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,4 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,5,11 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,2 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.2,7,12 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.5 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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Response

We thank Goldman et al and Lion for their letters to the editor in response to our commentary.1 We agree with both that polymerase chain reaction (PCR) technology has undoubtedly added valuable information to our current knowledge and understanding of minimal residual disease (MRD) in chronic myelogenous leukemia (CML) and other hematologic malignancies. We are also pleased that both letters address 2 basic issues regarding the clinical use of PCR: the operational (ie, clinical utility of such testing) and the biological (ie, the correlation of PCR results with the biology of MRD in CML). However, we believe that their enthusiasm for the clinical use of PCR is not supported by the available data.

Regarding the operational issue, even Goldman et al admit that PCR assays do not routinely achieve high sensitivity in practice and that their sensitivity can vary between specimens. Furthermore, qualitative PCR assays, especially at single time points, are not useful for predicting relapse. Meanwhile, quantitative PCR assays, such as those used for the serial evaluation of MRD in CML, have yet to meet the criteria expected of any laboratory test applied in clinical medicine: (1) standardization of the test across different laboratories; (2) reproducibility of positive and negative predictive values; and (3) reliable sensitivity and specificity. Because quantitative PCR testing is cumbersome, technically demanding, and highly dependent on internal standards and on the personnel conducting these tests,2 such assays should especially not be used to guide clinical treatment until they are standardized and proven to be acceptably reproducible, specific, and sensitive.

The biological nature of MRD in hematologic malignancies is intriguing. Residual disease is a dynamic process under the control of as yet unidentified mechanisms and characterized by significant variability among individuals. Furthermore, fluctuations in the level of measurable MRD do exist and, despite the technical pitfalls associated with PCR, such fluctuations have to be followed serially and quantitatively to allow any meaningful interpretation.3 However, does the lack of detection of BCR-ABL transcripts in a patient with MRD in CML mean that the patient is cured, or conversely, does a BCR-ABL-positive PCR assay herald imminent relapse? The answer is most likely no to both questions.1,3,4 Kurzrock et al3, for example, found that BCR-ABL transcripts were not detected by reverse transcriptase-PCR (RT-PCR) in 10 of 18 patients treated with interferon-α and in complete cytogenetic remission. Does PCR negativity correlate with improved survival? The answer is unclear. Talpaz et al12 analyzed patients with CML in complete cytogenetic remission who were being treated with interferon-α and were also BCR-ABL negative by RT-PCR. Using clonogenic assays combined with RT-PCR, Talpaz et al12 could still identify individual colonies that expressed BCR-ABL fusion transcripts. More recently, at the American Society of Hematology meeting in December 1998, Talpaz presented an update of these 7 patients with CML in whom long-term interferon-α treatment was discontinued and who were in unmaintained complete cytogenetic remission at a median follow-up duration of 10 years (range, 7 to 12 years). Four of the patients had detectable residual disease (30, 387, 73, and 20 BCR-ABL transcripts/µg protein, respectively). Other investigators reported similar observations.5,6 Thus, patients can apparently remain in long-term remission or probably even be cured and still express high levels of BCR-ABL transcripts (severalfold higher than those reported by Biernaux et al1 in healthy individuals).

Can a threshold of residual disease detected by PCR be defined above which relapse appears likely? Cross et al analyzed 91 patients by qualitative PCR. Twenty-eight patients with a positive PCR result were further studied by competitive PCR for quantitation of the BCR-ABL transcript. Among the 17 patients without cytogenetic relapse, BCR-ABL transcript levels ranged from 10 to 800 pg/mL. Among the 11 patients who relapsed, transcript levels ranged from 1,600 to 700,000 pg/mL, although these levels were determined at the time of cytogenetic relapse. In another study, Hochhaus et al10 quantified residual BCR-ABL transcripts by RT-PCR in 31 patients with CML who had achieved a complete cytogenetic remission after interferon-α therapy. BCR-ABL transcripts were detected in all patients with BCR-ABL transcript levels ranging from less than 10 to greater than 14,000/µg RNA. Moreover, BCR-ABL/ABL ratios for all patients ranged from 0.0007% to 3.6%, with a median of 0.024%. Among the 6 patients who later had a relapse (25 patients with residual disease did not have a relapse), BCR-ABL/ABL ratios were greater than 0.024%. Whether a similar threshold can be confirmed in other studies remains to be determined.

