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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).

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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

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).

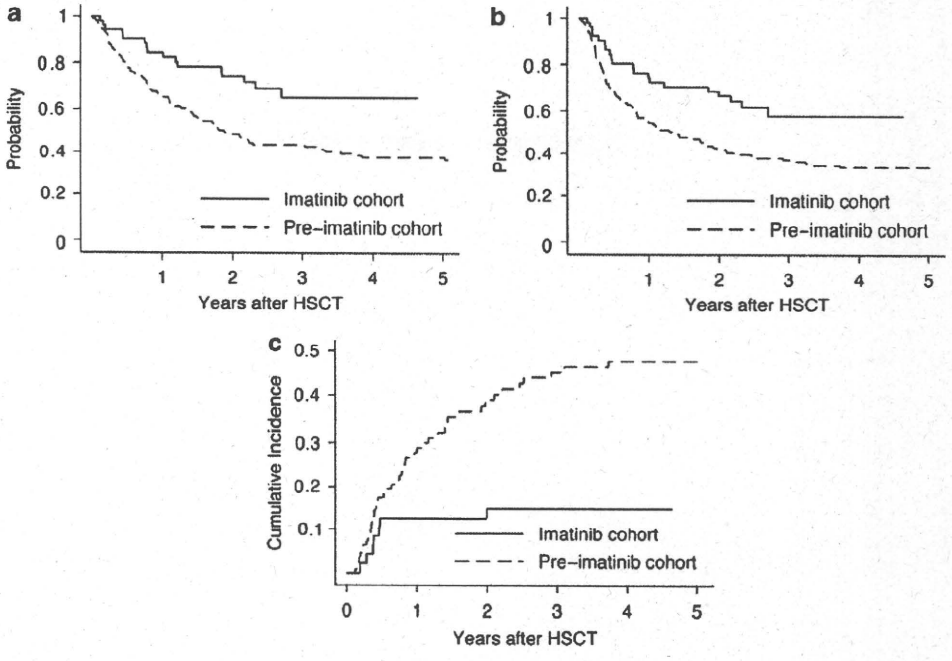

Figure 1 Transplantation outcomes of 51 patients who received imatinib-based therapy and 122 historical patients. (**a**) Overall survival, (**b**) disease-free survival and (**c**) cumulative incidence of relapse.

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

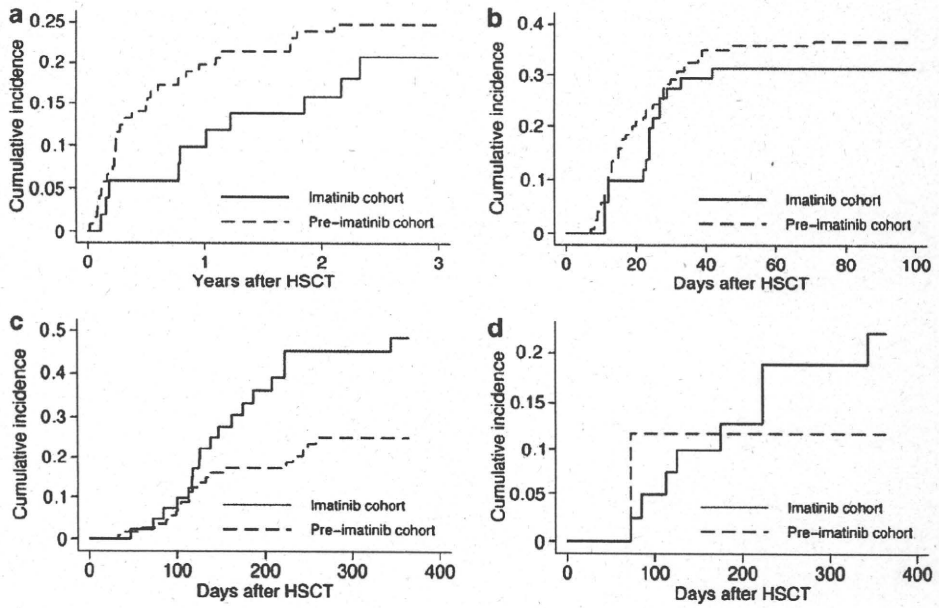

Figure 2 Cumulative incidence of GVHD or NRM. (**a**) Non-relapse mortality, (**b**) Grade 2–4 acute GVHD, (**c**) chronic GVHD and (**d**) extensive-type chronic GVHD.

