Molecular remission in chronic myeloid leukemia patients with sustained complete cytogenetic remission after imatinib mesylate treatment

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Background and Objectives. Imatinib mesylate induces a complete cytogenetic response (CCR) in many patients with chronic myeloid leukemia (CML). However, the ultimate goal of therapy for CML is complete elimination of Philadelphia chromosome positive cells or *BCR-ABL* rearrangements. We studied molecular responses in CML patients in CCR after imatinib treatment.

Design and Methods. Real-time quantitative reverse transcriptase polymerase chain reaction analysis were used to monitor *BCR-ABL* levels in 59 CCR patients. Negative results were confirmed by two different techniques performed in two different laboratories. Patients were considered in complete molecular remission if they had four undetectable analyses from two separate samples taken three months apart.

Results. The median follow-up was 41 months (17-53). The median BCR-ABL/ABL ratio at the time of CCR was 0.3 % (0-9.88). Patients were split into two groups: group A (n=43) comprised patients with a detectable BCR-ABL/ABL ratio throughout the follow-up and group B (n=16) included those with an undetectable level of BCR-ABL/ABL (< 10^{-5}) i.e. in complete molecular remission. No relapses were observed in group B, while 13 group A patients lost their CCR. The probability of losing CCR in this group was 33.2 % >±18.0. By Cox regression analysis the best factor for predicting the probability of achieving molecular remission was having a CCR at 6 months (p=0.038) or at 3 months (p=0.024).

Interpretation and Conclusions. Molecular remission after imatinib treatment, i.e. BCR-ABL/ABL< 10-5 in peripheral blood, is not a rare event, particularly in patients achieving CCR at 6 months.

Key words: chronic myeloid leukemia, imatinib mesylate, RQ-PCR, BCR-ABL

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by acquisition of the Philadelphia chromosome (Ph) in leukemic stem cells and their progeny.[1,2] The abnormal Ph chromosome is the result of a reciprocal translocation between chromosomes 9 and 22. The major consequence of this translocation is the fusion of the *ABL* gene to the *BCR* gene on chromosome 22.[3] The *BCR-ABL* fusion gene encodes a new protein of 190, 210 or 230 kd, depending on the breakpoint on the *BCR* gene.[4,5] All these Bcr-Abl fusion proteins have enhanced tyrosine kinase activity, which is crucial for the development of the disease.[6]

Imatinib mesylate is highly effective in the treatment and management of Ph-positive CML.[7,8] This drug is thought to bind competitively to the adenosine triphosphate (ATP)-docking site of tyrosine kinase proteins, including ABL itself and the hybrid BCR/ABL proteins.[9] A significant proportion of patients achieve complete cytogenetic response (CCR) with this therapy.[8] However, the ultimate goal of therapy for CML is the complete eradication of cells harboring the Ph chromosome or its molecular counterpart, the *BCR-ABL* rearrangement. Patients achieving CCR may still harbor up to 10^9 leukemic cells in their body.[10] Because the probability of disease acceleration is proportional to the number of Ph-positive cells, some of these patients may become resistant to imatinib.

Molecular biology techniques allow the quantitative assessment of leukemia-specific BCR-ABL transcripts and have already been widely used to monitor response in CML and to evaluate residual disease both after allogeneic hematopoietic stem cell transplantation and after interferon α and imatinib treatment. Real-time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) shows a persistent BCR-ABL transcript level in the peripheral blood of most patients who achieve CCR.[10] In the current study, we investigated the molecular response of patients who obtained a CCR after imatinib mesylate treatment in our department. Fifty-nine patients were evaluated and separated into two groups according to whether they had achieved molecular remission. Patients were considered in molecular remission when the level of BCR-ABL transcripts was

below our threshold of detection, i.e. when the value of BCR-ABL/ABL was $<10^{-5}$ in at least four different analyses from two separate samples taken 3 months apart, and when the result was confirmed by another laboratory using a different technology.

Compared to the response of interferon-α treatment and the achievement of CCR and because imatinib mesylate induces CCR in most patients, molecular remission is now considered the best surrogate marker to evaluate the efficiency of this treatment in CML.[11] Therefore we analyzed different clinical and biological factors for predicting the probability of achieving molecular remission.

