by searching with various keywords characteristic to each subcategory (such as DNA repair, regulation of chromatin structure, etc.), followed by manual inspection. The final gene list for the wafers is shown in Supplementary Table S6. Construction of the wafers, quality control analysis and data processing are described in Supplementary Text.

JAK3 analysis

Complementary DNAs for JAK3 mutants were generated using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) and ligated into the pMX retroviral vector (Onishi *et al.*, 1996). Ecotropic recombinant retroviruses encoding each mutant were produced in BOSC23 cells transfected with the corresponding pMX-based plasmid and were used to infect BA/F3 or 32D cells as described previously (Choi *et al.*, 2007). Both types of cell were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (both from Life Technologies, Carlsbad, CA, USA) and mouse IL-3 (Sigma, St Loius, MO, USA) at 10 Units/ml; differentiation of 32D cells was induced by culture in the presence of serum and mouse granulocyte colony-stimulating factor (Sigma) at 0.5 ng/ml. A concentrated preparation of a retrovirus with a VSV-G envelope and encoding both JAK3(M511I) and enhanced green fluorescent protein was used to infect $CD34^-$ c-Kit^+ $Sca\text{-}1^+$ $Lineage\text{-}marker^-$ ($CD34^-KSL$) hematopoietic stem cells isolated from the bone marrow of C57BL/6 mice, and the infected cells were transplanted into lethally irradiated mice congenic for the *Ly5* locus (Iwama *et al.*, 2004). *CD4*, *JAK2* and *JAK3* mRNAs were quantitated by reverse transcription and real-time PCR analysis using an ABI7900HT system (Life Technologies) and with the primers 5′-CTGGAATCCAACATCAAGGTTCTG-3′ and 5′-AATTGTAGAGGAGGCGAACAGGAG-3′ for CD4, 5′-CTCCAGAATCACTGACAGAGAGCA-3′ and 5′-CCAC TCGAAGAGCTAGATCCCTAA-3′ for *JAK2* and 5′-GAGC TCTTCACCTACTGCGACAAA-3′ and 5′-AGCTATGAAA AGGACAGGGAGTGG-3′ for JAK3; the cDNA for GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was also amplified with the primers 5′-GTCAGTGGTGGACC TGACCT-3′ and 5′-TGAGCTTGACAAAGTGGTCG-3′. The relative abundance of the cDNAs of interest was calculated from the threshold cycle (C_T) for each cDNA and that for *GAPDH* cDNA.

DNMT3A analysis

Recombinant His_6-tagged DNMT3A or DNMT3A(R882H) was expressed in SF9 cells using the Bac-to-Bac baculovirus expression system (Invitrogen, Carlsbad, CA, USA), and each protein was purified by stepwise column chromatography as described previously (Suetake *et al.*, 2003). The enzymatic activity of each protein was assayed with -*S*-adenosyl-L-methionine (GE Healthcare, Waukesha, WI, USA) and dIdC or dGdC as substrates (Suetake *et al.*, 2003). The association between Myc epitope-tagged human DNMT3L and wild-type or R882H forms of human DNMT3A in transfected HEK293 cells was examined by immunoprecipitation and immunoblot analyses.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Imatinib for newly diagnosed chronic-phase chronic myeloid leukemia: results of a prospective study in Japan

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Abstract Although imatinib has become the current standard treatment for chronic myeloid leukemia (CML), there is limited information regarding its efficacy and safety among Japanese patients. We therefore conducted a prospective multi-center open-label study of imatinib for Japanese patients with newly diagnosed chronic-phase CML (CP-CML). A total of 107 patients were enrolled and treated with imatinib at an initial daily dose of 400 mg. Eighty-three patients completed 3 years of study treatment. The cumulative rates of major cytogenetic response and complete cytogenetic response (CCyR) were 90.9 and 90.2% at 3 years, respectively. The safety profile was not very different from that reported in the IRIS study, although grade ≥3 neutropenia occurred relatively frequently (31.8 vs. 14.3%). Only seven patients discontinued the study due to adverse events, as did four patients due to

T. Nagai and J. Takeuchi contributed equally to this study, and the order in which they are listed should be considered arbitrary.

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insufficient efficacy. The 3-year probabilities of overall survival and progression-free survival were 93.2 and 91.4%, respectively. Higher average daily doses (i.e., ≥350 mg) were significantly associated not only with higher rates of achieving CCyR, but also with longer duration of CCyR. These findings confirm the clinical utility of imatinib in Japanese patients with newly diagnosed CP-CML, and suggest detrimental effect of low average daily dose on treatment results.

