an HLA-identical donor was available. The stem cell source for allo-HSCT was chosen in the following order: (1) matched-related allo-HSCT; (2) HLA-A, B and DRB1 allele matched (6/6) or DRB1 one-allele mismatched-unrelated allo-BMT, if patients had no HLA-matched-related donor and (3) unrelated CBT or HLA-mismatched-related allo-HSCT, if they had no donors described in (1) and (2). A prophylaxis for GVHD was determined by each institute, but did not include T-cell depletion. The study was approved by the institutional review board of each participating center and conducted in accordance with the Declaration of Helsinki.

Definition of engraftment and GVHD

Engraftment day was defined as the first day of three consecutive days when the absolute neutrophil count was $\geqslant 0.5 \times 10^9$/l. Graft failure was defined as the lack of any sign of neutrophil recovery. Engraftment that occurred after day 60 was also considered to be a graft failure. Patients who died early (<day 29) were excluded from the analysis of engraftment. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined according to previously described standard criteria.[11]

Quantitation of BCR–ABL transcripts

The copy number of *BCR–ABL* transcripts in BM was determined at a central laboratory using the RQ–PCR as described previously.[7] To minimize the variability in the results because of differences in the efficiency of cDNA synthesis and RNA integrity among the patient samples, the copy number of the *BCR–ABL* transcripts was converted to molecules per microgram RNA after being normalized by means of *GAPDH*. The normalized values of the *BCR–ABL* copies in each sample were reported as *BCR–ABL* number of copies. At least 5.7×10^5 copies/μg RNA *GAPDH* levels were required in a sample to consider a negative PCR result valid; otherwise, the sample was not useful for minimal residual studies. The threshold for quantification was 50 copies/μg RNA. The levels below this threshold were designated as 'not detected' or '<50 copies/μg'. In this study, the former was categorized as PCR negativity.

Minimal residual disease (MRD) at the time of HSCT was evaluated by the result of RQ–PCR within 30 days prior to transplantation.

Statistical considerations

The primary end point of this study was overall survival (OS) after allo-HSCT. Secondary end points included disease-free survival (DFS) and the incidence of aGVHD, cGVHD, NRM and relapse. We defined DFS events as relapse or death, whichever occurred earlier. The observation periods for OS were calculated from the date of transplantation until the date of the event or last known date of follow-up. The probabilities of OS and DFS were estimated using the Kaplan–Meier product limit method. The cumulative incidences of NRM, relapse, aGVHD and cGVHD were estimated as described elsewhere, taking the competing risk into account.[12] In each estimation of the cumulative incidence of an event, death without an event was defined as a competing risk. Risk factors for OS and DFS were evaluated by a combination of uni- and multivariate analyses. The following variables were evaluated for each analysis: imatinib-based therapy prior to HSCT, age group (under 40 versus 40 to 54 versus 55 and older), stem cell source (BM versus PB), HLA disparity (matched (HLA-identical siblings or 6/6 allele matched unrelated) versus mismatched), duration from diagnosis to HSCT and cGVHD as time-varying covariate (yes versus no). Univariate analysis was performed using Cox regression models or log-rank test. Multivariate analysis was performed using Cox proportional hazards regression model or competing risk regression model[13] as appropriate. For the evaluation of time-varying events, such as aGVHD or cGVHD, upon clinical outcomes, we treated these as time-varying covariates. Differences among groups in terms of demographic characteristics were tested using the χ^2 or Mann–Whitney tests as appropriate. All statistical analyses were conducted using STATA 11 (STATA Corp., College Station, TX, USA).

Results

Patient characteristics

In the imatinib cohort, there were 29 males and 22 females, with a median age of 38 years (range, 15–64 years). Regarding transcript types, 36 patients had minor *BCR–ABL* and 15 had major *BCR–ABL*. In 5 patients, pre-treatment cytogenetic data were not available, and of the remaining 46 patients, 8 showed t(9;22) only, 36 had additional chromosome aberrations and 2 showed normal karyotype. Of 48 patients who were evaluable for MRD analysis, 36 patients achieved PCR negativity at the time of HSCT.

Some of the clinical and biological features (such as presence of additional chromosome aberrations, *BCR–ABL* subtype, MRD status at HSCT and performance status at HSCT) were not available in the pre-imatinib cohort and not included in the present analysis.

Table 1 lists the characteristics of patients included in this comparative analysis. Some of the clinical features were significantly different between two cohorts: age distribution at HSCT ($P=0.048$), conditioning regimens ($P<0.001$), GVHD prophylaxis ($P<0.001$) and duration from diagnosis to HSCT ($P=0.041$). The majority of patients received the preparatory

Table 1 Patient characteristics ($N=173$)

Characteristic	*Imatinib cohort*	*Pre-imatinib cohort*	P
No. of transplantations	51	122	
Age, n (%)			0.048
–39	27 (53)	71 (58)	
40–54	17 (33)	49 (40)	
55–	7 (14)	2 (2)	
Median (range)	38 (15–64)	38 (15–57)	
Gender (male/female)	29/22	73/49	0.717
HSCT donor, n (%)			0.460
Related	24 (47)	73 (60)	
Unrelated	21 (41)	43 (35)	
HLA-mismatched related	6 (12)	6 (5)	
Hematopoietic cell source, n (%)			0.246
Bone marrow	35 (69)	94 (77)	
Peripheral blood	16 (31)	28 (23)	
Conditioning regimen, n (%)			<0.001
CY+TBI	24 (47)	26 (22)	
CY+CA+TBI	14 (27)	37 (31)	
CY+VP+TBI	2 (4)	21 (17)	
CY+TESPA+TBI	—	7 (6)	
CY+BU+TBI	—	6 (5)	
Flu+BU	3 (6)	—	
Flu+ LPAM ± TBI	2 (4)	—	
Others	6 (12)	25 (20)	
GVHD prophylaxis, n (%)			<0.001
Cyclosporine + sMTX	24 (47)	95 (80)	
Cyclosporine ± other	3 (6)	3 (2)	
Tacrolimus + sMTX	22 (43)	17 (14)	
Tacrolimus + other	—	4 (3)	
Median days from diagnosis to HSCT (range)	162 (67–512)	182 (66–834)	0.041

Abbreviations: BU, oral busulfan; CA, cytarabine; CY, cyclophosphamide; Flu, fuludarabine; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; LPAM, melphalan; sMTX, short-term methotrexate; TBI, total body irradiation; TESPA, tespamine; VP, etoposide.

regimen of total body irradiation followed by cyclophosphamide and/or cytarabine. Five patients aged >55 in the imatinib cohort were given a reduced intensity regimen consisting of fludarabine and melphalan or busulfan. In the pre-imatinib cohort, a combination of cyclosporine (CsA) and short-term methotrexate (sMTX) was mostly used in the prophylaxis of GVHD. On the other hand, both CsA + sMTX and tacrolimus (FK506) + sMTX combinations were commonly used in the imatinib cohort. In both cohorts, none of the patients received imatinib therapy after HSCT in their first CR. In the imatinib cohort, all patients who showed hematologic relapse after HSCT received salvage treatment comprising of imatinib and/or chemotherapy. As for the pre-imatinib cohort, 13 patients relapsed after the approval of imatinib by the Japanese government (beyond December 2001). However, we have no information on how many patients received imatinib-based therapy after their relapse. The median follow-up period for survivors was 2.6 years (range, 1.0–4.6 years) for the imatinib cohort and 6.9 years (range, 0.1–11.4 years) for the pre-imatinib cohort.

