

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

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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/m² 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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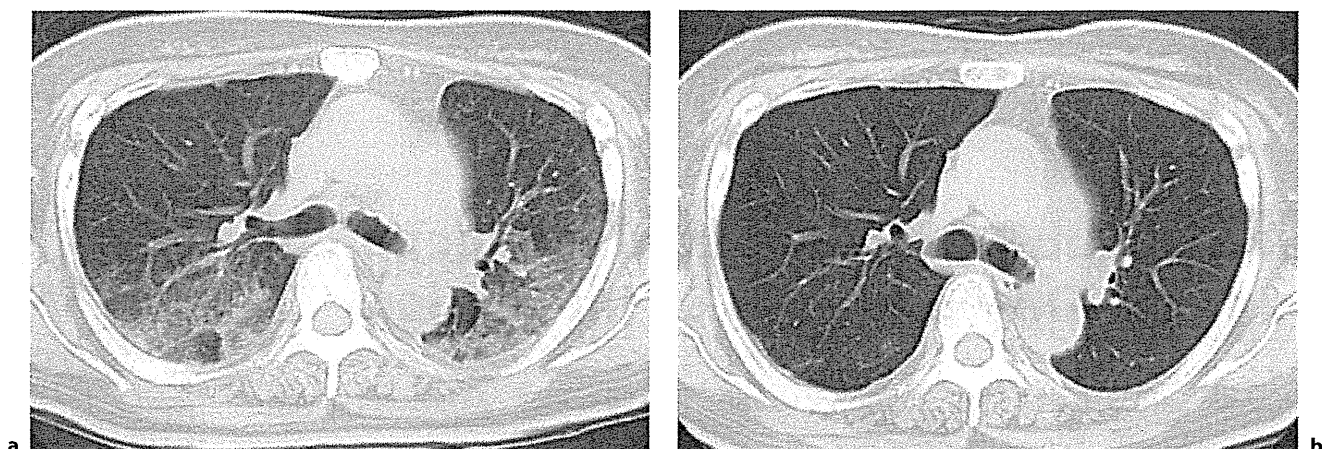


Fig. 1. CT shows bilateral alveolar and interstitial infiltration 3 weeks after completion of rituximab therapy (a) and complete disappearance without any additional therapy (b).

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

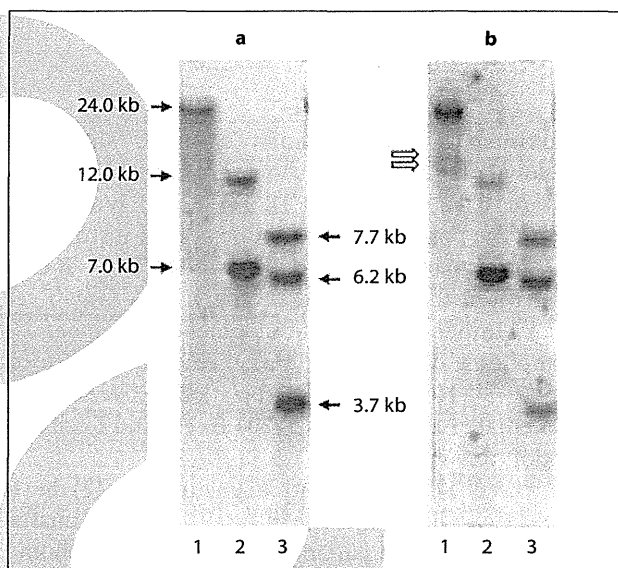


Fig. 2. Genomic Southern blot analysis shows rearrangement of the T cell receptor C β 1 gene. High-molecular-weight DNA was digested with *Bam*HI (lane 1), *Eco*RV (lane 2) or *Hind*III (lane 3). Two additional bands (open arrows) were obtained after digestion with *Bam*HI. **a** Germ-line control. **b**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

dritic 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

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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 gastroin-

testinal (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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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-2⁺, BCL-6⁺, and CD5⁻ and the other was CD10⁺, CD20⁺, CD23⁻, BCL-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	<i>P</i>
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
<i>κ/λ</i> ratios [#] (no.)				
<i>κ/λ</i> > 3.0	13.0 (23.4 ± 5.7)(19)	N/A	N/A	
0.5 ≤ <i>κ/λ</i> ≤ 3.0	N/A	1.3 (1)	1.3 (1.3 ± 0.1)(6)	
<i>κ/λ</i> < 0.5	0.03 (0.07 ± 0.02)(11)	N/A	N/A	
Chromosomal analysis (no.)				0.462
Normal karyotype	6	2	5	
Abnormal karyotype	8	0	0	
No metaphase	3	1	2	
Not examined	13	1	7	

