Molecular Responses in Patients with Chronic Myelogenous Leukemia in Chronic Phase Treated with Imatinib Mesylate

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Abstract

Purpose: To determine the clinical significance of molecular response and relapse among patients with chronic myelogenous leukemia (CML) treated with imatinib.

Experimental Design: We analyzed the results of quantitative PCR in 280 patients with CML in chronic phase who achieved complete cytogenetic remission with imatinib (117 after IFN-α failure and 163 previously untreated). Median follow-up was 31 months (range, 3-52 months).

Results: Median BCR-ABL/ABL ratio before the start of therapy was 39.44 (range, 0.252-170.53). A major molecular response (BCR-ABL/ABL ratio ≤0.05%) was achieved in 174 (62%), and transcripts became undetectable (complete molecular response) in 95 (34%). By multivariate analysis, only treatment with high-dose imatinib ($P = 0.02$) was associated with achievement of a major molecular response. Nine of 166 (5%) patients who achieved a major molecular response lost their cytogenetic remission, compared with 25 of 68 (37%) among those who did not achieve this response ($P < 0.0001$). Patients achieving a major molecular response 12 months after the start of therapy had significantly better complete cytogenetic remission duration than others. A >1-log reduction in transcript levels after 3 months of therapy predicted for an improved probability of achieving a major molecular response at 24 months. Increasing levels of BCR-ABL transcripts predicted for a loss of cytogenetic remission only among patients who did not achieve a major molecular response.

Conclusions: Achieving a major molecular response, particularly within the first year of therapy, is predictive of a durable cytogenetic remission and may be the future goal of therapy in CML.

Imatinib mesylate (Gleevec, Novartis, Basel, Switzerland) is a potent and selective tyrosine kinase inhibitor (1, 2). It was initially developed as an inhibitor of Bcr-Abl, the protein product of the Philadelphia chromosome (Ph) characteristic of chronic myelogenous leukemia (CML). The activity of imatinib in CML has made it the current standard therapy in this disease. Among patients who failed prior therapy with IFN-α, 45% to 60% of patients achieve a complete cytogenetic remission, and 80% of patients remain alive and free of progression after 4 years (3–5). A randomized trial in patients with previously untreated CML in early chronic phase showed the higher efficacy and better toxicity profile of imatinib compared with IFN-α (6). Over 75% of these patients achieved a complete cytogenetic remission with imatinib, >95% remained alive and free of progression at 18 months (6–8). Higher doses of imatinib improved the complete cytogenetic remission rates to 90% both in patients who failed prior IFN-α therapy (9) and in those previously untreated (10, 11).

As the rates of complete cytogenetic remission improve, monitoring of minimal residual disease by PCR has become more important. Patients are currently routinely followed with quantitative PCR techniques (e.g., real-time PCR) to determine the levels of BCR-ABL transcripts during the course of therapy (12). The prognostic significance of levels of minimal residual disease determined by PCR have been established in CML after bone marrow transplantation and IFN-α therapy (13–15). The clinical significance of molecular response among patients treated with imatinib is being investigated. The International Randomized Study of Interferon and STI571 (IRIS) trial showed that a significantly higher proportion of patients who were in complete cytogenetic remission at 6 months had at least a 3-log reduction of BCR-ABL/BCR levels if treated with imatinib compared with those treated with IFN-α (42% versus 13%, $P = 0.03$; ref. 16). Patients with at least a 3-log reduction of BCR-ABL/BCR levels after 12 months of therapy had a significantly better probability of disease-free survival compared with those in complete cytogenetic remission but with a less than 3-log reduction of BCR-ABL/BCR (16). This data has

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not been confirmed in independent series, and additional questions remain, including the factors predictive of a molecular response, the durability of response, and the significance of increasing transcript levels.

We analyzed the results of molecular monitoring in patients with CML in chronic phase treated with imatinib at our institution to determine the frequency of molecular response to imatinib and the long-term prognostic implications of molecular responses.