Outcome

OS and DFS. In the pre-imatinib cohort, 80 patients died after HSCT: 46 of disease recurrence and 34 of causes other than leukemia. In the imatinib cohort, 35 patients were alive, 32 of them were free of leukemia and 16 patients died after HSCT: 4 of disease recurrence and 12 of causes other than leukemia. The 3-year OS was 65% (95% confidence interval (CI), 49–78%) for the imatinib cohort and significantly higher than 44% (95% CI, 35–52%) for the pre-imatinib cohort ($P=0.0148$; Figure 1a). The 3-year DFS was 58% (95% CI, 41.8–70.9%) for the imatinib cohort and significantly higher than 37% (95% CI, 28.5–45.6%) for the pre-imatinib cohort ($P=0.039$; Figure 1b).

Table 2 shows the result of risk factor analysis for OS and DFS among all 173 patients. In the multivariate analysis, the only variable found to influence OS and DFS was the pre-transplant imatinib-based therapy (hazard ratio (HR) = 0.44 (95% CI, 0.25–0.77); $P=0.004$ and HR = 0.51 (95% CI, 0.31–0.86); $P=0.011$, respectively). The presence of cGVHD showed a tendency of favorable OS and DFS, but did not reach the statistical significance (HR = 0.66 (95% CI, 0.42–1.06); $P=0.085$ and HR = 0.75 (95% CI, 0.47–1.19); $P=0.217$, respectively).

Other outcomes of transplantation

Relapses. In the pre-imatinib cohort, 48 patients relapsed after HSCT with a median of 240 days (range, 42–2302 days).

In the imatinib cohort, 7 patients (3 of 36 with PCR negative and 4 of 12 with PCR positive at HSCT) relapsed after HSCT with a median of 137 days (range, 68–728 days). The estimated cumulative incidence of relapse at 3 years was 15.0% (95% CI, 6.6–26.7%), and significantly lower than that of the pre-imatinib cohort (50.4% at 3 years (95% CI, 39.6–60.2%); $P=0.002$; Figure 1c). Among patients in the imatinib cohort, patients with PCR negative showed significantly lower relapse rate compared with that of PCR positive (10.0% (95% CI, 2.5–23.6%) versus 41.3% (95% CI, 16.9–64.4%) at 3 years, respectively, $P=0.025$).

Non-relapse mortality. In the pre-imatinib cohort, 34 patients died of non-relapse causes at a median of 159 days (range, 5–2094 days) after HSCT. The estimated cumulative incidence of NRM in the pre-imatinib cohort was 28% (95% CI, 20–36) at 3 years (Figure 2a). In the imatinib cohort, 12 patients died of non-relapse causes at a median of 329 days (range, 41–850 days) after HSCT. The 3-year cumulative incidences of NRM were 21% (95% CI, 11–33%; Figure 2a). There were no significant differences between two cohorts ($P=0.265$).

Cause of death. Recurrence of the primary disease was the leading cause of death in both groups: 55% for the pre-imatinib cohort and 25% for the imatinib cohort. In the pre-imatinib cohort, the causes of NRM were organ failure (11%), infection (9%), GVHD (8%), transplantation-associated thrombotic microangiopathy (TMA) (4%), interstitial pneumonia (3%), graft failure (3%) and other causes (6%). In the imatinib cohort, the causes of NRM included infection (19%), bronchiolitis obliterans with organizing pneumonia (13%), TMA (13%), GVHD (13%), organ failure (6%) and other causes (12%).

Graft-versus-host disease. There was no significant difference in the cumulative incidence of Grades 2–4 aGVHD between two cohorts (31% (95% CI, 19–44%) versus 37% (95% CI, 29–46%), $P=0.391$; Figure 2b). The cumulative incidence of cGVHD at 1 year after HSCT was significantly higher in the imatinib cohort than in the pre-imatinib cohort (49% (95% CI, 31–64%) versus 27% (95% CI, 18–37%), $P=0.0261$; Figure 2c).

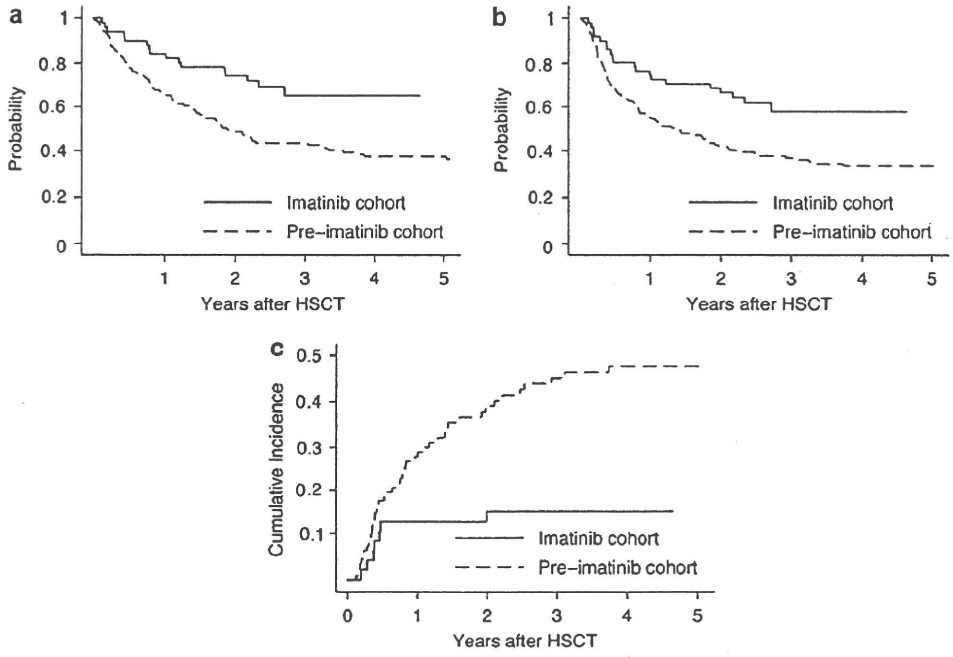

Figure 1 Transplantation outcomes of 51 patients who received imatinib-based therapy and 122 historical patients. (**a**) Overall survival, (**b**) disease-free survival and (**c**) cumulative incidence of relapse.

