BCR-ABL mRNA levels at and after the time of a complete cytogenetic response (CCR) predict the duration of CCR in imatinib mesylate–treated patients with CML

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Although most patients with chronic myeloid leukemia (CML) treated with imatinib mesylate achieve a complete cytogenetic response (CCR), some patients will relapse. To determine the potential of real-time quantitative BCR-ABL reverse transcriptase–polymerase chain reaction (RT-PCR) to predict the duration of continued CCR, we monitored 85 patients treated with imatinib mesylate who achieved a CCR. With a median follow-up of 13 months after CCR (29 months after imatinib mesylate; median 6 RQ-PCR assays), 23 patients (27%) had disease progression (predominantly loss of CCR). Compared with the median baseline level of BCR-ABL mRNA, 42% of patients achieved at least a 2-log molecular response at the time of first reaching CCR. Failure to achieve a 2-log response at the time of CCR was an independent predictive marker of subsequent progression-free survival (hazard ratio = 5.8; 95% CI, 1.7-20; $P = .005$). After CCR, BCR-ABL mRNA levels progressively declined for at least the next 15 months, and 42 patients (49%) ultimately achieved at least a 3-log reduction in BCR-ABL mRNA. Patients failing to achieve this 3-log response, at any time during therapy, had significantly shorter progression-free survival (hazard ratio = 8.1; 95% CI, 3.1-22; $P < .001$). The achievement of either a 2-log molecular response at the time of CCR or a 3-log response anytime thereafter is a significant and independent prognostic marker of subsequent progression-free survival. (Blood. 2006;107:4250-4256)

Introduction

The pathogenetic alteration in chronic myeloid leukemia (CML) is the presence of the BCR-ABL tyrosine kinase, the product of a reciprocal (9;22) chromosomal translocation. Targeted inhibition of this constitutively active kinase with imatinib mesylate is a highly effective, and now standard therapy for patients with CML. Imatinib mesylate induces a complete hematologic response in approximately 97% of patients with chronic-phase CML, and a complete cytogenetic response (CCR) in greater than 40% of patients in late-chronic phase[1] and greater than 70% of patients newly diagnosed in chronic phase.[2] Although responses are durable, relapse after an initial response occurs in a small percentage of patients treated in chronic phase.

In patients treated with imatinib mesylate who were resistant or intolerant of interferon, the percentage of bone marrow cells with a detectable Philadelphia chromosome (Ph) by conventional cytogenetic karyotyping inversely correlates with progression-free survival.[3,4] Similarly, in patients with newly diagnosed CML treated with imatinib mesylate, the achievement of a bone marrow without detectable Ph-positive cells, defined as a complete cytogenetic response (CCR), predicts superior progression-free survival.[5] The attainment of a CCR has thus become a specific therapeutic goal. Because only 20 to 30 metaphase nuclei are typically karyotyped, however, leukemia cell burdens below approximately 3% to 5% (often rapidly attained with imatinib mesylate) are not reliably detected by this method. In comparison, real-time quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) of fusion BCR-ABL mRNA (RQ-PCR), with its enhanced sensitivity, is typically able to detect and quantify residual leukemia cells down to a level of approximately 1 leukemia cell in 10^5 normal cells. The level of BCR-ABL mRNA at 12 months of imatinib mesylate therapy is a significant predictive laboratory marker of subsequent progression-free survival.[5] Although most patients with CML treated with imatinib mesylate achieve a CCR, the prognostic value of RQ-PCR at the time the CCR was achieved (or at specific times thereafter) has not been directly addressed. Despite the increasing use of RQ-PCR to monitor minimal residual disease, a critical unanswered question then remains the ability of BCR-ABL mRNA levels, measured at specific times at or after the achievement of a CCR, to predict subsequent relapse. Toward this goal, we have performed serial RQ-PCR and routine disease monitoring on a cohort of 85 patients with CML in whom imatinib mesylate had induced a CCR.