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-

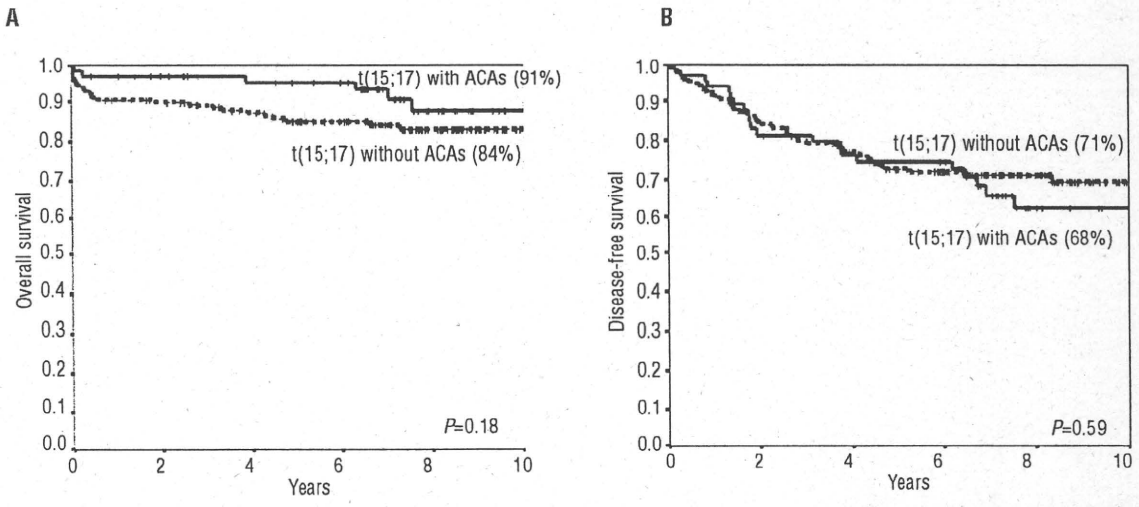

Figure 1. Overall survival and disease free survival of APL patients between with or without additional chromosomal abnormalities in addition to t(15;17). (A) Overall survival (91% *vs.* 84% at 10 years, *P*=0.18), (B) Disease-free survival (68% *versus* 71% at 10 years, *P*=0.59) were similar between 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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BCR-ABL1 mutations in patients with imatinib-resistant Philadelphia chromosome-positive leukemia by use of the PCR-Invader assay

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ABSTRACT

BCR-ABL1 kinase domain mutations were evaluated in 60 imatinib-resistant patients with Philadelphia-positive (Ph$^+$) leukemia using PCR-Invader assay and direct sequencing. In chronic myelogenous leukemia (CML) – chronic phase (CP), 5 had P-loop mutations and 3 had T315I mutations. CML-CP patients with high Sokal score showed significantly higher incidence of mutations. P-loop mutations were associated with higher risk of disease progression. In CML-advanced phase, P-loop mutations and T315I mutation were associated with significantly shorter survival. In Ph$^+$ acute lymphoblastic leukemia, overall survival was poor irrespective of mutational status. The PCR-Invader assay is useful for screening of mutations and prediction of prognosis.

1. Introduction

The introduction of imatinib has revolutionized the treatment of chronic myelogenous leukemia (CML). However, five-year follow-up results of the International Randomized Study of Interferon and STI571 (IRIS) on newly diagnosed CML showed that an estimated relapse rate was 17% at 60 months and an estimated 7% of all patients progressed to accelerated phase (AP) or blast crisis (BC) [1].

The most common cause of resistance to imatinib is point mutations in *BCR-ABL1* kinase domain (KD) [2]. However, the detection of mutations depends on the sensitivity of analysis methods [3]. In fact, the prevalence of *BCR-ABL1* KD mutations widely varies from 19% to 90% according to the reports, which used different analysis methods and various time points for sample collections [3–5]. Direct sequencing to detect *BCR-ABL1* KD mutations is commonly used, however its sensitivity is limited [4]. Denaturing high-performance liquid chromatography (D-HPLC)-based assay

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[5], PCR-restriction fragment length polymorphism (RFLP) [6] and allele specific oligonucleotide (ASO)-PCR assays [7] have been reported to be more sensitive methods. In the present study, we applied PCR-Invader assay [8,9], whose sensitivity is reportedly comparable to PCR-PFLP or ASO-PCR assay.

The prognostic significance of *BCR-ABL1* KD mutation is varied among the reports. Generally, P-loop mutations render less sensitivity to imatinib, and T315I mutation gives complete insensitivity to both imatinib and the second generation tyrosine kinase inhibitors (2nd TKIs) [10,11]. Several reports showed that patients with P-loop mutations had poor prognosis [3–5,11], while others found no difference in the disease progression [12]. Patients with CML-BC or Ph$^+$ acute lymphoblastic leukemia (ALL) developed *BCR-ABL1* KD mutation more frequently than those with CML-CP or AP [2].

