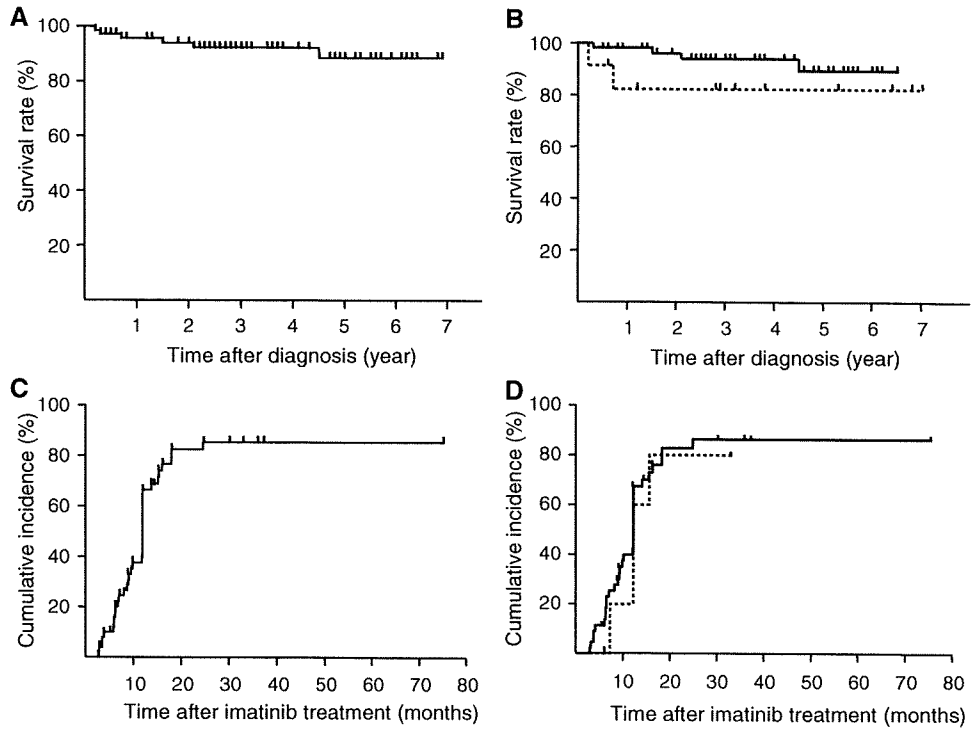

Fig. 1 Overall survival and cumulative cytogenetic response of patients whose initial treatment was imatinib. **a** Overall survival at 5 years was 88.7% in all cases ($n = 73$, 95% CI 79.3–98.1%), and (**b**) that of in chronic (*solid line*) and accelerated (*dotted line*) phases was 89.9 and 82.5%, respectively. **c** Cumulative cytogenetic response at 18 months was 82.5% in all cases ($n = 51$, 95% CI 70.7–94.3%), and (**d**) that of chronic (*solid line*) and accelerated (*dotted line*) phases was 82.9 and 80.0%, respectively

Table 2 Characteristics of patients whose imatinib concentration was measured

Male/female (number)	22/11
Initial imatinib treatment (yes/no)	22/11
Age at diagnosis, years (median)	17–82 (50)
Clinical phase at diagnosis	
CP	27
AP	6
BC	0
Time after diagnosis, years (median)	1.6–25.2 (4.9)
Duration of imatinib, years (median)	0.5–7.4 (4.2)
Dose of imatinib (mg/day)	
<200	3
200	1
300	9
400	19
600	1

CP Chronic phase, *AP* accelerated phase, *BC* blastic crisis

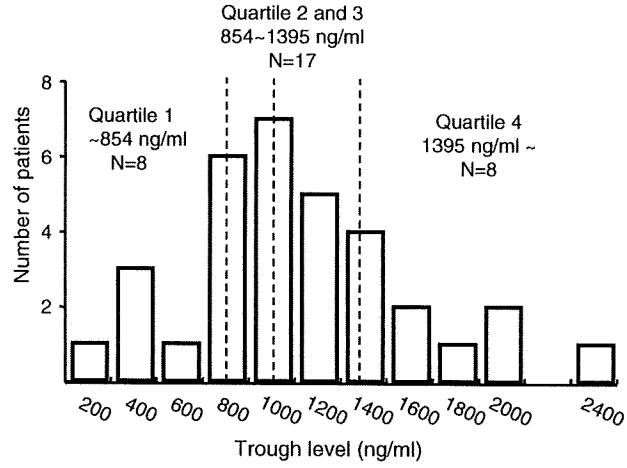

Fig. 2 Distribution of imatinib trough levels ($n = 33$). The *vertical dashed lines* represent the 25th, median, and 75th percentiles within quartiles 2 and 3

300 mg/day or less. As shown in Fig. 2, the median concentration was 1040 ng/ml (range 233–2420 ng/ml). We divided the patients into quartile groups (Q1–Q4) based on their imatinib trough level. The average of trough level of the lowest quartile (Q1) was 845 ng/ml, and that of upper quartile (Q4) was 1395 ng/ml. Trough concentration did not exhibit correlation with body weight of patients ($r^2 = 0.004$), or BSA ($r^2 < 0.001$), but it demonstrated significant correlation with the imatinib dose divided by BSA (dose/BSA, $r^2 = 0.28$, Fig. 3a–c) or by BW (dose/BW, $r^2 = 0.23$). However, even among those taking the same dose of 400 mg/day, the imatinib concentration was widely distributed (582–2420 ng/ml). Interestingly, the influence of body size on the plasma concentration of imatinib seemed stronger among those taking lower dose of imatinib: r^2 value in the concentration and dose/BSA among those with 200 mg was 0.65 ($P = 0.1922$) and those with 300 mg or more was 0.008 and 0.018 ($P = 0.8158$ and 0.5910, respectively, Fig. 3c). The similar tendency was observed in the relationship between BW

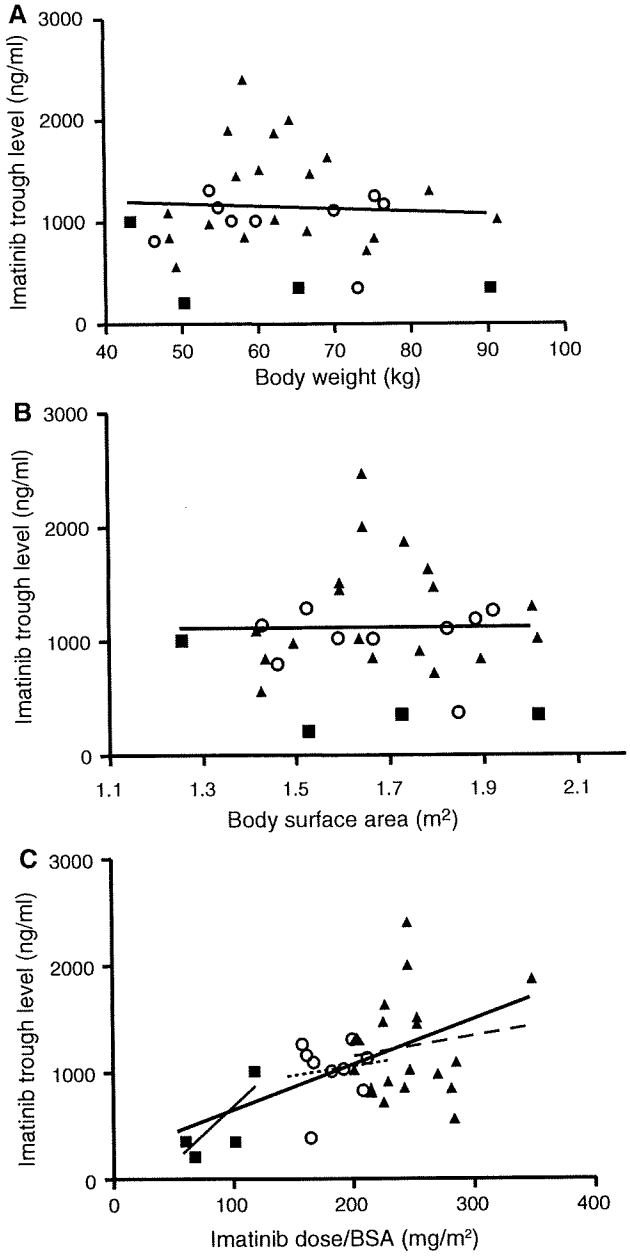

Fig. 3 Imatinib trough levels by body weight (**a**), body surface area, BSA (**b**), and daily imatinib dose divided by BSA (**c**). Imatinib trough and (**a**) body weight, $r^2 = 0.004$; **b** BSA, $r^2 < 0.001$; **c** imatinib dose/BSA, $r^2 = 0.28$. *Filled rectangles* represent cases taking imatinib 200 mg/day or less, *open circles* those taking 300 mg/day, *filled triangles* those taking 400 mg/day or more. *Bold line* represents a fit line in each figure. In **c**, *thin line* represents a fit line for those taking 200 mg/day or less, *dotted line* for those taking 300 mg/day, and *broken line* for those taking 400 mg/day or more. The imatinib trough level was significantly correlated with imatinib dose/BSA as total ($P = 0.0021$)

or BSA and imatinib concentration although not statistically significant (data not shown). There were eight out of thirteen patients (61.5%) whose imatinib concentration was higher than 1000 ng/ml despite taking 300 mg/day of imatinib or less. As shown in Fig. 4a, patients in the optimal-response category showed a significantly higher trough concentration than those in the suboptimal or failure categories ($P = 0.0087$). Similarly, 41% (10 out of 17) of the patients in the lower two quartiles (Q1 and Q2) and 94% (15 out of 16) in the upper two quartiles (Q3 and Q4) had an optimal response, demonstrating a significantly superior response (Table 3, $P = 0.04$) in the groups with a high-trough concentration. We also found a significant relationship between dose/BSA and the response ($P = 0.01$, Fig. 4b), and the dose and response ($P = 0.01$, Fig. 4c). These tendencies did not change even cases were divided into chronic and accelerated phases ($P = 0.0272$, Fig. 4d, e). Of note, there was no difference in the trough imatinib levels between patients with or without prior treatment (data not shown).