Is there any evidence that early intervention based on PCR monitoring is beneficial? Studies by van Rhee et al11 and Raanani et al12 suggest so, because both reported that molecular monitoring identified early relapse and that therapeutic intervention before the onset of hematologic relapse was associated with an increased probability of a response to donor lymphocyte infusions. However, both studies can be criticized, because they were not randomized, had small patient numbers, and did not clearly define early relapse. In fact, patients they considered to be in early relapse were, for the most part, patients in cytogenetic relapse, and only 2 in each study were in molecular relapse. Therefore, the studies by van Rhee et al11 and Raanani et al12 provide no evidence that initiation of therapy at molecular relapse is any better than initiation of therapy at cytogenetic relapse. We are still left with a lack of data as to the usefulness of therapy that is based on positive PCR results.

In conclusion, we again agree that PCR studies aimed at detecting, quantifying, and characterizing MRD have contributed valuable information to our understanding of MRD in hematologic malignancies. The
To the Editor:

In a recent report in *Blood*, Seyama et al.1 described 28 unique mutations of the *CD40 ligand* (*CD40L*) gene in 45 X-linked hyper IgM syndrome (XHIM) patients from 30 unrelated families. Generally, peripheral blood mononuclear cells (PBMC) are screened for CD40L expression after activation with phorbol myristate acetate (PMA) and ionomycin to acquire XHIM. Activated PBMC of most XHIM patients did not express functional CD40L (CD154); they failed to bind a CD40-Ig fusion protein (bCD40-Ig). The investigators discerned 5 different CD40L staining patterns on cultured T cells, using a polyclonal antiserum (pAb), 4 different monoclonal antibodies (McAb), and bCD40-Ig. The type 1 pattern showed weak staining of CD40L with all reagents mentioned; the type 2 through 5 patterns successively showed loss of functional activity (bCD40-Ig binding) and protein epitope expression (loss of 1 to 4 McAb and finally pAb binding). A relationship between genotype and phenotype was suggested, with “milder genotypes” (resulting in staining pattern type 1 or 2) showing milder clinical phenotypes: in 5 of 10 patients from 9 families with staining pattern type 1 or 2, symptoms started relatively late, and none of them suffered from opportunistic infections. Two patients with the 782C → T mutation, a missense mutation in exon 5, fit into this group.

We found the 782C → T XHIM mutation in 4 patients from 2 different families. Although they showed a favorable clinical course after Ig replacement therapy was started, the 2 index patients had symptoms from an early age onwards, including 1 who presented with Pneumocystis carinii pneumonia (PCP). The other 2 children were diagnosed shortly after birth because of the XHIM index patient in the family.

The first index patient, patient no. 1 (of family A), is a young Dutch man, now 19 years of age, who was treated with Ig replacement therapy from the age of 2 years onward because of hypogammaglobulinemia and frequent respiratory tract infections. Since then, the frequency of infections has normalized. He never had an infection with an opportunistic pathogen and never received prophylaxis with Co-trimoxazole. Recently, stimulation of his PBMC with PMA and ionomycin induced expression of the early activation marker CD69 (Leu-23; Becton Dickinson, San Jose, CA) on his CD3+ T lymphocytes, whereas CD40L (LL48; Schering-Plough, Dardilly, France) expression was absent (Fig 1). Fluorescent sequencing of the *CD40L* gene showed a 782C → T mutation (expected protein alteration T254M). The family was screened,

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The 782C → T (T254M) XHIM Mutation: Lack of a Tight Phenotype-Genotype Relationship

To the Editor:

The presence of *BCR-ABL* transcripts in patients who are probably cured is undoubtedly interesting. If these transcripts represent residual disease, then the identification of the mechanism responsible for its suppression is very important. However, we again assert that, thus far, it has not been established that detection of MRD in CML by PCR can be used clinically. Until PCR testing has been standardized and proven reliable and until randomized studies have proven that treating molecular relapse is indeed beneficial for patients with CML, PCR-based results should be interpreted only within the confines of well-defined clinical trials. Consequently, any generalized guidelines for using PCR tests to guide treatment appear premature and should be viewed critically.
Monitoring of Residual Disease in Chronic Myelogenous Leukemia by Quantitative Polymerase Chain Reaction and Clinical Decision Making

Thomas Lion