Patients and Methods

From December 1999 to August 2003, 377 patients with chronic phase CML were treated with imatinib. These included 190 patients receiving imatinib after failing therapy with IFN-α, and 187 who received imatinib as their first line of therapy for CML. All patients receiving at least one dose of imatinib were included in this analysis. The definitions of chronic phase and IFN-α failure were the same as those used in published studies using imatinib (3, 4, 7). Briefly, patients were considered to be in chronic phase if they had blasts <15%, blasts plus promyelocytes <30%, basophils <20%, platelets $\geq 100 \times 10^9$/L and no evidence of extramedullary disease. Patients with cytogenetic clonal evolution but without other features of accelerated phase were included in this analysis. IFN-α failure was defined as follows: hematologic failure included hematologic resistance (failure to achieve complete hematologic response after ≥6 months of IFN-α) or relapse (disease recurrence after achieving complete hematologic response), cytogenetic failure included resistance (Ph ≥65% after at least 12 months of IFN-α), or relapse (Ph increase >30% documented on two occasions, or a single increase to ≥65%); and intolerance defined as grades 3 to 4 non–hematologic toxicity (according to the National Cancer Institute Common Toxicity Criteria) not responding to adequate management.

Evaluation before and during treatment. Before the start of treatment, patients were evaluated with history an physical exam, complete blood cell count (CBC) with differential, and blood chemistry including total bilirubin, creatinine, and alanino aminotransferase. All patients had a pretreatment bone marrow evaluation for morphology and cytogenetic analysis, fluorescent *in situ* hybridization, and real-time PCR. After treatment was started, patients were evaluated with complete blood count and blood chemistry weekly during the first 1 to 3 months, then every 2 to 6 weeks. Bone marrow aspirations for morphology, cytogenetics (fluorescent *in situ* hybridization when cytogenetic analysis inevaluable) and PCR were repeated every 3 to 4 months for the first year, and every 6 months thereafter. Patients were followed for survival at least every 3 months. Drug toxicity was evaluated at each visit and graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

Response criteria. Response criteria were previously described (3). Briefly, a complete hematologic remission was defined as a WBC count of $<10 \times 10^9$/L, a platelet count of $<450 \times 10^9$/L, no immature cells (blasts, promyelocytes, myelocytes) in the peripheral blood, and disappearance of all signs and symptoms related to leukemia (including palpable splenomegaly) lasting for at least 4 weeks. A complete hematologic remission was further categorized by the best cytogenetic remission as complete (0% Ph-positive), partial (1-34% Ph-positive), and minor (35-90% Ph-positive). A major cytogenetic remission included complete plus partial cytogenetic remissions (i.e., Ph-positive <35%). Cytogenetic remission was judged by standard cytogenetic analysis; fluorescent *in situ* hybridization was used only when routine cytogenetic analysis was unanalyzable (i.e., insufficient metaphases). For the purpose of this analysis, a major molecular response was defined as a BCR-ABL/ABL ratio of <0.05%, a level that has been found to correlate with long-term remission after IFN-α-based therapy (14). A complete molecular response was defined as undetectable levels of BCR-ABL confirmed by nested PCR.

Cytogenetic analysis, fluorescent in situ hybridization, and PCR. Cytogenetic analysis was done in bone marrow cells by the G-banding technique. For chromosome analysis, at least 20 metaphases were analyzed and bone marrow specimens were examined on direct or short-term (24 hours) cultures. Bone marrow cells were analyzed by fluorescent *in situ* hybridization using the LSI BCR/ABL dual color extra-signal probe according to the manufacturer's instructions (Vysis, Inc., Downers Grove, IL).

BCR-ABL transcript levels was determined by real-time quantitative reverse transcription PCR (qRT-PCR), with negative results (i.e., undetectable transcript) confirmed by nested PCR. Total RNA from bone marrow aspirate (1-2 mL) and peripheral blood (10 mL) was isolated using Trizol reagent (Invitrogen Life Technologies, Gaithersburg, MD). The integrity of RNA was documented by gel electrophoresis prior to reverse transcription with samples rejected if rRNA bands were not visible. Reverse transcription was done using up to 14 μg of total RNA at a concentration of 0.5 μg/μL using Superscript II RT (Invitrogen). cDNA (5.0 μL representing ~1 μg of total RNA) was used as a template in a 25 μL qRT-PCR reaction using primers specific for *BCR-ABL* and *ABL*. Assays were done using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA).

A multiplex qRT-PCR assay was done in a single tube to simultaneously detect b2a2, b3a2, and e1a2 transcripts as well as total abl as a normalizing transcript, with each sample amplified in duplicate. Primers used are listed in Table 1, as are the fusion and normalizing abl detection probes which were labeled with 6-carboxyfluorescein (FAM) and VIC (ABI) fluorochromes, respectively. By mixing experiments with bcr-abl + cell lines (KBM-7, b2a2; K562, b3a2; and B15, e1a2), we had optimized the primer concentrations to allow for accurate quantitation of each of the fusion transcripts over the range of the assay. The bcr-abl levels for each sample were expressed as a percentage of BCR-ABL to ABL. For each run, the t(9;22)-positive cell lines KBM7, K562, and B15, that carry b2a2, b3a2, and e1a2 fusion genes, respectively, served as positive controls. The HL60 cell line was used as a negative control.

