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Early Prediction of Molecular Remission by Monitoring BCR-ABL Transcript Levels in Patients Achieving a Complete Cytogenetic Response after Imatinib Therapy for Posttransplantation Chronic Myelogenous Leukemia Relapse

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Imatinib induces a high complete cytogenetic response (CCR) rate in relapsed chronic myelogenous leukemia. By analyzing minimal residual disease (MRD) under the levels of CCR, we tried to assess the molecular response after imatinib therapy. By using real-time quantitative reverse transcriptase-polymerase chain reaction (Q-RT-PCR), MRD was evaluated in 23 patients (3 in cytogenetic relapse, 6 in chronic phase, 9 in accelerated phase, and 5 in blast crisis) who were treated with standard-dose imatinib for relapsed chronic myelogenous leukemia after allogeneic stem cell transplantation. With a median therapy time of 399 days (range, 35-817 days), 19 (83%) patients achieved a CCR. Meanwhile, 11 (58%) of them achieved a molecular remission (MR), which was associated with improved survival. The Q-RT-PCR data were compared according to the best response (MR, n = 11; CCR, n = 8) in the patients achieving a CCR. The BCR-ABL/ABL ratios were similar in 2 groups at 3 months but were significantly different at 6 months (median, 0.0000012 for MR and 0.00022 for CCR; $P = .003$). The probability of a subsequent MR was significantly higher in patients with a lower BCR-ABL/ABL ratio at 6 months (100% for <0.0001 versus 33% for ≥0.0001; $P = .006$) or a greater reduction in the level between 3 and 6 months (log-reduction ≥1.0;, 100%; <1.0, 17%; $P = .003$). Q-RT-PCR is a reliable method for monitoring MRD: the early trends in the BCR-ABL/ABL ratio may be clinically useful in discriminating patients who will achieve an MR from those who will remain in CCR.

KEY WORDS
Chronic myelogenous leukemia • Hematopoietic stem cell transplantation • Relapse • Imatinib mesylate • Minimal residual disease

INTRODUCTION

Allogeneic stem cell transplantation (SCT) is the only curative treatment for chronic myelogenous leukemia (CML), and 40% to 80% of these patients remain disease free for more than 5 years after SCT [1]. CML recurrence, which occurs in approximately 20% to 30% of patients who undergo transplantation in the first chronic phase (CP) and who receive non–T cell–depleted marrow [2], remains a major cause of treatment failure. Donor lymphocyte infusion (DLI) has been the treatment of choice for relapsed CML and results in complete remission in up to 75% of cases and in durable remission with a rate of almost 90% at 2 to 3 years [3,4]. However, it has been associated with fatal marrow aplasia and severe graft-versus-host disease, and it relies on the availability of the original donor [3-5].

Imatinib is a potent and selective inhibitor of

BCR-ABL tyrosine kinase, which is a product of the chimeric gene produced by the Philadelphia (Ph) chromosome [6,7]. Several studies have demonstrated that imatinib therapy induces a major cytogenetic response in up to 80% of cases with mild and reversible toxicities. In the post-SCT settings, it was suggested that imatinib should be used as a front-line treatment for relapsed CML, on the basis of the high response rate with relative safety [8]. Furthermore, severe graft-versus-host disease was observed in a relatively smaller portion of relapsed patients [9-13] compared with that observed after DLI [3,14].

Considering the high cytogenetic response rate after imatinib therapy, a more sensitive monitoring of minimal residual disease (MRD) under the levels of the cytogenetic response is needed, particularly in post-SCT settings. Real-time quantitative reverse transcriptase-polymerase chain reaction (Q-RT-PCR) correlates strongly with conventional cytogenetics, fluorescence in situ hybridization, RT-PCR, competitive RT-PCR, and Southern blotting [15-19], and its utility in MRD monitoring has been reported by many authors [15,20-22]. Because our previous study showed that the results of Q-RT-PCR were significantly different according to the positivity of RT-PCR [19], Q-RT-PCR studies of patients who achieve a complete cytogenetic response (CCR) after imatinib therapy may determine the level of molecular response, which correlates with a low risk of disease progression.

To our knowledge, none of the studies has addressed the important question of how to select patients who are unlikely to achieve molecular remission (MR) after a therapy with standard-dose imatinib for post-SCT CML relapse and therefore might be advised to consider alternative therapies. To answer this question, MRD was examined after imatinib therapy to determine whether it was possible to predict which patients will eventually achieve an MR by monitoring their early trends in the BCR-ABL/ABL ratio at 1, 3, and 6 months.

