incidence of these adverse events between time-points was observed.

Discussion

Here, we report the long-term efficacy and tolerability profiles of nilotinib in 34 patients with imatinib-resistant or -intolerant Ph+ CML or relapsed/refractory Ph+ ALL. In comparison with the findings obtained at 12 months [22], there were few occurrences of new adverse events during the 36-month study.

In the phase I/II clinical trial of nilotinib [22], the drug was found to be generally safe and well-tolerated in patients with imatinib-resistant or -intolerant CML, and those with relapsed/refractory Ph+ ALL. The tolerability of nilotinib up to doses of 400 mg twice daily was confirmed in Japanese patients. The dose intensity of nilotinib increased with increasing dose within the investigated dose range, and the 400 mg twice-daily dose regimen gave the highest exposure.

In the present extension study, in CML-CP patients, CCyR was achieved in 13/16 patients (81.3%) and CCyR was achieved rapidly, within a median of approximately 3 months. Furthermore, MMR (defined as a BCR-ABL/BCR ratio $\leq$0.1%) was also achieved in 13/16 patients (81.3%). These results compare favorably with those reported after 24 months of nilotinib treatment in another study of imatinib-resistant or -intolerant CML-CP [20]. In that study, 44% (141/321) of patients achieved CCyR and 28% (82/294) of patients achieved MMR. Comparable rates of HR, CyR and MMR during nilotinib therapy in CML-CP were reported in other studies. In this analysis, 13/16 patients achieved MMR and, in some patients, the BCR-ABL transcript level was undetectable by quantitative RT-PCR.

One CML-AP patient who responded well to nilotinib and achieved CCyR was treated with nilotinib for 3 years. This suggests that nilotinib has long-term benefits for the treatment of some patients with CML-AP. The findings in Ph+ ALL and CML-BC patients in this study are similar to those reported in other studies [26]. Although the sample size is small, the results obtained in 4 CML-BC patients and 7 Ph+ ALL patients suggest that, in some patients, nilotinib may be an effective drug for the treatment of imatinib-resistant or -intolerant CML-BC and Ph+ ALL. Further studies are needed in patients with advanced CML to verify these results. All 5 Ph+ ALL patients without MRD in this study were previously treated with imatinib, and only 1 patient (20.0%) achieved HR. The other 4 patients ultimately discontinued treatment because of disease progression. In contrast, both Ph+ ALL patients with MRD achieved HR. The small sample size in this study meant that patients with imatinib-resistant or -intolerant disease were considered together, not separately.

As reported previously [28], imatinib resistance or intolerance, or the presence of baseline BCR-ABL mutations associated with imatinib resistance, did not affect the response to nilotinib. We detected 5 new mutations in 7 patients after starting nilotinib treatment. T315I, which is the mutation associated with the most resistance to currently available TKIs, was detected in 3 patients (8.8%) with CML-BC or Ph+ ALL; these patients discontinued treatment because of disease progression. Three of the 4 patients who developed other mutations also discontinued treatment, and the remaining patient, who had an E255K mutation, achieved MMR. These findings are consistent with previous studies suggesting that patients with the T315I mutation have a poor response to nilotinib [12, 19].

Two types of amino acid substitution at F359, F359V and F359I, were detected in this study. A CML-CP patient with baseline M244V mutation later harbored an F359V mutation (detected on Day 174) and showed poor response to nilotinib treatment; this patient experienced disease progression, as seen in other patients with the F359V mutation described in previous reports [29]. On the other hand, another patient who had F359I mutation at baseline achieved MMR. A previous study [30] showed that the F359I mutation is moderately sensitive to nilotinib (IC_{90} value = 433 nM). Nevertheless, in the present study, nilotinib treatment was effective, and sustainable MMR was observed in the patient with F359I mutation at baseline.

A recent study also described that CML patients with baseline mutations on imatinib treatment were more likely to relapse because of the development of other mutations after receiving dasatinib or nilotinib as second-line treatment [31]. Although the sample size of our study was small, only one CML-CP patient with a BCR-ABL mutation showed disease progression while the others completed study treatment. The effects of BCR-ABL mutation on the efficacy of treatment may differ depending on not only the type of mutation, but also the disease type and stage.

Adverse events of any grade occurred in all of the patients, regardless of drug relationship, and adverse events of grade 3/4 occurred in 29/34 patients (85.3%). The most common hematologic or non-hematologic adverse events included rash, nasopharyngitis, nausea, headache, vomiting, leukopenia, neutropenia and thrombocytopenia. Hematologic adverse events were commonly of grade 3/4 severity, similar to previously reported findings [19–21, 25, 26, 28]. Abnormal biochemical findings included hyperbilirubinemia, hyperglycemia and increased lipase. The rates of abnormal hematologic/blood biochemical findings were similar to those reported in a 12-month study [22] and in a global phase II study [19–21]. Most of these events

were not serious. The majority of adverse events did not require treatment discontinuation, interruption or dose reduction. Taken together, these findings are comparable with those reported in global phase I and II clinical studies [19–21, 25, 26] and a retrospective multicenter analysis [28]. During the 36-month observation period, only one patient with CML-BC died. Death resulted from heart failure due to cardiac tamponade and pericardial effusion occurring after discontinuation of nilotinib treatment.

Hematological and cytogenetic effects of nilotinib have been already observed in studies of up to 12 months [22] or 24 months in duration [20]. We have extended these findings in Japanese patients with imatinib-resistant or -intolerant Ph+ CML (CP, AP, or BC) or relapsed/refractory Ph+ ALL treated with nilotinib 400 mg twice daily for up to 36 months in this study. Importantly, nilotinib was shown to be effective as a second-line treatment for patients who failed to respond to previous imatinib treatment and who were considered to have a poor prognosis, with many patients achieving HR and CyR, which were maintained until last observation. No safety concerns arose over 36 months of treatment that were not apparent during the first 12 months of treatment. Most adverse events resolved following nilotinib dose interruption, dose reduction or supportive care.

The median daily dose of nilotinib (750.7 mg; range 284.9–798.6 mg) was below the prescribed dose (800 mg), mainly as a result of dose reductions in response to adverse events. In a previous study of nilotinib in Japanese newly diagnosed CML patients [24], the median dose was 730 mg (range, 644–794 mg) in the group administered nilotinib 400 mg twice daily; this dose was not considered particularly low, providing dose intensities similar to those in the overall population. The dose reduction in that study [24] was similar to that in ours.

Nilotinib was approved in Japan for the treatment of patients with CML-CP or CML-AP, but not patients with CML-BC or Ph+ ALL. The results of this study update provide further evidence supporting the use of nilotinib in Japanese patients with CML-CP or CML-AP. Our results also suggest that nilotinib may be useful for the treatment of patients with CML-BC or Ph+ ALL. Indeed, efficacy was observed in some CML-BC and Ph+ ALL patients; however, it remains to elucidate for which patient populations this drug would be most suitable in CML-BC and Ph+ ALL.

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Brief Communication

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Rituximab-Induced Interstitial Pneumonia due to CD8-Positive T Cell Infiltration

Kazuya Sato[a] Tadashi Nagai[a] Tohru Izumi[c] Ken Ohmine[a] Katsutoshi Ozaki[a]
Kazuo Muroi[b] Keiya Ozawa[a]

Divisions of [a]Hematology and [b]Cell Transplantation and Transfusion, Jichi Medical University, and
[c]Division of Hematology, Tochigi Cancer Center, Tochigi, Japan

Rituximab, a mouse/human chimeric anti-CD20 monoclonal antibody, has shown remarkable clinical benefit in CD20-positive (CD20+) B cell lymphoma. However, the possibility of immune system disturbance due to rituximab-mediated depletion of CD20+ B cells has been discussed [1]. Indeed, proliferation of large T cell granular lymphocytes and development of peripheral T cell lymphoma during rituximab therapy have been reported [2, 3]. However, the association between rituximab-mediated depletion of CD20+ B cells and T cell dysregulation remains obscure.

We present a case of interstitial pneumonia (IP) due to CD8-positive (CD8+) T cell infiltration after administration of rituximab in a patient with follicular lymphoma. A 54-year-old male with systemic lymphadenopathy was diagnosed with stage IV follicular lymphoma (grade 2) by neck lymph node excisional biopsy. At the time of the first staging, computed tomography (CT) of the lungs revealed no pulmonary disorders. The patient underwent six courses of chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine and prednisolone. Unfortunately, the patient did not achieve complete remission and his condition rapidly deteriorated. Although the patient was given salvage chemotherapy consisting of dexamethasone, cytarabine and cisplatin, the abdominal mass remained. The patient was given four courses of treatment with rituximab at a dose of 375 mg/m^2 per month. The abdominal mass became smaller, and no adverse effects were observed during rituximab treatment. However, 3 months after the fourth administration of rituximab, the patient developed a nonproductive cough and progressive dyspnea. On admission, the patient was afebrile, and the results of almost all of the blood tests were within normal ranges. There was no evidence of progression of lymphoma. However, CT of the lungs demonstrated a diffuse bilateral infiltration shadow, suggesting IP complication (fig. 1a). *Pneumocystis carinii* was not detected in the patient's sputum, and bacterial and fungal cultures were also negative. His respiratory symptoms persisted for more than 2 weeks, and bronchoscopy was performed by bronchoalveolar lavage (BAL). The population of lymphocytes in BAL fluid was markedly increased to 87.2%, whereas almost all of the other cells were macrophages (atypical lymphocytes not observed). These lymphocytes showed normal morphology, and no enlarged cells with viral inclusion bodies were observed. Flow cytometry showed that the cells were positive for CD7, CD2, CD3, CD8 and human lymphocyte antigen and negative for CD4. All of the examined B cell and natural killer cell markers were negative. In addition, Southern blot analysis revealed that the cells had rearrangement of the T cell receptor gene Cβ1 (fig. 2), whereas rearrangement of immunoglobulin heavy chain was not found (data not shown). These results excluded the pos-

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Tadashi Nagai
3311-1 Yakushiji, Shimotsuke
Tochigi 329-0498 (Japan)
Tel. +81 285 58 7353, E-Mail t-nagai@jichi.ac.jp