Table 2 Results of uni- and multivariate analysis of overall survival and disease-free survival among 173 patients with Ph+ALL

Characteristic	*Overall survival*				*Disease-free survival*			
	Univariate analysis		*Multivariate analysis*		*Univariate analysis*		*Multivariate analysis*	
	RR (95% CI)	P	*RR (95% CI)*	P	*RR (95% CI)*	P	*RR (95% CI)*	P
Imatinib-imterim therapy before HSCT	0.45 (0.26–0.77)	0.004	0.44 (0.25–0.77)	0.004	0.51 (0.31–0.83)	0.007	0.51 (0.31–0.86)	0.011
Donor status (RE versus UR)	0.87 (0.57–1.32)	0.521	0.72 (0.40–1.30)	0.275	0.77 (0.51–1.16)	0.211	0.65 (0.37–1.16)	0.147
Age at HSCT (–39 versus 40–55 versus 55–)	1.03 (0.74–1.44)	0.852	1.12 (0.78–1.62)	0.536	0.98 (0.71–1.36)	0.914	1.03 (0.73–1.47)	0.862
HLA-disparity (matched versus mismatched)	0.90 (0.39–2.06)	0.800	0.76 (0.32–1.81)	0.531	1.11 (0.49–2.54)	0.800	1.06 (0.45–2.50)	0.895
Stem-cell source (BM versus PB)	1.15 (0.72–1.82)	0.565	1.23 (0.72–2.10)	0.451	1.30 (0.85–2.00)	0.228	1.34 (0.81–2.20)	0.254
Days from diagnosis to HSCT	1.00 (0.99–1.00)	0.217	1.00 (0.99–1.00)	0.141	1.00 (0.99–1.00)	0.415	1.00 (0.99–1.00)	0.125
cGVHD as time-varying covariate (yes versus no)	0.68 (0.43–1.08)	0.101	0.66 (0.42–1.06)	0.085	0.78 (0.50–1.23)	0.292	0.75 (0.47–1.19)	0.217

Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; CI, confidence interval; cGVHD, chronic graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hemtopoetic stem cell transplantation; PB, peripheral blood; Ph, Philadelphia chromosome; RE, related; RR, relative risk; UR, unrelated.

However, regarding the cumulative incidence of extensive-type cGVHD, there was no significant difference between two cohorts (22% (95% CI, 10–36%) versus 12% (95% CI, 6–20%), $P=0.119$; Figure 2d).

Association between cGVHD and OS/DFS/relapse. To examine the difference of impacts of cGVHD upon clinical outcome in the pre- and imatinib cohorts, we conducted stratified analysis by cohort, treating cGVHD as a time-varying covariate (Table 3). Multivariate analysis revealed that, in the imatinib cohort, there were no significant associations between cGVHD and OS/DFS/relapse ($P=0.707$, 0.332 and 0.713, respectively). On the other hand, in the pre-imatinib cohort, there was a significant association between cGVHD and OS (HR = 0.59 (95% CI, 0.35–1.00), $P=0.048$), but not between cGVHD and DFS/relapse ($P=0.234$ and 0.338, respectively).

Engraftment. In the pre-imatinib cohort, three patients experienced graft failure. The median periods to reach the neutrophil count of $>0.5 \times 10^6$/l and platelet count of 50×10^6/l were 15 days (range, 8–49 days) and 25 days (range, 9–120 days), respectively, for evaluable patients. In the imatinib cohort, all 51 patients were engrafted. The median period to reach a neutrophil count of $>0.5 \times 10^6$/l and platelet count of 50×10^9/l was 15 days (range, 5–41 days) and 25 days (range, 11–504 days), respectively, for evaluable patients. There was no

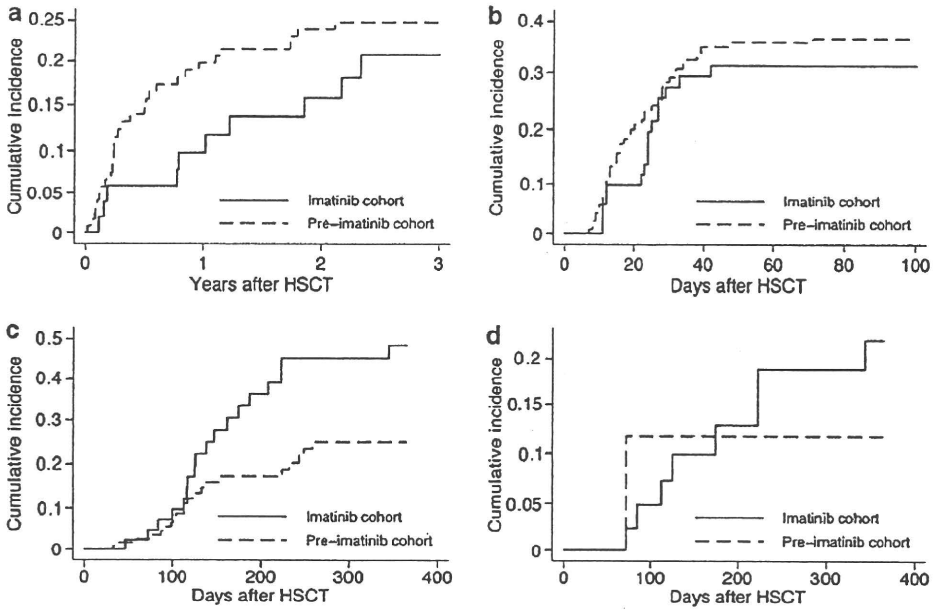

Figure 2 Cumulative incidence of GVHD or NRM. (**a**) Non-relapse mortality, (**b**) Grade 2–4 acute GVHD, (**c**) chronic GVHD and (**d**) extensive-type chronic GVHD.

Table 3 Impact of overall cGVHD on OS, DFS and relapse in multivariate analysis using cGVHD as a time-varying covariate

Cohort	*OS*			*DFS*			*Relapse*		
	Relative risk	*95% CI*	P	*Relative risk*	*95% CI*	P	*Relative risk*	*95% CI*	P
Imatinib cohort	0.80	(0.26–2.51)	0.707	0.59	(0.21–1.71)	0.332	0.74	(0.15–3.67)	0.713
Pre-imatinib cohort	0.59	(0.35–1.00)	0.048	0.73	(0.43–1.23)	0.234	0.75	(0.39–1.44)	0.388

Abbreviations: CI, confidence interval; cGVHD, chronic graft-versus-host disease; DFS, disease-free survival; HLA, human leukocyte antigen; OS, overall survival; PBSC, peripheral blood stem cell.
Data were adjusted for age categories, donors from unrelated subjects, HLA-matching status, PBSC graft and days to transplantation. Cox proportional hazard models were applied to OS and DFS, and a competing risk regression model was applied to relapse.

significant difference in neutrophil and platelet recovery between two cohorts ($P=0.201$ and 0.783, respectively).

