Thirty-one patients were enrolled into the phase II study and treated with nilotinib 400 mg twice daily (CML-CP: 14, CML-AP: 7, CML-BC: 3; Ph+ ALL: 7) and 3 patients were enrolled in the phase I study and treated with nilotinib 400 mg twice daily (CML-CP: 2; CML-BC: 1) [22]. The characteristics and disposition of patients are summarized in Tables 1 and 2, respectively. Fourteen patients (CML-CP: 13; CML-AP: 1) received nilotinib until the end of the study while 20 patients (CML-CP: 3, CML-AP: 6, CML-BC: 4; Ph+ ALL: 7) discontinued study treatment. The

Table 1 Patient characteristics (ITT population)

	CML-CP ($N = 16$)	CML-AP ($N = 7$)	CML-BC ($N = 4$)	Ph+ ALL ($N = 7$)	Total ($N = 34$)
Age (years)	57.0 (30–83)	61.0 (30–74)	53.0 (29–70)	62.0 (23–80)	61.5 (23–83)
Sex					
Male	9 (56)	5 (71)	2 (50)	6 (86)	22 (65)
Female	7 (44)	2 (29)	2 (50)	1 (14)	12 (35)
Body weight (kg)	61.2 (44.5–89.0)	64.8 (49.1–83.0)	63.3 (35.5–69.0)	55.8 (46.2–60.2)	60.5 (35.5–89.0)
WHO PS					
0	16 (100)	4 (57)	2 (50)	4 (57)	26 (76)
1	0 (0)	2 (29)	2 (50)	3 (43)	7 (21)
2	0 (0)	1 (14)	0 (0)	0 (0)	1 (3)
Time since first diagnosis (months)	30.4 (1.4–122.8)	108.6 (12.5–192.8)	65.3 (20.5–102.8)	16.2 (3.7–134.1)	30.4 (1.4–192.8)
Imatinib resistance	4 (25.0)	4 (57.1)	4 (100.0)	7 (100.0)	19 (55.9)
Imatinib intolerance	12 (75.0)	3 (42.9)	0 (0.0)	0 (0.0)	15 (44.1)
Highest imatinib dose (mg)	500 (200–800)	800 (400–800)	700 (600–800)	600 (600–600)	600 (200–800)

Values are *n* (%) or median (range)

ITT intention-to-treat, *WHO PS* World Health Organization performance status

Table 2 Patient disposition (ITT population)

	CML-CP ($N = 16$)	CML-AP ($N = 7$)	CML-BC ($N = 4$)	Ph+ ALL ($N = 7$)	Total ($N = 34$)
Completed the long-term study	13 (81)	1 (14)	0 (0)	0 (0)	14 (41)
Discontinued treatment and withdrawn from the study	3 (19)	6 (86)	4 (100)	7 (100)	20 (59)
Reason for discontinuation					
Adverse event(s)	0 (0)	1 (14)	1 (25)	1 (14)	3 (9)
Allo-HSCT performed	1 (6)	2 (29)	1 (25)	0 (0)	4 (12)
Disease progression	1 (6)	3 (43)	2 (50)	6 (86)	12 (35)
Withdrawal of consent	1 (6)	0 (0)	0 (0)	0 (0)	1 (3)
Dose reduction	15 (94)	5 (71)	3 (75)	4 (57)	27 (79)
Withdrawal from treatment	11 (69)	2 (29)	2 (50)	2 (29)	17 (50)
Drug administration recommenced at a reduced dose after withdrawal	10 (63)	1 (14)	0 (0)	2 (29)	13 (38)
Duration of exposure (days)[a]	1099.5 (176–1173)	84.0 (56–1099)	133.0 (15–247)	56.0 (13–644)	445.5 (13–1173)
Duration of administration (days)[b]	1084.5 (165–1173)	84.0 (28–1099)	126.5 (14–247)	56.0 (13–609)	428.0 (13–1173)
Daily dose (mg)[c]	612.9 (394.2–798.6)	789.6 (284.9–797.5)	742.6 (402.4–798.4)	785.7 (483.2–794.1)	750.7 (284.9–798.6)

Values are *n* (%) or median (range)

Allo-HSCT allogeneic hematopoietic stem cell transplantation, *ITT* intention-to-treat

[a] Includes drug interruptions

[b] Excludes drug interruptions

[c] Daily dose = total dose/duration of exposure (includes drug interruption)

Table 3 Best responses to nilotinib (ITT population)

	CML-CP ($N = 16$)	CML-AP ($N = 7$)	CML-BC ($N = 4$)	Ph+ ALL ($N = 7$)
Hematologic response (HR)	6 (100)[a]	5 (71)	2 (50)	3 (43)
Complete hematologic response	6 (100)	1 (14)	1 (25)	–
Complete response	–	–	–	3 (43)
Marrow response with no evidence of leukemia	–	3 (43)	0 (0)	–
Return to chronic phase	–	1 (14)	1 (25)	–
Stable disease	0 (0)	1 (14)	2 (50)	1 (14)
Progressive disease	0 (0)	0 (0)	0 (0)	3 (43)
Not evaluable/not assessable	10 (63)	1 (14)	0 (0)	0 (0)
Cytogenetic response (CyR)				
Major	15 (94)	1 (14)	2 (50)	–
Complete	13 (81)	1 (14)	2 (50)	–
Partial	2 (13)	0 (0)	0 (0)	–
Minor	0 (0)	0 (0)	1 (25)	–
Minimal	1 (6)	3 (43)	0 (0)	–
None	0 (0)	1 (14)	0 (0)	–
Not assessable	0 (0)	2 (29)	1 (25)	–
Molecular response (MR)				
Major[b]	13 (81)	1 (14)	2 (50)	1 (17)[c]
None	3 (19)	6 (86)	2 (50)	5 (83)[c]
Not evaluable	0 (0)	0 (0)	0 (0)	1 (14)

Values are *n* (%)

[a] Of which 6 were evaluable

[b] Major molecular response was defined as a BCR-ABL/BCR ratio ≤0.1%

[c] Of which 6 were evaluable

ITT intention-to-treat

most frequent reason for discontinuation was disease progression in 12 patients. Disease progression was seen in 1 patient with CML-CP, 3 patients with CML-AP, 2 patients with CML-BC and 6 patients with Ph+ ALL.

The median duration (range) of nilotinib exposure was 445.5 days (13–1173 days) and that of administration was 428.0 days (13–1173 days). The median daily dose (range) of nilotinib was 750.7 mg/day (284.9–798.6 mg/day) in all patients, consistent with the planned dose of administration (400 mg twice daily = 800 mg/day) in the study protocol. Dose reductions occurred in 27 patients (79.4%) because of adverse events in 19 patients (55.9%), in accordance with the study protocol in 14 patients (41.2%), incorrect administration in 10 patients (29.4%) or incorrect scheduling in 1 patient (2.9%) (multiple dose reductions were possible). Treatment interruption occurred in 17 patients (50.0%) because of adverse events in all 17 patients. Thirteen of these patients showed improvement of adverse events and were able to restart nilotinib administration at a lower dose.