A plasmid containing cloned sequences spanning abl exons 10 and 11 was used for generating the *ABL* standard curve. A plasmid containing clone *BCR-ABL* fusion sequences derived from K562 was used to generate the BCR-ABL standard curve. A 5-log dilution of each of these plasmid standards were included in each run as a test for assay sensitivity across the dynamic range. Dilutions of K562 RNA (1:10,000 and 1:100,000) into HL-60 RNA were also included in each run as a control for reverse transcription and PCR conditions. The sensitivity of each run was confirmed to be in the range of 1:100,000. PCR results were rejected if the sensitivity of the run was <10,000 or if the total level of normalizing ABL transcript in any given sample was <10,000 copies. This value sets a lower limit of sensitivity of 1:10,000 for detection of BCR-ABL transcripts in posttreatment samples. Interassay variability, assayed by repeated BCR-ABL/ABL determination starting from RNA from control samples, was estimated to be between 15% and 40% over the range of the assay, with highest variability noted at the high and low BCR-ABL copy numbers.

To determine the type(s) of BCR-ABL fusion transcripts that were amplified by qRT-PCR, capillary electrophoresis was subsequently done on all samples. The products were detected due to the abl exon 2 primer in the qRT-PCR reaction being labeled at the 5′ end with NED fluorescent dye (ABI). Specimens that were inadequate or negative for *BCR-ABL* fusion transcript detection by qRT-PCR/capillary electrophoresis were subsequently tested by nested PCR and then quantitated by competitive PCR, as previously reported (17, 18). The sensitivity of the nested PCR assay, established by dilution of control RNA samples is approximately 1:100,000 to 1:1,000,000. Given the differing methodologies, these values were not regarded as directly comparable to the qRT-PCR values and were thus not used for the statistical analyses below. The qRT-PCR methodology described above has been used routinely since October 2001. Before this date, a different methodology was used and the results cannot be extrapolated to current values.

Table 1. Sequence of primers and probes used in the TaqMan qRT-PCR

Primers	Final concentration (nmol/L)	
bcr-e1	400	GCAAGACCGGGCAGATCT
bcr-b2	400	ACAGCATTCCGCTGACCAT
abl-a2	400	NEDR-CGAGCGGCTTCACTCAGA
abl 10-F	50	AGAGCTGCAGAGCACAGAGACA
abl 11-R	50	GCTCTTTTCGAGGGAGCAATG
TaqMan probes		
bcrabl-2a	200	6FAMR-CCTGAGGCTCAAAGTCAGATGCTACTGG-TAMRA
abl 1011-P	100	VIC-ATGGTCCAGAGGATCGCTCTCTCCCT-TAMRA

Thus, for consistency, only those results obtained with this methodology are considered for this analysis.

Statistical analysis. Complete cytogenetic remission duration was considered from the time a complete cytogenetic remission was first achieved to the time when any Ph-positive metaphases were first detected again. Survival was calculated from the time the treatment began until death from any cause, or last follow-up. The probability of remission duration was estimated using the Kaplan-Mier product-limit method (19) and compared by means of the log-rank test (20). Associations between categorical variables were assessed via cross-tabulation and Fisher's exact test or the χ^2 test (21). Multivariate logistic regression analysis was used to assess the relationship between various predictors of interest and molecular response.

Results

Patients. A complete hematologic remission was achieved in 293 of 305 (96%) patients treated with imatinib for CML in active chronic phase (i.e., abnormal peripheral blood counts and/or splenomegaly). These included 177 of 181 (98%) in early chronic phase (i.e., within 12 months from diagnosis), and 116 of 124 (94%) in chronic phase after IFN-α failure. A major cytogenetic remission was achieved in 310 of 369 (84%) patients who had Ph-positive metaphases by standard cytogenetic analysis at the start of therapy, including 279 (76%) that achieved a complete cytogenetic remission. An additional seven patients had Ph-negative BCR-ABL-positive disease at the start of therapy. Among 286 patients in complete cytogenetic remission, 280 had at least one PCR test done for follow-up. These patients constitute the focus of this analysis. The median age of the study group was 50 years (range, 15-84 years). Among them, 117 (42%) patients received imatinib after IFN-α failure and 163 (58%) as first line of therapy. The starting dose of imatinib was 400 mg daily for 144 (51%) patients and 800 mg for 136 (49%) patients. The median BCR-ABL/ABL ratio before treatment with imatinib was started was 39.44% (range, 0.252-170.53%). The median follow up from the time treatment was started is 31 months (range, 3-52 months). Five patients have died while on therapy with imatinib, with an estimated overall survival of 95% at 48 months. Transformation while on therapy occurred in two patients.