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

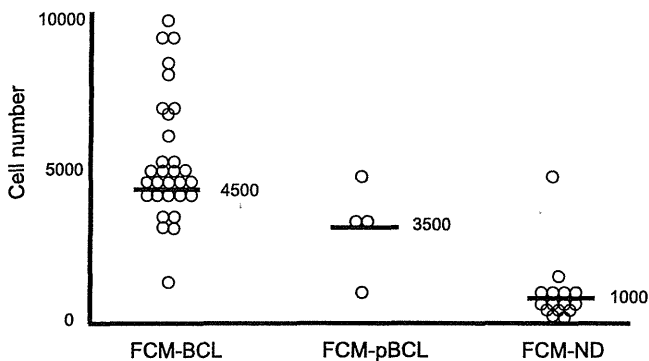


Fig. 1. Dot plots of analyzed cell number in each group. The bars indicate median cell number.

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 DLBCL 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.

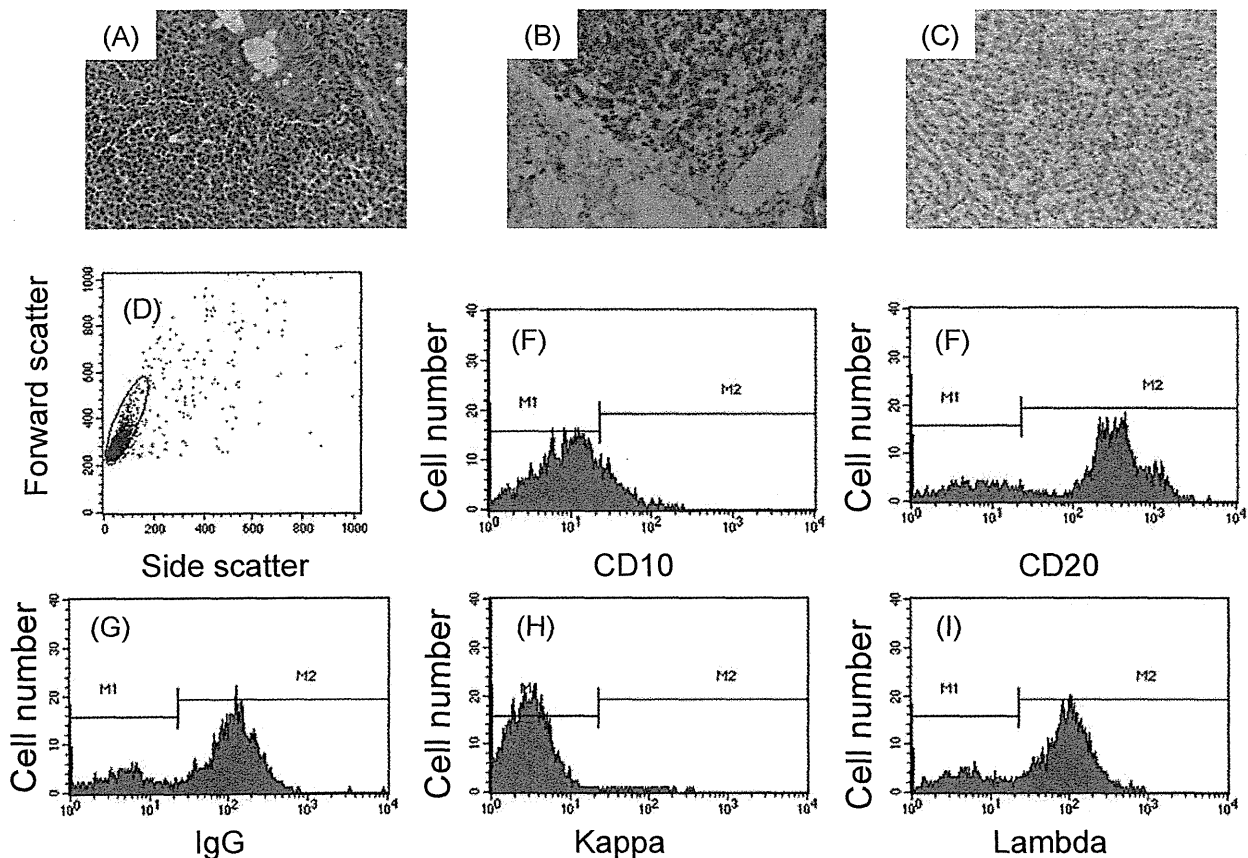


Fig. 2. Flow cytometric analysis of the biopsy specimen obtained from colon fiberscopy. (2A) Diffuse large B-cell lymphoma (DLBCL) in the colon mucosa (H&E stain, $\times 200$). (2B) Positivity of the leukocyte common antigen staining in the DLBCL cells ($\times 200$). (2C) Positivity of CD20 staining in the DLBCL cells ($\times 200$). (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

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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).

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.

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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-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	<i>p</i>