Efficacy

CML-CP

The best responses (HR, CyR and MR) in the ITT population are shown in Table 3. All 6 CML-CP patients without CHR at baseline achieved CHR. The median time

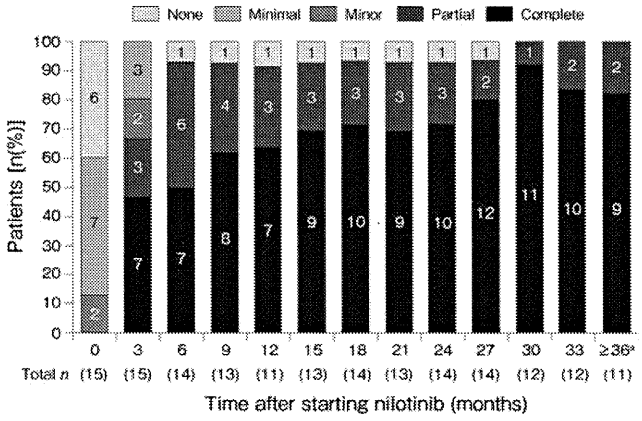

Fig. 1 Cytogenetic responses in CML-CP patients. [a]Including up to and beyond 36 months

(range) to CHR was 28 days (28–56 days). Of these, 5 patients showed sustained response up to the last evaluation, while the remaining patient discontinued treatment on Day 787 because of disease progression. The duration of CHR in that patient was 478 days. MCyR was achieved in 15 patients (93.8%) and the response was sustained at the last evaluation in 13 patients. CCyR was achieved in 13 patients (81.3%) and the response was sustained at the last evaluation in 11 patients. The median time (range) to MCyR or CCyR was 84 days (28–178 days) and 97 days (57–847 days), respectively. The rate of CyR in evaluable patients at each time point is shown in Fig. 1. Thirteen

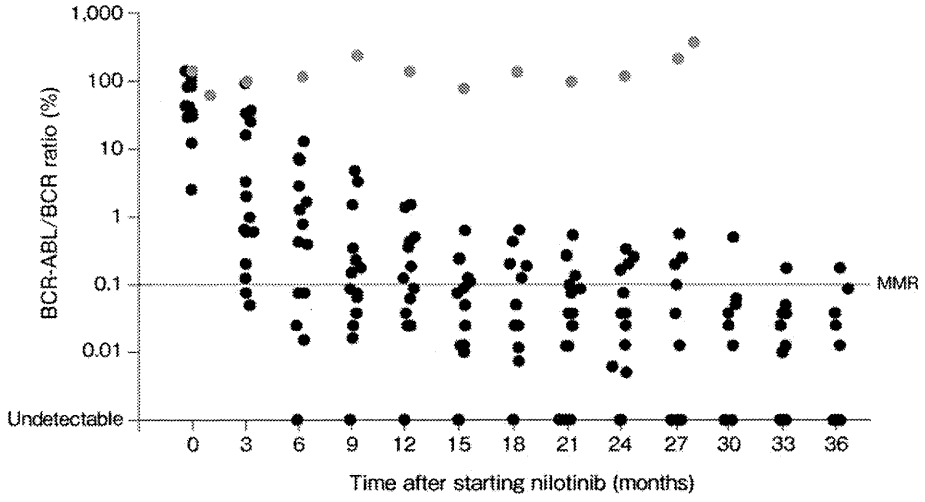

Fig. 2 Molecular responses during the 36-month study in patients with CML-CP. *MMR* major molecular response

patients continued treatment at 36 months or later. Among them, 11 patients were evaluated as showing cytogenetic response, all of whom achieved MCyR, including 9 with CCyR. The figure shows that the proportion of CCyRs increased with nilotinib treatment period.

The BCR-ABL/BCR ratio in CML-CP patients over time is shown in Fig. 2. The BCR-ABL/BCR ratio gradually decreased from baseline with long-term nilotinib treatment in all patients except one with baseline or newly detected mutations. An approximately 1-log reduction in BCR-ABL/BCR ratio from baseline at 6 months and an approximately 2-log reduction at 12 months were observed. MMR was achieved in 13 patients (81.3%) and was sustained at the last evaluation in 11 patients. The median time (range) to MMR was 248 days (84–852 days) in these CML-CP patients.

Among CML-CP patients, 3 patients discontinued nilotinib treatment. One patient discontinued treatment on Day 176 to undergo allogeneic hematopoietic stem cell transplantation (allo-HSCT). Another patient once achieved CCyR but discontinued treatment on Day 787 because of disease progression, as mentioned above. This patient had a newly detected mutation (F359V). Another patient withdrew consent on Day 931.

CML-AP

Among 7 CML-AP patients, 5 patients (71.4%) achieved HR, including CHR in 1 patient, marrow response with no evidence of leukemia in 3 patients, and return to chronic phase in 1 patient. Of the remaining 2 patients, 1 had stable disease and 1 was not evaluable. Of the 5 patients with HR, 1 patient with CHR and another 2 patients with HR experienced sustained response at the last evaluation or at discontinuation of treatment. In the remaining 2 patients, the duration of HR was 29 and 57 days, respectively. Minimal CyR was observed in 3 patients (42.9%). One patient with CHR achieved CCyR (14.3%). This patient also achieved MMR, which was sustained at the last evaluation.

CML-BC

Among 4 CML-BC patients, 2 patients (50.0%) achieved HR, including CHR in 1 patient and return to chronic phase in 1 patient. They also achieved CCyR and MMR. In both patients, MCyR was sustained until discontinuation of treatment to undergo allo-HSCT (on Day 247) in the first patient, or because of increasing blast numbers in bone marrow (on Day 168) in the second patient. The remaining 2 patients (50.0%) experienced stable disease and one of them achieved minor CyR.

Ph+ ALL

Among 7 patients with relapsed/refractory Ph + ALL, 1 of 5 patients (20.0%) without MRD experienced HR (complete response [CR]), which was sustained for 108 days. Three patients experienced disease progression and 1 experienced stable disease. Both patients with MRD achieved HR (CR). In one of these patients, CR was sustained for 58 days, but treatment was discontinued on Day 109 because of encephalitis. In the other patient, CR was sustained for 470 days, but treatment was discontinued on Day 644 because of disease progression. MMR was achieved in 1 patient with MRD, while the other patient with MRD achieved MMR at baseline and was thus considered not evaluable.

BCR-ABL mutations

Detection of new mutations

The development of new BCR-ABL mutations during the administration of nilotinib in this study is shown in

Table 4 Detection of new BCR-ABL mutations

Stage	Mutation	Day of detection	Baseline mutation	Achieved MMR	Outcome
CML-CP	F359V	174	M244V	No	Disease progression
CML-CP	E255K	340	None	Yes	Continued
CML-BC	T315I/Y253H	168	F317L	Yes	Disease progression
Ph+ ALL	T315I	16	E255K/E255V/G250E	No	Disease progression
Ph+ ALL	E255V	57	E459K	No	Disease progression
Ph+ ALL	T315I	43	None	No	Disease progression
Ph+ ALL	E255K/E255V	135	NA	No	Disease progression

MMR major molecular response, *NA* Not assessable

Table 4. New mutations were detected in 7 patients during nilotinib treatment. Among them, the T315I mutation occurred in 3 patients and nilotinib was discontinued in these patients because of disease progression. Three of the 4 patients with mutations other than T315I also discontinued treatment because of disease progression. The remaining patient continued treatment.

CML-CP

Among 16 CML-CP patients, MMR was observed in 4 of 5 patients (80.0%) with BCR-ABL mutations at baseline or emerging during the treatment period. As shown in Table 4, new mutations were detected in 2 patients.

One patient had a baseline M244V mutation and achieved minimal CyR on Day 87; however, an F359V mutation was also detected on Day 174. From Day 426, only the F359V mutation was detected and the M244V mutation was not; this patient was withdrawn from the study because of disease progression on Day 787 (see "CML-CP" under the heading Efficacy). In another patient without baseline mutation, E255K was detected only once on Day 340. This patient achieved MMR on Day 511, which was sustained at the last evaluation, and the mutation was not detected again after achievement of MMR. In 1 patient with an imatinib resistance-associated mutation (F359I) at baseline, the mutation could not be detected after commencing nilotinib treatment, which led to MMR that had been sustained for 666 days at the last evaluation.

