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Quantitative reverse transcription polymerase chain reaction should not replace conventional cytogenetics for monitoring patients with chronic myeloid leukemia during early phase of imatinib therapy

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A B S T R A C T

Background and Objectives. Imatinib is the new standard drug treatment for patients with chronic myelogenous leukemia (CML). Quantitative reverse transcription-polymerase chain reaction (qPCR) for detection of BCR-ABL transcripts is frequently used for monitoring patients in addition to or instead of conventional cytogenetics, although its place in the overall diagnostic framework is not yet clear. In this study, we compared qPCR and conventional cytogenetics for monitoring patients during the early phases of imatinib therapy.

Design and Methods. One hundred and seventeen patients treated with imatinib for CML in chronic or accelerated phase were prospectively followed with qPCR and karyotyping. Comparisons were made between both methods and between qPCR results from bone marrow and peripheral blood. To determine the prognostic impact of qPCR and cytogenetics during the early phase of imatinib treatment on subsequent cytogenetic response and progression-free survival (PFS), a multivariate model was generated that included established prognostic baseline variables.

Results. We found a significant correlation between the proportion of Philadelphia (Ph) chromosome-positive metaphases and qPCR in the bone marrow and peripheral blood. Low qPCR values after 3 months of therapy were correlated with major cytogenetic response (MCyR) at 6 months and PFS at 2 years. However, in multivariate analysis, the cytogenetic response at 3 months emerged as the only independent parameter predictive of MCyR at 6 months and PFS at 2 years.

Interpretation and Conclusions. Our data suggest that conventional karyotyping should remain the standard method for following patients on imatinib during the early phases of therapy.

Key words: CML, BCR-ABL, imatinib, quantitative PCR.

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Imatinib has become the standard drug treatment for patients with chronic myeloid leukemia (CML).[1-3] As in the case of interferon-α, patients are routinely monitored with conventional cytogenetics. However, quantitative reverse transcription polymerase chain reaction (qPCR) analysis for the detection of *BCR-ABL* transcripts is widely used to follow patients in addition to or instead of cytogenetics. Several studies have shown a good correlation between the proportion of Philadelphia (Ph) chromosome–positive bone marrow metaphases and qPCR of peripheral blood and bone marrow in patients on imatinib.[4-7] It was also demonstrated that the level of *BCR-ABL* transcripts in the peripheral blood after 4-12 weeks of therapy with imatinib is correlated with cytogenetic response at 6 months[5,7] and progression-free survival (PFS),[5] suggesting that following patients with qPCR during the early phase of therapy may provide independent prognostic information. Since all studies published thus far did not include other parameters with known prognostic significance, including the cytogenetic response at 3 months,[1,3] it is currently not known how much additional information can be derived from qPCR monitoring. We therefore used qPCR to monitor response to imatinib in a cohort of patients with CML in first chronic phase or accelerated phase. We then analyzed the results for their impact upon subsequent major cytogenetic response (MCyR) at 6 months and PFS after a median follow-up of 2 years in a multivariate model that included baseline parameters and the cytogenetic response at 3 months.

Design and Methods

Patients

The patients were treated with imatinib in successive Novartis-sponsored multi-institutional studies (protocols 0109, 0110, 106, 0113, and 0114). Approval for these studies was obtained from the institutional review board of the University of Leipzig, Germany. Informed consent was obtained according to the Declaration of Helsinki. A total of 117 patients were included (96 in first chronic phase and 21 in accelerated phase). Fifty-eight patients were female and 59 were male, with a median age of 51 (range, 23-71) years.

Definition of disease phase, response and disease progression

Accelerated phase and blast crisis were defined according to published criteria.[3,8] Disease progression was defined as (i) loss of complete hematologic response or (ii) progression to a more advanced disease phase. Loss of MCyR or a rise in the proportion of Ph-positive metaphases was not regarded as disease progression, unless accompanied by a loss of complete hematologic response.

Cytogenetic remission was categorized as follows: complete cytogenetic response (CCyR), Ph-positive metaphases 0%; partial cytogenetic response (PCyR), Ph-positive metaphases 1–34%; major cytogenetic response (MCyR) included CCyR and PCyR.