Design and Methods

Patients

Between January 2000 and June 2004, patients were treated in our center with 400-600mg imatinib according to the phase II trials or expanded access protocols (109, 110, 113, 114) and the phase III trial (106) supported by Novartis Pharmaceuticals. In our center, 59 patients (36 males and 23 females) achieved a CCR with a follow-up of more than one year. This study was focused on these complete cytogenetic responders. At the beginning of the treatment, all of these 59 patients were Ph positive and expressed either the b2a2 or the b3a2 BCR-ABL transcript, except one who expressed the e1a2 BCR-ABL transcript. At the start of imatinib therapy, 46 patients were in first chronic phase and 13 patients were in accelerated phase. Fifty of the 59 patients had failed to achieve a cytogenetic response to interferon-α or were intolerant, the others received imatinib as a front-line treatment. The median age at the start of therapy was 57 years (range 21 to 81). Response to therapy was determined by conventional cytogenetical analysis of bone marrow metaphases, molecular cytogenetics and real-time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) for BCR-ABL transcripts. CCR was defined according to standard criteria, i.e. 0% Ph^+ metaphases among at least 25 cells in metaphase in a bone marrow aspirate. Cytogenetic relapse after CCR was defined as the appearance of at least one Ph^+ metaphase on cytogenetic analysis or the loss of hematologic remission with leukocytosis > 10×10^9/L and/or immature precursors in peripheral blood.

Molecular studies

EDTA-anticoagulated peripheral blood was collected every three months during CCR. Total RNA was extracted from 10^6-10^7 peripheral blood cells of patients using the acid guanidinium thiocyanate and phenol-chloroform method and a commercially available extraction kit (Trizol, GibcoBRL, Life Technologies). After each extraction the quality of RNA was checked by gel or by station Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA). cDNA synthesis was performed according to the manufacturer's instructions using random hexamer priming and AMV reverse transcriptase (First Strand cDNA Synthesis Kit for RT-PCR, Roche Diagnostic, Mannheim, Germany). cDNA was amplified using a LightCycler (Roche Diagnostics, Mannheim, Germany); the primers and hybridization probes used in the study for BCR-ABL amplifications were those described by Emig *et al.*[12]

Standardization

Copy numbers of BCR-ABL and ABL (as an internal control) were calculated by using serial dilutions of standards, ranging from 10^5 to 10^1 copies of plasmid containing a BCR-ABL b3a2 insert (pGD 210). These were used to generate a standard curve after amplification in the Light Cycler. The BCR-ABL/ABL ratio was calculated as No. BCR-ABL/No. ABL where No. BCR-ABL and No. ABL were absolute copy numbers of BCR-ABL and ABL transcripts, respectively. The sensitivity of this technique is 10^{-5}. Normalizing the results to the ABL value compensated for variations in the quality of the RNA and for differences in the efficiency of the reverse transcription reaction.

Control

All samples (RNA or cDNA) that gave negative results were sent to another laboratory where they were checked by Taqman technology (Applied Biosystem, Foster City, USA). RQ-PCR was performed from 100 ng of cDNA as previously described,[13] using primer and probe sequences according to the Europe Against Cancer's protocol[14] for one-step amplification of the BCR-ABL target b2a2 and b3a2 fusion transcripts. RQ-PCR amplification of a standard reference gene was performed on TBP cDNA using primers TBPs: 5' CAC GAA CCA CGG CAC TGA TT 3', TBPas: 5' TTT TCT TGC TGC CAG TCT GGA C 3' and TBPp: 5' TGT GCA CAG GAG CCA AGA GTG AAG A 3' for forward, reverse and probe sequences, respectively. Under these experimental conditions, one CML cell could be detected among 10^6 normal cells. The BCR-ABL fusion transcript level was normalized by TBP transcript rate, then compared to the expression level of K562, a BCR-ABL positive cell line. For negative results nested PCR was also performed using two steps of amplification as already described.[15] Two negative controls (H_2O and bcr-abl-negative cDNA) and one positive control (M5: 10^{-5} dilution of cDNA of K562 cells in BCR-ABL negative cells) were used. The results were revealed on a 1.5% agarose gel with ethidium bromide.