4 Discussion

We analyzed the long-term results of 73 CML patients initially treated with imatinib in a practical clinical setting, and confirmed its excellent long-term efficacy as in our previous report [7]. Despite administration of a lower dose of imatinib as compared to the IRIS study (400 mg/day of imatinib or more in 92% of patients), the OS and CCR of our patients were comparable to those observed in the IRIS study [6]. These results were surprising considering that our patients were older and in a more advanced stage (i.e., 12 out of 73 were not in the chronic phase at diagnosis) than those in the IRIS study. To address why smaller amount of imatinib could provide an excellent response for patients in the Nagasaki Study, we measured the trough concentration of imatinib and found that it was comparable or higher than those reported in foreign studies (mean = 1058 and 1119 ng/ml in the French and Nagasaki Study, respectively, median = 979 and 1040 ng/ml in the IRIS and Nagasaki study, respectively) [8, 9]. Although our results are based on a relatively small number of patients, the mean imatinib trough concentration of patients administered with 400 mg/day was higher in our analysis (1244 ± 494 ng/ml) than that reported from a French group study (1058 ± 557 ng/ml) [8]. The trough imatinib concentration had the strongest relationship with imatinib dose/BSA compared to that with body weight or BSA alone, which might explain the paradoxical median trough concentration in patients taking 300 mg/day (1130 ng/ml) or 400 mg/day (1040 ng/ml).

It was demonstrated that the trough concentration of imatinib relates to the cytogenetic and molecular responses from two different groups [8, 9]. In accordance with these reports, despite the possibility of inappropriate inclusion of late responders, the clinical and molecular efficacy of

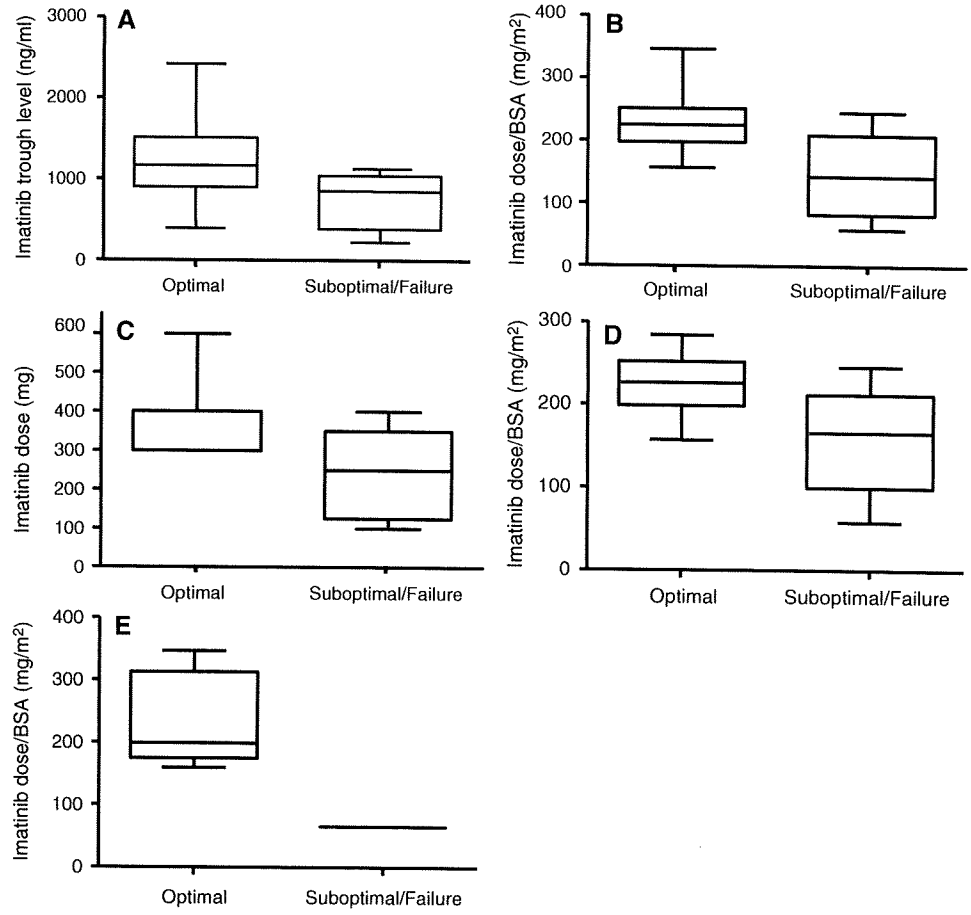

Fig. 4 Imatinib trough levels (**a**), imatinib dose/BSA (**b**), and imatinib dose (**c**) categorized as optimal response, and suboptimal response or failure based on European LeukemiaNet criteria. The trough level of cases with optimal response, mean = 1242 ng/ml, $n = 25$; suboptimal response/failure, mean = 736 ng/ml, $n = 8$; $P = 0.0087$. There were also significant differences in imatinib dose/BSA (**b**, $P = 0.01$) and in imatinib dose (**c**, $P = 0.01$) between the two response categories. This tendency remained even after cases were divided into chronic (**d**, Optimal, 18 cases; suboptimal/failure, 7 cases, $P = 0.0272$) and accelerated phase (**e**, Optimal, 5 cases; suboptimal/failure, 1 case). BSA was not available in 2 cases

Table 3 Number of patients in each quartile group

Quartile group	Total	Average dose (mg/day) of imatinib (range)	Response category[a]	
			Optimal	Suboptimal/ failure
Q1	8	275 (100–400)	4	4
Q2	9	361 (150–400)	6	3
Q3	8	325 (300–400)	7	1
Q4	8	425 (400–600)	8	0

[a] Distribution of patients in Q1/Q2 and Q3/Q4 is significantly different ($P = 0.04$)

imatinib as judged by the ELN criteria, was related to its trough concentration in the current analysis. These results strongly suggest that the high trough level in our patients resulted in an excellent imatinib response. Although body weight and BSA per se were not clinically significant determinants of the trough concentration [12, 13], the smaller body size of the Japanese population as compared to foreign populations might have influenced the results. The similar trough concentration despite smaller dose could be explained, at least in part, by the difference in the BSA between the IRIS (male 2.0 m^2, female 1.8 m^2) and the Nagasaki (male 1.77 m^2, female 1.45 m^2) studies [9].

The imatinib trough concentration is dependent on a variety of factors including prescribed dose, compliance, drug–drug interaction, serum-binding proteins, genetic differences in enzymatic pathways, and concomitant diseases [11–13]. Although not clearly mentioned previously, BSA might also affect trough imatinib concentration, in particular when BSA is small. It is well known that molecular monitoring of the *bcr-abl* fusion transcripts is necessary to manage CML patients for the appropriate choice of treatment: the conversion of tyrosine kinase inhibitors, or the indication of other treatment including IFN or stem cell transplantation [11, 14–17]. Because a plasma level above 1040 ng/ml (or 1000 ng/ml suggested by the IRIS study, or 1002 ng/ml reported from French study) seemed necessary to obtain a significant effect from imatinib, our results suggest that monitoring trough imatinib concentration in addition to molecular monitoring would be useful for the management of CML patients. For example, patients with an imatinib blood concentration lower than the optimal level could be candidates for an increased imatinib dose or for other treatment than imatinib

such as a second generation of BCR-ABL inhibitor. Given the fact that more than 30% of patients are treated with less than 400 mg/day in a practical setting, it would be useful to measure trough concentration of imatinib when it might be necessary to make a dosage change, such as to consider increase of imatinib with an unsatisfactory response, or to consider decrease of imatinib with a fair response but intolerable side effects. Maximizing the efficacy and minimizing the side effects of imatinib could be achieved by the dose adjustment based on its trough data, reducing the cost of treatment at the same time. Further research should include an evaluation of imatinib-binding proteins and genetic differences in metabolic enzymes, such as CYA3A5 [12, 13]. These types of studies would provide clinically important information for the prediction of imatinib efficacy in CML patients.