In the present study, we examined the emergence of *BCR-ABL1* KD mutations in Japanese patients with CML-CP, AP, BC and Ph$^+$ALL who had become resistant to imatinib, using PCR-Invader method for screening and subsequent direct sequencing for P-loop mutations and T315I mutation to confirm the result. We analyzed the prevalence, clinical efficacy of imatinib and prognosis of patients with *BCR-ABL1* KD mutations.

2. Materials and methods

2.1. Patients

Between January 2007 and July 2009, 60 Japanese patients with imatinib-resistant Ph or *BCR-ABL1*-positive leukemia who had received imatinib for at least 6 months, were enrolled in this study, including 29 patients with CML-CP, 2 with CML-AP, 12 with CML-BC and 17 with Ph$^+$ALL. Written informed consent was obtained from all patients. The protocol was reviewed and approved by the institutional review boards of all of the participating centers and was conducted in accordance with the Declaration of Helsinki.

AP, BC, complete hematologic response (CHR), complete cytogenetic response (CCyR), partial cytogenetic response (PCyR), and major cytogenetic response (MCyR) were defined by conventional criteria [13]. For CML-CP, primary imatinib resistance was defined as no CHR at or after 3 months of therapy; as no minimal cytogenetic response at or after 6 months; or as no MCyR at or after 12 months; or as no CCyR at or after 18 months. Secondary imatinib resistance was defined as loss of CHR, loss of minimal cytogenetic response, loss of MCyR, development of clonal evolution or progression to AP or BC [3]. Imatinib resistance to CML-AP was defined by one of the following criteria during treatment with at least 600 mg/day imatinib: disease progression defined at least a 50% increase in peripheral white blood count, basophils, or platelets during imatinib therapy; or lack of hematologic response in the bone marrow following a minimal of 4 weeks of imatinib therapy [14]. Resistance to CML-BC was defined as loss of MCyR, persistence of extramedullary disease, or a significant increase in the bone marrow blasts [15]. For Ph$^+$ALL, imatinib-resistance was defined as lack of response to treatment with imatinib after a minimum of 4 weeks of therapy at a dose of 600 mg/day or more [16].

2.2. PCR amplification of BCR-ABL1 transcript

Total RNA was extracted from peripheral blood or bone marrow aspirate samples using a commercial kit (QIAamp RNAeasy kit, QIAGEN, Hilden, Germany). c-DNA was synthesized from 1 μg of RNA in 20-μL reactions with commercial kit (SuperScript II c-DNA Synthesis Kit, Invitrogen, Carlsbad, CA, USA). The *BCR-ABL1* transcripts were amplified by PCR with primer pairs designed to generate 1617 bp fragment including *ABL1* KD.

2.3. Detection of BCR-ABL1 KD mutations by PCR-Invader assay

The detection of 25 mutations (M244V, L248V/R, G250E, Q252H/R, Y253F/H, E255K/V, E279K, F311L, T315A/I, F317L, M351T, F359I/V, V379I, L387M, H396P/R, S417Y, E459K and F486S) in *BCR-ABL1* KD was performed with PCR-Invader assay according to previous reports with minor modification [9]. Briefly, the primary probe and Invader oligo for detection of each mutation were designed with the Invader technology creator (TWT, Madison, WI, USA) and were based on *ABL1* (accession no. NM 005157) sequences. The Invader reactions were performed by using 384-well plates with reagents containing Cleavase XI Invader core reagent kit (Amplified DNA) (TWT, Madison, WI, USA), 70 nM primary probes, 7 nM Invader oligo, and 10^{-2} dilution PCR amplicon which was denatured at 95 °C for 5 min. Plates were incubated at 65 °C in the fluorescence micro plate reader (FluoDia-T70; Otsuka Electronics, Osaka, Japan). Fluorescence values of FAM (carboxyfluorescein) (wavelength/bandwidth: excitation, 485/20 nm; emission, 530/25 nm) for mutation and RED (REDmond RED) (excitation, 560/20 nm; emission, 620/40 nm) for wild type were measured 30 min later. The fold-over-zero (FOZ) values were used to normalize difference between the measurements. The FOZ values were calculated by dividing the fluorescence value of sample by that of the negative control. The mutations were identified by calculation of FOZ values.

When T315I mutation or P-loop mutation was detected by Invader assay, direct sequencing was performed to confirm the result. Four sequencing primers were designed for sequencing between aa240 and aa490 in *BCR-ABL1* KD. Purified PCR amplicon were sequenced by the dideoxy method with the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) in a model 3130 fluorescent DNA sequencer (Applied Biosystems).