Discussion

This study showed that patients with Ph + ALL who achieved CR by imatinib-based therapy and subsequently received allo-HSCT in their first CR showed significantly superior survival outcome to those in the pre-imatinib era. To our knowledge, our current report is the first to describe the superiority of imatinib-based therapy for this disease by analyzing a substantial number of patients with sufficient follow-up period. The treatment of Ph + ALL has changed dramatically since the introduction of imatinib and >90% of patients have achieved CR,[7,14,15] and allows SCT to be performed in a substantial proportion of patients in major or complete molecular remission.[8,16–18] Actually, in the imatinib cohort, 97 of 100 patients (97%) achieved CR and 60 (60%) could receive allo-HSCT in their first CR. Several studies reported improved OS rates compared with that in the pre-imatinib era by incorporation of imatinib-based therapy.[14,15,19,20] However, there had been few reports focusing on the clinical impact of pre-transplant imatinib administration on the outcome of HSCT. Lee *et al.*[8] reported superior outcome of HSCT by imatinib-based therapy compared with the historical control data, in which 29 patients with prior imatinib treatment showed better outcomes in terms of relapse, DFS and OS than the historical control patients. However, their comparative analysis included patients who received HSCT for refractory disease or beyond their first CR (4 of 29 patients in the imatinib group and 16 of 33 patients in the historical group). Several studies showed that remission status at the time of HSCT was one of the most important prognostic factors for outcome.[21,22] Therefore, we contend that it would be better to assess a greater number of patients and exclude patients with advanced stage at HSCT to accurately compare the clinical impact of imatinib-based therapy on the outcome of HSCT. To our knowledge, this study has the largest number of Ph + ALL patients receiving allo-HSCT in their first CR with the longest follow-up duration yet reported.

It is noteworthy from our findings that a lower rate of relapse was found in the imatinib cohort. Our results thus suggest that an imatinib-based therapy provides a survival benefit for newly diagnosed Ph + ALL patients by lowering the rate of subsequent relapse after HSCT. Despite the lack of comparative data of MRD in the pre-imatinib cohort, 75% of patients in the imatinib cohort achieved RQ-PCR negativity for *BCR/ABL* at the time of HSCT. Moreover, the relapse rate was significantly lower among

patients with PCR negative. From these, we believe that a powerful anti-leukemia activity of the imatinib-based therapy mostly contributed to the prevention of subsequent relapse after HSCT in the present analysis. Thinking of the reduced relapse rate after HSCT, impact of cGVHD should also be considered. Several studies in the pre-imatinib era reported beneficial impact of cGVHD on relapse incidence and survival.[23–25] In this study, the incidence of cGVHD was significantly higher in the imatinib cohort compared with that in the pre-imatinib cohort. In the imatinib cohort, more patients received PB as a stem cell source, which might have contributed to the high frequency of cGVHD. Besides, longer leukemia-free survival period in the imatinib cohort might have contributed to the increased frequency of cGVHD, which is a late complication often observed in the recipients of allo-HSCT who had survived without disease for at least 3 months after transplantation. One could argue that this observation could be related to a stronger graft versus leukemia effect and contribute to the lower relapse rate. However, the presence of cGVHD had no significant impact on the OS/DFS/relapse rate in our imatinib cohort by multivariate analysis.

To assist the proper interpretation of our current results, the strengths and limitations need to be considered. As discussed earlier, one of the strengths of this study is the large sample size for the imatinib cohort, which gives us a better estimation of the end points and also adds statistical power to the analyses. In addition, adjustments for potential confounders in the comparisons with the pre-imatinib cohort from a nationwide registry allow unbiased estimates to be made, at least in Japan. Given the evidence for a substantial impact of imatinib in Ph+ALL patients,[7,14–16] it is unrealistic to conduct a prospective study comparing treatments with or without imatinib. Hence, a retrospective cohort design could be suboptimal to address the key questions.

One of the possible limitations of our current analysis could be the presence of residual confounding factors both of known and unknown. Among the known factors, a difference in the conditioning regimens could be noted. The City of Hope National Medical Center reported a favorable result from the use of a fractionated TBI–etoposide regimen in the treatment of Ph+ALL.[26] However, in the comparative analysis, the clinical advantage of this approach seemed to be established mostly among patients transplanted in their second CR.[27] Moreover, this approach was commonly applied in our pre-imatinib cohort rather than in the imatinib cohort (22 and 4%, respectively). Differences in GVHD prophylaxes should also be considered. Tacrolimus was more frequently used in the imatinib cohort than in the pre-imatinib cohort, which reflects the change in practice within the field of allo-HCT in Japan as tacrolimus was widely used for unrelated allo-HSCT since 2000. Nevertheless, the lack of any differences in the incidence of aGVHD between two cohorts indicates that this factor had minimal impact in our analysis.

It may be argued that the improved outcome of the imatinib cohort have been influenced by the pre-transplant chemotherapy in the JALSG Ph+ALL 202 study. Although detailed information on the pre-transplant chemotherapy in the pre-imatinib cohort was not available, it was clear that the majority of patients were most likely treated by the JALSG ALL93 or JALSG ALL97 protocols as pre-transplant chemotherapy,[2] as these were widely used regimens in Japan at the time. The chemotherapeutic regimen in the JALSG Ph+ALL202 study was similar to those used in these protocols. Thus, the effectiveness on Ph+ALL would have been similar between the two cohorts. At least in JALSG, there had been neither remarkable progress in the chemotherapy of Ph+ALL until the clinical introduction of imatinib, nor in other groups including the MD Anderson Cancer Center.[28] Thus, in the present analysis, the influence of pre-transplant chemotherapy appears to be quite limited.

The difference of transplant year between the two cohorts (1995–2001 and 2002–2005, respectively) could have affected the outcome of HSCT, and the improvement of transplantation procedure might have contributed to the favorable outcome in the imatinib cohort. However, Nishiwaki *et al.*[29] analyzed the clinical outcome of 641 Japanese patients with Ph-negative ALL who had received allo-HSCT in their first CR in 1993–1997, 1998–2002 and 2003–2007, and reported that there was no statistical difference in OS and NRM between three periods. In this study, the incidence of NRM was lower in the imatinib cohort, but did not reach the statistical significance. Therefore, the influence of transplantation year is thought to be limited in this study.

Considering potential benefit by imatinib, the lack of information about post-transplant imatinib use in the pre-imatinib cohort might have led us to underestimate the difference between two cohorts.

In conclusion, we have found that there is a significant improvement in the OS and DFS of Ph+ALL patients who received allo-HSCT following imatinib-based therapy. Although further validation using larger cohorts from different populations is essential to confirm our findings, imatinib-based therapy is likely to be a useful strategy for not only giving patients with Ph+ALL more chance to receive allo-HSCT, but also for improving their outcome after allo-HSCT.

Conflict of interest

Dr Naoe has received research funding and honoraria from Novartis Japan. Dr Ohnishi has received research funding from Novartis Japan. Dr Miyazaki has received honoraria from Novartis Japan. The remaining authors declare no conflict of interest.