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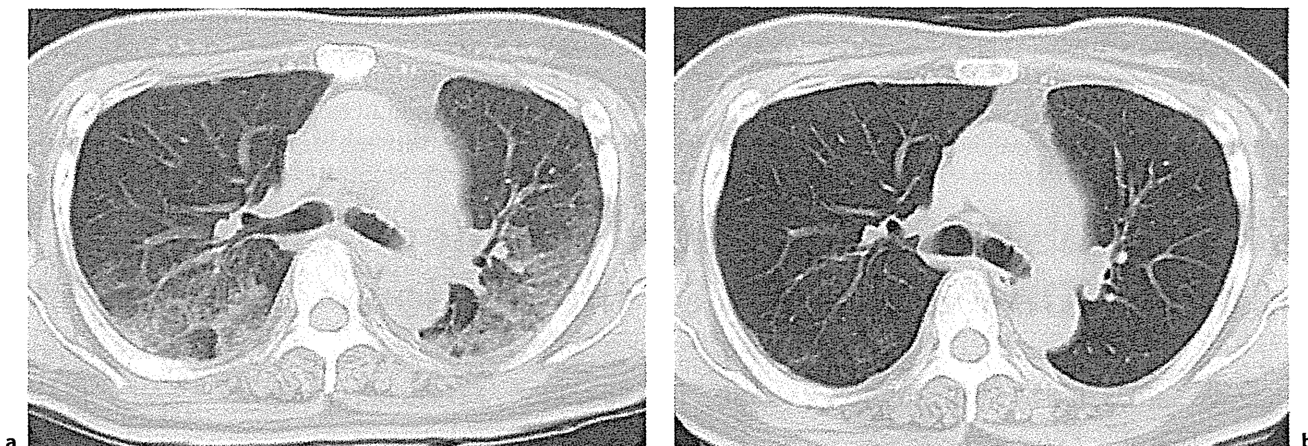

Fig. 1. CT shows bilateral alveolar and interstitial infiltration 3 weeks after completion of rituximab therapy (**a**) and complete disappearance without any additional therapy (**b**).

sibility of relapse of B cell lymphoma in the lung and suggested that IP developed after rituximab treatment due to infiltration of lymphocytes with a CD8+ T cell character. Since the severity of cough and dyspnea was reduced at that time, we did not use steroids for treatment of IP. Without any medications, the patient's condition gradually improved over a period of a few weeks after BAL, and CT of the lung showed complete disappearance of IP (fig. 1b). The patient achieved partial response with rituximab treatment, but the abdominal mass remained. Therefore, three courses of chemotherapy consisting of rituximab and fludarabine were given as an additional salvage therapy. Although the patient did not achieve complete remission with these therapies, no recurrence of IP has been observed. The reason why IP has not recurred despite repetition of rituximab treatment remains unknown. However, it is possible that fludarabine had a suppressive effect on IP development.

Causes of lymphocytic IP include infections, collagen diseases, radiation and drugs. Lymphocyte proliferation with CD8+ T cell predominance in BAL fluid is seen in drug-induced IP. However, rituximab-induced IP has several distinct features [4, 5]. Firstly, rituximab-induced IP sometimes occurs at and after the second rituximab infusion [4], whereas the onset of IP induced by other drugs is usually at the initial administration. Secondly, most patients with rituximab-induced IP are older than 55 years [5]. Thirdly, laboratory tests in rituximab-induced IP are generally nonspecific, except in severe cases. Therefore, it is likely that the mechanism of rituximab-induced IP is different from that of typical drug-induced

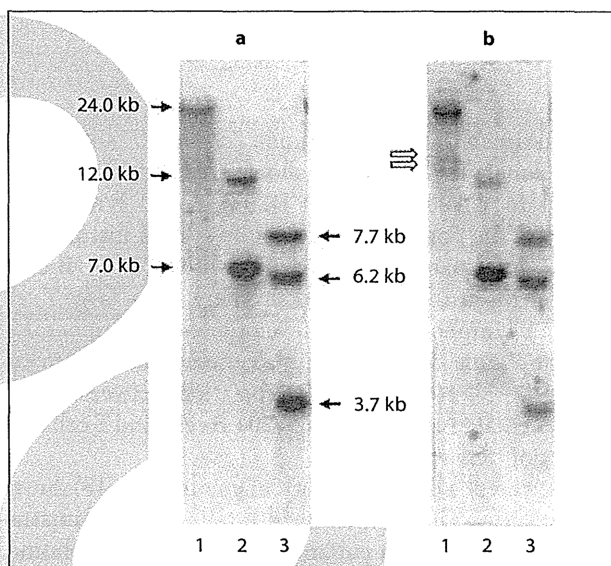

Fig. 2. Genomic Southern blot analysis shows rearrangement of the T cell receptor Cβ1 gene. High-molecular-weight DNA was digested with *Bam*HI (lane 1), *Eco*RV (lane 2) or *Hin*dIII (lane 3). Two additional bands (open arrows) were obtained after digestion with *Bam*HI. **a** Germ-line control. **b** Lymphocytes in BAL fluid.

IP. Furthermore, our patient had no other factors that could cause IP, and thus we concluded that rituximab was the cause of the development of IP. Hypersensitivity pneumonitis is also characterized by CD8+ T cell proliferation in the lung. However, clinical symptoms of hyper-

sensitivity pneumonitis such as fever and cough usually appear soon after exposure to specific antigens [6]. Therefore, we speculated that IP in this case was caused by CD20+ B cell depletion rather than hypersensitivity to rituximab, though the number of B cells in peripheral blood at the time of occurrence of IP was not examined.

There have been only a few reports on the underlying mechanism of clonal T cell promotion by CD20+ B cell depletion. An in vitro study showed that production of apoptotic lymphoma cells induced by rituximab treatment promoted phagocytosis by dendritic cells, resulting in cross-priming of CD8+ T cells [7]. Activation of dendritic cells may be one of the mechanisms that trigger monoclonal T cell proliferation. Furthermore, regulatory B cells, a specific and functionally important B cell subset, have been reported to be able to negatively regulate immune responses through IL-10 production. Since the regulatory B cell subset controls T cell expansion, differentiation and cytokine production [8, 9], rituximab may disturb T cell functions via abrogation of regulatory B cell function. Although rituximab-induced IP is rare, it is potentially fatal [1]. It is therefore important to elucidate the etiology and molecular mechanisms of this complication and to establish a definite diagnosis.

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Short Communication

Flow Cytometric Analysis of Kappa and Lambda Light Chain Expression in Endoscopic Biopsy Specimens before the Diagnosis of B-Cell Lymphoma

Satoko Oka,[1)] Kazuo Muroi,[2)] Kazuya Sato,[2)] Shin-ichiro Fujiwara,[2)] Iekuni Oh,[2)] Tomohiro Matsuyama,[2)] Ken Ohmine,[2)] Takahiro Suzuki,[2)] Katsutoshi Ozaki,[2)] Masaki Mori,[2)] Tadashi Nagai,[2)] Toshiaki Hanafusa,[1)] Noriyoshi Fukushima,[3)] Akira Tanaka,[3)] and Keiya Ozawa[2)]

Forty-eight patients with gastrointestinal (GI) tract B-cell lymphoma (BCL) were analyzed retrospectively. The diagnosis was based on the histological examination of specimens obtained by endoscopic biopsy. Before the diagnosis was made, single-color flow cytometry was performed to analyze the expression of light chains and B-cell antigens including CD10 in the specimens. Restricted light chain (RLC) expression, a marker of B-cell clonality, was defined as κ and λ ratios of either more than 3.0 or less than 0.5. The specimens from 30 patients (62.5%) showed RLC expression. No RLC expression or RLC expression not examined was divided into two groups : those showing CD10 positivity in more than 20% of cells (4 patients, 8.3%) and those showing no positivity (14 patients, 29.2%). The cell number analyzed in the latter group was significantly smaller than that in the other two groups. Abnormal karyotypes were found in the specimens from 8 patients (16.7%). These results indicate that the flow cytometric analysis of endoscopic biopsy specimens is useful when BCL is suspected if an adequate number of cells are obtained. 〔*J Clin Exp Hematopathol 52(2) : 127-131, 2012*〕

Keywords: flow cytometry, B-cell lymphoma, gastrointestinal tract, endoscopy

INTRODUCTION

Flow cytometry (FCM) is widely used for immunophenotyping of leukemia, lymphoma, myeloma, and myelodysplastic syndrome. FCM can determine B-cell clonality by analyzing κ and λ ratios in a B-cell population.[1,2] Usually, the samples for flow cytometric analysis are from peripheral blood and bone marrow ; however, fluid samples and fine needle aspiration biopsy samples can also be analyzed using FCM.[3] B-cell lymphoma (BCL) is diagnosed on the basis of histological examination including immunostaining in samples collected from nodal or extranodal lesions. The gastrointestinal (GI) tract is the most common site for extranodal BCL, which includes marginal zone B-cell lymphoma, diffuse large B-cell lymphoma (DLBCL), and other subsets of BCL.[4,5] If GI tract lesions are suspected, gastrointestinal endoscopy with biopsy is performed as routine practice in Japan.[6] Previously, we reported that FCM was useful for evaluating B-cell clonality in endoscopic biopsy specimens from 10 patients with GI tract BCL.[7] We extended our work to confirm these results in a large number of patients with GI tract BCL.

