

Karyotype at diagnosis is the major prognostic factor predicting relapse-free survival for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia treated with imatinib-combined chemotherapy

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ABSTRACT

To identify factors associated with relapse-free survival (RFS), 80 patients with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia, enrolled in a phase II study of imatinib-combined chemotherapy, were analyzed. The median follow-up of surviving patients was 26.7 months (maximum, 52.5 months). Twenty-eight out of 77 patients who had achieved CR relapsed. The probability of RFS was 50.5% at 2 years. Multivariate analysis revealed that the presence of secondary chromosome aberrations in addition to t(9;22) at diagnosis constitute an independent predictive value for RFS ($p=0.027$), and increase the risk of treatment failure by 2.8-fold.

Key words: acute lymphoblastic leukemia, Philadelphia chromosome, BCR-ABL, imatinib, karyotype.

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Introduction

The treatment for Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph⁺ ALL) has changed dramatically since imatinib, a selective inhibitor of the ABL tyrosine kinase, was introduced.^{1,2} Combined with chemotherapy, or even as a single agent, it can produce complete remission (CR) rates of 90% or higher in newly diagnosed patients.³⁻⁹ We previously reported the results of a phase II study by the Japan Adult Leukemia Study Group (JALSG) to test the efficacy and feasibility of imatinib-combined

chemotherapy for newly diagnosed Ph⁺ ALL.⁶ The rate of CR reached 96%, and that of BCR-ABL negativity in bone marrow 71%. However, despite a relatively short follow-up period, relapse occurred in a subset of the patients who had achieved CR.

On the other hand, remarkable progress is being made with the development of novel tyrosine kinase inhibitors with more potent *in vitro* and *in vivo* activities than imatinib.^{10,11} Given this, we investigated factors associated with relapse-free survival (RFS).

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Design and Methods

Patients and treatments

Eligibility criteria included newly diagnosed Ph⁺ ALL, age between 15 and 64 years, an Eastern Co-operative Oncology Group performance status between 0 and 3, and adequate liver, kidney and heart function. Written informed consent was obtained from all patients prior to registration.

For remission induction therapy, imatinib was administered from day 8 to day 63 in combination with daunorubicin, cyclophosphamide, vincristine (VCR) and prednisolone (PSL). Consolidation therapy consisted of an odd course (C1) comprising high-dose methotrexate, high-dose cytarabine and methylprednisolone, and an even course (C2) with single-agent imatinib for 28 days. C1 and C2 were alternated for 4 cycles each. After completion of the consolidation therapy, patients received maintenance therapy consisting of VCR, PSL and imatinib for up to 2 years from the date CR had been achieved.⁶ The daily dose of imatinib used in this study was 600 mg. Allogeneic hematopoietic stem cell transplantation (HSCT) was recommended if a matched sibling donor was available, and was allowed from an alternative donor.

The protocol was reviewed and approved by the institutional review board of each of the participating centers and was conducted in accordance with the Declaration of Helsinki.

Cytogenetic and molecular analysis

At diagnosis, bone marrow samples were examined for cytogenetic abnormalities with standard banding techniques. Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature.¹² The number of BCR-ABL copies in bone marrow was determined at a central laboratory with the real-time quantitative RT-PCR test according to the previously described method.¹³

Statistical analysis

Kaplan-Meier survival analysis was performed to estimate the probabilities of RFS, event-free survival (EFS), and overall survival (OS), with differences between the groups compared by the log-rank test. Cumulative incidences of relapse were calculated with non-relapse mortality considered as a competing risk, and differences between the groups were compared with the Gray's test. For risk factor analysis, a Cox proportional hazards model was constructed. In multivariate analysis, variables with p values of <0.10 determined by univariate analysis were included in the final model. A hazard ratio (HR) was calculated in conjunction with a 95% confidence interval (CI).

