So far, it has rarely been reported that this would result in significant numbers of circulating melanoma cells in peripheral blood. Usually, circulating melanoma cells can only be detected on the microscopic level by methods such as PCR for tyrosinase melanoma associated antigen. It is not clear whether the massive load of tumor cells observed in the peripheral blood is caused by dissemination of melanoma cells from bone marrow infiltration (Fig 1C) or whether the melanoma cells proliferated autochthonously in the peripheral blood compartment. Although the first possibility seemed to be more likely, we attempted to mimic the in vivo situation by culturing whole blood cells or Ficoll gradient-enriched mononuclear cells containing the tumor cells from this patient. Even by using different growth media, the tumor cells did not grow in suspension. However, when the cells were allowed to adhere to plastic of culture flasks, a stable cell line could be established that is now in culture for more than 1 year. This is the first report of a melanoma cell line established from the peripheral blood of a patient. To analyze the apparent reduced ability to transmigrate and to form solid metastases, we examined immunocytologically early passage melanoma cells from the cell line for molecules relevant for adhesion and migration. We found positive staining for integrin subunits α1 through 6 and β1 as well as intercellular adhesion molecule-1 (ICAM-1) and metastasis-promoting CD44 variant isoforms v5 and v6. There was no E-cadherin expression. Thus, the cells display a phenotype that is thought to be very compatible with progression of melanoma and other solid tumors by increasing proliferation, invasion, and/or metastasis.

It has been calculated in an animal model of melanoma that 80% of cells entering the microcirculation survive and extravasate by 24 hours. Because this patient had a calculated number of 5 \times 10^{10} circulating melanoma cells without macroscopically detectable metastases, it can be concluded that extravasation was significantly reduced, which therefore represents a key stage of metastatic control in this case. Alternatively, death of the patient might have occurred before solid metastases developed. Cancer cells of nonhematologic origin are only rarely seen on routinely prepared blood smears. The term carcinocythemia had been introduced to describe rare cases of disseminating carcinoma mimicking the clinical picture of acute leukemia. We suggest describing the phenomenon in the presented case as melanocythemia.

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Clinical Decision Making in Chronic Myeloid Leukemia Based on Polymerase Chain Reaction Analysis of Minimal Residual Disease

To the Editor:

In a recent commentary, Faderl et al posed the question “Should polymerase chain reaction analysis to detect minimal residual disease in patients with chronic myelogenous leukemia be used in clinical decision making?” We believe that the answer to this question is indubitably “yes.” Although we agree that nonquantitative assays that only indicate the presence or absence of BCR-ABL transcripts are of limited value, the results of serial quantitative reverse transcription-polymerase chain reaction (RT-PCR) studies may be highly informative. For chronic myeloid leukemia (CML) patients in cytogenetic remission after treatment by allografting, the results of serial quantitative RT-PCR analysis can alert the clinician to the need for further antileukemia therapy. For CML patients treated with interferon-α (IFN-α), BCR-ABL transcript numbers very seldom fall below the level of detection, but their actual level correlates with the probability that remission will be maintained.

The principal aim of residual disease analysis in patients with CML is to assess a patient’s response to treatment and to recognize early signs of relapse. RT-PCR for BCR-ABL mRNA is by far the most sensitive assay in this context and can detect a single leukemia cell in a background of 10^6 to 10^8 normal cells. However, it is important to note that this high sensitivity may not be achieved routinely, and variations in the actual sensitivity for each specimen almost certainly account for many of the apparent discrepancies in the literature. Because nonquantitative RT-PCR analysis gives only limited information, several groups have developed quantitative or semiquantitative RT-PCR assays that enable the kinetics of residual disease to be monitored over time. The number of BCR-ABL transcripts in a specimen is typically normalized to the number of transcripts of a housekeeping gene, which serves to
control for the integrity and quantity of patient cDNA. For RT-PCR–negative specimens, the control gene gives a clear indication of the sensitivity with which residual disease can be ruled out for that particular specimen and thus provides meaning to a result that previously had been difficult to interpret. Because of the technical complexities of these acts, quantitative RT-PCR has only been performed in a limited number of centers. Nevertheless, several studies have clearly indicated the clinical utility of this approach.

After allogeneic bone marrow transplantation (BMT), serial quantitative RT-PCR analysis of peripheral blood specimens can effectively distinguish those patients who are destined to remain in remission from those who are destined to relapse. Patients who remain in remission after BMT have persistently undetectable, low, or falling BCR-ABL levels on sequential analysis. After 6 to 9 months, BCR-ABL is undetectable in most cases and remains so indefinitely. Such patients may never require any further examination of their bone marrow, a fact for which they are very grateful. Other patients may remain intermittently or persistently RT-PCR positive for prolonged periods of time without evidence of cytogenetic relapse. The level of detectable BCR-ABL transcripts in these individuals is usually very low. In contrast, for patients destined to relapse, increasing or persistently high levels of BCR-ABL mRNA can be detected on sequential analysis, often several months before the cytogenetic detection of the Philadelphia (Ph) chromosome in bone marrow metaphases. The recognition of relapse at the molecular level provides a window for therapeutic intervention while the burden of disease is still relatively low. To take account of the fact that low-level RT-PCR positivity after allografting may not herald clinically relevant disease recurrence, we have established specific criteria for molecular relapse. Patients who meet these criteria receive treatment by donor lymphocyte infusions (DLI), if possible before the onset of hematologic relapse, because the evidence suggests that early use of DLI is more effective than if the treatment is delayed. The great majority of patients who respond to DLI achieve durable molecular remission (RT-PCR negativity), with a median follow-up of more than 2 years.