Molecular response. A major molecular response was achieved in 174 patients (62%; 46% of all patients treated) and 95 patients (34%; 25% of all patients treated) had at least one analysis showing undetectable BCR-ABL (i.e., complete molecular response). Among these, 81 have had subsequent PCR analyses and 44 (54%) have had at least two consecutive undetectable assays. The median time from start of treatment to achievement of a major molecular response was 10 months (range, 2.8-46 months), and 16.7 months (range, 3-48 months) for achievement of a complete molecular response. However, as shown in Fig. 1, these responses may occur late and there is no clear evidence of a plateau for time to molecular response.

We then analyzed the pretreatment clinical characteristics associated with achieving a molecular response. Table 2 shows the clinical characteristics at the start of imatinib therapy for all 280 patients. Characteristics associated with a higher probability of achieving a major molecular response included a lower percentage of peripheral blood blasts, Sokal low-risk group, treatment with high-dose imatinib, and treatment in early chronic phase. Achievement of undetectable levels of BCR-ABL was associated with Ph-positive metaphases ≤90% at the start of therapy. In a multivariate analysis, treatment with high-dose imatinib was the only variable independently associated with an increased probability of achieving a major molecular remission (P = 0.02). Multivariate analysis for complete molecular remission identified a lower percentage of Ph-positive metaphases (<90%) as the only variable associated with this response (P = 0.01); treatment with high-dose imatinib was marginally significant (P = 0.08).

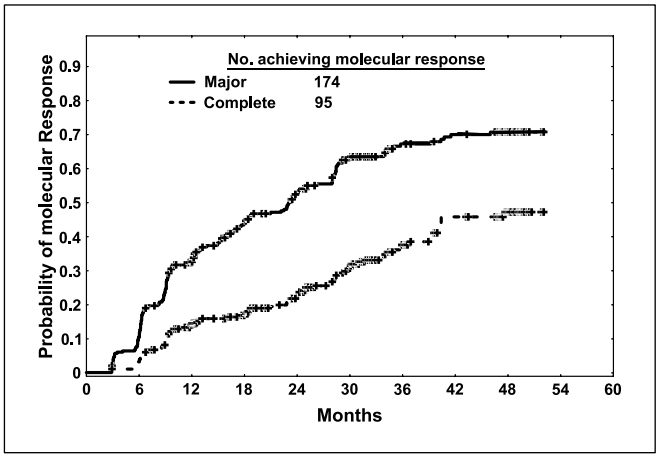

Fig. 1. Cumulative probability of achieving a molecular response on imatinib for 280 patients with CML in chronic phase who achieved a complete cytogenetic remission on imatinib. All times are calculated from the start of therapy with imatinib.

This analysis does not take into account the time difference in follow-up between patients. Thus, to adjust for the time to achieving molecular response, we repeated the multivariate analysis considering achieving a major or complete molecular remission at 12 months as the end point. For this analysis, we focused on patients treated in early chronic phase as this is the setting where most patients are currently being treated with imatinib, and for these patients, the percentage of Ph-positive metaphases <90% rarely applies. In this analysis, treatment with high-dose imatinib was the only variable associated with an increased probability of achieving a major ($P = 0.01$) or complete molecular response ($P = 0.02$).

Durability of cytogenetic response by molecular response. We then investigated the long-term clinical significance of achieving a molecular response (Table 3). Because of the small number of deaths and transformations during therapy, the analysis of the clinical implications of molecular response focused on duration of complete cytogenetic remission. Patients achieving a major molecular response were followed for a median of 31 months (range, 7.5-52 months) from the start of imatinib therapy. Eight patients achieved a major molecular response only on their last evaluation and therefore have no follow-up at the time of this report. Among the 166 patients who had at least one additional cytogenetic analysis after achieving a major molecular response, 9 (5%) lost their complete cytogenetic remission, compared with 25 of 68 (37%) who did not achieve a major molecular response ($P = 0.0001$). Two and four patients eventually re-gained a complete cytogenetic remission, respectively (one after increasing the dose of imatinib, all others without additional intervention). Patients with undetectable levels of BCR-ABL were followed for a median of 14 months (range, 3-27 months) after reaching this milestone, not considering 13 who became undetectable only on their latest follow-up