Results and Discussion

A total of 80 patients were recruited between September 2002 and January 2005. The median age was 48 years (range 15-63), with 49 males and 31 females. CR was achieved by 77 (96.2%) patients. During a median follow-up of 26.7 months (maximum 52.5 months), 28 patients relapsed. Of the 17 relapses observed during the consolidation therapy, 13 occurred during the imatinib course. The probabilities of EFS and OS were $48.5 \pm 5.7\%$ and $58.1 \pm 5.7\%$ at 2 years (Figure 1). For patients who had achieved CR, the probability of RFS was $50.5 \pm 5.9\%$ at 2 years. Allogeneic HSCT was performed for 60 patients, including 24 from a sibling donor, 1 from a related donor other than a sibling, 25 from an unrelated donor, and 10 from unrelated cord blood. Disease status at the time of transplantation was first CR for 44 patients, second CR for 4 and non-CR for 12. The 2-year RFS for those who had undergone allogeneic HSCT during first CR was $62.6 \pm 7.5\%$ and $62.1 \pm 12.3\%$ for those who had not undergone allogeneic HSCT. When allogeneic HSCT was considered as a time-dependent covariate, it was shown to have no significant effect on RFS (HR, 1.03; 95% CI, 0.51-2.09; $p=0.934$). Major and minor BCR-ABLs were detected in 23 and 56 patients respectively. The transcript type of the remaining patient could not be determined because fluorescent *in situ* hybridization analysis was used instead of the PCR test. Neither transcript types nor copy numbers at diagnosis were associated with RFS ($p=0.763$ and 0.912). Pre-treatment cytogenetic results were not available for 4 patients because analysis was not performed ($n=2$) or was not successful ($n=2$). Of the remaining 76 patients, 22 showed only $t(9;22)$ or variant translocations, 51 showed additional chromosome aberrations, and 3 showed normal karyotype. Additional aberrations exceeding a frequency of 10% comprised $+der(22)t(9;22)$ in 17 patients, abnormalities involving the short arm of chromosome 9 [$abn(9p)$] in 17, monosomy 7 in 10, and trisomy 8 in 10. Figure 2 compares RFS for patients with and without additional chromosome aberrations. The presence of additional aberrations was significantly associated with shorter RFS ($p=0.003$). The relapse rate was also higher in patients with additional aberrations (41% vs. 20% at 2 years, $p=0.0414$). Analyses of the 4 recurrent abnormalities mentioned above demonstrated a statistically significant negative impact on RFS for $+der(22)t(9;22)$ and $abn(9p)$ ($p<0.001$ and $p=0.005$). Even after allogeneic HSCT, patients with additional aberrations appeared to have a trend for shorter RFS than those without ($p=0.080$), but this might reflect a larger proportion of transplantation beyond first CR in the former (31% vs. 17%). In patients allografted during first CR, there was no difference in cumulative incidences of relapse dated from the day of transplantation between the 2 groups

(16.5% vs. 12.5% at 2 years, $p=0.546$). Variables that showed a significant effect on RFS in the univariate Cox model included additional chromosome aberrations ($p=0.005$), peripheral blood blasts % ($p=0.024$) and sex ($p=0.03$). Results of multivariate analysis are shown in Table 1. The presence of additional chromosome aberrations was identified as the only independent prognostic factor for RFS ($p=0.027$). These updated data strongly support recent reports showing the feasibility and remarkable efficacy of imatinib-combined chemotherapy for newly diagnosed Ph⁺ ALL.^{3-9,14,15} The main objective of this report was to identify factors affecting RFS, an issue of rapidly increasing importance given the development of novel tyrosine kinase inhibitors which are expected to further expand the treatment options for this disease. Our data indicated that additional chromosome aberrations, particularly +der(22)t(9;22) and abn(9p), were associated with shorter RFS. It is well known that additional chromosome aberrations are seen frequently in Ph⁺ ALL. Before the imatinib era, some groups reported the prognostic relevance of additional aberrations.¹⁶⁻¹⁸ By contrast, from a large series of 204 patients, Moorman *et al.*¹⁹ recently showed no significant effect of specific additional aberrations, including +der(22)t(9;22) and del(9p), on survival. In this study, analyzing patients treated with imatinib-combined chemotherapy, the 2-year RFS rate exceeded 80% for those without additional aberrations, whereas outcomes for those with additional aberrations were relatively unfavorable.

