Ph clone recurrence after imatinib discontinuation for chronic myelogenous leukemia patients who had complete molecular remission.

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ABSTRACT Imatinib (IM) is a tyrosine kinase inhibitor that blocks the kinase activity of BCR-ABL fusion protein, thereby inhibiting Ph-positive leukemic progenitor cell proliferation. Imatinib has produced excellent clinical and cytogenetic/molecular responses against chronic myelogeneous leukemia (CML), and is now the first-line treatment of CML. A few to 34% of patients reaches a complete molecular response (CMR) by IM treatment, however it is not known whether patients who have a CMR require continued therapy to maintain it or whether IM can safely be discontinued. We analyzed the cytogenetic and molecular outcomes of 7 patients with CML, for whom IM therapy had been discontinued after a CMR. From January 2002 to September 2006, 16 patients reached CMR by IM therapy at our institute. Because of economic reasons or adverse events, 7 of the 16 patients had IM discontinued and all had an early relapse. The median time to relapse was 4.9 months (range, 2-6 months). One factor for relapse was that the duration of molecular remission before IM was discontinued was short (4.3 months; range,1-12 months). Based on these results, discontinuation of IM therapy for patients who have a CMR can not be recommended. More information about cases of Japanese CML must be gathered in order to devise effective CML treatment strategies for patients who achieved a CMR.

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Key words : **Imatinib, Chronic myelogenous leukemia, Molecular response, Cytogenetic response, Discontinuation**

INTRODUCTION

Chronic myelogenous leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of the *bcr-abl* fusion gene due to reciprocal translocation between chromosomes 9 and 22. It is visible cytogenetically as a shortened chromosome 22 (Philadelphia [Ph] chromosome)[1,2]. The molecular consequence of this translocation

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is the novel fusion gene, *bcr-abl*, which encodes a constitutively active protein tyrosine kinase[3]. The resulting BCR-ABL fusion protein confers enhanced proliferative activity and decreased sensitivity to apoptotic cell death in CML cells. A tyrosine kinase inhibitor, imatinib (IM) was designed to bind specifically to the adenosine triphosphate (ATP)-binding site of the tyrosine kinase protein which causes Ph-positive leukemia[4]. It has high, relatively specific, biochemical activity and an acceptable pharmacokinetic and toxicity profile, and has been rapidly introduced into clinical practice[4-8]. IM has produced encouraging results in all CML phases[4,9-11]. This has resulted in a revolutionary advance in the management of CML and, by extension, a shift in the paradigm for cancer management in general.

It has had excellent clinical and cytogenetic/molecular effects in Japanese CML patients[9,10,12-15]. For IM-treated patients newly diagnosed with CML, the complete hematologic response (CHR) rate after 5 years was 98%, the complete cytogenetic response (CCR) rate 92%, and the major molecular response (MMR) rate 87%[10]. In addition to having a CCR, a few to 34% of the patients had a complete molecular response (CMR), defined as an undetectable level of *bcr-abl* transcripts as measured by a quantitative or qualitative polymerase chain reaction (PCR)[16,17]. However, it is not known if continued therapy is necessary to maintain this level of response or if IM can be safely discontinued.

We analyzed the cytogenetic and molecular outcomes of 7 patients with CML, for whom IM therapy had been discontinued after having a bone marrow CMR.

PATIENTS AND METHODS

Patients

Statuses of consecutive patients at our institute who had a confirmed diagnosis of Ph-positive CML, were analyzed. Patients were followed from January 2002 to September 2006, 19 with CML being treated with IM. Two of them had accelerated phase(AP)-CML, 17 chronic phase(CP)-CML. The 19 included 10 patients who received IM after failed IFN α therapy and 9 who received IM as the first line of therapy for CML. A CCR occurred in 17 (89.5% of the 19 patients treated), and 16 (84.2% of those treated) had undetectable *bcr-abl* transcripts in at least one nested-PCR analysis (i.e., CMR). Of these, 7 who had a CMR had IM discontinued.

We analyzed their cytogenetic and molecular outcomes. Imatinib was discontinued because of economic reasons or adverse events. IM was discontinued with the patients' informed consent and monitoring of their cytogenetic and molecular outcomes was continued.

Evaluation and criteria for cytogenetic responses

Patients' hematological, cytogenetic, and molecular responses were evaluated. Bone marrow cells underwent cytogenetic analysis by the G-banding technique and fluorescence-in-situ-hybridization (FISH). For chromosome analysis, 20 mitotic cells in metaphase and 1,000 interphase cells were analyzed by FISH.