Table 2. Associations of patient and disease characteristics with molecular response ($n = 280$)

Variables	Category	*n*	MMR	*P*	CMR	*P*
Age (y)	<60	199	122 (61)	0.75	65 (33)	0.57
	≥60	81	52 (64)		30 (37)	
Splenomegaly	No	232	148 (64)	0.25	77 (33)	0.62
	Yes	48	26 (54)		18 (38)	
Hemoglobin (g/dL)	<12	110	63 (57)	0.23	36 (33)	0.80
	≥12	169	110 (65)		59 (35)	
WBC ($\times 10^9$/L)	≤20	159	97 (61)	0.39	61 (38)	0.20
	20.1-50	64	44 (69)		17 (26)	
	>50	56	32 (57)		17 (30)	
Platelets ($\times 10^9$/L)	≤450	205	120 (58)	0.06	63 (31)	0.08
	>450	75	54 (72)		32 (43)	
Peripheral basophils (%)	<7	238	151 (63)	0.30	79 (33)	0.60
	≥7	42	23 (55)		16 (38)	
Peripheral blasts (%)	0-1	254	163 (64)	0.04	89 (35)	0.35
	2	18	9 (50)		5 (28)	
	≥3	8	2 (25)		1 (12)	
Marrow basophils (%)	<4	220	140 (64)	0.40	79 (36)	0.22
	≥4	60	34 (57)		16 (27)	
Marrow blasts (%)	<5	261	166 (64)	0.08	91 (35)	0.32
	≥5	19	8 (42)		4 (21)	
Cytogenetic clonal evolution	No	258	159 (62)	0.82	84 (32)	0.23
	Yes	21	14 (67)		10 (48)	
Sokal risk*	Low	98	76 (78)	0.008	34 (35)	0.53
	Intermediate	69	41 (59)		26 (38)	
	High	34	18 (53)		9 (26)	
% Ph at pretreatment	≤90	56	39 (70)	0.28	27 (48)	0.02
	>90	221	134 (61)		67 (30)	
Duration of chronic phase (months)	<12	175	112 (64)	0.49	54 (31)	0.30
	12-35	65	36 (55)		27 (42)	
	≥36	40	26 (65)		14 (35)	
Study group	ECP	163	110 (67)	0.04	52 (32)	0.47
	IFN failure	117	64 (55)		43 (37)	
Starting dose of imatinib (mg)	400	144	77 (53)	0.003	42 (29)	0.11
	800	136	97 (71)		53 (39)	

Abbreviations: MMR, major molecular response; CMR, complete molecular response; ECP, early chronic phase.
*Two hundred and one patients evaluable.

Table 3. Significance of achieving molecular response in sustaining a complete cytogenetic remission

Best Bcr-Abl/Abl ratio	No. evaluable	No. who lost complete cytogenetic remission to PR/Minor/>90%	Total (%)	*P*	Median follow-up from molecular response (range)
≥0.05%	68	12 + 4*/5+1*/3	25 (37)	<0.0001	11.2 (3.0-28.9)
<0.05%	166	6 + 2*/0/1	9 (5)		18.4 (3-33.4)
>0 and <0.05%	74	5/0/0	5 (7)	0.48	15.0 (3.4-30.6)
Undetectable	82	1 + 1*/0/1	3 (4)		14.3 (3-27)

*Patients lost complete cytogenetic remission only transiently.

evaluation. Among the 82 patients with subsequent evaluations, only 3 (4%) lost their complete cytogenetic remission (one of them later regained a complete cytogenetic remission), compared with 5 of 74 (7%) who had detectable BCR-ABL levels but <0.05%. These differences are not due to a difference in follow up, as there is no difference in follow-up time between patients who did and did not achieve a major or complete molecular response.