Statistical analysis

Baseline and pre-treatment characteristics of the two groups were compared using descriptive statistics (n, proportion, median, range) and the Mann-Whitney test for quantitative variables or the χ^2 test or Fisher's exact test for qualitative variables. Global survival and achievement of molecular remission were estimated by the Kaplan-Meier method. The probability of remaining in CCR was calculated using the log-rank method. The duration of CCR was defined as the time interval between the day of achieving CCR and the day of the appearance of at least one Ph metaphase on cytogenetic analysis or the loss of hematologic remission with leukocytosis >10×10^9/L and/or immature precursors in peripheral blood, or the

Table 1. Clinical characteristics of the 59 patients at diagnosis. Group A corresponds to patients with a BCR-ABL/ABL > 10^{-5} throughout the follow-up of the study, and group B corresponds to those with a BCR-ABL/ABL < 10^{-5}.

	Group A (n=43)			*Group B (n=16)*			
	Median	*Number of patients*	*Range*	*Median*	*Number of patients*	*Range*	*p value*
Age (y)	53		19-81	54		31-77	0.93
Sex (M/F)		27/16			9/7		0.76
Enlarged spleen		23			6		0.37
White blood cell count (x10^9/L)	130.0		9.3-421.3	48.8		15.7-450	0.07
Platelet counts (x10^9/L)	421		43-1230	497		280-1020	0.11
Peripheral blood blast cells (%)	1		0-16	1		0-9	0.63
Peripheral basophils (%)	4		0-26	4		0-20	0.88
Hemoglobin level (g/L)	121		69-155	122		80-158	0.74
Chronic phase/Accelerated phase		39/4			14/2		0.66
		Sokal classification, Number of patients (%)					
Low risk <0.8		14 (32%)			5 (31%)		0.11
Intermediate risk 0.8<risk<1.2		13 (30%)			9 (56%)		
High risk >1.2		16 (37%)			2 (12%)		

day of last contact for those without cytogenetic relapse and who were censored on that day. Univariate analysis was used to analyze and define variables influencing the achievement of molecular remission. The significant variables were entered into a Cox regression model and a forward stepping procedure was used to obtain the best model for finding the independent prognostic factors for molecular remission.

Results

Definition of the two groups of CCR patients

All the patients in this study were in CCR and the median follow-up was 41 months (17-53) after achievement of first CCR. Two groups of patients were defined retrospectively: group A or *PCR <10^{-5}* was composed of 43 patients (73%) with a BCR-ABL/ABL >10^{-5} throughout the follow-up period; group B or *PCR <10^{-5}* included 16 patients (27%) with a BCR-ABL/ABL<10^{-5} i.e. undetectable using our technique. This undetectable BCR-ABL load was confirmed on at least four different analyses from two samples taken 3 months apart.

There was no significant difference in follow-up between the two groups, with a median follow-up of 40 months (17-53) for group A and 43 months (23-53) for group B (p=0.52). Moreover, there were no clinical differences at diagnosis between the groups, with a sex ratio of 16F/27M versus 7F/9M (p=0.76) and a median age at diagnosis of 53 years (21-81) versus 54 years (31-77) (p=0.93). Four patients were in accelerated phase at diagnosis in group A (9%) versus two in group B (p=0.66). There was no difference between the two groups for Sokal score, with 14 low risk patients in group A versus 5 in group B, 13 intermediate risk patients in group A versus 9 in group B, and 16 high risk in group A versus 2 in group B (p=0.108). The main clinical and biological characteristics of the groups at diagnosis are shown in Table 1.

Effect of imatinib doses on the response

Among the patients belonging to group A, three started treatment with 600 mg imatinib for accelerated phase. For 11 patients an increasing dose from 400 to 600 mg was proposed during the course of the treatment: three before the achievement of the CCR, five after CCR had been achieved but then lost; there were three responses and two relapses during the follow-up. For two patients the levels of BCR-ABL were very stable and an increase of the dose was followed by a decrease of BCR-ABL load. In one case, the dose was increased because the patient lost his molecular response. In most cases the increased dose improved the response. In group B, with undetectable levels of transcripts, no increase of imatinib dose was proposed during the follow-up, however two patients started treatment with 600 mg because of accelerated phase disease and continued with 600 mg of imatinib throughout the study.

Sensitivity, accuracy and controls of RQ-PCR for negative results

When negative results were obtained, the samples (RNA or cDNA) were sent to a hematology laboratory (Calmette, Lille, France). There they were checked by Taqman technology which, as explained above, has a sensitivity of 10^{-6}.[13] The level of ABL was high enough i.e. the copy number of ABL was at least 5×10^4. The mean value of ABL copies in group B was 87352 with a mean cycle threshold (C_T) of 25.16. In group A the mean value of ABL copies was 69784 and the mean value of C_T was 25.35.