PATIENTS AND METHODS

Patients

Patients admitted to Jichi Medical University Hospital from January 1992 to August 2010 were retrospectively surveyed. Ten patients previously reported were included in this study.[7] Gastrointestinal endoscopy with biopsy was performed on patients complaining of GI tract symptoms at initial presentation. Biopsy specimens collected from each patient were used for both histological examination including immunostaining and FCM.[7] In cases where sufficient num-

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[1)] Department of Internal Medicine (I), Osaka Medical College, Takatsuki, Osaka, Japan
[2)] Division of Hematology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan
[3)] Department of Pathology, Jichi Medical University, Shimotsuke, Japan
Address correspondence and reprint requests to : Kazuo Muroi, M.D., Division of Hematology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan
E-mail : muroi-kz@jichi.ac.jp

bers of cells from the biopsy specimens could be obtained, chromosomal analysis was performed. Histological subtypes were defined according to the World Health Organization's Classification.[8]

Flow cytometry

Single-color FCM was performed as reported previously.[7] Specimens were dissected and suspended in phosphate-buffered saline containing 1% bovine serum albumin to obtain single-cell suspensions. Then, cells were stained with a panel of fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies : CD10, CD19, CD20, CD2, CD3, CD4, CD5, CD7, CD8, CD25, TCR$\alpha\beta$, TCR$\gamma\delta$, CD33, CD34, CD117, CD56, HLA-DR, κ light chain, and λ light chain. For the negative controls, cells were stained with isotype-matched control antibodies. Stained cells were analyzed using a flow cytometer (FACSCalibur, Becton Dickinson Biosciences, San Jose, CA). Flow cytometric data were evaluated by two independent FCM specialists before the diagnosis of BCL was made. B-cell clonality was determined by the quantification of the κ and λ light chain expression in a gated cell population. B-cell clonality was defined as κ and λ ratios of either more than 3.0 or less than 0.5.[1,2]

Statistical analysis

A Kruskal-Wallis test was used to investigate the mean differences between three dependent populations. Values of $P < 0.05$ were considered significant. Calculations were performed with the JMP version 5 program Stat View 512 (Berkley Software ; Berkley, CA).

RESULTS AND DISCUSSION

Forty-eight patients were diagnosed as having GI tract BCL. Twenty-seven were males and 21 were females, and the median age was 65.7 (range, 33-88). Endoscopic biopsy specimens were obtained from the stomach (18 patients), ileum (19), and colon (11). The histological subtypes were as follows : 31 patients, DLBCL ; 6, follicular lymphoma (FL) ; 4, lymphoma involving mucosa-associated lymphoid tissue ; 1, mantle cell lymphoma ; 1, Burkitt lymphoma ; and 5, unclassified BCL. Patients were divided into three groups according to FCM (Table 1) : patients with κ and λ ratios outside the normal range in endoscopic biopsy specimens were designated as FCM-BCL. When κ and λ ratios were within the normal range or not examined, but CD10 positivity was more than 20% in the specimens, patients were designated as FCM-probable BCL (FCM-pBCL). Patients having other flow cytometric patterns including insufficient analysis were designated as FCM-ND. Table 1 shows the characteristics of the patients with GI tract BCL. There were no differences in age, sex, specimen site, histological diagnosis, and chromosomal analysis among the three groups. The most common BCL was DLBCL in the three groups. Analyzed cell number was significantly different among the groups ; the FCM-ND group had the lowest cell number (Table 1, Fig. 1). In the FCM-BCL group, 19 patients showed κ light chain restriction, while 11 showed λ light chain restriction (Table 1, Fig. 2). Both FCM-BCL and FCM-pBCL groups showed high positivity for CD19, CD10, and CD20, while the FCM-ND group showed low positivity for the antigens. Specimens from one patient in the FCM-pBCL group and 6 patients in the FCM-ND group did not show RLC expression. In the FCM-pBCL group, CD10 positivity was confirmed in specimens from two patients by immunostaining : one sample was $CD10^+$, $CD20^+$, $BCL\text{-}2^+$, $BCL\text{-}6^+$, and $CD5^-$ and the other was $CD10^+$, $CD20^+$, $CD23^-$, $Bcl\text{-}2^+$, and $CD5^-$. These patients were diagnosed as having FL. The remaining specimens showed CD10 negativity by immunostaining : one was $CD10^-$, $CD20^+$, $CD23^-$, and $CD5^-$ and the other was $CD10^-$, $CD20^+$, $CD23^-$, and $CD5^-$. These patients were diagnosed as having DLBCL. The evaluation of light chain expression by immunostaining was not performed in all three groups. The highest cell number in the FCM-ND group (5,000) was from a specimen from a patient with DLBCL. In this specimen, CD19 positivity, CD20 positivity, and κ/λ ratio were 32%, 39%, and 2.1, respectively. Chromosomal analysis could be performed in the specimens from 21 patients. Of these 21 patients, only specimens from 8 patients designated as having FCM-BCL showed abnormal karyotypes.

RLC expression in B-cells has been used as a traditional marker of BCL evaluated by FCM.[1-3] In our study, specimens from 7 patients did not show RLC expression. Several reasons for the negative results can be considered. The first is the single-color FCM used in this study. It is difficult to isolate neoplastic B-cells from residual T-cells and monocytes by single-color FCM. The second is the distribution of neoplastic B-cells in the specimens. If samples do not contain abundant neoplastic B-cells, FCM cannot detect RLC expression. This is the case of the highest cell number in the FCM-ND group. No RLC expression in this specimen may have been due to an insufficient number of DLBCL cells in it. The third is the sensitivity of the antibodies that reacted to the light chains used in this study. Recently, Horna *et al.* reported that it is important to evaluate κ and λ light chain expression with both monoclonal and polyclonal antibodies.[9] Patients with BCL sometimes show a lack of surface immunoglobulin light chain expression.[10,11] In our study, immunostaining to light chains in specimens was not performed. Therefore, it cannot be ruled out that BCL with a lack of surface immunoglobulin light chain expression was present in the FCM-ND group. In addition, RLC expression can be found in the germinal center B-cells in reactive follicular hyperplasia of lymph nodes.[12] We do not know whether RLC

Table 1. Characteristics of the patients with gastrointestinal tract B-cell lymphoma

	FCM-BCL	FCM-pBCL	FCM-ND	p
Patients (no.)	30	4	14	
Age (years)#	65 (33-88)	63 (49-77)	65 (50-83)	0.458
Sex (no. ; male/female)	17/13	1/4	9/5	0.987
Specimen				0.991
Stomach	13	1	4	
Ileum	12	1	6	
Colon	5	2	4	
Histological diagnosis (no.)				0.744
Diffuse large B-cell lymphoma	20	2	9	
Follicular lymphoma	4	2	0	
MALT lymphoma	1	0	3	
Mantle cell lymphoma	1	0	0	
Burkitt lymphoma	1	0	0	
Unclassified	3	0	2	
Analyzed cell numbers#	4,500 (1,500-10,000)	3,500 (1,000-5,000)	1,000 (200-5,000)	< 0.001
Phenotypes of the cells (%)#				
$CD19^+$	77.9 (90.7 ± 4.6)	88.5 (90.1 ± 9.3)	23.4 (25.1 ± 8.3)	< 0.01
$CD10^+$	85.6 (94.0 ± 4.4)	47.0 (53.3 ± 15.5)	11.2 (4.2 ± 6.4)	< 0.05
$CD20^+$	74.2 (83.3 ± 4.3)	73.4 (74.8 ± 14.8)	14.7 (13.9 ± 2.9)	< 0.05
κ/λ ratios# (no.)				
$\kappa/\lambda > 3.0$	13.0 (23.4 ± 5.7)(19)	N/A	N/A	
$0.5 \leq \kappa/\lambda \leq 3.0$	N/A	1.3 (1)	1.3 (1.3 ± 0.1)(6)	
$\kappa/\lambda < 0.5$	0.03 (0.07 ± 0.02)(11)	N/A	N/A	
Chromosomal analysis (no.)				0.462
Normal karyotype	6	2	5	
Abnormal laryotype	8	0	0	
No metaphase	3	1	2	
Not examined	13	1	7	

no., number ; #, median ; N/A, not applicapable ; MALT lymphoma, lymphoma involving mucosa-associated lymphoid tissue.

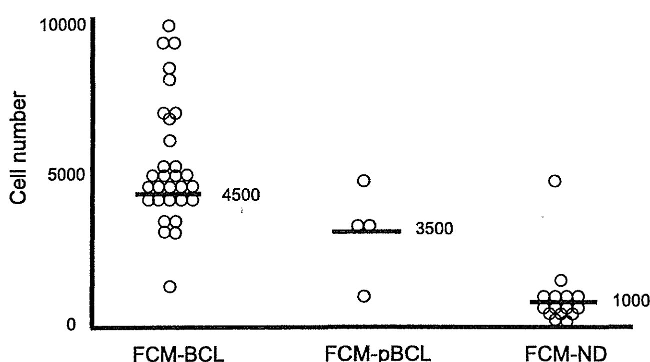

Fig. 1. Dot plots of analyzed cell number in each group. The bars indicate median cell number.

expression is found in B-cells in the reactive GI tract lesions.

CD10 is a proteolytic enzyme expressed on the surface of germinal center B-cells and lymphomas derived from these cells, that is, FL.[13,14] High-intensity CD10 expression is associated with FL and DLBLC can also express the antigen.[13,14] We used 20% as the cut-off value of CD10 positivity for identifying BCL because mature B-cells do not express CD10 and reactivity is generally defined as positive when more than 20% of cells are stained with a monoclonal antibody.[15] This cut-off value was adapted to only 4 patients designated as having FCM-pBCL. Of these patients, two specimens showed CD10 positivity by immunostaining, while the other two did not. The reason for the discrepancy in CD10 positivity between FCM and immunostaining is not yet understood. It may be due to the difference of sensitivity to detect antigen expression between the two methods. It is necessary to verify whether this cut-off value is appropriate to identify BCL.

Recently, endoscopic ultrasound-guided fine needle aspiration biopsy has been introduced and this technique combined with FCM has been used to diagnose BCL.[16,17] This approach is particularly important to diagnose deep-seated lymphoma, the lesions of which occur in the mediastinum, para-aorta, pancreas, spleen, and so on.[18] In Japan, GI tract lesions are usually examined by GI endoscopy with biopsy as routine practice.[6] To our knowledge, there are only a few reports of the flow cytometric analysis of endoscopic biopsy specimens for the diagnosis of BCL.[7,19,20] FCM is accurate and quantitative. Its data can be reanalyzed easily. FCM analysis provides significant information on B-cell clonality associated with BCL before a morphological assessment is completed.