Keywords Chronic myeloid leukemia · Chronic phase · Newly diagnosed · Imatinib

1 Introduction

Imatinib is a molecule-targeting drug that inhibits BCR-ABL tyrosine kinase and exerts a selective proliferation-inhibitory effect in chronic myeloid leukemia (CML) [1, 2]. Several international trials have documented excellent clinical efficacy of imatinib in patients with chronic-phase CML (CP-CML) [3–5], as well as in patients in accelerated phase (AP) [6] and blast crisis (BC) [7]. Based on those studies along with Japanese phase I and phase II studies [8], imatinib was approved in Japan in November 2001, and has been available in clinical practice since December 2001. However, there is very limited information regarding efficacy and safety of imatinib among Japanese patients. We therefore conducted a post-marketing study to confirm clinical utility of imatinib in Japanese patients with newly diagnosed CP-CML.

2 Patients and methods

2.1 Study design

This was a prospective, multi-center, non-controlled study to evaluate efficacy and safety of imatinib in Japanese patients 15–74 years of age with Philadelphia chromosome positive (Ph+) CP-CML. Eligible patients were those with Eastern Cooperative Oncology Group performance status 0–3 who had been previously untreated with interferon (IFN) or imatinib. Patients were excluded if serum bilirubin or serum creatinine levels were ≥3 times the upper limit of the normal range, if serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels were ≥5 times the upper limit of the normal range, if they received hydroxycarbamide within a week prior to enrollment or any other antileukemic drug within 2 weeks, or if there was any evidence of AP or BC in association with any of the following conditions: ≥15% blasts in the peripheral blood or bone marrow; ≥30% blasts plus promyelocytes in the peripheral blood or bone marrow; ≥20% basophils in the peripheral blood; or extramedullary leukemic infiltrates with the exception of spleen or liver. Women who were pregnant or possibly pregnant were also excluded.

Patients were treated with imatinib at a daily dose of 400 mg. Dose escalation to 600 mg was implemented if they had failed to achieve complete hematologic response (CHR) at 3 months or major cytogenetic response (MCyR) at 6 months. If the patient had failed to achieve MCyR at 9 months, IFN was started at a daily dose of 300 million unit per body two or three times a week while on imatinib. Dose modification of imatinib was generally based on the following guidelines. For grade ≥3 liver dysfunction (elevated bilirubin, AST, or ALT), administration was interrupted until recovery to grade <2, and then resumed at 300 mg/day. For grade ≥3 neutropenia or thrombocytopenia, administration was interrupted until recovery to grade <2, and then resumed at 400 mg/day. If grade ≥3 toxicity recurred after resuming, dose reduction to 300 mg/day was implemented. The study was discontinued in the event of failure to achieve CHR at 6 months, intolerance to imatinib, disease progression to AP or BC, death, patient request, and lost to follow-up, or at the discretion of the investigator. Patients were followed up to 3 years from the day of starting imatinib.

2.2 Endpoints

The primary endpoints were overall survival (OS) and progression-free survival (PFS). OS was defined as the time from the day of first dose of imatinib to death or last follow-up, and PFS was defined as the time from the day of first dose of imatinib to progression to AP or BC, death or last follow-up. Secondary endpoints were hematologic, cytogenetic and molecular response, and adverse events. Cytogenetic response was assessed by using bone marrow cells every 3 months until 12 months and every 6 months thereafter until 36 months. Complete cytogenetic response (CCyR) was defined as complete disappearance of the Philadelphia chromosome. MCyR was defined as decrease in Philadelphia chromosome to 35% or lower. Adverse events were assessed according to the National Cancer Institute Common Toxicity Criteria version 2.0.

Cumulative rates of hematologic and cytogenetic response, PFS, event-free survival (EFS), and OS were evaluated in accordance with the IRIS study reports [3, 9]. EFS was defined as the time from the day of first dose of imatinib to death, progression to AP or BC, loss of CHR, loss of MCyR, increase in white blood cell count to 20000/μL, or last follow-up.

This study was conducted in compliance with the Declaration of Helsinki and was approved by local Institutional Review Boards. All patients provided written informed consent prior to initiation of study medication.

2.3 Statistical methods

OS, PFS, and EFS were estimated by using the Kaplan–Meier method. Efficacy endpoints were compared by patient age (≥60 years and <60 years), and by average daily dose of imatinib (i.e., ≥350 mg/day, 250 to <350 mg/day and <250 mg/day). The Wilcoxon two-sample test was used to compare the average daily dose between the age groups. In patients who had achieved CCyR, CCyR duration was compared by average daily dose of imatinib after achieving CCyR. Average daily dose was calculated as cumulative dosage divided by the total days on study.