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Appendix

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ORIGINAL ARTICLE

Phase 1/2 clinical study of dasatinib in Japanese patients with chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia

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Abstract A phase 1/2 study was conducted to assess the safety and efficacy of dasatinib in Japanese patients with chronic myelogenous leukemia (CML) or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL) resistant or intolerant to imatinib. In phase 1, 18 patients with chronic phase (CP) CML were treated with dasatinib 50, 70, or 90 mg twice daily to evaluate safety. Dasatinib $\leq$ 90 mg twice daily was well tolerated. In phase 2, dasatinib 70 mg was given twice daily to CP-CML patients for 24 weeks and to CML patients in accelerated phase (AP)/blast crisis (BC) or Ph^+ ALL for 12 weeks. In the CP-CML group ($n = 30$) complete hematologic response was 90% and major cytogenetic response (MCyR) 53%. In the AP/BC-CML group ($n = 11$) major hematologic response (MaHR) was 64% and MCyR 27%, whereas in the Ph^+ ALL group ($n = 13$) MaHR was 38% and MCyR 54%. Dasatinib was well tolerated and most of the nonhematologic toxicities were mild or moderate. Dasatinib therapy resulted in high rates of hematologic and cytogenetic response, suggesting that dasatinib is promising as a

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new treatment for Japanese CML and Ph+ ALL patients resistant or intolerant to imatinib.

Keywords CML · Ph+ ALL · Dasatinib · Imatinib resistant · Imatinib intolerant

1 Introduction

Chronic myeloid leukemia (CML) is a disease attributable to abnormalities of hematopoietic stem cells involving uncontrolled proliferation of cells originating from the bone marrow. The Philadelphia (Ph) chromosome is formed by translocation between chromosomes 9 and 22. The *BCR-ABL* fusion gene on this chromosome produces BCR-ABL, which constitutively activates ABL tyrosine kinase and is thus responsible for CML and 20–30% of adult patients with acute lymphoblastic leukemia (ALL) [1]. Imatinib (Glivec®) is a selective BCR-ABL inhibitor effective against CML and Ph-positive (Ph+) ALL. Currently, imatinib is the only tyrosine kinase inhibitor indicated in newly diagnosed CML and Ph+ ALL [2–4]. However, resistance to imatinib gradually develops in many patients with CML and Ph+ ALL, particularly those with advanced disease. Among CML patients treated with imatinib, 31% discontinue the drug within 5 years because of insufficient responses or unacceptable toxicity [5]. As a major factor responsible for development of resistance to imatinib, numerous point mutations in BCR-ABL have been reported [6–8]. Additional factors including *BCR-ABL* gene amplification [6, 9], excretion of the drug through a P-glycoprotein efflux pump [10, 11], and activation of the signal transduction pathway for SRC family kinase and other signals [12, 13] have also been implicated. Therefore the development of new treatments is desirable for patients with insufficient response to imatinib and in whom imatinib cannot be continued at effective doses due to toxicity.

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Dasatinib (BMS-354825) is a novel oral tyrosine kinase inhibitor that exerts inhibitory activity against BCR-ABL and SRC family kinase. In vitro, dasatinib binds to both active and inactive BCR-ABL and is 325 times more potent than imatinib and 16 times more potent than nilotinib against wild-type BCR-ABL-expressing cells [14]. Dasatinib has demonstrated activity against all reported types of imatinib-resistant mutant BCR-ABL, except for T315I [14–18]. Five phase 2 studies collectively known as START (SRC/ABL Tyrosine kinase inhibition Activity Research Trials of dasatinib) studies demonstrated that dasatinib is safe and elicits hematologic and cytogenetic response at all stages of CML and Ph+ ALL resistant or intolerant to imatinib [18–22]. Against chronic phase (CP)-CML, dasatinib was highly effective with 91% of patients showing complete hematologic responses (CHR) and 62% major cytogenetic responses (MCyR). Efficacy for CP-CML was durable and duration of MCyR was 88%, progression-free survival was 80% and overall survival was 94% at 2-year follow-up [23]. Dasatinib (Sprycel®) was initially approved in the United States in June 2006 and has received marketing approvals in numerous other countries world-wide.

We conducted an open-label phase 1/2 study of dasatinib in Japanese patients with CP-CML, accelerated phase (AP)/blast crisis (BC)-CML or Ph+ ALL resistant or intolerant to imatinib. This study comprised two parts. Phase 1 evaluated the safety of dasatinib at escalating doses in patients with CP-CML. Phase 2 evaluated the efficacy and safety of dasatinib in patients with all-stage CML or Ph+ ALL.

2 Methods

2.1 Patients

Adult CML or Ph+ ALL patients aged 20–75 years who were resistant or intolerant to imatinib were conducted from 2005 to 2007. Because imatinib had no registered indication for Ph+ ALL in Japan at the start of this study, patients with Ph+ ALL resistant to or intolerant of prior therapies were eligible. Treatment and analysis were conducted in three cohorts with CP-CML, AP/BC-CML and Ph+ ALL (Table 1).

CP-CML was considered to be resistant to imatinib when given at a dose level ≥ 400 mg/day if the following occurred: (1) white blood cell count (WBC) showed a ≥2-fold increase from nadir to >20000/mm^3 or rose from nadir to ≥50000/mm^3; (2) CHR was not achieved despite ≥3-month treatment with imatinib; (3) cytogenetic response was not achieved despite ≥6-month treatment with imatinib; (4) MCyR was not achieved despite ≥12-month

Table 1 Definition of CML phases

Phase	Description
	Patients satisfying all the following requirements:
CP	• Percentage of blasts in peripheral blood and bone marrow <15%
	• Percentage of basophils in peripheral blood or bone marrow <20%
	• Total percentage blasts and promyelocytes in peripheral blood and bone marrow <30%
	• Platelet count ≥100,000/mm^3 (rated at chronic stage if thrombocytopenia due to prior therapy is present)
	• Extramedullary leukemia absent
AP	• Nonacute patients satisfying ≥1 of the following requirements:
	• Percentage blasts in peripheral blood or bone marrow ≥15 and <30%
	• Percentage basophils in peripheral blood or bone marrow ≥20%
	• Total percentage blasts and promyelocytes in peripheral blood or bone marrow ≥30% and percentage blasts <30%
	• Platelet count <100,000/mm^3 (not associated with treatment)
BC	• Patients satisfying ≥1 of the following requirements:
	• Percentage blasts in peripheral blood or bone marrow ≥30%
	• Extramedullary leukemia, excluding that affecting liver or spleen

treatment with imatinib; (5) relapse after MCyR or CHR; or (6) mutation in *ABL* gene suggestive of resistance to imatinib (L248V, G250E, Q252H/R, Y253H/F, E255K/V, T315I/D, F317L or H369P/R) was noted in patients of chronic CML. AP-CML was considered as resistant to imatinib if the following occurred in patients treated with imatinib at a dose level ≥600 mg/day, or ≥400 mg/day if the initial diagnosis was CP-CML intolerant to imatinib: (1) progressed to BC; (2) hematologic response was not achieved in ≤4 weeks; or (3) progressed to AP after hematologic response. BC-CML was considered as resistant to imatinib if the following patients occurred: (1) the condition progressed into BC after hematologic response; or (2) the condition remained BC-CML despite ≥4-week treatment. Ph$^+$ ALL was considered as resistant to prior therapies if the following occurred: (1) CHR was not achieved at least 2 weeks after the start of treatment; or (2) progressed from CHR.

Patients with CP-CML were assessed as intolerant to imatinib if grade ≥3 nonhematologic toxicity was observed or grade 4 hematologic toxicity persisted ≥7 days. Patients with AP/BC-CML were considered intolerant to imatinib if treatment had to be discontinued or the dosage kept <400 mg/day for reasons of toxicity. Ph$^+$ ALL patients were considered intolerant to prior therapy if grade ≥3 nonhematologic toxicity was noted, grade 4 hematologic toxicity persisted ≥7 days, or existing therapy could not be given for other reasons. This study was carried out in accordance with the principles of the Declaration of Helsinki, ICH-GCP, and requirements set forth by Japanese Good Clinical Practice. Prior to the study, written informed consent was obtained from each subject. The study was approved by the Institutional Review Board at each participating institution. The study was designed by academic investigators in conjunction with representatives from the sponsor, Bristol-Myers K.K. Both parties contributed to the collection and analysis of the data. This study was registered at http://www.clinicaltrials.gov as NCT00227454.

2.2 Two-part study design: phases 1 and 2

Phase 1 was designed as a dose-escalation study in patients with CP-CML, evaluating the safety of dasatinib. Phase 2 was designed as a fixed-dose study in patients with CP or AP/BC-CML resistant or intolerant to imatinib and Ph$^+$ ALL resistant or intolerant to prior therapies, evaluating the efficacy and safety of dasatinib. In this phase, the primary endpoint was cytogenetic response in patients with CP-CML and hematologic response in those with AP/BC-CML and Ph$^+$ ALL.

