and RIC recipients. To further clarify the clinical significance of preconditioning in allogeneic BMT or PBSCT for ATL, we analyzed the interactions of preconditioning with age, disease status, and PS. There was a clear trend indicating that RIC contributed to better OS in older patients compared with MAC. In contrast, the associations between MAC and RIC to OS were almost similar even if ATL patients at transplantation were in CR or not. In general, when considering allogeneic HSCT for many other types of leukemia/lymphoma patients who are in non-CR, it seems more usual to apply MAC for those patients because MAC should have the more potent effect in eradicating residual leukemia/lymphoma cells than RIC. However, the present study does not support this strategy at least in HSCT for ATL. The associations between MAC and RIC to OS were almost similar even when the PS at transplantation was 0, 1, or 2 to 4. In general, considering allogeneic HSCT for patients who have a worse PS, it seems to be more usual to apply RIC because RIC should be less toxic for recipients than MAC. However, the present study also does not support this strategy, at least in HSCT for ATL.

In the subgroup analyses stratified by MAC or RIC, older age was an independent unfavorable prognostic factor in MAC recipients, but not in RIC recipients. Female sex, good ATL disease status, and PS significantly contributed to better OS in both groups. Among MAC recipients, there was no significant difference in OS according to the type of MAC, but among RIC recipients, a Flu + Mel–based regimen contributed to better OS compared with a Flu + BU–based regimen. Although RIC regimens that contain alemutuzumab have been widely used in various parts of the world,[31] we had no data available as to whether any of the regimens used included alemtuzumab. Thus, we were not able to clarify the significance of the inclusion of alemtuzumab as a conditioning agent.

Multivariate analysis of variables contributing to mortality demonstrated that there was significantly more ATL-related mortality in RIC recipients. Although not statistically significant, a clear trend showed an association of increased TRM but not ATL-related mortality in older patients. Male sex was significantly associated with increased TRM, which might contribute to the better OS of female recipients. ATL patients not in CR had greater ATL-related mortality, but not TRM. A poor PS was significantly associated with both ATL-related mortality and TRM, but the association was closer with TRM. HSCT from unrelated donors was significantly associated with increased TRM but not with ATL-related mortality.

Cumulative incidence curves of TRM and ATL-related mortalities in MAC and RIC recipients showed characteristic features as illustrated in Figure 3. In comparison with the black lines indicating ATL-related mortality, the red lines showing TRM rise in the early phase after transplantation. Two solid lines for MAC had quite different trajectories, with TRM being greater than ATL-related mortality at any time after transplantation. In contrast, the 2 dotted lines for RIC nearly joined at 24 months after transplantation and were almost identical thereafter. Both lines for RIC were between those for MAC TRM and ATL-related mortality.

Currently, several promising new agents for ATL are being developed.[32-35] These novel treatments should increase the number of ATL patients with a sufficient disease control status and who have maintained a good PS who could become suitable candidates for transplantation. This would require further improvement in allogeneic HSCT for ATL as well as better rescue strategies for patients relapsing after HSCT. Although treatment by AZT/IFN-α[6] and/or alemutuzumab[34,36] are applied for ATL patients in many countries, none of these agents are currently approved in Japan for the treatment of ATL under the national health insurance. Therefore, there are currently no data on their clinical impact on outcome after allogeneic HSCT for ATL. We do expect, however, that the application of AZT/IFN and alemutuzumab would contribute to improved outcomes of HSCT for ATL.

Although this study reports significant novel findings for allogeneic HSCT for ATL patients, it also has inherent limitations common among observational retrospective studies. Eligibility for transplantation as well as choice of transplantation protocol, including the selection of MAC or RIC, was determined by the physicians at each institution. Regarding mortality analysis, it is not easy to determine whether death of an ATL patient after allogeneic HSCT is TRM or ATL-related mortality. This is partially because relapsed ATL patients sometimes achieve partial or complete remission on decreasing or discontinuing immunosuppressive agents, donor lymphocyte infusions, or chemotherapy, which can result in long-term remission and survival.[9,13,18]

In conclusion, allogeneic BMT or PBSCT not only with conventional MAC but also RIC is an effective treatment that results in long-term survival of selected patients with ATL. Posttransplantation outcomes are influenced by the recipient's age, sex, PS, disease status at transplantation, and the relationship between recipient and donor. Although no significant difference in OS between MAC and RIC recipients was observed, there was a clear trend that RIC contributed to better OS in older patients. Regarding results of analysis of mortality, RIC was more significantly associated with ATL-related mortality in comparison with MAC. More definitive conclusions on the role of allogeneic HSCT in the therapeutic algorithm for ATL will need to be drawn from well-designed prospective clinical trials.

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Authorship

Contribution: T.I., M.H., K.K., R.T., and A.U. designed the research, organized the project, and wrote the paper; T.I. and T.N. performed statistical analysis; H.S. and R.S. collected data from JSHCT; Y.M. collected data from JMDP; K.K. collected data from JCBBN; and all authors interpreted data, reviewed, and approved the final manuscript.

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ORIGINAL ARTICLE

Randomized comparison of fixed-schedule versus response-oriented individualized induction therapy and use of ubenimex during and after consolidation therapy for elderly patients with acute myeloid leukemia: the JALSG GML200 Study

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Abstract We conducted a multicenter prospective randomized study to compare a fixed-scheduled induction therapy with a response-oriented individualized induction therapy for elderly patients with acute myeloid leukemia (AML). Newly diagnosed AML patients, aged between 65 and 80, were randomly assigned to receive fixed or individualized induction. Both groups received daunorubicin (DNR) 40 mg/m^2 for 3 days and behenoyl cytarabine (BHAC) 200 mg/m^2 for 8 days. In the individualized group, bone marrow biopsy was done on days 8 and 10, and according to the cellularity and blast ratio, the patients received additional DNR and BHAC for two to four more days. All patients achieving complete remission (CR) were randomized a second time to determine whether they would receive ubenimex. CR was obtained in 60.1 % of the fixed group and 63.6 % of the individualized group.