CML-AP/-BC and Ph+ ALL

Among 7 CML-AP patients, no new mutations were detected. As shown in Table 4, among 4 CML-BC patients, new mutations were detected in 1 patient with the F317L mutation at baseline. This patient achieved CCyR and MMR on Day 56; however, Y253H and T315I mutations were detected on Day 168 followed by disease progression on Day 171. Among 7 Ph+ ALL patients, new mutations were detected in 4 patients, all of whom experienced disease progression.

Safety analysis

All adverse events regardless of drug relationship occurring at a frequency ≥20% and those of grade 3/4 are summarized in Table 5 (adverse events and adverse drug reactions occurring in ≥10% of subjects are shown in Supplemental Tables 1 and 2, respectively, while all adverse events of grade 3 or worse are shown in Supplemental Table 3). Adverse events occurred in all of the patients. The most common non-hematologic events were rash (64.7%), nasopharyngitis (58.8%), nausea and headache (47.1% each), and vomiting (41.2%). Hematologic events included leukopenia (47.1%), neutropenia (47.1%), thrombocytopenia (47.1%) and anemia (38.2%).

Adverse events of grade 3/4 occurred in 29/34 patients (85.3%). The most frequent grade 3/4 non-hematologic events were abnormal hepatic function, hyponatremia and pneumonia (11.8% each). Grade 3/4 hematologic events included neutropenia (47.1%), leukopenia (41.2%), thrombocytopenia (32.4%), anemia (29.4%) and lymphopenia (11.8%). The most common biochemical grade 3/4 events were decreased blood phosphorus levels (14.7%), hyperglycemia and increased lipase levels (11.8% each).

Serious adverse events

Thirty-four serious adverse events occurred in 19 patients. Among these, 21 events in 12 patients were considered possibly related to nilotinib. Two of these patients discontinued nilotinib treatment because of serious adverse events considered to be related to the drug. One, with CML-BC, developed back pain (non-serious) and discontinued treatment. Two days later, this patient developed cardiac tamponade and pericardial effusion, and died because of heart failure. The other, with Ph+ ALL, developed encephalitis and also discontinued treatment. Furthermore, one CML-CP patient developed acute pancreatitis reported as a serious adverse event that resolved

Table 5 Non-hematologic, hematologic and biochemical adverse events with a frequency ≥20% for all grades

Total $N = 34$	All grades					Grade 3/4				
	CML-CP *n* (%)	CML-AP *n* (%)	CML-BC *n* (%)	Ph+ ALL *n* (%)	Total *n* (%)	CML-CP *n* (%)	CML-AP *n* (%)	CML-BC *n* (%)	Ph+ ALL *n* (%)	Total *n* (%)
Non-hematologic events										
Rash	9 (56.3)	5 (71.4)	3 (75.0)	5 (71.4)	22 (64.7)	1 (6.3)	0 (0.0)	1 (25.0)	0 (0.0)	2 (5.9)
Nasopharyngitis	15 (93.8)	3 (42.9)	2 (50.0)	0 (0.0)	20 (58.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Headache	7 (43.8)	2 (28.6)	3 (75.0)	4 (57.1)	16 (47.1)	0 (0.0)	0 (0.0)	1 (25.0)	1 (14.3)	2 (5.9)
Nausea	6 (37.5)	3 (42.9)	4 (100.0)	3 (42.9)	16 (47.1)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (2.9)
Vomiting	6 (37.5)	3 (42.9)	2 (50.0)	3 (42.9)	14 (41.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pyrexia	4 (25.0)	1 (14.3)	4 (100.0)	4 (57.1)	13 (38.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Constipation	8 (50.0)	2 (28.6)	1 (25.0)	1 (14.3)	12 (35.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hyperbilirubinemia	5 (31.3)	3 (42.9)	1 (25.0)	1 (14.3)	10 (29.4)	2 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (5.9)
Hyperglycemia	8 (50.0)	1 (14.3)	1 (25.0)	0 (0.0)	10 (29.4)	2 (12.5)	1 (14.3)	1 (25.0)	0 (0.0)	4 (11.8)
Malaise	8 (50.0)	0 (0.0)	0 (0.0)	2 (28.6)	10 (29.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Back pain	6 (37.5)	0 (0.0)	2 (50.0)	1 (14.3)	9 (26.5)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (2.9)
Pruritus	3 (18.8)	2 (28.6)	1 (25.0)	3 (42.9)	9 (26.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abnormal hepatic function	5 (31.3)	0 (0.0)	1 (25.0)	2 (28.6)	8 (23.5)	1 (6.3)	0 (0.0)	1 (25.0)	2 (28.6)	4 (11.8)
Conjunctivitis	7 (43.8)	1 (14.3)	0 (0.0)	0 (0.0)	8 (23.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Diarrhea	3 (18.8)	2 (28.6)	1 (25.0)	2 (28.6)	8 (23.5)	0 (0.0)	0 (0.0)	1 (25.0)	1 (14.3)	2 (5.9)
Anorexia	5 (31.3)	1 (14.3)	0 (0.0)	1 (14.3)	7 (20.6)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Arthralgia	5 (31.3)	2 (28.6)	0 (0.0)	0 (0.0)	7 (20.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Eczema	6 (37.5)	0 (0.0)	1 (25.0)	0 (0.0)	7 (20.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hypokalemia	1 (6.3)	2 (28.6)	2 (50.0)	2 (28.6)	7 (20.6)	0 (0.0)	1 (14.3)	0 (0.0)	1 (14.3)	2 (5.9)
Insomnia	2 (12.5)	2 (28.6)	1 (25.0)	2 (28.6)	7 (20.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pharyngitis	4 (25.0)	0 (0.0)	0 (0.0)	3 (42.9)	7 (20.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hematologic events										
Leukopenia	7 (43.8)	3 (42.9)	2 (50.0)	4 (57.1)	16 (47.1)	5 (31.3)	3 (42.9)	2 (50.0)	4 (57.1)	14 (41.2)
Neutropenia	7 (43.8)	3 (42.9)	2 (50.0)	4 (57.1)	16 (47.1)	7 (43.8)	3 (42.9)	2 (50.0)	4 (57.1)	16 (47.1)
Thrombocytopenia	7 (43.8)	3 (42.9)	2 (50.0)	4 (57.1)	16 (47.1)	3 (18.8)	3 (42.9)	2 (50.0)	3 (42.9)	11 (32.4)
Anemia	5 (31.3)	2 (28.6)	3 (75.0)	3 (42.9)	13 (38.2)	3 (18.8)	2 (28.6)	2 (50.0)	3 (42.9)	10 (29.4)
Biochemical events										
Increased bilirubin	6 (37.5)	1 (14.3)	1 (25.0)	2 (28.6)	10 (29.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Increased alanine aminotransferase	3 (18.8)	0 (0.0)	2 (50.0)	3 (42.9)	8 (23.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	1 (2.9)
Increased lipase	5 (31.3)	1 (14.3)	1 (25.0)	1 (14.3)	8 (23.5)	3 (18.8)	1 (14.3)	0 (0.0)	0 (0.0)	4 (11.8)

The table includes drug-related and non-related adverse events combined

following nilotinib dose interruption. This patient restarted nilotinib at 400 mg once daily, which was then increased to 400 mg twice daily, and the subject completed study treatment. QT interval prolongation occurred in 1 CML-CP patient and nilotinib treatment was interrupted. This patient restarted nilotinib at 400 mg once daily and continued treatment without QT interval prolongation.