2.3 Dasatinib treatment

During phase 1, dasatinib was orally administered twice daily at 50, 70, or 90 mg/dose for 24 weeks. Dose-limiting toxicity (DLT) defined as grade ≥3 nonhematologic toxicity, grade 3–4 QTc interval prolongation, grade 4 neutropenia lasting ≥7 days, grade 4 thrombocytopenia, bleeding requiring platelet transfusion, and other toxicity requiring discontinuation of the drug was evaluated during the first 4 weeks of treatment.

Phase 2 was started after the safety of 70 mg twice daily was confirmed. During phase 2, dasatinib was orally administered at 70 mg twice daily for 24 weeks in the CP-CML group and for 12 weeks in the AP/BC-CML and Ph$^+$ ALL groups. Upon completion of the observation period, an extension study involving continued treatment was planned.

The dose level of dasatinib was reduced if the following occurred: (1) grade ≥2 nonhematologic toxicity (grade ≥3 nonhematologic toxicity in patients of CP-CML); or (2) grade 4 neutropenia in patients of AP/BC-CML and Ph$^+$ ALL when bone marrow cell density and percentage of blasts were checked ≥15 days after the start of treatment. The dose level of dasatinib for CP-CML patients was increased if: (1) progression of disease (PD) was noted; (2)

Table 2 Criteria for efficacy evaluation

Hematologic response[a]	
(1) CP-CML	
CHR	
• WBC count less than or equal to institutional upper limit of normal	
• Platelet count <450,000/mm^3	
• Absence of blasts or promyelocytes in peripheral blood	
• Total percentage myelocytes and metamyelocytes in peripheral blood <5%	
• Percentage basophils in peripheral blood <20%	
• Absence of extramedullary leukemia (including hepatomegaly and splenomegaly)	
(2) AP/BC-CML and Ph$^+$ ALL	
Major HR	
(a) CHR	
• WBC count less than or equal to institutional upper limit of normal	
• Neutrophil count ≥1000/mm^3	
• Platelet count ≥100,000/mm^3	
• Absence of blasts/promyelocytes in peripheral blood	
• Percentage of blasts in bone marrow <5%	
• Total percentage myelocytes and metamyelocytes in peripheral blood <5%	
• Percentage basophils in peripheral blood <20%	
• Absence of extramedullary leukemia (including hepatomegaly and splenomegaly)	
(b) NEL	
• WBC count less than or equal to institutional upper limit of normal	
• Absence of blasts or promyelocytes in peripheral blood	
• Percentage blasts in bone marrow <5%	
• Total percentage myelocytes and metamyelocytes in peripheral blood <5%	
• Percentage basophils in peripheral blood <20%	
• Absence of extramedullary leukemia (including hepatomegaly and splenomegaly)	
• Platelet count ≥20,000/mm^3 and <100,000/mm^3 and/or neutrophil count ≥500/mm^3 and <1000/mm^3	
Minor HR	
• Percentage blasts in bone marrow/peripheral blood <15%	
• Total percentage blasts/promyelocytes in peripheral blood <30%	
• Percentage basophils in peripheral blood <20%	
• Absence of extramedullary leukemia other than in spleen and liver	
Cytogenetic response	
Percentage Ph$^+$ cells in bone marrow	
MCyR	
(a) CCyR	0%
(b) PCyR	>0 and ≤ 35%
Minor CyR	>35 and ≤65%
Minimal CyR	>65 and ≤95%
No response	>95 and ≤100%

CHR Complete hematologic response, *NEL* no evidence of leukemia, *MCyR* major cytogenetic response, *CCyR* complete cytogenetic response, *PCyR* partial cytogenetic response

[a] Hematologic response is confirmed if the remitted state lasts ≥4 weeks

CHR was not achieved despite ≥8 weeks of treatment; and (3) MCyR was not achieved despite ≥12 weeks of treatment. For AP/BC-CML and Ph$^+$ ALL patients, the dose level of dasatinib was increased if: (1) PD was noted; (2) the percentage of blasts in peripheral blood showed an increase from that recorded ≥1 week previously; and (3) CHR was not achieved despite ≥4-week treatment. During the study period, concomitant use of anticancer drugs other

than dasatinib was prohibited in both CML and Ph^+ ALL patients, except for short term ($\leq$14 days) use of hydroxycarbamide in patients in whom WBC was >50000/mm^3.

2.4 Patient evaluation

Evaluation of peripheral blood findings was performed every week during the first 4 weeks in phase 1, every other week during the first 4 weeks in phase 2, and every 4 weeks thereafter. Evaluation of bone marrow findings was made at the end of the study. Table 2 shows the criteria for efficacy evaluation. Cytogenetic response was evaluated in bone marrow by G-band test and in bone marrow and peripheral blood samples by fluorescence in situ hybridization (FISH) for *BCR-ABL* at baseline and at week 12 in AP/BC-CML and Ph^+ ALL patients and at week 24 in those with CP-CML. *BCR-ABL* point mutation was assessed by direct sequencing of PCR products of peripheral blood cells before the start of treatment. Adverse events were graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0.

3 Results

3.1 Patient demographics and dasatinib treatment

A total of 55 patients were registered for this trial, of whom dasatinib was administered to 54 (18 and 36 patients during phases 1 and 2, respectively). Median age was 43 (range 27–66) and 60 (29–73) years in patients entered in phases 1 and 2, respectively. Of the 54 patients, 35 were males and 19 females. Thirty-five patients were resistant to imatinib at daily dose of 400 mg or more, and 19 patients were intolerant to imatinib. Table 3 shows patient characteristics. Phase 1 involved 18 patients of CP-CML (12 resistant/6 intolerant); phase 2 involved 12 patients of CP-CML (6 resistant/6 intolerant), 11 AP/BC-CML (8 resistant/3 intolerant), and 13 Ph^+ ALL (9 resistant/4 intolerant). Major causes for intolerance to imatinib were rash ($n = 6$), myalgia and vomiting ($n = 3$ each), and hepatic dysfunction ($n = 2$). Although prior treatment with imatinib was not a requirement for enrollment in the Ph^+ ALL group, all patients enrolled had a history of imatinib therapy and were either resistant or intolerant to imatinib.

The duration of prior imatinib therapy was 1–3 years in 19 patients (35%), and >3 years in 19 patients (35%). The dosage of imatinib during prior therapy was $\geq$400 mg/day in all patients. Forty-three patients (80%) had previously received therapy other than imatinib, seven patients (13%) had undergone hematopoietic stem cell transplantation.

In phase 1, dose reduction was performed for 3 of 7 patients in the 70 mg group and 3 of 4 patients from the 90 mg group because of hematologic toxicity in 5 patients and nonhematologic toxicity in one patient. In phase 2, dose reduction was performed for 10 of 12 patients in the CP-CML group, 3 of 11 patients in the AP/BC-CML group, and 5 of 13 patients in the Ph^+ ALL group because of hematologic toxicity in 10 patients and nonhematologic toxicity in 8 patients. Dose increase was performed in one

Table 3 Patients' baseline characteristics

	CP-CML, phase 1 ($n = 18$)	CP-CML, phase 2 ($n = 12$)	AP/BC-CML ($n = 11$)	Ph^+ ALL ($n = 13$)
Median age, range (years)	43 (27–66)	60 (30–68)	57 (31–73)	64 (29–70)
Median time after diagnosis, range (years)	6.9 (0.3–19)	3.6 (0.7–15)	1.6 (0.0–14)	1.1 (0.2–6.3)
Imatinib resistant, *n* (%)	12 (67)	6 (50)	8 (73)	9 (69)
Imatinib intolerant, *n* (%)	6 (33)	6 (50)	3 (27)	4 (31)
Length of prior imatinib therapy, *n* (%)				
<1 years	3 (17)	4 (33)	2 (18)	7 (54)
1–3 years	4 (22)	3 (25)	6 (55)	6 (46)
>3 years	11 (61)	5 (42)	3 (27)	0
Prior imatinib dosage, *n* (%)				
400–600 mg/day	16 (89)	11 (92)	5 (45)	13 (100)
>600 mg/day	2 (11)	1 (8)	6 (55)	0
Prior chemotherapy, *n* (%)	12 (67)	9 (75)	9 (82)	13 (100)
Prior IFN therapy, *n* (%)	9 (50)	6 (50)	3 (27)	0
Prior HSCT, *n* (%)	0	1 (8)	3 (27)	3 (27)
BCR-ABL mutation, *n* (%)	4 (22)	1 (8)	2 (18)	4 (31)

IFN Interferon, *HSCT* hematopoietic stem cell transplantation

patient with Ph+ ALL because of insufficient response. The median treatment period was 24 weeks in phase 1 and 24, 12, and 11 weeks in the CP-CML, AP/BC-CML, and Ph+ ALL groups, respectively, in phase 2. Median dose was 96.20 (range 46.5–179.5) mg/day in phase 1 and 99.05 (44.7–141.8) mg/day in phase 2.

