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Molecular Monitoring of BCR-ABL Transcripts after Allogeneic Stem Cell Transplantation for Chronic Myeloid Leukemia

Mario Arpinati*, Giulia Tolomelli, Maria Teresa Bochicchio, Fausto Castagnetti, Marilina Amabile, Giuseppe Bandini, Francesca Bonifazi, Marta Stanzani, Gianantonio Rosti, Giovanni Martinelli, Michele Baccarani

Department of Hematology and Oncological Sciences "Seragnoli," University of Bologna, Italy

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ABSTRACT

The monitoring of minimal residual disease (MRD) through low sensitivity real-time (RT) polymerase chain reaction (PCR) analysis of BCR-ABL transcripts allows early detection of chronic myeloid leukemia (CML) relapse after allogeneic hematopoietic stem cell transplantation (HSCT). The introduction of more sensitive techniques, such as RT quantitative (Q)-PCR, may lead to an overestimation of the risk of CML relapse. In this study, we reviewed the results of peripheral blood RT Q-PCR in CML patients who underwent allogeneic HSCT from 1983 to 2007. In our laboratory, RT Q-PCR analysis was routinely performed since 2002. Eighty-seven of 189 patients had available RT Q-PCR data; 63 patients had at least 3 RT Q-PCR analyses assessable. Fifty-two of 63 patients (83%) had, at least once, detectable transcript levels, but with an BCR-ABL/ABL ratio $<.1\%$ defined as $<$MR3 (molecular remission $<0,1\%$), whereas 11 (17%) had persistent undetectable BCR-ABL transcripts. Six of 52 patients with $<$MR3 relapsed, defined as BCR-ABL transcript numbers $>.1\%$ confirmed by the finding of Ph+ cells in bone marrow. No patients with persistent undetectable transcripts relapsed ($P = .19$). Relapse did not correlate with the number of occurrences of $<$MR3 or with the time to the first $<$MR3 result. Finally, of 46 patients with detectable transcripts who did not relapse, 35 had undetectable transcripts at last contact. RT Q-PCR analysis had low specificity (19%) and low positive predictive value (12%) in predicting relapse of CML patients after allogeneic HSCT. Our data suggest that detection of low BCR-ABL transcript levels by RT Q-PCR analysis has a poor accuracy in defining the risk of CML relapse and should not be considered as the unique indication to treatment. Fluctuation of BCR-ABL transcripts levels is common as late as $\geq$10 years post-transplantation, possibly suggesting the long-term persistence of CML stem cells.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has been used to treat patients with chronic myeloid leukemia (CML) in the chronic phase for over 30 years. The incidence of clinical relapse has been low, but some patients have relapsed more than 10 years after an otherwise "successful" transplantation [1]. Indeed, data collected by the Center for International Blood and Marrow Transplant Research show that the cumulative incidence of relapse at 15 years for patients in remission at 5 years posttransplantation has been as high as 17% [2]. Thus, the issue of whether patients transplanted for CML continue to harbor in their body small quantities of residual leukemia after allogeneic HSCT is still open.

We and others have previously observed that the detection of residual disease by polymerase chain reaction (PCR) analysis correlates with relapse after bone marrow transplantation [3]. The methodology used for detecting BCR-ABL transcripts by PCR analysis has evolved over the years. Previous low sensitivity PCR techniques, such as qualitative single-step [4] or older quantitative competitive PCR techniques [5], could predict the risk of relapse post-transplantation [6,7]. However, the sensitivity of these assays was similar to conventional metaphase cytogenetics (approximately 10^{-2}). After 2000, the Taqman methodology increased the sensitivity to 10^{-4} or more. Moreover, in 2003 a Europe Against Cancer program established standardized protocols for BCR-ABL quantitation in multiple centers using this methodology. Taqman-based real-time (RT) quantitative (Q)-PCR is currently used in the follow-up of patients treated with tyrosine kinase inhibitors (TKIs) and has improved treatment strategies [8]; notably, RT Q-PCR may be positive in many patients in complete cytogenetic remission. However, the predictive value of RT Q-PCR analysis on the risk of relapse after allogeneic HSCT is still debated [9]. This is particularly important as treatment of early (molecular or cytogenetic) relapses after allogeneic HSCT with either donor lymphocytes infusion [9-11] or TKIs has been shown to be more effective than late (advanced) relapses and to induce durable molecular responses [12-14]. In this article, we review the results of RT Q-PCR analysis of BCR-ABL transcripts in all patients with CML who underwent allogeneic HSCT at our institute, between 1983 and 2007, and correlate these results with the risk of relapse.