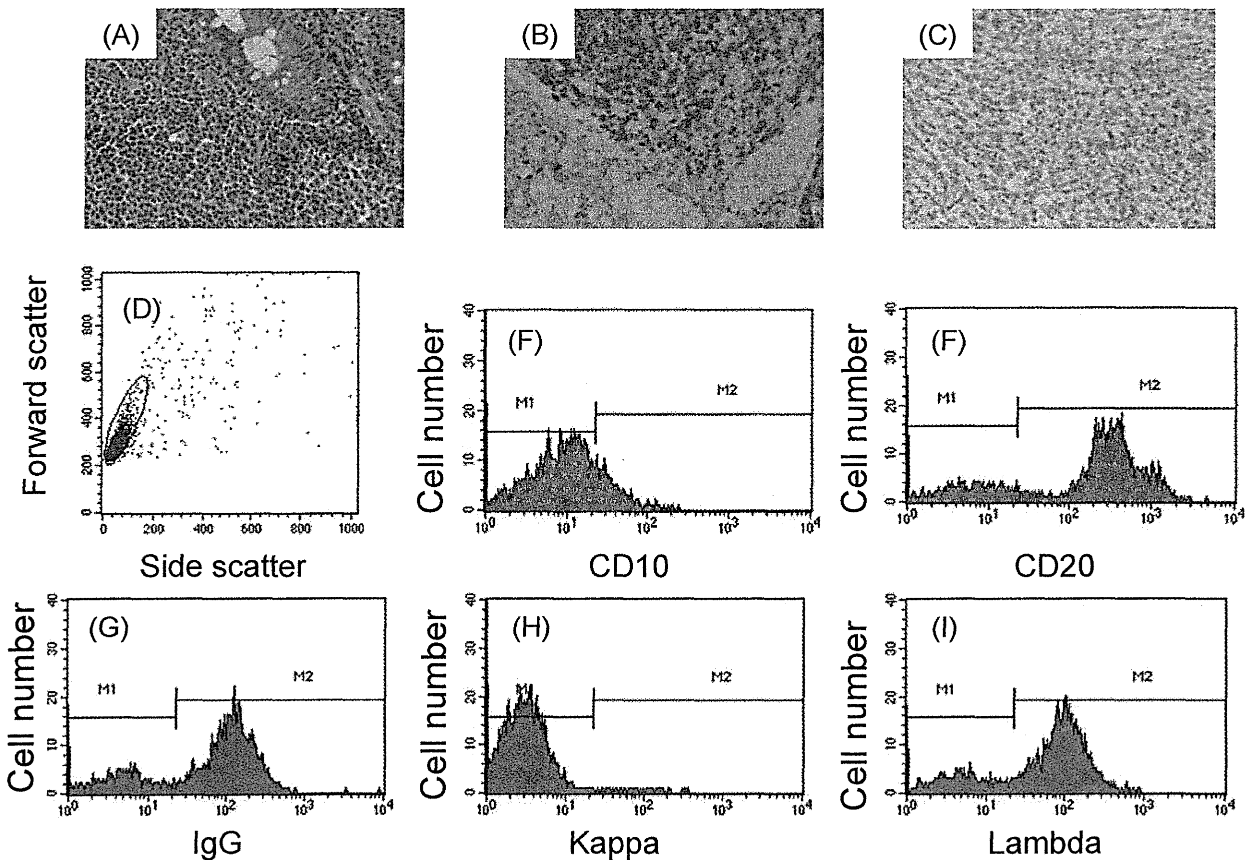

Fig. 2. Flow cytometric analysis of the biopsy specimen obtained from colon fiberscopy. (*2A*) Diffuse large B-cell lymphoma (DLBCL) in the colon mucosa (H&E stain, × 200). (***2B***) Positivity of the leukocyte common antigen staining in the DLBCL cells (× 200). (***2C***) Positivity of CD20 staining in the DLBCL cells (× 200). (***2D***) The gated region is indicated by a circle ; from *2E* to *2I*, DLBCL cells express CD20, IgG, and λ light chain, while the cells express CD10 at a low level.

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Short Communication

Prediction of Progression from Refractory Cytopenia with Unilineage Dysplasia by Analysis of Bone Marrow Blast Cell Composition

Satoko Oka,[1] Kazuo Muroi,[2] Shin-ichiro Fujiwara,[2] Iekuni Oh,[2] Tomohiro Matsuyama,[2] Ken Ohmine,[2] Takahiro Suzuki,[2] Katsutoshi Ozaki,[2] Masaki Mori,[2] Tadashi Nagai,[2] Keiya Ozawa,[2] and Toshiaki Hanafusa[1]

A retrospective analysis of 71 patients newly diagnosed with refractory cytopenia with unilineage dysplasia (RCUD) revealed that 12 developed refractory anemia with an excess of blasts or acute myeloblastic leukemia. Before the diagnosis of RCUD was made, phenotypes of cells in the bone marrow (BM) blast region were analyzed using flow cytometry. Patients with RCUD were divided into two groups ; those with no progression (Group A) and those with disease progression later on (Group B). The cell composition in the BM blast region differed significantly between the groups : Group A showed higher percentages of B lymphoid cells but lower percentages of myeloid cells. A cut-off value of 20 for the CD33/CD10 ratio in the BM blast region clearly separated Group A from Group B. These results suggest that cell composition in the BM blast region evaluated by flow cytometry may indicate the progression of RCUD. 〔*J Clin Exp Hematopathol 52(1) : 63-66, 2012*〕

Keywords: myelodysplastic syndrome, bone marrow, flow cytometry, blast

INTRODUCTION

Refractory anemia (RA) is a type of myelodysplastic syndrome (MDS), which was first defined in the French, American, and British classification.[1] In the revised (2008) World Health Organization (WHO) classification, RA was reclassified into refractory cytopenia with unilineage dysplasia (RCUD), which includes several cytopenias.[2] Gene expression arrays showed that the progression of MDS from RA to advanced phases is associated with the aberrant expression of genes associated with proliferation and differentiation.[3] In a clinical setting, simplified methods to predict the progression from RCUD are needed. We analyzed the cell composition in a blast cell region of the bone marrow (BM) in RCUD patients by employing flow cytometry (FCM).

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[1]Department of Internal Medicine (I), Osaka Medical College, Takatsuki, Osaka, Japan
[2]Division of Hematology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan
Address correspondence and reprint requests to : Kazuo Muroi, M.D., Division of Hematology, Department of Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan
E-mail : muroi-kz@jichi.ac.jp

PATIENTS AND METHODS

During the period from June 1996 to February 2008, 71 patients who were newly diagnosed with RA according to the WHO classification (2002) were reclassified according to the revised WHO classification (2008).[2] Three-color FCM combined with two-color FCM was performed to evaluate the phenotypes of cells in the BM blast region on initial presentation (Table 1).[4] Blasts are characterized by intermediate CD45 expression and low side scatter properties and most of the cells in the region are lymphoblasts and myeloblasts.[4] Stained cells were analyzed using a flow cytometer (FACSCalibur ; BD Biosciences, San Jose, CA, USA). Progression was defined as the appearance of blasts in the peripheral blood (PB) after the diagnosis of RCUD. Once blasts appeared in the PB, BM aspiration was performed to determine whether the disease progressed to advanced stages. Follow-up was conducted until determination as progression or a recent examination of PB in which blasts were not observed. The follow-up period ended on February 1, 2009. Therapy was started on the basis of doctors' decisions regarding their patients' conditions. *P*-values below 0.05 were considered significant using Student's *t*-test and the χ^2 test.

RESULTS AND DISCUSSION

Patients who did not show disease progression were categorized as Group A, while patients who showed disease progression were categorized as Group B. In Group A, 44, 11, and 4 patients were diagnosed as having RA, refractory neutropenia, and refractory thrombocytopenia, respectively. In Group B, 8, 2, and 2 patients were diagnosed as having RA, refractory neutropenia, and refractory thrombocytopenia, respectively. Of the 59 patients with RCUD in Group A, 6 patients had been incorporated in the previously reported study.[4] In Group B, disease progression was confirmed by BM aspiration as follows : 7 patients, RA with excess blasts-1 ; 4, RA with excess blasts-2 ; and 1, overt leukemia. The median follow-up periods in Groups A and B were 36 and 11 months, respectively. As shown in Table 2, there were no differences between the two groups in terms of age, sex, PB cell count, BM blast percentage, chromosomal risk factors, and International Prognostic Scoring System (IPSS) scores. By the end of the follow-up, about half of the patients in Group A had not received any medications including cyclosporine, steroid, anabolic steroid, antithymocyte globulin, or a combination thereof. In Group B, only 3 of 12 patients underwent watchful waiting, the other patients receiving the medications described above. In the flow cytometric analysis, Group A showed higher percentages of B lymphoid cells but lower percentages of myeloid cells in the BM blast region than Group B (Table 2, Fig. 1). The percentages of $CD34^+$ and $CD117^+$ cells in the BM blast region were significantly higher in Group B than in Group A ($p < 0.05$). There were no differences between the two groups in the percentages of $CD15^+$, $CD14^+$, $CD11b^+$, $CD11c^+$, $CD5^+$, $CD2^+$, $CD7^+$, $CD25^+$, $CD36^+$, $CD41^+$, and $HLA\text{-}DR^+$ cells in the region (data not shown). To enhance the balance of myeloid cells and B lymphoid cells in the region, ratios for the myeloid cell percentages to the B lymphoid cell percentages were calculated; significant differences in CD33/CD19, CD33/CD10, CD13/CD19, and CD13/CD10 ratios were noted between the

Table 1. Antibody combinations

PE	FITC	PerCP
Control	Control	CD45
CD34	CD7	CD45
CD13	CD10	CD45
CD33	CD19	CD45
CD117	CD5	CD45
CD56	CD2	CD45
	CD41	CD45
	CD11b	CD45
	CD11c	CD45
	CD14	CD45
	CD15	CD45
	CD20	CD45
	CD25	CD45
	CD36	CD45
	CD235a	CD45
	HLA-DR	CD45
	MPO	CD45
	TdT	CD45

PE, phycoerythrin ; FITC, fluorescein isothiocyanate ; PerCP, peridinin chlorophyll protein ; MPO, myeloperoxidase ; TdT, terminal deoxynucleotidyl transferase