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Impact of additional chromosomal abnormalities in patients with acute promyelocytic leukemia: 10-year results of the Japan Adult Leukemia Study Group APL97 study

The t(15;17) chromosome translocation in acute promyelocytic leukemia is classified as a favorable cytogenetic feature among acute myeloid leukemia patients.[1-4] However, the prognostic impact of additional chromosomal abnormalities (ACAs) in acute promyelocytic leukemia has been debated.[5-9] We analyzed the clinical features, biological markers and clinical outcome of Japanese acute promyelocytic leukemia patients with or without ACAs who were treated by all-*trans* retinoic acid (ATRA) and chemotherapy, and tried to determine the role of ACAs on a 10-year follow up.

Adult patients with previously untreated *de novo* acute promyelocytic leukemia were registered consecutively into the JALSG APL97 study.[4] This study was approved by the institutional review boards of each participating institution and registered at *http://www.umin.ac.jp/ctrj/* under C000000206. Informed consent was obtained from patients before registration in the study in accordance with the Declaration of Helsinki.

Chromosomes analyzed by G-banding on bone marrow samples from patients before treatment were classified according to the 1995 International System for Human Cytogenetic Nomenclature (ISCN). Patients were categorized into two groups: those with t(15;17) and ACAs, and those with t(15;17) but without ACAs. Patients with der(17)t(15;17), der(15)t(15;17) or three-way translocation were placed in the group with ACAs.

Details of treatment protocol have been described previously.[4] In brief, remission induction consisted of ATRA and chemotherapy including idarubicin and cytarabine. Dose and duration of chemotherapy were based on initial leukocyte count. After completion of consolidation chemotherapy, patients negative for the *PML-RARA* tran-

Table 1. Clinical features of patients.

Parameters	Total N.(%)	Total Median (range)	t(15;17) N.(%)	t(15;17) Median (range)	t(15;17) with ACAs N.(%)	t(15;17) with ACAs Median (range)	P
N. of patients	225		158		67		
Age, years		48 (15-70)		49 (15-70)		45 (19-70)	0.08
15-29	39 (17%)		21 (13%)		18 (27%)		
30-49	84 (37%)		62 (39%)		22 (33%)		0.06
50-70	102 (46%)		75 (48%)		27 (40%)		
Gender							0.24
Male	122 (54%)		90 (57%)		32 (48%)		
Female	103 (46%)		68 (43%)		35 (52%)		
Leukocyte count, ×10⁹/L		1.7 (0.03-256)		1.65 (0.03-256)		1.7 (0.4-70.9)	0.77
Less than 3.0	135 (60%)		93 (59%)		42 (63%)		
3.0-10.0	48 (21%)		31 (20%)		17 (26%)		0.21
10.0 or higher	42 (19%)		34 (21%)		8 (12%)		
Platelet count, ×10⁹/L		29 (2-238)		30 (2-238)		29 (3-180)	0.69
Less than 10	31 (14%)		26 (16%)		5 (7.4%)		
10-40	10 (48%)		71 (45%)		38 (57%)		0.12
40 or higher	85 (38%)		61 (39%)		24 (36%)		
DIC score*	n = 213	6 (0-12)	n = 151	6 (0-12)	n = 62	6 (0-11)	0.46
3 or higher	198		139 (92%)		59 (95%)		
10 or higher	12		16 (11%)		5 (8%)		
FAB subtype							0.04
Typical	210 (93%)		144 (91%)		66 (99%)		
Variant	15 (7%)		14 (9%)		1 (1%)		
CD56 expression	n = 192		n = 128		n = 64		0.45
positive	19 (10%)		11 (9%)		8 (13%)		
negative	173 (90%)		117 (91%)		56 (87%)		
Peripheral blood count, ×10⁹/L							
leukocyte < 10, platelet > 40	72 (32%)		51 (32%)		21 (31%)		
leukocyte < 10, platelet < 40	112 (50%)		74 (47%)		38 (57%)		0.22
leukocyte > 10	41 (18%)		33 (21%)		8 (12%)		
Incidence of secondary							
MDS/AML	5 (2%)		4 (3%)		1 (1%)		0.63

*FAB: French-American-British; EFS: event free survival; RFS: relapse free survival. NA: not applicable; *DIC score, Score 3 indicates suspected DIC; scores from 4 to 10, definitive DIC; score 10 or more, severe DIC.*

script were randomly allocated either to receive 6 courses of intensified maintenance chemotherapy or to observation. Patients who were positive for the *PML-RARA* fusion transcript received late ATRA therapy followed by maintenance therapy, and received allogeneic hematopoietic stem cell transplantation if they had a human leukocyte antigen-identical donor.

Hematologic response was evaluated by standard criteria according to a previous report.[2] Hematologic and molecular relapse detected by RT-PCR analysis of *PML-RARA* was considered a relapse event.

The primary end point of the JALSG APL97 study was overall survival and disease free survival of patients who achieved complete remission. Overall survival for all patients was calculated from the first day of therapy to death or last visit. Disease free survival was measured from the date of complete remission to relapse, death from any cause or last visit. We also evaluated overall and disease free survival from the time of randomization to maintenance chemotherapy or observation.

Clinical and biological characteristics were compared between patients with or without ACAs by the χ^2 test or Fisher's exact test for categorical data, and Wilcoxon's rank-sum test for continuous data. Overall and disease free survival were estimated by the Kaplan-Meier method and then compared by the log rank test. Clinical outcomes were updated on January 2009 and the median follow-up period is 7.3 years. Statistical analyses were performed using SPSS 11.0 software (SPSS Inc, Chicago, IL, USA).

Among 302 patients enrolled between May 1997 and June 2002, 283 patients were evaluable.[4] Of these, 58 patients were excluded because of insufficient data for ACAs status. Thus, the present analysis was carried out on 225 patients.

Sixty-seven (30%) of 225 patients had ACAs. Trisomy 8 was the most frequently observed ACA and detected in 21 cases (31%). Seven cases (11%) had ACAs in chromosome 15 in addition to t(15;17), 6 (9%) in chromosome 9, 6 (9%) in chromosome 7, 4 (6%) in chromosome 15, and 4 (6%) in chromosome 6. There was no significant difference in clinical or biological characteristics between the two groups, except the frequency of M3v (1% *vs.* 9%, *P*=0.04) (Table 1).

Complete remission rates in patients with or without ACAs were 97% and 95%, respectively (*P*=0.72). There was no difference in cumulative incidence of early death at 50 days, severe hemorrhagic complication or retinoic acid syndrome between the two groups (*P*=0.16, *P*=0.46 and *P*=0.16, respectively). There was also no difference in overall survival, disease free survival or cumulative incidence of relapse between the two groups (91% *vs.* 84%, *P*=0.18; 68% *vs.* 71%, *P*=0.59; 26% *vs.* 22%, *P*=0.51, respectively). Overall and disease free survival are shown in Figure 1A and B. In addition, clinical outcome was analyzed among subgroups of patients with ACAs. However, ACAs including chromosome 8, 7, 9, 15 and 17 did not influence outcomes.