3 Results

3.1 Patients

A total of 107 patients were enrolled in the study between November 2002 and June 2004, and administered imatinib. All patients were evaluable for efficacy and safety, and the median duration of imatinib exposure was 1091 days (range, 82–1156 days). Among these patients, 83 completed 3 years of study treatment, whereas 24 discontinued the study due to adverse events ($n = 7$), withdrawal of consent ($n = 5$), insufficient efficacy ($n = 4$), allogeneic bone marrow transplantation ($n = 4$), and other reasons ($n = 4$). Demographic characteristics of patients in the full analysis set are summarized in Table 1. The median age was 47 years (range, 16–74 years), with 71 males and 36 females. Prior therapies for CML included hydroxycarbamide ($n = 7$), and leukapheresis ($n = 1$). The median time from diagnosis of CML to initiation of imatinib was 8.0 days (range, 1–1526 days).

The initial dose of imatinib was 400 mg/day for all patients. The mean (±standard deviation) dose administered during the study was 343 (±90) mg/day. Dose modification was required in 70.1% of patients mainly due to adverse events. Details of dose modification are summarized in Table 2. There were no patients in whom IFN was added to imatinib. Average daily doses were ≥350 mg, 250 to <350 mg, and <250 mg in 68 (63.6%), 21 (19.6%) and 18 patients (16.8%), respectively. As shown in Table 3, the percentage of patients who continued imatinib at 400 mg/day without any dose modification was 48.6% during week 1–13, 57.5% during week 14–26, and was around 60% thereafter.

3.2 Treatment results

The cumulative rate of CHR was 99.1% at 1 year, and the cumulative rates of MCyR and CCyR were 90.9 and 90.2% at 3 years, respectively (Fig. 1). The median time to CHR

Table 1 Patient characteristics

Characteristics	Category	Number of patients
Total number of subjects		107
Sex	Male	71 (66.4)
	Female	36 (33.6)
Age	10s	4 (3.7)
	20s	10 (9.3)
	30s	24 (22.4)
	40s	18 (16.8)
	50s	26 (24.3)
	60s	19 (17.8)
	70s	6 (5.6)
	Mean ± SD	47.1 ± 14.7
	Minimum–maximum	16–74
	Median	47.0
Body weight	40 to <50 kg	13 (12.1)
	50 to <60 kg	34 (31.8)
	60 to <70 kg	40 (37.4)
	70 to <80 kg	13 (12.1)
	80 to <90 kg	5 (4.7)
	≥90 kg	2 (1.9)
	Mean ± SD	61.66 ± 10.88
	Minimum–maximum	43.0–103.0
	Median	61.50
Body surface area	1.2 to <1.4 m^2	5 (4.7)
	1.4 to <1.6 m^2	31 (29.0)
	1.6 to <1.8 m^2	53 (49.5)
	1.8 to <2.0 m^2	15 (14.0)
	≥2.0 m^2	3 (2.8)
	Mean ± SD	1.6705 ± 0.1670
	Minimum–maximum	1.307–2.151
	Median	1.6800
Previous CML therapy	No	99 (92.5)
	Yes	8 (7.5)
	Hydroxycarbamide	7 (6.5)
	Leukapheresis	1 (0.9)
ECOG performance status	0	95 (88.8)
	1	10 (9.3)
	2	2 (1.9)
Time elapsed from the first day of CML diagnosis to the start of study treatment	<4 weeks	92 (86.0)
	4 to <13 weeks	14 (13.1)
	≥52 weeks	1 (0.9)
	Mean ± SD	27.0 ± 146.9
	Minimum–maximum	1–1526
	Median	8.0

Values within parenthesis are given in percentage

SD standard deviation, *CML* chronic myeloid leukemia, *ECOG* Eastern Cooperative Oncology Group

and CCyR were 92.5 days (range, 75–207 days) and 179.5 days (range, 70–589 days), respectively. In 92 patients who had achieved CCyR, 77 patients remained in CCyR until the end of 3 years of imatinib treatment. All of the 15 patients who hadn't achieved CCyR discontinued the study. Among them, 4 patients progressed to AP or BC, and 5 patients proceeded to hematopoietic stem cell transplantation.

Of 107 patients, progression to AP or BC and death occurred in nine and seven patients, respectively. One death, which was because of pneumonia, was reported during the study and the remaining six deaths were reported after patients discontinued the study. The probabilities of OS, PFS and EFS at 3 years were 93.2% [95% confidence interval (CI) 88.3–98.1%), 91.4% (95% CI 86.1–96.8%), and 81.9% (95% CI 74.6–89.3%), respectively (Fig. 2).

3.3 Response and survival by average daily dose

Next, we evaluated cumulative CCyR rate, OS, PFS, and EFS according to the average daily dose of imatinib (≥350 mg/day, 250 to <350 mg/day, and <250 mg/day). As shown in Figs. 3, 4, CCyR and EFS were significantly associated with the average daily dose ($p < 0.001$, respectively). In particular, patients with the average daily dose <250 mg had low rates of CCyR and EFS. CCyR duration was also significantly different according to the average daily dose ($p < 0.001$, Fig. 5). OS and PFS seemed lower in those with lower average daily dose, although the differences did not reach statistical significance.

