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Correlation Between Imatinib Pharmacokinetics and Clinical Response in Japanese Patients With Chronic-Phase Chronic Myeloid Leukemia

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Despite the outstanding results generally obtained with imatinib mesylate (IM) in the treatment of chronic myeloid leukemia (CML), some patients show a poor molecular response. To evaluate the relationship between steady-state trough plasma IM concentration (IM-C_{min}) and clinical response in CML patients, we integrated data from six independent Japanese studies. Among 254 CML patients, the mean IM-C_{min} was 1,010.5 ng/ml. Importantly, IM-C_{min} was significantly higher in patients who achieved a major molecular response (MMR) than in those who did not ($P = 0.002$). Multivariate analysis showed that an MMR was associated with both age (odds ratio (OR) = 0.97 (0.958–0.995); $P = 0.0153$) and with IM-C_{min} (OR = 1.0008 (1.0003–1.0015); $P = 0.0044$). Given that patients with IM-C_{min} values >1,002 ng/ml had a higher probability of achieving an MMR in our large cohort ($P = 0.0120$), the data suggest that monitoring of IM levels in plasma may improve the efficacy of IM therapy for CML patients.

Imatinib mesylate (IM) is a potent and selective inhibitor of the BCR-ABL tyrosine kinase and the autophosphorylation of the tyrosine kinase receptor c-KIT, and it has been approved for the treatment of Philadelphia chromosome–positive chronic myeloid leukemia (CML)[1] and gastrointestinal stromal tumors.[2] Despite the outstanding results generally achieved with IM in CML, there have been cases of treatment failure, as well as cases in which the response to IM was suboptimal.[3] Factors that might be associated with suboptimal responses to IM include (i) biological factors, such as the baseline presence or later emergence of BCR-ABL mutations and other genetic variants; (ii) clinical features, such as the disease status of the patient or the Sokal risk score at baseline; (iii) pharmacokinetics-related interindividual pharmacogenetic variations and/or drug–drug interactions affecting IM metabolism; and (iv) adherence.[4–6]

Several previous studies have investigated whether variations in the concentration of IM in plasma influence the clinical response of IM-treated patients; however, the studies produced varied results (for a summary, see **Table 1**).[6–12] For example, data from three studies suggested a correlation between the trough plasma IM concentration (IM-C_{min}) and clinical response among CML patients.[6–8] Larson *et al.* reported that the IM-C_{min} was significantly higher in patients who achieved a complete cytogenetic response (CCyR) than in patients without a CCyR.[7] In addition, Picard *et al.* reported that an IM-C_{min} of 1,002 ng/ml should be set as an efficacy threshold because this concentration was significantly associated with a major molecular response (MMR) in 68 chronic-phase CML patients.[8] In contrast, Forrest *et al.* did not find any correlation between IM-C_{min} and clinical response among 78 CML patients after a minimum of 12 months of IM therapy.[9] However, as stated by the authors, their results

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Table 1 Correlation of imatinib pharmacokinetics with clinical response

Authors	Disease	No. of patients	IM daily dose (mg)	IM-C_{min} (ng/ml)	Correlation with response
Picard *et al.*[8]	CML	50	400	1,058 ± 557	Yes (CCyR, MMR)
		18	600	1,444 ± 710	
Larson *et al.*[7]	CML	351	400	979 ± 530	Yes (CCyR)
Forrest *et al.*[9]	CML	78	400	999 (203–2,910)	No (CCyR, MMR)
Widmer *et al.*[12]	CML	20	400	NA	No (AUC_u vs. HR)
	GIST	38	600	NA	Yes (AUC_u vs. OR)
Kawaguchi *et al.*[10]	CML	13	400	1,400 ± 570	NA
		9	300	1,150 ± 440	
Demetri *et al.*[11]	GIST	36	400	1,530 ± 666	Yes (TTP, OOBR)
		37	600	1,752 ± 794	
Marin *et al.*[6]	CML	84	400	900 (400–1,600)	Yes (MMR)

AUC_u, free area under the curve; CCyR, complete cytogenetic response; CML, chronic myeloid leukemia; GIST, gastrointestinal stromal tumor; HR, hematologic response; IM, imatinib mesylate; MMR, major molecular response; NA, not available; OOBR; overall objective benefit rate; OR, overall response; TTP, time to progression.

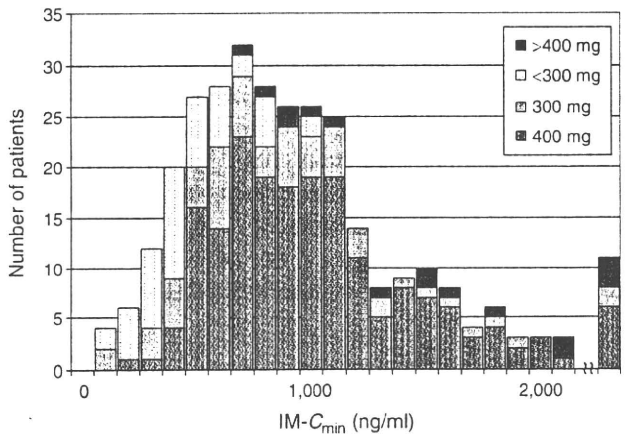

Figure 1 Distribution of steady-state trough plasma IM concentration (IM-C_{min}; $n = 314$, data obtained from all doses). The mean and median values of IM-C_{min} were 1,010.5 ± 564.6 and 900 ng/ml (range, 111–3,620 ng/ml), respectively.

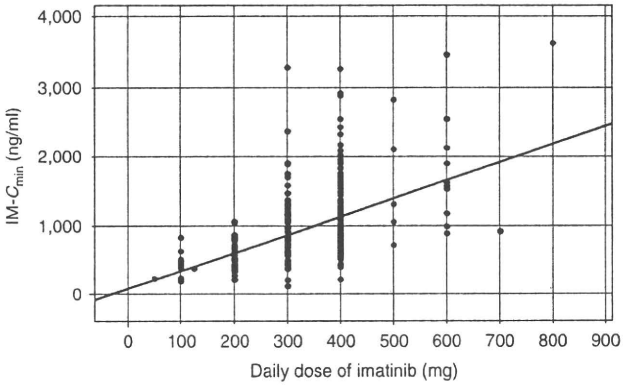

Figure 2 Steady-state trough plasma IM concentration (IM-C_{min}) achieved with the indicated daily dosages ($n = 314$). The IM-C_{min} predicted using linear regression analysis was related to dose as follows: 76.29 + 2.624 × dose ($r^2 = 0.232$; $P < 0.00001$).

may have been influenced by sample size and heterogeneous sampling times.

In Japan, the relationship between IM-C_{min} and clinical response has been independently studied by six groups.[13–18] Although two of these studies identified a significant correlation between IM-C_{min} and clinical response,[13,17] the others did not; however, these latter four studies that detected no correlation may have been insufficiently powered because of their modest sample sizes. In this study, our aim was to investigate the usefulness of monitoring IM-C_{min} in a large cohort of CML patients by integrating the data from these six Japanese studies.

RESULTS

Data for 314 Japanese patients with chronic-phase CML (189 men and 125 women) were integrated in this analysis. The median age of the patients was 60 years (range, 16–91 years), the mean body weight was 61.3 ± 12.1 kg (median, 60.0 kg; range, 37–103 kg), and the mean body surface area was 1.64 ± 0.19 m^2 (median, 1.65 m^2; range, 1.19–2.25 m^2). Among the study participants, 190 (60.5%) received 400 mg of IM daily, 59 (18.8%) received 300 mg, 48 (15.3%) received <300 mg, and 17 (5.4%) received >400 mg. The median duration of IM therapy was 1,435 days (range, 56–2,582 days).

The distribution of IM-C_{min} values across all doses is shown in **Figure 1**. The mean and median IM-C_{min} values were 1,010.5 ± 564.6 and 900 ng/ml (range, 111–3,620 ng/ml), respectively. Although there was substantial interpatient variability among patients treated with the same dose of IM, IM-C_{min} increased significantly and proportionately across doses ranging from 50 to 800 mg (**Figure 2**). Of these 314 patients, 60 patients were excluded from further analysis aimed at correlating IM-C_{min} with clinical response because the duration of IM therapy was <12 months or because a molecular response was not evaluated. The clinical characteristics of the 254 patients included are summarized in **Table 2**. There were no correlations between IM-C_{min} and age, body weight, body surface area, or the duration of IM therapy ($P = 0.343$, $P = 0.073$, $P = 0.075$, and $P = 0.931$, respectively), gender (Student's t-test: $P = 0.648$), or Sokal risk score (analysis of variance: $P = 0.399$).