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Patients, materials, and methods

Patients

The 85 patients enrolled in this study represent all of the patients with CML followed at our institution who have both achieved a CCR on imatinib mesylate therapy and have undergone monitoring with BCR-ABL RQ-PCR with the first sample obtained no later than the time of first achieving CCR. The study protocol was approved by the Institutional Review Board at Oregon Health and Science University (OHSU). Informed consent was provided according to the Declaration of Helsinki. Table 1 describes the key demographic features of this patient cohort, including several established CML prognostic factors. Thirty-nine patients (46%) were newly diagnosed and treated with imatinib mesylate within 6 months of diagnosis. Forty-six patients (54%) were beyond 6 months from their diagnosis at the time of starting imatinib mesylate. Thirty-nine of these patients with "late-stage" CML had been previously treated with interferon-α (IFN-α) and had stopped this therapy because of intolerance (n = 12), cytogenetic failure (n = 17), or hematologic failure (n = 10).[1] Six patients had previous allogeneic hematopoietic stem cell transplantations (4 with previous IFN-α therapy) and 2 had autologous stem cell transplantations (both with previous IFN-α therapy), at a median of 66 months prior to starting imatinib mesylate. The dose of imatinib mesylate was initially 400 mg/day in patients with chronic-phase CML (n = 72) and 600 mg/day in those with accelerated phase (n = 13). The imatinib mesylate dose was adjusted, when necessary, according to response and tolerance. The average daily imatinib mesylate dose was 450 mg for patients in chronic phase and 590 mg in those with accelerated phase (Table 1). For this study, disease progression was defined as the occurrence of any of the following events after achieving a CCR (defined as no detectable Ph-positive metaphases in routine karyotype analysis of in vitro–cultured bone marrow cells [median 20 cells examined; 25th-75th percentile, 20-20]): death, the development of accelerated-phase or blast-phase CML,[1] loss of a complete hematologic response (defined by a peripheral blood white blood cell count above 20×10^9/L [20 000/mm^3], a platelet count of at least 600×10^9/L [600 000/mm^3], at least 5% myelocytes and metamyelocytes, or any detectable blasts or promyelocytes), or loss of a CCR (defined as the appearance in the bone marrow of any cell with a detectable Philadelphia chromosome by routine metaphase karyotyping).

Cytogenetics and BCR-ABL FISH

Karyotyping and fluorescence in situ hybridization (FISH) were performed on bone marrow aspirates at regular intervals, typically every 3 to 4 months. Karyotyping was performed using routine G-banding methods.[6] The t(9;22) was also routinely assessed with interphase BCR-ABL FISH probes (bone marrow samples only; Ventana Medical Systems, Tucson, AZ) using standard methods.[6]

Real-time quantitative RT-PCR (RQ-PCR)

RQ-PCR was performed on either peripheral blood (25% of 506 total samples) or bone marrow aspirate specimens. Sixteen subjects had a paired blood and bone marrow sample drawn on the same day, and the BCR-ABL mRNA levels within these 2 compartments were not significantly different [mean log-drop RQ-PCR = 1.4 in bone marrow (95% CI, 0.62-2.2) and 1.3 in blood (95% CI, 0.51-2.1)] and were highly correlated ($R = 0.98$, regression line slope = 0.99, $P < .001$). After lysis of red blood cells, total leukocyte pellets were lysed (at 10 million cells per mL) (MagnaPure LC mRNA isolation kit lysis buffer; Roche Applied Science, Indianapolis, IN) and stored at −80°C until the subsequent batch processing of mRNA on a MagNA Pure LC automated robotic instrument using the reagents and protocols supplied by the manufacturer (Roche MagnaPure LC mRNA isolation kit I). From the 50 μL total mRNA (from 3 million cells), 10 μL was reverse transcribed into cDNA in a 20-μL total volume using random

Table 1. Demographic characteristics of CML study patients with CCR

Characteristic	Value
Median age, y (range)	51.8 (20-74)
Sex, % male	60
Median time from leukemia diagnosis to start of imatinib mesylate, mo (range)	8.9 (0-165)
"Early" CML diagnosis*, %	46
Baseline CML diagnosis, % chronic phase/% accelerated phase	85/15
New CML (Hasford) risk score† at diagnosis,[20] %	
Low risk	55
Intermediate risk	36
High risk	9
Additional cytogenetic abnormalities at baseline, %	13
Median percentage of Ph-positive metaphases at baseline (25%-75% levels)	100 (95-100)
Average imatinib mesylate dose, mg/d	
Chronic phase	450
Accelerated phase	590
Other concomitant therapies, with imatinib mesylate, %	
None	65
IFN or Ara-C	31
Tipifarnib	1
Donor leukocytes	1
Hsp70 vaccine	1
Median time from start of imatinib mesylate to CCR, mo (range)	9.5 (2.4-57)
Median time from start of imatinib mesylate to major cytogenetic response‡, mo (range)	5.5 (1-57)
Median time of follow-up after imatinib mesylate, mo (range)	29 (8-59)
Median time of follow-up after CCR, mo (range)	13 (0-32)
Median interval between monitoring visits, mo (range)	3.2 (0.1-21)
Median number of laboratory monitoring visits (range)	6 (1-16)

N = 85. No significant difference was observed between patients with durable CCR (n = 62) and patients with disease progression (n = 23) for all listed variables. Percentages given in table are percent of total patients.