After treatment with IFN-α, virtually all patients remain RT-PCR positive despite the fact that many achieve complete cytogenetic remission. For patients in continuing cytogenetic remission, quantitative RT-PCR has demonstrated that the levels of detectable residual disease may vary between patients by as much as 10,000-fold. The actual level of residual BCR-ABL transcripts is related to the probability of relapse, opening up the possibility that molecular monitoring may identify a subset of patients for whom treatment may be safely withdrawn.

Based on current evidence, we believe that quantitative RT-PCR has clear utility for early detection of relapse after allogeneic BMT and is useful to gauge patient response after other treatments. Some of the theoretical arguments that have been used against the use of RT-PCR are, in fact, spurious. For example, although variant BCR-ABL fusions are seen in a small proportion of CML patients, there is no evidence for the evolution of subclones expressing different fusion products from the original clone. Similarly, the evidence for a neoplastic clone before the acquisition of the BCR-ABL fusion, although provocative, is weak and not widely accepted. Moreover, although it is true that very low levels of BCR-ABL can be detected in some normal individuals using modified and extremely sensitive PCR assays, this is not expected to impinge significantly on the routine analysis of minimal residual disease.

New real time quantitative RT-PCR procedures promise to greatly simplify the cumbersome protocols that are currently in use. They also offer a unique opportunity to standardize the assay and to develop rigorous standards and controls. We believe that quantitative RT-PCR will shortly become a routine and robust basis for clinical decision making in CML and other malignancies.

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**Monitoring of Residual Disease in Chronic Myelogenous Leukemia by Quantitative Polymerase Chain Reaction and Clinical Decision Making**

To the Editor:

In the May 1, 1999 issue of Blood, Faderl et al. discuss the possible clinical role of polymerase chain reaction (PCR) analysis in the surveillance of residual disease in patients with chronic myelogenous leukemia (CML). Faderl et al. raise questions regarding the significance of positive PCR reactions in patients who are in clinical and cytogenetic remission and express doubts about the possibility of using PCR analysis as a basis for therapy decisions. They indicate that a major drawback of current PCR studies is the lack of quantification of PCR data and that the short follow-up duration of published studies makes it difficult to interpret the results. The conclusions made are questionable in view of the fact that the investigators do not discuss any of the reports published by different groups on the monitoring of the dynamics of residual disease in CML patients by serial quantitative PCR analysis.2-7 Numerous investigators have demonstrated that detection of persisting residual cells expressing the chimeric BCR-ABL message by sensitive techniques such as PCR does not provide reliable information on impending relapse in individual patients with CML. During treatment with interferon-α (IFN-α), sensitive PCR assays generally show continuous presence of cells expressing the BCR-ABL fusion transcripts, and it is not possible to predict imminent relapse by qualitative PCR testing. Patients after allogeneic bone marrow transplantation (BMT) have a statistically increased risk of relapse if PCR positivity persists beyond 6 months after allografting,8 but several patients have been reported who remained in unmaintained remission for several years after BMT, despite continuous detectability of BCR-ABL mRNA by PCR. Taken together, the data available indicate that mere detection of PCR positivity in CML does not permit reliable prediction of the course of disease in individual patients. This fact has been appreciated by several investigators already a number of years ago and has provided an impetus for the development of quantitative PCR (Q-PCR) assays facilitating the assessment of disease activity. The first techniques based on competitive PCR permitting quantification of BCR-ABL transcripts were published approximately 7 years ago,5-7,12 and different groups have addressed the clinical impact of serial Q-PCR analysis in CML. In the first report on the surveillance of residual disease by a quantitative PCR approach in CML after allogeneic BMT,28 patients were presented who had been monitored in a largely retrospective study over a period of up to 106 months (median, 37 months). It has been shown that detection of increasing levels of BCR-ABL expression in serial samples from peripheral blood preceded hematologic relapse by a median of 6 months. Introduction of the term “PCR relapse” has been proposed based on the dynamics of BCR-ABL expression. PCR relapse has been defined as a 10-fold or greater increase in the relative expression of the marker gene detected and confirmed by a minimum of 3 independent, consecutive Q-PCR analyses. The proposed definition has been designed to account for the possibility of transient changes, such as fluctuating BCR-ABL expression, and the inaccuracy inherent in the technique. The possibility of predicting relapse in CML patients after BMT by the detection of increasing levels of BCR-ABL transcripts by serial Q-PCR analysis has been strengthened by a prospective study in 91 CML patients.3 The observations indicating that quantitative monitoring of BCR-ABL mRNA expression permits early prediction of disease recurrence have been further confirmed by prospective studies performed by the above-noted groups and by other investigators.5,7,12

Similar findings were also reported in CML patients on IFN-α therapy.4 We have shown that increasing levels of BCR-ABL transcripts before disease progression do not necessarily reflect an increase in the proportion of leukemic cells in the samples analyzed, but show elevated steady-state levels of the chimeric mRNA in the malignant cells.13 The fact that increasing BCR-ABL expression can be detected before proliferation of the leukemic clone underlines the value of quantitative analysis of the chimeric BCR-ABL mRNA for early prediction of relapse.

Based on the results of the early studies using serial quantitative PCR, the European Group of Investigators on CML (EICML) has provided guidelines for the clinical use of Q-PCR monitoring in CML patients.14 Hence, a number of investigators regard the quantification of BCR-ABL transcripts directed at the monitoring of the dynamics of residual disease as a useful parameter for therapy decisions. It seems, therefore, that the data provided by the reports discussed above should be considered when the potential benefit of PCR analysis in CML is critically evaluated.

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