Table 2 Association between potential predictive factors and imatinib trough concentration

Variable (n = 254)	Mean or no. of patients	Correlation with imatinib concentration (r)	P-value
Quantitative features[a]			
Age (years)	59.2	0.060	0.343
Weight (kg)	61.1	−0.113	0.073
Body surface area (m^2)	1.6	−0.112	0.075
Duration of IM therapy (days)	1,454.2	−0.005	0.931
Qualitative features			
Sex (male/female)[b]	151/103		0.648
Sokal risk group (low/intermediate/high)[c]	91/91/52		0.399

IM, imatinib mesylate.

[a]Compared using Pearson's product-moment correlation analysis. Data are presented as correlation coefficient (r) or mean values. [b]Compared using Student's t-test. [c]Compared using analysis of variance.

Table 3 Patient characteristics and clinical response to imatinib therapy

Characteristic (no. of patients)	MMR (166)	No MMR (88)	P-value	CCyR (218)	No CCyR (36)	P-value
Quantitative features						
Imatinib concentration (ng/ml)	1,107.4 ± 594.4	872.7 ± 528.5	0.002	1,057.8 ± 585.0	835.0 ± 524.3	0.033
Age (years)	57.1 ± 15.4	62.8 ± 14.2	0.004	58.3 ± 15.2	64.3 ± 14.4	0.029
Body weight (kg)	61.1 ± 12.1	61.5 ± 12.0	0.808	61.1 ± 12.0	61.6 ± 12.5	0.818
Body surface area (m^2)	1.643 ± 0.195	1.645 ± 0.188	0.926	1.638 ± 0.200	1.643 ± 0.191	0.888
Daily imatinib dose (mg)	367.5 ± 79.6	323.6 ± 135.4	0.006	365.1 ± 94.0	272.0 ± 127.0	0.0002
Duration of imatinib therapy (days)	1,459 ± 623	1,458 ± 697	0.986	1,450 ± 643	1,482 ± 705	0.786
Qualitative features						
Sex (male/female)	95/71	56/32	0.349	128/90	23/13	0.572
Sokal risk group (low/intermediate/high)	63/61/33	28/30/19	0.779	81/81/43	10/11/9	0.529

Data are presented as mean values (±SD) for quantitative features. Quantitative variables were compared using Student's t-test. Qualitative variables were compared using the χ^2 or Fisher's exact test.

CCyR, complete cytogenetic response; MMR, major molecular response.

Among all the patients evaluated, 166 (65.3%) achieved an MMR, and 218 (85.8%) achieved a CCyR (**Table 3**). IM-C_{min} values were significantly higher in patients with an MMR than in those without an MMR; the mean values were 1,107.4 ± 594.4 ng/ml (median, 986 ng/ml) and 872.7 ± 528.5 ng/ml (median, 719.5 ng/ml), respectively (P = 0.002). In addition, there were significant differences in age and daily dosage between patients with an MMR and those without an MMR (P = 0.004 and 0.006, respectively). Importantly, when we subclassified all the patients according to their IM-C_{min} as previously reported,[8] we found that patients with an IM-C_{min} ≥1,002 ng/ml had a higher probability of achieving an MMR than those with an IM-C_{min} <1,002 ng/ml (P = 0.0120, **Figure 3**).

In addition, IM-C_{min} was significantly higher in patients with a CCyR (n = 219) than in those without a CCyR (n = 36); the mean values were 1,057.8 ± 585.0 ng/ml (median, 916 ng/ml) and 835.0 ± 524.3 ng/ml (median, 688 ng/ml), respectively (P = 0.033). There were also significant differences in age and daily dosage between the group with a CCyR and those without a CCyR (P = 0.029 and 0.0002, respectively, **Table 3**).

In a stepwise forward-selection multiple logistic analysis, MMR was associated with both the age of the patient (odds ratio (OR) = 0.97 (0.958–0.995); P = 0.0153) and the IM-C_{min} value (OR = 1.0008 (1.0003–1.0015); P = 0.0044), whereas a CCyR was associated with only daily dosage (OR = 1.0073 (1.0036–1.0110); P = 0.0001). The association between IM-C_{min} and CCyR was not observed in the stepwise forward-selection multiple logistic analysis.

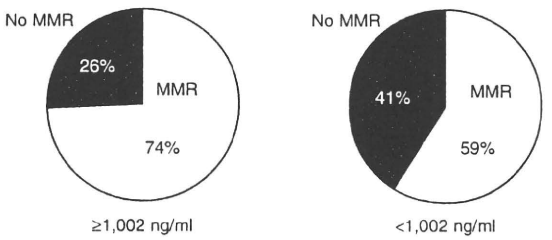

Figure 3 Correlation of trough plasma IM concentration (IM-C_{min}) with MMR (n = 254). IM-C_{min} values ≥1,002 ng/ml had a significantly higher probability of achieving an MMR (Fisher's exact test; P = 0.0120). MMR, major molecular response.

DISCUSSION

IM has favorable pharmacokinetic characteristics, including complete bioavailability and a proportionate dose–response relationship.[4,19] However, we found that, although there was a

linear relationship between IM-C_{min} and the daily dose of IM, there was also substantial interpatient variability. Factors that could underlie this interpatient variability include body size, age, gender, liver function, renal function, interaction with other medications given concomitantly, adherence to medication regimens, and polymorphisms of enzymes or transporters related to IM pharmacokinetics and/or pharmacodynamics. In this analysis, we did not observe any correlation between IM-C_{min} and body weight, body surface area, or age. Moreover, the eligibility criteria of each of the integrated studies ensured that there were no patients with serious renal or hepatic dysfunction, and no patients who were taking other drugs that might interact with IM. In addition, adherence to medication regimens was monitored by self-report for at least 7 days prior to blood sampling.

In our study of 254 evaluated IM-treated CML patients, steady-state IM-C_{min} correlated significantly with both MMR and CCyR. Among those who achieved an MMR, the mean IM-C_{min} (1,107.4 ng/ml from all doses, 1,154.3 ng/ml from 400 mg) was >1,002 ng/ml, previously shown to be an effective IM-C_{min} threshold.[8] Moreover, we found that patients with an IM-C_{min} <1,002 ng/ml had a lower probability of achieving an MMR ($P = 0.0120$, **Figure 3**), thereby supporting the previously reported 1,002 ng/ml plasma concentration efficacy threshold for Japanese CML patients.

Prior to data integration, two of the six individual studies revisited in this report found a statistically significant difference in plasma IM concentrations between patients with an MMR ($n = 34$) and those without an MMR ($n = 28$) ($P = 0.010$ by Student's t-test)[13] and between patients with an optimal response ($n = 25$) and those with a suboptimal or failed response ($n = 8$) ($P = 0.0087$ by Student's t-test).[17] In the other four Japanese studies, it is possible that the number of patients per study was too small to achieve a statistically significant correlation between IM-C_{min} and outcome, as was previously suggested by Forrest *et al.*[9]

Our results suggest that higher IM-C_{min} is associated with an increased likelihood of achieving MMR and CCyR ($P = 0.002$ and 0.033, respectively, in univariate analysis). Additionally, in a multivariate analysis, both IM-C_{min} and the age of the patient were independently predictive of achieving an MMR. Age is one of the clinical factors that is included in the Sokal risk score as a baseline characteristic. Although we could not find a significant difference in Sokal risk score between patients with an MMR and those without, previous studies have reported that the Sokal risk score predicts outcome for IM-treated CML patients.[9,20] In contrast, by multivariate analysis, daily dosage of IM was the only independent predictive factor for achieving a CCyR. Together, these findings suggest that variability in IM exposure has clinical implications and, probably more so, implications for a molecular response.

Several clinical trials have established that a molecular response is a surrogate marker for predicting the likelihood of disease progression and/or survival in patients with CML.[20] In this study, although we could not directly compare IM-C_{min} with long-term outcomes, higher IM-C_{min} was associated with an increased likelihood of achieving an MMR. Given that an MMR may indicate a decreased risk for progression to accelerated phase or blastic crisis, we speculate that increased IM-C_{min} would be associated with longer survival; however, further study is necessary to test this hypothesis.