Forty-four patients completed the trial (17 in phase 1 and 27 in phase 2). One patient in phase 1 and 9 patients (2 patients of AP/BC-CML and 7 of Ph+ ALL) in phase 2 discontinued study treatment prematurely, because of insufficient response in 6 patients and adverse events in 4 patients.

3.2 DLT evaluation: phase 1

In phase 1, DLT was evaluated in 15 patients (6 each in the 50 and 70 mg groups and 3 in the 90 mg group). One patient in the 50 mg group was not evaluated who was diagnosed as AP-CML after registration, one in the 70 mg group had violated the protocol, and one in the 90 mg group reduced dosage. One patient in each of the 50 and 70 mg groups developed grade 4 thrombocytopenia as DLT, whereas no patient in the 90 mg group developed DLT. Two patients in the 50 mg group exhibited grade 3 elevation of ALT, but this change was not deemed DLT since it was transient and subsided without requiring treatment. There was no dose level at which DLT appeared in $\geq$2 patients; thus dasatinib was well tolerated at dose levels $\leq$90 mg twice daily.

Following this finding, dasatinib 70 mg twice daily, which was previously demonstrated safe and effective in an overseas phase 1 and 2 studies, was adopted as the regimen for the second phase of this study.

3.3 Efficacy: phases 1 and 2

3.3.1 CP-CML

Table 4 shows the efficacy results for 30 patients with CP-CML in phase 1 (n = 18) and 2 (n = 12). A high response rate was achieved, with 90% of CP-CML patients achieving a CHR (83% in imatinib-resistant and 100% -intolerant). CHR was achieved rapidly and median time to CHR was 10 days. Fifty-three percent of CP-CML patients exhibited a MCyR following dasatinib therapy. The rate of CCyR was 43%. MCyR was achieved in 33% of imatinib-resistant and 83% of -intolerant patients. In phase 1, CHR, MCyR and CCyR were 89, 50 and 44%, respectively. In phase 2, CHR, MCyR, and CCyR were 92, 58 and 42% respectively. Dasatinib therapy was not discontinued in any CP-CML patient due to insufficient response.

3.3.2 AP/BC-CML

MaHR was achieved in a high percentage (64%) of AP/BC-CML patients (63% imatinib-resistant, 67% -intolerant). Median time to MaHR was 34 days. MCyR was achieved in 27% of AP/BC-CML patients, whereas CCyR was observed in 9%. MCyR was achieved in 38% of imatinib-resistant and 0% -intolerant patients. Dasatinib therapy was not discontinued in any AP/BC-CML patient due to insufficient response.

3.3.3 Ph+ ALL

MaHR was achieved in 38% of Ph+ ALL patients (33% imatinib-resistant, 50% -intolerant). Median time to

Table 4 Treatment response

	CP-CML			AP/BC-CML			Ph+ ALL		
	Imatinib resistant n = 18	Imatinib intolerant n = 12	Total n = 30	Imatinib resistant n = 8	Imatinib intolerant n = 3	Total n = 11	Imatinib resistant n = 9	Imatinib intolerant n = 4	Total n = 13
Hematologic response, n (%)									
Major	–	–	–	5 (63)	2 (67)	7 (64)	3 (33)	2 (50)	5 (38)
Complete	15 (83)	12 (100)	27 (90)	2 (25)	0	2 (18)	0	1 (25)	1 (8)
NEL	–	–	–	3 (38)	2 (67)	5 (45)	3 (33)	1 (25)	4 (31)
Minor	–	–	–	1 (13)	0	1 (9)	2 (22)	2 (50)	4 (31)
Cytogenetic response, n (%)									
Major	6 (33)	10 (83)	16 (53)	3 (38)	0	3 (27)	3 (33)	4 (100)	7 (54)
Complete	5 (28)	8 (67)	13 (43)	1 (13)	0	1 (9)	2 (22)	4 (100)	6 (46)
Partial	1 (6)	2 (17)	3 (10)	2 (25)	0	2 (18)	1 (11)	0	1 (8)
Minor	3 (17)	1 (8)	4 (13)	2 (25)	0	2 (18)	0	0	0
Minimal	3 (17)	1 (8)	4 (13)	1 (13)	1 (33)	2 (18)	0	0	0

CHR + NEL = Major hematologic response, CCyR + PCyR = major cytogenetic response, NEL = no evidence of leukemia

MaHR was 57 days. CCyR was achieved in 46% of Ph^+ ALL patients. MCyR was seen in 33% of imatinib-resistant and 100% -intolerant patients. Dasatinib treatment was discontinued because of insufficient response in 6 patients.

3.3.4 Efficacy by baseline BCR-ABL mutation status

Of the 54 subjects, 11 (20%; 5 CP-CML; 2 AP/BC-CML; 4 Ph^+ ALL) showed 8 different *BCR-ABL* point mutations (L248V, G250E, Y253H, E255K, F311I, T315I, E355A, and H396R) at baseline. All these 11 patients were resistant to imatinib (Table 3). Seven patients (64%) had mutation of kinase domain P-loop (amino acids 244–255) and one that of T315I, which are highly resistant mutations to imatinib. Nonetheless, even in patients with various BCR-ABL point mutations, dasatinib conferred a MaHR in 5 (45%; 3 CP-CML; 1 AP/BC-CML; 1 Ph^+ ALL) of 11 patients and MCyR in 4 patients (36%; 2 CP-CML; 1 AP/BC-CML; 1 Ph^+ ALL), comparable to the MaHR and MCyR rates for patients without *BCR-ABL* mutation. Six patients had no hematologic or cytogenetic response; 2 patients early discontinued dasatinib due to adverse events, 1 patient had T315I mutation at baseline and 2 patients had additionally emerging T315I mutation during dasatinib treatment period.

3.4 Safety

Overall, dasatinib was well tolerated. Most of the nonhematologic adverse events were mild or moderate and required no intervention or disappeared following dose interruption or reduction of dasatinib. Frequently observed adverse events possibly related to dasatinib were headache (41%), fever (33%), diarrhea (33%), rash (31%), edema (31%), and malaise (30%) (Table 5). Pleural effusion was seen in 14 patients (26%), but was mostly mild or moderate except for one patient with grade $\geq$3. In all patients, the adverse events recovered to a level that allowed resumption of study treatment upon administration of diuretics or dose interruption/reduction of dasatinib. Hematologic toxicity was observed in a high percentage of patients, as expected, but was often reversible and subsided following dose interruption or reduction. Grade $\geq$3 thrombocytopenia was seen in 50% of CP-CML, 64% of AP/BC-CML, and 62% of Ph^+ ALL patients. Neutropenia was observed in 47, 73, and 77%, respectively (Table 6). The incidence of grade >3 anemia was highest in Ph^+ ALL patients.

Treatment was discontinued in 4 (7%) of the 54 patients because of adverse events; pneumonia in 2 patients, neutropenia in 1 patient and arrhythmia and heart failure in 1 patient.

Table 5 Cumulative possibly dasatinib related adverse events in the total treated population (n = 54) at 24 weeks (CP-CML) or 12 weeks (AP/BC-CML, Ph^+ ALL) of follow-up

Adverse event	Cumulative incidence rate, n (%)	
	All grade	Grades 3–4
Headache	22 (41)	0
Fever	18 (33)	0
Diarrhea	18 (33)	1 (2)
Rash	17 (31)	1 (2)
Edema	17 (31)	0
Malaise	16 (30)	0
Pleural effusion	14 (26)	1 (2)
Weight gain	14 (26)	0
Nausea	11 (20)	0
Constipation	11 (20)	0
Anorexia	10 (19)	0
Cough	10 (19)	0
Stomatitis	7 (13)	0
Weight loss	7 (13)	0
Pain in extremity	6 (11)	1 (2)
Vomiting	6 (11)	0
Arthralgia	6 (11)	0

4 Discussion

This two-part study was designed to evaluate the safety of escalating doses of dasatinib in Japanese patients with CP-CML (phase 1) and its safety and efficacy in patients with CP-CML, AP/BC-CML, and Ph^+ ALL (phase 2).

Although the results shown in this paper cover relatively short treatment periods of 6 and 3 months in CP-CML and AP/BC-CML or Ph^+ ALL, respectively, dasatinib demonstrated clinical efficacy in Japanese patients in all stages of CML and Ph^+ ALL resistant or intolerant to imatinib. Among patients with CP-CML, more than half achieved MCyR and most retained their cytogenetic response throughout the study period. These observations are clinically significant in view of reports that long-term prognosis may be improved in patients with CP-CML achieving MCyR [24, 25]. Also, in patients with AP/BC-CML and Ph^+ ALL, dasatinib monotherapy resulted in rapid achievement of a high rate of MaHR (64 and 38%, respectively) and the percentage of patients showing hematologic response among imatinib-resistant patients was comparable to that of imatinib-intolerant patients. The rate of cytogenetic response seemed to be higher in imatinib-intolerant patients than in imatinib-resistant patients in this study. Most patients enrolled in this study had a history of long-term imatinib therapy and of many other therapies such as interferon and chemotherapy, and were therefore expected to have a poor prognosis.

