Detection of Minimal Residual Disease in Acute Lymphoblastic Leukemia

To the Editor:

We appreciate the comprehensive review on minimal residual disease in patients with acute lymphoblastic leukemia (ALL) written by Campana and Pui, but we believe the discussion relevant to polymerase chain reaction (PCR) assays and immunologic methods used to detect residual disease in ALL requires comment.

The authors suggest that the realistic sensitivity for multi-parameter flow cytometric analysis is about 1 target cell in 10⁴ normal mononucleated marrow cells. They cite four examples as evidence that PCR is not consistently more sensitive than this level, despite the abundance of studies that clearly show reliable detection in the 10⁻⁷ range. The first example, a "fingerprinting" method, does have a lower sensitivity limit, but recently, because of this characteristic, the assay was chosen to screen for marrow samples with high leukemia cell concentration (>10³³). Fingerprint-negative samples were subsequently analyzed with an allele-specific oligonucleotide in a two-step PCR procedure that increased overall sensitivity to the 10⁻⁴ range. The second and third cited examples of PCR studies were performed on the same DNAs isolated from archival bone marrow smears. The samples in about half of the cases yielded less than 10⁴ intact genomes. Thus, the samples directly limited the sensitivity of residual disease detection rather than the PCR methods used for these studies.

The last example is from the studies performed in Ulm. It is remarkable to note that this group has previously reported a comparison between immunologic methods and their PCR technique (as reported by Campana himself). Despite the suggestion that some cases may be screened with suboptimal sensitivity, the results from the comparison showed that all samples were positive for residual leukemia when tested by PCR. By contrast, only 25% were positive using immunologic methods, resulting in false-negative results for half of the patients analyzed. Since the submission of the review by Campana and Pui, the Ulm group has developed a strategy to further improve their PCR sensitivity to uniformly meet or exceed the 10⁻⁷ level.

Taking all of the results together, it can be concluded that PCR is routinely capable of detecting residual leukemia at a significantly greater sensitivity than immunologic methods. Two additional considerations lead to the conclusion that PCR is preferable to immunologic techniques for studying residual disease in childhood ALL.

First, in addition to lack of absolute specificity for a particular leukemia cell population by immunologic techniques, differentiation, characterized by changes in antigen expression, occurs in some ALL...
cases. In such cases, antibody combinations may detect only a certain subpopulation of the total residual leukemia cells and not more differentiated or undifferentiated malignant cells, thereby underestimating the extent of disease. This would be most critical when leukemia progenitor cells, the small population of cells that propagates and maintains the entire population of more-differentiated leukemia cells, lack one or more of the antigens identified by a panel of antibodies. Second, it is important to consider that over 90% of patients with either B-precursor or T-cell immunophenotypes can be studied by PCR whereas only about 50% of childhood ALL cases have a “leukemia-associated” antigen phenotype satisfactory for immunologic analysis.

Does the increased sensitivity for detecting residual disease by PCR actually inhibit the accuracy for predicting relapse? The authors suggest that the presence or absence of residual disease as detected by PCR is not consistently predictive of clinical outcome. However, the technical factors that are critical to interpreting each of these studies are not discussed. We suggest that an entirely different interpretation can be reached when key factors such as patient population, specimen source and quality, and PCR methodology are considered. The combined data from the 20 studies published to date indicates that a consistent pattern for residual disease disappearance over many months exists for patients who remain in extended complete remission and a pattern of residual disease persistence and reappearance preceding clinical findings exists for the majority of those who ultimately relapse. Furthermore, as suggested by Campana and Pai, accurate estimates of residual disease are more likely to be informative than simply detecting either the presence or absence of occult leukemia cells. In this regard, we have recently developed a limiting-dilution PCR method for estimating residual disease in B-precursor ALL that is highly accurate (standard deviation averages 0.5, an order of magnitude) without compromising sensitivity (5 x 10^-10). We are conducting a prospective study of residual disease in childhood and adult ALL using both this quantitative PCR method and a clonogenic assay performed in parallel on marrow samples obtained serially during remission.

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REFERENCES

Response

The letter of Roberts et al compels us to reiterate some of the concepts that we and many of our colleagues thought were clearly stated in our report. As we extensively discussed, there are several methods that can be used to detect minimal residual disease (MRD) in cases of acute lymphoblastic leukemia (ALL), each with its own advantages and limitations. Because none of these techniques is either perfect or universally applicable, we recommended simultaneous use of different approaches. In our laboratory, for example, we apply both immunologic methods and polymerase chain reaction (PCR) to study MRD in children with ALL.

Roberts et al contend that the published studies of MRD detection by PCR collectively indicate consistent "patterns" of residual disease appearance and disappearance. If so, why have they undertaken a study to assess, yet again, the informative value of PCR? Would it not be logical, based on their interpretation of the literature, to select treatment for ALL by PCR findings? We would argue that the patterns emerging from published studies of MRD are not nearly as clear as Roberts and his colleagues suggest, and that PCR findings during treatment remain difficult to interpret, hindering their clinical application.

They also question our statement that PCR has not consistently surpassed the 10^-4 sensitivity level in published studies comparing MRD findings with clinical outcome. Besides the four reports we cited to support this statement, at least five others, in which DNA was obtained from fresh or cryopreserved samples (rather than smears) and the "fingerprinting" technology was not used, can be provided as corroborating evidence (see also review by Potter et al). However, this is not to say that PCR methods are incapable of
higher sensitivities; to the contrary, the MRD detection limit may be as high as 10^{-4}, as indicated in Tables 2 and 5 of our review. We would stress that it was not our intention to downplay the potential usefulness of PCR assays, which we are using ourselves.

Roberts et al refer to our earlier comparative analysis of immunologic and PCR methods as an example of the superior sensitivity of PCR. However, more important than sensitivity is the clinical usefulness of the MRD assay, which may depend on how well it discriminates viable from apoptotic leukemic cells. As we have pointed out, PCR could amplify DNA sequences from dying cells (eg, those undergoing apoptosis due to chemotherapy), whereas immunologic techniques would identify only viable cells.9 Other potential limitations of PCR have been noted by Estrov and Zipf themselves, who pointed out that PCR also fails to determine either the cells' proliferative activity or stage of differentiation.9 In one of their reports they state that "... measuring residual disease by PCR in the entire mononuclear cell fraction of a remission bone marrow specimen could lead to incorrect conclusions regarding that status of the patient's disease."10 Thus, the authors have developed a new assay incorporating assessment of colony growth in an attempt to overcome this limitation of PCR,11 although neither the percentage of cases that can be studied with this technique nor its relative sensitivity is known. We are also concerned that rapidly dividing clonogenic cells studied in vitro may not correspond to the leukemic lymphoblasts that survive and proliferate in the patient.

Attempts to improve MRD quantitation by PCR methods are commendable, but the PCR-based limiting dilution method developed by their group11 appears to be too cumbersome for daily use with multiple samples. Moreover, possible discrepancies in specimen integrity between diagnostic reference sample and test sample could well limit the accuracy of this approach. Although we endorse efforts to test the validity of PCR-based assays in a prospective fashion, we cannot predict which of the techniques discussed in our review will prove to be the most robust and clinically informative.

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REFERENCES


Detection of minimal residual disease in acute lymphoblastic leukemia [letter; comment]

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