Kinetics of Increasing BCR-ABL Transcript Numbers in Chronic Myeloid Leukemia Patients Who Relapse After Bone Marrow Transplantation

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We prospectively studied 98 chronic myeloid leukemia (CML) patients after bone marrow transplantation by competitive polymerase chain reaction to detect and quantify leukemia-specific BCR-ABL mRNA. Of 69 patients who had persistently undetectable, decreasing, or low BCR-ABL levels (<50 transcripts/μg RNA) on sequential analysis, one (1%) subsequently relapsed. Of 29 patients who had increasing or persistently high BCR-ABL (>50 transcripts/μg RNA) on sequential analysis, 21 (72%) have relapsed before relapse, indicating a constant BCR-ABL transcript doubling time. The doubling time for patients relapsing cytogenetically or into chronic phase (median, 24.7 days) was significantly longer than that of patients relapsing into advanced phases (median, 14.7 days; P = .005). Eight patients were treated for relapse by donor leukocyte transfusions. The doubling time of responders was significantly longer than that of nonresponders (P = .017). We conclude that quantification of BCR-ABL transcripts after bone marrow transplantation (BMT) is valuable in predicting relapse: a more rapid BCR-ABL transcript doubling time before relapse might indicate a more aggressive disease.

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CHRONIC MYELOID LEUKEMIA (CML) is characterized in approximately 95% of the cases by the chimeric fusion protein, p210 BCR-ABL, which results from a translocation involving the BCR gene on chromosome 22 and the ABL gene on chromosome 9. This translocation is usually visible cytogenetically as the Philadelphia (Ph) chromosome.

CML can be treated by allogeneic bone marrow transplantation (BMT). This treatment apparently eradicates the disease in the majority of cases, but some patients do relapse. The probability of relapse for patients transplanted in first chronic phase who receive unmanipulated marrow from an HLA-identical sibling donor after high-dose chemotherapy is 10% to 20% at 5 years. The outlook is worse for patients receiving transplants in advanced phases of the disease, and the leukemia-free survival for those undergoing BMT in blast crisis is 5% to 15%. Patients who relapse after BMT provide the opportunity to study the growth kinetics of CML cells in vivo.

Because the BCR-ABL fusion gene is specific to the leukemia cells, it is an excellent marker to identify residual disease. Several groups have demonstrated that BCR-ABL mRNA may be specifically and efficiently detected by reverse transcriptase–polymerase chain reaction (RT-PCR) and that this technique is routinely capable of detecting a single CML cell in a background of 10^6 to 10^9 normal cells. However, many patients have detectable BCR-ABL mRNA (PCR-positive) for many months after BMT without relapsing, and this qualitative PCR technique is therefore of limited value in the clinical management of individual patients (reviewed in Miyamura et al). We and others have developed a competitive PCR assay to quantify BCR-ABL transcripts and have shown that increasing levels of the fusion gene mRNA can be observed before cytogenetic detection of relapse. Furthermore, such evidence of early relapse may be used to initiate therapy in the form of leukocyte transfusions from the original marrow donor.

In this study, we examined the utility of quantitative RT-PCR for BCR-ABL in the management of CML patients after BMT, and used the technique to gain insight into the kinetics of relapse.

MATERIALS AND METHODS

Patients. Ninety-eight CML patients who had undergone allogeneic BMT were studied. Patients were between 1 and 65 months (median, 16 months) after transplantation with either unmanipulated marrow from HLA-identical sibling donors (n = 66) or matched unrelated donors (n = 32). Recipients of sibling marrow over the age of 45 (n = 9) and all recipients of unrelated donor marrow underwent in vivo T-cell depletion with the use of a Campath 1G (MRC/Wellcome Therapeutic Antibody Centre, Cambridge, UK), and all patients received cyclosporin A plus methotrexate post-BMT. At the time of transplant, 81 patients were in first chronic phase, 10 were in second chronic phase, and seven were in accelerated phase. The conditioning regimen in all groups included cyclophosphamide 120 mg/kg and fractionated total-body irradiation to a total dose of 10, 12, or 13.2 Gy administered at a rate of 15 cGy/min. During the period of study, 24 patients had cytogenetic or hematologic evidence of relapse. Of these, 15 (63%) had undergone in vivo T-cell depletion.

Cytogenetics. Cytogenetic analysis of marrow cells was performed routinely on all patients at 3, 6, 9, and 12 months post-BMT and subsequently at 6-month intervals whenever possible. Usually, 30 metaphases were studied. All patients were Ph-positive before BMT.