PATIENTS AND METHODS

Patients

Adults with Ph-positive or BCR-ABL–positive CML who had relapsed after a transplantation were enrolled in this study. The eligibility criteria included disease status with cytogenetic or hematologic evidence of a relapse and treatment with standard-dose imatinib for at least 1 month. Written, informed consent was received from each patient before enrollment in this study.

Treatment with Imatinib

Imatinib was started at an oral dose of 400 mg/d for cytogenetic relapse or CP CML and 600 mg/d for accelerated phase or blast crisis CML, and the doses were adjusted according to the previously published guidelines for pretransplantation CML [10,23]. The treatment was continued until the disease was considered unresponsive (no hematologic response after at least 4 weeks or no cytogenetic response after at least 6 months of treatment) to imatinib, until the patient died, until there was a change to another treatment (including a dose escalation of imatinib), or until the appearance of unacceptable adverse events that did not respond to dose modification.

Response Analysis

Every 3 months or more frequently, the responses to imatinib were analyzed from the bone marrow samples by using conventional cytogenetics, nested RT-PCR, and Q-RT-PCR assays in all cases. The cytogenetic responses, which were analyzed with the standard Giemsa banding method with a minimum of 20 metaphases examined each time, were categorized as being complete (no Ph-positive metaphases), partial (1%-35% Ph-positive metaphases), minor (36%-65% Ph-positive metaphases), minimal (66%-95% Ph-positive metaphases), and no response (>95% Ph-positive metaphases). MR was defined as the absence of BCR-ABL transcripts in nested RT-PCR and Q-RT-PCR, as well as full donor chimerism.

PCR Assays

The nested RT-PCR and Q-RT-PCR were performed as previously described [19,24]. Briefly, the total RNA was extracted by using an RNAqueous Kit (Ambion, Austin, TX). Reverse transcription was performed with 1 μg of RNA, and samples with less than 1 μg of RNA were discarded. The 2-step nested procedure in triplicate was used for the nested RT-PCR amplification (sensitivity, 10^{-6}), and Q-RT-PCR was performed in triplicate by using iCycler software 2.1 (Bio-Rad, Hercules, CA) under the standard conditions (95°C for 10 minutes, 50 cycles at 95°C for 15 seconds, and 60°C for 1 minute). The plasmid standard titrations with the defined copy numbers for the BCR-ABL and the reference ABL were analyzed simultaneously with the patient samples, and the 50-μL PCR reaction mixture contained 5 μL of 10× PCR buffer, 4.5 mmol/L $MgCl_2$, 0.2 mmol/L deoxynucleoside triphosphate, 0.2 μmol/L primers, 140 nmol/L TaqMan probe (Tibmolbiol, Berlin, Germany), 1.25 U of AmpliTaq Gold (PE Applied Biosystems, Foster City, CA) DNA polymerase, and 4 μL of target complementary DNA. The quantity of the BCR-ABL transcript was normalized to the ABL expression level (sensitivity, 10^{-5}).

Chimerism Analysis

Chimerism analysis using a multiplex short tandem repeat was performed with the AmpF/STR Identifiler PCR Amplification Kit (PE Applied Biosystems, Foster City, CA), and the fragments were analyzed on an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems), as previously described [25].

Statistical Analysis

The end points for response analysis were calculated on the basis of the date of the last bone marrow examination and for the survival on the date of the last follow-up. The outcome probabilities, such as CCR, MR, and overall survival, were calculated with the Kaplan-Meier method; the comparison was made by using the log-rank test. The Mann-Whitney test was used to compare the normalized BCR-ABL transcripts levels between appropriate groups. All quoted P values were 2 sided, and a P value $<.05$ was considered significant.

RESULTS

Patients

A total of 23 patients with a median age of 33 years, in whom CML had relapsed at a median of 13.6 months after the SCT, were enrolled in this study (Table 1). The duration of imatinib therapy was a median of 399 days (range, 35-817 days). Before imatinib therapy, immunomodulatory treatment such as immunosuppressant withdrawal, DLI, or interferon was given to 19 of 23 patients, and the median interval from salvage treatment to imatinib therapy was 6.5 months (range, 3.1-59.3 months). Among them, 16 patients had refractory disease (defined as no hematologic response at 3 months or no cytogenetic response at 6 months after treatment) to these treatments: disease progression at a median of 6.7 months after various treatments (n = 10); no cytogenetic responses at 6.5 months after immunosuppressant withdrawal (n = 2); or no hematologic responses at a median of 7.7 months after DLI and/or interferon (n = 3) or at 4.4 months after immunosuppressant withdrawal (n = 1). The disease phase at the initiation of imatinib therapy was a cytogenetic relapse in 3 patients, CP in 6 patients, accelerated phase in 9 patients (all classified only because of the presence of acquired clonal aberrations), and blast crisis in 5 patients.