METHODS

Patients

We studied 189 consecutive patients with Philadelphia chromosome (Ph) and BCR-ABL–positive CML who underwent allogeneic HSCT from 1983 through 2007 at the Department of Hematology and Oncology Sciences "Seragnoli," University of Bologna. Follow-up was updated as of January 2010. Because in our laboratory RT Q-PCR was routinely performed since 2002, only 87 patients had at least 1 BCR-ABL transcript quantitation available. Of 87 patients, only 63 had at least 3 separate assessable RT Q-PCR

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* Correspondence and reprint request: Dr. Mario Arpinati, Dipartimento di Ematologia e Scienze Oncologiche "L.&A.Seragnoli," Universita' di Bologna, Via Massarenti, 9 40138 Bologna, Italy.
E-mail address: mario.arpinati@unibo.it (M. Arpinati).

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measurements. Of the remaining 24 patients, 11 had 2 measurements, whereas 13 had only 1 measurement. Only 2 more events could be observed if the analysis was extended to these 24 patients (2 relapses, 1 in the subgroup with 2 and 1 in the subgroup with only 1 measurement). Additional analyses were performed, including patients with 2 measurements, and the results (not shown) were similar to those reported in the current study. Therefore, the final analysis included only 63 patients with 3 available measurements. Fifteen patients were monitored with RT Q-PCR analysis from the date of transplantation; for the remaining 48 patients, the median time between the date of transplantation and the first RT Q-PCR analysis was 2607 days (range, 729 to 5321). The median number of Q-PCR measurements per patients was 6; 14 patients had ≥10 quantifications.

The characteristics of the study group are summarized in Table 1. The study group comprised 44 males and 19 females with a median age of 36 years (range, 13 to 56). At the time of transplantation, 68% patients were in chronic phase and 32% in accelerated phase. Accelerated phase was defined based on standard European group of Blood and Marrow Transplantion criteria by any of the following: anemia (hemoglobin <8 g/dL), leukocytosis (white blood cell count $> 100 \times 10^9$/L), thrombocytopenia (platelets $< 100 \times 10^9$/L), thrombocytosis (platelets $> 1000 \times 10^9$/L), or splenomegaly unresponsive to BU busulfan or hydroxyurea; extramedullary disease; clonal marrow cytogenetic abnormality(ies) in addition to the original Ph chromosome; blood or marrow blasts >10%; and blood or marrow blasts plus promyelocytes >20% and/or blood basophils+ eosinophils >20% [15]. Sixty-four percent of patients had been previously treated with interferon-α and 22% with TKIs. All patients received a myeloablative conditioning regimen (67% busulfan based and 33% total body irradiation based). Sixty percent of patients received low-dose antithymocyte globulin (ATG; Fresenius, Bad Homburg, Germany) in the conditioning regimen, as previously described [16]; 38% received the transplantation from a unrelated donor; and 38% received peripheral blood stem cells. All patients received cyclosporine and methotrexate as graft-versus-host disease (GVHD) prophylaxis. We classified acute and chronic GVHD according to standard criteria [17,18].

Molecular Monitoring of Minimal Residual Disease

BCR-ABL transcript level assessment was performed using RT Q-PCR according to suggested procedures and recommendations, as previously described [19]. The cycle threshold was defined as the cycle associated with a significant increase in fluorescence and was correlated with the amount of mRNA by using increasing amounts of plasmid RNA containing the mRNA of interest, leading to the definition of a standard curve. Samples yielding an ABL threshold cycle >30, corresponding to <1000 ABL transcript copies, were considered as having degraded RNA and discarded. BCR-ABL transcript levels were expressed as a percentage of the amount of BCR-ABL/ABL ratio according to the International Scale, taking advantage of the ongoing international initiatives that standardize the quantitation of BCR-ABL transcripts through the use of a conversion factor [20]. The lower detection limit of the assay is 10^{-4}. The reference laboratory that performed all molecular analysis on this study was located in Bologna (conversion factor, .6), and the data were checked and validated (10 samples at 3, 6, 9, and 12 months) in Turin and Naples.