Table 2. Characteristics of the patients

Clinical findings	Group A	Group B	p
Patients (no.)	59	12	
Age (years)#	65 (35-87)	64 (45-77)	0.685
Sex (no. ; male/female)	33/26	7/5	0.878
White blood cells ($\times 10^9$/L)#	3.0	3.2	0.793
Hemoglobin (g/dL)#	9.3	7.8	0.186
Platelets ($\times 10^9$/L)#	141	134	0.833
BM blasts (%)#	2.9	2.8	0.630
IPSS chromosome (no.)			0.608
Good	51	11	
Intermediate	6	0	
Poor	2	1	
IPSS score (no.)			0.387
Low	31	3	
Intermediate-1	28	9	
Intermediate-2	0	0	
High	0	0	
Phenotypes of the cells in the BMBCC (%)#			
$CD19^+$	27.7	7.3	< 0. 001
$CD10^+$	14.4	4.2	< 0. 01
$CD13^+$	51.2	68.4	< 0. 05
$CD33^+$	64.4	78.2	< 0. 01
$CD34^+$	40.6	60.9	< 0. 05
$CD117^+$	24.0	59.7	< 0. 001
Ratios of the cells in the BMBCC#			
CD33/CD19	3.2 (0.8-4.8)	23.1 (20.5-37.1)	< 0. 01
CD33/CD10	3.2 (0.9-5.8)	31.8 (27.8-42.6)	< 0. 001
CD13/CD19	3.0 (0.5-5.0)	21.9 (11.6-32.7)	< 0. 001
CD13/CD10	2.5 (0.6-6.3)	39.9 (19.2-50.8)	< 0. 001
Treatment (no.)			< 0. 05
No medications	35	3	
Medications	24	9	

Group A, no progression after the diagnosis of refracory anemia ; Group B, progression after that ; no., number ; #, mean ; BM, bone marrow ; IPSS, International Prognostic Scoring System ; BMBCC, bone marrow blast cell composition ; Medications include cyclosporine, steroid, anabolic steroid, antithymocyte globulin, or a combination thereof.

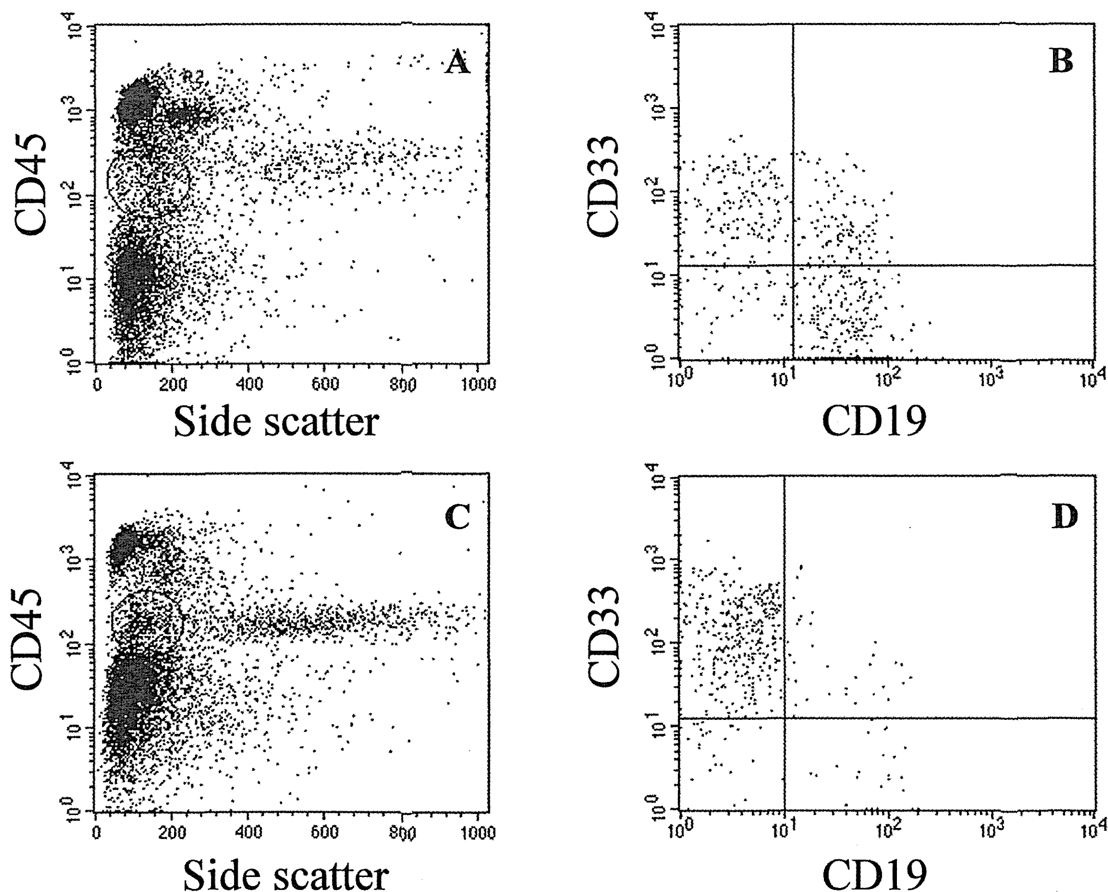

Fig. 1. Flow cytometric analysis of bone marrow cells. (*1A*) & (*1B*), a case in Group A ; (*1C*) & (*1D*), a case in Group B. The circle indicates the bone marrow (BM) blast gate region. The cell composition in the region is different between the two patients. Co-expression of CD34 and CD117 in the BM blasts was not measured.

two groups (Table 2, Fig. 2). Differences in the mean values of CD33/CD10 ratios between the two groups were large and variations of the ratios in Group B were small. A cut-off value of 20 for the CD33/CD10 ratio clearly separated Group A from Group B. At the end of October 2011, patients in Group A and Group B were evaluated by medical charts. In Group A, 22 patients were alive, 4 patients died due to ovarian cancer, cardiac sudden arrest, hepatoma or cholecystitis, and 33 patients were lost to follow-up. Only one patient underwent bone marrow transplantation and she was alive. In Group B, 3 patients were alive, 6 patients died due to hypoglycemia, pneumonia or sepsis, and 3 patients were lost to follow-up. All of the surviving patients underwent bone marrow transplantation.

Abnormalities in antigen expression in $CD34^+$ cells have been reported, such as the overexpression of CD33, CD117, and HLA-DR, asynchronous expression of CD11b, CD15, and CD16, and aberrant expression of CD7 and CD56.[5] Tavil *et al.* reported that an increase of the $CD34^+$ $CD117^+$ cells in BM blasts is associated with MDS progression.[6] Abnormal antigen expression has been detected in granulocytes, monocytes, and erythroblasts.[5,7,8] Recently, Xu *et al.* reported a flow cytometric scoring system for the diagnosis of MDS.[9] To identify abnormalities in antigen expression in MDS patients by FCM, it is necessary to have a clear understanding of antigen expression profiles along with normal hematopoietic stem cell differentiation, complicating the use of FCM for the diagnosis of MDS as a routine practice. It is not clear whether abnormal antigen expression profiles in MDS can be used to predict the progression of the disease. Previously, Sternberg *et al.* showed that B precursor cells in the BM of patients with MDS decrease in number.[10] This report suggests that B lymphoid cells in the BM blast cell region evaluated by FCM reflect disease progression from RCUD. Taking these findings together, CD33 and CD10 ratios in the BM blast region may be useful markers for predicting the

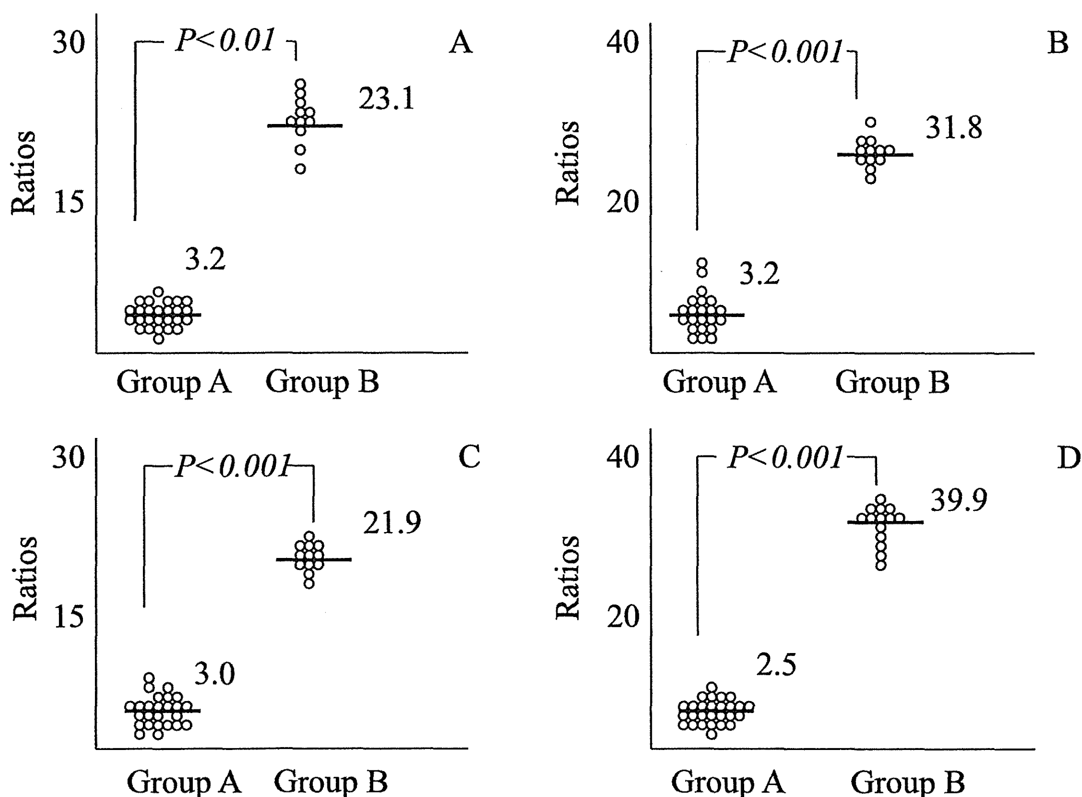

Fig. 2. Ratios of antigen expression in the blast region of bone marrow. (*2A*), CD33/CD19 ; (*2B*), CD33/CD10 ; (*2C*), CD13/CD10 ; (*2D*), CD13/CD19. The values are means.

progression of RCUD. To confirm our results, prospective studies are needed.