A CCR was defined as the absence of Ph-positive metaphases or as being below the detection limit of a *bcr-abl* signal by FISH analysis.

Evaluation and criteria for molecular responses

Bone marrow cells underwent molecular analysis by an RT-nested PCR. Total cellular RNA was extracted from mononuclear bone marrow cells with SepaGene RV-R(Sankojyunyaku). The reverse transcription reaction was run with up to 1 μg of total RNA in an RT mixture [5×RT buffer, 2.5mM dNTP, reverse primer, 100mM DTT, RNassin (40U/μl), M-MLV RTase (200U/μl)], at 37℃ for 60 minutes, then at 99℃ for 5 minutes, followed by cooling down to 20℃. The cDNA then was amplified by a nested PCR that used two sets of primers.

The sequences of the 1st PCR primer pair were 5'-GCTTCTCCCTGACATCCGTG-3' (forward) and 5'-GGCCCATGGTACCAGGAGTG-3' (reverse), those of the 2nd PCR primer pair were 5'-GGAGCTGCAGATGCTGACCAAC-3' (forward) and 5'-GTTTCTCCAGACTGTTGACTG -3' (reverse). The first PCR which used cDNA with the 1st PCR Mixture [DEPC-treated water, 10×PCR buffer, 2.5mM dNTP, forward and reverse primers, Taq polymerase(5U/μl)] was run using 35 cycles of 94℃ for 30 seconds, 63℃ for 30 seconds, and 72℃ for 30 seconds. The second PCR was run on the 1st PCR products and the 2nd PCR mixture [DEPC-treated water, 10×PCR buffer, 2.5mM dNTP, forward and reverse primers, Taq polymerase (5U/μl)], for 35 cycles of : 94℃ for 30 seconds, 63℃ for 30 seconds, and 72℃ for 30 seconds.

A CMR was defined as an undetectable level of *bcr-abl* transcripts confirmed by an RT-nested PCR.

RESULTS

From January 2002 to September 2006, 19 patients in various stages of CML were treated with IM at our institute. The median follow-up time from start of treatment was 42.6 months (range, 10-56 months). Seventeen (89.5%) had a CCR, and 16 (84.2%) had at least one analysis showing a CMR (data not shown). The median time from start of treatment to a CCR was 5.6 months (range, 1-12 months) and to a CMR 10.6 months (range, 4-37 months). For 7 of the 16 patients who had a CMR IM was discontinued. Patients' characteristics and responses to IM therapy are summarized in Table 1. Of those 7 patients, 6 had CP-CML and one AP-CML. Median age was 55.3 years old (range, 31-73 years old), and 5 were women. The median follow-up period from the time treatment started was 50.3 months (range, 30-56 months). It included 5 patients who received IM after IFN-α therapy failed, and

Table 1. Patients characteristics and responses to IM therapy

Patient	Sex	Age	CML status at diagnosis	Previous therapy	Time from IM itiation (months)	CML tatus at IM initiation	Best response to IM	Time to CCR (months)	Time to CMR (months)	Follow up time (months)
1	F	31	CP	IFN	18	CP	CMR	3	6	56
2	F	53	CP	IFN	90	CP	CMR	6	6	56
3	M	58	CP	IFN	124	CP	CMR	2	4	55
4	F	49	CP	IFN	38	CP	CMR	4	4	52
5	F	61	CP	IFN	24	AP	CMR	4	10	50
6	M	73	CP	-	0	CP	CMR	7	18	53
7	F	62	CP	-	2	CP	CMR	3	6	30

IM: imatinib mesylate CML: chronic myelogenous leukemia F: female M: male CP: chronic phase CML AP: accelerated phase CML IFN: interferon-alpha CCR: complete cytogenetic response CMR: complete molecular response(RT-nested PCR negative)

Table 2. Outcomes of the 7 patients who discontinued IM therapy after having molecular remission

Patient	Sex	Age	Previous therapy	Number of IM discontinu-ations (Times)	Time from CCR to IM discontinuation (Months)	Time from CMR to IM discontinuation (Months)	Time to relapse (Months)	Present status	Present IM dose
1	F	31	IFN	2	4/4	1/4	6(cy)/6(cy)	CMR	400mg
2	F	53	IFN	3	1/nd/7	1/12/7	2(cy)/3(cy)/4(cy)	CMR	400mg
3	M	58	IFN	1	5	1	3(cy)	CMR	400mg
4	F	49	IFN	2	5/1	1/1	6(mol)/6(mol)	CMR	100mg
5	F	61	IFN	1	26	7	6(cy)	CMR	300mg
6	M	73	-	1	17	6	6(cy)	CMR	400mg
7	F	62	-	1	9	6	6(cy)	CMR	200mg