2.4. Statistical analysis

Progression free survival (PFS) was measured from the start of imatinib until disease progression to AP/BC or death from any cause in CML-CP. Overall survival (OS) was defined from the start of imatinib until the date of death. Patients were censored at the time when imatinib was switched to new TKIs including nilotinib and dasatinib, or they underwent hematopoietic stem cell transplantation (HSCT). These analyses were performed using the Kaplan–Meier method, with statistical significance (*P*-values) assessed by log-rank test. The Fisher's exact-test, Mann–Whitney *U*-test or chi-square test was used for paired group comparisons. All analyses were performed using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. BCR-ABL1 KD mutations

BCR-ABL1 KD mutations were detected in 30 (50%) of 60 patients. Characteristics of patients are shown in Tables 1 and 2. Twelve different mutations were found among 25 types of mutations selected in this study (Table 3). KD mutations were detected in 14 (48%) of 29 patients in CP, 7 (50%) of 14 patients in AP or BC, 7 (53%) of 17 patients with Ph$^+$ALL. To analyze correlation between mutations and prognostic significance, mutations were categorized into three groups; T315I, mutations involving residues 245–255 (the ATP phosphate-binding loop: P-loop), and other mutations (non-P-loop).

In 29 patients with CML-CP, 13 were primary resistant and 16 were secondary resistant. Median duration of imatinib therapy from the start to the emergence of mutation was 33 months (range, 6–91 months). Three of 29 patients progressed to AP and one to BC (Table 1). Among 15 different mutations detected in 14 patients with CML-CP, 6 (40%) mutations were located in P-loop, 6 (40%) in non-P-loop and 3 (20%) were T315I (Table 3).

In 14 patients with CML-AP ($n=2$) or CML-BC ($n=12$), median duration of imatinib therapy was 6.7 months (range, 1–67 months) (Table 2). Among 9 different mutations found in 7 patients with CML-AP/BC, 3 (33%) were located in P-loop, one (11%) in non-P-loop and 5 (56%) were T315I (Table 3).

In 17 patients with Ph$^+$ALL, median duration of imatinib therapy was 8 months (range, 1–32 months) (Table 2). Among 11 different mutations found in 9 patients with Ph$^+$ALL, 7 (64%) were located in P-loop and 4 (36%) were T315I (Table 3). The common mutations detected in this study were T315I ($n=12$), Y253 H/F ($n=8$) and E255V/K ($n=7$). Six patients (1 in CP, 3 in AP/BC, and 2 in Ph$^+$ALL) had two different mutations (Table 3).

Moreover, we sequenced 24 samples of RNA in which the P-loop mutation or T315I mutation was detected by PCR-Invader assay. In all patients but one, the same mutations were detected by direct sequencing (Table 4).

3.2. Patient characteristics associated with mutations

Clinical features of CML-CP patients are listed in Table 1. In patients with CML-CP, 5 (100%) of 5 patients with high Sokal risk score and 5 (26%) of 19 with intermediate/low risk developed the mutations ($P=0.004$) (Table 1). During imatinib therapy, other feature associated with increased frequency of mutations was disease progression to AP or BC ($P=0.03$). In patients with CML-AP/BC

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Table 1
Clinical characteristics of imatinib resistant patients with CML-CP.

Characteristics	No. of patients			*P*-value
	Total	Mutation		
		Present	Absent	
Patients no.	29	14	15	
Age				
Median (range)	57 (20–87)	55 (24–71)	59 (20–87)	
Sex				
M/F	20/9	9/5	11/4	0.56
Sokal score				
High/intermediate–low	5/19	5/5	0/14	0.004
ACAs	6	1/13	5/10	0.08
Imatinib start from diagnosis				
Median month (range)	0.5 (0–106)	0.2 (0–106)	3 (0–103)	
Duration of imatinib therapy				
Median month (range)	33 (9–93)	21 (10–70)	32 (9–93)	
Best response to imatinib				0.13
MMR	5	1	4	
CCyR	10	6	4	
PCyR	4	1	3	
CHR	8	6	2	
Non-HR	3	0	2	
Resistance				0.09
Primary	13	5	8	
Secondary	16	9	7	
Loss of HR	4	3	1	
Loss of MCyR	1	0	1	
Loss of CCyR	4	2	2	
Progression to AP/BC	4	4	0	0.03
Loss of MMR	3	0	3	

CP: chronic phase; HR: hematologic response; CHR: complete hematologic response; PCyR: partial cytogenetic response; CCyR: complete cytogenetic response; MMR: major molecular response; ACAs: additional chromosomal abnormalities.

or Ph⁺ALL, there were no significant features associated with the emergence of mutations.