The average daily doses were significantly different by age group, with 360 (±81) mg in patients aged <60, and 287 (±97) mg in patients aged ≥60 years ($p < 0.001$). Patients aged <60 had statistically non-significant better EFS than those aged ≥60 years (85.3 vs. 70.6% at 3 years, $p = 0.101$). In terms of OS or PFS, there were no significant differences between the age groups.

Table 2 Summary of dose modification

	n	%
No dose change	32	29.9
Dose change	75	70.1
Reduction only	7	6.5
Reduction and interruption	43	40.2
Reduction and increase	1	0.9
Increase only	1	0.9
Increase and interruption	4	3.7
Interruption only	19	17.8
Total	107	100.0

3.4 Adverse events

Adverse events were reported in all of the 107 patients. Serious adverse events which developed in ≥2 patients included neutropenia ($n = 4$), blast crisis ($n = 3$), anemia, intestinal obstruction, gastric antral vascular ectasia, appendicitis, herpes zoster, thrombocytopenia, and leukocytopenia ($n = 2$, each). Grade ≥3 adverse events were reported in 31 patients (29.0%, 47 episodes). As listed in Table 4, grade ≥3 adverse events reported in >5% of patients were neutropenia (31.8%), leukocytopenia (19.6%), lymphocytopenia (17.8%), thrombocytopenia (14.0%), and rash (8.4%). When frequencies of adverse events were compared between this study and the IRIS study [3], nasopharyngitis, rash, upper respiratory tract infection, pyrexia, and grade ≥3 neutropenia seemed more frequent, while nausea, muscle cramp, joint pain seemed less frequent in our study.

4 Discussion

Although it is widely accepted that imatinib is the standard treatment for CP-CML, published experiences of imatinib

Table 3 Average daily dose of imatinib over time

Week:	1–13		14–26		27–39		40–52		53–78		79–104		105–130		131–156	
No. of patients (*n*):	107		106		102		95		92		90		88		88	
Average daily dose (mg)	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
<200	3	2.8	12	11.3	9	8.8	7	7.4	6	6.5	4	4.4	3	3.4	1	1.1
200 to <300	24	22.4	16	15.1	11	10.8	7	7.4	9	9.8	9	10.0	9	10.2	14	15.9
300 to <350	13	12.1	12	11.3	14	13.7	18	18.9	12	13.0	12	13.3	16	18.2	12	13.6
350 to <400	15	14.0	4	3.8	4	3.9	0	0.0	5	5.4	5	5.6	4	4.5	6	6.8
400	52	48.6	61	57.5	61	59.8	61	64.2	58	63.0	58	64.4	54	61.4	52	59.1
>400	0	0.0	1	0.9	3	2.9	2	2.1	2	2.2	2	2.2	2	2.3	3	3.4

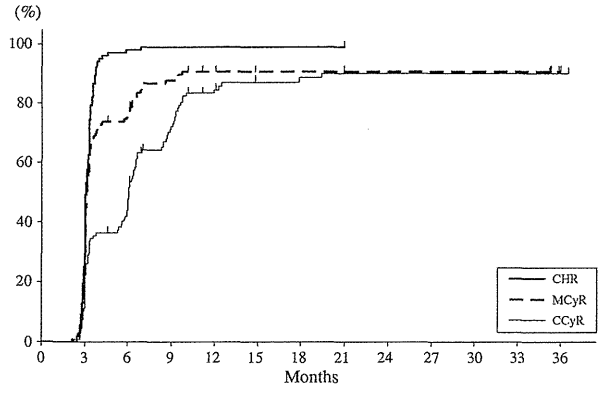
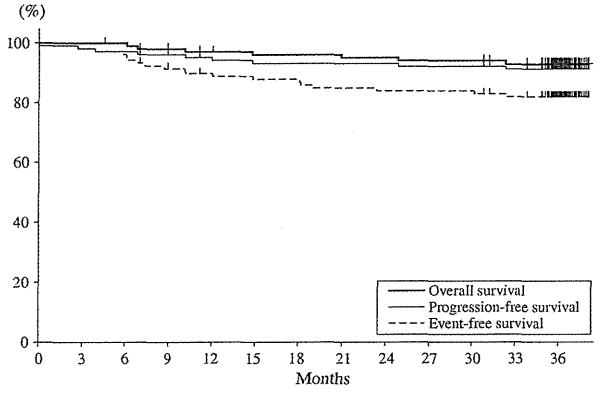


Fig. 1 Kaplan–Meier curves of cumulative rates of complete hematologic response (*CHR*), major cytogenetic response (*MCyR*) and complete cytogenetic response (*CCyR*)