IFN indicates interferon-α; Ara-C, cytarabine.

*< 6 months before imatinib mesylate.

†Euro score.

‡Ph < 35%.

hexamer primers and AMV reverse transcriptase as per the manufacturer's protocol (Roche). BCR-ABL and G6PDH transcripts in 5 μL cDNA were quantified using real-time quantitative PCR and fluorescent resonance energy transfer hybridization probes (20 μL reaction volume) in a LightCycler instrument (Roche) as previously described[7] with some minor modifications. The BCR-ABL RQ-PCR reaction used 2 forward bcr primers (exons b2 and e1) and an abl exon a4 reverse primer. The 2 hybridization probes, both within abl exon a3, were labeled, respectively, at the 5′ end with the fluorophore LC-Red 640 or at the 3′ end with fluorescein (IT Biochem, Salt Lake City, UT). All primer and probe sequences have been previously described[7] with the exception of a modified bcr exon b2 forward primer (B2A) that was one base shorter than the previously published sequence (lacking the 3′ terminal T nucleotide). This semi-multiplexed PCR reaction amplified both the p210 (b2a2 and b3a2) and p190 (e1a2) BCR-ABL products. To control for both the integrity of the sample and any intersample variation in the preparation of mRNA, the reference gene *G6PDH* was amplified under identical reaction conditions in a different tube.[7] After the instrument software determined the crossing points of the real-time PCR amplification curves (using the second derivative maximum method), the relative BCR-ABL transcript quantity was calculated using routine real-time PCR relative expression analysis methods[8] and was reported as a ratio of BCR-ABL mRNA to G6PDH mRNA (in percentage). The relative BCR-ABL mRNA level was also expressed as a "log-drop from baseline" value[5] that represented the change in the relative transcript ratio (on a base-10 log scale) from a median baseline value. The laboratory-specific median baseline that was used to calculate the log-drop value (4.0% BCR-ABL/G6PDH ratio) was generated as the median transcript ratio of all the patients with chronic-phase CML followed at our institution (n = 38) with a first available baseline sample containing 100% Ph-positive metaphases. Replicate analyses of the identical low-level (1 in 10 000 diluted) CML control sample on 68 consecutive RQ-PCR batch runs (including repeat mRNA preparations) revealed an interassay coefficient of variation of 5.9% (including the variation from both the BCR-ABL and G6PDH transcripts).

Complete molecular response

If BCR-ABL mRNA was undetectable using 5 μL cDNA, a larger-scale 200-μL preparation of cDNA (from 6 million cells) was volume-reduced approximately 15-fold with an Amicon centrifugal filter (YM-50; Millipore, Billerica, MA), and 5 μL of this concentrated cDNA was used for real-time PCR. If BCR-ABL mRNA was still undetectable, 5 μL cDNA was subjected to a more sensitive BCR-ABL–nested PCR procedure[9] (in triplicate). Samples were classified as "complete molecular response" only if each of 3 replicate nested PCR reactions was BCR-ABL negative and if the mRNA was of adequate quality to ensure that a minimal 4.0 log-drop in BCR-ABL mRNA levels, if present, would have been detectable. Given the 25-fold higher baseline level of G6PDH mRNA relative to BCR-ABL mRNA (equivalent to 4.6 real-time PCR cycles) and the assay's low-level detection limit in fresh, undegraded samples of one CML cell diluted into 100 000 normal cells (maximal crossing point = 40.5), a G6PDH crossing point below 22.5 ensured the detection of a BCR-ABL mRNA level at least 4 logs below the baseline. This 22.5 crossing point threshold represented the 10th percentile (1 tailed) of the entire distribution of G6PDH crossing points obtained from the first 1092 BCR-ABL–positive samples referred to our laboratory since 2002. In comparison, among the smaller group of 85 locally managed patients in this particular study (without prolonged sample transit times), a smaller 5.7% fraction of more than 500 samples was defined as having "poor quality" mRNA (with maximal achievable sensitivity below 4 logs) and was not considered in any subsequent data analysis.