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Predicted 4-year relapse-free survival (RFS) was 9 % for the fixed group and 18 % for the individualized group. There were no statistically significant differences in CR and RFS between the fixed and individualized groups. In the ubenimex group, prolonged RFS was observed. Notably, gender was a prognostic factor in this study, as 102 female patients had a significantly higher CR rate (72.5 vs. 54.3 %, $p = 0.0048$) and better OS (24 vs. 14 % at 4 years, $p = 0.018$), compared with 140 male patients.

Keywords Acute myeloid leukemia · Elderly · Response-oriented individualized induction therapy · Daunorubicin · Behenoyl cytarabine (enocitabine, BHAC)

Introduction

With the extension of life-span, elderly patients with acute myeloid leukemia (AML) are increasing in number, and the median age of AML is presently around 65–70. Prognosis of these patients is poorer, compared with younger patients, as their complete remission (CR) rate is around 50 % and overall survival (OS) is <20 % at 5 years, showing no remarkable progress during the past decades, despite every possible effort by many investigators. Regrettably, there is no recommendable standard regimen effective enough for the treatment of elderly AML [1–6].

In Japan, a response-oriented individualized induction therapy has been employed for AML since the DCMP two-step therapy, using daunorubicin (DNR), cytarabine (Ara-C), 6-mercaptopurine (6MP) and prednisolone (PSL) by Uzuka et al. in the mid 1970s, reporting more than 80 % CR rate, which is not surprisingly high today but was remarkable at that time even for a single institutional study [7]. Subsequently, a response-oriented individualized BHAC-DMP induction therapy, using behenoyl Ara-C (BHAC, enocitabine), DNR, 6MP and PSL, was developed by Ohno et al. [8], reporting more than 80 % CR in adult AML by a single institutional study. A multi-institutional AML87 study, conducted by the Japan Adult Leukemia Study Group (JALSG), confirmed the high CR rate of BHAC-DMP therapy for adult AML, resulting in 80 % CR rate [9]. Succeeding JALSG studies, AML89 [10] and AML92 [11] also employed the response-oriented individualized induction therapy and reported 81 and 77 % CR rates, respectively, for younger adult patients with non-M3 type AML. These CR rates were around 10 % higher than those reported from cooperative study groups in the USA and Europe, where fixed-scheduled induction therapies were employed [3, 12–14].

However, after clinical introduction of idarubicin (IDR), a more potent derivative of DNR, the JALSG AML95 study which prospectively compared the two treatment schedules, using Ara-C and IDR instead of DNR, could not demonstrate any advantage of the response-oriented individualized induction therapy over the fixed-scheduled induction therapy for younger patients with AML of age <65 [15].

In the present study, with elderly AML patients of age from 65 to 80, we compared a response-oriented individualized induction therapy with a fixed-scheduled induction therapy using BHAC and DNR. Additionally, we randomly compared the effectiveness of ubenimex among patients who had achieved CR by these two induction regimens. Ubenimex, a dipeptide immunostimulator, reportedly prolonged OS and disease-free survival in adult AML patients when used during and after consolidation therapy [16–18].

Materials and methods

Patients

From August 2000 to December 2005, all newly diagnosed elderly patients with AML were consecutively registered from 55 institutions which participated in this study. Informed consent was obtained from all the patients before registration in accordance with the Declaration of Helsinki. AML was first diagnosed by the French–American–British (FAB) classification at each institution. Peripheral blood and bone marrow smears from all registered patients were sent to Nagasaki University, and examined with May-Giemsa, peroxidase and esterase staining. Then, diagnosis was reevaluated by the central review committee. Eligibility criteria for the randomization study included age from 65 to 80 years, AML by FAB classification except M3, adequate functioning of the liver (serum bilirubin level <2.0 mg/dL), kidney (serum creatinine <2.0 mg/dL), heart (ejection fraction >50 %) and lungs, an Eastern Cooperative Oncology Group performance status between 0 and 2,

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and written informed consent for the randomized study. Patients were not eligible if they had pre-diagnosed myelodysplastic syndromes (MDS), but were eligible if they had no definite diagnosis of MDS, even when they had previous history of hematological abnormality. Patients with ill-controlled diabetes mellitus, angina pectoris, infectious episodes and liver cirrhosis were not eligible, as well as those with positive HIV antibody, HCV antibody and HB antigen. Patients who did not meet the eligibility criteria or did not agree to the randomization study were included also for the initial evaluation and survival. Cytogenetic analysis was performed by standard methods of G-banding, and abnormalities were grouped according to the MRC classification [19]. The protocol was approved by the institutional review board of each hospital.

Treatment regimens

Eligible patients who had given their informed consent for the randomized study were assigned to receive either a fixed-scheduled induction therapy or a response-oriented individualized induction therapy through a centralized computer system. All assigned patients received DNR 40 mg/m^2/day by 30-min infusion on days 1–3 and BHAC 200 mg/m^2/day by 3-h infusion on days 1–8. For patients of age 70 or older, the dose of DNR was reduced to 30 mg/m^2/day. In the individualized group, bone marrow aspiration was performed on day 8, and if the marrow was not severely hypoplastic and had more than 20 % blasts, additional BHAC was given on days 9 and 10. If 20–50 % of blasts remained, DNR was added on day 8, and if more than 50 % of blasts remained, DNR was added on days 8 and 9. Another bone marrow aspiration was performed on day 10, and if the marrow was not severely hypoplastic and had more than 20 % blasts, additional BHAC was given on days 11 and 12. If 20–50 % of blasts remained, DNR was added on day 11, and if more than 50 % of blasts remained, DNR was added on days 11 and 12 (Fig. 1). If patients had documented infection or other complications on day 8 or day 11, cancellation of additional chemotherapy was permitted by the attending physician's judgment. In the fixed-scheduled group, patients did not receive additional doses, regardless of their marrow status at day 8. If patients did not achieve CR by the first course, the same induction therapy was repeated at approximately 3- to 4-week interval. If patients did not achieve CR with two courses, these cases were judged as failure.