Monitoring of patients on treatment

Full blood counts were done at intervals of 4 weeks or less. Cytogenetics and qPCR were performed prior to initiating treatment and then at 3-6 month intervals, unless the clinical situation necessitated more frequent testing. All studies were done as per protocol or as part of routine follow-up of CML patients at the Department of Hematology, University of Leipzig, Germany.

Quantitative RT-PCR

Total white cells from 20 mL of heparinized peripheral blood or 5 mL of bone marrow were isolated by red-cell lysis followed by 2 washes in phosphate buffered saline. Samples were processed within 24 hours, usually within 8 hours. Between 0.5 and 2.2×10^7 cells were lyzed in guanidine isocyanate solution[9] and stored at -2° C until used. RNA was extracted with the RNAeasy kit (Qiagen, Hilden, Germany) and 0.5 mg were reversely transcribed into cDNA with random hexamer primers, as described elsewhere.[10]

The probe and primers for amplification of BCR-ABL were designed using the Primer Express software program (Perkin Elmer, Foster City, CA, USA). Primers were 5´CATCCGTGGAGCTGCAGAT-3` (BCR exon 13, forward primer) and 5`AGTCAGATGCTACTGGCCGC-3` (ABL exon 2, reverse primer). The probe was 5`-CCAACTCGTGTGT-GAAACTCCAGACTGTCC-3` (BCR exon13). 5'-labeling was with 6-carboxyfluorescein (FAM, reporter dye) and 3'-labeling with 6-carboxytetramethylrhodamine (TAMRA, quencher dye). Primers and probe were purchased from Roboscreen, GmbH, Leipzig, Germany. The reactions were set up with premixed real-time PCR reagents, as described previously.[11] The threshold cycle (C_T) was determined in the patient's sample and compared with the CT of the standard curve. In order to generate the respective standard reference curves for each run, the amounts of *BCR-ABL* were calculated from a 8-well ready-to-use reference DNA strip containing 8 defined amounts of *BCR-ABL* as a quantification control in a range of 5 to 10^5 molecules/run. This standard was found to be storage-stable, with an inter-serial variation regarding the quality assurance parameters slope of the reference curve of less than 6%. All samples were analyzed in duplicate or triplicate and subsequent calculations were done using means. Values were normalized for expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the RoboGene® *GAPDH* cDNA Quantification Module (Roboscreen®, GmbH, Leipzig, Germany). Samples with GAPDH levels < 0.01 amol/μL (equivalent to 6022 molecules/μL) were excluded from the analysis, as previously described.[12] Samples with undetectable expression of *BCR-ABL* were assigned a value of 0.00001%. The lower limit of sensitivity of the qPCR assay is 0.00002%.

Karyotyping

The percentage of Philadelphia chromosome-positive metaphases was determined by conventional R-banding or by fluorescence *in situ* hybridization (FISH) of at least 25 bone marrow metaphases. FISH was done with the LSI bcr/abl ES probe (Vysis, Stuttgart, Germany), according to the instructions of the manufacturer. Six patients, who failed to grow sufficient numbers of metaphases, were followed with FISH on bone marrow interphases.

Statistical analysis

Baseline variables at the start of imatinib therapy, cytogenetics after 3 months and BCR-ABL transcripts in peripheral blood and bone marrow prior to imatinib therapy and after 3 months were studied in univariate analysis for their impact on cytogenetic response at 6 months. Comparisons were performed with the χ^2 test in the case of categorical variables, and with the Mann-Whitney test or the Kruskal-Wallis test in the case of continuous variables. Probabilities of PFS at 2 years were compared with the log rank test for categorical variables and with the Cox regression model (Wald χ) for continuous variables. Factors significant at a level

Table 1. Results of karyotyping and qPCR from BM or PB at baseline and 3 and 6 months after the start of imatinib.

	Baseline	*3 month*	*6 month*
Available results	117	83	96
Ph+ metaphases (%), median	100	44	22
range	16-100	0-100	0-100
Available results	84	76	89
Q-PCR (BM, BCR-ABL/GAPDH in %), median	0.70	0.23	0.12
range	0.033-19.7	0.000017-3.4	0.000017-13.8
Available results	61	55	45
Q-PCR (PB, BCR-ABL/GAPDH in %), median	0.86	0.42	0.10
range	0.027-11.6	0.000017-17.7	0.000017-4.0

Ph+: Philadelphia chromosome positive; q-PCR: quantitative PCR for BCR-ABL; BM0: bone marrow; PB: peripheral blood.

of $p < 0.1$ in univariate analysis were included in the multivariate model (MCyR at 6 months: logistic regression model, Wald χ; PFS: Cox regression model, Wald χ). Factors in the multivariate model were sequentially removed in order of least significance until the final model included only factors with $p < 0.05$. All p values reported are two-sided. Spearman's rank test was used to assess correlations between qPCR values in the peripheral blood and bone marrow and between qPCR and cytogenetics. All calculations were done with the SPSS software package.