	No. of Events	Estimated 3-year rate (%)
OS	7	93.2
PFS	9	91.4
EFS	19	81.9

Fig. 2 Kaplan–Meier curves of overall survival, progression-free survival and event-free survival of all patients

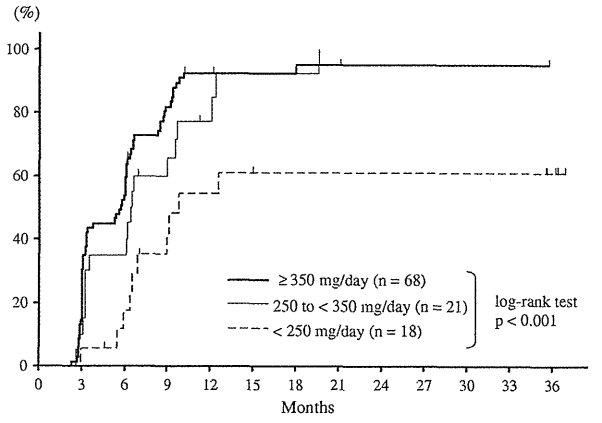


Fig. 3 Kaplan–Meier curves of cumulative rates of complete cytogenetic response (CCyR) by average daily dose of imatinib

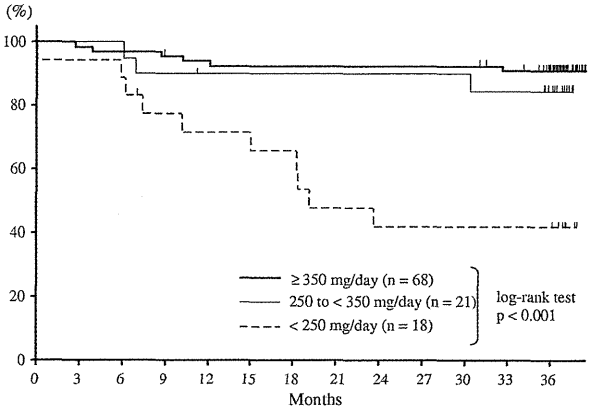


Fig. 4 Kaplan–Meier curves of event-free survival by average daily dose of imatinib

in Japanese patients are limited [8, 10–16]. Under such circumstances, a nationwide registration system for CML has been established by the Japanese Society of Hematology since 2003, and early results were published [15]. To further clarify the clinical utility of imatinib among Japanese patients, we conducted a prospective study of imatinib in 109 patients with newly diagnosed CP-CML. MCyR and CCyR rates at 12 months were 90.9 and 84.8%, which were comparable or even superior to those in the IRIS study (85 and 69%, respectively) [9]. Likewise, long-term outcomes were not different between both studies, because the OS rate in our study was 93.2% at 3 years, whereas, in the IRIS study, it was reported to be 97.2% at 18 months and 89% at 5 years [3, 9]. The safety profile observed in our study was almost comparable with that of the IRIS study, although grade ≥3 neutropenia occurred relatively frequently in our study than in the IRIS study (31.8 vs. 14.3%), while the incidences of neutropenia of all grades were not different (53.3% in our study versus 60.8% in the IRIS study). In both studies, imatinib was initiated at a daily dose of 400 mg and interrupted in the event of grade ≥3 neutropenia or thrombocytopenia until the toxicity resolved to grade <2. The reason for this observation was not clear; however, the finding that only seven of our patients discontinued the study due to adverse events showed feasibility of the treatment. Some non-hematological adverse events like nausea, muscle cramp, and joint pain were less frequent in Japanese than in Caucasians. These efficacy and safety results, taken together, confirmed the clinical utility of imatinib in Japanese patients with newly diagnosed CP-CML.

Based upon observations in a relatively small number of Japanese patients, some authors have suggested the possibility that the daily dose of imatinib could be reduced to less than 400 mg without significant disadvantage, partly

due to smaller body size as compared with Caucasians [12, 13]. Analyses of cumulative rate of CCyR and EFS by average daily dose in our study showed that patients given higher average daily doses of imatinib (≥350 mg) not only achieved higher CCyR rate but also had longer CCyR duration than those given lower average daily doses. EFS was also superior among patients who were treated with higher average daily doses of imatinib. Matsuo et al. [10] reported similar findings of a clear dose–response relationship between imatinib daily dose and treatment results. In that study, CCyR rate at 30 months was higher in patients receiving daily dose of imatinib >300 mg than in those receiving 250–300 mg, or <250 mg. Sugita et al. [16] also reported that mean daily doses of ≥300 mg led to higher CCyR rate, longer CCyR duration, and improved OS as compared to 200–300 mg. These results, taken together, suggest detrimental effect of low average daily dose on treatment results. Our observation that EFS was relatively lower in patients aged ≥60 years than in those aged <60 years might be explained partly by the difference in the average daily dose. To achieve and maintain better response, it would be beneficial to avoid excessive dose