Patient samples. Peripheral blood or bone marrow cells were studied prospectively at various intervals post-BMT. Informed consent was obtained as required by the Declaration of Helsinki. All patient samples considered herein were obtained after August 1992, i.e., the date we introduced the competitive PCR assay on a routine basis. Some patients had PCR assays before this time; these results were not quantified and have been published elsewhere.

RT-PCR for BCR-ABL. After extraction of leukocyte RNA, 313 samples from 98 CML patients were analyzed by RT-PCR for BCR-ABL mRNA (median, 2.5 samples per patient; range, one to 11). One hundred twenty-six samples were PCR-negative, and 187 were PCR-positive. BCR-ABL transcripts in all PCR-positive samples were quantified by competitive PCR. Results were standardized by deriving the number of BCR-ABL transcripts per microgram.

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leukocyte RNA. We have previously shown that the levels of residual disease detected by PCR in bone marrow and peripheral blood of samples from CML patients are essentially identical, 13 that the assay yields reproducible results at both high and low levels of residual disease (coefficient of variation = 17%), and that the results are linear over at least four orders of magnitude. 15 High BCR-ABL levels were defined as 50 transcripts/μg RNA or greater; low BCR-ABL levels were less than 50 transcripts/μg RNA. Increasing or decreasing BCR-ABL levels were defined as differences of more than a half order of magnitude on sequential analysis. Fluctuating BCR-ABL levels were defined as differences of more than a half order of magnitude. Transient molecular relapse was defined as increasing levels of BCR-ABL in patients who subsequently became PCR-negative without any treatment.

Analysis of results. The number of months after BMT was plotted against the log10 value of the number of BCR-ABL transcripts per microgram RNA for the period in which a clear increase in the number of fusion transcripts was evident (Table 1). This was necessary to exclude periods when a patient was PCR-negative or had stable BCR-ABL levels, eg, at relapse, or decreasing BCR-ABL levels, eg, shortly after transplant. 6 The equation of the regression line and the correlation coefficient (r) was calculated using least-squares analysis. The gradient of the regression line ("a" in the formula, y = ax + b) is the rate of increase in the number of log [BCR-ABL transcripts] per month, and the doubling time is calculated as the interval in which the number of log[BCR-ABL transcripts] increased by 0.30 (log 2). Groups were compared by Student's t test if standard deviations were similar, and otherwise by the Mann-Whitney test.

RESULTS

Rate of relapse. The median number of BCR-ABL transcripts in patients with established chronic-phase CML is approximately 5 \times 10^5/μg RNA. 6,14 Although PCR assays were not usually performed once relapse had been established by cytogenetic or hematologic criteria, it is clear that at relapse BCR-ABL transcripts level off at between 10^7 and 10^8/μg RNA (eg, patients no. 160, 159, 211, and 263). This plateau is achieved because it is the number of BCR-ABL transcripts per microgram RNA that is measured, not the total number of BCR-ABL transcripts per unit volume, ie, the measured value is maximal at 100% CML cells irrespective of the leukocyte count. Rates of relapse were therefore derived from regression lines of plots of time after BMT versus log [BCR-ABL transcripts] before the plateau phase when this was apparent, or before hematologic relapse. Of 24 patients who had either hematologic or cytogenetic evidence of relapse, 19 had increasing BCR-ABL levels on sequential analysis. Clinical details and RT-PCR results for these patients are shown in Tables 1 and 2. Initially, the analysis was performed for 12 patients for whom three or more data points were assessable. In each case, the correlation coefficient (r) was close to 1, indicating a constant logarithmic increase in the number of BCR-ABL transcripts before relapse, which is tantamount to a constant BCR-ABL transcript doubling time. Since the value of r was so high for each of these cases (> .947), another seven patients were included for whom data on only two competitive PCR assays were assessable. Plots for all 19 patients are shown in Fig 1. The calculated doubling times ranged from 9.3 days for patient no. 321 to 43.2 days for patient no. 297 (median, 23.9).

BCR-ABL transcript doubling time and phase of relapse. Of these 19 patients, nine relapsed into chronic-phase CML...
Abbreviations: NIA, not applicable; <, less than.

Results obtained when an exponential increase in BCR-ABL transcript levels was evident.
months post-BMT. Over the following year, BCR-ABL levels were approximately static and the patient remained in cytogenetic remission. Subsequently, BCR-ABL levels increased again before hematologic relapse at 56 months post-BMT.