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 BMBC [#]			
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 BMBC [#]			
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 refractory anemia; Group B, progression after that; no., number; #, mean; BM, bone marrow; IPSS, International Prognostic Scoring System; BMBC, bone marrow blast cell composition; Medications include cyclosporine, steroid, anabolic steroid, antithymocyte globulin, or a combination thereof.

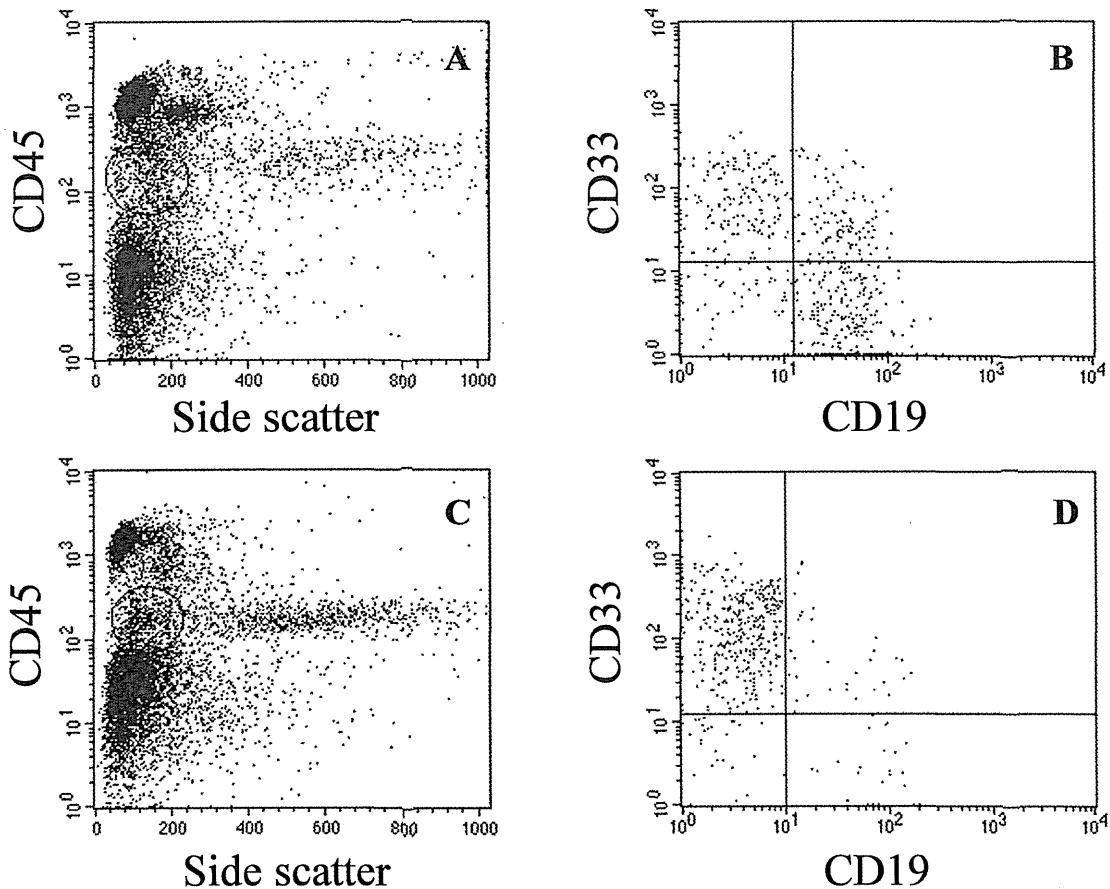


Fig. 1. Flow cytometric analysis of bone marrow cells. (1A) & (1B), a case in Group A ; (1C) & (1D), a case in Group B. The circle indicates the bone marrow (BM) blast gate region. The cell composition in the region is different between the two patients. Co-expression of CD34 and CD117 in the BM blasts 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, Stemberg *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