Clinical and biological characteristics have been compared between patients with or without ACAs. ACAs have been detected in 26% to 33% of newly diagnosed acute promyelocytic leukemia patients in whom trisomy 8 was consistently the most frequent ACA.[5-9] In this study, 67 patients (30%) had ACAs, and trisomy 8 was the most frequent (31%). There was no significant difference in overall survival, disease free survival or relapse rate between patients with or without trisomy 8.

The frequency of M3v was significantly lower among our patients with ACAs. This agrees with the report by Schoch *et al.*,[10] although several previous studies showed that the morphology of M3v was not related to the presence of ACAs.[5,6,8] The inconsistency of these results may be caused by a considerably smaller number of M3v cases (16% to 27% of APL). Some authors have reported that the morphology of M3v is related to fms-like tyrosine kinase 3 mutations.[8,11,12] Future analysis of this with ACAs is needed.

Several authors have discussed the clinical importance of ACAs in acute promyelocytic leukemia patients treated with ATRA and chemotherapy. Cervera *et al.*[9] found in the LPA99 trial that ACAs were associated with lower relapse free survival in univariate analysis but not in mul-

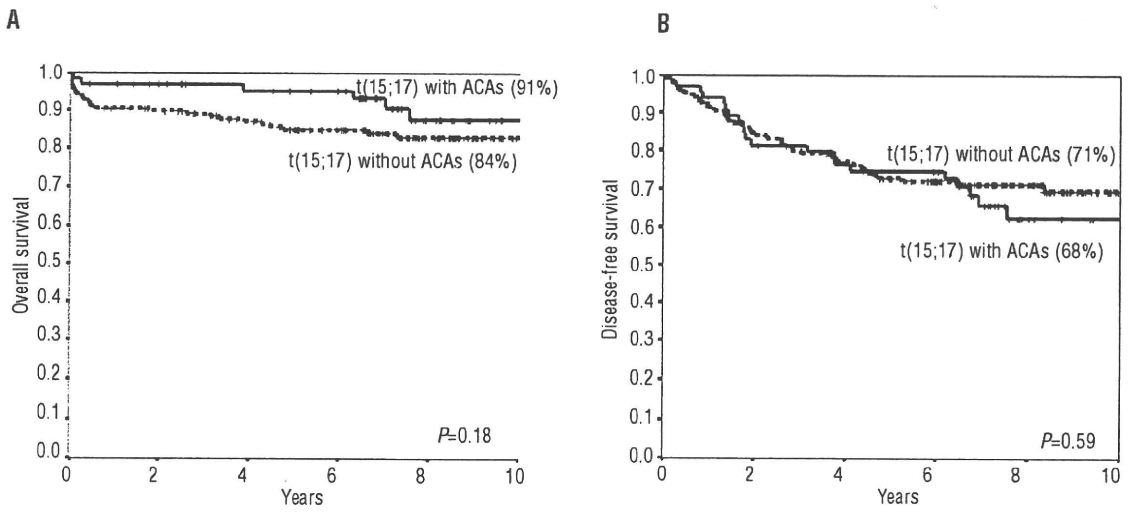

Figure 1. Overall survival and disease free survival of APL patients between with or without additional chromosomal abnormalities in addition to t(15;17). (A) Overall survival (91% *vs.* 84% at 10 years, *P*=0.18), (B) Disease-free survival (68% *versus* 71% at 10 years, *P*=0.59) were similar between two groups.

tivariate analysis. Schlenk *et al.*[8] analyzed 82 patients and reported that ACAs were an unfavorable prognostic marker for overall survival due to early death during the induction therapy. On the contrary, Botton *et al.*[6] and Hernandez *et al.*[7] reported that ACAs had no impact on clinical outcome. In our study, ACAs also did not show any prognostic significance. One of the reasons for this discrepancy would be that the clinical outcome of acute promyelocytic leukemia has recently improved dramatically. The outcome of each subgroup has also been greatly improved, although with some limitations, because patients have been stratified according to risk factors and consequently recent studies have used risk-adapted therapies. Thus, it may become more difficult to identify prognostic factors in acute promyelocytic leukemia.

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ORIGINAL ARTICLE

Analysis of bacteremia/fungemia and pneumonia accompanying acute myelogenous leukemia from 1987 to 2001 in the Japan Adult Leukemia Study Group

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Abstract We analyzed the incidence and prognosis of bacteremia/fungemia and pneumonia during remission induction therapy of a newly diagnosed acute myelogenous leukemia (AML) in the Japan Adult Leukemia Study Group treated with individual protocols of AML-87/-89 (1987–1991), AML-92 (1992–1995), AML-95 (1995–1997), and AML-97 (1997–2001). Bacteremia/fungemia was present in 251 of 2585 cases (9.7%); the causative microorganism was gram-positive bacteria (GPB) in 122 cases (49%), gram-negative bacteria (GNB) in 90 cases (36%), fungi (F) in 31 cases (12%), and polymicrobes (P) in 8 cases (3%). Particularly prevalent were *Pseudomonas aeruginosa* in 49 cases (20%), *Staphylococcus epidermidis* in 29 cases (12%), and *Staphylococcus aureus* in 25 cases (10%). With AML-87/-89, incidence of bacteremia/fungemia was 11.8% while it was 9.4% with AML-92, 8.7% with AML-95, and 9.2% with AML-97. The proportion of GPB, GNB, F, and P was 40, 41, 16, and 3% in AML-87/-89, 46, 40, 11, and 3% in AML-92, 48, 39, 11, and 2% in AML-95, and 59, 26, 11, and 4% in AML-97. The mortality rate by period was 26.5, 16.4, 14.0, and 6.8%, respectively. Pneumonia was found in 433 cases (16.8%); microbiological research covered 359 cases of

For the Japan Adult Leukemia Study Group.

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AML-87/-89, AML-92, AML-97 and excluded AML-95 as there was no listing for the causative microorganism on questionnaires. Microbiologically documented pneumonia was found in 123 cases (34.3%), with GPB in 33 cases (27%), GNB in 28 cases (23%), F in 44 cases (36%), and P in 18 cases (15%); particularly prevalent were *Aspergillus* in 23 cases (19%), *Staphylococcus aureus* in 16 cases (13%), and *Pseudomonas aeruginosa* in 15 cases (12%). The incidence of pneumonia overall was 24.6% with AML-87/-89, 16.9% with AML-92, 13.9% with AML-95, and 12.9% with AML-97, with a mortality rate of 28.9, 33.3, 16.7, and 16.7%, respectively. Incidence of bacteremia/fungemia and pneumonia complicating AML has tended to decline in recent years, and mortality has also tended to improve.