3.3. Impact of *BCR-ABL1* mutation in survival

In CML-CP at a median follow-up of 43 months (range, 13–100 months), PFS from the start of imatinib therapy until disease progression to AP or BC was significantly worse in the patients with mutations than those without mutations ($P = 0.03$, Fig. 1A). There was significant difference in PFS among P-loop mutations, other mutations and no mutation ($P = 0.004$, Fig. 1B). On the other hand, neither the presence ($P = 0.81$) nor the type of mutations ($P = 0.39$) was associated with OS from the start of imatinib therapy (data not shown). Three patients with T315I sustained CML-CP for 7–20 months after the detection of T315I. In 29 patients with chronic phase, 9 received nilotinib or dasatinib, and 2 underwent HSCT after imatinib resistance.

In patients with CML-BC, 2 of 3 with P-loop mutation and 3 of 4 with T315I died at a median follow-up of 16 months (range, 1–68 months). On the other hand, only 2 of 7 patients without mutation died, and none with CML-AP died even though one had T315I mutation. Median OS was 10 months in patients with mutation and 51 months in those without mutation ($P = 0.002$). According to the type of mutation, median OS was 13 months for patients with P-loop mutation, 9 months for T315I mutation, and 51 months for no mutations, respectively. P-loop or T315I mutations were significantly associated with poor prognosis ($P = 0.005$, Fig. 1C). In patients with CML-AP/BC, 2 patients received dasatinib and 3 patients underwent HSCT after imatinib resistance.

Table 2
Clinical characteristics of imatinib resistant patients with CML-AP/BC and Ph⁺ALL.

Characteristics	CML-AP/BC	Ph⁺ALL
No. of patients	14	17
Age		
Median (range)	58 (18–73)	56 (29–76)
Sex		
M/F	7/7	10/7
ACAs	2	5
Duration of imatinib therapy		
Median month (range)	6.7 (1–67)	8 (1–32)
Stage		
AP	2	–
BC	12	–

AP: accelerated phase; BC: blast crisis; ACAs: additional chromosomal abnormalities.

Table 3
BCR-ABL1 KD mutations according to disease phase.

Mutation	CML-CP	CML-AP/BC	Ph⁺ALL	Total (%)
No. of patients registered	29	14	17	60
No. of patients with mutations	14*	7**	9***	30
No. of mutations	15	10	11	36
P-loop	6	4	7	17 (47%)
G250E			1	1 (3%)
Q252H	1			1 (3%)
Y253H	2		4	6 (17%)
Y253F	1	1		2 (5%)
E255V	2	1		3 (8%)
E255K		2	2	4 (11%)
T315I	3	5	4	12 (33%)
Non-P-loop	6	1	0	7 (20%)
M244V	2			2 (5%)
F311I	1			1 (3%)
F359V	1			1 (3%)
E459K	2			2 (5%)
E486S		1		1 (3%)

The values in the parentheses represent the percent of each mutation in all 36 mutations detected.
Multiple mutations detected in one patient among each disease phase as follows: *Q252+Y253H, **Y253F+T315I, E255K+E255V, T315I+F486S, and ***E255K+T315I, G250E+Y253H.

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Table 4
T315I mutation and P-loop mutations detected by direct sequencing and PCR-invader assay.

Disease phase of patients	PCR-Invader assay	Direct sequencing
CML-CP	Y253F	Y253F
CML-CP	Y253H	Y253H
CML-CP	E255V	E255V
CML-CP	Q252H, Y253H	Q252H, Y253H
CML-CP	E255V	E255V
CML-CP	T315I	T315I
CML-CP	T315I	T315I
CML-CP	T315I	T315I
CML-AP	T315I	T315I
CML-BC[a]	Y253F, T315I	Y253F
CML-BC	E255K, E255V	E255K, E255V
CML-BC	E255K	E255K
CML-BC	T315I	T315I
CML-BC	T315I	T315I
CML-BC	T315I, F486S	T315I, F486S
Ph⁺ALL	G250E, Y253H	G250E, Y253H
Ph⁺ALL	Y253H	Y253H
Ph⁺ALL	Y253H	Y253H
Ph⁺ALL	Y253H	Y253H
Ph⁺ALL	E255K, T315I	E255K, T315I
Ph⁺ALL	E255K	E255K
Ph⁺ALL	T315I	T315I
Ph⁺ALL	T315I	T315I
Ph⁺ALL	T315I	T315I

We sequenced 24 samples with T315I mutation and/or P-loop mutations (8 in CP, 1 in AP, 6 in BC, 9 in Ph⁺ALL) detected by PCR-invader assay.
[a] In only one patient with CML-BC, T315I was not detected by direct sequencing.