Table 6 Hematologic adverse events grade 3–4

	Cumulative incidence rate, *n* (%)		
	CP-CML ($n = 30$)	AP/BC-CML ($n = 11$)	Ph+ ALL ($n = 13$)
Leukopenia	8 (27)	5 (45)	10 (77)
Neutropenia	14 (47)	8 (73)	10 (77)
Thrombocytopenia	15 (50)	7 (64)	8 (62)
Anemia	5 (17)	2 (18)	4 (31)

However, these patients without effective treatment options showed favorable responses to dasatinib. The observation period was short in this study to be able to fully assess the efficacy of dasatinib in CML and Ph+ ALL patients and it would be expected that the response rate would be higher than the result in the present study.

At baseline, 20% of the subjects had *BCR-ABL* point mutations reported associated with resistance to imatinib [26]. Moreover, 64% of mutations observed were P-loop mutations, which are associated with high resistance to imatinib. Even these highly resistant patients achieved hematologic and cytogenetic responses. It is known that mutations associated with imatinib resistance reduce the potential of imatinib to bind to the ATP-binding site of BCR-ABL. Since the mode of binding by dasatinib differs from that by imatinib, dasatinib retains its activity even in the presence of mutation associated with imatinib resistance.

Although 35 (65%) of the 54 subjects in the present study were resistant to imatinib, mutation associated with imatinib resistance was seen in only 31% of the 35 imatinib-resistant subjects. This finding suggests that resistance to imatinib involves not only BCR-ABL point mutation but also other mechanisms. Since dasatinib exerted clinical efficacy even in patients without *BCR-ABL* point mutation, treatment with dasatinib is expected to overcome resistance to imatinib attributable not only to BCR-ABL mutation but also to other mechanisms.

In phase 1 of this study, dasatinib was shown to be safe in patients with chronic CML with dose escalations up to 90 mg twice daily. The only DLT observed in this study was grade 4 thrombocytopenia in 2 patients. Cytopenia is common adverse events in leukemia patients who have long-term and intensive prior therapy. Although cytopenia following dasatinib treatment could be controlled by dose interruption or reduction, close monitoring of blood cell counts is advisable during use of this drug.

Treatment had to be discontinued in 4 (7%) of the 54 patients because of adverse events. These results indicate that dasatinib is safe in patients with all phases of CML and Ph+ ALL resistant or intolerant to imatinib. Pleural effusion was noted in 14 (26%) patients, but the incidence of edema (a frequent toxicity of imatinib) was low in the present study. Grade ≥ 3 pleural effusion was seen in only one patient, and treatment did not have to be discontinued. The mechanism by which dasatinib induces pleural effusion is likely related to off-target kinase inhibition, platelet-derived growth factor receptor beta (PDGFRβ) in particular [27]. Pleural effusion was successfully treated by interruption of dasatinib and was reversible. There was low incidence of rash, muscle cramp, and nausea, which are frequent toxicities associated with imatinib. There was no apparent difference in the safety profile of dasatinib among Japanese and non-Japanese CML and Ph+ ALL patients [18–22]. It was rare that patients who had been intolerant to imatinib experienced the same severe nonhematologic toxicity following treatment with dasatinib. Therefore it is possible to treat imatinib-intolerant patients safely with dasatinib.

It has been reported that most Japanese CML patients are treated with lower dosages of imatinib than the standard recommended dosage, because of toxicities [28–31]. Imatinib treatment at low dosage is related with low rate of cytogenetic response [30]. Dasatinib is a meaningful option for those patients intolerant to the standard dosage of imatinib.

In the overseas phase 3 study designed to determine the optimal dose level and dosing method of dasatinib in patients with CP-CML [32], the efficacy of dasatinib 100 mg once daily in terms of hematologic response and cytogenetic response was comparable to that of 70 mg twice daily while the incidence of adverse events was lower. Dasatinib 100 mg once daily is currently being evaluated in Japanese patients with CP-CML. A multinational study (including Japan) is underway to access the efficacy and safety of dasatinib in newly diagnosed CML patients. In the past, only limited options were available for the treatment of imatinib-resistant or -intolerant CML and Ph+ ALL and patients often had a poor prognosis. The results of the present study indicate that dasatinib is promising as a new treatment for Japanese CML and Ph+ ALL patients resistant or intolerant to imatinib.

Conflicts of interest statement The authors indicated no potential conflicts of interest. T. S. is employee of Bristol-Myers K.K.

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ORIGINAL ARTICLE

A Phase I/II study of nilotinib in Japanese patients with imatinib-resistant or -intolerant Ph+ CML or relapsed/refractory Ph+ ALL

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Abstract Nilotinib is a second-generation BCR-ABL kinase inhibitor with improved potency and selectivity compared to imatinib. A Phase I/II dose-escalation study was designed to evaluate the efficacy, safety, and pharmacokinetics of nilotinib in Japanese patients with imatinib-resistant or -intolerant Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) or relapsed/refractory Ph+ acute lymphoblastic leukemia (ALL). A total of 34 patients were evaluated in this analysis and had a median duration of drug exposure of 293 (range 13–615) days. All 6 CML-CP patients without complete hematologic response (CHR) at baseline rapidly achieved CHR. A major cytogenetic response was achieved in 94% of patients with CML-CP, including a complete cytogenetic response in 69%. A major molecular response was achieved by 56%. These responses were also observed in patients with CML in advanced stages and Ph+ ALL. Non-hematologic adverse events were mostly mild to moderate. Grade 3 or 4

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neutropenia and thrombocytopenia occurred in 50 and 28% of patients, respectively. Overall, the results of this study suggest that nilotinib induced significant responses in imatinib-resistant or -intolerant patients with CML-CP and CML in advanced stages and Ph+ ALL. The results of this study confirmed the efficacy and safety of nilotinib in Japanese patients.

Keywords Nilotinib · CML · BCR-ABL · Imatinib resistant · Ph+ ALL

1 Introduction

The Philadelphia chromosome (Ph), which results from a reciprocal translocation between the long arms of chromosomes 9 and 22, is detected in more than 90% of chronic myeloid leukemia (CML) and 20–30% of adult acute lymphoblastic leukemia (ALL). The Philadelphia chromosome carries the *BCR-ABL* fusion gene, which encodes a constitutively active protein tyrosine kinase [1, 2]. Without BCR-ABL-targeted therapy, CML generally progresses within several years from a stable chronic phase (CP) to an accelerated phase (AP), and terminates in blast crisis (BC) [3]. Ph+ ALL is the most aggressive form of ALL and carries a poor prognosis comparable to CML-BC [4].

Imatinib (Gleevec®, Glivec®; Novartis Pharmaceuticals, Florham Park, NJ), a BCR-ABL tyrosine kinase inhibitor (TKI), has greatly improved the outcome in CML. In newly diagnosed CML patients, treatment with imatinib has shown a complete cytogenetic response (CCyR) rate of 87%, a progression rate to AP or BC of 7%, and an estimated 5-year survival rate of 89% [5, 6]. At the 6-year follow-up, the CCyR rate was 82% with 0% transformation to AP or BC between years 5 and 6, and an estimated 6-year overall survival of 88% [7]. However, resistance and intolerance to imatinib does occur in some patients and, therefore, additional treatment options are necessary to address these unmet medical needs.

Nilotinib (Tasigna®; Novartis Pharmaceuticals) is a second-generation TKI with improved potency and target specificity [8]. Like imatinib, nilotinib binds to and stabilizes an inactive conformation of the kinase domain of the ABL protein, thus preventing the enzyme from adopting the catalytically active conformation and blocking the tyrosine phosphorylation of proteins involved in BCR-ABL signal transduction [8, 9]. Nilotinib has been approved for the treatment of patients with CML-CP and -AP resistant to or intolerant of prior therapy, including imatinib, in 50 countries, including the United States and Europe based on the pivotal Phase II registration study. Responses to nilotinib were rapid and durable, with the vast majority of patients with CML-CP or -AP remaining alive at 12 months. Nilotinib is generally well tolerated, with a minimal occurrence of grade 3/4 drug-related adverse events and a favorable hematologic adverse event profile compared to other second-generation TKIs. Nilotinib also displayed significant activity in imatinib-resistant or -intolerant CML-BC, and relapsed/refractory Ph+ ALL, with significant rates of complete hematologic response (CHR), major cytogenetic response (MCyR), and CCyR [10–14].

This Phase I/II dose-escalation study, including an extension portion of the study, was conducted to confirm the efficacy, safety, and pharmacokinetic profiles of nilotinib in Japanese patients with imatinib-resistant or -intolerant CML or relapsed/refractory Ph+ ALL.

2 Methods

2.1 Study design and patient population

A Phase I/II dose-escalation study with an extension portion of the study was designed to evaluate the efficacy, safety, and pharmacokinetics of nilotinib. Patients who completed at least three 28-day cycles of treatment in the Phase I/II study without discontinuation were enrolled into the extension study. Tolerability up to the dose levels 400 mg BID, clinical dose approved in the US and Europe was confirmed in Japanese patients in a Phase I component [15].