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Long-term outcome following imatinib therapy for chronic myelogenous leukemia, with assessment of dosage and blood levels: the JALSG CML202 study*

Kazunori Ohnishi,[1,17] Chiaki Nakaseko,[2] Jin Takeuchi,[3] Shin Fujisawa,[4] Tadashi Nagai,[5] Hirohito Yamazaki,[6] Tetsuzo Tauchi,[7] Kiyotoshi Imai,[8] Naoki Mori,[9] Fumiharu Yagasaki,[10] Yasuhiro Maeda,[11] Noriko Usui,[12] Yasushi Miyazaki,[13] Koichi Miyamura,[14] Hitoshi Kiyoi,[15] Shigeki Ohtake,[16] Tomoki Naoe[15] and for the Japan Adult Leukemia Study Group

[1]Oncology Center, Hamamatsu University School of Medicine, Hamamatsu; [2]Department of Hematology, Chiba University Hospital, Chiba; [3]Department of Hematology and Rheumatology, Nihon University School of Medicine, Tokyo; [4]Department of Hematology, Yokohama City University Medical Center, Yokohama; [5]Division of Hematology, Jichi Medical University Hospital, Shimotsuke; [6]Cellular Transplantation Biology, Kanazawa University Graduate School of Medical Science, Kanazawa; [7]Department of Hematology, Tokyo Medical University, Tokyo; [8]Department of Hematology, Institute for Artificial Organs, Transplantation & Gene Therapy, Sapporo Hokuyu Hospital, Sapporo; [9]Department of Hematology, Tokyo Women's Medical University School of Medicine, Tokyo; [10]Department of Hematology, International Medical Center, Saitama Medical University, Hidaka; [11]Department of Hematology, Kinki University Faculty of Medicine, Osakasayama; [12]Division of Oncology and Hematology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo; [13]Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki; [14]Hematology Division, Japanese Red Cross Nagoya Daiichi Hospital, Nagoya; [15]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya; [16]Department of Clinical Laboratory Science, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

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A prospective multicenter Phase II study was performed to examine the efficacy and safety of imatinib therapy in newly diagnosed Japanese patients with chronic-phase CML. Patients were scheduled to receive imatinib 400 mg daily. Plasma imatinib concentrations were measured by liquid chromatography–tandem mass spectrometry. In 481 evaluable patients, estimated 7-year overall survival (OS) and event-free survival (EFS) at a median follow-up of 65 months were 93% and 87%, respectively. Because imatinib dosage was reduced in many patients due mainly to adverse events, subgroup analysis was performed according to the mean daily dose during the first 24 months of treatment: ≥360 mg (400-mg group; *n* = 294), 270–359 mg (300-mg group; *n* = 90) and <270 mg (200-mg group; *n* = 67). There were no significant differences in OS and EFS between the 300- and 400-mg groups; however, cumulative rates of complete cytogenetic and major molecular responses differed significantly between the two groups. There were no significant differences in mean imatinib trough levels between these two groups for the patients in whom trough levels had been measured. Survival and efficacy in the 200-mg group were markedly inferior to the former two groups. These results suggest that, although a daily dose of 400 mg imatinib is associated with better outcomes, 300 mg imatinib may be adequate for a considerable number of Japanese patients who are intolerant to 400 mg imatinib. Blood level monitoring would be useful to determine the optimal dose of imatinib. (*Cancer Sci* 2012; 103: 1071–1078)

Imatinib mesylate, a selective BCR-ABL1 kinase inhibitor, has demonstrated remarkable long-term efficacy in the treatment of chronic-phase (CP) CML[1] and now is the standard therapy for this disease.[2] An 8-year follow-up during the International Randomized Study of Interferon and STI571 (IRIS) on newly diagnosed CP CML demonstrated that continuous imatinib therapy exhibited superior efficacy and improved survival.[3] In Japan, imatinib was approved for the treatment of CML in 2001, and a multicenter prospective Phase II study of imatinib therapy (CML202 study) for newly diagnosed CP CML was immediately initiated by the Japan Adult Leukemia Study Group (JALSG). Herein, we report on the results of this study after a median follow-up period of 65 months.

In the present study, although the daily dose of imatinib was set at 400 mg, because of adverse events in many patients the dosage was reduced to less than 400 mg. Nevertheless, the overall efficacy and outcomes were excellent compared with that reported in other studies.[1,4,5] The relatively smaller body size of Japanese patients may explain why a daily dose of < 400 mg imatinib was adequate in some patients.[6] To confirm this assumption, we measured plasma trough levels of imatinib in patients receiving 400 or 300 mg imatinib daily and evaluated the association between plasma concentrations of imatinib and the efficacy, as well as long-term outcome, in these patients.

Materials and Methods

Study design and treatment. The present study was a prospective multicenter Phase II study on previously untreated, newly diagnosed patients with CP CML, with patients receiving a daily dose of 400 mg imatinib. The primary endpoint was overall survival (OS). Secondary endpoints included the rate of a complete hematologic response (CHR), the rate of a cytogenetic response, progression-free survival (PFS), event-free survival (EFS), and safety. The study was registered with the UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctr/index/htm, accessed 10 Sep 2005; registration no. C000000153, the JALSG CML202 study).

Patients. Patients were eligible for inclusion in the study if they were 15 years or older, had de novo Philadelphia (Ph)-chromosome positive CP CML and had not received interferon-α treatment for CML. Further eligibility criteria were adequate liver function (serum bilirubin level ≤2.0 mg/dL and serum liver aminotransferase less than threefold the upper limit of normal), kidney function (serum creatinine ≤2.0 mg/dL), heart and lung function, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–3, and no prior

[17]To whom correspondence should be addressed.
E-mail: kohnishi@hama-med.ac.jp
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or concurrent malignancy. Written informed consent was obtained from all patients prior to registration. The study protocol was reviewed and approved by the institutional review board of all the participating centers and the study was conducted in accordance with the Declaration of Helsinki.

Dose modification of imatinib. Patients were scheduled to receive imatinib at an oral daily dose of 400 mg. Lower dose of < 400 mg daily were permitted at the start of imatinib therapy in patients who were old and/or had a small body size, but it was planned to increase the dose of imatinib to 400 mg within the first month if patients tolerated the reduced dose. Dose escalation to 600 mg was implemented if patients failed to achieve a complete hematologic response (CHR) at 3 months or a major cytogenetic response at 6 months in the absence of dose-limiting adverse events. If patients did not exhibit a CHR at 6 months, they were switched to alternative therapy. If patients achieved a major cytogenetic response within 9 months, imatinib at 400 mg or the adjusted dose was maintained until disease progression.

If Grade 2 non-hematologic toxicities occurred and did not resolve spontaneously, imatinib was interrupted until the toxicities had been ameliorated to Grade 1 or less, and then resumed at the preceding dose. If Grade 3 or 4 non-hematologic or hematologic toxicities occurred, imatinib was interrupted until the toxicities had been ameliorated to Grade 1 or less, and then resumed at a reduced daily dose of 300 mg. Imatinib therapy was discontinued in the event of failure to achieve a CHR at 6 months, intolerance to imatinib, or disease progression to an accelerated phase (AP) or blast crisis (BC).

Definitions. The phases of CML (i.e. CP, AP, or BC) were defined as described previously in the IRIS study.[7] A CHR was defined as a reduction in the leukocyte count to $<10 \times 10^9$/L and a reduction in the platelet count to $<450 \times 10^9$/L that persisted for at least 4 weeks. Cytogenetic responses were evaluated by G-banding of at least 20 marrow cells in metaphase and were categorized as complete (CCyR; no cells positive for the Ph chromosome) and partial (PCyR; 1–35% of cells positive for the Ph chromosome). A major cytogenetic response (MCyR) was defined as complete or partial responses.[2] A major molecular response (MMR) was defined as a 3-log reduction or more in *BCR-ABL1* transcripts compared with median baseline levels, as measured by reverse-transcription real-time quantitative polymerase chain reaction (RQ-PCR)[8,9] or the transcription-mediated amplification and hybridization protection assay (TMA-HPA)[10,11] (For details, refer to Fig. S1 and Data S1, which are available as online Supplementary Material for this paper).

Event-free survival was defined as the time between registration and the earliest occurrence of any of the following events: death due to any cause, progression to AP or BC, and/or loss of MCyR or CHR. Progression-free survival was defined as the time between registration and the earliest occurrence of any of the following events: death due to any cause or progression to AP or BC. Overall survival was defined as the time between the date of registration and death due to any cause. Hematopoietic stem cell transplantation (HSCT) was not censored. Adverse events were assessed according to the National Cancer Institute–Common Toxicity Criteria version 2.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm, accessed 15 Mar 2012). The mean daily dose of imatinib in a designated period was defined as the total of the doses administered divided by the total number of days on which it was administered.

Measurement of trough plasma levels of imatinib. Blood samples were obtained within 24 ± 2 h after the last imatinib administration from patients who had been receiving 300 or 400 mg imatinib daily without any dose modification for at least 2 years. Plasma was immediately separated at 4°C and at 5000*g* for 10 min by centrifugation and stored at −80°C until measurement. Plasma imatinib concentrations were measured at the Toray Research Center (Tokyo, Japan), as reported previously.[12] Briefly, sample extracts were analyzed using reverse-phase chromatography with a Waters Symmetry column (Waters, Milford, MA, USA), followed by detection with a Sciex API 3000 mass spectrometer (PE Biosystems, Foster City, CA, USA). The lower limit of quantification was 4 ng/mL imatinib mesylate and the assay was fully validated. The precision from validation ranged from 99 ± 5% to 108 ± 5% over the concentration range 4–10 000 ng/mL.[13] The internal standard, imatinib mesylate, was provided by Novartis Pharma (Basel, Switzerland) and the assay system was approved by Novartis Pharma.