To further define the long-term prognostic implications of molecular response and account for the potential lead-time bias, we investigated the complete cytogenetic remission duration according to molecular response at different time points. For this analysis, only patients who had both a molecular analysis and were still on therapy and in complete cytogenetic remission at the specified time points were evaluable. Patients who achieved a major molecular remission by 12 months after the start of therapy have significantly longer cytogenetic remission durations than those without this magnitude of molecular response (Fig. 2C). Molecular response at earlier time points (i.e., 3 and 6 months) shows a similar trend (Fig. 2A and B). Most patients who lost their complete cytogenetic remission still have at least a partial cytogenetic remission. Thus, we repeated the analysis considering only loss to at least 35% Ph-positive metaphases. Because of the few number of events, the results are not statistically significant. In fact, the analysis could not be done for response at 12 months as no patients lost their major cytogenetic response in either group (with or without major molecular response). A trend could be observed in favor of the earlier responders at 3 ($P = 0.29$) and 6 months ($P = 0.11$; data not shown).

Other studies used a 3-log reduction rather than an absolute value as a measure of molecular response for patients treated with imatinib (16). We thus repeated the analysis using a 3-log reduction as a measure of major molecular response. For this purpose, published reports have used the median calculated from measuring pretreatment values from a small number of patients as baseline levels. For our analysis we calculated a 3-log reduction in two different ways. First, we used the median of the total population (i.e., 39.44%) as the baseline value. Thus, a 3-log reduction would be considered achieving a ratio of ≤0.039%. The results are nearly identical to those considering a value <0.05% as a major molecular response. We then used each individual patients' pretreatment BCR-ABL/ABL value as their own baseline to determine log reduction. Data for this analysis is available for 79 patients. Although the difference is not statistically significant, the same trend is observed in favor of patients achieving a molecular response at each of these early time points.

Since we identified that achieving a major molecular response after 12 months of therapy is predictive of cytogenetic remission duration, we investigated the probability of achieving this response at 12 months based on the response achieved at 3 months. Patients who achieve ≤1-log reduction after 3 months of therapy have a significantly lower probability (55%) of achieving a major molecular response at 24 months, compared with those with greater than 1-log or 2-log reduction (84% and 95%, respectively; $P = 0.0002$).

Durability of molecular response. We next investigated the durability of molecular responses. Among the 174 patients who achieved a major molecular response, 165 (95%) have had repeated molecular testing (median, five follow-up tests, range, two to eight). The molecular response was sustained in 118 (72%) of them, for a median of 19 months (range, 3-33 months). Subsequent evaluation is available for 81 of the 95 (85%) patients who achieved undetectable levels of BCR-ABL, with a median of two follow-up tests (range, one to six). In 43 patients (53%), BCR-ABL/ABL became detectable again in subsequent testing, whereas 38 (47%) had sustained undetectable BCR-ABL levels over a median of 18 months (range, 3-27 months). Among 47 patients who lost a major molecular response, 13 (28%) did so after having therapy interrupted temporarily or permanently. The most common reasons for treatment interruptions were noncompliance in six, toxicity in five [liver ($n = 3$), gastrointestinal ($n = 1$), and weight gain ($n = 1$)], and hospitalization for other reasons ($n = 2$).

To determine the significance of an increasing BCR-ABL/ABL ratio while on therapy, we investigated the clinical significance of a <1-log ($n = 23$), 1-log ($n = 30$), or 2-log ($n = 8$) increase in the BCR-ABL/ABL ratio from the lowest level achieved. Only four (17%), four (13%), and two (25%) patients with each of these magnitudes of increasing transcript levels, respectively, lost their complete cytogenetic remission ($P = 0.72$). However, 9 of the 10 patients who lost the response had the lowest BCR-ABL/ABL ratio of >0.05, whereas only 1 of 37 patients with nadir levels of ≤0.05% had lost their response, regardless of their log increase ($P = 0.04$). If the absolute increase is considered instead of the log increase, an increment of BCR-ABL/ABL of more than 1.0 percentage point from the nadir is associated with an increased risk of cytogenetic relapse (5 of 11, 45%)