Japanese patients were eligible for this multi-center, open label study when having imatinib-resistant/intolerant Ph+ CML or relapsed/refractory Ph+ ALL who were at least 20 years of age. Patients also needed to have adequate performance status (World Health Organization [WHO] Performance Score [PS] $\leq$ 2) and normal hepatic, renal, and cardiac functions.

CML-CP, -AP, -BC, Ph+ ALL and Ph+ ALL with minimal residual disease (MRD) were defined as previously described [10–14]. Imatinib resistance in patients with CML-CP was defined as failure to achieve CHR after 3 months, cytogenetic response (CyR) after 6 months, MCyR after 12 months, or loss of a hematologic or cytogenetic response at any time during treatment with imatinib following a minimum of 3 months of imatinib therapy with at least 600 mg/day. Imatinib resistance for CML-AP and -BC was defined by one of the following criteria during treatment with at least 600 mg/day of imatinib: (1) disease progression from chronic phase to accelerated or blast phase occurring during imatinib therapy; (2) disease progression defined as at least a 50% increase in peripheral white blood cell count, blast count, basophils, or platelets during imatinib therapy for accelerated or blast phase; or (3) lack of hematologic response (HR) in the bone marrow following a minimum of 4 weeks of imatinib therapy for

accelerated or blast phase. In addition, patients receiving less than 600 mg/day of imatinib were eligible for participation if *BCR-ABL* mutations were found present by sequencing any one of the following amino acids: L248, G250, Q252, Y253, E255, T315, F317, H396, M237, M244, D325, S348, M351, E355, A380, L387, M388, F486, and F359.

Imatinib intolerance for CML patients was defined as the discontinuation of imatinib therapy due to any of the following: grade 3 or 4 adverse events that persisted in spite of optimal supportive care measures, or grade 2 adverse events related to imatinib therapy in spite of optimal supportive care measures that persisted for at least 1 month or that recurred more than 3 times whether the dose was reduced or discontinued. In addition, the protocol definition of imatinib intolerance included the lack of a MCyR with imatinib.

Nilotinib (400 mg) was administered twice daily (BID; every 12 h) with water, while fasting 2 h before and 2 h after dosing. Dose reductions to 400 mg daily and subsequently 200 mg daily were permitted for the management of toxicity. If administration of a dose was delayed for more than 21 days for the management of toxicity (or more than 42 days for grade 3 or 4 hematologic toxicity), the patient was discontinued from the study. Treatment with nilotinib was continued until the patient experienced disease progression, developed unacceptable toxicity that precluded any further treatment, withdrew consent, and/or if the patient no longer benefited from the treatment (at the investigator's discretion).

2.2 Statistical analysis

The intent-to-treat (ITT) population and the safety population were included in the efficacy (including analyses for biomarker) and the safety analyses, respectively. The ITT population included all patients who received at lease one dose of nilotinib 400 mg BID. The safety population included all the patients in the ITT population who had at least one safety assessment. Pharmacokinetic analyses were performed for the pharmacokinetic population that included all patients who had available pharmacokinetic sample data. All analyses presented in this paper are based on the data obtained with the cut-off date of 3 October 2007 in all patients who received nilotinib 400 mg BID in any component of the study, including the Phase I, II, and its extension portions.

The rates on overall best hematologic response were summarized by disease phases and type (CML-CP, -AP, -BC, relapsed/refractory Ph+ ALL, and Ph+ ALL with MRD). The rates on overall best cytogenetic response were summarized for all CML patients. For CML-CP group only, 95% confidence intervals (95% CIs) using Clopper–Pearson limits were determined. Other efficacy analyses included the time-to-first response and duration of response using either the descriptive statistics or the Kaplan–Meier method.

2.3 Efficacy parameters

Hematologic and cytogenetic response criteria have been described in detail previously [10–14]. Criteria for cytogenetic responses are as follows: complete (0% Ph+ cells), partial (1–35% Ph+ cells), minor (36–65% Ph+ cells), and minimal (66–95% Ph+ cells). A MCyR includes both complete and partial cytogenetic responses. Cytogenetic responses were based on the percentage of Ph+ cells among 20 or more cells in metaphase in each bone marrow sample. Results obtained from fluorescent in situ hybridization (FISH) were also used to determine cytogenetic response only if fewer than 20 cells in metaphase were examined or bone marrow sample was not adequate on a particular assessment date due to other reasons. Only evaluable patients in the ITT population were included in the analysis for overall best hematologic and cytogenetic response rates. Patients with Ph+ CML who had a CHR at baseline were not included in the efficacy analysis for best hematologic response rates. Similarly, Ph+ CML patients who had CCyR at baseline were excluded from the analysis for best cytogenetic response. Evaluable patients who discontinued the study with no valid efficacy assessment were not included in the analysis for best responses.

2.4 Biomarkers

Peripheral blood samples were obtained prior to the first dose of nilotinib and every 3 months during nilotinib therapy. The BCR-ABL kinase domain (amino acid 230–490) was amplified from total blood RNA and mutations identified by direct sequencing that allowed for detection of more than 20% minor alleles. BCR-ABL transcript levels in blood were also monitored by a real-time quantitative RT-PCR (qRT-PCR) assay. The BCR-ABL mutational and qRT-PCR analyses were performed by Institute of Medical and Veterinary Science, Adelaide, Australia.

Patients were grouped based on their baseline mutational status: no mutation, any mutation, or multiple mutations. The number and percentage of patients who achieved a HR and CyR and major molecular response (MMR) were calculated for each mutation category in order to investigate the correlation between clinical responses and baseline BCR-ABL mutation status. A MMR was defined as BCR-ABL/control gene ratio of $\leq 0.1\%$ based on international scale, equivalent to ≥ 3 log reduction in BCR-ABL transcripts from the standardized baseline as determined in the international randomized study of

interferon and STI571 (IRIS) study. The number and percentage of patients who had at least one MMR post-baseline were calculated by disease phase. Patients who had a MMR at baseline were excluded from the analysis.

2.5 Safety parameters

Safety assessments included evaluation of adverse events, hematologic and biochemical testing, urinalysis, cardiac enzyme assessment, blood coagulation test, WHO PS scores, vital signs, physical examinations, 12-lead ECG, echocardiography, and chest X-rays. All adverse events were recorded with grades based on the Common Terminology Criteria for Adverse Events (CTCAE, version 3.0) of the National Cancer Institute, and monitored for at least 28 days after the last dose of nilotinib in patients who discontinued the study. Laboratory measurements were evaluated based mainly on the calculated CTC grades at baseline and post-baseline.

2.6 Pharmacokinetic parameters

The pharmacokinetic parameters were calculated by the standard non-compartmental method using WinNonlin Professional Edition 5.0 (Pharsight Corporation). Serum concentrations of nilotinib below the limit of quantitation (2.5 ng/mL) were treated as zero for the calculation of pharmacokinetic parameters. The following parameters were obtained: maximum serum concentration of nilotinib (C_{max}), time to reach C_{max} (T_{max}), area under the serum nilotinib concentration time curve from time 0 to 12 h post-dosing (AUC_{0-12}), and minimum serum concentration of nilotinib (C_{min}), defined as the concentration immediately before nilotinib administration.

2.7 Study conduct

The study was conducted in accordance with the Declaration of Helsinki. Patients gave written informed consent, according to institutional guidelines. The study was approved by the institutional review board at each study center.

3 Results

3.1 Patient demographics

Results are presented for 34 patients with at least 12 months of follow-up or those who prematurely discontinued study treatment. These include 31 patients enrolled in the Phase II component of the study (14 CML-CP, 7 CML-AP, 3 CML-BC, 7 Ph+ ALL) and 3 patients enrolled in the Phase I component of the study who received nilotinib 400 mg BID (2 CML-CP, 1 CML-BC). Disposition of patients is shown in Table 1. Of the 34 patients, 25 were enrolled in the extension study (16 CML-CP, 3 CML-AP, 3 CML-BC, 3 Ph+ ALL). At the time of data cut-off, 17 (50%) patients remained in the study. The most frequent reason for treatment discontinuation was disease progression.

The median duration of exposure (293 days; range 13–615) closely approximates the median duration of treatment (291 days; range 13–615) indicating minimal duration of treatment interruption. Nilotinib was well tolerated as indicated by the administration of median dose intensity (756 mg/day; range 285–799) which was close to the planned dose (400 mg BID = 800 mg/day) for the study.