To compare the two techniques, dilutions of pGD210 plasmids were analyzed in both laboratories. The different values of C_T are shown in Table 2. A shift of two cycles between the two techniques was observed in each dilution, corresponding to a difference in sensitivity i.e. roughly a difference of less than 1 decimal logarithm (log). This was confirmed using samples from

Table 2. C_T values for the plasmid pGD210 (dilutions 100, 10, 1) with the two techniques.

	Hybridization probes (Bordeaux) C_T	*Taqman technology (Lille)* C_T
ABL (copies)		
100	33.5	32.42
10	37	36.36
1	40.5	ND
BCR-ABL (copies)		
100	33.3	31.39
10	36.5	34.67
1	39.8	37.9

The C_T values are lower after Taqman technology (Lille) than Lightcycler (Bordeaux) for BCR-ABL amplification.

patients. Indeed, for all 16 group B samples, the residual disease was also undetectable with the other technique (Taqman technology). When samples were negative with Light Cycler technology for the first time, they were positive between 10^{-5} and 10^{-6} with Taqman technology. However, the subsequent follow-up samples, i.e. 3 months later in most cases, were confirmed to be negative. This difference was also due to the decrease in the BCR-ABL/ABL ratio during the 3 months the patients were receiving therapy. For this reason, we considered this category of patients as having achieved a *complete molecular remission*. In addition after confirmation by Taqman technology the PCR-negative results were also checked by nested PCR as described in the Methods section and confirmed for all the last points of the follow-up patients i.e, for group B. The same number of analyses per patient was performed in the two groups of patients.

Kinetics of BCR-ABL/ABL ratio after CCR

The evolution of the BCR-ABL/ABL ratio differed among patients, as shown in Figure 1. The median ratio of BCR-ABL/ABL in peripheral blood for the 59 patients was 0.37% (range 0-9.88) at the first CCR, with a value of 0.57% (0.07-9.88) in group A and 0.16% (0-2.74) in group B (p=0.024). The median value 3 months later was 0.22% (0-5.2) in group A and 0.01% (0-0.58) in group B (p=0.027). This detectable BCR-ABL/ABL ratio decreased dramatically during the first 6 months to reach a plateau at 9 months with a median value of 0.14% (0-9.32) in group A, while BCR-ABL transcripts became undetectable in group B. The median value remained around 0.1-0.2% in group A throughout the follow-up, as shown in Figure 1. Among CCR patients, the probability of achieving a molecular remission, i.e. belonging to group B, was 27.95%±11.94% (Figure 2). The median follow-up after achievement of the complete molecular remission is 29 months (6 to 37).

Evolution of disease

Among the 43 group A patients, 13 (30%) developed a cytogenetic relapse, i.e. loss of CCR, including four who had disease progression. Mutations in the kinase domain of BCR-ABL, which is one of the most frequent mechanisms of imatinib resistance, were found in two

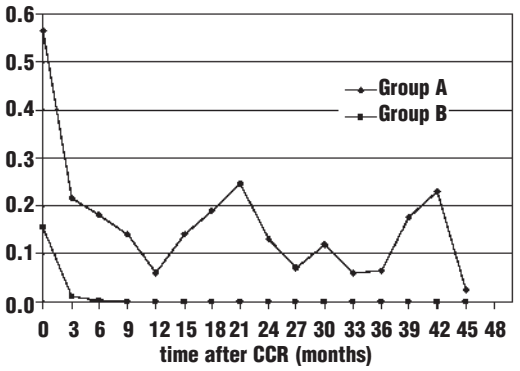

Figure 1. Kinetics of residual disease in patients with CCR under imatinib treatment. This graph shows median values of BCR-ABL/ABL (%) for the patients during follow-up after CCR, for groups A (PCR >10^{-5}) and B (PCR <10^{-5}). Each patient was studied every 3 months after CCR. In group A the median BCR-ABL/ABL ratio decreased during the first 9 months and remained around 0.1-0.2%. In group B the median value was undetectable from 9 months after CCR. The number of patients was 59 from the start of the study to 1 year after CCR, and then decreased: 39 at 24 months and 10 at 36 months.