Results

Correlation between qPCR and cytogenetics

Median values of cytogenetics and qPCR and the availability of results at baseline, 3 and 6 months are shown in Table 1. Overall, the median number of cytogenetic results per patient was 3 (range 1-3), the median number of qPCR results from the bone marrow was 2 (range 0-3) and the median number of qPCR results from the peripheral blood was 1 (range, 0-3).

Contemporaneous results of cytogenetics and qPCR were available for 249 bone marrow and 150 peripheral blood samples. There was a significant correlation between the degree of Ph-positivity in the bone marrow and *BCR-ABL* transcripts in the bone marrow ($r = 0.731$, $p < 0.001$) and peripheral blood ($r = 0.684$, $p < 0.001$, Spearman's rank test). *BCR-ABL* transcript levels were significantly different between patients with CCyR, PCyR and without MCyR (Figure 1). There was no significant difference between the *BCR-ABL* levels in the bone marrow and peripheral blood in any of the three cytogenetic response categories. Analysis of *BCR-ABL* transcripts in contemporaneously obtained bone marrow and peripheral blood samples (n = 120) showed a good cor-

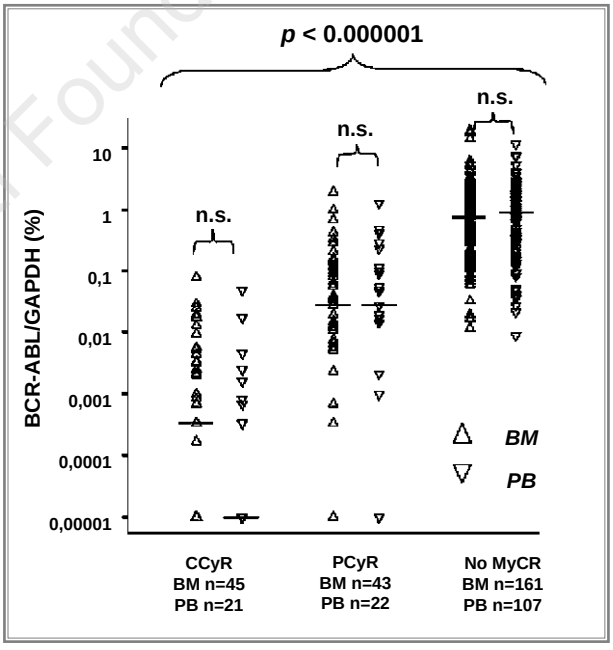

Figure 1. *BCR-ABL/GAPDH* ratio in samples from bone marrow (BM) and peripheral blood (PB), according to cytogenetic response (CCyR: complete cytogenetic response; PCyR: partial cytogenetic response; no McyR: no major cytogenetic response). Horizontal bars represent the position of the median values. Negative results are given as a ratio of 0.00001% (the limit of sensitivity of the assay is 0.00002). There were significant differences according to the cytogenetic response groups ($p < 0.000001$).