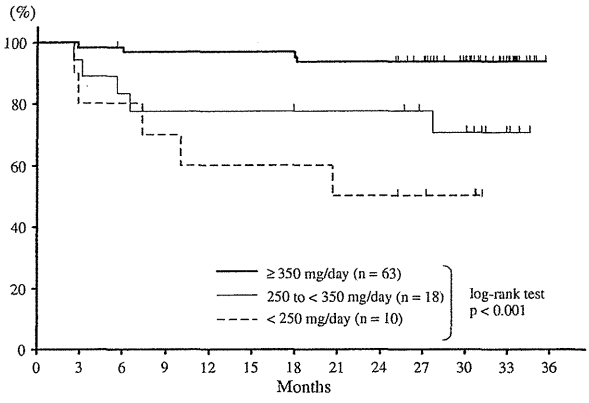


Fig. 5 Kaplan–Meier curves of duration of complete cytogenetic response (CCyR)

Table 4 Comparison of adverse events between this study and the IRIS study

	This study (n = 107)				IRIS study (n = 533) [3]			
	All grades		Grade 3/4		All grades		Grade 3/4	
	n	%	n	%	n	%	n	%
Hematological								
Neutropenia	57	53.3	34	31.8	324	60.8	76	14.3
Leukocytopenia	51	47.7	21	19.6	NR	NR	NR	NR
Lymphocytopenia	48	44.9	19	17.8	NR	NR	NR	NR
Thrombocytopenia	44	41.1	15	14.0	302	56.6	42	7.8
Anemia	33	30.8	3	2.8	238	44.6	17	3.1
Nonhematological								
Surficial edema	71	66.4	0	0.0	296	55.5	5	0.9
Nasopharyngitis	70	65.4	0	0.0	117	22.0	0	0.0
Rash	64	59.8	9	8.4	181	33.9	11	2.0
Diarrhea	44	41.1	3	2.8	175	32.8	10	1.8
Gastroenteritis	37	34.6	3	2.8	NR	NR	NR	NR
Nausea	35	32.7	0	0.0	233	43.7	4	0.7
Malaise	29	27.1	0	0.0	184	34.5	6	1.1
Myalgia	27	25.2	2	1.9	114	21.4	8	1.5
Upper respiratory tract infection	27	25.2	0	0.0	77	14.5	1	0.2
Muscle cramps	26	24.3	0	0.0	204	38.3	7	1.3
Pyrexia	26	24.3	0	0.0	70	13.1	4	0.7
Headache	23	21.5	0	0.0	166	31.2	2	0.4
Dizziness	17	15.9	0	0.0	77	14.5	5	0.9
Vomiting	16	15.0	0	0.0	90	16.9	8	1.5
Joint pain	14	13.1	0	0.0	151	28.3	13	2.4
Cough	13	12.1	0	0.0	77	14.5	1	0.2
Anorexia	11	10.3	0	0.0	28	5.3	0	0.0
Pruritus	11	10.3	0	0.0	39	7.3	1	0.2

NR not reported

reduction and interruption with careful monitoring of safety in individual patients. A similar concept was advocated by a study reported by Kanda et al. [14].

In summary, this prospective study confirmed remarkable efficacy and safety of imatinib in Japanese patients with newly diagnosed CP-CML. It also suggested a clear relationship between higher daily doses of imatinib (i.e., ≥350 mg) and better treatment results.

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Randomized study of induction therapy comparing standard-dose idarubicin with high-dose daunorubicin in adult patients with previously untreated acute myeloid leukemia: JALSG AML201 Study

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Randomized study of induction therapy comparing standard-dose idarubicin with high-dose daunorubicin in adult patients with previously untreated acute myeloid leukemia: JALSG AML201 Study

Running Title: High-dose DNR compared with IDR in AML

Scientific heading: Clinical Trials and Observations

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For a complete list of Japan Adult Leukemia Study Group (JALSG) participants, please see the supplemental appendix.

Abstract

We conducted a multi-institutional randomized study to determine whether high-dose daunorubicin (DNR) would be as effective as standard-dose idarubicin (IDR) in remission induction therapy for newly diagnosed adult patients aged younger than 65 with acute myeloid leukemia (AML). Of 1,064 patients registered, 1,057 were evaluable. They were randomly assigned to receive either DNR (50 mg/m^2 daily for 5 days) or IDR (12 mg/m^2 daily for 3 days), in combination with 100 mg/m^2 cytarabine by continuous infusion daily for 7 days, as induction therapy. Complete remission (CR) was achieved in 407 (77.5%) of 525 patients in the DNR group and 416 (78.2%) of 532 in the IDR group (P = 0.79). Patients achieving CR received intensive post-remission therapy consisting of either 3 courses of high-dose cytarabine or 4 courses of standard-dose therapy. Overall survival rates at 5 years were 48% for the DNR group and 48% for the IDR group (P = 0.54), and relapse-free survival rates at 5 years were 41% and 41% (P = 0.97), respectively. Thus, high-dose DNR and standard-dose IDR are equally effective for the treatment of adult AML, achieving a high CR rate and good long-term efficacy. This study is registered at http://www.umin.ac.jp/ctrj/ as C000000157.