Statistical analysis. The Kaplan–Meier method and 95% confidential intervals (CI) were used to analyze OS, PFS, and EFS. Differences between subgroups of patients were evaluated using the log-rank test. Cumulative rates of CHR and cytogenetic responses were estimated according to the competing risk method, in which discontinuation of imatinib was evaluated as competing risk. Comparisons of baseline characteristics in the subgroups were made using the chi square test or Fisher's exact test for categorical variables, and with the Mann–Whitney *U*-test for continuous variables. All statistical analyses were performed using JMP software (SAS Institute, Cary, NC, USA) and R software (http://www.r-project.org, accessed 15 Feb 2011). Two-sided $P < 0.05$ was considered significant.

Results

Patients. Between April 2002 and April 2006, 489 patients from 86 hospitals belonging to the JALSG were enrolled in the CML202 study. Of these patients, three were deemed to be ineligible for inclusion because they were in AP, and a further five were excluded because of insufficient data. The characteristics of the remaining 481 evaluable patients at the time of registration are given in Table 1. The median follow-up time was 65.2 months (range 0.4–95.1 months). Eighty-two of 481 patients (17%) discontinued imatinib therapy or were switched to other therapy (Table 2).

Efficacy. For all 481 evaluable patients, the estimated cumulative rate of CHR was 96% at 7 years, whereas the rates for MCyR and CCyR were 94% and 90%, respectively (Fig. 1a). The *BCR-ABL1* transcript was measured in 428 patients using TMA-HPA and/or RQ-PCR. Levels of the *BCR-ABL1* transcript decreased to <100 copies/μg mRNA (i.e. MMR) in 39% of patients at 18 months and in 79% of patients after 7 years from the start of imatinib (Fig. 1b). According to the Sokal scoring system,[14] the cumulative rates of CCyR were 93%, 84%, and 82% in the low-, intermediate-, and high-risk groups, respectively. There was a significant difference in the rates of CCyR between the low- and intermediate/high-risk groups ($P = 0.006$).

Long-term outcomes. The estimated 7-year rates (with 95% CI) of OS, PFS, and EFS were 93% (90–96%), 93% (90–95%), and 87% (84–91%), respectively (Fig. 1c). The estimated rate of freedom from progression to AP/BC was 97% (95% CI 96–99%) and the estimated 7-year rates of OS according to the Sokal scoring system for patients in the low-, intermediate-, and high-risk groups were 95%, 90%, and 91%, respectively. Patients in the low-risk group exhibited significantly better OS ($P = 0.016$) and EFS ($P = 0.022$) than those in the intermediate- or high-risk groups. In the landmark analysis, patients who had achieved a CCyR at 12 months or an MMR at 18 months exhibited significantly better PFS than

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Table 1. Patient characteristics

Total no. patients	489
No. evaluable patients	481
Age (years)	52 (15–88)
No. patients ≥60 years of age (%)	141 (29)
Sex (M/F, %)	310/171 (64/36)
ECOG PS	
0	441 (92)
1	36 (8)
2	4 (1)
3	0 (0)
Duration from diagnosis (months)	0.4 (0–8.3)
Sokal risk group (%)	
Low	253 (53)
Intermediate	163 (34)
High	65 (14)
Hasford risk group (%)	
Low	202 (42)
Intermediate	227 (47)
High	39 (8)
Unknown	13 (3)
Additional chromosomal abnormalities (%)	
Yes†	51 (11)
Trisomy 8	4 (0.8)
Double Ph	3 (0.6)
Loss of sex chromosome	3 (0.6)
Others	41 (8.5)
Splenomegaly (%)	
Yes	127 (27)
≥10 cm below the costal margin	29 (6)
WBC ($\times 10^9$/L)	36.7 (4.5–634.7)
Hb (g/dL)	12.9 (4.8–19.1)
Platelets ($\times 10^9$/L)	473 (96–2916)
PB blast (%)	0 (0–13.0)
PB basophils (%)	5.0 (0–19.0)
Body weight (kg)	
All patients	61.8 ± 12.1
Men	66.9 ± 10.9
Women	52.6 ± 8.2
BSA (m^2)	
All patients	1.621 ± 0.187
Men	1.714 ± 0.148
Women	1.453 ± 0.121

Data are presented as the mean ± SD, as the median with the range given in parentheses, or as the number of patients in each group with percentages given in parentheses, as appropriate. †The presence of additional chromosomal abnormalities was not an exclusion criterion for the present study. BSA, body surface area; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; PB, peripheral blood; WBC, white blood cells.

Table 2. Patients' treatment status

	No. patients (%)
Continued imatinib treatment	399 (83.0)
Discontinued imatinib treatment	82 (17.0)
Reasons for discontinuation and/or change in therapy	
Adverse events	34 (7.1)
Disease progression	11 (2.3)
Unsatisfactory therapeutic effect	12 (2.5)
HSCT	6 (1.2)
Death	2 (0.4)
Lost to follow-up	7 (1.5)
Withdrawal of consent	8 (1.7)
Unknown	2 (0.4)

HSCT, hematopoietic stem cell transplantation.

those without CCyR or MMR ($P = 0.0005$ and $P = 0.012$, respectively).

Safety. The adverse events observed in all patients are listed in Table 3. Grade 3 or 4 hematologic adverse events were neutropenia (18%), thrombocytopenia (12%), and anemia (6%). Grade 3 or 4 non-hematologic adverse events included skin eruption (8%) and peripheral edema (0.6%). Grade 3 or 4 liver dysfunction was reported in 4% of patients. Congestive heart failure (Grade 3) developed in one patient and interstitial pneumonitis (Grade 3) developed in another patient. Grade 3 or 4 thrombocytopenia and skin eruptions occurred more frequently in the present study than in the IRIS study.[7]

Efficacy and outcomes in relation to imatinib dosage. Although it was planned to administer imatinib to patients at a dose of 400 mg daily, 82 patients (17%) discontinued imatinib or were switched to other treatment mainly because of adverse events or unsatisfactory efficacy (Tables 2, 3). Dose reduction or interruption were required in 223 (46%) patients, with escalated doses given to 10 patients (2%) during the first 24 months. Among all 481 patients, the initial dose of imatinib was 400 mg in 458 patients (95.2%), 300 mg in 10 patients (2.1%), 200 mg in 11 patients (2.3%), 100 mg on one patient, and 600 mg in one patient. The mean daily dose during the first 24 months of treatment was ≥360 mg in 294 patients (61%; designated the "400-mg group"), 270–359 mg in 90 patients (19%; designated the "300-mg group"), and < 270 mg in 67 patients (14%; designated the "200-mg group"). Thirty patients (6%) discontinued imatinib during the first 24 months. Regarding the safety profile, Grade 3 or 4 neutropenia, thrombocytopenia, liver dysfunction, and skin eruptions tended to be observed more frequently in the 300- and 200-mg groups because dose reductions from the scheduled dose of 400 mg imatinib daily were mostly made for patients in these groups because of adverse events (Table 3). The patients in the 300-mg group were significantly more likely to be female, older, have a lower body weight (BW), and a smaller body surface area (BSA) than patients in the 400-mg group (Table 4). Patients in the 300- and 200-mg groups had significantly higher Sokal risk than patients in the 400-mg group ($P = 0.001$). Of the patients in the 400- and 300-mg groups, age ($P = 0.0024$) and sex ($P = 0.0077$) were significant independent predictors for OS, as determined by multivariate analysis; however, dosage was not a significant predictor of OS ($P = 0.64$).

Efficacy and survival were analyzed according to the mean daily dose during the first 6, 12, and 24 months. During each period, the estimated cumulative rate of CCyR or MMR was significantly higher for patients in the 400- and 300-mg groups than for patients in the 200-mg group ($P < 0.001$ and $P < 0.0001$, respectively). There was a significant difference in achieving CCyR or MMR between the 400- and 300-mg groups ($P = 0.018$ and $P = 0.017$, respectively; Fig. 2a,b). There were no significant differences in OS and EFS between the 400- and 300-mg groups during the first 24 months ($P = 0.77$ and $P = 0.49$, respectively). However, the OS and EFS of the 200-mg group were significantly inferior to those of the 400- and 300-mg groups during the same periods ($P = 0.009$ and $P = 0.002$, respectively; Fig. 3a,b). Survival was analyzed according to the mean daily dosage of imatinib during the first 24 months per BW (Table 5). Patients who received a mean dose of imatinib per BW that was >5.0 mg/day/kg showed significantly superior OS and EFS than those receiving ≤5.0 mg/day/kg ($P = 0.0012$ and $P = 0.0016$, respectively; Fig. 4). These results indicate that patients who had relatively high daily dosage per BW had better OS and EFS, although the actual daily dose had been lower than 400 mg imatinib.

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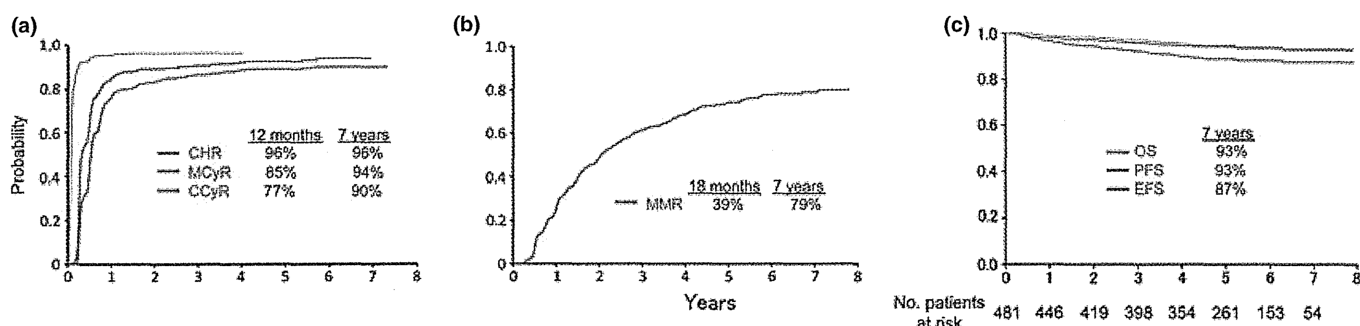

Fig. 1. Cumulative best (a) cytogenetic and (b) molecular responses and (c) survival of patients on imatinib therapy for chronic phase CML. Cumulative rates of responses were estimated according to the competing risk method. Discontinuation of imatinib was evaluated as a competing risk. CHR, complete hematologic response; MCyR, major cytogenetic response; CCyR, complete cytogenetic response; MMR, major molecular response; OS, overall survival; PFS, progression-free survival; EFS, event-free survival.