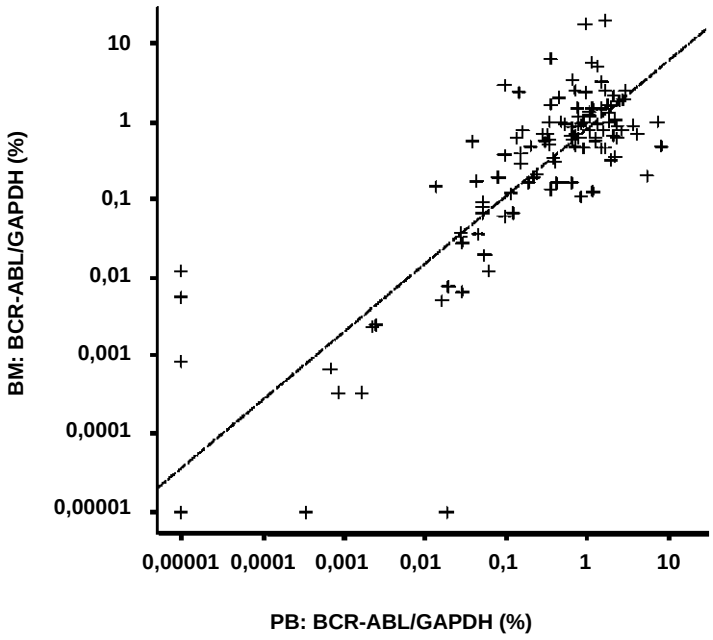

Figure 2. Correlation between *BCR-ABL/GAPDH* ratios in peripheral blood (PB) and bone marrow (BM). Contemporaneously obtained samples were available in 120 cases. Negative results are given as a ratio of 0.00001. There was a significant correlation between peripheral blood and bone marrow (R = 0.719, $p < 0.001$, Spearman's rank test).

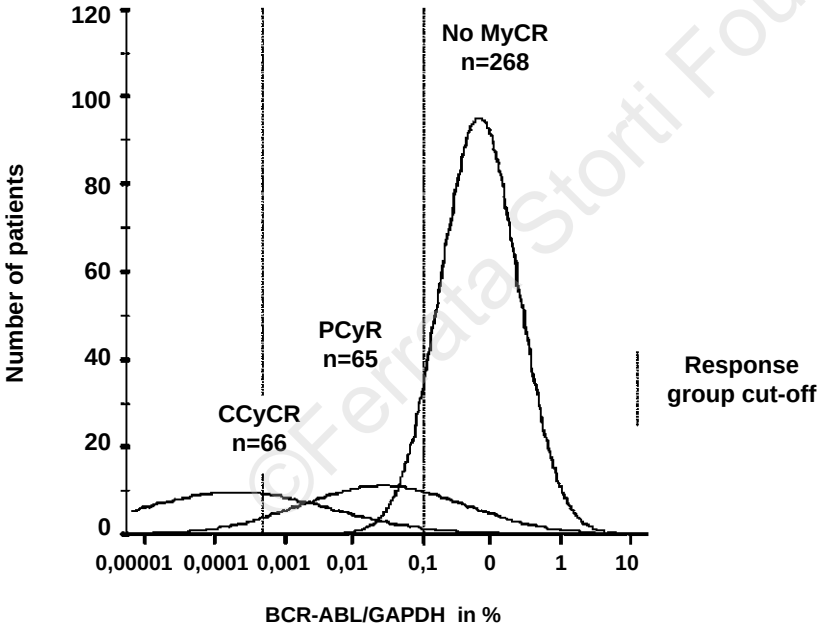

Figure 3. Overlap between molecular response categories. Logarithmic expression of the *BCR-ABL/GAPDH* ratios led to near normal distributions. The cut-offs between the response groups (—-) were chosen such that an identical number of values was *wrongly* assigned to the lower or higher response group. This model represents an optimal fit of the data but still leads to 17% misclassifications.

relation (r = 0.719, $p < 0.001$, Spearman's rank test) (Figure 2). Next, the qPCR values were grouped into 3 molecular response categories. Cut-offs between adjacent categories were chosen such that an equal number of results were *wrongly* assigned to the higher or lower category. With this approach, the cut-off between CCyR and PCyR was set at 0.006 and that between PCyR and no MCyR at 0.1% *BCR-ABL/GAPDH* (Figure 3). We found that 83% of all results were concordant; in 17%, there was a minor discordance, i.e. the response categories assigned by the two tests were adjacent. No major discordances were observed (Table 2).

Correlation between qPCR and subsequent cytogenetic response

The expression of BCR-ABL is a marker of leukemic cell burden and its course over time reflects the efficacy of a given therapy. We therefore hypothesized that qPCR results prior to therapy might be predictive of subsequent cytogenetic response at 6 months. qPCR

Table 2. Correlation between molecular and cytogenetic response group (n=399).