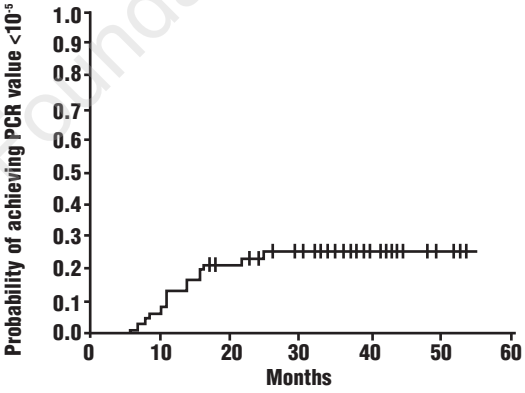

Figure 2. Probability of achieving a molecular remission (PCR value <10^{-5}) for the 59 patients. The PCR value is the BCR-ABL/ABL ratio obtained by RQ-PCR. The probability of obtaining a molecular remission is 27.95%±11.94%.

of those four patients (*data not shown*). One was in accelerated phase and the other in chronic phase. Of the other two, one is still in chronic phase in major cytogenetic remission, i.e. with more than 66% Ph-negative metaphases and the other has a minor cytogenetic response. No mutations were found in the other nine cases. One of the 13 patients stopped treatment because of hepatotoxicity and then lost CCR.[16] The remaining eight patients (18% of group A) went through a period of fluctuation but then were able to increase their imatinib dose and regained CCR. The probability of losing CCR in group A was 33.2±18.0% (Figure 3). Unsurprisingly, no cytogenetic relapse was observed in group B with a median follow-up of 43 months (23-53). Among the 16 patients in this group, only one exhibited a molecular relapse with a BCR-ABL/ABL ratio of 0.023% at 30 months. Therefore, belonging to group B was correlated with a good prognosis and the probability of losing CCR was significant-

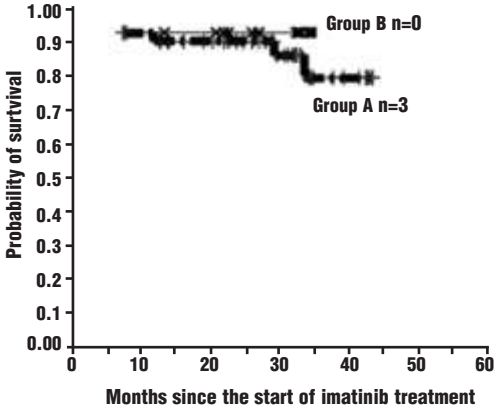

Figure 4. Probability of survival according to molecular response. Total survival in this study is 90.39±11.38%.

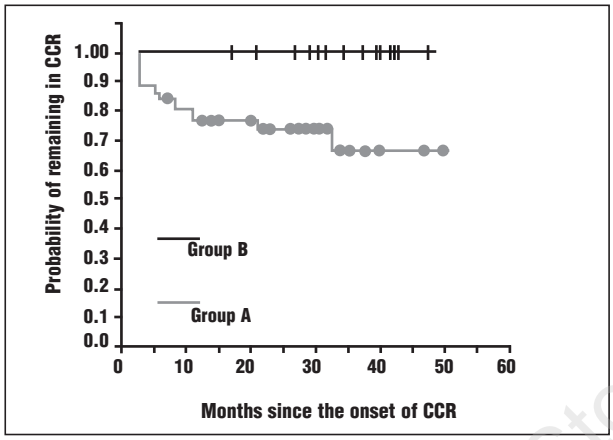

Figure 3. Probability of remaining in CCR according to molecular response. No cytogenetic relapses were observed in group B (16 patients), while 13 patients in group A relapsed (p=0.017).

ly different between the groups (p=0.0172) (Figure 3).

The total probability of survival in the current study was 90.39±11.38 (Figure 4). During the follow-up, three patients in group A died: one developed resistance to imatinib with mutations in the BCR-ABL kinase domain, another developed hepatotoxicity and stopped treatment and the third committed suicide.