Keywords Bacteremia/fungemia · Pneumonia · Acute myelogenous leukemia

1 Introduction

Bacteremia/fungemia and pneumonia are serious infectious complications in patients with neutropenia. Classically, enteric gram-negative rods have been the most frequent isolates in patients with bacteremia/fungemia and have a high mortality rate especially in case of *Pseudomonas aeruginosa*. In recent years, an increase in gram-positive bacteria like Methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative *staphylococci* (CNS) has been noted [1–3]. With regard to pneumonia, the relative frequency among febrile neutropenic episodes ranges between 10 and 30%, and reported mortality range between 20 and more than 60% [1, 4–6]. Although the etiology of pneumonia in neutropenic patients can be defined in only 10–45%, an increase of filamentous fungi such as *Aspergillus* spp. has been recently emphasized.

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The most marked neutropenia occurs in patients with acute myelogenous leukemia (AML) as a result of the disease itself, together with intensive chemotherapy. Analysis of infections of AML in the Japan Adult Leukemia Study Group (JALSG) treated with the AML-87/-89 protocol has been reported [7]. In that analysis, complications of bacteremia/fungemia and pneumonia were prevalent (11.8 and 24.6%), and the mortality rates were high (26.5 and 31.9%). Substantial progress in infection control was seen early in the 1990s. As example, antibacterial prophylaxis using fluoroquinolones and antifungal prophylaxis using fluconazole (FLCZ) are recommended during remission induction therapy for AML [8–10]. Clean rooms equipped with high-efficiency particulate air (HEPA) filters and/or portable clean beds with HEPA filters are used to prevent pneumonia [11, 12]. Moreover, various antibacterial and antifungal agents or colony stimulating factors such as granulocyte-colony stimulating factor have been developed, and guidelines on their use have also been published [8–10, 13, 14]. Thus, the incidence of and prognosis for bacteremia/fungemia and pneumonia complicating AML may change considerably. We have retrospectively analyzed the data of 2585 adult patients with newly treated AML during 1987 and 2001.

2 Patients and methods

2.1 Patients and chemotherapy regimens

The incidence of bacteremia/fungemia and pneumonia during remission induction, the causative microorganism, and prognosis were analyzed. Data regarding infection were reviewed based on the case cards specified by each protocol. With regard to pneumonia, the incidence alone was analyzed in patients treated with AML-95 because there was no listing for the causative microorganism on the case cards in AML-95 protocol. Only the death caused by infection was included in this study.

Patients with newly diagnosed AML were treated with individual protocols of AML-87/-89 (1987–1991), AML-92 (1992–1995), AML-95 (1995–1997), and AML-97 (1997–2001). The treatment outcomes of individual protocols of AML-87/-89, AML-92, AML-95, and AML-97 have previously been reported [15–19]. Two-hundred fifty-two patients were enrolled for AML-87 remission induction therapy and administered daunorubicin (DNR), behenoyl cytarabine (BHAC), 6-mercaptopurine (6MP), and prednisolone (PSL); patients were randomized as to whether vincristine was added to these drugs [15]. Three-hundred twenty-five patients were enrolled for AML-89, and those who were administered DNR, 6MP, PSL, and BHAC or cytarabine were randomized [16]. Infection analysis of the

two together has already been reported [7], so they were also analyzed together in the current work. Six-hundred ninety-nine patients were enrolled for AML-92, and all were administered DNR, BHAC, and 6MP. Patients were randomized as to whether etoposide was added [17]. Five-hundred thirty-one patients were enrolled for AML-95; patients were administered idarubicin and cytarabine, and set therapy and individualized therapy were compared [18]. Eight-hundred eight patients were enrolled for AML-97, and they were treated with set therapy of idarubicin and cytarabine [19]. Respective remission induction rates for AML-87, AML-89, AML-92, AML-95, and AML-97 were 78, 77, 76, 81, and 79%, respectively. The mean of leukocyte nadir in each regimen ranged from 328 to 424/μl. The mean of duration of leukopenia (less than 1000/μl) ranged between 16.2 and 19.6 days in each regimen. Thus, the intensity of each regimen was estimated to be almost equally strong from the clinical viewpoint.

2.2 Supportive care

Supportive therapy for infection was left to the discretion of each institution. Patients were treated in separate rooms under laminar air-flow with HEPA filters whenever possible; about 60% of patients were treated in such a protective environment in AML-87/-89 [7]. It rose to 77% in 2001, the final year of AML-97, according to the questionnaire distributed by the Committee of supportive care of the JALSG [11, 12]. Most patients received antibacterial prophylaxis with oral polymyxin B or oral fluoroquinolones in AML-87/-89. Two-thirds of patients received polymyxin B. In 2001, the ratio was fluoroquinolones in 38%, polymyxin B in 31%, trimethoprim–sulfamethoxazole in 16%, other agents in 10%, and only 6% were without prophylaxis [11, 12]. Antifungal prophylaxis was also performed with oral amphotericin B (AMPH) or fluconazole (FLCZ). More than 80% of patients received AMPH in AML-87/-89. In 2001, the ratio was AMPH in 42%, FLCZ in 41%, itraconazole (ITCZ) capsules in 10%, and other agents in 4%, and only 3% were without prophylaxis [11, 12].

If patients were febrile, broad spectrum antibiotics were given empirically using guidelines of Infectious Diseases Society of America (IDSA) [8, 9]. Vancomycin (VCM) was not routinely used in initial empiric therapy in Japan [11, 12], but was used only if patients were suffering from microbiologically documented infection due to MRSA. The continuation of initial antibiotics or changes to other antibiotics and the addition of antifungal agents were based on each institutional practice.

The administration of G-CSF was also left to the discretion of each institution. Initially, there was some hesitation regarding the use of G-CSF, due to fear of the possible stimulating activity of G-CSF on AML cells in vitro [20]. Only 20.6% of bacteremia/fungemia and 4.4% of pneumonia received G-CSF in AML-87/-89 [7]. Thereafter, the principles of physicians to use G-CSF by condition in patients with AML was changed as follows: 12% in cases of febrile neutropenia (FN), 20% in FN refractory to empirical antibiotics, 26% in clinically/microbiologically documented infection, and 27% in life-threatening infection. Six percent of physicians do not use G-CSF and 9% were in the case by case [11, 12]. Unfortunately, the exact percentage of the use of G-CSF was unknown except for AML-87/-89.

This study was approved by the institutional IRB of chief investigator.

3 Results

3.1 Bacteremia/fungemia

Analysis of infection was possible in a total of 2585 patients, including 577 from AML-87/-89, 669 from AML-92, 531 from AML-95, and 808 from AML-97. Bacteremia/fungemia was found in 251 patients (9.7%); the causative microorganism was gram-positive bacteria (GPB) in 122 patients (49%), gram-negative bacteria (GNB) in 90 patients (36%), fungi (F) in 31 patients (12%), and polymicrobes (P) in 8 patients (3%). Particularly prevalent were *Pseudomonas* (*Ps*) *aeruginosa* in 49 patients (20%), *Staphylococcus* (*S*) *epidermidis* in 29 patients (12%), and *S. aureus* in 25 patients (10%) (Table 1). Sixty-four percent of *S. aureus* were Methicillin-resistant.