	Cytogenetic response group N (%)		
Q-PCR (BCR-ABL/GAPDH in %)	no MCyR (N = 268)	PCyR (N = 65)	CCyR (N = 66)
> 0.1	245 (61.4)	**22 (5.5)**	0
0.006-0.1	**23 (5.8)**	31 (7.8)	**11 (2.7)**
<0.006	0	**12 (3.0)**	55 (13.8)

no MCyR: no major cytogenetic remission; PCyR: partial cytogenetic remission; CCyR: complete cytogenetic remission. Bold face: discordant results.

Table 3. Correlation between qPCR and major cytogenetic response (McyR) after 6 months.

Factor/category	*N. of patients*	*N. (%) of MCyR*	*N. (%) no MCyR*	*p*
BCR-ABL/GAPDH (%) in BM, initial				
As a continuous variable	80			0.051*
< 7.0×10^{-1} (median)	40	26 (65)	14 (35)	0.025**
≥ 7.0×10^{-1}	40	16 (40)	24 (60)	
BCR-ABL/GAPDH (%) in BM after 3 months				
As a continuous variable	72			0.002*
< 2.3×10^{-1} (median)	37	29 (78)	8 (22)	0.000006**
≥ 2.3×10^{-1}	35	11 (31)	24 (69)	
Relative compared to baseline				
< 0.5	32	26 (81)	6 (19)	< 0.000001**
≥ 0.5	26	4 (15)	22 (85)	
BCR-ABL/GAPDH (%) in PB after 3 months				
As a continuous variable	54			0.001*

**Mann-Whitney-test, **χ^2-test.*

results from bone marrow prior to imatinib were available for 80 patients (Table 3). When analyzed as a continuous variable, the qPCR values prior to imatinib therapy did not correlate with MCyR at 6 months, although there was a trend towards higher rates of MCyR in patients with low levels of *BCR-ABL* transcripts (p = 0.051, Mann-Whitney test). This correlation was somewhat improved when the initial qPCR values were stratified according to the median and analyzed as a categorical variable. Using this approach, 26/40 patients (65%) with *BCR-ABL* expression below the median compared to 16/40 (40%) with expression above the median achieved MCyR at 6 months (p = 0.025, χ^2). The correlation between initial *BCR-ABL* levels in peripheral blood and MCyR was not investigated, since qPCR results were only available for 61 patients (Table 1). Next, we analyzed whether the qPCR results at 3 months (n = 72) might predict subsequent MCyR (Table 3). We found a significant correlation between lower BCR-ABL transcripts in bone marrow (p = 0.002) and peripheral blood (p = 0.001) at 3 months and achievement of MCyR at 6 months. When the qPCR values in the bone marrow were stratified according to the median, 29/37 (78%) patients with values below the median achieved MCyR compared to 11/35 (31%) of those with values above the median (p = 0.000006). Thus, using this criterion, prediction of MCyR at 6 months was accurate in 74% of patients. Stratification according to relative reduction of BCR-ABL expression (> and < 50% of initial values) led to similar results: of 32

Table 4. Correlation between qPCR and progression-free survival (PFS) at 2 years.

Factor/category	*N. of patients*	*N. (%) loss of CHR*	*N. (%) continuing CHR*	*2-year PFS (± SD)*	*p*
Transcripts (BCR-ABL/GAPDH %) in BM, initial					
As a continuous variable	84				0.782*
$< 7.0\times10^{-1}$ (median)	42	4 (10)	38 (90)	0.90 (±0.05)	0.063**
$\geq 7.0\times10^{-1}$	42	10 (24)	32 (76)	0.76 (±0.07)	
Transcripts (BCR-ABL/GAPDH %) in BM after 3 months					
As a continuous variable	76				< 0.0005*
$< 2.3\times10^{-1}$ (median)	38	2 (5)	36 (95)	0.94 (±0.04)	0.007**
$\geq 2.3\times10^{-1}$	38	11 (29)	27 (71)	0.72 (±0.07)	
Relative compared to baseline					
< 0.5	32	3 (9)	29 (91)	0.90 (±0.05)	0.078**
≥ 0.5	28	7 (25)	21 (75)	0.78 (±0.08)	
Transcripts (BCR-ABL/GAPDH %) in PB after 3 months					
As a continuous variable	55				< 0.0005*

**Cox regression model, **log-rank-test; BM: bone marrow.*

patients whose levels of BCR-ABL at 3 months were < 50% of initial levels, 26 (81%) achieved MCyR at 6 months, compared to 4/26 with levels >50% ($p < 0.000001$). The overall accuracy using this criterion for prediction of MCyR at 6 months was thus 83%. However, since this calculation was only possible for 58 patients (50%), the result must be interpreted with caution.