All patients who had achieved CR received 3 courses of consolidation therapy, and were randomly assigned either to receive daily 30 mg of ubenimex (Bestatin, Nippon Kayaku, Tokyo, Japan) or not, concomitantly during the consolidation therapy. The first course of consolidation consisted of BHAC (200 mg/m^2 by 3-h infusion on days 1–5) and mitoxantrone (MIT, 7 mg/m^2 by 30-min infusion on days 1–3). The second consisted of BHAC (200 mg/m^2 on days 1–7), DNR (30 mg/m^2 by 30-min infusion on days 1–2) and etoposide (ETP; 100 mg/m^2 by 1-h infusion on days 1–3). The third consisted of BHAC (200 mg/m^2 on

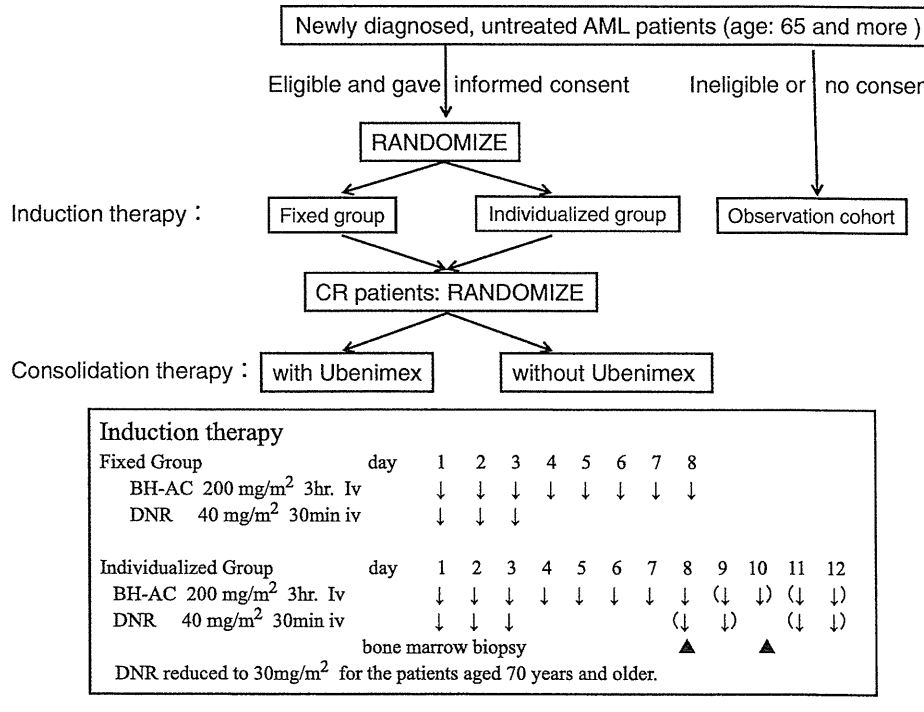

Fig. 1 Consort diagram and treatment schedule of induction therapy. Eligible patients were randomized to fixed group or individualized group. Patients achieved complete remission were done second randomization to with ubenimex or without ubenimex. Induction therapy in individualized group, BHAC dosage should be escalated up to twelve doses and up to seven doses for daunorubicin according to the bone marrow state

days 1–5) and aclarubicin (ACR; 14 mg/m^2 by 30-min infusion on days 1–5). For patients of age 70 or more, the dose of MIT, DNR, ETP and ACR was reduced to 5, 25, 75 and 10 mg/m^2, respectively. Each consolidation course was given as soon as possible after the leukocyte and platelet counts had recovered to more than 3,000 and 100,000/μL, respectively. Intrathecal methotrexate (15 mg), Ara-C (40 mg) and PSL (10 mg) were given after the third consolidation therapy for the prophylaxis of central nervous system leukemia. Patients assigned to be given ubenimex received it for 3 more months after the completion of consolidation therapy, but no further chemotherapy was given to either group. For non-eligible patients or for those who did not give informed consent for the randomized study, no intervention was specified and the therapy was left to the decision of attending physicians. However, their OS data were reported.

Best supportive care, including administration of antibiotics and platelet transfusion from blood cell separators, was given if indicated. When patients had life-threatening infections during neutropenia, the use of granulocyte colony-stimulating factor (G-CSF) was permitted.

Response criteria and statistical analysis

CR was defined as the presence of all the following criteria: <5 % of blasts in bone marrow, no leukemic blasts in peripheral blood, recovery of peripheral neutrophil counts over 1,000/μL and platelet counts over 100,000/μL, and no evidence of extramedullary leukemia. CR had to continue for at least 4 weeks, but the date of CR was defined as the first day when these criteria were fulfilled. Relapse was defined as the presence of at least one of the following: recurrence of more than 10 % leukemic cells in bone marrow, any leukemic cells in peripheral blood, and appearance of extramedullary leukemia.

Overall survival (OS) was calculated from the diagnostic day to death by any cause, and censored at the last follow-up. Relapse-free survival (RFS) for patients who achieved CR was measured from the date of CR to relapse or death by any cause, and censored at the last follow-up.

This was a multi-institutional randomized phase 3 study with a 2 × 2 factorial design. The primary end point of the first randomization was CR rate, and the secondary endpoints were OS and RFS. For the second randomization, the primary end point was RFS and the secondary endpoint was OS, and Kaplan–Meier product limit estimation was used to determine OS and RFS. A sample size of 98 patients per group was estimated to have a power of 70 % at a 5 % level of significance (single-sided) to demonstrate 10 % non-inferiority in CR rate (60 vs. 55 %). Statistical testing for the non-inferior trial was performed according to the method of Blackwelder [20]. To test the factors to predict CR, χ^2 test and Wilcoxon rank-sum test were used for univariate analysis, and the multiple logistic regression model was used for multivariate analysis. For comparison of OS and RFS, the log-rank test and the generalized Wilcoxon test were used for univariate analysis and Cox's proportional hazard model was used for multivariate analysis. SAS ver. 8.2 (SAS Institute Inc., Cary, NC, USA) was used for the analysis. p values <0.05 (two-sided) were considered statistically significant. Analysis was done on an intent-to-treat basis. This study is registered at http://www.umin.ac.jp/ctrj/ as C000000220 for the randomization study on eligible patients and C000000224 for the observation study on non-eligible patients.