In patients with Ph⁺ALL, 3 of 6 patients with P-loop mutation and one of 3 patients with T315I died at a median follow-up of 22 months (range, 6–34 months). One of 8 patients without mutation died. Median OS was 18 months for patients with P-loop mutation and 19 months for those with T315I. Neither the presence ($P = 0.46$, Fig. 1D) nor the type of mutations ($P = 0.71$) was associated with OS. In patients with Ph⁺ALL, 9 patients received dasatinib, and 7 underwent HSCT after imatinib resistance.

4. Discussion

The clinical resistance to imatinib has been ascribed to several possible mechanisms [2], and the emergence of *BCR-ABL1* KD mutations is the most common mechanism. Recent studies have clarified that the mutations influence the response to 2nd TKIs [3]. Y253H, E255K/V and F359V/C are less sensitive to nilotinib, and F317L and V299L are less sensitive to dasatinib. T315I is insensitive to both. Therefore, mutation analysis is required for the treatment decision in imatinib resistant patients. We established a new method using PCR-Invader assay to screen known mutations of *BCR-ABL1* KD and confirmed that this method was appropriate to detect the mutations and useful in clinical practice.

The reliability of mutation analysis depends on the methods and the quality of RNA. The direct sequencing method for mutation analysis of the complete *BCR-ABL1* KD enables the detection of single or multiple mutations in imatinib resistant patients. However, 20–30% of mutant clones are required to detect mutation in this assay [7]. The analyses of the mutations have been reported using several other methods. The sensitivity of each method, however, varied depending on primers and the type of mutations. Soverini et al. reported that D-HPLC-based method reached lower detection limit between 2% and 5% for Y253F, E255K and T315I but between 5% and 7% for M351T [5]. The ASO-PCR assay exhibited higher sensitivity when the percentage of mutant was less than 1% of the total [17].

In this study, we combined reverse transcriptase PCR (RT-PCR) with Invader technology developed by Third Wave Technologies, Inc. (Madison, WI, USA) [8,9], as a new diagnostic tool for detecting mutations of *BCR-ABL1* transcripts. Invader assay-combined RT-PCR was developed at first to serially and quantitatively monitor the emergence of T315I transcripts, since this approach is simpler to perform than direct sequencing or PCR-RFLP [6]. The PCR-Invader method used in the present study could detect mutated sequence of T315I when the proportion of mutant was 1–5%. Sensitivity of this assay is comparable to PCR-RFLP or ASO-PCR [9]. Moreover, this assay requires only small amounts of peripheral blood for analysis and has clear advantages owing to its simplicity and target-specific reaction. Willis et al. indicates that high sensitivity screening for KD mutation is not recommended at least in imatinib-naive patients because it is impossible to predict whether the mutant clone will expand and cause relapse [18]. Some mutations detected in this PCR-Invader method were correlated with poor prognosis, suggesting that the sensitivity of this assay may be appropriate for clinical use and, therefore, this assay is applicable to routine monitoring of CML patients, even though it cannot detect mutations except selected ones.

For imatinib resistant CML-CP patients, it was reported that 27% to 55% had mutations [3,5,12,19]. Soverini et al. showed that mutations were found in 14% treated with imatinib frontline and 31% treated with imatinib post interferon failure [5]. On the other hand, Jabbour et al. reported that mutations were highly detected in late CP as compared with early CP (42% *vs.* 24%) [12]. In the present study, *BCR-ABL1* KD mutations were detected in 48% of patients with CML-CP, and 17% of CML-CP patients were treated with imatinib post interferon failure. Mutations were observed in 56% of late CP patients.

A number of variables associated with a higher incidence of detectable mutations and imatinib resistance have been identified. Branford et al. demonstrated that these variables were a longer time from diagnosis to imatinib start and failure to achieve a MCyR by 6 months [4]. Khorashad et al. identified predictors before the start of imatinib therapy for the development of KD mutations, and, by multivariate analysis, late CP and high Sokal score were independent predictors for the development of mutations [11]. Soverini et al. showed that no differences emerged between patients with or without mutation regarding sex, median age and disease history [5]. In the present study, among patients' characteristics before imatinib therapy, high Sokal score was only significant predictor for the emergence of mutation.

In this study, CML-CP patients with P-loop mutations had a significantly shorter time to progression to AP or BC. However, the type of mutations, including P-loop mutation, was not associated with OS from the start of imatinib in CML-CP patients. The prognostic impact of P-loop mutations in CML-CP patients is controversial. Jabbour et al. reported that P-loop mutations were not associated with poor outcome [12]. However, they analyzed M244V as P-loop mutation and 46% of patients with P-loop mutations received therapy with 2nd TKIs. M244V is relatively innocuous and had favorable responses to 2nd TKIs [11]. Therefore, this factor favorably affects the prognosis of the group with P-loop mutations. On the other hand, several reports showed poor prognosis of CML-CP patients with P-loop mutations [4,5,11]. However, Khorashad et al. analyzed prognostic impact of mutations in CML-CP patients without resistance to imatinib [11] and 2nd TKIs were not administered in two reports [4,5]. The prognosis of CML patients with P-loop mutations is dependent on several factors. We should continue to analyze clinical outcomes of patients with P-loop mutation in larger prospective studies using 2nd TKIs therapies, because some P-loop mutations might increase the oncogenicity of *BCR-ABL1* [20].