Correlation between qPCR and progression-free survival

Since cytogenetic response is only a surrogate marker of therapeutic efficacy, we analyzed the correlation between qPCR and PFS. At a median follow-up of 24 months, 19 patients (16%) had progressed to accelerated phase or blast crisis or had lost complete hematologic response. Ten of these patients had been in chronic phase and 9 in accelerated phase at the initiation of imatinib therapy. *BCR-ABL* transcripts in the bone marrow prior to the therapy and PFS were not correlated if analyzed as a continuous ($p = 0.782$, log rank) or categorical variable ($p = 0.063$, log rank) (Table 4).

Similarly, the relative reduction of *BCR-ABL* mRNA at three months compared to initial levels was not predictive of PFS at 2 years ($p = 0.078$, log rank). By contrast, low levels of *BCR-ABL* transcripts at 3 months were significantly correlated with progression-free survival in logistic regression analysis ($p < 0.0005$ for bone marrow and peripheral blood, Table 4).

Cytogenetics is the dominant prognostic factor in multivariate analysis

Having established that low levels of BCR-ABL transcripts at 3 months are predictive of MCyR at 6 months and progression-free survival at 2 years, we asked whether these correlations would hold up in a multivariate model that incorporated other known prognostic baseline factors.[1] We also included the cytogenetic response at 3 months as a variable, since this has been correlated with PFS in several studies.[1,3]

In addition to initial and 3-month BCR-ABL transcript levels, factors positively associated ($p < 0.1$) with MCyR at 6 months were less advanced disease, response to interferon, shorter time from diagnosis, absence of splenomegaly, ECOG performance status < 1, hemoglobin > 100 g/L and the percentage of Ph-positive metaphases at 3 months (Table 5). In multivariate analysis, this last proved to be the only variable that predicted response at 6 months. This result was independent of how the cytogenetic response was considered (i.e. as a continuous or categorical variable).

A similar analysis was performed for PFS (Table 5). In addition to lower qPCR values, less advanced disease, previous response to interferon, absence of splenomegaly, ECOG performance status < 1, peripheral blood basophils < 2%, bone marrow blasts < 5% and the percentage of Ph-positive metaphases at 3 months were all associated with longer PFS. However, as with MCyR, only the cytogenetic response at 3 months retained significance in multivariate analysis. In fact, no patient

Table 5. Univariate and multivariate analysis of prognostic factors associated with major cytogenetic response at 6 months and progression-free survival at 2 years.

Factor/category	*Major cytogenetic response at 6 months*		*Progression-free survival*	
	Univariate analysis (p)	*Multivariate analysis (p, Wald χ) logistic regression model*	*Univariate analysis (p)*	*Multivariate analysis (p, Wald χ) Cox regression model*
BCR-ABL/GAPDH (%) in BM initial as a continuous variable				
<7.0×10^{-1} (median)	0.051*	n.s.	0.782^{+}	–
≥ 7.0×10^{-1}	0.025**	n.s.	0.063^{++}	n.s.
BCR-ABL/GAPDH (%) in BM after 3 months as a continuous variable	0.002*	n.s.	< 0.0005^{+}	n.s.
< 7.0×10^{-1} (median)	0.000006**	n.s.	0.007^{++}	n.s.
≥ 7.0×10^{-1}				
< 0.5 (relative)	< 0.000001**	n.s.	0.078^{++}	n.s.
≥ 0.5				
BCR-ABL/GAPDH (%) in PB after 3 months as acontinuous variable	0.001*	n.s.	< 0.0005^{++}	n.s.
% Ph^{+} after 3 month as a continuous variable	< 0.000003*	< 0.000003	0.005^{+}	0.005
CCyR	< 0.000001°	0.002	0.008^{++}	
PCyR				
No MCyR				
MCyR	< 10^{-8}**	0.004	< 0.002^{++}	
No MCyR				
Phase of CML				
CP, newly diagnosed	0.011**	n.s.	0.082^{++}	n.s.
CP, hematologically resistant to IFN				
CP, cytogenetically resistant to IFN				
CP, intolerant to IFN				
AP				
Other chromosomal abnormalities (yes/no)	0.214**	–	0.538^{++}	–
Time from diagnosis to imatinib start as a continuous variable	0.004*	n.s.	0.121^{+}	–
B symptoms (yes/no)	0.947**	–	0.124^{++}	–
Splenomegaly (yes/no)	0.003**	n.s.	0.015^{++}	n.s.
ECOG performance status (<1 or ≥1)	0.085**	n.s.	0.037^{++}	n.s.
Hemoglobin g/dL (< 10 or ≥ 10)	0.077**	n.s.	0.230^{++}	–
WBC count ×10^{9}/L (< 10×10^{9}, 10-50×10^{9}, >50×10^{9})	0.254**	–	0.909^{++}	–
Platelet count ×10^{9}/L (< 450, 450-700, > 700)	0.384**	–	0.195^{++}	–
Basophils in PB (%) (≤ 2, ≥ 2)	0.310**	–	0.082^{++}	
Blasts in PB (%) (< 2, ≥ 2)	0.314**	–	0.184^{++}	–
Blasts in BM (%) (< 5, ≥ 5)	0.214**	–	0.054^{++}	–