IM: imatinib mesylate CML: chronic myelogenous leukemia F: female M: male CP: chronic phase CML AP: accelerated phase CML IFN: interferon-alpha CCR: complete cytogenetic response CMR: complete molecular response(RT nested-PCR negative) partial CR: partial complete response nd: not done cy: cytogenetic relapse mol: molecular relapse

2 who received IM as the first line of therapy for CML. The median period from diagnosis to IM initiation was 42.3 months (range, 0-124 months). Imatinib was started at 400mg/day for all the patients. The median period to CCR was 4.1 months (range, 2-7 months) and to CMR 7.7 months (range, 4-18 months). The median duration of CMR status until IM discontinuation was 4.3 months (range, 1-12 months).

Totally, IM was discontinued 11 times for 7 patients. All who had IM discontinued after gaining a CMR experienced early relapse. The median time to relapse was 4.9 months (range, 2 to 6 months). Their cytogenetic and molecular outcomes after stopping IM are summarized in Table 2. After discontinuation, one patient experienced molecular relapse (but not cytogenetic or hematologic ones). Cytogenetic relapses occurred in 6 patients after 2 to 6 months.

Imatinib therapy re-introduced for all 7 patients produced a new decline in the disease. Three had the IM dose reduced to 100mg-300mg/day because of adverse events. All of them reached CMR, and none acquired resistance to IM. It should be noted that one patient who received 100mg/day low dosage IM had a 3rd CMR (patient No.4).

DISCUSSION

The introduction of IM has revolutionized the treatment of CML in Japan. Imatinib has produced encouraging results for all phases of CML[9-11]. Current studies have shown that a 75% to 90% of patients treated with IM who had early CP-CML had a CCR[9,18,19]. In addition, up to 34% of patients studied had a CMR[9,16,17].

These findings confirm the superiority of IM therapy to conventional drug therapy (IFN α with or without cytosine arabinoside) for patients with CML. Today in Japan IM is the standard therapy for patients with CML[5,12], but it is not known whether those who have a CMR, require continued therapy to maintain it or IM can be safely discontinued. At present this is the most important concern for CML management.

Because the CCR frequency is very high in IM-treated patients, PCR monitoring of minimal residual disease has become more important. Currently, patients are routinely followed by means of quantitative or qualitative PCR techniques during therapy to determine *bcr-abl* transcript levels. About 70% of CP-CML patients who had a CCR, were considered to have a major molecular response (MMR)[17]. Complete and major molecular responses are associated with excellent prognoses due to IM therapy[9,10,16,17,19,20]. The MMR incidence (about 30% to 70%) is reasonable but CMRs have been reported at lower frequencies of 7% to 10% for standard-dose IM given patients with newly diagnosed CML[16,19]. Several updates have shown a higher frequency of CMR. Cortes et al[17] reported that of 280 CP-CML patients (117 after IFN failure, 163 previously untreated) who had had a CCR with IM, 174 (62%) also had an MMR, and 95 (34%) at least once had undetectable *bcr-abl* transcripts. Moreover, the median period from start of treatment for an MMR to occur was 10 months (range, 2.8-46 months) and for a CMR 16.7 months (range, 3-48 months)[17]. Rosti et al[21] reported 22 of 191 patients who had a CMR (12%) in late CP-CML who had been treated with IM after IFN-α failure (median follow-up time, 45 months). In Japan, Matsuo et al[13] evaluated the cytogenetic and molecular effects of IM in a practical setting in Nagasaki Prefecture. In his group I, consisting of patients with newly diagnosed CML (n=43), 16 (61.5%) of 26 had had an MMR by 24 months. In group II, consisting of patients with previously diagnosed CML (n=56), 14 (58.3%) of 24 had had an MMR owing to IM treatment. Of the evaluatable patients, 86.1% in group I(n=37) and 77.9% in group II(n=31) had had a CCR by 30 months. These findings are comparable to those reported in the IRIS study[9]. In summation, the

MMR rate was 30% to 70%, and the CMR rate 7% to 34% for CP-CML patients who underwent IM treatment. Patients who have a CMR with IM therapy are predicted to form an upward trend[17].