Statistics

Samples with "undetected" BCR-ABL mRNA were assigned a real-time PCR crossing point 1 log lower than the assay's low-level detection limit to generate a numeric log-drop value for quantitative analyses. Continuous "time after CCR" values were rounded to the nearest 3-month integer to allow categorization for comparing BCR-ABL mRNA levels at various time points. Categoric variables were compared with the chi-square test, and continuous variables were compared with either the Wilcoxon rank sum test (for comparing 2 groups) or the Kruskal-Wallis test (for comparing 3 or more groups). A mixed effects model was fit to the RQ-PCR kinetic data to assess the linear trend between log-drop RQ-PCR and time from CCR. The data analyzed included RQ-PCR values between CCR and disease progression (or last available RQ-PCR). The main effects of time and disease progression were considered, as well as the interaction between these variables. The Kaplan-Meier method was used to estimate progression-free survival and time to major and complete molecular responses. Differences in progression-free survival were compared by using the log-rank test. A Cox proportional hazard regression model was used to determine hazard ratios and to identify the variables independently associated with progression-free survival (using a stepwise procedure). Diagnostic sensitivity and specificity for the achievement of a 2-log molecular response at the time of CCR were adjusted for censored observations and calculated as per Heagerty et al.[10] All reported *P* values are 2-sided, and *P* values less than .05 were considered statistically significant.

Results

Patients

The demographic and risk factor characteristics of the 85 subjects are shown in Table 1. The patients have been monitored for disease status for a median of 29 months following the initiation of imatinib mesylate therapy and a median of 13 months after achieving CCR. Monitoring consisted of peripheral blood counts, bone marrow morphology, metaphase karyotyping of bone marrow cells, interphase FISH (bone marrow samples only), and BCR-ABL RQ-PCR (bone marrow and blood samples). The first RQ-PCR sample was drawn 5.6 months (median) after the start of imatinib mesylate (median, 5.7 months before, and never later than, the first CCR), and the subsequent interval between successive molecular monitoring visits (median 6 samples per patient) was 3.2 months (median). During follow-up, 23 patients (27%) experienced disease progression a median of 6 months (range, 3-19 months) after achieving CCR (median, 19 months [range, 5-49 months] after starting imatinib mesylate). Twenty-two of the events defining disease progression were the loss of a CCR, and 1 was a hematologic relapse. The patients with disease progression did not significantly differ from the patients with durable remission with respect to any of the variables listed in Table 1. In particular, once CCR was attained, the 39 patients treated with imatinib mesylate within 6 months of diagnosis (11 of whom experienced disease progression) had no significant difference in progression-free survival than did the 46 patients with late-stage CML (12 of whom experienced disease progression).

RQ-PCR kinetics at and after CCR

At the time of first achieving CCR, 34 patients had undetectable BCR-ABL by interphase FISH, and the remaining 51 patients had low-level FISH positivity (median, 4% of interphase cells). At this time of first CCR, the median BCR-ABL mRNA level had fallen 1.9 logs (25th-75th percentile, 1.4-2.4) below the median baseline value in the 78 patients with evaluable RQ-PCR data at this time point. Following CCR, the levels of BCR-ABL mRNA progressively declined until at least 15 months after CCR, at which time the molecular response reached 3.7 logs (median) below the median baseline (25th-75th percentile, 2.2-4.7) ($P < .001$; linear trend between 0 and 15 months, $P = .008$) (Figure 1). There was no significant change in the level of BCR-ABL mRNA at later time points (linear trend between 15 and 33 months, $P = .35$, data not shown). However, because the sample numbers became increasingly small beyond the median follow-up after CCR of 13 months,

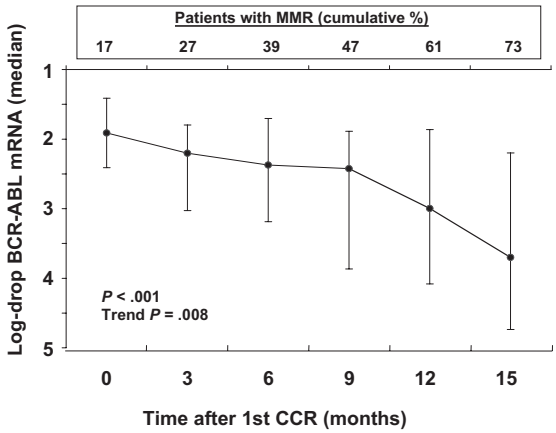

Figure 1. BCR-ABL mRNA levels progressively decline after CCR. Plotted are the median BCR-ABL mRNA levels (log-drop from a median baseline) at specific time points after first achieving CCR. The brackets show the interquartile range (25th-75th percentile levels). There was a significant time-dependent difference in BCR-ABL mRNA levels ($P < .001$) and a significant trend toward better responses with increasing times (trend $P = .008$). The cumulative percentage of patients achieving a major molecular response (MMR; 3 log-drop or greater) is also shown (Kaplan-Meier estimate). Samples drawn at or after the time of disease progression were excluded.