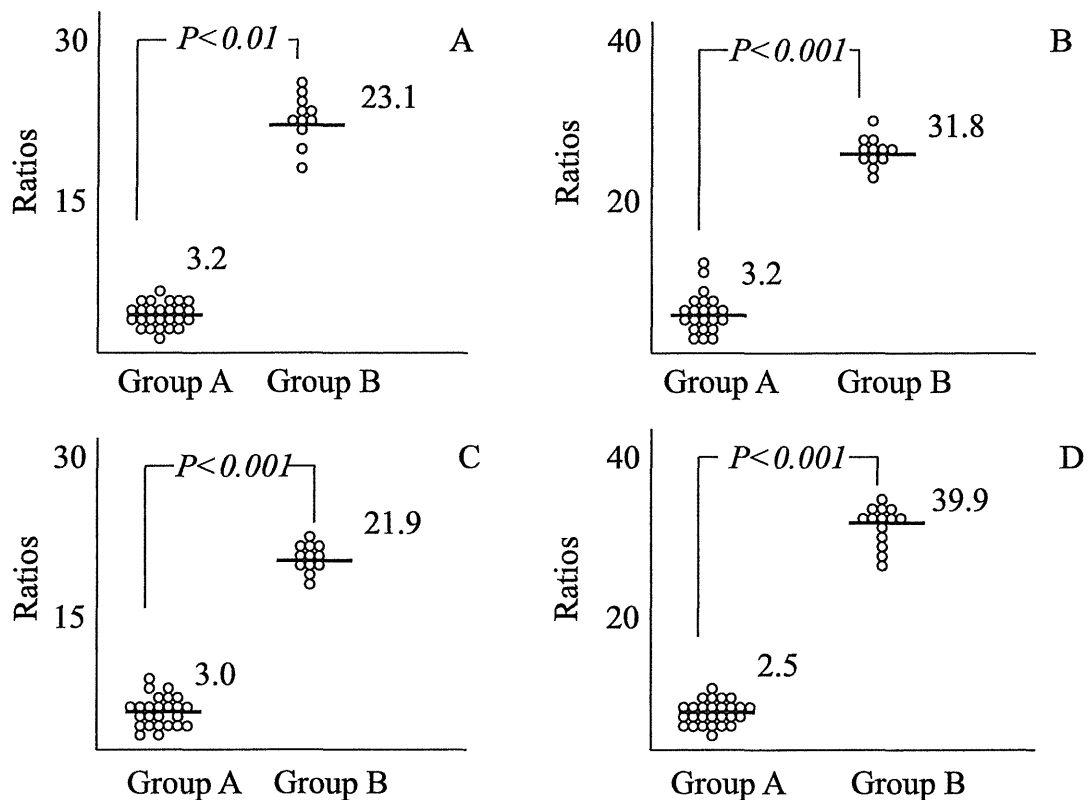


Fig. 2. Ratios of antigen expression in the blast region of bone marrow. (2A), CD33/CD19 ; (2B), CD33/CD10 ; (2C), CD13/CD10 ; (2D), CD13/CD19. The values are means.

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*

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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

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*Name of trial register: JALSG CML202. Registration no. C000000153, UMIN Clinical Trials Registry.

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 5000g 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 $99 \pm 5\%$ to $108 \pm 5\%$ 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/ μ g 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

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 (×10 ⁹ /L)	36.7 (4.5–634.7)
Hb (g/dL)	12.9 (4.8–19.1)
Platelets (×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.