Introduction

The combination of anthracycline and cytarabine (Ara-C) with or without other antileukemic drugs is a standard induction therapy for acute myeloid leukemia (AML)[1-3], and a combination of daunorubicin (DNR) at a dose of 45 to50 mg/m^2 given daily for 3 days and Ara-C at a dose of 100 to 200 mg /m^2 given daily for 7 days was generally used. In late 1980s, however, idarubicin (IDR) was introduced into clinics and 3 randomized studies comparing IDR with DNR reported significantly higher complete remission (CR) rates in favor for IDR.[4-6] A meta-analysis also confirmed a superior effect of IDR at a dose of 10 to 12 mg/m^2 for 3 days to DNR at a dose of 45 to 60 mg/m^2 for 3 days in the achievement of CR. Nevertheless, the long-term follow-up of above mentioned 3 randomized studies comparing IDR with DNR revealed that, in only one study, the IDR group resulted in better overall survival (OS) compared with the DNR group.[8]

Japan Adult Leukemia Study Group (JALSG) employed IDR and Ara-C as induction therapy in the AML95 and AML97 studies,[9-11] after IDR was registered and approved for the national health insurance system in 1995. Both studies resulted in satisfactorily high CR rates, 80% and 79%, respectively. However, these CR rates were not superior to those of our earlier AML87, AML89, and AML92 studies, which used DNR in combination with other anti-leukemia drugs.[12-14] In these 3 earlier studies, DNR and other drugs were administered in a response-oriented individualized manner, that is, additional drugs were given for a few days when the bone marrow at day 8 was not hypoplastic, containing a substantial number of blasts. Therefore, the total doses of DNR administered during the first course

of induction therapy were 240 to 280 mg/m^2 given over 5 to 7 days, which were more than the conventional dose of 40 to 60 mg/m^2 for 3 days. Usui et al. also reported that the optimal dose of DNR in their induction therapy for newly diagnosed adult AML was approximately 280 mg/m^2 (40 mg/m^2 for 7 days).[15]

Since there had been no prospective randomized study comparing a higher dose of DNR with the standard dose of IDR (12mg/m^2) in adult AML, in the present multi-institutional randomized study, we prospectively compared IDR (12 mg/m^2 for 3 days) with DNR (50 mg/m^2 for 5 days), in combination with Ara-C (100 mg/m^{2}for 7 days), as induction therapy of previously untreated adult AML. High-dose DNR resulted in the same CR rate and predicted 5-year OS compared with standard-dose IDR.

Patients and methods

Patients

From December 2001 to December 2005, 1,064 newly diagnosed adult patients aged 15 to 64 years with "de novo" AML were consecutively registered from 129 participating institutions. 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 by May-Giemsa, peroxidase, and esterase staining. Then, diagnosis was reevaluated by the central review committee. FAB-M3 was not registered in this study. Eligibility criteria included adequate function of liver (serum bilirubin level < 2.0 mg/dL), kidney (serum creatinine < 2.0 mg/dL), heart, and lung, and an Eastern

Cooperative Oncology Group performance status between 0 and 3. Patients were not eligible if they had prediagnosed myelodysplastic syndrome (MDS), but were eligible if they had no definite diagnosis of MDS confirmed by bone marrow histological analysis even when they had a previous history of hematological abnormality. Cytogenetic abnormalities were grouped by standard criteria and classified according to the Medical Research Council (MRC) classification.[16] The study was approved by the Institutional Review Boards at each participating institution. Written informed consent was obtained from all patients before registration in accordance with the Declaration of Helsinki. The study was registered at http://www.umin.ac.jp/ctr/ as C000000157.

Treatments

Patients were randomly assigned to receive either IDR or DNR using a centralized computer system. Randomization was stratified by age (younger or older than 50 years) and type of AML (FAB classification). All patients received 100 mg/m^2/day Ara-C, by 24-hour continuous infusion from days 1 to 7. In the IDR group, patients received 12 mg/m^2/day IDR for 3 days, and in the DNR group, 50 mg/m^2/day DNR for 5 days. If patients did not achieve CR by the first course, the same induction therapy was repeated after an approximately 3- to 4-week interval. If patients did not achieve CR with two courses, they were judged as failure cases.