Given the importance of the duration of IM treatment in an evaluation of clinical response,[20] 60 of the 314 patients (19%) were excluded from further analysis of the correlation between IM-C_{min} and clinical response, either because the duration of IM therapy was <12 months or because a molecular response was not evaluated. Although there was no significant difference in C_{min} values between the excluded patients ($n = 60$; 994.5 ± 481.9 ng/ml) and the included patients ($n = 254$; 1,026.1 ± 582.2 ng/ml) per Student's t-test ($P = 0.315$), a potential source of bias cannot be entirely ruled out in this retrospective analysis.

In conclusion, on the basis of our data, we propose that, in addition to BCR-ABL mutation analysis for CML patients, it may be useful to assay plasma IM levels when making decisions related to IM therapy. Further study is necessary to prospectively confirm the link between IM-C_{min} and clinical response, including survival, in large multiethnic patient populations.

METHODS

Patients. The 314 patients included in this analysis were those who had previously been enrolled in six independent Japanese clinical studies.[13–18] All the patients had Philadelphia chromosome (Ph)-positive chronic-phase CML, and all were treated orally with IM for >2 months. Informed consent was obtained from each participant in accordance with the Declaration of Helsinki. Each study protocol was reviewed and approved by the ethics committees or institutional review boards of the participating centers.

Clinical parameters including response to the therapy. A CCyR was defined as the absence of Ph^+ metaphase cells among 20 or more bone marrow cells examined. In some cases, fluorescent *in situ* hybridization was also carried out for detection of bcr-abl fusion genes in neutrophils from peripheral blood.[21] An MMR was defined as a threefold log reduction in bcr-abl transcripts measured using real-time reverse transcriptase–mediated quantitative PCR and/or AMP-CML. The samples used to evaluate IM response and those for measurement of IM-C_{min} were collected from patients on the same day.

Measurement of IM concentrations in plasma. Blood samples were collected by venipuncture 24 h (± 2 h) after oral administration of IM. Plasma was isolated by centrifugation at 1,900*g* for 15 min and stored at −40 °C until analysis. IM-C_{min} values were determined using high-performance liquid chromatography coupled to electrospray-ionization tandem mass spectrometry[22] at the TORAY Research Center, (Nihonbashi, Tokyo, Japan), which is the only assay system in Japan authorized by Novartis Global.

Statistical analyses. Statistical analyses were carried out using SPSS statistical software (version 17.0; SPSS Japan, Tokyo, Japan). Data are presented as mean values ± SD unless indicated otherwise. Pearson's product moment correlation was applied to assess the relationship between IM-C_{min} and clinical variables (age, body weight, body surface area, and duration of IM therapy). A linear regression analysis was applied to assess the correlation between IM-C_{min} and the daily dose of IM. Differences in IM-C_{min} between two patient groups were evaluated using the Student's t-test. Comparison of IM-C_{min} among three groups was made using one-way analysis of variance with *post hoc* Tukey's multiple-comparison procedure. The χ^2 test or Fisher's exact test was used to compare the proportions of patients with an MMR or a CCyR and to compare groups. Stepwise forward-selection multiple logistic analyses

were performed for MMR and CCyR in order to determine the effects of the factors examined in the univariate analysis. Values of $P < 0.05$ were considered significant.

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CONFLICT OF INTEREST

The authors declared no conflict interest.

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ORIGINAL ARTICLE

Intensified consolidation therapy with dose-escalated doxorubicin did not improve the prognosis of adults with acute lymphoblastic leukemia: the JALSG-ALL97 study

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Abstract We designed a treatment protocol for newly diagnosed adult acute lymphoblastic leukemia (ALL) in the pre-imatinib era, employing intensified consolidation therapy with a total of 330 mg/m^2 doxorubicin and adopting slightly modified induction and maintenance regimen of the CALGB 8811 study. Of 404 eligible patients (median age 38 years, range 15–64 years), 298 (74%) achieved complete remission (CR). The 5-year overall survival (OS) rate was 32%, and the 5-year disease-free survival (DFS) rate was 33%. Of 256 Philadelphia chromosome (Ph)-negative patients, 208 (81%) achieved CR and the 5-year OS rate was 39%, and 60 of them underwent allogeneic-hematopoietic stem cell transplantation (allo-HSCT) from related or unrelated donors during the first CR, resulting in 63% 5-year OS. Of 116 Ph-positive patients, 65 (56%) achieved CR and the 5-year OS rate was 15%, and 22 of them underwent

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allo-HSCT from related or unrelated donors during the first CR, resulting in 47% 5-year OS. In Ph-negative patients, multivariate analysis showed that older age, advanced performance status and unfavorable karyotypes were significant poor prognostic factors for OS and higher WBC counts for DFS. The present treatment regimen could not show a better outcome than that of our previous JALSG-ALL93 study for adult ALL.

Keywords Acute lymphoblastic leukemia · A multiinstitutional trial · Doxorubicin · Prognostic factors · The Japan Adult Leukemia Study Group (JALSG)

1 Introduction

The emergence of imatinib therapy for acute lymphoblastic leukemia (ALL) with Philadelphia chromosome (Ph) has markedly changed the therapeutic strategy for ALL [1, 2]; however, the treatment outcome of adult ALL without Ph, which comprises 70–75% of adult patients, is still poorer than that of childhood Ph-negative ALL. Although complete remission (CR) rate exceeds 80% in adult Ph-negative ALL, overall survival (OS) rate decreases below 50% within 5 years in most cooperative group studies [3–9]. Since there was no new breakthrough agents for ALL in 1997, we employed a modification of post-remission therapy as one of the treatment strategies to improve overall therapeutic outcomes of this leukemia in the present study.

ALL is very heterogeneous regarding the underlying genetic abnormality, which is associated with its biological features and treatment outcome. In addition, other prognostic factors, such as age, performance status (PS) and disease progression status at the time of diagnosis, influence the treatment outcome, resulting in complicated evaluation of these factors. Among Ph-negative ALL, there are many types of genetic abnormalities and the proportion of each subset is small, which has hindered the evaluation of prognostic risk by cytogenetics. Recently, the Medical Research Council (MRC) and Eastern Cooperative Oncology Group (ECOG) reported the prognostic impact of more than 20 specific chromosomal abnormalities on the outcome of adult ALL [10]. The Southwest Oncology Group (SWOG) also demonstrated the importance of cytogenetics on the outcome by combining subgroups with similar risk [11]. Although their findings will greatly contribute for the planning of treatment strategy on this leukemia, further clarification of the relationship between cytogenetics and other risk factors is necessary.

In the present JALSG-ALL97 study, which started in the pre-imatinib era, we employed a consolidation therapy similar to that of aggressive non-Hodgkin lymphoma, including frequent administration of vincristine (VCR), glucocorticoid, cyclophosphamide (CPM) and doxorubicin (DOX). The total dose of DOX was 330 mg/m^2 in the consolidation phase. As for induction and maintenance therapy, we adopted the CALGB 8811 study [12], one of the standard regimens for adult ALL, with a slight modification. The primary aim of this study was to evaluate a new treatment protocol with intensified consolidation therapy, and to examine the impact of clinical and biological characteristics, including cytogenetics, on the therapeutic outcome in adult ALL. This report mainly focuses on the outcome of Ph-negative patients. Approximately 30% of Ph-negative patients who achieved CR underwent allogeneic-hematopoietic stem cell transplantation (allo-HSCT) during their first CR; thus, we also added an assessment of its results.

2 Patients and methods

2.1 Patient eligibility criteria

Adult patients with previously untreated ALL were consecutively registered to the JALSG-ALL97 study. Eligible criteria were a diagnosis of ALL (excluding mature B-cell ALL); age from 15 to 64 years; ECOG PS between 0 and 3; and adequate function of heart (no severe abnormalities detected on ECGs and echocardiographs), lung ($PaO_2 >$ 60 mmHg or $SpO_2 > 93\%$), liver (serum bilirubin level $<$ 2.0 mg/dL), and kidney (serum creatinine level $<$ 2.0 mg/dL). ALL was diagnosed according to the French–American–British (FAB) classification [13] using morphology, cytochemistry and immunophenotyping studies at each institution, which was later reevaluated by the Central Review Committee. Surface markers were considered positive when more than 20% of blasts expressed antigens.

Cytogenetic studies on pretreatment bone marrow or unstimulated blood samples were performed using standard banding techniques. Karyotypes were interpreted using the

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International System for Human Cytogenetic Nomenclature [14]. Evaluable cases were classified according to the modified MRC UKALLXII/ECOG E2993ALL cytogenetic subgroups reported by the SWOG [11].

The protocol was approved by institutional review board of each hospital. Written informed consent was obtained from all patients before registration in accordance with the Declaration of Helsinki.