Acquisition of resistance to imatinib is an emerging problem in the treatment of chronic myeloid leukemia. One of the most common mechanisms of resistance is the mutation involving the ABL kinase domain. Although it has not been confirmed whether such mutations compromise the clinical outcome of Ph⁺ ALL patients treated with imatinib-combined chemotherapy, our observation that most of the early relapses occurred during the consolidation courses consisting of imatinib alone implies possible imatinib resistance. If that is the case, switching from imatinib to other novel tyrosine kinase inhibitors based on the pre-treatment cytogenetic results soon after achieving CR or even ear-

lier could be an alternative treatment approach for further improving outcome in Ph⁺ ALL. Lack of mutation analysis is a major limitation of this study. Recently, Pfeifer *et al.*²⁰ studied the ABL kinase domain mutation status in newly diagnosed Ph⁺ ALL patients who were treated with imatinib-combined chemotherapy, and showed that even before exposure to imatinib, mutations were detected in 38% of patients. Importantly, the frequency of the mutant allele was low in such patients. However, at the time of relapse, the same mutation was present as the dominant clone in 90% of the relapsing cases.²⁰ Altogether, further insights will be provided by investigating the association between karyotype and mutation status at diagnosis.

Despite such limitations, the analysis of 80 patients entered into a single trial identified karyotype at diagnosis as a significant prognostic factor for RFS in newly diagnosed Ph⁺ ALL patients treated with imatinib-combined chemotherapy. Although our results need to be confirmed regarding kinase domain mutation status, these findings may play a critical role in the future treatment of Ph⁺ ALL.

Table 1. Multivariate analysis of factors associated with relapse-free survival.

P-value	HR (95% CI)*	Factors
Additional chromosome aberrations	0.027 2.84 (1.12-7.19)	Present Absent 1.00
Peripheral blood blasts%	0.051 1.12 (1.00-1.22)	Per 10% increase
Sex	0.148 1.73 (0.82-3.64)	Male Female

HR, hazard ratio; 95% CI, 95% confidence interval. *Values higher than unity indicate higher risk for failure.

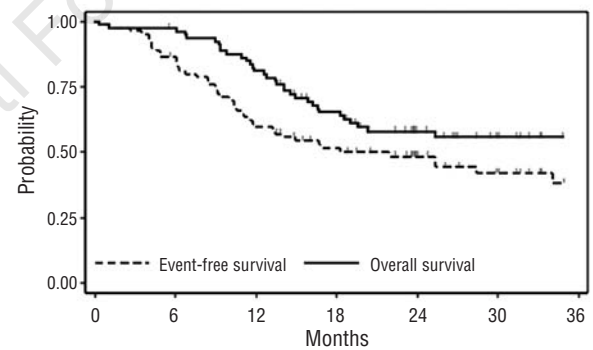


Figure 1. Kaplan-Meier curves for event-free and overall survival. The probabilities of event-free and overall survival at 2 years were 48.5% and 58.1% respectively (n=80).

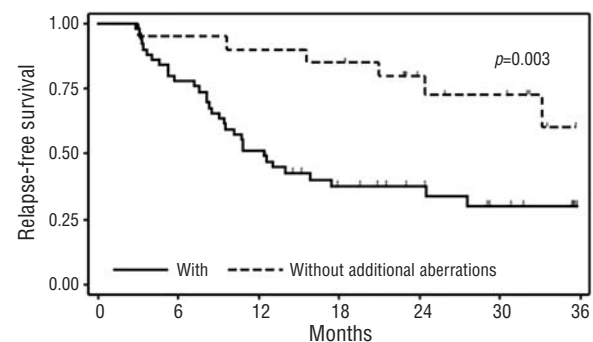


Figure 2. Relapse-free survival for patients with and without additional cytogenetic aberrations. Patients with additional cytogenetic aberrations (n=50) had significantly shorter relapse-free survival than those without (n=20).

Authorship and Disclosures

MY designed and co-ordinated the study, analyzed the data, and wrote the paper; JT, NU, FY, SM, and IJ designed the study, and provided patient sample and clinical data; IS, HA, KN, YU, MT, and AM provided patient sample and clinical data; HN co-ordinated the study, and revised the paper. YM provided patient sample and clinical data, and engaged in data manage-

ment. SO designed the study, provided patient sample and clinical data, and engaged in data management; KM designed the study, and analyzed the data; TN chaired the study group, co-ordinated the study, and revised the paper; RO served as the principal investigator, chaired the study group, and revised the paper. All authors reviewed the paper, interpreted the results, and approved the final version. The authors reported no potential conflicts of interest.

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