Adverse events by time-points

Among the CML-CP patients, the incidences of blood/lymphatic system disorders, gastrointestinal disorders, laboratory abnormalities, and skin/subcutaneous tissue disorders in Cycles 1–12 in the first year of treatment were 68.8, 87.5, 62.5 and 75.0%, respectively. The incidences of these events were much lower during Cycles 13–24 (20.0, 40.0, 40.0 and 53.3%, respectively) and Cycles 25 or later (20.0, 73.3, 26.7, 46.7%) in the second year of treatment. Gastrointestinal disorders showed higher incidence in Cycles 25 or later (3 years or more of treatment) and, in particular, the incidence of constipation was as high as 26.7%. Fewer patients with CML-AP, CML-BC, and Ph+ ALL continued treatment beyond Cycle 24, so no significant difference in the

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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Conflict of interest Taro Amagasaki and Aira Wanajo are employees of Novartis Pharmaceuticals. The other authors have no conflicts of interest to disclose.

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ORIGINAL ARTICLE

Lack of non-hematological cross intolerance of dasatinib to imatinib in imatinib-intolerant patients with Philadelphia chromosome positive chronic myeloid leukemia or acute lymphatic leukemia: a retrospective safety analysis

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Abstract The aim of this retrospective study was to evaluate the toxicity profiles of dasatinib in patients with Philadelphia chromosome positive chronic myeloid leukemia (CML) or acute lymphatic leukemia (ALL) who were intolerant to imatinib, and who had been enrolled in our previous clinical trials to evaluate efficacy of dasatinib in patients resistant or tolerant to imatinib therapy. Twenty-four patients with CML and four with ALL were enrolled in the clinical studies to evaluate the efficacy according to the eligibility criteria related to intolerance to imatinib therapy. The toxicities reported during imatinib therapy were non-hematological toxicities in 23 patients and hematological toxicities in six patients. Patients were administered dasatinib 50–70 mg BID or 100 mg QD. Cross intolerance was observed in four patients who showed hematological toxicity after dasatinib treatment. However, it was possible to successfully continue therapy with only temporary interruption. No cross intolerance in

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non-hematological toxicity was found with the exception of one patient who showed cross intolerance, which did not result in treatment interruption. Dasatinib can be safely administered to imatinib-intolerant CML or Ph-positive ALL patients.

Keywords Dasatinib · Cross intolerance · CML · ALL

1 Introduction

The introduction of imatinib mesylate (Gleevec®) has dramatically changed the management and prognosis of patients with chronic myeloid leukemia (CML) [1–3]. Despite the outstanding results achieved by imatinib, approximately 31 and 45% of patients in the IRIS study discontinued imatinib treatment within 5 and 8 years, respectively, because of insufficient responses or unacceptable toxicities [4, 5]. The most commonly reported adverse events of imatinib were edema (including peripheral and periorbital edema), muscle cramps, diarrhea, nausea, muscloskeletal pain, rash and other skin problems, abdominal pain, fatigue, joint pain, headache and pancytopenia.

Dasatinib, a novel BCR-ABL tyrosine kinase inhibitor, possesses 325-fold greater potency than imatinib in inhibiting BCR-ABL kinase in vitro [6], and is effective in treating patients with CML resistant or intolerant to imatinib [7–12]. Fluid retention, gastrointestinal toxicities, and myelosuppression are the most common adverse events of dasatinib, and have been shown to be dose- and schedule-dependent in a global study [13]. The efficacy and safety profiles of dasatinib were also evaluated in Japanese patients and compared with the global data [7, 14]. This retrospective analysis was performed to evaluate whether toxicity profiles demonstrated by imatinib therapy influenced those shown by dasatinib therapy in patients enrolled in our previous studies.

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2 Patients and methods

Data from 2 studies in CML or acute lymphatic leukemia (ALL) patients (1 phase I/II and 1 phase II) were analyzed. Intolerance to imatinib was defined as Grade 3 non-hematologic toxicity and/or Grade 4 hematologic toxicity lasting 7 days or longer. Dasatinib doses were either 50, 70, 90 mg BID or 100 mg QD, depending on the study in which the patient was enrolled. Intolerance to imatinib was defined as either non-hematological toxicity greater than or equal to Grade 3, hematological toxicity greater than or equal to Grade 3 [CA180-138 study [15]], or hematological toxicity of Grade 4 lasting 7 days or longer [CA180-031/036 studies [14]]. Cross intolerance was defined as the same adverse events greater than or equal to Grade 3 occurred due to dasatinib therapy.

3 Results

A total of 77 patients were enrolled in our clinical studies and 28 imatinib-intolerant CML and Ph(+) ALL patients were included in this analysis (Table 1). The dose of prior imatinib therapy was 400–600 mg per day in 25 patients (89%). Imatinib therapy was administered for over 3 years in 5 patients (18%). The eligibility criteria according to which patients were enrolled in our clinical studies were hematologic toxicities of imatinib in 6 patients and non-hematologic toxicities in 23 patients (1 patient was enrolled in the study with a combination of both criteria) (Table 2).

Hematologic toxicities of imatinib developed in 6 patients with chronic phase CML (CML-CP) (Table 3). These included thrombocytopenia in 2 patients, anemia in 2 patients, and neutropenia and pancytopenia in 1 patient, respectively. Of the 6 patients, 2 patients (1 patient with anemia and 1 patient with pancytopenia) did not develop the same toxicities, however, the other 4 patients again developed the same toxicities at Grade 3 or higher. The latter 4 patients had developed thrombocytopenia (2 patients), anemia (1 patient), and neutropenia (1 patient) due to prior imatinib therapy. Two patients who had developed thrombocytopenia had the same toxicities. However, dasatinib was temporarily interrupted for 21 and 14 days, and, after re-starting, toxicity was resolved without dose reduction in the former patient, and in the latter with dose reduction to 80 mg QD.

Toxicity was resolved without discontinuation in 1 patient with anemia due to prior imatinib therapy, which again developed anemia with dasatinib therapy. In addition, neutropenia recurred in 1 patient and the patient had to have temporary interruption for 28 days, however, the toxicity was resolved following dose reduction of dasatinib at 80 mg QD. In total, only 2 patients required dose

Table 1 Patient characteristics

	CP-CML P I/II ($N = 12$)	CP-CML P II ($N = 9$)	AP/BC-CML ($N = 3$)	Ph+ALL ($N = 4$)
Median age (range)	38 (27–67)	55 (32–74)	59 (31–73)	62 (29–70)
Median years from diagnosis (range)	1.6 (0.3–14.7)	0.8 (0.2–6.3)	2.5 (0.9–10.4)	1.2 (0.2–2.2)
Intolerance				
Hematologic toxicity	1	3	2[a]	0
Non-hematologic toxicity	11	6	2[a]	4
Prior imatinib therapy				
400–600 mg/day (%)	92	100	67	100
>600 mg/day (%)	8	0	33	0
>3 years (%)	33	0	33	0
BCR-ABL mutation (%)	0	0	0	0

[a] One case showed both hematological and non-hematological toxicity

Table 2 Patient characteristics

Intolerance	CML-CP ($N = 21$)	CML-AP/BC ($N = 3$)	Ph+ALL ($N = 4$)	Total ($N = 28$)
Hematological toxicity				
Thrombocytopenia	1	1	0	2
Anemia	2	0	0	2
Neutropenia	1	0	0	1
Pancytopenia	0	1	0	1
Non-hematological toxicity				
Skin eruption	10	0	1	11
Nausea/vomiting	1	2	1	4
Myalgia	2	1	0	3
Sensory organ disturbance	2	0	0	2
Hepatotoxicity	1	0	1	2
Pulmonary toxicity	1	0	0	1
Arthralgia	1	0	0	1
Fluid retention	1	0	0	1
Neuropathy	0	0	1	1

Table 3 Hematological intolerance

	Imatinib intolerance	Cross intolerance[a]	Dasatinib temporary interruption	Dasatinib dose reduction	Dasatinib discontinuation
Thrombocytopenia	2	2	2	1	0
Anemia	2	1	0	0	0
Neutropenia	1	1	1	1	0
Pancytopenia	1	0	0	0	0
Total	6	4	3	2	0

[a] Adverse events grade greater than or equal to 3, by which imatinib intolerance was defined

reduction of dasatinib, but all 4 patients were able to have further dasatinib therapy administered.