Table 3. Adverse events associated with imatinib therapy

Adverse event†	No. patients (%)				
	All patients (n = 481)		400-mg group‡ (n = 294)	300-mg group‡ (n = 90)	200-mg group‡ (n = 67)
	All grades	Grade 3 or 4	Grade 3 or 4	Grade 3 or 4	Grade 3 or 4
Non-hematologic					
Superficial edema	234 (48.6)	3 (0.6)	0	3 (3.3)	0
Nausea/vomiting	106 (22.0)	4 (0.8)	2 (0.7)	1 (1.1)	1 (1.5)
Anorexia	94 (19.5)	5 (1.0)	2 (0.7)	2 (2.2)	1 (1.5)
Muscle cramps	81 (16.8)	1 (0.2)	0	1 (1.1)	0
Musculoskeletal pain (myalgia)	100 (20.8)	5 (1.0)	2 (0.7)	0	2 (3.0)
Arthralgia	47 (9.8)	1 (0.2)	0	0	0
Rash	192 (39.9)	37 (7.7)	7 (2.4)	10 (11.1)	14 (20.9)
Fatigue	114 (23.7)	0 (0)	0	0	0
Diarrhea	75 (15.6)	2 (0.4)	1 (0.3)	0	0
Headache	36 (7.5)	1 (0.2)	0	0	0
Hemorrhage	24 (5.0)	3 (0.6)	2 (0.7)	0	1 (1.5)
Pyrexia	49 (10.0)	1 (0.2)	1 (0.3)	0	0
Depression	25 (5.2)	0 (0)	0	0	0
Infection	35 (7.3)	8 (1.7)	5 (1.7)	0	2 (3.0)
Interstitial pneumonitis	3 (0.6)	1 (0.2)	0	0	1 (1.5)
Hematologic					
Anemia	197 (41.0)	28 (5.8)	12 (4.1)	4 (4.4)	10 (14.9)
Neutropenia	188 (39.1)	85 (17.7)	36 (12.2)	25 (27.8)	18 (26.9)
Thrombocytopenia	199 (41.4)	59 (12.3)	19 (6.5)	20 (22.5)	16 (23.9)
Biochemical					
Elevated ALT/AST	99 (20.6)	18 (3.7)	3 (1.0)	6 (6.7)	7 (10.4)
Renal dysfunction	37 (7.7)	1 (0.2)	1 (0.3)	0	0

†Adverse events were assessed according to the National Cancer Institute–Common Toxicity Criteria version 2.0. ‡Mean daily doses in the 400-, 300-, and 200-mg groups were ≥360, 270–359, and < 270 mg imatinib, respectively. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Plasma trough levels of imatinib according to the daily dose. Plasma trough levels (C_{min}) of imatinib were determined in 50 patients who continuously received imatinib at a daily dose of 300 mg (n = 24) or 400 mg (n = 26) without any dose modification (Table 6). The patients receiving 300 mg imatinib tended to be older and to have a smaller BSA than patients in the 400-mg group. These tendencies did not different from those of the entire study population (Tables 4 and 6). There was no significant difference in mean C_{min} between the two groups (P = 0.673). The C_{min} in 15 of 24 patients (63%) receiving 300 mg imatinib and in 15 of 26 patients (58%) receiving 400 mg imatinib were distributed above 1000 ng/mL, and the ratio of patients >1000 ng/mL C_{min} did not differ significantly between the two groups (P = 0.10). However, the C_{min} in patients receiving 300 mg imatinib was distributed towards lower concentrations compared with those receiving 400 mg imatinib. There was a significant correlation between C_{min} and age only in the 400-mg group (P = 0.034), with weak correlations between C_{min} and BW or BSA. These results indicate that small, elderly, and/or female patients receiving 300 mg imatinib daily had almost the same C_{min} as patients receiving 400 mg daily.

Discussion

In the present study (CML202), the best cumulative rates of MCyR and CCyR 7 years after the start of imatinib were 94% and 90%, respectively, and the estimated 7-year OS and EFS

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Table 4. Patient characteristics in each of the mean daily dose groups during the first 24 months of treatment

	Imatinib daily dose group†				*P*-value
	400 mg	300 mg	200 mg	Discontinued	
No. patients	294	90	67	30	
Daily dose (mg)	398 ± 17	310 ± 23	187 ± 68	NA	
No. men/women	212/82	46/44	30/37	22/8	<0.0001
Age (years)	48 (16–81)	57 (19–79)	63 (19–87)	52.5 (15–88)	<0.0001
Body weight (kg)	64.6 ± 11.8	57.6 ± 10.5	55.3 ± 10.0	61.8 ± 15.3	<0.0001
BSA (m^2)	1.67 ± 0.18	1.55 ± 0.16	1.51 ± 0.17	1.61 ± 0.22	<0.0001
Sokal risk group (*n*)					
Low	180	39	23	11	<0.0001
Intermediate	84	30	32	13	
High	30	21	12	6	
Dose reduction (*n*)	1	69	59	NA	
Interruption (*n*)	65	21	8	NA	
Dose escalation (*n*)	10	0	0	NA	

Unless indicated otherwise, data are given as the mean ± SD or as the median with the range given in parentheses. †Mean daily doses in the 400-, 300-, and 200-mg groups were ≥360, 270–359, and <270 mg imatinib, respectively. BSA, body surface area; NA, not applicable.

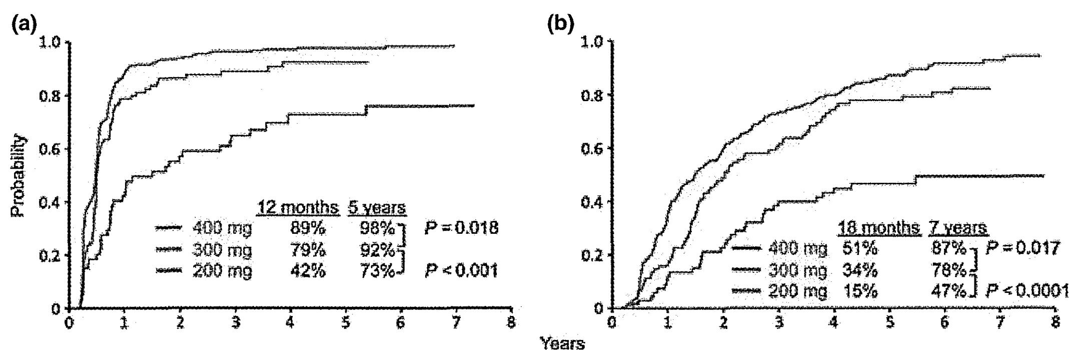

Fig. 2. Cumulative rates of best responses according to the mean daily dose during the first 24 months of treatment with imatinib. (a) Cumulative rates for complete cytogenetic responses (CCyR). (b) Cumulative rates of major molecular responses (MMR). Mean daily doses in the 400- (*n* = 294), 300- (*n* = 90), and 200-mg (*n* = 67) groups were ≥360, 270–359, and <270 mg imatinib, respectively.

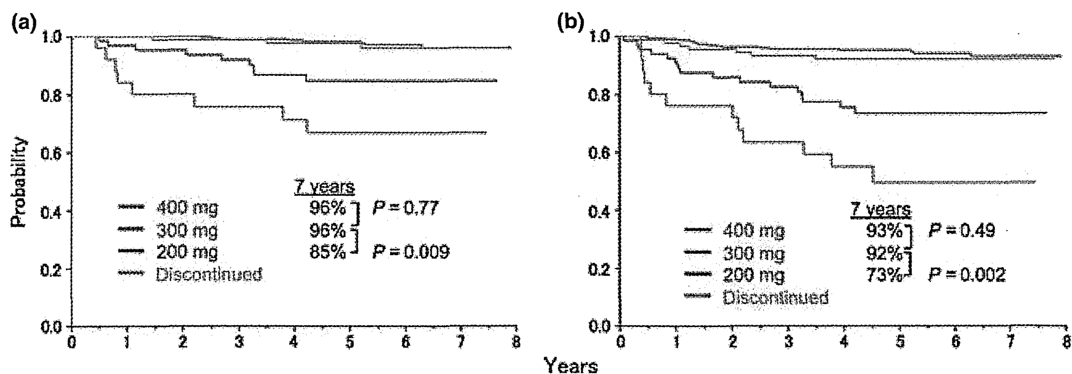

Fig. 3. (a) Overall and (b) event-free survival according to the mean daily dose during the first 24 months. Mean daily doses in the 400- (*n* = 294), 300- (*n* = 90), and 200-mg (*n* = 67) groups were ≥360, 270–359, and < 270 mg imatinib, respectively.

rates were 93% and 87%, respectively. The Sokal risk showed favorable prognostic significance in low-risk patients compared with intermediate- or high-risk patients. These results are comparable to those reported in the IRIS trial and others studies in Western countries.[3–5] In terms of baseline characteristics, there was a tendency for fewer patients with a high-risk Sokal score in the present study compared with the IRIS study. We believe this is due to the Japanese medical system, in which a considerable number of people undergo annual medical check-ups.

Imatinib is currently established as the first-line therapy for patients with CP CML. Nevertheless, several controversial issues remain,[15] with the dose of imatinib as one of the most important.[6,16–21] In the present study, many patients received a lower dose of imatinib than the planned initial dose of 400 mg. Therefore, we performed subgroup analysis according

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