All patients who achieved CR were again randomized to receive either 4 courses of conventional consolidation therapy or 3 courses of high-dose Ara-C therapy. In the conventional consolidation group, the first course consisted

of mitoxantrone (MIT; 7 mg/m^2 by 30-minute infusion on days 1 to 3) and Ara-C (200 mg/m^2 by 24-hour continuous infusion on days 1 to 5). The second consisted of DNR (50 mg/m^2 by 30-minute infusion on days 1 to 3) and Ara-C (200 mg/m^2 by 24-hour continuous infusion on days 1 to 5). The third consisted of aclarubicin (ACR; 20 mg/m^2 by 30-minute infusion on days 1 to 5) and Ara-C (200 mg/m^2 by 24-hour continuous infusion on days 1 to 5). The fourth consisted of Ara-C (200 mg/m^2 by 24-hour continuous infusion on days 1 to 5), etoposide (ETP; 100 mg/m^2 by 1-hour infusion on days 1 to 5), vincristine (VCR; 0.8 mg/m^2 by bolus injection on day 8), and vindesine (VDS; 2 mg/m^2 by bolus injection on day 10). Each consolidation was administered as soon as possible after the neutrophils, white blood cells (WBC), and platelets recovered to over 1.5 x 10^9/L, 3.0 x 10^9/L, and 100 x 10^9/L, respectively. In the high-dose Ara-C group, 3 courses of 2.0 g/m^2 Ara-C were given by 3-hour infusion every 12 hours on days 1 to 5. Each course was administered one week after the neutrophils, WBC, and platelets recovered to the above counts.

The best supportive care, including administration of antibiotics and platelet transfusions, was given as indicated. When patients had life-threatening documented infections during neutropenia, the use of granulocyte colony-stimulating factor was permitted.

After completion of consolidation therapy, no patients received further chemotherapy. Allogeneic stem cell transplantation (SCT) was offered during the first CR to patients aged 50 years or less and with a histocompatible donor in the intermediate or adverse cytogenetic risk groups.

Definition and study end points

Responses were evaluated by the recommendations of the International Working Group.[17] CR was defined as the presence of all of the following: less than 5% blasts in bone marrow, no leukemic blasts in peripheral blood, recovery of peripheral neutrophil counts over 1.0 x 10^9/L and platelet counts over 100 x 10^9/L, and no evidence of extramedullary leukemia. Relapse after CR was defined as the presence of at least one of the following: reappearance of leukemic blasts in the peripheral blood, recurrence of more than 5% blasts in the bone marrow not attributable to any other cause (e.g., bone marrow regeneration after consolidation therapy), and appearance of extramedullary leukemia.

This was a multi-institutional randomized phase 3 study with a 2 x 2 factorial design. The primary end point of the first randomization was CR rate. The result of the second randomization is partially reported here but will be presented fully in a separate paper. OS was calculated from the date of entry to the study until death from 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 until the date of AML relapse or death from any cause and censored at the last follow-up. Patients who underwent allogeneic SCT were not censored at the date of SCT.

Statistical methods

This study was prospectively powered to demonstrate non-inferiority of DNR compared with IDR. With a sample size of 420 patients per group (840 in total), the study had a power of 90% at a 1% level of significance to

demonstrate non-inferiority (assuming 80% CR rate for both groups). Statistical testing for the non-inferior trial was performed according to the method of Blackwelder.[18] The Kaplan-Meier method was used to estimate probabilities of OS and RFS.[19] To test factors to predict CR, the χ^2 test and the Wilcoxon rank-sum test were used for univariate analysis and the multiple logistic regression model for multivariate analysis. For comparison of OS and RFS, the log-rank test was used for univariate analysis and the proportional hazard model of Cox for multivariate analysis.[20, 21] Cumulative rates of CR, neutrophil recovery and platelet recovery were estimated according to the Kaplan–Meier method and were evaluated with the log-rank test. JMP program (SAS Institute Inc., Cary, NC) was used for these analyses. All analyses were performed according to the intent-to-treat principle. All statistical tests except the method of Blackwelder were 2-sided, and the significance level was set at 0.05.

Results

Patient characteristics

Among 1,064 registered patients, 7 did not meet the inclusion criteria (misdiagnosis: one, infectious complication: one, without therapy: one, and withdrawal of consent: 4). The study population thus comprised 1,057 patients (Fig. 1). Patient characteristics are presented in Table 1. Median age was 47 years (range, 15 to 64). Cytogenetics data were available for 1,021 (96.6%) patients. Among these, 247 (24.2%) were classified in the favorable-risk group, 681 (66.7%) in the intermediate-risk group, and 93 (9.1%) in the high-risk group. Five hundred and thirty-two patients were assigned to the