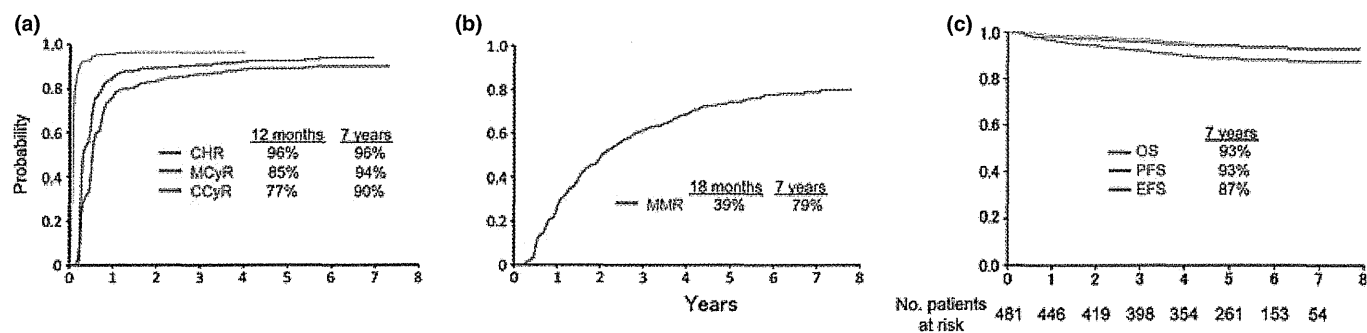


Fig. 1. Cumulative best (a) cytogenetic and (b) molecular responses and (c) survival of patients on imatinib therapy for chronic phase CML. Cumulative rates of responses were estimated according to the competing risk method. Discontinuation of imatinib was evaluated as a competing risk. CHR, complete 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 differ 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

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.

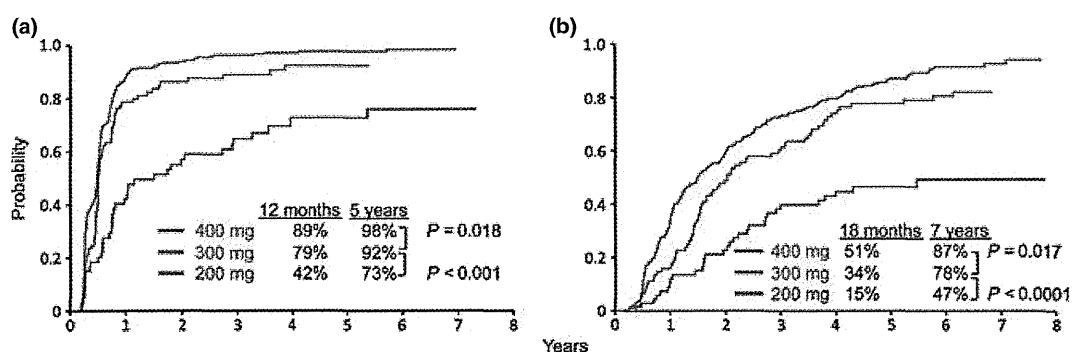


Fig. 2. Cumulative rates of best responses according to the mean daily dose during the first 24 months of treatment with imatinib. (a) Cumulative rates for complete cytogenetic responses (CCyR). (b) Cumulative rates of major molecular responses (MMR). Mean daily doses in the 400- (*n* = 294), 300- (*n*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

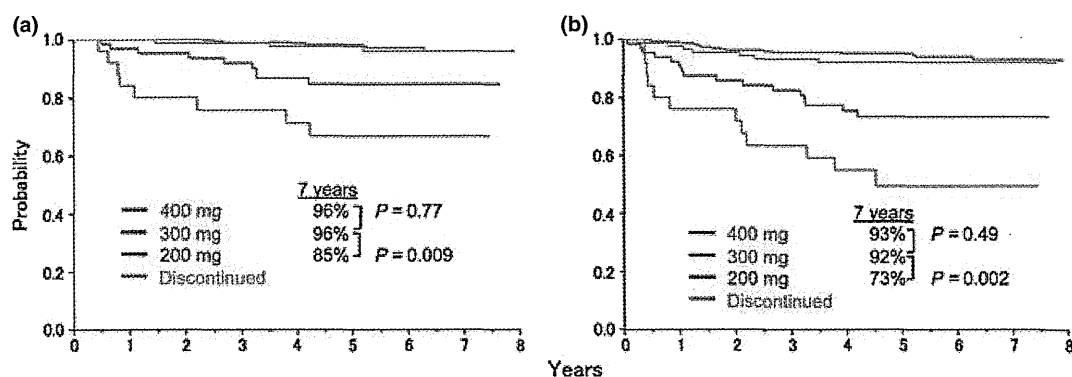


Fig. 3. (a) Overall and (b) event-free survival according to the mean daily dose during the first 24 months. Mean daily doses in the 400- (*n* = 294), 300- (*n* = 90), and 200-mg (*n* = 67) groups were ≥360, 270–359, and <270 mg imatinib, respectively.