Results

Patient population and characteristics

Of 375 patients registered, 130 patients were either judged as non-eligible by the attending physicians because of various reasons listed in eligibility criteria, including 6 patients with FAB-M3, or eligible but gave no informed consent to enter the randomized study. Of 245 eligible and consented patients, 122 were assigned to the fixed-scheduled therapy and 123 to the individualized therapy. One in the former group and two in the latter were unevaluable due to insufficient data. Pretreatment characteristics of 242 evaluable patients are presented in Table 1. Overall, the median age was 71, and 47 patients (19 %) were of age 75 or older. Successful cytogenetic data were reported in 231 patients (95 %), including 113 patients (91 %) in 124 observation cohort excluding M3. There were no major imbalances between the two randomized groups, although there were fewer patients with favorable cytogenetics and more with adverse cytogenetics in the fixed-scheduled group ($p = 0.1338$) (Table 1).

In the individualized therapy group, during the first course of the induction therapy, 45 patients received additional doses of DNR and BHAC from day 9, and 13 patients received the additional doses from day 11, and, during the second course, 11 patients received additional doses from day 9 and 2 from day 11.

Overall treatment results

Of 242 evaluable patients, 150 (62.0 %) achieved CR. Of 121 patients in the fixed-scheduled group, 73 (60.3 %) obtained CR, and of 121 in the individualized group 77 (63.6 %) achieved CR ($p = 0.6913$). In the fixed-scheduled group, 56 patients (46.3 %) achieved CR after the first course, while in the individualized group 56 patients (46.3 %) achieved CR after the first course. Of 53 (43.8 %)

Table 1 Patient characteristics

	Fixed-scheduled	Individualized	Non-randomized	Total
No. of patients	121	121	124	366
Age (years)				
65–69	54	51	29	134
70–74	42	48	36	126
75–79	25	22	32	79
80–	0	0	27	27
Median (range)	70 (65–79)	71 (65–79)	74 (65–92)	
Chromosome				
Favorable	6	14	7	27
Intermediate	91	92	91	274
Adverse	18	10	15	43
Unknown	6	5	11	22
FAB classification				
M0	10	8	10	28
M1	24	23	32	79
M2	48	52	45	145
M4	18	18	17	53
M5	13	16	12	41
M6	5	3	5	13
M7	3	1	3	7
Sex				
Male	75	65	72	212
Female	46	56	52	154
PS				
0	110	113	103	326
1	6	8	9	23
2	5	0	4	9
3			6	6
4			2	2

patients who had received additional chemotherapy during the first course of the individualized therapy, 22 (41.5 %) achieved CR (Table 2). There was no statistically significant difference in CR rates between the two groups regarding cytogenetics, gender, age, PS or FAB classification (data not shown).

The individualized group received significantly larger dosages of BHAC ($p < 0.001$) and DNR ($p < 0.001$) during the first course of induction therapy (Table 3). Myelosuppression judged by the period of leukocyte count <1,000/μL after the first course of induction therapy was significantly severer in the individualized group ($p = 0.040$) (Table 4). Early death within 30 days occurred in 5 (4.1 %) patients in the fixed-scheduled group and 4 (3.3 %) in the individualized group. There was no statistically significant difference in the incidence of complications between the two groups (Table 4).

Significant prognostic factors for the achievement of CR in all patients were cytogenetic risk group and gender (Table 2). Eighteen (90 %) of 20 patients with favorable risk cytogenetics, 120 (65.6 %) of 183 patients with intermediate risk, and 7 (25 %) of 28 with adverse risk achieved CR, respectively ($p < 0.0001$). Seventy-four (72.5 %) of 102 female patients achieved CR, while 76 (54.5 %) of 140 male patients attained it ($p = 0.0048$). These 2 factors were statistically significant and independent prognostic factors by the multivariate analysis (Table 5). Since this randomized study only included elderly patients who had met the eligibility criteria and agreed to enter the study, PS was 0 in 223 patients (92 %), 1 in 14 (6 %) and 2 in 5 (2 %). Paradoxically, patients with PS 1 or 2 had higher CR rate (84.2 %) compared with those with PS 0 (60.1 %) by the univariate analysis ($p = 0.0478$), but the difference was not statistically significant by the multivariate analysis ($p = 0.0998$).

Table 2 Response to induction therapy

Response by induction	Fixed-scheduled	Individualized	Total	*p* value
CR	73 (60.3 %)	77 (63.6 %)	150 (61.9 %)	0.6913
Non CR	48 (39.7 %)	44 (36.4 %)	92 (38.0 %)	
CR after first course	56 (46.3 %.)	56 (46.3 %)	114 (47.1 %)	
CR after second course	17 (14.0 %)	21 (17.4 %)	39 (16.1 %)	
Response by age group	65–69 years	70–74 years	75–79 years	
CR	64/106 (61.0 %)	56/90 (62.2 %)	30/47 (63.8 %)	0.9429
Response by PS	PS 0	PS 1	PS 2	
CR	134/223 (60.1 %)	11/14 (78.6 %)	5/5 (100.0 %)	0.0804
Response by PS	PS 0	PS 1 + 2		
CR	134/223 (60.1 %)	16/19 (84.2 %)		0.0478
Response by gender	Male	Female		
CR	76/140 (54.3 %)	74/102 (72.5 %)		0.0048
Response by cytogenetic risk[a]	Favorable	Intermediate	Adverse	
CR	18/20 (90.0 %)	120/183 (65.6 %)	7/28 (25.0 %)	<0.0001

[a] Cytogenetic data of 11 patients were not available

Table 3 Total administered dosage of behenoyl cytarabine (BHAC) and daunorubicin (DNR)

	BHAC (mg/m^2)		DNR (mg/m^2)	
	Average	Mean (range)	Average	Mean (range)
First course				
Fixed-scheduled (n = 121)	1,605	1,600 (200–3,000)	109	120 (40–240)
Individualized (n = 121)	1,851	1,600 (160–3,840)	139	120 (12–440)
p value	<0.001		<0.001	
Second course				
Fixed-scheduled (n = 42)	1,633	1,600 (1,600–2,400)	106	105 (60–180)
Individualized (n = 44)	1,732	1,600 (160–4,200)	123	120 (12–315)
p value	0.234		0.026	