Monitoring of molecular disease (minimal residual disease [MRD]) was planned to be performed every 3 months until 2 years, then every 6 months until 5 years, and then yearly afterward. The first analysis was performed at 3 months posttransplantation. Only peripheral blood samples were included in the analysis [21]. When detectable BCR-ABL occurred, monitoring was performed more frequently, as clinically indicated. Patients were included in the low-level (<MR3 [molecular remission <0,1%]) detectable transcript group when the BCR-ABL/ABL ratio was <.1%, according to the International Scale in at least 1 sample during the follow-up [4]. All patients with detectable transcript but with BCR-ABL/ABL ratio <.1% were included in the <MR3 group. A single <MR3 result was sufficient to include a patient in the <MR3 group. Two or more consecutive results of <MR3 were grouped together for purposes of analysis as "occurrences" (Table 2). Patients with stable undetectable BCR-ABL were included in the undetectable BCR-ABL group. Patients with confirmed loss of the molecular response (BCR-ABL/ABL ratio >.1% International Scale) and evidence of Ph+ cells in bone marrow upon cytogenetic analysis underwent preemptive treatment in accordance with our local policy.

Statistical Analysis

Categorical variables were compared among groups by the chi-square test. Continuous numeric variables were expressed as medians and compared among groups by the Wilcoxon test. Statistical significance was set at .05. Multiple logistic regression analysis was used to identify the independent prognostic factors for the outcomes. Variables with a $P < .1$ were included in the multivariate model. Curves representing the time to clinical relapse were drawn according to the Kaplan-Meier method starting from the time of the first <MR3 result and compared by the log-rank test. For patients with persistent undetectable BCR-ABL transcripts, the time to relapse was calculated starting from the time of the first Q-PCR analysis. Sensitivity, specificity, and positive predictive and negative predictive values were determined as follows:

$$\text{Sensitivity}:\ T/D \times 100$$

$$\text{Specificity}:\ (\text{all} - T)/(\text{all} - D) \times 100$$

$$\text{Negative predictive value}:\ \text{all} - (T \text{ and } D)/(\text{all} - T) \times 100$$

$$\text{Positive predictive value}:\ T \text{ and } D/T \times 100$$

where T is patients with an abnormal test result and D is patients with disease.

Statistical analyses were performed by using Graph Pad Prism version 4.02 (GraphPad Software Inc. La Jolla, CA). Multivariate analyses were performed using SPSS version 9.1 (IBM Italia, Segrate, MI).

RESULTS

Patients' Molecular Status after Allogeneic Transplantation

Of 63 patients, after a median follow-up of 3693 days (range, 898 to 9405), 60 were still alive and 3 had died without relapsing. Fifty-two patients had at least 1

Table 1
Patient Characteristics (N = 63)

Age, yr (range)	36 (20-56)
Gender	
Male	44 (69.8%)
Female	19 (30.2%)
Female-to-male	16 (25.4%)
Donor	
Related	39 (61.9%)
Unrelated	24 (38.1%)
Source	
BM	39 (61.9%)
PBSC	24 (38.1%)
Conditioning	
Busulfan-based	42 (66.7%)
TBI-based	21 (33.3%)
ATG-containing	38 (60.3%)
Disease phase	
Chronic phase	43 (68.3%)
Accelerated phase	20 (31.8%)
Previous treatments	
Interferon-α	40 (63.5%)
Imatinib	14 (22.2%)
Acute GVHD	
Grade I	11 (17.5%)
Grades II-IV	12 (19.1%)
Chronic GVHD	
Limited	21 (33.3%)
Extensive	18 (28.6%)

BM indicates bone marrow; PBSC, peripheral blood stem cells; TBI, total body irradiation.