Cytogenetic Response

CCR was observed in 19 (83%) of the 23 patients. Among the remaining 4 patients, 1 patient achieved a partial cytogenetic response, and the others did not achieve any cytogenetic response. The time to CCR was a median of 97 days (range, 27-285 days) after the initiation of imatinib therapy. According to the disease phase at the initiation of imatinib therapy, the CCR rate was 100% (3/3) in cytogenetic relapse, 83% (5/6) in CP, 89% (8/9) in accelerated phase, and 60% (3/5) in blast crisis. The probability of overall survival after imatinib therapy was significantly different between those who had experienced CCR (83%) and those who had not (0%) ($P = .002$).

Molecular Response among the Patients Achieving CCR

Nineteen patients with CCR were divided into 2 groups (MR group versus CCR group) according to the best response to imatinib therapy and the Q-RT-PCR data were compared between the 2 groups. Eleven (58%) patients were classified as being in the MR group, in whom an MR was achieved at a median of 279 days (range, 90-559 days) and persisted for a median of 190 days (range, 30-370 days), and none of the MR patients lost their response until the time of data analyses. Of 8 patients in the CCR group, 6 patients remained in continuous CCR for a median of 356 days (range, 144-700 days), whereas 2 patients lost their CCR after 52 and 281 days. The overall survival probability after imatinib therapy was significantly higher in the MR group (100%) than in the CCR group (60%) ($P = .03$; Figure 1).

Kinetics of BCR-ABL Transcript Levels among the Patients Achieving CCR

Quantitative monitoring by Q-RT-PCR showed a rapid decline in the normalized BCR-ABL transcripts during 3 months of imatinib therapy in both the MR and CCR groups (Figure 2). After then, the median BCR-ABL/ABL ratio progressively declined in the MR group, whereas it slowly increased after 6 months in the CCR group. The BCR-ABL/ABL ratios were not significantly different between groups at 1 month (median, 0.03 for MR and 0.03 for non-MR) and 3 months (median, 0.00004 for the MR group and 0.00099 for the CCR group). However, they were significantly different after 6 months of therapy (median, 0.0000012 for the MR group and 0.00022 for the CCR group; $P = .003$; Table 2).

Prediction of MR with BCR-ABL Transcript Levels among the Patients Achieving CCR

No significant levels were identified to be useful for discriminating the subsequent MR when using the Q-RT-PCR results at 1 month or 3 months. When the Q-RT-PCR results at 6 months were analyzed in 10 patients who remained in CCR until 6 months, the probability of a subsequent MR was significantly higher in patients with BCR-ABL/ABL ratios <0.0001 (100% versus 33% for ≥ 0.0001; $P = .006$; Figure 3a). Because there were significant differences

Table 1. *Characteristics and the Responses to Imatinib Therapy of the Enrolled Patients*