Table 4 Toxicity during induction therapy

	Fixed-scheduled (n = 121)	Individualized (n = 121)	
Leukopenia			
G3/4	119 pts. (98.3 %)	117 pts. (96.7 %)	$p = 0.513$
G4	107 pts. (88.4 %)	111 pts. (91.7 %)	
Median duration of leucocytes <1,000/μl in G4 pts.			
1st course	14 days (2–52)	17 days (2–78)	$p = 0.04$
2nd course	15.5 days (2–32)	17.5 days (2–35)	$p = 0.24$
Use of G-CSF			
1st course	40 pts. (33.1 %)	44 pts. (36.4 %)	$p = 0.686$
2nd course	15 pts. (34.9 %)	11 pts. (24.4 %)	$p = 0.352$
Hemorrhage (CNS, pulmonary, GI): G3/4,	3 pts. (2.5 %)	3 pts. (2.5 %)	$p = 1$
Infection: G3/4	13 pts. (10.7 %)	11 pts. (9.1 %)	$p = 0.72$
Febrile neutropenia: G3/4	45 pts. (37.2 %)	48 pts. (39.7 %)	$p = 0.696$

Table 5 Multivariate analysis for achievement of complete remission and overall survival

Variable	Classification	No. of patients	Odds ratio	95 % CI	p value
Multivariate analysis for complete remission					
Treatment group	Fixed/individualized	115/116	0.973	0.543–1.744	0.9263
Age		231	1.000	0.925–1.081	0.9979
Sex	Male/female	133/98	2.192	1.200–4.003	0.0106
PS	0/1 + 2	213/18	3.065	0.808–11.634	0.0998
Cytogenetic risk	Favorable/Adverse	20/28	28.435	5.108–158.288	0.0001
	Intermediate/Adverse	183/28	4.764	1.878–12.086	0.0010
Multivariate analysis for overall survival					
Treatment group	Fixed/Individualized	115/116	1.037	0.751–1.430	0.8264
Age		231	1.003	0.961–1.047	0.8924
Sex	Male/female	133/98	0.747	0.530–1.052	0.0952
PS	0/1 + 2	213/18	0.833	0.365–1.902	0.6650
Cytogenetic risk	Favorable/adverse	20/28	0.390	0.185–0.820	0.0130
	Intermediate/adverse	183/28	0.422	0.261–0.680	0.0004

Analyzed in 231 patients by excluding 11 patients whose cytogenetic data were not available

Of 150 patients who had achieved CR, 63 patients were randomly assigned to receive ubenimex during 3 courses of consolidation therapy, plus 3 more months thereafter, and 60 received no ubenimex. All courses of consolidation therapy were administered to 65 (84.4 %) of 77 patients in the individualized group and 58 (79.5 %) of 73 patients in the fixed-scheduled group (p = 0.5248). There was no significant difference between patients receiving ubenimex or none, regarding myelosuppression and non-hematological toxicity, as well as complications during the consolidation therapy.

At a median follow-up of 39 months (range 2–76 months), predicted 4-year OS was 18.3 % for the fixed-scheduled group

and 17.1 % for the individualized group ($p = 0.807$) (Fig. 2a), and predicted 4-year RFS for patients who had achieved CR was 8.8 % for the former group and 17.9 % for the latter ($p = 0.467$) (Fig. 2b). Significant prognostic factors for OS were cytogenetic risk group and gender (Table 5). Predicted 2-year OS for patients with favorable cytogenetic risk was 56.4 %, while that for patients with intermediate risk was 35.8 % and for patients with adverse risk was 12.6 % ($p < 0.0001$ both for favorable and intermediate risk groups vs. adverse risk group) (Fig. 3). Predicted 4-year OS for female patients was 24.4 %, while that for male patients was 13.5 % ($p = 0.0018$) (Fig. 4). By the multivariate analysis, cytogenetic risk group was a significant prognostic factor ($p < 0.0001$), but the significance regarding gender was marginal ($p = 0.0106$) (Table 5). It is of note that there was no significant difference in OS among patients of age 65–69, 70–74 and 75–79 (Fig. 5). For 124 patients in the non-randomized observation cohort, predicted 1-, 2-, 3- and 4-year OS was 46.5, 33.7, 26.5 and 21.6 %, respectively, which did not differ from the randomized cohorts (Fig. 2a).

Among patients who had obtained CR, predicted 4-year OS was 32.3 % for 63 patients in the ubenimex group, and 18.7 % for 60 patients in the control group ($p = 0.111$) (Fig. 6a). Predicted 4-year RFS was 16.4 % for the former group and 10.4 % for the latter, in favor of the ubenimex group ($p = 0.061$ by the log-rank test and $p = 0.014$ by the generalized Wilcoxon test) (Fig. 6b).

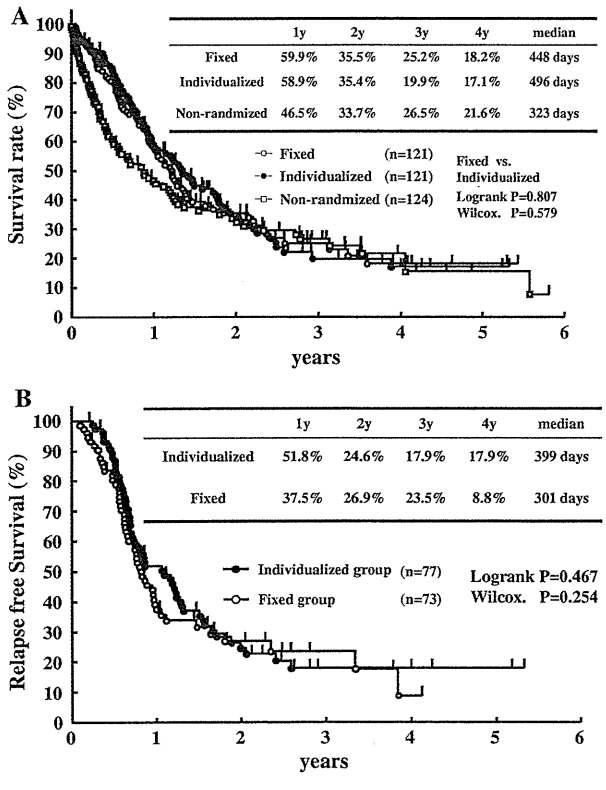

Fig. 2 Overall survival and relapse-free survival. Overall survival rate in three groups (**a**). There was no significant difference in each group. Relapse-free survival in fixed and individualized group (**b**). There was no significant difference in each group