we cannot confidently determine whether BCR-ABL mRNA will continue to decline or plateau with additional follow-up. At each specific time point after CCR, there was no significant difference in the BCR-ABL mRNA levels between samples drawn from blood versus bone marrow. The cumulative frequency of patients with at least a 3-log reduction in BCR-ABL mRNA levels progressively increased with longer times after CCR, from 17% at the time of achieving a CCR, to 27% at 3 months after CCR, and to 73% at 15 months after CCR (Figure 1). An analogous RQ-PCR kinetic analysis restricted to the 62 patients with sustained CCR revealed a similar result, namely, a progressively increasing molecular response from the time of CCR (median log-drop = 2.1; 25th-75th percentile, 1.6-2.7) to 15 months later (median log-drop = 3.8; 25th-75th percentile, 2.2-4.7) ($P = .002$; linear trend $P < .001$). In contrast, in the 23 patients with disease progression, there was no significant linear trend toward progressively increasing molecular responses with increased times after CCR (linear trend $P = .2$). The progressive time-dependent decline in leukemic disease burden thus applies only to patients with sustained CCR.

Prognostic value of the BCR-ABL mRNA level at the time of first CCR

We next evaluated the effect of BCR-ABL mRNA levels on the time to disease progression. At the time of first achieving CCR, the median BCR-ABL mRNA level was significantly lower in the sustained responders (median log-drop = 2.1; 25th-75th percentile, 1.6-2.7) than in the patients with subsequent disease progression (median log-drop = 1.5; 25th-75th percentile, 1.2-1.9; $P = .003$). The distribution of RQ-PCR values at the time of CCR extended over a more than 5-log range (0.7-5.9), suggesting that the level of molecular response could stratify relapse risk in patients within the same cytogenetic response category. This heterogeneity of molecular responses was best demonstrated by the observation that, at the time of CCR, 3 patients (3.8% of those with available data) had minimal molecular responses below 1 log, whereas 13 patients (17%) had already achieved at least a 3-log reduction, and 2 patients (2.6%) had undetectable BCR-ABL transcripts. Because the overall median BCR-ABL log-drop at the time of first CCR was 1.9, we chose 2.0 as a convenient threshold value to stratify the risk of subsequent relapse. Of the 78 patients with available RQ-PCR data at this time point, 33 (42%) achieved at least a 2-log reduction in BCR-ABL mRNA levels, and their subsequent progression-free survival was significantly longer than that of the 45 patients not achieving this level of response ($P > .001$) (Figure 2). In these 33 patients with at least a 2-log molecular response at the time of CCR, the median progression-free survival was not reached. The hazard ratio for disease progression was 5.9 (95% CI, 1.7-20; $P = .005$) in the 45 CCR patients failing to achieve (versus those achieving) a 2-log molecular response at the time of CCR, and the median progression-free survival was 14 months. The overall diagnostic sensitivity (for predicting disease progression) of not achieving a 2-log molecular response at the time of CCR was 80%, and the diagnostic specificity was 59%. To further determine whether the prognostic capability of this molecular marker (a minimal 2-log molecular response at the time of CCR) was dependent on other established risk-related variables, we performed a multivariate analysis in which each of the possible risk-stratifying variables in Table 1 was included in the model. The multivariate analysis confirmed that failure to achieve a 2-log molecular response at the time of CCR was the only independent prognostic marker of subsequent disease progression (hazard ratio = 5.8; 95% CI, 1.7-20; $P = .005$).