Of eight patients in the group of 29 who did not relapse, four had increasing or high BCR-ABL levels that did not exceed $10^3$ BCR-ABL transcripts/μg RNA; two had transient cytogenetic relapses with either persistently high or fluctuating BCR-ABL levels, and two had transient molecular relapse, with BCR-ABL levels increasing to a maximum of 50 and 100 transcripts/μg RNA at 9 and 29 months post-BMT, respectively, before molecular remission.

Fig 1. Plots of log [BCR-ABL transcripts] before relapse versus months post-BMT for 19 patients who had ≥2 quantitative PCR assays before relapse. (------) Patients who relapsed into chronic phase or who only had cytogenetic evidence of relapse; (-----) patients who relapsed into accelerated phase (no. 160 and 263) or blast crisis (no. 159 and 325).

Fig 2. Doubling time versus phase of disease at relapse for all 19 patients. CP/cyto, chronic phase or cytogenetic evidence of relapse only; AP/BC, accelerated phase or blast crisis. Median doubling times (24.7 and 14.7 days, respectively) are indicated. The doubling time for CP/cyto is significantly longer than that for advanced phases ($P = .005$, Mann-Whitney test).

DISCUSSION

We have used quantitative PCR for BCR-ABL in patients relapsing after allogeneic BMT to assess the growth kinetics of CML at a much earlier stage than previously possible using cytogenetic or morphologic analysis. In the majority of patients, there is a constant logarithmic increase in the number of detectable BCR-ABL transcripts in the period before overt relapse. This implies that the CML cells are themselves growing exponentially during this period and that the disease has a constant bulk doubling time. This doubling time is not the same as the cell generation time and indeed is likely to be much longer, since some malignant cells must be dying through senescence or apoptosis, and many would not be proliferating.16,17

Despite the fact that the number of patients who relapsed was relatively small, we have found that advanced-phase CML has a significantly faster transcript doubling time (median, 14.7 days) than chronic-phase CML (median, 24.7 days). This is not unexpected, since doubling times may be much more rapid in acute leukemia, eg, 4 to 6.5 days in relapsing children,18,19 than in chronic-phase CML. We also found that patients with rapidly doubling transcript numbers
are less likely to respond to donor leukocyte transfusion than those with long doubling times, even if the relapse was into chronic phase. Since donor leukocyte transfusion is less effective for CML patients in advanced phases, this suggests that relatively rapid doubling times may identify patients whose disease has progressed even though conventional signs of acceleration are not present. This is supported by the fact that all four patients (no. 321, 389, 327, and 238) who relapsed into chronic phase but whose disease rapidly progressed had relatively short doubling times. An alternative explanation for response to donor leukocyte transfusion is that patients with longer doubling times have some residual graft-versus-leukemia effect that slows the rate of relapse, and that these patients respond to “reinforcement” of graft-versus-leukemia by infusion of donor leukocytes. A prospective analysis with frequent competitive PCR assays would be of value in determining the clinical importance of different doubling times.

Recently, Radich et al reported that PCR positivity at 6 to 12 months post-BMT is an independent predictor of relapse. Positivity at more than 36 months post-BMT was associated with a much lower risk of relapse, or possibly a considerably slower rate of relapse. However, in our series, late PCR positivity was associated with a high risk of relapse. Nineteen patients had at least one PCR result at more than 36 months post-BMT. Of these, 13 (68%) had only negative assays and six (32%) had at least one positive assay. Of six that were positive, four relapsed and another had a transient relapse. Furthermore, we found that the rate of relapse was not related to the time after transplant. However, some patients may have a persistently low level of residual disease for prolonged periods before having an increase in BCR-ABL mRNA levels and subsequent relapse (eg, patient no. 337).

Of 29 patients with increasing or persistently high BCR-ABL levels, 21 (72%) have relapsed at the time of analysis and others are still at risk. It may be reasonable therefore to consider therapeutic intervention in any patient who shows BCR-ABL transcript levels increasing or persistently above 100 transcripts/µg RNA, ie, the highest number of BCR-ABL transcripts reached in those patients who had increasing levels of BCR-ABL mRNA on sequential analysis but who subsequently became PCR-negative. This level is about 2 to 2 log values less than that at which Ph-positive metaphases typically become detectable in the bone marrow. Using these criteria, 27 patients in this series of the group of 29 with high or increasing BCR-ABL levels could have been treated before the onset of cytogenetic relapse. This includes the three patients with ‘transient’ cytogenetic relapse, two of whom might or might not have been treated unnecessarily.

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