Case	Age/ Sex	SCT Type	Disease Status at SCT	Relapse Status (Months after SCT)	Salvage Treatment (Months after Relapse)	Status at IMTx (Months after Relapse)	Best Response to IMTx (Duration; d)	Survival (d)*	Log (3m/6m)
1	45/F	Mismatched-related	AP	CyRel (11.6)	IS-W (0.2)†	CyRel (6.7)	MR (190+)	Alive, 370+	>3
2	35/M	Matched-related	CP	CyRel (18.0)	None	CyRel (4.6)	MR (126+)	Alive, 216+	>3
3	31/F	Matched-related	CP	CP (56.9)	None	CP (0.6)	MR (139+)	Alive, 418+	1.4
4	31/M	Matched-unrelated	CP	CP (28.2)	IS-W (1.7)	CP (2.2)	MR (293+)	Alive, 643+	0.6
5	32/F	Matched-related	CP	CP (33.8)	DLI (0.8)†	CP (5.2)	MR (370+)	Alive, 817+	1.56
6	33/F	Matched-related	AP	CP (60.7)	DLI + IFN (0.2)†	CP (7.9)	MR (30+)	Alive, 399+	2.1
7	45/M	Matched-related	AP	AP (4.9)	DLI + INF (1.6)	AP-CE (4.0)	MR (327+)	Alive, 640+	1.5
8	46/F	Matched-related	CP	CyRel (18.9)	DLI (1.7)†	AP-CE (9.1)	MR (63+)	Alive, 622+	2.0
9	22/M	Matched-related	CP	CyRel (9.3)	DLI + INF (0.5)†	AP-CE (15.0)	MR (209+)	Alive, 404+	>3
10	30/F	Matched-unrelated	CP	CyRel (23.1)	IS-W (0.6)†	AP-CE (6.6)	MR (299+)	Alive, 397+	>3
11	36/F	Matched-related	CP	CyRel (7.6)	IS-W (0.8)†	AP-CE (5.0)	MR (70+)	Alive, 250+	>3
12	32/M	Matched-related	CP	CyRel (11.5)	IS-W (0.6)†	CyRel (7.1)	CCR (242+)	Alive, 332+	−0.3
13	56/M	Matched-related	CP	CP (18.8)	DLI + IFN (5.1)†	CP (8.2)	CCR (700+)	Alive, 800+	0.1
14	33/M	Matched-unrelated	CP	CyRel (3.6)	IS-W (0.3)†	AP-CE (3.5)	CCR (52)	Died of relapsed disease, 79	NA
15	28/M	Matched-related	CP	CyRel (11.0)	DLI + IFN (0.9)†	AP-CE (10.5)	CCR (306+)	Alive, 411+	1.4
16	30/F	Syngeneic	CP	CyRel (5.4)	DLI + IFN (0.3)†	AP-CE (10.0)	CCR (486+)‡	Alive, 583+	0.5
17	43/M	Matched-related	CP	CyRel (67.3)	DLI (1.5)	BC (2.0)	CCR (281)	Died of relapsed disease, 525	−0.9
18	55/M	Autologous	CP	CP (15.8)	Chemotherapy (5.1)†	BC (28.1)	CCR (406+)	Alive, 550+	0.66
19	22/F	Matched-related	BC	BC (6.3)	None	BC (0.1)	CCR (144)	Died of relapsed disease, 211	−2.8
20	45/F	Matched-related	BC	CyRel (13.6)	DLI (1.0)†	BC (4.2)	PCR	Died of relapsed disease, 72	NA
21	37/M	Matched-related	CP	CP (3.0)	IFN (1.1)†	CP (60.4)	NR	Died of refractory disease, 474	0.58
22	36/M	Matched-unrelated	CP	CyRel (13.0)	IS-W (0.6)†	AP-CE (4.2)	NR	Alive, 272+	0.60
23	19/F	Matched-related	CP	CP (21.2)	None	BC (0.2)	NR	Died of refractory disease, 259	NA

SCT indicates stem cell transplantation; IMTx, imatinib therapy; CP, chronic phase; AP, accelerated phase; AP-CE, AP with clonal evolution alone, BC, blast crisis; Rel, relapse; CyRel, cytogenetic relapse; IFN, interferon; DLI, donor lymphocyte infusion; IS-W, immunosuppressant withdrawal; CCR, complete cytogenetic response; PCR, partial cytogenetic response; NR, no response; NA, not available.

*Days after the initiation of imatinib therapy.

†Refractory to salvage treatment, defined as no hematologic response at 3 months or no cytogenetic response at 6 months while receiving immunomodulatory treatment.

‡MR induced after 4 months of high-dose imatinib therapy.

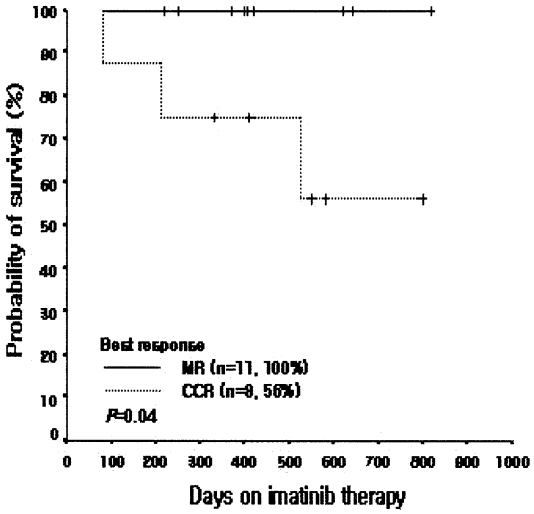

Figure 1. Probability of overall survival according to the best response after imatinib therapy in patients achieving a complete cytogenetic response.