Demographic and other baseline characteristics of patients are shown in Table 2. The median age of all

Table 1 Disposition of patients (ITT population)

	n (%)				
	CML-CP $N = 16$	CML-AP $N = 7$	CML-BC $N = 4$	Ph+ ALL $N = 7$	Total $N = 34$
Patients who enrolled in the extension study	16 (100)	3 (43)	3 (75)	3 (43)	25 (74)
Patients with treatment ongoing at cut-off date	15 (94)	1 (14)	0 (0)	1 (14)	17 (50)
Discontinued treatment at cut-off date	1 (6)	5 (71)[a]	4 (100)	6 (86)	16 (47)[a]
Reason for discontinuation					
Adverse event(s)	0 (0)	1 (14)	1 (25)	1 (14)	3 (9)
alloHSCT performed	1 (6)	2 (29)	1 (25)	0 (0)	4 (12)
Disease progression	0 (0)	2 (29)	2 (50)	5 (71)	9 (27)

The ITT population included all patients in the Phase I and Phase II studies who were administered a 400 mg BID dose at least once

alloHSCT allogeneic hematopoietic stem cell transplantation

[a] Does not include one patient who completed three cycles of the initial therapy but did not move to the extension study

Table 2 Demographic and other baseline characteristics (ITT population)

	CML-CP $N = 16$	CML-AP $N = 7$	CML-BC $N = 4$	Ph+ ALL $N = 7$	Total $N = 34$
Age (years)					
Median	57	61	53	62	62
Range (min–max)	30–83	30–74	29–70	23–80	23–83
Sex, *n* (%)					
Male	9 (56)	5 (71)	2 (50)	6 (86)	22 (65)
Female	7 (44)	2 (29)	2 (50)	1 (14)	12 (35)
Weight (kg)					
Median	61	65	63	56	61
Range (min–max)	45–89	49–83	36–69	46–60	36–89
WHO performance status, *n* (%)					
Grade 0	16 (100)	4 (57)	2 (50)	4 (57)	26 (76)
Grade 1	0 (0)	2 (29)	2 (50)	3 (43)	7 (21)
Grade 2	0 (0)	1 (14)	0 (0)	0 (0)	1 (3)
Grade >2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Time since first diagnosis (months)					
<6 months	2 (13)	0 (0)	0 (0)	1 (14)	3 (9)
≥6 months to <1 year	2 (13)	0 (0)	0 (0)	1 (14)	3 (9)
≥1 year to <2 years	3 (19)	2 (29)	1 (25)	4 (57)	10 (29)
≥2 years to <5 years	3 (19)	0 (0)	1 (25)	0 (0)	4 (12)
≥5 years	6 (38)	5 (71)	2 (50.0)	1 (14)	14 (41)
Number of patients, *n* (%)					
Imatinib resistant	4 (25)	4 (57)	4 (100)	7 (100)	19 (56)
Imatinib intolerant	12 (75)	3 (43)	0 (0)	0 (0)	15 (44)
Highest imatinib dose					
Mean ± SD	519 ± 210	686 ± 157	700 ± 115	600 ± 0	591 ± 178
Median	500	800	700	600	600
Range (min–max)	200–800	400–800	600–800	600–600	200–800

patients was 62 years (range 23–83 years), and approximately 65% of all patients were male. The median weight of all patients was 61 kg (range 36–89 kg). Imatinib-intolerant patients constituted 75% (12/16) of CML-CP, though all CML-BC and Ph+ ALL patients were imatinib resistant. One of 4 imatinib-resistant CML-CP patients was primary resistant.

3.2 Pharmacokinetic analysis

Table 3 shows pharmacokinetic parameters determined following the administration of nilotinib 400 mg BID. Absorption of nilotinib was relatively rapid with median T_{max} of 3 h with large inter-individual variability. Steady state was achieved by day 6 after repeated dosing since C_{min} had been almost constant after day 6. The accumulation ratio calculated by ratio of AUC_{0-12} on days 15 to 1 was 2.64 ± 1.07 (mean ± SD). The serum concentrations of nilotinib did not differ among the phases of CML and Ph+ ALL group on the first day of treatment. On day 15, nilotinib exposure in the CML-BC group and Ph+ ALL group appeared to be slightly higher than in other groups, but this difference is most likely due to the small patient number and relatively large inter-individual variability. Steady-state nilotinib concentrations observed on day 15 in this study were similar to those observed previously in non-Japanese patients [16].

3.3 Efficacy

3.3.1 Hematologic response

Table 4 details the HR rates. Six of the 16 patients with CML-CP patients without a CHR at baseline were included in the efficacy analysis for HR. All 6 patients achieved a CHR (100%; 95% CI: 54.1–100.0%). In the CML-AP patients, a HR was achieved in 5/7 (71%) patients, including 1 CHR, 3 marrow responses with no evidence of leukemia (NEL), and 1 return to CP. In the CML-BC patients, a HR was achieved in 2/4 (50%) patients,

Table 3 Pharmacokinetic parameters following administration of 400 mg BID of nilotinib (ITT population)

	N	T_{max} (h) [median (range)]	Mean ± SD		
			C_{max} (ng/mL)	AUC_{0-12} (ng h/mL)	C_{min} (ng/mL)
Day 1					
Total	33	3.0 (2.0–23.0)	1070 ± 458	7850 ± 2790	NA
CML-CP	15	3.0 (2.0–7.0)	942 ± 276	7110 ± 1800	NA
CML-AP	7	3.0 (2.9–23.0)	1120 ± 614	7550 ± 3150	NA
CML-BC	4	5.5 (3.0–7.0)	1150 ± 458	8880 ± 2700	NA
Ph+ ALL	7	3.0 (2.2–7.0)	1220 ± 618	9150 ± 4010	NA
Day 15					
Total	28	3.0 (1.8–8.0)	2320 ± 1070	19000 ± 9090[a]	1170 ± 588
CML-CP	13	3.0 (1.9–8.0)	2010 ± 652	17200 ± 6030[b]	1051 ± 410
CML-AP	6	3.0 (1.8–3.0)	1760 ± 884	15000 ± 6770	885 ± 349
CML-BC	3	2.1 (1.9–5.0)	3210 ± 1340	30300 ± 15200	1890 ± 893
Ph+ ALL	6	3.0 (1.9–8.0)	3140 ± 1310	21200 ± 10400[c]	1350 ± 732

NA not applicable

[a] $N = 26$

[b] $N = 12$

[c] $N = 5$

including 1 CHR and 1 return to CP. The rate of HR confirmed at 2 consecutive visits at least 4 weeks apart was achieved in 2/7 (29%) CML-AP patients, 2/4 (50%) CML-BC patients. A CR was achieved in 1/5 (20%) relapsed/refractory Ph+ ALL and both 2 Ph+ ALL patients with MRD.

The median time to CHR was 1 month (range 1–2 months) for CML-CP patients. Time to HR was 1 month for all of the CML-AP, BC and Ph+ ALL patients but 2 months in one CML-BC patient. The duration of CHR was not determined because all CML-CP patients were still responding to treatment at the data cut-off date. The range of duration of CHR in 6 CML-CP patients who achieved CHR up to the data cut-off date was 11.6–13.6 months. All ten patients with CHR at baseline were maintaining response at the time of data cut-off. In 3 of the 5 patients with CML-AP who achieved a HR, the response continued until either the time they discontinued study treatment or the data cut-off date. The duration of the other 2 CML-AP patients was 1 and 2 months each. Of the 2 patients with CML-BC who achieved a HR, one was still in HR at data cut-off and one had a duration of HR of 2 months. For patients with relapsed/refractory Ph+ ALL, 1 who achieved a HR continued to show a response for 3.9 months. One of the two Ph+ ALL patients with MRD was still in HR at data cut-off and the other lasted for 2 months.

3.3.2 *Cytogenetic response*

A MCyR was achieved in 15 of the 16 CML-CP patients (94%; 95% CI: 70–100.0%), and a CCyR in 11 (69%) of these patients (Table 5). A CCyR was achieved in 1 (14%) of the 7 CML-AP patients. In the other 6 patients, 3 achieved a minimal CyR, 1 no CyR, and 2 patients were considered not assessable for response due to dry-tap bone marrow. As for the patients with CML-BC, 2 of the 4 patients (50%) achieved a CCyR.

Time to MCyR or CCyR was evaluated in CML-CP patients. The median time to MCyR was 3 months (range 1–6.6 months) and the time to CCyR was also 3.2 months (range 2–11.9 months). The MCyR continued, in all patients achieving MCyR, until the data cut-off date or discontinuation from study treatment, so the median duration of MCyR has not been reached at the time of data cut-off.

3.3.3 *Molecular response*

A MMR was achieved in 9 (56%) of 16 CML-CP patients. The median time to MMR was 6.3 months (range 3–18.3 months). These 9 patients are still in MMR at data cut-off. A MMR was achieved in 1 (14%) of the 7 CML-AP patients, 2 (50%) of the 4 CML-BC patients. None achieved MMR in relapsed/refractory Ph+ ALL. One of 2 Ph+ ALL with MRD achieved MMR and the other one was considered as not evaluable due to MMR at baseline.

3.3.4 *Response by BCR-ABL mutation status*

BCR-ABL mutations at baseline were detected in 4 (25%) of 16 CML-CP patients, in 6 (86%) of 7 CML-AP patients, in 2 (50%) of 4 CML-BC patients, and in 4 (57%) of 7 Ph+ ALL patients. A total of 14 different BCR-ABL mutations