**Mann-Whitney test; **χ^2-test; °Kruskal-Wallis-test; $^{+}$Cox regression model; $^{++}$log-rank-test; AP: accelerated phase; CP: chronic phase; IFN: interferon α; n.s.: not significant.*

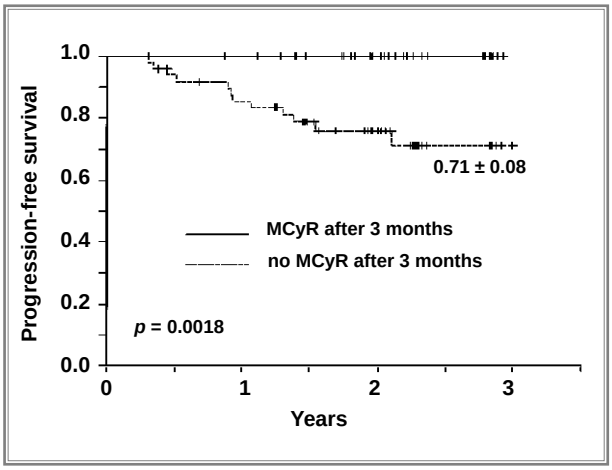

Figure 4. Progression-free survival at a median follow-up of 2 years according to major cytogenetic response at 3 months.

with MCyR at 3 months had progressed at 2 years (Figure 4).

Discussion

Quantitative RT-PCR to detect *BCR-ABL* transcripts is a widely used method to follow CML patients after allogeneic stem cell transplantation. High qPCR levels less than 6 months after transplant are associated with a high risk of relapse.[13] Similarly, rising qPCR values predict subsequent cytogenetic and hematologic relapse and are thus considered an indication for donor lymphocyte infusion.[14] In patients in CCyR to interferon-α, PFS is correlated the level of residual disease.[15] In current clinical practice, qPCR of peripheral blood is frequently used to follow patients on imatinib, even outside of clinical trials, although only limited information is available on how it fits into the overall diagnostic framework. Several studies have demonstrated that the level of expression of *BCR-ABL* transcripts in the peripheral blood and bone marrow is correlated with the percentage of Ph-positive metaphases in the bone marrow, suggesting that qPCR may be a substitute for cytogenetics.[4–7,16] Our data confirm these results in principle. However, in spite of a generally acceptable linear correlation, there is an overlap between the qPCR values of the cytogenetic response groups. We, therefore, decided to define the cut-off between CCyR and MCyR, and between MCyR and no MCyR such that an equal number of patients would be wrongly assigned to the next higher or lower category, respectively. Using this approach, the rate of minor discordance was 17%, and there were no major discordances. In contrast, using BCR-ABL/ABL ratios of 2 and 10% as cut-offs, Kantarjian *et al.* found major discordances in 10% and minor discordances in 24% of their patients.[6] The higher rate of discordances is likely to reflect the fact that our model represents the optimal fit of the data, while the cut-off values used by Kantarjian *et al.* probably represent data obtained from patients on interferon therapy. It is clear, however, that a certain rate of discordant results is inevitable. Several groups reported that the early response to imatinib, as assessed by qPCR, is predictive of subsequent cytogenetic response. Merx *et al.* found a correlation between the level of *BCR-ABL* transcripts in the peripheral blood after 8 and 12 weeks of imatinib with cytogenetic response at 6 months.[7] Wang *et al.* reported that a reduction of *BCR-ABL* transcripts in the peripheral blood to < 50% of initial values after 4 weeks on imatinib was predictive of MCyR at 6 months; conversely, a reduction to < 10% of initial values at 3 months predicted MCyR at 6 months.[5] They found similar correlations with PFS. Our results are in agreement with these data: low levels of *BCR-ABL* transcripts in bone marrow and peripheral blood at 3 months were predictive of MCyR at 6 months and PFS at 2 years. We also found a trend towards better cytogenetic responses at 6 months in patients with lower levels of *BCR-ABL* prior to therapy. This trend became of borderline significance if patients were stratified according to expression above and below the median. Since the level of *BCR-ABL* expression appears to increase with disease progression,[17,18] higher initial levels may indicate more advanced disease, regardless of other criteria that define disease stage.