Non-hematologic toxicities of imatinib included Grade 3–4 rash [11 patients (39%)], nausea and vomiting [4 patients (14%)], and myalgia [3 patients (11%)]. Of these, only 1 patient, who had developed Grade 3 hepatotoxicity of AST, ALT, and γ-GTP due to prior imatinib therapy and was enrolled in the study, again suffered Grade 3 hepatotoxicity upon starting dasatinib therapy (Table 4). This patient developed Grade 2 AST and ALT elevation. On Day 3, this patient experienced Grade 3 AST elevation, and was administered ursodeoxycholic acid and glycyrrhizin.

Table 4 Non-hematological intolerance

	Imatinib intolerance	Cross intolerance[a]	Dasatinib temporary interruption	Dasatinib dose reduction	Dasatinib discontinuation
Skin eruption	11	0	0	0	0
Nausea/vomiting	4	0	0	0	0
Myalgia	3	0	0	0	0
Sensory organ disturbance	2	0	0	0	0
Hepatotoxicity	2	1	0	0	0
Pulmonary toxicity	1	0	0	0	0
Arthralgia	1	0	0	0	0
Fluid retention	1	0	0	0	0
Neuropathy	1	0	0	0	0
Total	26	1	0	0	0

[a] Adverse events grade greater than or equal to 3, by which dasatinib intolerance was defined

The elevated AST levels became Grade 2 on Day 8, and were resolved completely on Day 27. The ALT levels were also resolved by day 30.

In the other 21 patients, no toxicities occurred after switching to dasatinib. It is notable that toxicity that was observed relatively commonly during imatinib therapy, such as skin eruption, was rarely observed to recur with dasatinib.

4 Discussion

In this analysis, we investigated whether patients who had suffered toxicities due to imatinib therapy could be safely switched to dasatinib therapy. This is the first report to examine the cross intolerance between imatinib and dasatinib in detail except for those that have been published only in abstract form [16].

We found that among 6 patients with hematologic toxicities of imatinib, 4 patients again experienced the same toxicities after starting dasatinib. However, after a temporary interruption, the toxicities subsided, as was also reported by Khoury et al. [16], who found that among patients who discontinued imatinib for hematologic toxicities, only 13% discontinued dasatinib because of cytopenia. This phenomenon may be specific to the subset of this treatment group, and might have been related to the progressed and resistant condition of the disease, and not a reflection of the toxicity of the drug. These cases might have been responding to imatinib therapy, and normal suppressed clones could have recovered if imatinib therapy was continued.

Thus, myelosuppression may be an indicator of therapeutic advantage, as indicated by the much greater cytotoxic effect against Ph-bearing tumor cells with dasatinib than with imatinib [17, 18]. Even if cross hematological intolerance of myelosuppression should appear, switching to dasatinib is warranted.

In terms of non-hematologic toxicities, it was clearly shown that there was no cross intolerance except for in 1 patient who again developed hepatotoxicities. Even in that patient, the toxicities regressed without discontinuation. Imatinib is generally tolerated, however, it is not tolerated by a certain subset of patients. Since continuation of imatinib administration is required to achieve a molecular response [19], switching to dasatinib might be one of the currently demonstrated two ways to achieve remission after abandoning imatinib [20, 21]. In fact minimal non-hematological cross resistance of nilotinib has also been reported although only in abstract form [22].

In conclusion, we have demonstrated that dasatinib can be safely administered to imatinib-intolerant CML or Ph(+) ALL patients. Switching to dasatinib should be considered if discontinuation due to toxicity is observed during treatment of CML with imatinib.

Appendix: Participants

National Cancer Center Hospital, Tokyo Metropolitan Komagome Hospital, Kyoto Prefectural University of Medicine, Tohoku University, Aichi Cancer Center, Hyogo College of Medicine, Yokohama City University Medical Center, Tokai University School of Medicine, The Jikei University School of Medicine, Saiseikai Maebashi Hospital, Tokyo Medical University, Sapporo Hokuyu Hospital, Japanese Red Cross Medical Center, Jiaikai Imamura Branch Hospital, Matsushita Memorial Hospital, Jichi Medical University, Nagoya University, Tokyo Women's Medical University, Saitama Medical University, Okayama University, Nagasaki University, Hamamatsu University School of Medicine, Nagoya City University, Keio

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Molecular Target Therapy in Hematological Malignancy: Front-runners and Prototypes of Small Molecule and Antibody Therapy

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Molecular-targeted drugs were first introduced for the treatment of hematological malignancies. Herein, the success stories of small molecule-targeted drugs, such as imatinib for the treatment of chronic myeloid leukemia and the tumor-specific antibody rituximab for the treatment of CD20-positive lymphoma, will be introduced. The introduction of imatinib and rituximab has changed the mortality rates associated with chronic myeloid leukemia and CD20-positive lymphoma, respectively. In particular, the therapeutic outcomes of imatinib treatment have been so good that clinical trials to assess the feasibility of treatment discontinuation after remission are ongoing. Methods for developing new anti-cancer agents have changed, and structure-based chemical compounds are now screened *in silico*. Second- and third-generation anti-cancer agents have already been successfully identified, and resistance mechanisms have been explained based on the interaction of these chemical structures with their target molecules. In the area of antibody development, the introduction of humanized antibody has been successful, and the use of antibody carriers has resulted in the development of potent second-line antibody drugs. The discovery of new target surface markers is also being reported, and trials for both chimeric and humanized antibodies against these molecules are ongoing. Through these efforts, disease mortality rates have begun to decline. We are facing a brilliant future in which we can strive to eliminate cancer-related mortality.

Key words: chemo-hematopoietic – hematol-leukemia/lymphoma – targeted therapy

INTRODUCTION

Molecular-targeted drugs were first introduced for the treatment of hematological malignancies. In this mini-review, the contribution of hematologists to the development of molecular-targeted drugs, including small molecule-targeted drugs and tumor-specific antibodies, will be introduced, so that this knowledge can be shared with oncologists specializing in other types of malignancies.

DEVELOPMENT OF IMATINIB THERAPY AGAINST CHRONIC MYELOGENOUS LEUKEMIA

Imatinib, which is used for the treatment of chronic myeloid leukemia (CML), was the very first molecular-targeted drug to be developed successfully (1). The molecular target of imatinib is the *abl* gene, which is activated in nearly all cases of CML because CML itself is defined by the presence of *bcr–abl* gene fusion derived from the translocation of chromosomes (2), otherwise known as the Philadelphia chromosome. Consequently, the fused *bcr–abl* gene is activated and expressed in all patients with CML. The fused gene works as a constitutively activated form of the *abl* gene and induces downstream signals. Therefore, the suppression of these signals by inhibitors was expected to exert a suppressive effect on growth and survival signals, and the development of such inhibitors was anticipated to suppress *abl* gene activity completely in normal cells. The advantage of this situation was that even if the normal *abl* gene was suppressed, it would not affect other cellular functions or cell survival. This line of reasoning was demonstrated in an experiment using knockout mice (3). The resulting mice

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were normal with minimal bone changes, presumably because of the redundancy of tyrosine kinases and the bypassing effects of other kinases.