Prognostic value of major molecular response

Forty-two patients (49%) ultimately achieved at least a 3 log-drop in BCR-ABL mRNA levels at any time during therapy. This level of response is referred to as a major molecular response (MMR). In these 42 patients, the median time to reach this response level was 14 months after starting imatinib mesylate therapy and 4.5 months after CCR. Of the 43 patients who never achieved a 3-log molecular response, 15 patients (18%) achieved a best molecular response of greater than 2 logs below the median baseline, and 28 patients (33%) never reached a 2-log drop. This best achievable molecular response level was a significant prognostic marker of subsequent disease progression ($P < .001$; Figure 3). In particular, compared with patients achieving a 3-log molecular response, the risk of disease progression progressively increased for patients with a greater than 2- but less than 3-log molecular response (hazard ratio = 3.8; 95% CI, 0.92-16; $P = .049$) and those with a best molecular response below 2 logs (hazard ratio = 10; 95% CI, 3.8-28; $P < .001$). If all of the patients with a best molecular response below 3 logs were grouped together and compared with the patients that did achieve this 3-log level of response, the

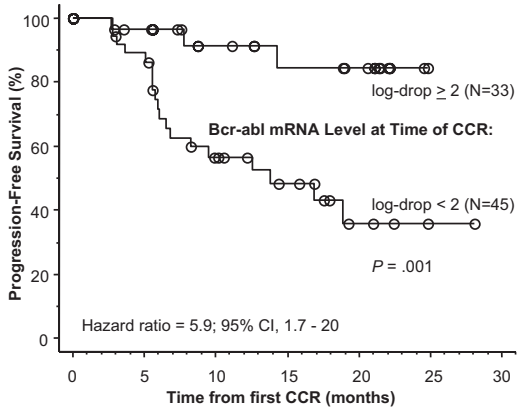

Figure 2. A 2-log drop in BCR-ABL mRNA at the time of CCR predicts better progression-free survival. The cumulative rate of progression-free survival (Kaplan-Meier method) is shown for those subjects achieving, versus not achieving, a minimal 2.0 log-drop in BCR-ABL mRNA at the time of first CCR.

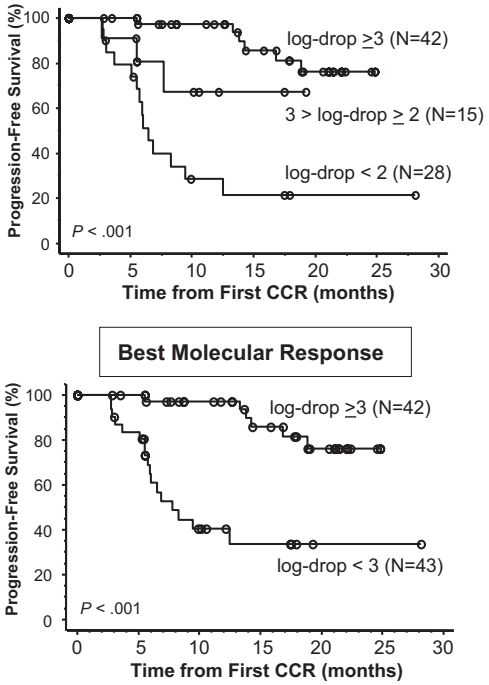

Figure 3. A best molecular response of at least a 3-log reduction in BCR-ABL mRNA levels predicts better progression-free survival. Kaplan-Meier survival curves are shown for patients achieving a best RQ-PCR level (at any time) above various thresholds. The top panel compares 3 patient groups: those with a best molecular response of at least 3 (log-drop units), at least 2 (but below 3), and below 2. The bottom panel compares patients with a best molecular response of at least 3 versus those below 3. These are landmark analyses starting at the time of CCR.

patients failing to achieve a 3-log reduction had a significantly shorter progression-free survival ($P < .001$) and an accompanying hazard ratio for disease progression of 7.3 (95% CI, 2.8-19) (Figure 3). In multivariate analysis in which each of the variables in Table 1 was included in the regression model, the failure to achieve a 3-log molecular response (at any time) was the only independent predictor of progression-free survival (hazard ratio = 8.1; 95% CI, 3.1-22; $P < .001$).