Table 2. *Comparison of the Median BCR-ABL/ABL Ratio at 1, 3, and 6 Months According to the Best Responses in Patients Achieving a Complete Cytogenetic Response*

Variable	Best Response		*P* Value*
	MR	CCR	
n	11	8	
Median duration of IM-Tx, d (range)	404 (216-817)	450 (35-800)	
BCR-ABL/ABL ratio			
Before transplantation	0.25	0.54	.74
Month 1	0.03	0.03	.95
Month 3	0.0004	0.00099	.62
Month 6	0.0000012	0.00033	.003

MR indicates molecular remission; CCR, complete cytogenetic remission, IM-Tx, imatinib therapy.
*Mann-Whitney test.

in the BCR-ABL/ABL ratio between the 2 groups during third and sixth months, the prediction of an MR was evaluated with a log-reduction during that time in the 15 patients remaining in CCR until 3 months. A significant difference in the probability of a subsequent MR was observed at the log-reduction level of 1.0 (log-reduction ≥1.0, 100%; <1.0, 17%; $P = .003$; Figure 3b).

DISCUSSION

This study monitored the BCR-ABL/ABL ratio after imatinib therapy in patients with relapsed CML after transplantation. The patients, who at some point had an undetectable BCR-ABL, were identified to be associated with an improved survival. This study also showed that normalized BCR-ABL transcript levels in patients who eventually achieved an MR significantly decrease within the first 6 months of imatinib therapy; therefore, the early monitoring of BCR-ABL by Q-RT-PCR may indicate a subsequent MR.

Cytogenetic assays continue to be regarded as the standard method for assessing the response to various therapies for CML. Obtaining a major response or a CCR has been associated with excellent long-term survival after interferon treatment [26]. Therefore, the cytogenetic response to imatinib therapy may provide useful prognostic information. A significant number of patients achieve a CCR on imatinib therapy. Approximately 75% of newly diagnosed CP CML patients who were initially treated with imatinib and approximately 40% of late CP patients who were treated with imatinib after interferon therapy failure achieved a CCR [27,28]. In imatinib therapy for post-transplantation relapse, limited series with a small number of cases have been analyzed for cytogenetic response. The results of the 8 literature reviews showed that 51 (45%) of 114 evaluable patients achieved a CCR [13]. Our recent experience with 13 patients with CP with or without clonal aberrations

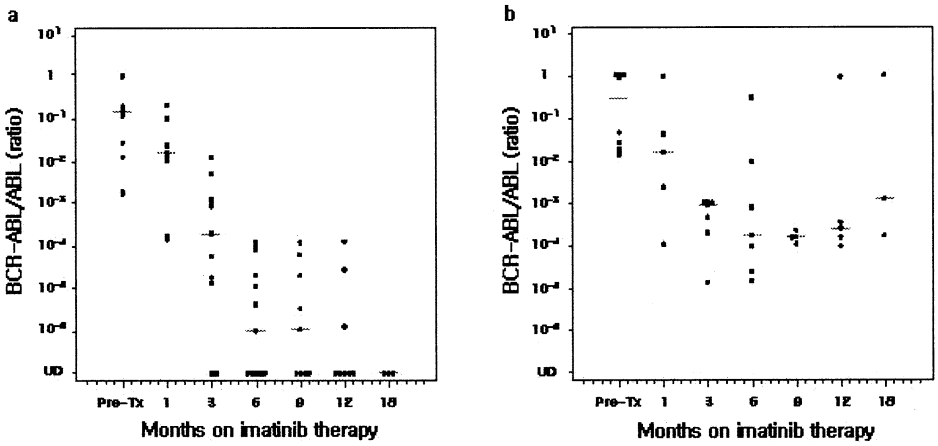

Figure 2. Kinetics of the normalized BCR-ABL transcript levels in patients achieving a molecular remission (a) or a complete cytogenetic response (b) as a best response to imatinib therapy.