2.2 Treatment

Details of the treatment schedule are described in Table 1. We slightly modified the induction therapy used in the CALGB 8811 study [12] by decreasing the dose of L-asparaginase (L-ASP). In the 1990s, there were two different commercial L-ASP preparations from *E. coli* (L-ASP Medac and L-ASP Bayer) in the United States, and the enzyme activities of the two were significantly different [15]. In Japan, L-ASP Kyowa is the only available preparation and its enzyme activity is much higher than L-ASP Bayer [16].

Induction therapy consisted of five drugs: VCR, daunorubicin, CPM, prednisolone (PSL), and L-ASP. When patients were 60 years or older, the doses of daunorubicin and CPM were reduced and PSL therapy was shortened. If patients did not achieve CR with the first course of induction therapy, consolidation I in Table 1 was applied as the second course of induction therapy. If this also failed, the patients were regarded as failure cases for remission induction.

Consolidation therapy included 8 courses featuring dose-intensified DOX (60 mg/m^2), which was administered by continuous infusion for 24 h on day 1, CPM, and intermediate-dose methotrexate (MTX). Central nervous system prophylaxis was given by intrathecal injection of MTX, cytarabine (Ara-C) and dexamethasone during the consolidation courses. Patients with high initial WBC counts of 50 $\times$ 10^9/L and/or a high LDH level above 5 times of the upper normal limit received prophylactic whole cranial irradiation at a total dose of 20 Gy after 8 consolidation courses. Patients with symptomatic or cytological evidence of central nervous system leukemia received additional intrathecal injections and whole cranial irradiation was given at a total dose of 20 Gy. Subsequent consolidation courses were started immediately after neutrophil counts surpassed 1.5 $\times$ 10^9/L and platelet counts were more than 100 $\times$ 10^9/L. After consolidation, maintenance therapy with daily 6-mercaptopurine, weekly MTX and monthly pulses of VCR and PSL was given until 24 months after the start of induction. All patients were given trimethoprim/sulfamethoxole for pneumocystis prophylaxis. Prophylactic granulocyte-colony stimulating factor was recommended after chemotherapy.

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 (PB), recovery of PB values to a neutrophil count of at least 1.5 $\times$ 10^9/L and a platelet count of at least 100 $\times$ 10^9/L, and no evidence of extramedullary leukemia. Relapse was defined as the presence of at least one of the following: recurrence of more than 10% leukemic cells in bone marrow or of any leukemic cells in PB or extramedullary sites.

2.3 HSCT

For patients with Ph or t(4;11) who achieved CR, allo-HSCT was recommended during their first CR if a human leukocyte antigen-matched sibling was available, and allo-HSCT from an alternative donor was allowed. For patients with other types, HSCT was not mandatory. Preparative and post-transplant regimens for HSCT were decided by the institutional guidelines at each hospital.

2.4 Statistical analyses

The cutoff date for analysis was January 1, 2007. The median duration of follow-up was estimated with the reverse Kaplan–Meier method [17]. Continuous data were described as the median and ranges, and compared using the Wilcoxon rank-sum test. Categorical data were compared using the Chi-square test or Fisher's exact test. The main endpoint of this study was OS. The probability of OS was calculated using the Kaplan–Meier estimator, death from any cause was considered an event, and surviving patients were censored at last follow-up [18]. Patients undergoing transplantation were not censored. Statistical comparison of time-to-event curves was completed by the log-rank test. An additional outcome evaluated was disease-free survival (DFS), which was calculated as survival without relapse or death (whichever came first) from the date of first CR. Patients undergoing transplantation were not censored. Univariate and multivariate Cox proportional hazards model [19] was used to determine prognostic factors for OS and DFS and the hazard ratio (HR) estimate was calculated with 95% confidence intervals (CIs). Statistical analyses were performed using SAS (version 9; SAS Japan Institute Inc., Tokyo, Japan). All statistical tests were two sided and conducted at the 5% significance level.

3 Results

3.1 Patient entry and characteristics

Between May 1997 and December 2001, 432 patients from 90 hospitals participating in the JALSG were

Table 1 Treatment schedule for the JALSG-ALL97

Agent	Route	Dose	Day number
Induction			
Vincristine	IV	1.3 mg/m^2	1, 8, 15, 22
Daunorubicin	IV	45 mg/m^2 (30 mg/m^2 [a])	1, 2, 3
Cyclophosphamide	IV	1,200 mg/m^2 (800 mg/m^2 [a])	1
Prednisolone	PO	60 mg/m^2	1–14 (1–7[a]), then tapered
L-Asparaginase	IV	3,000 U/m^2	9, 11, 13, 16, 18, 20
Consolidation(C)-1			
Vincristine	IV	1.3 mg/m^2	1
Doxorubicin	CI for 24 h	60 mg/m^2	1
Cyclophosphamide	IV	1,000 mg/m^2	1
Prednisolone	PO	60 mg/m^2	1–3
CNS prophylaxis (MD[b])	IT		1
C-2			
Methotrexate[c]	CI for 24 h	500 mg/m^2	1
Vincristine	IV	1.3 mg/m^2	2
Doxorubicin	IV	45 mg/m^2	2
Prednisolone	PO	60 mg/m^2	2–4
CNS prophylaxis (MD)	IT		1
C-3			
Vincristine	IV	1.3 mg/m^2	1
Doxorubicin	CI for 24 h	60 mg/m^2	1
Cyclophosphamide	IV	1,000 mg/m^2	1
Prednisolone	PO	60 mg/m^2	1–3
CNS prophylaxis (MAD[d])	IT		1
C-4			
Etoposide	IV	100 mg/m^2	1–4
Cytarabine	CI	200 mg/m^2	1–4
6-Mercaptopurine	PO	60 mg/m^2	1–4
Prednisolone	PO	60 mg/m^2	1–4
CNSprophylaxis (MAD)	IT		1
C-5			
Same as C-1 except for substituting dexamethasone 10 mg/m^2 PO × 3 for prednisolone			
C-6			
Same as C-2 except for substituting dexamethasone 10 mg/m^2 PO × 3 for prednisolone			
C-7			
Same as C-3 except for substituting dexamethasone 10 mg/m^2 PO × 3 for prednisolone			
C-8			
Mitoxantrone	IV	8 mg/m^2	2, 3
Cytarabine	CI	200 mg/m^2	1–4
6-Mercaptopurine	PO	60 mg/m^2	1–4
Dexamethasone	PO	10 mg/m^2	1–4
CNSprophylaxis (MAD)	IT		1
Maintenance			
Vincristine	IV	1.3 mg/m^2	1[e]
Prednisolone	PO	60 mg/m^2	1–5[e]
6-Mercaptopurine	PO	60 mg/m^2	1–28[e]
Methotrexate	PO	20 mg/m^2	1, 8, 15, 22[e]

Maximum dose of vincristine was 2.0 mg/body

IV intravenously, *PO* per os, *CI* continuous infusion, *IT* intrathecally

[a] Doses or schedule for patients 60 y.o. or older

[b] *MD*, methotrexate 15 mg/body + dexamethasone 4 mg/body for IT

[c] 50 mg/m^2 of MTX was administered as IV for 30 min and 450 mg/m^2 of MTX as IV for 23.5 h. After 36 h from the start of MTX infusion, 15 mg/body of leucovorin was administered 8 times every 6 h by IV, subcutaneously (SC), intramuscularly (IM) or PO. When the plasma concentration of MTX at 48 h was 1×10^{-6} M or more, 60 mg/body of leucovorin was added 8 times every 6 h by IV, SC, IM or PO, and when it was $5–10 \times 10^{-7}$ M, 15 mg/body of MTX was added by the same schedule

[d] *MAD* MD + cytarabine 40 mg/body used for IT

[e] Every 4 weeks

enrolled in this study. Sixteen patients were excluded because 13 had been misdiagnosed (6 with acute myeloid leukemia, 4 with mature B-cell leukemia, 2 with blastic crisis of chronic myeloid leukemia and one with non-Hodgkin lymphoma), 2 were not consistent with the eligible criteria and one died before treatment. Evaluable data from 12 were incomplete at the time of analysis; thus, here, we report outcome of 404 eligible patients. Median age was 38 years and there were 208 men (51%) and 196 women. Pretreatment characteristics are summarized in Table 2.