Predictive factors in molecular responders

As mentioned above, there was no clinical difference at diagnosis between the two groups. Various clinical and biological characteristics were studied at the beginning of treatment (age, sex, splenomegaly, white blood cell count, platelet count, peripheral blood blast cells, peripheral basophils, hemoglobin level, phase of the disease) and during it (complete cytogenetic response at 6 months) to predict for a molecular response. The potential risk factors studied are shown in Table 3. Pre-treatment variables and cytogenetic response were evaluated by univariate analysis using the Cox regression model to identify factors predictive of achieving a molecular remission before and under treatment. In this univariate analysis, three variables were found to be significant at the p<0.2 level: CCR at 6 months (p=0.038, HR=8.59), white cell count (p=0.100, HR=0.97), and %Ph$^+$ cells in metaphase (p=0.183, HR=0.99). The BCR-ABL/ABL ratio at the time of the CCR was also significantly different with a median value of 0.57% (0.07-9.88) in group A and 0.16% (0-2.74) in group B, but this was not included in the regression model to avoid a statistical bias. There was also a difference between the two groups regarding the time to achieve CCR after starting imatinib: the 43 patients in group A achieved CCR after imatinib treatment in a median time of 5.56 months (2.76-34.95) versus 2.79 months (2.76-13.74) in group B (p=0.0151).

Early CCR achievement is a good predictor of molecular response

From these three covariables retained in a multivariate analysis, the stepwise Cox regression selected CCR

Table 3. Characteristics of the 59 patients at the start of imatinib therapy.

	Group A (n=43)			Group B (n=16)			
	Median	Number of patients	Range	Median	Number of patients	Range	p value
Age (y)	56		21-81	61		32-78	0.45
Sex (M/F)		27/16			9/7		0.76
Enlarged spleen		11			3		0.74
White blood cell count (×10^9/L)	17.20		3.72-230	6.7		3.3-59.5	0.010
Platelet counts (×10^9/L)	267		51-1495	293		130-926	0.43
Peripheral blood blast cells (%)	0		0-6	0		0-3	0.91
Peripheral basophils (%)	3		0-28	2.7		0-25	0.92
Hemoglobin level (g/L)	119		64-149	122		90-167	0.80
Chronic phase/Accelerated phase		32/11			14/2		0.48
Previous interferon therapy/*de novo*		35/8			15/1		0.42
% Ph$^+$	100		0-100	90		3-100	0.055
% BCR-ABL/ABL CCR	0.57		0.07-9.88	0.16		0-2.74	0.024
CCR at 6 months/not CCR at 6 months		26/17			15/1		0.044

Group A corresponds to patients with a BCR-ABL/ABL > 10^{-5} throughout the follow-up of the study, and group B corresponds to those with a BCR-ABL/ABL < 10^{-5}. At the start of imatinib therapy, the white blood cell count, the percentage of Ph+ chromosomes and the BCR-ABL/ABL ratio were significantly lower in group B than in group A.

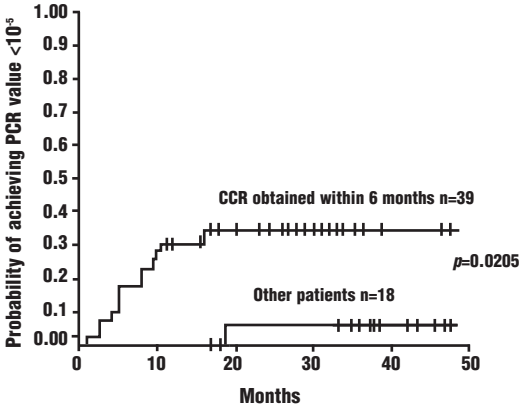

Figure 5. Probability of achieving molecular remission after imatinib therapy for the patients who achieved CCR 6 months after starting the treatment (n=43) and for the others (n=14). The probability of being a molecular responder is 34.4% ± 15.6 % for the patients who obtained CCR 6 months after the start of imatinib versus 6.2% ± 12.1% for those who did not (p=0.02). Two patients are missing from this figure because they achieved molecular remission before 6 months of treatment.

at 6 months as the best independent predictive factor for molecular response (p=0.038, HR=8.59, 95% CI [1.13-65.20]). Among the patients who obtained CCR at 6 months after starting treatment, the probability of being a molecular responder, i.e. belonging to group B, was 34.4±15.6% versus 6.2±12.1% for patients who did not achieve CCR at 6 months (p=0.02) (Figure 5).

From the clinical point of view, we chose CCR at 6 months as a risk factor. However, when CCR was tested as a categorical qualitative variable (CCR ≤3 months, 3 months <CCR ≥6 months, CCR >6 months) using Cox regression, CCR at 3 months was also significant (p=0.024, HR=11.083, [1.379-89.079]). When CCR was tested as a time-dependent variable using Cox regression by univariate analysis, the p value was 0.096 but the HR was 6.058. Therefore, early CCR is a prognostic factor for achieving molecular remission.