Table 2
Characteristics of Patients in the <MR3 Group (N = 52)

Characteristics	Value
Number of occurrences of <MR3 results*	
1	29 (55.8)
2	17 (32.7)
≥3	6 (11.5)
Time to first occurrence of a <MR3 result†	
<1	10 (19.2)
1-5	14 (26.9)
>5	28 (53.9)

* An occurrence is defined as ≥1 consecutive positive results.
† Measured in years from transplantation.

occurrence of detectable low-level BCR-ABL transcripts (<MR3) and were therefore included in the <MR3 group. Eleven patients had persistent undetectable BCR-ABL transcripts. As shown in Table 2, 29 patients had only 1 occurrence of a <MR3 result, as defined in Methods; 17 patients had 2 occurrences and 5 patients had 3 occurrences, whereas 1 patient had 4. Fifteen patients were monitored starting from the date of transplantation: 10 patients had their first detectable BCR-ABL transcript within 1 year posttransplantation; in 7 of them, BCR-ABL transcripts were detectable at the first analysis. In the remaining 37 patients, the first Q-PCR analysis was performed after a median of 1841 days (range, 729 to 5331) posttransplantation. In 28 patients, the first occurrence of a <MR3 result was observed at ≥5 years after transplantation, with only 9 of 28 patients positive at their first RT Q-PCR analysis. Among the other 9 patients, the first occurrence of a <MR3 result was observed at <5 years posttransplantation, with 6 patients positive at their first PCR analysis.

In univariate analysis, the occurrence of a <MR3 result positively correlated with the administration of ATG during conditioning and was more common in patients transplanted after 2002 (i.e., patients who were monitored with RT Q-PCR starting at the time of transplantation) (Table 3). In multivariate analysis, we observed a trend in which patients who had received ATG pretransplantation (Table 3) were more frequent in the <MR3 group. Other factors, including the stage of the disease, the presence or absence of GVHD, the type of donor, or the type of source, did not correlate with the results of BCR-ABL analysis posttransplantation (Table 3).

Impact of the Molecular Status on the Risk of Relapse

Six patients had relapsed at a median of 2142 days (range, 1419 to 3746) posttransplantation. At relapse, 2 patients were in the blastic phase, 2 were in chronic phase, and 2 had only cytogenetic evidence of the disease (UPN [Unique Patient Number] 379 had 13%, whereas UPN 553 had 50% Ph+ metaphases in the bone marrow). The characteristics of relapsed patients are described in Table 4.

When we analyzed the risk of relapse on the basis of RT Q-PCR, we observed that 6 of 52 patients in the <MR3 group relapsed after a median follow-up of 1094 days (range, 561 to 1384) after their first positive result, resulting in an actuarial probability of relapse of 15.8% as determined by Kaplan-Meier analysis. Instead, none of the 11 patients with persistent undetectable results relapsed during the follow-up period (Figure 1). However, the difference between the two groups was not significant ($P = .19$), as determined by the log-rank test. The risk of relapse was not influenced by the number of occurrences of a <MR3 result or by the time since transplantation when a <MR3 result was first detected (Figure 2). We then tested whether detectable BCR-ABL transcripts correlated with relapse in patients with accelerated phase (n = 20) at the time of transplantation, a subset of patients with a particularly high risk of relapse. However, only 1 of 20 patients with accelerated phase relapsed during follow-up, although 18 of them had detectable BCR-ABL transcripts during follow-up (not shown). As MRD in CML has been reported to be common early posttransplantation, we performed a separate analysis on patients who were monitored starting at the time of transplantation (n = 15). However, all 15 patients transplanted after 2002 had detectable BCR-ABL transcripts, and 3 of them relapsed, making it not possible to detect the impact of molecular status on relapse.

To determine the outcome of patients with detectable BCR-ABL transcripts who did not relapse, we reviewed their molecular status at last follow-up. After a median of 1595 days (range, 22 to 2298) from the first <MR3 result, only 11 of 46 patients (21%) still had detectable BCR-ABL transcripts (not shown).

Figure 3 describes the results of molecular monitoring in the 6 patients who relapsed. All these patients had detectable BCR-ABL transcripts before relapse. One-half of them (UPN 273, 279, and 383) had their first <MR3 result at 3 to 8 years posttransplantation, which was followed by relapse within 1 to 3 years. The remaining 3 patients (UPN 536, 547, and 553) were followed starting at the time of transplantation and had fluctuating BCR-ABL transcript amounts (at least 2 separate <MR3 results) before relapse.