Imatinib was originally developed to block the suppression of PDGFα and the differentiation of 12-*O*-tetradecanoylphorbol 13-acetate (TPA), and not as a drug for the treatment of CML. Thus, imatinib was intended for the treatment of tumors in which the PDGF receptor was abundantly expressed, such as gliomas (4). The commercial name for imatinib, 'Gleevec', reflects this initial intended use for the treatment of gliomas. However, the compound was also found to exert an inhibitory effect against the *abl* gene, which led to a switch in the direction of drug development toward the treatment of CML (5).

This switch in the direction of drug development was a great success. The reported 7-year overall survival rate of CML patients treated with this drug was 86% (1). This significantly better survival outcome reflected a sustained remission throughout the observation period. The occurrence of events such as blast transformation and the need to discontinue treatment decreased toward the end of the protocol study. In the clinical trial examining the use of imatinib for the treatment of CML with the longest follow-up period to date, the IRIS study, the incidence of events [loss of complete hematological response, loss of major cytogenetic response, development of accelerated phase (AP)/blastic phase (BC) and death from any cause] occurring during each post-treatment year steadily decreased, with recorded incidences in the first, second, third, fourth, fifth and sixth years of 3.3, 7.5, 4.8, 1.7, 0.8 and 0.3%, respectively (6). Furthermore, the rates for the development of AP/BC during the same follow-up period were 1.5, 2.8, 1.6, 0.9, 0.5 and 0%, respectively (6).

The depth of remission can be evaluated by the quantity of fusion gene expression, and the number of patients achieving complete molecular response (CMR), defined as the absence of fusion gene as detected using nested polymerase chain reaction (PCR), has been shown to increase even after 4 and 5 years of therapy (7). Together with the decrease in the AP/BC rate, the above observation has led to the idea that imatinib therapy may be a curative therapy for CML.

Mahon et al. reported the occurrence of long-term remission after the discontinuation of imatinib therapy in CML patients (8). They identified 50 cases of CML in which a CMR had been achieved and followed these cases after the discontinuation of therapy. They reported that 15 of these patients were still in remission at 2 years after the discontinuation of therapy. The outcome was particularly remarkable among the patients who had received interferon a. This report prompted the start of a study to examine the feasibility of treatment discontinuation.

In many countries, clinical trials to demonstrate the feasibility of the discontinuation of imatinib treatment after remission have been ongoing for many years. However, the eligibility criteria vary among the studies. Goh et al. (9) recruited 26 patients that had achieved not only a CMR, but also exhibited major molecular remission and conducted a retrospective review of the effect. The results were disappointing: almost all the cases showed an increase in the expression level of the *bcr/abl* gene. However, all the patients again achieved the same expression level as observed at the start of discontinuation once treatment was resumed, resulting in the imatinib treatment being 'saved.'

The Japan Adult Leukemia Study Group (JALSG) and our group are also planning a trial to examine the feasibility of discontinuing imatinib treatment in cases with molecular remission. The eligibility criteria would include cases with documented complete remission at a minimum of two time points. In the planned protocol, imatinib would be incrementally discontinued: 1 month of discontinuation would be followed by 3 months of treatment, followed by 2 months of discontinuation and 2 months of treatment and so on. The endpoint would be the complete discontinuation of treatment, and re-induction would be undertaken if an increase in the *bcr/abl* level was subsequently observed. The institutions involved in the study will be limited to certain subsets of JALSG facilities, and careful molecular monitoring will be performed.

The success of imatinib therapy has led to a change in clinical practice. For example, treatment with imatinib has resulted in an annual decrease in the number of patients requiring transplantations. This outcome is remarkable, since prior to the use of imatinib, transplantation was the only way to cure patients with CML. Younger patients were previously recommended to receive allogeneic stem cell transplantations as soon as suitable HLA-compatible donors could be found; this practice has now changed, and the current practice is to recommend transplantation only if/when oral chemotherapy including imatinib therapy fails.

The success obtained with imatinib has also brought the molecular monitoring of CML into practice. Using molecular methods, the disease status of CML can be precisely monitored (10). The remission level must be closely monitored to evaluate the depth of remission. The results of such assessments can then be used in decisions regarding treatment strategies, with treatment re-initiated if an increase in the expression level of the *bcr/abl* gene is observed using PCR.

The success of imatinib has also changed the manner in which pharmaceutical companies develop new agents (11). Imatinib was the first molecular-targeted drug with a known mechanism of efficacy. Three-dimensional structural analysis can be used to reveal the contact of a drug with its target molecule. Better structural fitting can lead to the discovery of improved drugs. Using this method, a new drug, nilotinib, was discovered for the treatment of CML; nilotinib has a precise and specific contact with the abl protein. Recently the results of a head-to-head comparison of this second-generation abl inhibitor with imatinib in a phase III trial were reported, and the primary endpoint of molecular response at 1 year was better than that obtained with imatinib (12).

The mechanism of imatinib resistance has been intensively studied, and the discovery of a mutation in the *abl* gene has led to the discovery of yet another drug, dasatinib. This drug is effective against the mutated *abl* gene, as it binds to the

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alternative form of the *abl* gene, namely, the inactive src-kinase-like form. The resistance induced by the mutation of the *abl* gene can be overcome using this new agent, which binds to the *abl* gene more precisely, although the spectrum of abl mutations may vary. With these alternative agents, nilotinib and dasatinib, patients with CML can successfully achieve a second long-lasting molecular remission even after imatinib-resistant clones have emerged (13).

Recently, CML stem cells have been identified and have been shown to be resistant to imatinib therapy. A mechanism for maintaining leukemic stem cells has also been proposed. In the proposed model, FOXP3 activity, which is up-regulated by a reduced level of AKT, can be suppressed by the blockage of TGF beta signaling (14); therefore, stem cell sensitization can be achieved using a TGF beta inhibitor. An AKT inhibitor may maintain CML stem cells, and a combination of inhibitors might contribute to unpredictable interference. However, various kinase inhibitors are being pursued to improve the results of treatment for CML (Table 1).

Another issue that should be mentioned is the reappraisal of the concept of the area under the curve as a sensitivity test for predicting response (15). As the inhibition of a kinase is determined by the concentration of the agent, the concentration of the drug must be greater than the target level to be effective, and achieving the target concentration is directly related to the effect of the drug. The situation is comparable to that of antibiotic therapy for the treatment of bacterial infections, in which it is necessary to keep the drug concentration above the sensitivity level.

The serum levels of imatinib in Japanese patients were rather high, compared with previously reported levels, because the body surface area (BSA) and the body weight of Japanese subjects are less than the values of populations in the USA and the UK. The results of a phase II study in Japan were recently published, and the actual dose used was approximately 300 mg, which is less than the previously reported dose of 400 mg. Nonetheless, the reported molecular effect was the same. In the phase II JALSG CML-202 study, the estimated complete cytogenetic response rate in 489 patients at 48 months was 92%, consistent with the results of the IRIS study, in which the rate was also 92% (Table 2). Therefore, the observation of the same effect at a lower drug dosage prompted us to measure the serum concentration of the drug, and the serum levels in the Japanese patients were comparable to those observed in populations in the USA and UK (16).