The incidence of bacteremia/fungemia was 68 of 577 patients (11.8%) for AML-87/-89, 63 of 669 patients (9.4%) for AML-92, 46 of 531 patients (8.7%) for AML-95, and 74 of 808 patients (9.2%) for AML-97. The incidences of bacteremia due to GPB out of all of the enrolled patients were 4.7, 4.3, 4.1, and 5.4%; substantial changes were not found. In contrast, the incidences of GNB were 4.9, 3.7, 3.4, and 2.4%, and those of F were 1.9, 1.0, 0.9, and 1.0%; a decline was found in both microbials. The proportion of GPB, GNB, F, and P among bacteremia/fungemia was 40, 41, 16, and 3% in AML-87/-89, 46, 40, 11, and 3% AML-92, 48, 39, 11, and 2% in AML-95, and 59, 26, 11, and 4% in AML-97 (Fig. 1). Among bacteremia/fungemia, the percentage of GPB has tended to increase in recent years. *S. aureus*, CNS, and *Streptococcus/Enterococcus* accounted for 10.9, 21.6, and 17.6%, respectively in AML-97. Bacteremia due to MRSA appeared starting in 1992, accounting for 87.5% (7 of 8) of the *S. aureus* bacteremia in AML-97. Although a decline was found in gram-negative bacteremia, *Ps. aeruginosa* still accounted for 13.5% in AML-97 (Fig. 1).

Table 1 Pathogens causing bacteremia/fungemia

Organism	Total (%)
Gram-positive bacteria (GPB)	122 (48.6)
Staphylococcus aureus	9 (3.6)
Staphylococcus aureus (MRSA)	16 (6.4)
Staphylococcus epidermidis	29 (11.6)
Coagulase-negative *staphylococci* (CNS)	11 (4.4)
Staphylococcus not specified	4
Streptococcus mitis	6 (2.4)
Streptococcus sanguis	3
Other Viridans streptococci	9 (3.6)
Streptococcus agalactiae	4
Enterococcus faecalis	11 (4.4)
Bacillus cereus	5 (2.0)
Propionibacterium	2
Other GPB	5
GPB not identified	8 (3.2)
Gram-negative bacteria (GNB)	90 (35.9)
Pseudomonas aeruginosa	49 (19.5)
Pseudomonas cepacia	2
Other *Pseudomonas* sp.	3
Enterobacter cloacae	6 (2.4)
Other *Enterobacter* sp.	4
Escherichia coli	8 (3.2)
Klebsiella pneumoniae	2
Serratia marcescens	2
Other GNB	8
GNB not identified	6 (2.4)
Fungi	31 (12.4)
Candida not specified	12 (4.9)
Candida albicans	3
Candida glabrata	5 (2.0)
Other candida	2
Trichosporon beigelii	3
Other fungi	3
East like fungi	3
Polymicrobial[a]	8 (3.2)
Total	251 (100)

[a] *S. sanguis + Ps. aeruginosa, B. cereus + S. epidermidis, Ps. aeruginosa + Enterococcus + C. glabrata, K. pneumoniae + E. coli + Enterococcus avium, S. sanguis + Flavobacterium, Ps. aeruginosa + C. albicans, B. cereus + S. epidermidis, E. coli + S. epidermidis,* in one each

The mortality rate for a typical causative microorganism is shown in Table 2. Overall, there was a mortality rate of 15.9%, but by period it was 26.5, 16.4, 14.0, and 6.8%, so improvement was apparent. Especially, 6 of 11 patients (54.5%) with fungemia died in AML-87/-89, but afterward the mortality rate was 0/7 (0%), 0/4 (0%), and 1/8 (12.5%), so prognosis has clearly improved.

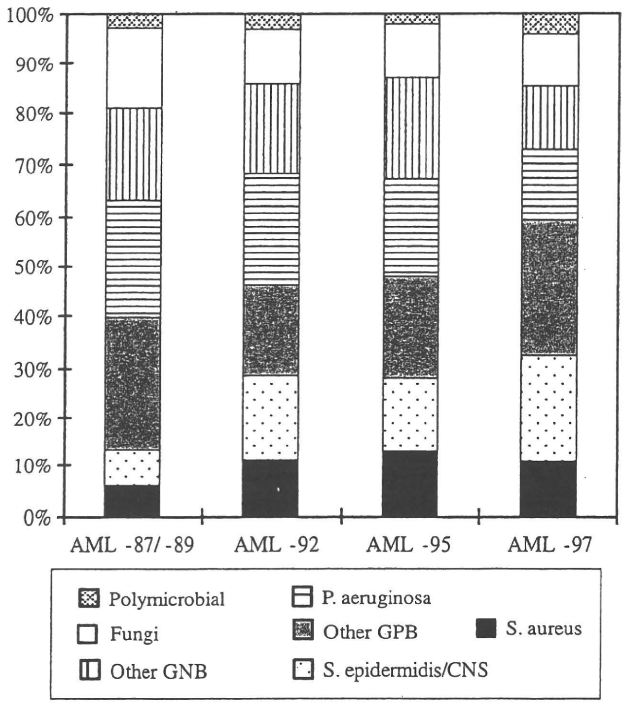

Fig. 1 Isolated microorganisms causing bacteremia/fungemia

3.2 Pneumonia

Pneumonia was found in 433 of a total of 2585 patients (16.8%). The incidence of pneumonia overall was 24.6% in AML-87/-89, 16.9% in AML-92, 13.9% in AML-95, and 12.9% in AML-97, so there has been a decline. Analysis of the causative microorganism was possible in 359 patients, excluding AML-95. Microbiologically documented pneumonia was found in 123 patients (34.3%), attributed to GPB in 32 patients (26.0%), GNB in 29 patients (23.6%), F in 44 patients (35.8%), and P in 18 patients (14.6%) (Table 3). By bacterial/fungal strain, most prevalent were *Aspergillus* in 23 patients (18.7%), *S. aureus* in 16 patients (13.0%), and *Ps. aeruginosa* in 15 patients (12.2%). The proportion of GPB, GNB, F, and P by period was 25.5, 25.5, 38.3, and 10.6% in AML-87/-89, 26.7, 22.2, 31.1, and 20.0% in AML-92, and 29.0, 19.4, 38.7, and 12.9% in AML-97 (Fig. 2). Invasive pulmonary aspergillosis (IPA) in particular clearly increased from 7 of 47 patients (14.9%) in AML-87/-89 and 6 of 45 patients (13.3%) in AML-92 to 10 of 31 patients (32.3%) in AML-97.

Overall mortality rate was 25.1%, but by period it was 28.9, 33.3, 16.7, and 16.7%. The mortality rates of GPB, GNB, F, and P were 25.0, 42.9, 37.2, and 27.8%, respectively (Table 4). The mortality rate of IPA was 34.8%. Clinically documented pneumonia was found in 236 patients. The mortality rate for these patients by period was 27.4, 27.9, and 12.5%, respectively (Table 4). The mortality rate of clinically documented pneumonia was better