Finally, we determined the diagnostic accuracy of the detection of BCR-ABL transcripts, by RT Q-PCR, in the prediction of relapse. Although the detection of BCR-ABL transcripts showed high sensitivity with no false-negative results, resulting in a negative predictive value of 100%, its specificity was low (19%), resulting in a poor positive predictive value (Table 5).

Table 3
Risk of a <MR3 Result Posttransplantation (N = 52)

	Univariate Analysis	Multivariate Analysis
Female-to-male	OR .32 (.08-1.26) $P = .19$	n.p.
MUD	OR 3.3 (.6-16.8) $P = .13$	n.p.
PBSC	OR 1.8 (.4-7.6) $P = .42$	n.p.
ATG	OR 5.5 (1.3-23) $P = .01$	OR 3.5 (.8-15) $P = .09$
BMT after 2002	OR 9.5 (.5-172) $P = .04$	Not significant*
Previous interferon-α	OR .13 (.01-1.1) $P = .04$	Not significant
Accelerated phase	OR 2.4 (.5-12.2) $P = .29$	n.p.
Acute GVHD grades II-IV†	OR .6 (.1-2.8) $P = .54$	n.p.
Extensive chronic GVHD†	OR 2 (.4-10.3) $P = .40$	n.p.

MUD indicates matched unrelated donor; PBSC, peripheral blood stem cell; BMT, bone marrow transplantation; n.p., not performed (because *P* not statistically significant).
Univariate analysis was performed with the chi-square test, as described in Methods, whereas multivariate analysis was performed by step-wise logistic regression analysis, as described in Methods. Values are odds ratio (OR), with 95% confidence intervals in parentheses.
* $P > .5$ for both factors in multivariate analysis.
† Acute GVHD grades I-IV and all chronic GVHD were also tested and were not associated with the detection of BCR-ABL.

DISCUSSION

In this study of long-term follow-up of patients with CML after allogeneic HSCT, we observed that low levels of BCR-ABL transcripts, consistent with <MR3 (BCR-ABL/ABL ratio <.1% International Scale), were detected in most patients after allogeneic HSCT. We observed that none of the patients with stable undetectable BCR-ABL relapsed, whereas patients with <MR3 results had a low relapse probability of 15.8%. With the limits of the small number of patients, the difference was not statistically significant. Therefore, the detection of BCR-ABL transcripts by RT Q-PCR posttransplantation in CML patients may not effectively predict clinical relapse with sufficient specificity.

Competitive RT-PCR and RT-PCR with older techniques, such as “LightCycler,” was estimated to have about 10^{-2} sensitivity and a good correlation with the results of FISH (Fluorescent In Situ Hybridization) analysis [7], which has often been reported to predict relapse of CML posttransplantation. However, Kitzis et al. reported that BCR-ABL

Table 4
Characteristics of the 6 Relapsed Patients

UPN	Age (yr)	Status	Interval	Interferon	Glivec	Conditioning Regimen	Acute GVHD	ChronicGVHD	Donor	Source
273	37	Chronic phase	23	Y	N	Cy+ATG+TBI	0	L	MUD	BM
379	30	Chronic phase	11	Y	N	Cy+ATG+TBI	0	No	MUD	BM
383	40	Chronic phase	14	N	N	Cy+Bu	0	No	Family	BM
536	50	Accelerated phase	38	Y	Y	Cy+Bu	0	E	Family	PBSC
547	49	Chronic phase	13	N	Y	Cy+Bu+ATG	0	L	Family	BM
553	28	Chronic phase	24	Y	Y	Cy+ATG+TBI	0	No	MUD	BM

Cy indicates cyclophosphamide; Bu, busulfan; TBI, total body irradiation; MUD, matched unrelated donor; BM, bone marrow; PBSC, peripheral blood stem cell; L, limited; E, extensive.