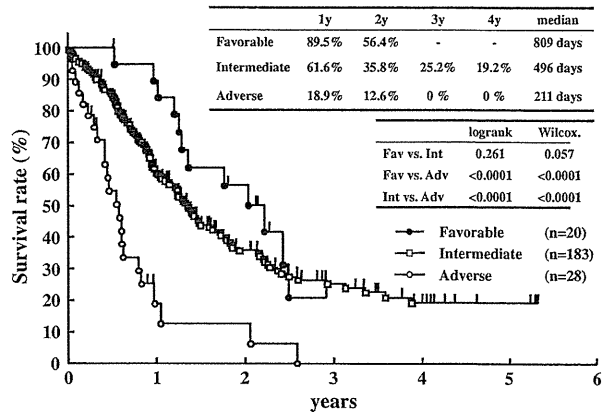

Fig. 3 Overall survival according to cytogenetics. Survival rate decreases down according to cytogenetics group

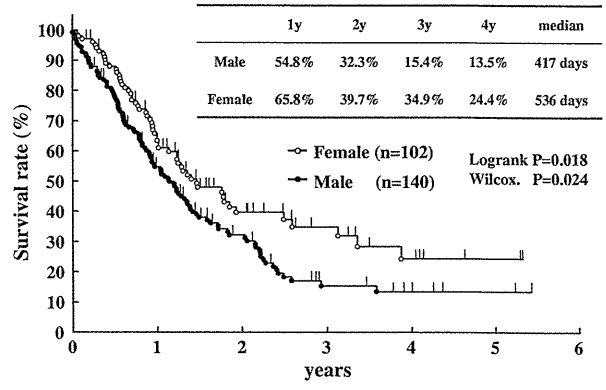

Fig. 4 Overall survival according to gender. There was significant difference between male and female

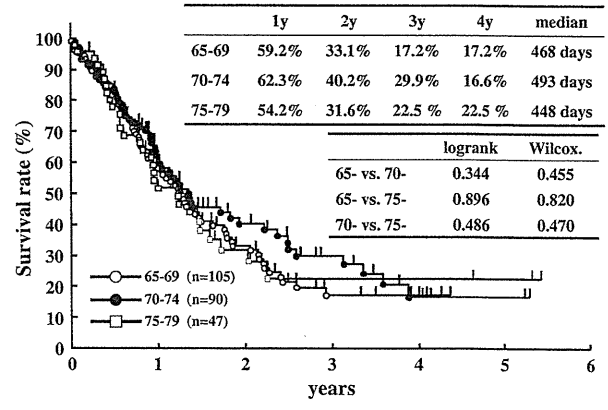

Fig. 5 Overall survival according to age. There was no significant difference in three age groups

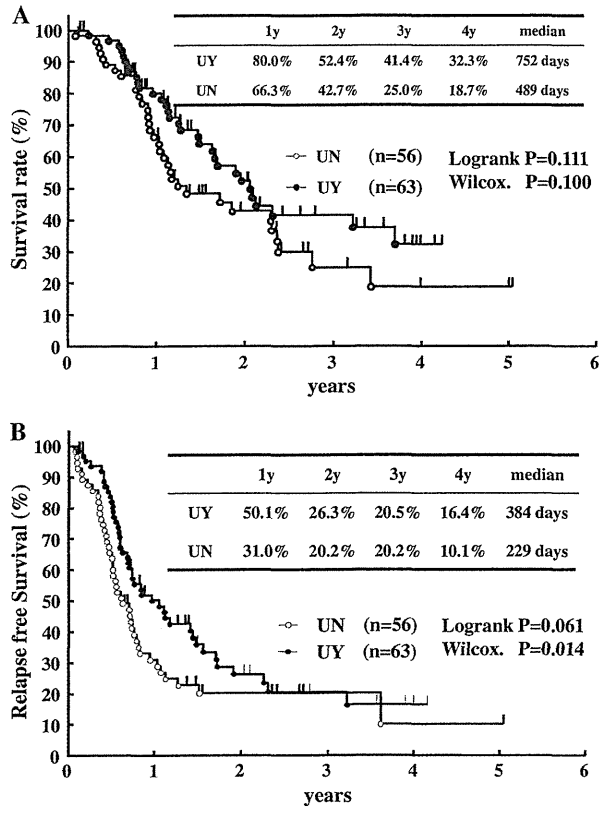

Fig. 6 Overall survival and relapse free survival for with ubenimex group and without ubenimex group. Overall survival (**a**). There was no significant difference between two groups. Relapse-free survival (**b**). There was significant difference by Wilcoxon analysis. UY is with ubenimex group, UN is without ubenimex group

Discussion

Aging generally causes comorbidity, poor performance status, decreased immune competency, deficient stem cell reservoir in bone marrow and so on, and inevitably puts patients at a great disadvantage for receiving intensive chemotherapy. Additionally, elderly AML is biologically associated with higher frequency of adverse karyotypes such as complex abnormalities and aberrations of chromosomes 5 or 7, MDR1 expression, antecedent MDS and secondary AML. Thus, the treatment outcome of elderly patients with AML is much poorer than that of younger patients, when treated with currently available intensive therapy using cytotoxic drugs [1–6].

Recently, HOVON/AMLSG/SAKK group reported that escalation of the dose of DNR to twice the conventional dose to elderly patients of age 60–83 (median 67) with AML or high-risk refractory anemia resulted in higher CR rate (64 vs. 54 %, $p = 0.0002$) without additional toxic effects, but that OS did not differ significantly between the two groups. Subset analysis, however, revealed that only patients of age 60–65 in the escalated-treatment group had significantly higher CR rate (73 vs. 51 %), event-free survival (29 vs. 14 %), and OS (38 vs. 23 %) than patients of the same age range in the conventional dose group, indicating that there was no advantage in the escalated treatment to patients older than 65 [21].