The T315I mutation is one of the most resistant mutation in vitro [21]. In patients with CML-CP, several studies showed that patients with T315I have a shorter survival [19,22]. However, other report suggested a lack of impact of T315I mutation on survival [23].

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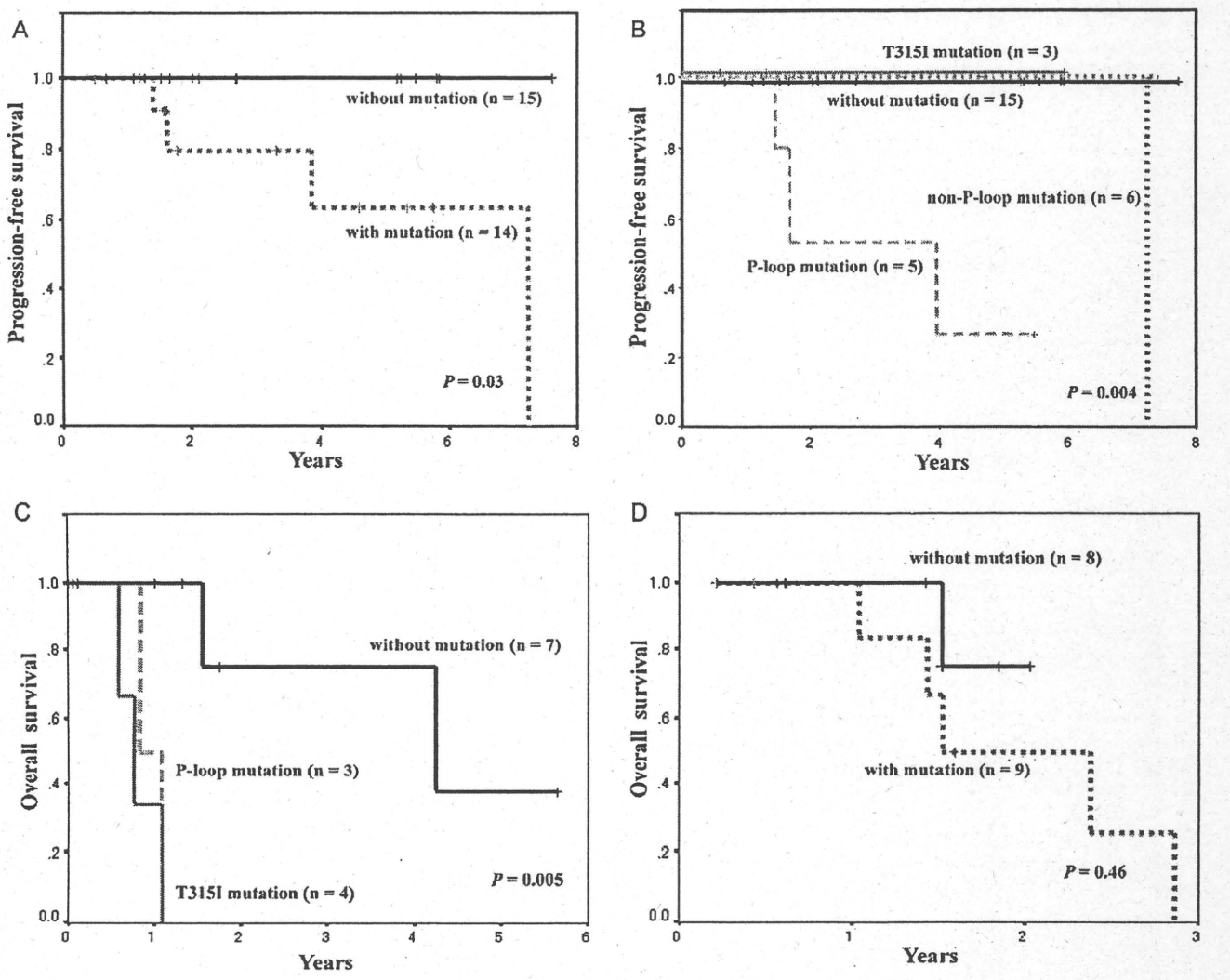