Discussion

BCR-ABL load in CML patients with sustained complete cytogenetic remission after imatinib treatment is very variable. However, we decided to constitute two groups of patients: those with an undetectable or very low BCR-ABL/ABL ratio and those with a sustained positive ratio. In group A, with a positive BCR-ABL ratio, 30% of the patients developed a cytogenetic relapse, while none of the group B patients did, with a median follow-up of more than three years. Therefore, an undetectable BCR-ABL/ABL ratio would seem to be a good prognostic factor for a sustained CCR. All the negative results obtained during the evaluation of minimal residual disease were checked.

This is the first report of a study comparing two quantitative RT-PCR techniques: LightCycler technology using hybridization probes with fluorescence resonance energy transfer and TaqMan technology using double-labeled probes for hydrolysis. The latter was more sensitive with a difference of less than one log (roughly 10^{-5} versus 1^{-6}) but this was probably due to the difference in fragment length (size of PCR product) after BCR-ABL amplification, as shown by the difference in C_T values for BCR-ABL amplification (Table 2). Both techniques are very reliable and have already been used to evaluate minimal residual disease in CML patients after imatinib treatment.[17,18]

Fewer than 5% of patients in the IRIS study reported by Hughes had undetectable levels of BCR-ABL transcripts.[11] The PCR used in that study had a maximal sensitivity of one leukemic cell per 10^5 normal cells. Our technique had the same sensitivity with a gain of one log (one leukemic cell per 10^6 normal cells) when the results were checked by the second laboratory. Surprisingly, 16 out of 59, i.e. 27% of our patients, had undetectable levels of BCR-ABL with our technique which has the same sensitivity and the total probability of achieving a molecular remission was 27.95±11.94% in our study. This difference is probably due to the median follow-up which was longer in our study (41 months) than in the IRIS study (19 months). In addition, it is now widely accepted that residual disease continues to decrease during the period after achieving CCR. Rosti *et al.* reported comparable results in CML patients previously treated with interferon but who had a shorter follow-up (median 26 months). They evaluated 85 CCR patients, of whom 22 (25%) had undetectable transcript levels.[19] Our results are similar to those reported recently by the MD Anderson group who observed that 24% of CCR patients (40/162) had an undetectable BCR-ABL/ABL ratio.[20] The same team also reported a significant number of molecular remissions in *de novo* patients treated with high doses of imatinib. In RQ-PCR studies, 71 (63%) of 112 patients showed BCR-ABL/ABL percentage ratios decreasing to less than 0.05%, and 31 (28%) to undetectable levels.[21]

To the best of our knowledge, ours is the first study to analyze factors predictive of complete molecular remission. Various clinical and biological factors were tested, including white blood cell count at diagnosis, the proportion of Ph chromosome positive metaphases at diagnosis, the BCR-ABL/ABL ratio at the time of CCR, and the rapidity of CCR achievement after starting imatinib treatment. A low BCR-ABL/ABL ratio at the time of CCR was well correlated with complete molecular remission 12 months after CCR and continued to be a good prognostic factor. Moreover, the time to achieve CCR after the start of treatment very important. Indeed, a rapidly achieved CCR after the beginning of treatment was correlated well with the attainment of undetectable BCR-ABL transcripts. In addition, the achievement of CCR at 6 months was a factor that remained significant after Cox regression analysis.

The present findings confirm that quantification of BCR-ABL transcripts is an essential non-invasive technique for the optimal management of CML patients who achieve CCR on imatinib. Moreover, molecular remission after imatinib treatment, i.e. undetectable BCR-ABL in peripheral blood (with a sensitivity of 10^{-5}), is not a rare event in this series with a relatively long

median follow-up of 41 months. Although imatinib is well tolerated in most cases, the drug is expensive and the long-term side effects remain unknown. In most patients a low load of BCR-ABL persists over time, highlighting the need for careful follow-up examinations.

MC performed the experiments, analyzed the data and wrote the manuscript; CC and CR performed experiments; M-PF performed the statiscal analysis; GM, JR, VP provided samples from CML patients; CP assisted in the design of the study and manuscript preparation; FXM designed and supervised the work and manuscript preparation. Authors declare that they have no potential conflict of interest.

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