These results clearly show the importance of drug concentration monitoring in the era of molecular-targeted drugs. The serum levels of imatinib were previously reported to be unrelated to the BSA, body weight or age, and they appear to vary among individuals (17): as the serum level of imatinib is directly related to the treatment efficacy, serum concentrations should be monitored to ensure that they exceed the minimal tumor suppressive concentrations (18).

From a marketing viewpoint, the number of patients with CML is too small to launch a profitable drug discovery project. Following the success of imatinib, however, the development of cytostatic drugs has emerged as a new trend in drug development. With cytostatic drugs, even if the number of patients is small, drug development may proceed since such drugs will be used over prolonged periods. This approach has led to an enormous number of 'maintenance' drugs, such as drugs for maintaining the remission of acute myelogenous leukemia and malignant lymphoma, for the induction of myelodysplastic syndrome, and for prolonging remission after the resection of solid cancers. Some of these drugs are oral medications that have never before been pursued (19). The effectiveness of these drugs must be confirmed in phase III randomized clinical trials.

Table 1. New kinase inhibitors under development for the treatment of CML

Targeted kinase	Drug
Bcr-Abl	Adaphostin, AZD0530,CNS-9,I NNO-406, SKI606 (bosutinib)
VEGF	AEE778, AG01376, sorafenib, CHIR258, PTK787, sunitinib
mTOR	RAD001 (everolimus), CCI-779 (temsirolimus), AP23573
Raf, Mek, Erk	AAL881, BAY 43-9006, levostatin, PD98059
Src	AP23464, dasatinib, PD166326, SU6656, AZD0530
Aurora kinase	MK0457
Akt, PI3K	Perifosine
IGF-1R	AEW541, ADW742, tyrophostins
Jak2, Stat 3	AG490, WP-1066
MAPK	SD-282, SCIO-469

Table 2. Results of the JALSG CML202 study

Response	JALSG-CML202 (%)	IRIS (%)
MCR	98	98
CCR	92	92
MMR	55	70

CCR, complete cytogenetic response; CML, chronic myeloid leukemia; JALSG, Japan Adult Leukemia Study Group; MCR, major cytogenetic response; MMR, major molecular response.

DEVELOPMENT OF DRUGS TARGETING CD20-EXPRESSING MALIGNANT TUMORS

Follicular lymphoma (FL) is the most common subtype of indolent lymphoma. With the mass production of monoclonal antibodies on a commercial scale, remarkable progress has been made in the treatment of this disease. In 1997, the US Federal Drug Administration (FDA) approved rituximab for the treatment of CD20-positive B cell malignancy; if the

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aim of treatment for FL is assumed to be prolonging the disease-free survival period, this antibody is an ideal tool, considering its low toxicity.

Rituximab was subsequently approved for the treatment of diffuse large B-cell lymphoma by the FDA in 2002 and by the Japanese government in 2003. The survival rate of patients with this disease has been prolonged by the addition of rituximab to cyclophosphamide, hydroxydaunorubicin, vincristine and prednisone (CHOP) therapy. The success of anti-CD20 monoclonal antibody therapy has prompted efforts to identify new target molecules and to increase the killing activity of antibodies, and a new generation of antibody is now being developed.

The anti-CD20 antibody rituximab exerts its killing activity via three mechanisms: complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and the induction of apoptosis (20). Resistance to this agent is also generated through resistance to these three mechanisms: low ADCC activity, low CDC activity and resistance to apoptosis. In addition, pharmacokinetic factors, such as decreased serum concentrations, must also be considered.

ADCC is triggered by the binding of the Fc receptor and immunoreceptor tyrosine-based activation and phosphorylation (21). A polymorphism at the 158th amino acid residue is involved in the first step of this binding: if the amino acid residue is Val, the binding is weaker than when the residue is Phe. Thus, polymorphisms might also be an important factor determining individual sensitivities.

NEWER-GENERATION ANTI-CD20 ANTIBODIES (Table 3)

One of the newer generations of anti-CD20 antibodies that is expected to be developed is ofatumumab (also known as HuMax-CD20; Genmab) (22). The antibody is a completely humanized IgG1 that recognizes different epitopes from that recognized by rituximab. Preclinical data show a stronger binding activity than that of rituximab, and the drug is anticipated to be effective against chronic lymphocytic leukemia (CLL), as higher doses of antibodies are required to overcome relative resistance. Recently, the FDA approved its use for the treatment of CLL in patients who are resistant to fludarabine and alemtuzumab (23).

The recognition site of ocrelizumab is the same as that of murine 2H7 monoclonal antibody. The advantage of ocrelizumab, however, is that infusion reactions are rare, and this agent can be injected subcutaneously for the treatment of autoimmune diseases (24). A phase I/II study has been completed (25), and a phase III trial targeting non-malignant disease is now ongoing (26).

Veltuzumab (Immu-106, hA20) is another humanized antibody that recognizes the same epitope recognized by ocrelizumab. The humanized part is the same as that of epratuzumab, an anti-CD22 humanized antibody manufactured by the same company. Veltuzumab is expected to offer a lower frequency/severity of infusion reactions (27,28). A phase II study has been completed (29).

Table 3. Newer generation anti-CD20 antibodies

Generic name	Code name	Company	Potential benefits	Phase
Ofatumumab	HuMax-CD20	Genmab	Complete humanized form; different epitope from that of rituximab; more CDC activity	I/II
Ocrelizumab	H7	Genentech	Humanized form and less infusion toxicity; applicable to autoimmune diseases	III; (RA, SLE)
Veltuzumab	Immu-106, hA20	Immunomedics	Humanized form; less toxicity	I/II
	AME-133	Eli Lilly	Works with lesser FcgRIIIa activity; (10× Rituximab)	I/II
Obinutuzumab	GA101	Genentech	Different epitope from that of rituximab; more ADCC activity by modification of the sugar chain	I/II

ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity.

The Fc portion has been engineered to achieve a high efficacy, even in patients with a low Fcγ binding affinity. AME-133 was developed to work even in cases with the low affinity-type FcγRIIIa, and a clinical trial is ongoing (30).

Another antibody, obinutuzumab (formerly known as GA101), recognizes a different epitope. The variable portion is that of the murine B1 antibody, and the remaining part is humanized. The Fc portion is bound to sugar chains, and is, therefore, more flexible and fits better at the hinge portion. The binding capacity of this formulation to FcγRIIIa is 50 times more potent than that of the current antibody (31,32). Thus, the development of anti-CD20 antibodies has been bi-directional: the newer agents are less toxic (ocrelizumab and veltuzumab) and more effective (ofatumumab, AME-133, and GA101).

Antibodies work additively when combined with radioisotopes and toxins. Two types of radioimmunotherapy and one type of immunotoxin therapy have been developed for this purpose. Ibritumomab tiuxetan (Zevalin®) was approved by the FDA in 2002 for the treatment of relapsed or refractory

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FL, low grade non-Hodgkin lymphoma (NHL) and transformed NHL. The manufacturing of this drug was approved in Japan in January 2008 (33). The murine monoclonal antibody 2B8 is conjugated with ^{90}Y through the chelator tiuxetan (MX-DTPA). For this agent, a murine monoclonal antibody is used instead of a chimeric or humanized antibody because multiple injections are rarely needed.

The cytocidal activity of radiation is mainly achieved by beta emission, and the selection of the radionuclear material has a direct bearing on the antitumor effect. If the half-life is too long, protection is difficult, meanwhile, if the half-life is too short, the radiation treatment can be difficult to perform in a timely manner. In this sense, ^{90}Y is an ideal radionucleus, with an energy intensity of 2.3 MeV, a range of 5 mm and a half-time of 64 h, also, ^{90}Y is a pure beta emitter.