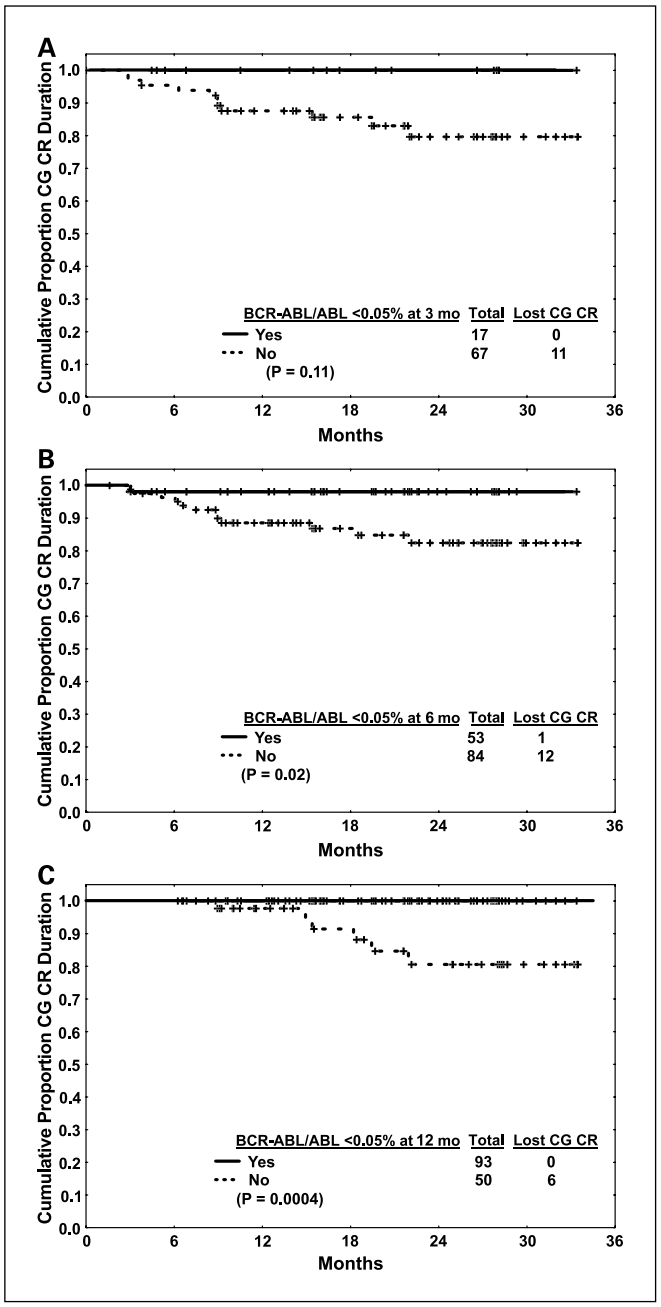

Fig. 2. Landmark analysis of duration of complete cytogenetic remission according to the molecular response at (*A*) 3 months, (*B*) 6 months, and (*C*) 12 months. Patients are grouped based on their BCR-ABL/ABL ratio <0.05% versus ≥0.05% at the specified time point. An event is considered the recurrence of any Ph-positive metaphases after achieving a complete cytogenetic remission. For this analysis, the starting point in the horizontal axis is the time when patients achieved complete cytogenetic remission.

compared with 6 of 33 (18%) of those with percentage point increments of 0.05 to 1, and 0 of 44 of those with increments of <0.05 ($P = 0.0001$). Again, patients with nadir levels of ≥0.05 had the highest probability of relapse.

Discussion

Imatinib has become the standard therapy for patients with CML. A complete cytogenetic remission is achieved in 75% to 90% of patients treated with imatinib in early chronic phase (6, 7, 11). With standard-dose imatinib, ~40% of patients have been reported to achieve at least a 3-log reduction in BCR-ABL/BCR levels, a reduction associated with an increased probability of progression-free survival (16). In these studies, 4% to 10% of patients have achieved undetectable levels of BCR-ABL (16). Studies using higher doses of imatinib have reported major molecular responses (i.e., 3-log reduction or <0.05%) in ~65% of patients, and undetectable levels in 35% to 40% (9, 11). In this study, we investigated the clinical significance of these molecular responses among patients in chronic phase treated with imatinib. Among 280 patients who achieved a complete cytogenetic remission with imatinib, 174 (62%) achieved a major molecular response, and 95 (34%) had undetectable BCR-ABL transcripts at least once. It should be recognized that variations in the assay sensitivity may account for differences in the rate of patients with undetectable transcripts among different reports. The relevance of the sensitivity of the assay is further emphasized by the fact that approximately half of the patients that had undetectable transcript levels on at least one occasion later had low levels detectable. Similar results have been reported from a subset of patients from the IRIS trial. In that study, 10 of 26 (38%) patients had undetectable transcripts on at least one occasion, but only 4 (14%) had consistently undetectable levels for more than 6 months (22).