In Japan, where people enjoy the longest life expectancy in the world, JALSG has regarded patients as elderly when they were 65 years or older, since the AML95 study started in 1995, after the analysis of the treatment outcomes of preceding AML87, AML89 and AML92 studies in which patients of age 65 or older were included [9–11]. Thus, even in the HOVON/AMLSG/SAKK study, the question of recommendable treatment for elderly patients older than 65 remains unsettled.

Most drug therapies are generally carried out in a response-oriented and individualized manner, and physicians adjust dosage and treatment period depending on the response of patient's symptoms to administered drugs. However, cancer chemotherapy is generally carried out by fixed dosage and period, because the nadir of myelosuppression, the most important toxic effect of cytotoxic drugs, appears 7–10 days after the discontinuation of drugs. Myelosuppression is usually judged by leukocyte or platelet counts in the peripheral blood, but, if it is judged by bone marrow itself, it is possible to obtain information on myelosuppression directly and earlier.

We attributed the higher CR rates of our previous JALSG studies for adult AML: AML87 [9], AML89 [10] and AML92 [11], to response-oriented individualized therapy, which administered highly intensive but not too toxic doses of anti-leukemia drugs, especially DNR. Disappointingly, however, a prospective randomized study for AML of younger patients of age <65, the JALSG-AML95 failed to demonstrate that response-oriented individualized therapy was superior to the fixed-scheduled therapy, although IDR instead of DNR was used in combination with Ara-C in this study [15]. Both regimens resulted in very high CR rates: 79 and 82 %, respectively, but leukocytopenia was significantly severer and its duration significantly longer, and early death within 30 days tended to occur more frequently in the individualized group. We speculated that, if DNR instead of IDR had been used, the CR rate of the fixed-scheduled group might have been lower like around 70 % as reported from other large scale multicenter studies.

In the present study with elderly patients, we again prospectively compared a fixed-scheduled therapy with a response-oriented individualized therapy, utilizing DNR and BHAC. BHAC has been chosen because this analogue of Ara-C is administered by 3-h infusion, instead of 24-h continuous infusion required for Ara-C, and thus is more conveniently given especially to elderly patients, and also

because BHAC in combination with DNR, 6MP and PSL produced over 70 % CR rates in adult AML in the previous JALSG studies.

Again, however, we could not demonstrate that the response-oriented individualized therapy was not inferior to the fixed-scheduled therapy. CR rate and OS were almost the same in both groups. Patients in the individualized therapy group, being given additional drugs on day 8 and thereafter, showed severer myelosuppression, but the 30-day mortality rates were almost the same in both groups.

Ubenimex is a small molecule inhibitor of leucine aminopeptidase and has various immunomodulatory properties via macrophage or T cell activation. A myeloid lineage marker, CD 13, has been identified as aminopeptidase N36. Ubenimex inhibits aminopeptidase N, and increases the sensitivity of leukemia cells to apoptosis through the inhibition of cell-surface aminopeptidase N activities by hampering the degradation of endothelial cell-derived interleukin 8 [16, 22–24]. In the JALSG AML89 study for younger patients with AML, however, we could not demonstrate that ubenimex given after the end of maintenance chemotherapy improved DFS of AML patients [25]. In this study for elderly AML, ubenimex given orally during and after the consolidation therapy did not clearly improve OS, although RFS in the ubenimex group was longer than that in no-ubenimex control group ($p = 0.061$ by the log-rank test and $p = 0.014$ by the generalized Wilcoxon test).

Cytogenetic risk factor was the most important prognostic factor in this study. Although the number of cytogenetically favorable risk group was small (9 %), 90 % achieved CR and predicted 2-year OS was 56 %. Of patients with intermediate risk cytogenetics, 66 % achieved CR and predicted 4-year OS was 19 %. Of patients with adverse risk cytogenetics, only 25 % achieved CR and predicted 3-year OS was 0 %. Thus, elderly patients with favorable and intermediate risk karyotypes seemed to be benefitted from the present chemotherapy, but not those with adverse risk cytogenetics.

One interesting observation from this study was that female elderly patients had significantly higher CR rate and better OS compared with male patients. Although our female patients tended to have less adverse risk cytogenetics, gender was an independent significant factor for the achievement of CR ($p = 0.0106$) and marginal one for OS ($p = 0.0952$) by the multivariate analysis. In our past adult AML studies, there has been no such observation. ECOG reported that female gender was one of the independent prognostic factors to predict a long-term survival of more than 3 years among 1,414 adult AML patients, but karyotypes were not included in their analysis [26]. German Study Alliance Leukemia recently proposed a novel prognostic model for elderly patients with AML, based on the data of 909 patients entered into the prospective trial, but female gender was not a prognostic factor in achievement of CR, or in OS, either [27]. On the other hand, in childhood leukemia, female patients generally have better prognosis, although no clear explanation has been provided so far. The average remaining life expectancy of Japanese female of age 65 in 2002 was 22.4 years which is 5 years longer than Japanese male of the same age (17.4 years), and this may apply to leukemia patients. However, the higher CR rate is not explainable by this statistics of average life expectancy. Another notable observation was that age was not prognostic factor in the present setting. If patients are eligible for rather strict inclusion criteria as in this study, chronological age alone should not be regarded as a single bad prognostic factor.

In conclusion, we could not demonstrate that the response-oriented individualized therapy gave a better treatment outcome in elderly AML of age 65 or older. Ubenimex given concomitantly during consolidation therapy and thereafter showed a marginal benefit in RFS, but was not impressive. The treatment of elderly AML is still being explored, and new effective therapeutic drugs, especially pathogenic molecule-specific target drugs, are desperately awaited for the treatment of this leukemia, which is increasing in number all over the world.

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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.

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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.