Cytogenetic evaluation was performed in 344 patients (85%); 130 (32%) had normal karyotypes, 214 (53%) showed abnormal karyotypes and 96 (28%) Ph based on conventional banded studies. The fusion gene of *BCR-ABL* was analyzed in 191 patients and 72 (38%) were positive. Twelve patients without Ph had the fusion gene of *BCR-ABL* (9 with normal karyotype; one with monosomy 7; 2 with other karyotypes). We defined patients with Ph and/or *BCR-ABL* fusion gene as Ph-positive (116 patients), and patients without Ph or *BCR-ABL* fusion gene as Ph-negative (256). Thirty-two patients were not assessable for Ph status. Pretreatment characteristics of the Ph-negative group and the Ph-positive one are summarized in Table 2. Age and WBC count were significantly higher in the Ph-positive group ($P < 0.0001$ for both variables). Ph-negative patients were classified according to the modified MRC UKALLXII/ECOG E2993ALL cytogenetic subgroups [11]: the very high risk group ($n = 32$) included t(4;11) ($n = 8$), complex karyotype defined as more than 5 abnormalities without known translocations ($n = 20$), or low hypodiploidy/near triploidy ($n = 4$); the high risk group ($n = 10$) included other *MLL* translocations ($n = 4$), monosomy 7 with less than 5 abnormalities ($n = 2$) or t(1;19) ($n = 4$); the standard-risk group included high hyperdiploidy ($n = 9$); the intermediate risk group ($n = 185$) included normal karyotype ($n = 121$) or other miscellaneous abnormal karyotypes ($n = 64$).

3.2 Response to induction therapy

The results of therapy are summarized in Table 3. Overall, 298 (74%) of 404 evaluated patients achieved CR:. 276 (68%) after the first treatment and 22 after additional consolidation course 1. Twenty-one patients (5%) died within 4 weeks after the start of induction therapy before their remission status could be ascertained. The causes of death were sepsis ($n = 14$), pneumonia ($n = 2$), intracranial hemorrhage ($n = 2$), and others ($n = 3$). Eighty-five patients (21%) failed to respond. Among 256 Ph-negative patients, 208 (81%) achieved CR, 12 (5%) died during the induction phase and 36 (14%) were refractory, whereas only 65 (56%) of 116 Ph-positive patients achieved CR.

3.3 Survival

After a median follow-up of 5.8 years (range 2 days to 8.6 years), 146 of 404 eligible patients were alive and 104 were disease-free. The median OS was 23.8 months and the estimated probability of the OS rate at 5 years was 32% (95% CI 27–37%), as shown in Fig. 1a. Among 298 CR patients, 24 died in remission and 170 relapsed. The median DFS was 18.8 months, and the estimated 5-year DFS rate was 33% (95% CI 27–38%), as shown in Fig. 1b. The outcome by Ph status is shown in Table 3. The 5-year OS rates for 256 Ph-negative patients and 116 Ph-positive patients were 39% (95% CI 32–45%) and 15% (95% CI 9–23%), respectively (Fig. 1c).

3.4 Prognostic factors for Ph-negative patients

Univariate analyses for the effects of clinical and biological features on outcome among Ph-negative patients are summarized in Table 4. PS and WBC count were significantly related to CR achievement. The 5-year OS rate for patients who achieved CR was 45% (95% CI 38–52%), whereas that for those who did not reach CR after 2 induction courses was 10% (95% CI 3–21%). Older age, PS 2 or 3, hepatomegaly, WBC count (30×10^9/L or higher) and cytogenetics (the very high/high risk or other miscellaneous abnormal karyotypes) were significantly related to OS. Hepatomegaly, WBC count (30×10^9/L or higher) and cytogenetics (the very high/high risk) were significantly related to DFS. Figure 2a shows OS for Ph-negative patients by age group. Although the OS rate decreased with advancing age, there was no difference between patients of 15–24 and 25–34 years old. When we compared OS between those older and younger than 35 years old, survival of older patients was significantly poorer (HR 1.54, 95% CI 1.12–2.12; $P = 0.008$). In 236 Ph-negative patients with evaluable cytogenetics, there was highly significant heterogeneity of OS among the 5 cytogenetic subgroups ($P = 0.0064$, Fig. 2b). Because of the small number of patients in the high risk group or the standard-risk group, the former was combined with the very high risk group, and the latter with the normal karyotype group. Patients with the very high/high risk karyotype or other miscellaneous abnormal karyotype had significantly poorer OS than those with the standard/normal karyotype (Table 4). DFS of the very high/high risk group was significantly worse than that of the standard/normal karyotype group. Immunophenotype was not a significant prognostic factor for OS (Table 4). The 5-year OS rates for B-lineage patients and for T-lineage were 42% (95% CI 35–49%) and 33% (95% CI 17–49%), respectively ($P = 0.43$). Time to CR was not a risk factor, either. The 5-year OS rate for 191 patients who achieved CR after one course of

Table 2 Clinical and biological features of patients at diagnosis

Parameters	No. (%) or median (range)		
	All	Ph-negative	Ph-positive
No. of patients evaluated	404	256	116
Sex			
Male	208 (51)	120 (47)	69 (59)
Female	196 (49)	136 (53)	47 (41)
Age (years)			
Median (range)	38 (15–64)	30 (15–64)	48 (15–64)
15–24	120 (29)	98 (38)	13 (11)
25–34	63 (16)	43 (17)	11 (10)
35–54	144 (36)	70 (27)	64 (55)
55 or older	77 (19)	45 (18)	28 (24)
Performance status			
0, 1	359 (89)	230 (90)	102 (88)
2, 3	45 (11)	26 (10)	14 (12)
Hepatomegaly			
Yes	87 (22)	58 (23)	25 (22)
No	317 (78)	198 (77)	91 (78)
Splenomegaly			
Yes	75 (19)	49 (19)	20 (17)
No	329 (81)	207 (81)	96 (83)
Lymphadenopathy			
Yes	111 (27)	80 (31)	25 (22)
No	293 (73)	176 (69)	91 (78)
Fever over 38°C			
Yes	126 (31)	78 (30)	43 (37)
No	278 (69)	178 (70)	73 (63)
CNS involvement			
Yes	4 (1)	3 (1)	1 (1)
No	399 (99)	253 (99)	114 (98)
Missing	1 (0.2)		1 (1)
WBC count ($\times 10^9$/L)			
Median (range)	12.6 (0.3–810)	10.5 (0.3–718)	29.2 (1.0–810)
Less than 3	62 (15)	48 (19)	9 (8)
3–10	115 (29)	75 (29)	25 (22)
10–30	90 (22)	62 (24)	25 (22)
30 or higher	136 (34)	71 (28)	56 (47)
Missing	1 (0.2)		1 (1)
FAB classification			
L1	75 (19)	55 (21)	15 (13)
L2	325 (80)	199 (78)	100 (86)
Unknown	4 (1)	2 (1)	1 (1)
Immunologic classification			
B-lineage	330 (82)	199 (77)	108 (94)
T-lineage	38 (9)	35 (14)	0 (0)
Others	36 (9)	22 (9)	7 (6)

CNS central nervous system, *Ph* Philadelphia chromosome

chemotherapy was 48%, compared with 28% for 17 who did after the additional chemotherapy, but this difference was not statistically significant ($P = 0.16$).

Multivariate analyses revealed that advanced age, PS 2 or 3, and cytogenetics (the very high/high risk or other miscellaneous abnormal karyotypes) were independent

prognostic factors for OS and only WBC count (30×10^9/L or higher) was an independent prognostic factor for DFS (Table 4). We developed a simple scoring system for predicting outcome based on the HR of these risk factors for OS of CR patients. A score of one was allocated to each of the following parameters: age ≥ 35 years, PS 2 or 3, WBC counts ≥ 30×10^9/L and other miscellaneous abnormal karyotype, and a score of 2 to the very high/high risk karyotype. OS curves of patients scoring 0, 1, 2, 3, and 4 or more are shown in Fig. 2c. The 5-year OS rate for patients scoring 0 was 60% (95% CI 45–73%). OS decreased with an increasing total score, and 4-year OS rate for patients scoring 4 or more was only 10% (95% CI 1–35%; Table 5).