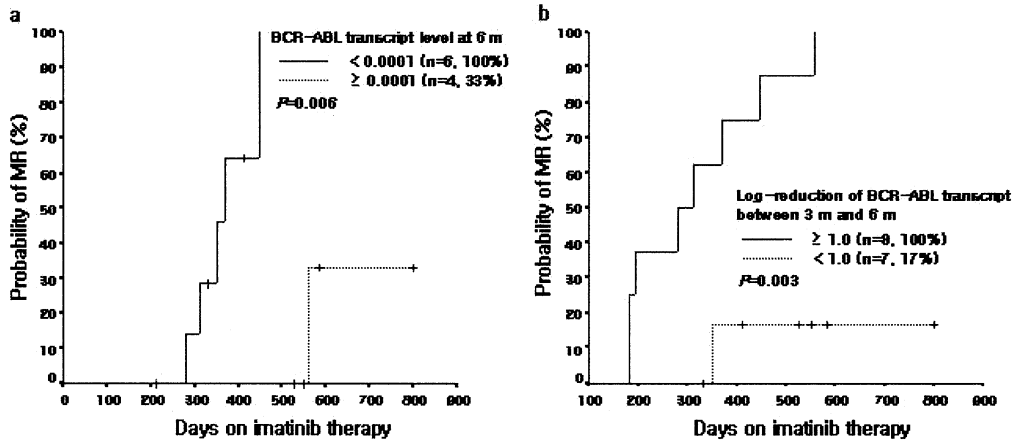

Figure 3. Probability of molecular remission according to the BCR-ABL transcript levels at 6 months (a) and to the log-reduction of the BCR-ABL transcript levels between 3 and 6 months (b).

showed the CCR rate to be >90% [8]. In this study, it was observed that 78% of the enrolled patients achieved a CCR, but the reason for the higher CCR rate in our study is not obvious. Among the various possible factors, such as disease or chimeric status and time from transplantation to relapse, partial influences of prior salvage treatment should be considered, although most enrolled patients had diseases that progressed or did not respond after long-term observation. Although this study was conducted on a small group of patients, we suggest that achieving CCR is an important prognostic factor for survival.

Regarding the high CCR rate after imatinib therapy, the next concern focuses on the response under the cytogenetic level. It is known that achievement of MR after DLI is highly predictive of better survival in patients with relapsed CML [4]. Whether the achievement of MR on imatinib therapy also correlates with the long-term outcome is not yet known. Although heterogeneity in the molecular response was found in the complete cytogenetic responders after interferon or imatinib, low levels of MRD were associated with continuous remission [29-32]. This study showed that the probability of survival for patients who achieved an MR was significantly better than for those who did not. Whether imatinib can replace DLI depends on the rate and duration of MR, which should be revealed in larger series. To define the role of imatinib in comparison with DLI for post-SCT CML relapse, studies on how long imatinib therapy should be continued in patients achieving MR should also be performed.

The fact that approximately 40% of patients who achieved a CCR in this study did not achieve an MR, even with the continuation of imatinib therapy, underlies the need for a more sensitive method for predicting an MR and for recognizing the early warning signs of imatinib resistance. Q-RT-PCR has been used in monitoring BCR-ABL expression after imatinib therapy [11,12,31,33,34]. Recent reports suggest that a normalized BCR-ABL transcript level with Q-RT-PCR correlates with the contemporary or subsequent marrow cytogenetic response [35-37]. Similarly, a good correlation between the BCR-ABL transcript levels and the cytogenetic response was observed in this study (data not shown). In all patients achieving a CCR, a rapid decline in the BCR-ABL/ABL ratio was observed during the initial 3 months of imatinib therapy in both groups. However, the decrease in the BCR-ABL/ABL ratio during the next 3 months was significantly greater in the patients who were destined to achieve an MR. This finding suggests that poor responders can be predicted and approached with other therapeutic options by early molecular monitoring with Q-RT-PCR. Alternatively, hazardous therapies can be avoided for the patients who are likely to acquire an MR and subsequent long-term disease control.

Extremely poor end results in blast crisis were observed in this study; 4 of 5 patients died of refractory or relapsed disease after a transient response. This finding suggests that more potent therapies should be considered. In addition, therapeutic strategies should be established for patients who do not achieve an MR. Possible approaches could be a dose escalation of imatinib or combinations of imatinib with DLI, low-dose cytarabine, or interferon. Although high-dose imatinib led to more favorable outcomes and was reported to be beneficial for overcoming the resistance to standard-dose treatment [38,39], it is not known whether there is dose-dependent responsiveness in patients with relapsed CML. However, one patient (case 16), in whom imatinib was increased to 800 mg/d after the CCR had continued for more than 12 months of standard-dose imatinib, achieved an MR after 4 months of high-dose therapy. This finding illustrates the possible role of dose escalation even in a posttransplantation setting.

Although standardization of the Q-RT-PCR method will be needed in the near future, it is believed that quantitative determination of residual disease with Q-RT-PCR is a reliable and sensitive method for monitoring the MRD. It was also shown that the early trend in the BCR-ABL/ABL ratio might be clinically useful for the early identification of patients who are destined to achieve an MR on imatinib.

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