From January 2002 to September 2006, 7 of the 16 patients who had a CMR IM was discontinued, and then all of them experienced early relapse. One reason for stopping IM treatment was adverse events, typified by facial edema or itching eruption. Such adverse events showed the same tendency in previous reports[22-24]. Molecular and cytogenetic relapses occurred rapidly in all our patients. This is partly because the period of molecular remission before IM discontinuation was too short (4.3 months). IM was re-started for all 7 patients at a low dose (100-300mg/day). All had a CMR, and significantly, one had a 3rd CMR on receiving a 100mg/day low dose. This suggests that CML patients treated with IM who have a CMR can be maintained at that level with a low drug dose. In all the reported cases, including ours, patients who re-started IM responded rapidly, indicative that there was no acquired resistance to it [22-25].

Other case reports of patients with a CMR who stopped IM have been published[22-25]. The conclusions of those reports are shown in Table 3. Twenty-one patients in molecular remission who stopped IM have been documented. In 12 relapse was rapid. The median period from discontinuation of IM to relapse was 4.3 months (range, 1-10 months), but 9 patients continued to have a CMR. The median follow-up time from IM discontinuation

Table 3. Recent reports on discontinuation of IM therapy after complete molecular remission.

Patient	Age	Sex	CML status	IFN/Response at IM	Time from CMR to IM discontinuation (months)	Relapse/Follow-up time (months)
Rousselot P, et al.; Blood 2007						
1	42	F	CP	+/NR	26	+/3
2	54	M	CP	+/NR	30	+/2
3	75	F	CP	+/NR	45	+/1
4	83	F	CP	+/NR	45	+/2
5	73	M	AP	+/mCR	42	+/1
6	58	F	CP	+/NR	27	+/5
7	76	M	CP	+/NR	32	−/+22
8	68	M	CP	+/NR	44	−/+9
9	58	F	CP	+/NR	24	−/+24
10	71	M	CP	+/pCR	34	−/+21
11	66	F	CP	+/pCR	32	−/+15
12	78	M	CP	+/NR	32	−/+9
Merante S, et al.; Haematologica 2005						
13	63	M	CP	+/NR	19	+/7
14	51	F	CP	+/CCR	17	+/10
15	60	M	CP	+/NR	13	−/+15
16	52	F	CP	+/NR	14	−/+14
Mauro MJ, et al.; Leukemia Res 2004						
17	36	F	CP	+/NR	8	+/3
18	36	F	CP	-	8	−/+12
Cortes J, et al.; Blood 2004						
19	34	F	CP	+/NR	12	+/3
20	70	M	CP	-	12	+/6
21	40	F	AP	+/NR	18	+/8

IM: imatinib mesylate CMR: complete molecular response NR: no response CCR: complete cytogenetic response
mCR: minor cytogenetic response pCR: partial cytogenetic response

was 15.7 months (range, 9-24 months). No significant difference was found between relapsing and non-relapsing patients. Rousselot et al[25] reported six patients (50%) in persistent molecular remission after a median follow-up of 18 months (range, 9-24months), and a trend for a shorter time to *bcr-abl* negativity (8.5 months in non-relapsing patients versus 11 months in relapsing ones, *P*=.05). Moreover, the relapse rate was lower (50%) in his study, as compared with previous studies, because the median period of molecular remission before IM interruption was longer (32 months; range, 24-45 months)[22-25]. In our study, the cytogenetic and molecular responses of Japanese CML patients to IM therapy showed equivalent therapeutic effects when compared with previous overseas reports. In all the reported cases of discontinued IM therapy, however, early relapse occured. In addition, the median period to relapse (4.9 months) was similar to that in previous reports (4.3 months)[22-25].

To conclude, we found that Japanese CML patients treated with IM who were in CMR had early relapses once IM therapy was discontinued. Therefore, at present it is not recommended to discontinue IM treatment for patients who are in molecular remission. On the one hand, as reported previously[25], patients who stopped receiving IM who had been in CMR for more than 2 years remained in remission after 18 months of follow up. Our study found that 3 patients who received 100-300mg/day low dose IM had a CMR (Table 2). These findings suggest that the discontinuation or reduction of IM is beneficial in some instances for CMR patients. We need gather more information on Japanese CML patients, in order to devise better CML treatment strategies for Japanese patients who have a CMR with IM.

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