Table 3 Summary of therapy results

	All patients	Ph-negative	Ph-positive
Patients eligible	404	256	116
Early deaths	21 (5%)	12 (5%)	9 (8%)
Refractory	85 (21%)	36 (14%)	42 (36%)
Dead	70	28	38
Alive	15	8	4
CR achievement (% of all)[a]	298 (74%)	208 (81%)	65 (56%)
Died in CR	24	14	6
Relapse[b]	170	121	38
Dead	143	99	36
Alive	27	22	2
CCR	104	73	21
Total dead	258	153	89
Total alive	146	103	27

CCR continuous complete remission, *CR* complete remission, *Ph* Philadelphia chromosome

[a] CR achievement includes those reached CR by induction therapy and 1st consolidation therapy

[b] Relapse indicates the first relapse after CR achievement including the first relapse after hematopoietic stem cell transplantation (HSCT) among those who received HSCT during CR

3.5 HSCT for Ph-negative patients

Among 208 Ph-negative patients who achieved CR, 60 (29%) underwent allo-HSCT during their first CR (37 from a related donor and 23 from an unrelated donor). The median duration from the time of achieving CR to transplantation was 7.5 months (range 3.1–34.6 months). Patients who received allo-HSCT were significantly younger than those who did not [median (range) 25.5 years (16.0–55.0) vs. 31.0 years (15.0–64.0), $P = 0.02$]. Among 60 patients who received allo-HSCT, 8 (13%) died in remission, 16 (27%) relapsed, and 36 (60%) were in continuous CR (CCR). The 5-year OS rate was 63% (95% CI 49–74%; Fig. 3a), 68% (95% CI 50–81%) from a related donor and 55% (95% CI 32–73%) from an unrelated donor, showing no significant difference ($P = 0.43$). Patients scoring 0 or 1 had significantly better OS [75% (95% CI 55–86%)] than those scoring 2 or more [48% (95% CI 26–67%)] ($P = 0.02$; Fig. 3b).

Among 148 patients who did not receive allo-HSCT during their first CR, 37 (25%) were in CCR, 6 (4%) died in remission (2, therapy-related death; one, other disease; 3, unknown) and 105 (71%) relapsed. Of 105 relapsed, 46 received allo-HSCT for salvage therapy, and 10 were alive in remission after transplantation with a median duration of 3.9 years (range 7 months to 7.1 years). The 5-year OS

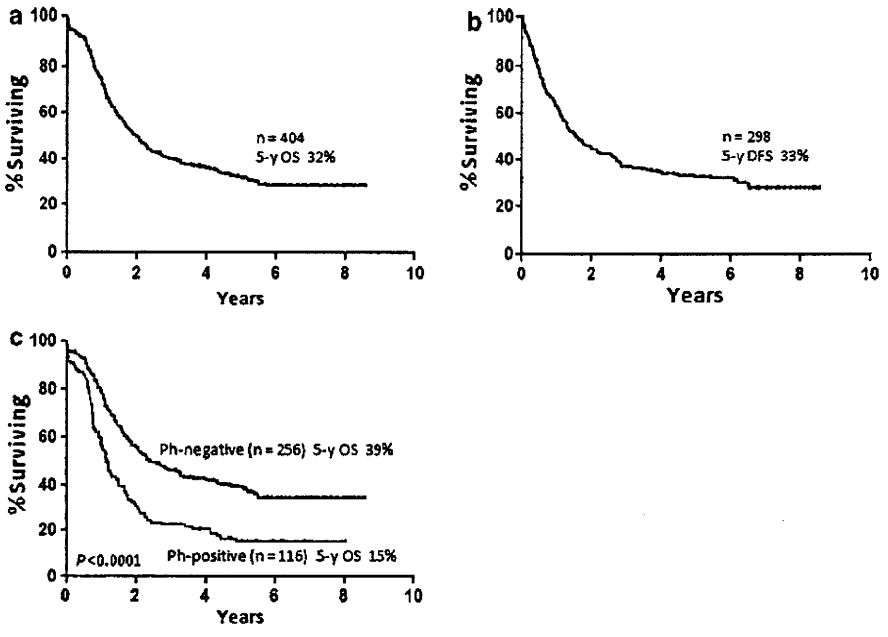

Fig. 1 Survival analysis. **a** Overall survival (OS) of 404 eligible patients. **b** Disease-free survival (DFS) of 298 patients who achieved complete remission. **c** OS of 116 Philadelphia chromosome (Ph)-positive patients and 256 Ph-negative patients

Table 4 Effects of clinical and biological features on outcome among Ph-negative ALL (univariate analyses)

Parameters	No. of patients at diagnosis	CR		OS		DFS	
		%	*P*	No. of events[a]	Hazard ratio (95% CI)	No. of events[b]	Hazard ratio (95% CI)
Total	256	81		153		135	
Sex							
Female	136	85	0.08	82	Ref	80	Ref
Male	120	77		71	1.08 (0.78–1.48)	55	0.86 (0.60–1.23)
Age (years)							
15–24	98	85	0.72	52	Ref	50	Ref
25–34	43	79		23	0.93 (0.57–1.52)	22	0.96 (0.57–1.61)
35–54	70	80		44	1.31 (0.87–1.96)	36	1.06 (0.68–1.65)
55 or older	45	78		34	1.87 (1.21–2.90)	27	1.37 (0.84–2.21)
Performance status							
0, 1	230	83	0.03	133	Ref	123	Ref
2, 3	26	65		20	2.00 (1.25–3.21)	12	1.56 (0.84–2.90)
Hepatomegaly							
No	198	81	0.74	111	Ref	99	Ref
Yes	58	83		42	1.50 (1.05–2.15)	36	1.71 (1.15–2.53)
Splenomegaly							
No	207	83	0.12	120	Ref	110	Ref
Yes	49	74		33	1.29 (0.88–1.91)	25	1.37 (0.88–2.14)
Lymphadenopathy							
No	176	89	0.30	106	Ref	94	Ref
Yes	80	78		47	0.98 (0.69–1.39)	41	1.13 (0.77–1.65)
Fever over 38°C							
No	178	81	0.83	104	Ref	96	Ref
Yes	78	82		49	1.12 (0.80–1.58)	39	0.84 (0.57–1.24)
CNS involvement							
No	253	82	0.09	150	Ref	134	Ref
Yes	3	33		3	3.01 (0.96–9.46)	1	1.40 (0.20–9.99)
WBC count ($\times 10^9$/L)							
Less than 30	185	86	0.002	105	Ref	97	Ref
30 or higher	71	69		48	1.66 (1.17–2.33)	38	1.80 (1.22–2.65)
Immunologic classification							
B-lineage	199	84	0.14	115	Ref	107	Ref
T-lineage	35	74		22	1.20 (0.76–1.92)	19	1.30 (0.78–2.17)
Chromosome category (n = 236), unknown = 20							
Standard risk	9	89	0.49	3	Ref[c]	3	Ref[c]
Normal	121	79		65		58	
Miscellaneous	64	78		44	1.68 (1.14–2.46)	35	1.47 (0.95–2.26)
High risk	10	90		5	1.87 (1.21–2.89)[c]	5	1.82 (1.15–2.89)[c]
Very high risk	32	91		25		24	
Days from treatment start to CR achievement							
≤30 days	85			44	Ref	54	Ref
>30 days	120			66	1.00 (0.68–1.46)	78	1.02 (0.71–1.46)

ALL acute lymphoblastic leukemia, *CNS* central nervous system, *CR* complete remission, *DFS* disease-free survival, *OS* overall survival, *Ph* Philadelphia chromosome

[a] Death

[b] Relapse or death

[c] The standard-risk group was combined with the normal karyotype group, and the high risk group with the very high risk group