Complete molecular response

Of the 506 samples with acceptable mRNA quality, 50 (9.9%) had undetectable BCR-ABL mRNA by real-time quantitative RT-PCR (from 20 different patients). However, as the evolving consensus for defining a "complete molecular response" requires an undetectable BCR-ABL mRNA determination using the more sensitive nested PCR method,[5] only 32 of these samples (6.2%), from 13 patients, tested negative for BCR-ABL mRNA by a subsequent nested PCR reaction (in triplicate). In the 13 patients with a complete molecular response, the median time to first achieving this response was 18 months after starting imatinib mesylate and 12 months after CCR. No patient achieved a complete molecular response before completing 12 months of imatinib mesylate therapy. Thereafter, the cumulative rate of PCR negativity progressively increased, with no evidence of a time-dependent plateau, to 9% of patients at 18 months, 16% at 24 months, and 20% (maximum) after more than 32 months of imatinib mesylate therapy (Figure 4). In comparison, the cumulative frequency of patients with a major molecular response (of 3 logs or greater) also progressively increased with longer treatment times to reach a maximum of 87% after more than 57 months of imatinib mesylate therapy (Figure 4). Two of the 13 patients with undetectable BCR-ABL transcripts had subsequent disease progression (with loss of a complete hematologic response at 13 months after CCR and loss of a CCR at 17 months after CCR). The small number of patients with a complete molecular response makes statistical comparisons of progression-free survival to other groups unreliable.

Forty patients (47%) had molecular disease monitoring performed, at least once, on a peripheral blood sample. Using a Cox proportional hazard regression model, we found that these 40 patients with partial peripheral blood monitoring had no significant difference in the time to achieve either a major molecular response or a complete molecular response as compared with the 45 patients with every RQ-PCR sample drawn from bone marrow.

Discussion

Imatinib mesylate therapy now allows most patients with CML to achieve a CCR, a confirmed good prognostic indicator. Further stratification of the risk of relapse at and after achieving CCR would then be attainable only with the enhanced sensitivity of RQ-PCR. The clinical need for this additional risk stratification is evident from the significant fraction of patients who lose a CCR during imatinib mesylate therapy. For example, in studies of patients with late chronic-phase disease, 20% to 30% of these patients have lost a CCR after 2 to 4 years of imatinib mesylate therapy.[3,11-13] For patients with newly diagnosed chronic-phase CML treated with imatinib mesylate, this rate is lower.[2,14] In our study, which included a slight majority of patients with late chronic-phase disease, 27% of patients lost a CCR after 2.4 years (median) of imatinib mesylate therapy. Accordingly, it would be useful to have additional means to stratify patients for their risk of relapse at or after obtaining a CCR during imatinib mesylate therapy. Toward that goal, we have shown that BCR-ABL mRNA levels are a significant and independent prognostic marker of progression-free survival when measured either at the time of first CCR or at any time that the molecular response exceeds a 3-log reduction from baseline.

At the time when CCR was first recognized, there was a wide range of BCR-ABL mRNA levels of greater than 3 to 5 logs in this study and others.[5,12] Because the patients were not continually monitored, however, but rather sampled with a median interval of 3.2 months, the precise "first CCR" event could have occurred at any time after the collection of the immediately preceding sample (that, by definition, was Ph positive). The observed heterogeneity of responses at the time when CCR was first recognized (and at later times) could then be the result of

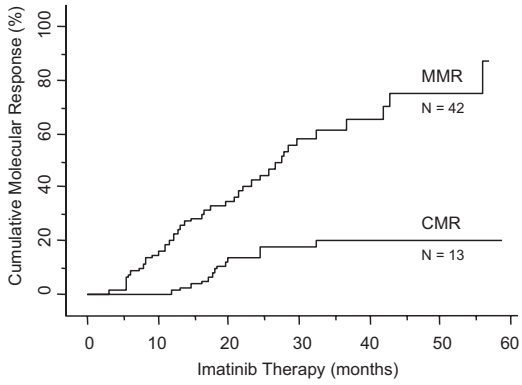

Figure 4. Molecular response level versus imatinib mesylate treatment time. Shown is the cumulative percentage of patients achieving either a major molecular response (MMR; BCR-ABL mRNA log-drop ≥ 3) or a complete molecular response (CMR; undetected BCR-ABL mRNA).