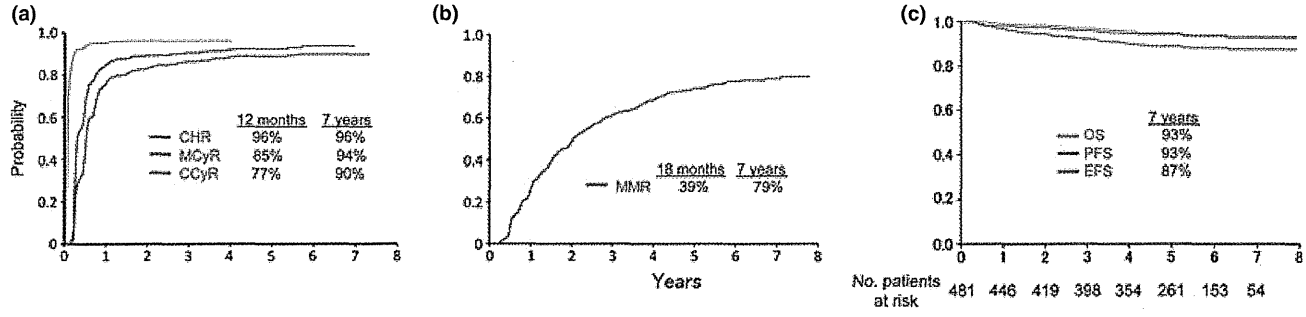

Fig. 1. Cumulative best (a) cytogenetic and (b) molecular responses and (c) survival of patients on imatinib therapy for chronic phase CML. Cumulative rates of responses were estimated according to the competing risk method. Discontinuation of imatinib was evaluated as a competing risk. CHR, complete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

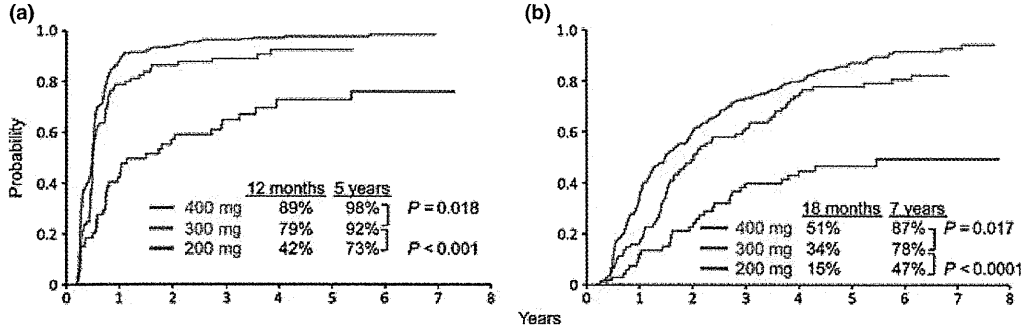

Fig. 2. Cumulative rates of best responses according to the mean daily dose during the first 24 months of treatment with imatinib. (a) Cumulative rates for complete cytogenetic responses (CCyR). (b) Cumulative rates of major molecular responses (MMR). Mean daily doses in the 400- (*n* = 294), 300- (*n*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

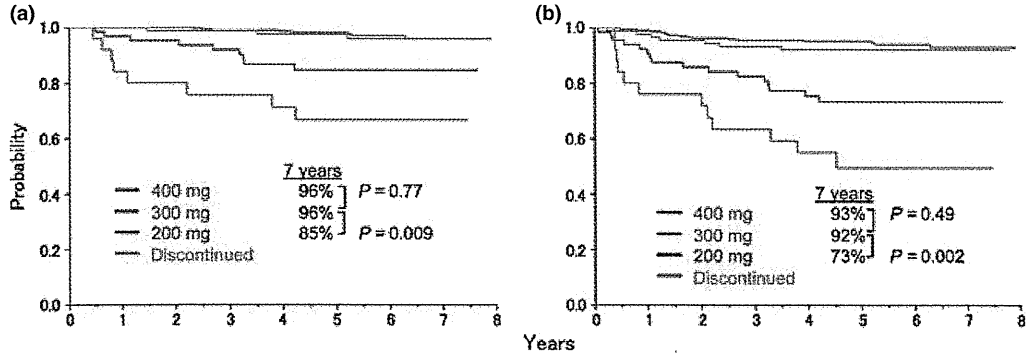

Fig. 3. (a) Overall and (b) event-free survival according to the mean daily dose during the first 24 months. Mean daily doses in the 400- (*n* = 294), 300- (*n* = 90), and 200-mg (*n* = 67) groups were ≥360, 270–359, and < 270 mg imatinib, respectively.

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

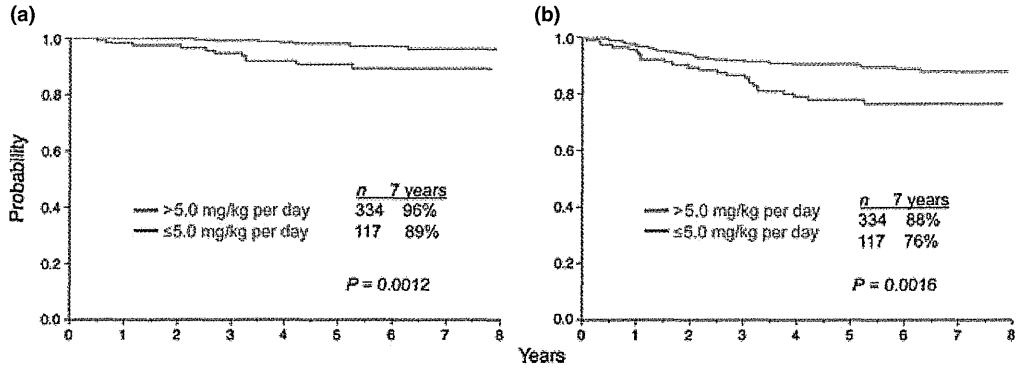

Fig. 4. (a) Overall and (b) event-free survival according to the mean daily dose during the first 24 months per body weight. The cut-off value was set at >5.0 mg/day/kg (e.g. if a patient whose body weight was <60 kg received imatinib at a mean daily dose of 300 mg).

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)				
	>5.0†		≤5.0		*P*-value
	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

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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 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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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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