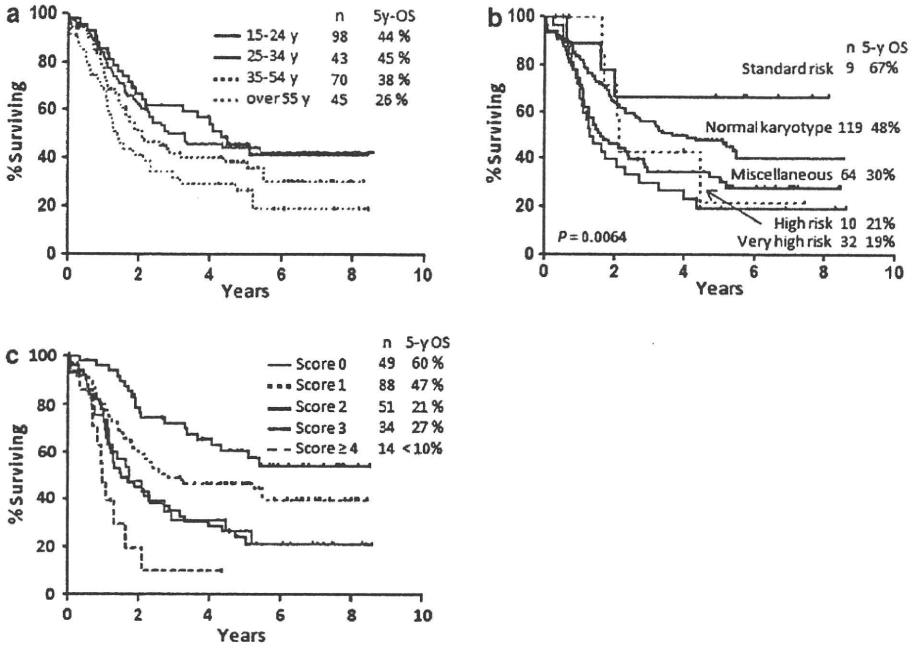

Fig. 2 Survival analysis of Philadelphia chromosome-negative patients. **a** Overall survival (OS) by age group. **b** OS by karyotype category according to the modified MRC UKALLXII/ECOG E2993ALL cytogenetic subgroups: the very high risk group included t(4;11), complex karyotype defined as more than 5 abnormalities without known translocations, or low hypodiploidy/near triploidy; the high risk group included other *MLL* translocations, monosomy 7 with less than 5 abnormalities or t(1;19); the standard-risk group included high hyperdiploidy; other miscellaneous abnormal karyotypes were categorized as intermediate risk. **c** OS by a scoring system that we developed. A score of one was allocated to each of the following parameters; age ≥ 35 years, performance status 2 or 3, WBC counts ≥ 30 × 10^9/L and other miscellaneous abnormal karyotype, and a score of 2 to the very high/high risk karyotype

Table 5 Effects of clinical and biological features on survival among Ph-negative ALL (multivariate analyses)

Parameters	HR (95% CI)		
	OS	OS of CR patients	DFS
Age (years old)			
35 or older (vs. 15–34)	1.74 (1.24–2.44)	1.64 (1.11–2.43)	1.21 (0.83–1.74)
Performance status			
2, 3 (vs. 0, 1)	2.06 (1.26–3.37)	1.94 (1.02–3.69)	1.43 (0.74–2.77)
Hepatomegaly			
Yes (vs. no)	1.26 (0.86–1.85)	1.43 (0.91–2.23)	1.44 (0.94–2.21)
WBC count (×10^9/L)			
30 or higher (vs. less than 30)	1.42 (0.98–2.01)	1.16 (0.73–1.82)	1.63 (1.08–2.48)
Chromosome category			
Miscellaneous group (vs. SR + NK[a])	1.55 (1.05–2.29)	1.56 (0.98–2.50)	1.26 (0.81–1.97)
High and very high risk (vs. SR + NK[a])	1.60 (1.02–2.50)	2.25 (1.37–3.70)	1.49 (0.92–2.41)

ALL acute lymphoblastic leukemia, *CR* complete remission, *DFS* disease-free survival, *HR* hazard ratio, *OS* overall survival, *Ph* Philadelphia chromosome

[a] Standard risk + normal karyotype

rate for all Ph-negative patients who did not receive allo-HSCT during the first CR was 37% (95% CI 29–46%; Fig. 3c). Among those, the 5-year OS rate for patients scoring 0 or 1 was 53% (95% CI 41–63%) and that for patients scoring 2 or more was 19% (95% CI 10–31%), showing a significantly better OS in the former than the latter ($P < 0.0001$; Fig. 3d).

3.6 HSCT for Ph-positive patients

Among 65 Ph-positive patients who achieved CR, 22 (34%) underwent allo-HSCT during their first CR (19 from a related donor and 3 from an unrelated donor). The median duration from the time of achieving CR to transplantation was 4.6 months (range 2.6–12.1 months).

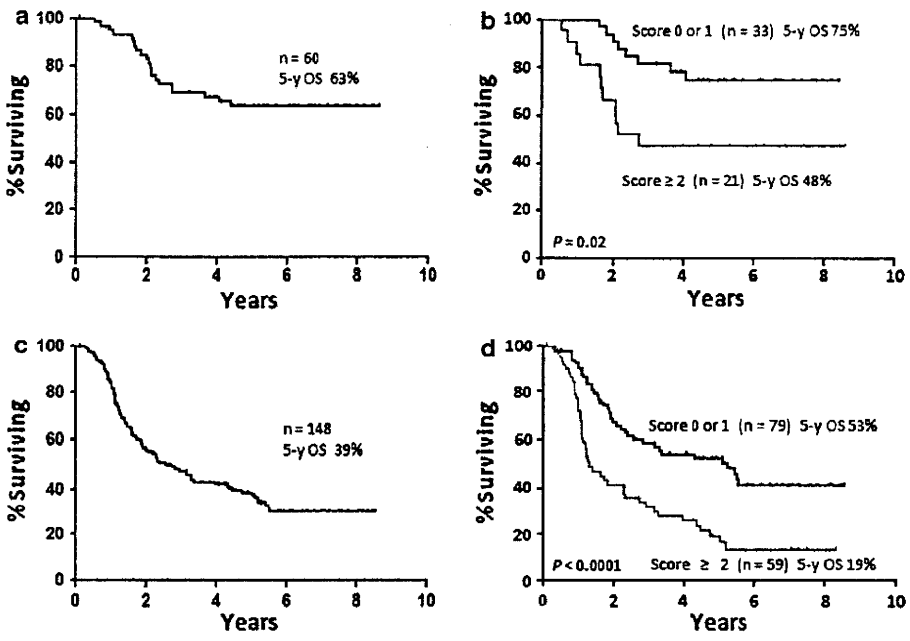

Fig. 3 Survival analysis of Philadelphia chromosome-negative patients with/without allogeneic-hematopoietic stem cell transplantation (allo-HSCT) in first complete remission. **a** Overall survival (OS) in those who received allo-HSCT. **b** OS in those who received allo-HSCT by dichotomized prognostic score group. **c** OS in those who did not received allo-HSCT. **d** OS in those who did not received allo-HSCT by dichotomized prognostic score group

Patients who received allo-HSCT were significantly younger than those who did not [median (range) 41.5 years (15–56) vs. 49.0 years (24–63), $P = 0.02$]. Among 22 Ph-positive patients who received allo-HSCT, 5 (23%) died in remission, 6 (27%) relapsed, and 11 (50%) were in CCR. The 5-year OS rate was 47% (95% CI 24–67%; Fig. 4).

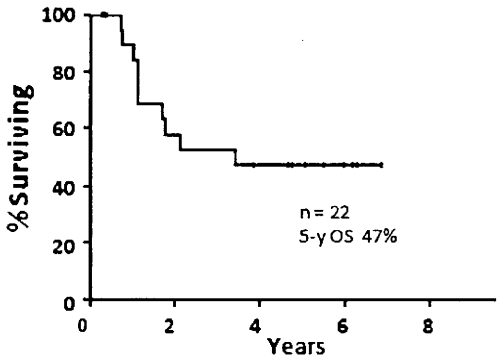

Fig. 4 Overall survival (OS) of Philadelphia chromosome-positive patients who received allo-hematopoietic transplantation in first complete remission

4 Discussion

In the present study, although the CR rate of all 404 evaluable patients did not exceed 80%, the rate was greater in Ph-negative patients (81%) than Ph-positive patients (56%). These results are not so different from our preceding JALSG-ALL93 study [4] (Ph-negative, 83%; Ph-positive, 51%) and from the CALGB 8811 study [12] (Ph-negative, 84%; Ph-positive, 70%). In the JALSG-ALL93 study, we tested an intensified induction therapy mainly using DOX. In the present study, we asked whether a benefit could be achieved by intensifying the consolidation phase of the CALGB 8811 study protocol, mainly using DOX. However, DFS of CR patients did not differ much from that of the CALGB 8811 study or that of the CALGB 9111 study [3] in which the same chemotherapy regimen was used. Besides, the 5-year OS of 45% for Ph-negative patients who achieved CR was similar to that in the MRC UKALL XII/ECOG E2993 study [7], suggesting that the present intensified consolidation therapy resulted in a similar outcome to the standard consolidation regimen, and had little impact on the survival improvement of adult Ph-negative ALL.

Age is a major prognostic factor in ALL. When we compared by age, OS of patients younger than 35 years was significantly better than that of patients aged 35 years or older (5-year OS; 44 vs. 32%, $P = 0.008$); however, there was no significant difference between patients of 15–24 and 25–34 years old. A similar outcome was seen in the MRC UKALL XII/ECOG E2993 study [7], i.e., the 5-year OS rates for Ph-negative patients aged 15–19 and 20–29 years old were 44 and 45%, respectively.