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

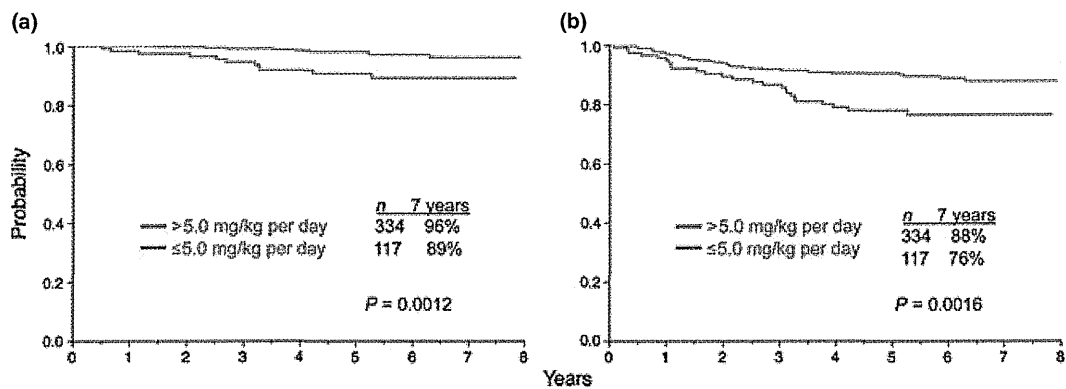


Fig. 4. (a) Overall and (b) event-free survival according to the mean daily dose during the first 24 months per body weight. The cut-off value was set at >5.0 mg/day/kg (e.g. if a patient whose body weight was <60 kg received imatinib at a mean daily dose of 300 mg).

Table 5. Number of patients and survival according to the mean daily dose of imatinib during the first 24 months per body weight

	Mean daily dose/body weight (mg/day/kg)				P-value
	>5.0†		≤ 5.0		
	Actual bodyweight (kg)	No. patients	Actual bodyweight (kg)	No. patients	
Imatinib daily dose group‡					
400 mg	<80	266	≥ 80	28	
300 mg	<60	63	≥ 60	27	
200 mg	<40	5	≥ 40	62	
Estimated 7-year OS		96%		89%	0.0012
Estimated 7-year EFS		88%		76%	0.0016

†The cut-off value was set at >5.0 mg/day/kg (e.g. the mean daily dose of imatinib during the first 24 months (300 mg) divided by body weight (<60 kg)). ‡Mean daily doses in the 400-, 300-, and 200-mg groups were ≥360, 270–359, and <270 mg imatinib, respectively. Patients who discontinued imatinib were not included in the analysis. EFS, event-free survival; OS, overall survival.

to the mean daily dose during the first 6, 12, and 24 months of treatment. The rate of achieving CCyR or MMR differed significantly between the 300- and 400-mg groups during the first 24 months. Even so, there were no significant differences in OS, PFS, and EFS between the 300- and 400-mg groups during the first 6, 12, or 24 months of treatment. Conversely, the 200-mg group showed markedly inferior cytogenetic and/or molecular responses, as well as inferior survival, compared with the 300- and 400-mg groups. We also analyzed outcomes according to the mean daily dosage during the first 24 months per BW, with the results suggesting that patients who had relatively high daily dosage per BW were likely to have better OS and EFS even though the actual daily dose had been lower than 400 mg imatinib. The OS and EFS in the 300-mg group in the present study were not inferior compared with rates reported in the IRIS study (85% at 7 years vs. 83% at 6 years), which suggests that a considerable number of Japanese patients who received doses lower than 400 mg demonstrated an adequate response. A prospective comparative study would be necessary to confirm this observation.

Two recent studies showed a correlation between the plasma trough levels (C_{min}) and response, suggesting that maintaining C_{min} above approximately 1000 ng/mL was associated with improved outcomes.^(22,23) In the present study, the mean daily dose was 331 ± 108 mg during the first 24 months and the relatively high dosage of imatinib per BW was associated with better OS and EFS, whereas in the IRIS study the mean daily dose among the patients who continued receiving imatinib was 382 ± 50 mg.⁽¹⁾ On the basis of our results, we assume that