rearrangement, as based on FISH analysis, was less reliable than RT Q-PCR analysis [22]. A review of the literature published in 2000 suggested that repeated detectable BCR-ABL mRNA levels, as determined by RT Q-PCR, could have been predictive of relapse after allogeneic HSCT [6], although its value was higher if associated with the presence of mixed chimerism by VNTR (Variable Number of Tandem Repeats)-PCR analysis, moreover if both mixed chimerism and MRD were increasing [23]. We also previously observed that persistent high amounts of BCR-ABL transcripts were associated with relapse, particularly in patients with accelerated phase disease [3]. Moreover, Kaeda et al. suggested that fluctuating BCR-ABL transcript levels correlated with a higher probability of relapse in CML patients who underwent T cell–depleted allogeneic HSCT [9]. Several other studies pointed out that high levels of BCR-ABL transcripts, particularly if detected early (within 1 year) posttransplantation, confer a significantly higher risk of relapse [24-26]. It is reasonable that such different results are mostly related to different methods used to monitor MRD among populations composed of patients with different characteristics and treated with different types of GVHD prophylaxis (e.g., use of ATG or of in vitro T cell depletion) or conditioning regimen (e.g., total body irradiation). Moreover, our method of RT Q-PCR has a sensitivity of up to 1 in 10^6 cells, possibly higher than previously described.

However, the results of our study are also consistent with other reports that did not confirm the association between detection of BCR-ABL and relapse. A meta-analysis on qualitative PCR published in 1994 confirmed persistent PCR positivity within 2 years as not predictive of relapse in CML patients after allogeneic HSCT [27], later confirmed by Santini et al. [28] using nested PCR. Furthermore, Otazú et al. also showed 30 patients with detectable BCR-ABL transcripts both early and late posttransplantation, reporting only 1 relapse among them [29].

The biological significance of persistent detectable BCR-ABL transcripts after allogeneic HSCT is still a matter of debate. It could be explained by transient or intermittent BCR-ABL positivity related to the detection of nonmalignant BCR-ABL–positive leukocytes, as previously described for RT PCR analysis as well [30,31]. Indeed, in our study, of 52 patients, only 11 patients had still detectable BCR-ABL transcripts at last follow-up, after a median of 1595 days from the first event. Otherwise, the graft-versus-leukemia effect can play a role in maintaining remission and in controlling potential malignant recipient cells [32]. It would be interesting, in future studies, to determine whether the frequency of leukemia-specific T cells [33] may correlate with the risk of clinical relapse in the patients with detectable BCR-ABL transcripts.

The factors predicting persistent <MR3 posttransplantation are still poorly characterized. First, the occurrence of acute GVHD grades II to IV did not appear to correlate with undetectable BCR-ABL transcripts, contrary to that previously suggested [9] in patients receiving T cell–depleted transplantations; in our study, we could not find any correlation between chronic GVHD and molecular relapse, as previously

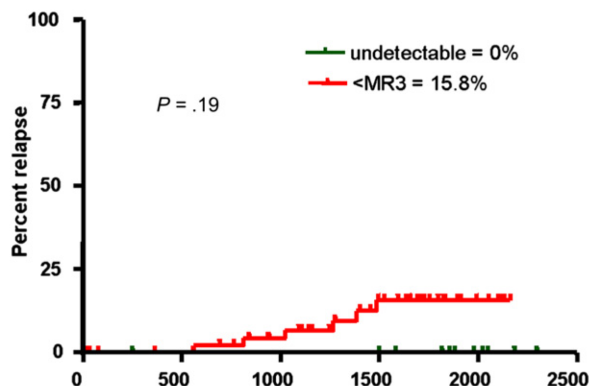

Figure 1. Kaplan-Meier analysis of the time from first detection of BCR-ABL to relapse of <MR3 patients (n = 52) compared with patients with persistent undetectable results (n = 11). The significance of the difference between the 2 groups of patients, as indicated in the plot, was determined by the log-rank test.