Several retrospective analyses reported improved outcomes for adolescent and young adult ALL treated by the pediatric regimens [20, 21]. Stock et al. [21] reported the outcomes of 321 adolescents and young adults who underwent pediatric (Children's Cancer Group) or adult (CALGB) trials, and the 7-year OS rates were 67 and 46%, respectively. As one potential explanation for these differences, they suggested dose intensification of nonmyelosuppressive drugs, such as glucocorticoids, VCR and

L-ASP, which have been the mainstay of pediatric ALL therapy. The outcome of adolescents and young adults in our study was similar to that of the same cohort in the CALGB study, including the 8811 trial, and we did not use L-ASP during the post-remission therapy. Therefore, to improve the therapeutic outcome of adult ALL, particularly that of adolescent and young adult ALL, pediatric regimens using dose-intensified nonmyelosuppressive drugs should be prospectively tested. Such studies are already underway in several adult cooperative study groups, including the JALSG-202 study, showing promising results [22, 23].

The outcome of T-ALL patients in JALSG-ALL97 study has previously been reported together with T-ALL patients in other JALSG ALL studies [24]. Reportedly, the T-cell phenotype is generally a favorable prognostic factor in adult ALL; however, the outcome of T-ALL patients in our present study was not better than that of Ph-negative precursor B-ALL. T-ALL was said to be benefited from Ara-C and CPM [25]. In our consolidation phase, high doses of anthracycline and CPM were used, but not Ara-C. Thus, T-ALL may not have been benefitted from anthracycline in consolidation therapy. T-ALL therapy may need a higher dose of Ara-C and/or a new drug such as nelarabine, a promising drug for T-cell malignancies [26, 27].

In the present study, we were able to confirm the impact of cytogenetics on the outcome of adult ALL based on the grouping by MRC UKALL XII/ECOG E2993 study [10] and SWOG 9400 study [11]. In addition to Ph, the very high risk group in the present study was t(4;11), complex type and low hypodiploidy/near triploidy, and the outcome (5-year OS, 19%) of this group was very similar to the SWOG 9400 study (22%) and the MRC UKALL XII/ECOG E2993 study (22–28%), suggesting that this grouping is useful for the prediction of poor prognostic group. Normal diploidy is the most frequent karyotype among Ph-negative ALL. In the present study, the 5-year OS rate of patients with a normal karyotype was 48%, which was similar to that of the MRC UKALL XII/ECOG E2993 study (48%) and the SWOG 9400 study (50%). In contrast, the prognosis of other miscellaneous types was worse in the present study than in the SWOG 9400 study. This group includes numerous cytogenetic abnormalities, and the prognostic risk of each type has not been defined because the number of each type is very small. In fact, in the MRC UKALL XII/ECOG E2993 study, the largest study of adult ALL, most other miscellaneous types did not show any significant association with disease outcome, and only a few karyotypes exceeded 45% 5-year OS, showing no conflict to our results. Since the high risk group in the present study, comprising other *MLL* translocations, monosomy 7 or t(1;19), showed a poor prognosis, we combined this group with the very high risk group for statistic analysis, although the outcome of the high risk group in the SWOG 9400 study was not particularly detrimental. It seems difficult to discuss the difference because of the small number of patients in each study (SWOG study, 12 patients vs. present study, 10).

In our previous JALSG-ALL93 study, CR patients under 40 years old with human leukocyte antigen-matched siblings were scheduled to receive allo-HSCT during the first CR. In this study, however, we did not incorporate recommendation for HSCT except for patients with Ph or t(4;11), because the ALL93 study showed no survival difference between patients of age under 40 years with and without a sibling donor, except for Ph-positive patients who benefited from allo-HSCT. However, if patients without a sibling wished to have HSCT, most of them can obtain an unrelated donor through the Japan Marrow Donor Program. Approximately 30% of Ph-negative patients who achieved CR underwent allo-HSCT in their first CR, and 38% of them from unrelated donors. The 5-year OS rate in Ph-negative patients who received allo-HSCT during the first CR was 63% and the transplantation-related mortality rate was only 13%. Notably, the 5-year OS of patients without risk factors, such as older age, advanced PS, a higher WBC count and unfavorable karyotypes, was 75% and very satisfactory despite of marked selection bias in the choice of treatment. Recently, the MRC/ECOG group reported that matched related allo-HSCT for adult ALL in the first CR provided survival benefit for standard-risk patients in prospective sibling donor versus no-donor comparison [28]. The HOVON Cooperative Group also stated that standard-risk ALL patients showed favorable survival following allo-HSCT, due to both a strong reduction of relapse and a modest transplantation-related mortality, although their standard-risk criteria did not include age [29]. These results suggest that allo-HSCT is the most promising treatment modality for adult ALL patients who have achieved CR and have few risk factors.

Multivariate Cox analysis in our Ph-negative patients showed that older age (35 years old or more), advanced PS (PS 2 or 3) and unfavorable karyotypes (very high/high risk or other miscellaneous abnormalities) were independent adverse prognostic factors for OS, and a higher WBC count (30×10^9/L or more) for DFS. The 5-year OS of patients without these risk factors was 60%, whereas that of patients with multiple risk factors was under 30%. Our scoring system worked well for both patients who received HSCT or did not in their first CR. This demonstrates importance to assess prognostic factors, including cytogenetics, when making a treatment plan. Further studies on this scoring system should be performed to prove its usefulness in the individualized therapy on Ph-negative ALL possessing different prognostic scores.

Regretfully, the present study could not show the benefit of intensified consolidation with myelosuppressive drugs in

adult ALL. Dose intensification of nonmyelosuppressive agents such as glucocorticoids, VCR and L-ASP like pediatric regimens and/or incorporation of new agents such as molecule-targeting drugs and monoclonal antibodies would be the next step to be tested in order to increase the cure rate of adult ALL.

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Relationship between Regulatory T Cells and the Combination of Pegylated Interferon and Ribavirin for the Treatment of Chronic Hepatitis Type C

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Key Words
Chronic hepatitis C · Hepatitis C virus · Interferon · Ribavirin · Regulatory T cells

Abstract
Background/Aim: The frequency of regulatory T cells (Tregs) may be related to persistent hepatitis C virus (HCV) infection. We studied the alteration of the Treg ratio in peripheral blood mononuclear cells (PBMCs) from chronic hepatitis C patients during combination therapy compared with the Treg ratio in liver-infiltrating lymphocytes (LILs) before therapy. ***Method:*** The study group consisted of 20 patients who were treatment-naive and had high virus titers of HCV genotype 1. Blood samples were collected prior to treatment and at several time points during treatment. All patients received a liver biopsy prior to treatment. Forkhead box P3 (Foxp3)+, CD3+, CD4+ and CD8+ cells in PBMCs and LILs were stained by specific antibodies. ***Results:*** Ten patients had a sustained virological response (SVR), and 10 patients were non-responders. The SVR group had a significant increase in the Foxp3+/CD4+ ratio in PBMCs at 8 and 12 weeks as well as a significant decrease in the Foxp3+/CD4+ ratio and increase in the CD8+/Foxp3+ ratio in LILs. ***Conclusion:*** The evaluation of Tregs, a potentially significant factor for persistent HCV infection, in LILs prior to treatment and in PBMCs during treatment could predict the result of combination therapy.

Introduction

Chronic infection with the hepatitis C virus (HCV) causes chronic hepatitis (CH), liver cirrhosis and hepatocellular carcinoma [1–3]. Naive HCV infection causes self-limited, acute hepatitis; however, 50–80% of these acute cases develop into CH [4, 5]. One of the causes of persistent infection is the immunological modulation of HCV [6–8]. In fact, patients with chronic HCV infection have weak HCV-specific T-cell responses [9]. The CD4+CD25+ regulatory phenotype T cell (Treg) contributes to the mechanism of evasion of immunological surveillance [10–12]. During persistent HCV infection, Tregs in the peripheral blood mononuclear cells (PBMCs) and liver-infiltrating lymphocytes (LILs) suppress the proliferation and cytokine production of HCV-specific CD8– and CD4– T cells [13–16]. We also found that intrahepatic Tregs restricted the supply of infiltrated CD8– T cells in chronic hepatitis C (CHC) patients [17]. Tregs contribute to the development of CH in cases of persistent hepatitis B virus infection [18], autoimmune hepatitis [19] and primary biliary cirrhosis [20].

The most common method of treatment for CHC is pegylated interferon (peg-IFN) combined with ribavirin (Rib). In recent years, the combination of peg-IFN and Rib has achieved a 50% or greater sustained virological response (SVR) rate [21, 22]. Cases that have achieved an

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