the relatively small body size of Japanese patients compared with their Western counterparts may have affected C_{min} , although differences in the metabolism of imatinib because of ethnicity cannot be ruled out either. Therefore, we measured the C_{min} of imatinib in a group of patients who had received imatinib continuously at a daily dose of either 300 or 400 mg. The patients from whom blood samples were collected showed almost similar background characteristics to the entire study population. There was no significant difference in the mean C_{min} between patients receiving 300 or 400 mg imatinib, and there was no significant difference in the ratio of patients whose C_{min} was higher than 1000 ng/mL between the two groups. When pharmacokinetic analyses of patients receiving 400 mg imatinib in the present study are compared with the IRIS study, the C_{min} in the present study was distributed at higher concentrations than in the IRIS study (mean C_{min} 1165 vs. 979 ng/mL, respectively); however, the distribution of C_{min} in patients receiving 300 mg imatinib was similar between the studies.⁽²³⁾ Larson *et al.* reported a weak correlation between C_{min} and age, BW, or BSA in the IRIS study, but also suggested that the effects of body size and age on C_{min} were not likely to be of clinical significance because C_{min} showed large interpatient variability.⁽²³⁾ However, the C_{min} in their female patients was significantly higher than that in male patients, and they speculated that this may be due to the small body size of the female patients. The same tendency was seen in the present study, especially in terms of age and gender. Therefore, a small body size among Japanese old and/or female patients may partly account for the higher C_{min} of imatinib. Regarding

Table 6. Patient characteristics and plasma trough levels of imatinib according to the daily dose of imatinib

	Imatinib daily dose†		P-value
	400 mg	300 mg	
No. patients	26	24	
No. men/women	19/7	12/12	0.092
Age (years)	49 (17–79)	58 (33–76)	0.012
Body weight (kg)	65.2 ± 10.6	59.5 ± 10.7	0.062
BSA (m ²)	1.68 ± 0.17	1.57 ± 0.17	0.034
Sokal risk group (n)			
Low	18	13	0.357
Intermediate	6	6	
High	2	5	
C _{min} (ng/mL)			
Mean ± SD	1165 ± 445	1113 ± 426	0.673
Median (range)	1035 (710–2420)	1130 (439–2140)	
% Patients on >1000 ng/mL imatinib	57.7 (15/26)	62.5 (15/24)	0.1
Best response (%)			
MCyR	26 (100)	23 (96)	
CCyR	26 (100)	22 (92)	
MMR	24 (92)	23 (96)	

Unless indicated otherwise, data are given 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. †Imatinib at a daily dose of 400 or 300 mg without any dose modification. BSA, body surface area; CCyR, complete cytogenetic response; C_{min}, plasma trough level; MCyR, major cytogenetic response; MMR, major molecular response.

the plasma concentration of imatinib in Japanese patients, there are other reports showing sufficient C_{min} in patients receiving imatinib at doses lower than 400 mg,^(6,24) but it remains uncertain whether there are any individual or ethnic differences in the metabolism of imatinib.^(24,25)

Another possible reason for the satisfactory outcomes seen for patients in the 300-mg group could be that, at this dose, imatinib could be administered continuously to some patients

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without serious adverse events. A recent study regarding imatinib dosage in Japanese patients reported that, based on multivariate analysis, older age and lower BW are significant risk factors for the discontinuation of imatinib therapy and that patients with these factors were less likely to achieve a CCyR.⁽¹⁸⁾ Continuous and adequate dosage is essential for optimal outcome, and adherence to imatinib therapy is critical.^(26,27)

In conclusion, the long-term follow-up of the JALSG CML202 study revealed almost similar excellent outcomes to those of the IRIS study and others. There were no significant differences in OS and EFS between the 300- and 400-mg imatinib groups. However, cumulative rates of cytogenetic or molecular responses in the 300-mg group were inferior to those in the 400-mg group. The results of the present study suggest that imatinib at a dose of 400 mg may be optimal for Japanese patients, but that 400 mg imatinib is not tolerable in a considerable number of patients, and that the measurement of C_{min} is useful in finding the optimal dose, especially in elderly and/or female patients. Nevertheless, excessive dose reductions to <300 mg imatinib should be avoided even in patients who are intolerant to 400 mg imatinib or have a small body size. We hope our findings are useful for the treatment of CML patients in other Asian countries.

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Disclosure Statement

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Correlation between Amp-CMLTM (FUJIREBIO Inc., Tokyo, Japan) and Fusion Quant M-BCRTM (Ipsogen, Marseille, France).

Data S1. Measurement of major *BCR-ABL1* transcript.

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