^{131}I-tositumomab (Bexxar®) (34) was approved by the FDA in 2003. This agent also recognizes a novel epitope of CD20. The radioactive material is not conjugated through linkers, but the tyrosine residues of the antibody are iodinated. To protect the thyroid gland from the radiation, the patient is given iodine in advance. Compared with ibritumomab tiuxetan, the radiation dose to the kidney is higher, whereas the dose to the liver is lower. Despite approval in the USA, the company is not preparing for a clinical trial of this agent to file for approval in Japan.

The above-mentioned successes have also induced the development of antibodies against other surface target molecules. The loss of CD20 has been reported to be a mechanism of resistance to antibody therapy (35). In such cases, therapy targeting other surface molecules would be more effective.

CD22 is an adhesion molecule that is rapidly internalized after binding. Its activity is related to the activation of B cells, modifying antigen signaling. The anti-CD22 antibody epratuzumab is a modified and humanized form of the murine mLL2 antibody. Its efficacy has been demonstrated not only against low-grade lymphomas, but also against aggressive lymphomas, although with lesser efficacy (36). Rituximab combined with this antibody was more effective than rituximab alone in patients with relapsed lymphoma (37).

Inotuzumab ozogamicin (CMC-544) also recognizes CD22, and this antibody is linked to a toxin, calicheamicin. Once the antibody binds to CD22, it is internalized and cleaved within the cell. The process after internalization is the same as that of Mylotarg, an anti-CD33 antibody that is also combined with calicheamicin. A Japanese phase I/II study on this agent has been completed (38).

NEW AGENTS UNDER CONSIDERATION

Table 4 lists the molecular-targeted drugs that are being developed for the treatment of hematological malignancies. The targets of these drugs are not limited to B cells. Alemzumab was originally used to treat T cell suppression in allogeneic transplantation settings. Some of these drugs have already been used for the treatment of solid cancers. Bevacizumab for VEGF has been widely used to treat colon cancer and is being examined in a phase III trial to compare the effect of its addition to R-CHOP therapy with that of R-CHOP therapy alone.

Table 4. Antibody therapy against B cell malignancy (43)

Antibody	Antigen	Phase in the USA	References
Alemtuzumab	CD52	Approved	(44)
Lumiliximab	CD23	Phase III	(45,46)
Anti-TRAIL	DRD	Phases I–II	(47)
Bevacizumab	VEGF	Phase III	(48)
Galiximab	CD80	Phases II–III	(49)
Epratuzumab	CD22	Phase II	(36,37)
SGN-40	CD40	Phases II–III	(49,50)
Anti-CD74	CD74	Phase I	(51)
Apolizumab (HID10)	HLA-DR	Phase II	(52)

DRD, death receptor domain.

Table 5. New agents under development for leukemia

Monoclonal antibodies	Lintuzumab
MDR modulators	Cyclosporine, PSC-833, zosuquidar
FLT3 inhibitors	PKC-412, CEP-701, MNL-518, SU-11248
Farnesyl transferase inhibitors	Tipifarnib, lonafarnib
Antiangiogenesis agents	SU-5416, PTK-787, bevacizumab
Histone deacetylase inhibitors	Depsipeptide, valproic acid, SAHA

Hematological malignancies include acute leukemia and multiple myeloma. Treatment advances for this disease have been achieved using new agents. Among them, a humanized anti-CD33 antibody conjugated with calicheamicin, gemtuzumab ozogamicin, has been developed for use against CD33-positive acute myelogenous leukemia (39). Other drugs being investigated for the treatment of leukemia are shown in Table 5. The introduction of the proteasome inhibitor bortezomib has changed the survival outcome of multiple myeloma (40).

INTRODUCTION OF BIOMARKERS

The use of these drugs for hematological malignancies requires biomarkers for identifying populations that can benefit from these drugs to be established. Identifying patients who are likely to be sensitive to the newer agents is problematic. In 1991, when the efficacy of tretinoin (an all-trans retinoic acid, ATRA, which is a retinoic acid alfa

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derivative) was reported, the detection of the gene fusion of PML/RARA was used as a diagnostic test (41). If the fusion was found to exist in an individual, the patient was almost guaranteed to be sensitive to tretinoin. This test was performed using a chromosomal analysis or reverse transcription PCR (RT–PCR), and, more recently, using fluorescent in situ hybridization. The Philadelphia chromosome, which is the hallmark of CML and acute lymphoblastic leukemia, is the target of imatinib therapy. CD20 positivity is also an indicator of the efficacy of rituximab. Thus, the introduction of molecular-targeted drugs has produced changes in the approval process for diagnostic tests: approval for an assay system for molecular markers and the corresponding molecular-targeted drugs should be sought simultaneously.

In Japan, however, the approval process for molecular diagnostic tests is slow, creating a serious issue for the introduction of newer agents. In Japan, if the test is approved by the Government, the cost is covered by the national health insurance program; however, the extent of coverage is far less than the global standard, and the actual cost is not fully covered. Some hospitals send the invoice to the government insurer for the diagnostic test as a pre-determined cost. The nature of this system leads to hesitation in filing with the Japanese regulatory agent for the approval of such tests.

Even if the test is not covered by health insurance, hospitals can pay for the cost. However, the test is not for clinical use, and the cost should be covered by the insurer. In this situation, combined payment by the insurer and an extra-insurance payment is not allowed. Thus, new molecular-targeted drugs are generally approved without the approval of the corresponding biomarker test. The approval process for molecular-targeted drugs and corresponding biomarker tests should be simultaneous, otherwise patients will not benefit from the new treatments. In fact, the FDA is planning a newer simultaneous approval process for biomarkers and drugs (42).

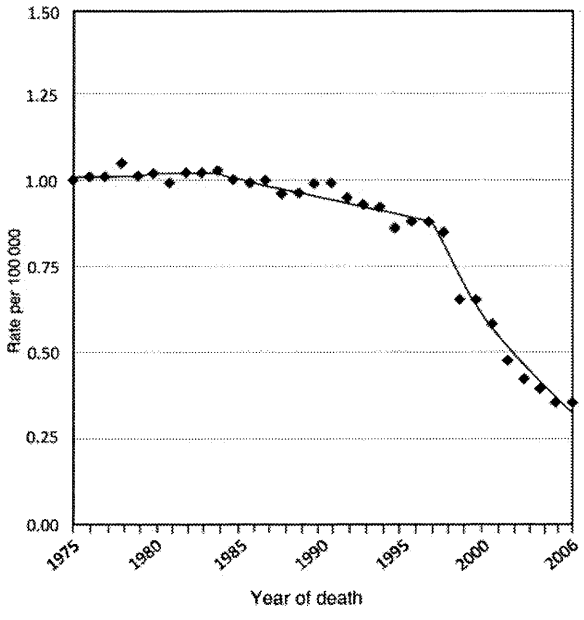

Figure 1. Age-adjusted mortality of patients with CML in the US Data from the SEER (Surveillance, Epidemiology and End Results) Program were retrieved on 1 October 2009 (http://www.seer.cancer.gov/).

CONCLUDING REMARKS

In the field of molecular-targeted therapy, hematological malignancies are the front-runners ×for drug development. Figure 1 shows the annual changes in the mortality rate of CML, adjusted for age. The first down-slope represents the change in the mortality rate that occurred during the introduction of allogeneic bone marrow transplantation. The second decline, starting in 1999, clearly reflects the introduction of imatinib therapy. The mortality rate has been reduced by half and continues to decline. This figure can be interpreted as depicting the triumph of molecular-targeted therapy.

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Conflict of interest statement.

None declared.

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