Several studies have analyzed the impact of baseline disease characteristics on the rate of MCyR. In the largest series of patients with chronic phase CML after failure of interferon-α, a favorable previous response to interferon, a short time from diagnosis, normal hemoglobin, the absence of blasts in the peripheral blood and no excess of blasts in the bone marrow were all independently predictive of MCyR.[1] In landmark analysis, the achievement of MCyR at 3 and 6 months was shown to predict PFS in accelerated phase and chronic phase patients after failure of interferon-α.[1,19] We, therefore, determined the predictive value of baseline characteristics as well as cytogenetic response at 3 months and qPCR in multivariate analysis. In this model, the proportion of Ph-positive metaphases at 3 months emerged as the only independent factor that predicted MCyR at 6 months and PFS at 2 years. Introduction of cytogenetic response and/or qPCR as categorical rather than continuous variables had no impact on the results of the multivariate model. Similarly, when the qPCR values were introduced as the ratio between the values prior to imatinib and at 3 months, cytogenetic response was still the predominant factor. These findings suggest that the proportion of Ph-positive

metaphases at 3 months may reflect the biology of the disease and its responsiveness to imatinib more accurately than the level of *BCR-ABL* transcripts. As discussed above, the qPCR values of the cytogenetic response groups show an overlap, despite a good correlation in general. This is a consistent finding in several studies on this subject.[6,7] One possible explanation is that qPCR measures the average level of *BCR-ABL* mRNA in all cells, including those that are terminally differentiated and not capable of causing relapse. In contrast, cytogenetics measures the Philadelphia chromosome status of cells as well as their capacity for cell division, and thus may more reliably reflect their proliferative potential. Whatever the precise mechanism, our results suggest that cytogenetics should remain the diagnostic standard for assessment of response to imatinib, at least in the early stages of treatment. By analogy to patients on interferon-α and after allografting,[13,20] qPCR is crucial for monitoring patients with CCyR.[21] In addition, clonal cytogenetic abnormalities have been observed in the Ph-negative cells of some patients treated with imatinib, sometimes with progression to a myelodysplastic syndrome.[22] If the follow-up of patients relied entirely on qPCR, such abnormalities would be missed. This is an additional indication that karyotyping should remain an essential part of monitoring response in patients on imatinib.

Contributions: TL and TB were the principal investigators: they designed the study and analyzed the data. SO was responsible for data base management. H-K A-A was responsible for the clinical care of most of the patients. IK, DK and TK analyzed the samples. RK did the statistical analysis and generated the figures. DN contributed to patients' care and organized the institutional requirements. MWND supervised the establishment of the PCR assay, wrote the manuscript and gave final approval for submission. He is taking primary responsibility for the paper. The order of authorship reflects the contribution given to the study. Responsibility for figures and tables: tables 1, 2, 3, 4: TL; table 5: TB; all figures: RK. The authors reported no conflict of interest. The authors are grateful to Scarlet Musiol, Gerlinde Patzer, Sabine Leiblein, Christel Müller, Evelin Hennig and Christina Franke for expert technical support and Ute Hegenbart and Leanthe Grommisch for dedicated care of patients.

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