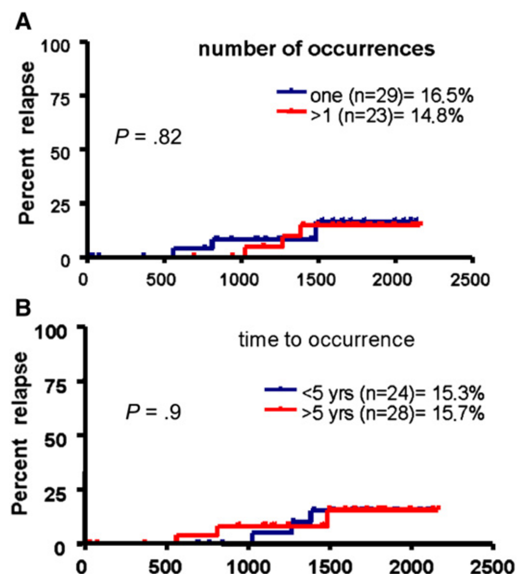

Figure 2. Kaplan-Meier analysis of the time from first detection of BCR-ABL to relapse of patients in the <MR3 group as based on the indicated factors: (A) number of events and (B) time to event. The significance of the difference between the 2 groups of patients, as indicated in the plot, was determined by the log-rank test.

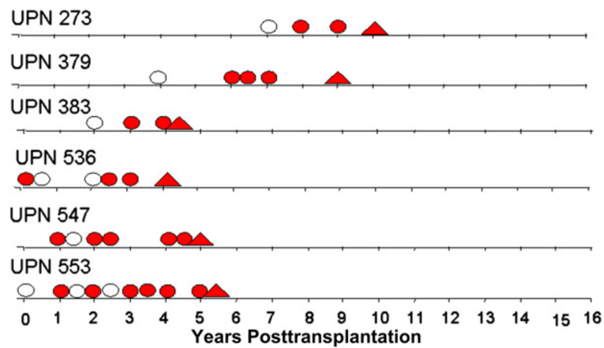

Figure 3. Results of molecular monitoring in the 6 relapsed patients. Red circles represent <MR3 results, white circles represent undetectable values, and red triangles represent relapse.

suggested [30]. Second, ATG appeared to be the only significant ($P = .01$) risk factor in univariate, but not in multivariate, analysis. In our study, ATG was administered at a low dose to 60% of patients. We and others previously described that low-dose ATG in the preparative regimen for both related [34] and unrelated [35,36] bone marrow transplantation for CML was not associated with a higher risk of relapse but only with a lower incidence of acute and chronic GVHD [16]. Presently, no reports specifically address the effect of ATG on MRD after allogeneic HSCT for CML patients. Third, positive BCR-ABL transcripts by RT Q-PCR analysis may be related to the disease status at transplantation. Our study involved patients both in chronic and accelerated phases (43 and 20, respectively), but we could find no evidence that the accelerated phase was significantly associated with persistent <MR3 results, possibly due to the low number of cases. Indeed, detectable transcripts are reported early posttransplantation irrespective of CML phase [28], although in 2000 Martinelli et al., by using RT Q-PCR, showed a possible link between BCR-ABL positivity and accelerated phase [3].

Maintenance of CR [complete remission] after ≥5 years, appears to be associated with a 15% to 20% long-term risk of relapse [2] and with mortality rates approaching those of the general population, consistent with our results. In our study, among 28 patients (54%) with low-level BCR-ABL positivity at >5 years posttransplantation, only 3 relapsed after a median follow-up of 2783 days (range, 1659 to 5289). However, our study does not give any significant information on the risk of relapse of the general CML population, as most patients who received transplantation during the observational time were not included because of early death or relapse.

Therefore, our study does not suggest that a low amount of detectable BCR-ABL transcript after allogeneic HSCT should indicate a preemptive treatment. Based on our data, >8 patients should be treated to prevent 1 relapse. Many different therapies (donor lymphocytes infusion, TKIs, immunosuppressant withdrawal) may be used to obtain a complete remission or to maintain low-level BCR-ABL transcript levels in patients at risk of relapse [37,38]. The role of immunotherapy and imatinib (or other TKIs) in obtaining a molecular or cytogenetic response in relapsed patients still needs to be better defined [39-41]. Because most of these treatments are costly or potentially toxic, we deem that a larger prospective study is needed to evaluate preemptive treatment in selected patients to avoid overtreatment. Moreover, the best choice between different possible treatments is still under investigation. At the moment, the recommendation is to simply watch patients with MRD positivity by increasing the frequency of molecular monitoring.

Table 5
Accuracy of a <MR3 Result in Predicting Relapse

Sensitivity	100%
Specificity	19%
Positive predictive value	12%
Negative predictive value	100%

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