potential bias in this "lead time," defined as the time interval between CCR recognition and the immediately preceding sample. To exclude this possibility, however, we have found that the lead time variable was not significantly related to either disease progression, achievement of MMR, or the magnitude of the molecular response at the time of CCR recognition (data not shown). Instead, this response heterogeneity suggested that the depth of the molecular response, at the time of CCR, might predict the durability of CCR. To confirm this hypothesis, we have shown that patients who fail to achieve at least a 2-log reduction in BCR-ABL transcript levels at the time that a CCR is first recognized have a significantly shorter time to disease progression. In addition, our kinetic analysis after CCR showed that, in patients with sustained CCR (but not those with disease progression), the molecular response continued to progressively improve until reaching a maximal 3.7 log-drop at 15 months after CCR. The depth and kinetics of this molecular response after CCR were comparable to that observed in the large IRIS (International Randomized Study of Interferon versus STI571) study (restricted to patients newly diagnosed)[5] despite the use of different real-time PCR detection methods and different normalization reference genes. Similar BCR-ABL kinetic findings, specifically a progressive decline in BCR-ABL mRNA levels with continued follow-up, have also recently been reported by Marin et al,[13] with a cytogenetic relapse rate (25%) comparable to that in our study (27%). We have also used the criteria of Marin et al[13] to categorize the patients with sustained CCR into either of 2 subgroups according to whether their last evaluable BCR-ABL mRNA level had either "declined" (n = 20) or "plateaued" (n = 21) as compared with the average patient-specific BCR-ABL mRNA level after 18 months of imatinib mesylate therapy. Although 21 patients did not have sufficient follow-up to allow subclassification by these criteria (and 23 patients had disease progression), the percentage of our classifiable patients without a relapse and with a plateau in BCR-ABL mRNA levels (51%) is similar to that reported by Marin et al[13] (57%).

Consistent with these observations of a progressive decline in BCR-ABL mRNA levels after CCR, we found that, at 3 months after CCR, the achievement of a threshold molecular response slightly above 2.0 logs (2.3 logs) was also significantly predictive of longer progression-free survival (data not shown). This suggests that, after achieving at least a 2-log decrease in BCR-ABL mRNA levels at the time of CCR, BCR-ABL mRNA levels need to continue to further decline to maintain optimal disease control. Thus, the failure to achieve either a 2-log decrease in BCR-ABL mRNA levels at the time of CCR or a subsequent continued decrease in transcript levels might suggest the need for more frequent molecular monitoring and/or alterations of therapy.

Other investigators have previously shown that BCR-ABL mRNA levels have prognostic significance when measured early after starting imatinib mesylate therapy, before a CCR is attained.[15-18] In contrast, we focused on the prognostic significance of BCR-ABL mRNA levels at time points at and after a CCR was achieved and demonstrated that, at these later time points, RQ-PCR remained a significant predictive marker of disease progression. In the only other report of the prognostic relevance of BCR-ABL mRNA levels at the time of CCR, Paschka et al,[12] in a study of 41 patients with CML, found a trend for greater molecular responses at the time of first CCR in patients with continuous remission versus those who subsequently lost their response, although this trend did not reach statistical significance.

Previous studies in patients with CCR have shown that a 3-log reduction of BCR-ABL mRNA, a major molecular response, predicts for prolonged progression-free survival, but only when measured at a specific 12-month time point after imatinib mesylate.[5,14] At other times at and after CCR, the prognostic significance of a 3-log reduction in BCR-ABL mRNA levels has not previously been evaluated. In this study, we demonstrate that the achievement of a 3-log reduction in BCR-ABL transcript levels, attained at any time during therapy, was a significant and independent predictor of progression-free survival. By extending the time range for achieving a 3-log transcript decrease, we now show that it is the molecular response itself, and not the time to achieve that response, that carries the prognostic relevance.

Of note, BCR-ABL mRNA levels, measured at and after CCR, were significantly predictive of disease progression independent of the traditional prognostic markers that have long been clinically useful in the era before imatinib mesylate. Although our study size was perhaps limiting (n = 85), we found that factors such as age, disease phase, disease duration, baseline cytogenetics, pretreatment clinical and hematologic parameters, and time to achieve a major or complete cytogenetic response were not significant predictors of relapse, at least after the achievement of CCR. In support of this conclusion, each of these same variables has also been shown to lack independent prognostic significance in another recent study of patients with chronic-phase CML achieving an imatinib mesylate–induced CCR.[14] Thus, once a CCR is achieved, the major prognostic factor for patients with CML treated with imatinib mesylate is the depth of the antileukemic response, as measured by cytogenetic or molecular methods.[3-5,14-19] However, as cytogenetic methods lack adequate sensitivity for minimal residual disease detection after CCR (by definition), the only remaining prognostic marker with practical utility for risk stratification after CCR is RQ-PCR. As most patients with CML treated with imatinib mesylate reach CCR (after a median of 9.5 months in our cohort), the ability of RQ-PCR to stratify relapse risk at several time points at and after CCR has significant practical clinical relevance. For imatinib mesylate–treated patients with CML, the routine clinical use of serial molecular monitoring may then allow improved risk stratification and improved disease management, by identifying those patients with the highest risk of relapse.

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