Fig. 1. Survivals in imatinib-resistant patients with Ph⁺ leukemia according to the mutational status in *BCR-ABL1* KD. (A) PFS in imatinib-resistant patients with CML-CP with or without mutations in *BCR-ABL1* KD. PFS was measured from the start of imatinib until disease progression to AP/BC or death from any cause in CML-CP. Median PFS was 87 months in patients with mutation and did not reach in patients without mutation ($P = 0.03$). A solid line; CML-CP patients without mutation ($n = 15$), A dashed line; CML-CP patients with mutation ($n = 14$). (B) PFS in patients with CML-CP according to the mutational status in *BCR-ABL1* KD. Median PFS was 49 months in patients with P-loop mutations and did not reach in patients with other mutations or without mutation ($P = 0.004$). A solid line; CML-CP patients without mutations ($n = 15$), a dotted line; CML-CP patients with T315I mutation ($n = 3$), a bold dashed line; CML-CP patients with non-P-loop mutation ($n = 6$) and a light dashed line; CML-CP patients with P-loop mutation ($n = 5$). (C) OS in imatinib-resistant patients with CML-AP/BC according to the mutational status in *BCR-ABL1* KD. Patients were censored at the time when they were switched to new tyrosine kinase inhibitors or underwent HSCT. Median OS was 13 months in patients with P-loop mutation, 9 months in patients with T315I and 51 months without mutation, respectively ($P = 0.005$). A solid line; CML-AP/BC patients without mutation ($n = 7$), a light solid line; CML-AP/BC patients with T315I mutation ($n = 4$), and a dashed line; CML-AP/BC patients with P-loop mutation ($n = 3$). (D) OS in imatinib-resistant patients with Ph⁺ALL with or without mutations in *BCR-ABL1* KD. There was no difference in OS between with or without mutations ($P = 0.46$). Patients were censored at the time when they were switched to new tyrosine kinase inhibitors or underwent HSCT. A solid line; Ph⁺ALL patients without mutation ($n = 8$), A dashed line; Ph⁺ALL patients with mutation ($n = 9$).

Recently, Nicolini et al. reported that median OS from detection of T315I was 22.4, 28.4, 4.0, and 4.9 months, and median PFS was 11.5, 22.2, 1.8 and 2.5 months, respectively, for CP, AP, BC and Ph⁺ALL patients [24]. In consistence with their report, 3 patients in CML-CP had T315I mutation but none of them died, while almost all patients with CML-BC or Ph⁺ALL who had T315I mutation died in short duration after the start of imatinib in this study.

These findings suggest that survival of patients with T315I is mostly dependent on the phase of disease.

In conclusion, the PCR-Invader assay used in this study is an appropriate tool for the screening of mutations during TKI therapy. High Sokal score is only predictive factor for emergence of mutation in CML-CP. P-loop mutations were associated with poor PFS in CML-CP. Patients with T315I or P-loop mutations showed poor OS in CML-AP/BC. The monitoring of mutational status is necessary to identify candidate patients suitable for 2nd TKIs or HSCT.

Conflicts of interest

K.O. had served as a consultant for Banyu Pharmaceutical; K.O. also received research funding from Novartis, Bristol–Myers Squibb and Banyu Pharmaceutical, honoraria from Novartis and Bristol–Myers Squibb. The other authors reported no potential conflict of interest.

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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

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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.

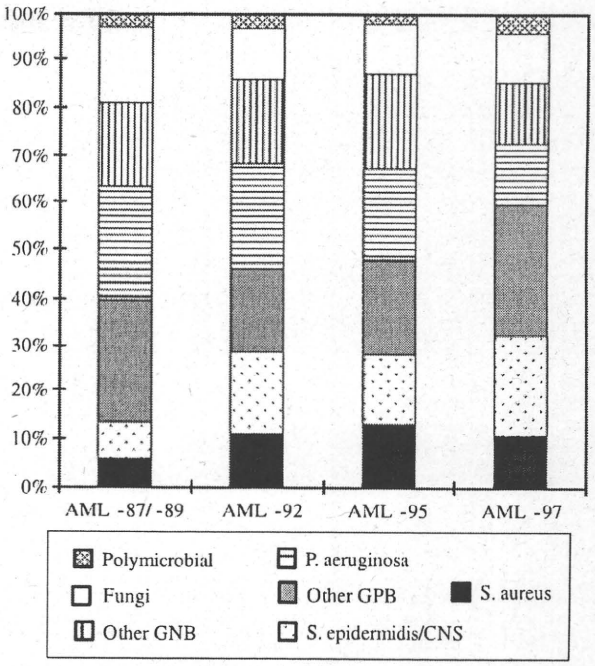

Fig. 1 Isolated microorganisms causing bacteremia/fungemia

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