Treatment with high-dose imatinib (i.e., 800 mg) was the most significant factor associated with in increased probability of achieving a molecular response, particularly at earlier time points (i.e., 12 months). However, the overall rate of major and complete molecular response is not different between patients treated with standard versus high-dose imatinib. Because the follow-up is significantly longer for patients treated with standard dose and patients may continue to improve their cytogenetic and molecular response with continuation of therapy, further follow-up is required to determine whether there is an absolute difference in molecular response with high-dose imatinib or if it is a means of getting earlier to the same end point.

The definition of molecular responses is still evolving. For this analysis, we considered a major molecular response as reaching an absolute value of BCR-ABL/ABL ratio <0.05%, a value that has been found predictive of duration of cytogenetic response after IFN-α-based therapy by our group and others. Interestingly, it is also the median value after 12 months of therapy for the study population in this report. Other studies have reported the log decrease of BCR-ABL transcripts and considered a 3-log reduction as a major response associated with improved outcome (16). The studies have used a standardized baseline value for patients derived from the median value obtained from 30 patients studied before the start of therapy (16). Thus, it was not necessary to know the BCR-ABL level of a patient at baseline to calculate the subsequent reduction (16). The median baseline value for our patient population was 39.44. If we were to use a 3-log reduction from this value instead of an absolute ratio of <0.05% to determine a major molecular response, only five patients would not be considered to have achieved a major molecular response, and the results of the analysis would be similar.

Our results suggest that major (i.e., BCR-ABL/ABL <0.05%) or complete molecular remissions (i.e., undetectable BCR-ABL) were associated with more durable cytogenetic remissions. Only 5% of patients who have achieved a major molecular response and 4% of those reaching undetectable levels of BCR-ABL/ABL have lost their complete cytogenetic remission, compared with 37% of patients not reaching these degrees of response ($P < 0.0001$). This difference is not due to a lead-time bias as patients in both groups were followed for a similar period of time. A similar trend was reported in a smaller series by Rosti et al. (23). One of 18 patients who had a 2-log reduction in BCR-ABL/B2M ratio (6%) lost a complete cytogenetic remission, compared with 3 of 9 patients who had a <2-log reduction.

The importance of achieving an early molecular response was first reported from the IRIS trial. Among patients who achieved a complete cytogenetic remission after 12 months of therapy, those with at least a 3-log reduction in BCR-ABL transcript levels had a significantly better progression-free survival compared with those with less than a 3-log reduction (16). Other smaller series' and subsets of the larger IRIS cohort have reported that early declines in transcript levels are associated with achievement of cytogenetic response (24–26). In our series, patients who achieved a major molecular remission by 12 months after the start of therapy had an improved probability of a sustained complete cytogenetic remission. A similar trend was seen at earlier time points (i.e., 3 and 6 months) but it was not statistically significant, probably because of the relatively small number of patients who reach these levels at earlier time points. However, if patients are divided by quartiles of BCR-ABL transcript levels at 3 months, those with the lowest levels (i.e., <0.0847) have a significantly better probability of a sustained cytogenetic remission. Branford et al. (27) reported similar results for patients reaching a 2-log reduction at 6 months. Patients with greater than a 1-log reduction (our series) or 2-log reduction (27) at 3 months have an improved probability of later achieving a molecular response. These results emphasize the need for frequent and early molecular monitoring of patients treated with imatinib, and the importance of achieving the best response as early as possible.

There is little available data on the durability of molecular responses. In our study, major molecular responses have been sustained in 72% of patients, whereas complete responses have been sustained in nearly half of the patients. In nearly a third of these patients, the cause for loss of response was treatment interruption. Despite the loss of molecular responses, this has not translated into loss of cytogenetic remissions in most patients. An important question then is the significance of increasing levels of BCR-ABL transcripts during therapy. Our results suggest that there is a high risk of loss of cytogenetic remission with greater increases of measurable molecular disease (i.e., >2-log increase or an absolute increase >1), but the risk is particularly high for patients who did not achieve a major molecular remission. However, it is possible that patients that achieved lower transcript levels will only require longer times to show a loss of their cytogenetic response. Continued follow-up will answer this question. Different treatment strategies, such as adding other agents to imatinib, should be investigated in these cases of increasing molecular disease.

In summary, molecular monitoring provides valuable information for the follow-up of patients with CML in chronic phase treated with imatinib. Achieving a major molecular response correlated with an improved probability of a durable cytogenetic remission, particularly when achieved early during the course of therapy. Most major molecular remissions have been durable, and increases in BCR-ABL transcripts have not translated in loss of cytogenetic remission for most patients with molecular response. Thus, a major goal of therapy with imatinib should be to achieve an early major molecular response.

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