To the editor:

Optimizing fusion transcript monitoring in CML

The recently published recommendations for the harmonization of methodology for BCR-ABL fusion transcript monitoring in chronic myeloid leukemia (CML) patients responding to treatment with tyrosine kinase inhibitors are timely and pertinent for all laboratories undertaking such testing. The authors provide practical guidelines for laboratories to help optimize their testing and reporting procedures in a manner designed to improve assay performance and the generation of reliable quantitative data across different molecular laboratories.

One of the consequences of the current lack of standardization of quantitative BCR-ABL testing relates to the reliability of detection of a rise in the level of fusion transcript that indicates potential disease progression. That threshold level of transcript rise remains contentious, since some have suggested a 2-fold rise in transcript is significant, while others have argued for a 5-fold rise or more. In clinical practice, what matters is the trend in polymerase chain reaction (PCR) value and confirmation of an increase in transcript. Certainly, the clinical implications of a confirmation of disease progression, as indicated by a rise in transcript. Certainly, the clinical implications of a confirmation of disease progression, as indicated by a rise in transcript, are substantial and include undertaking kinase domain mutation screening, imatinib dose escalation, a switch to an alternative tyrosine kinase inhibitor, or even allogeneic stem cell transplantation.

Accordingly, these clinical consequences underscore the importance of accurate and reliable analysis of BCR-ABL transcript values in these patients. However, the inherent interassay and intra-assay variability can lead to considerable uncertainty in the confirmation of any putative transcript rise. Furthermore, any variability in testing depends upon the level of detection. That is, as the level of fusion transcript declines progressively, and approaches the limit of detection of the assay, so the variability in assay performance rises. In fact, a 2-fold rise (or greater) in transcript that occurs at relatively low transcript levels may well lie within the calculated coefficient of variation (CV) of the quantitative PCR assay. Suggested assay modifications to help minimize this inherent assay variability include the use of duplicate or triplicate samples or even inclusion of the previous sample in the assay run. However, this can set up a complex and expensive testing scenario, which may still not achieve the requisite improvements in testing accuracy.

Of most importance, the planned international assay standardization procedures for quantitative BCR-ABL testing, involving exchange of reference standards and quality control samples, are likely to be pivotal in reducing this assay variability. It is anticipated that these standardization measures will maximize the reliability of quantitative BCR-ABL testing for clinical decision making particularly among patients for whom there may be concerns about potential escape from disease control.

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References


Response:

Reliability of PCR for BCR-ABL transcripts

We agree with the statements made by Hertzberg and McDonald regarding the reliability of the measurements at different levels that are acceptable over the dynamic range to account for the wider variability at very low levels of BCR-ABL transcripts. A stratification of assay variability has previously been demonstrated over the dynamic range; it was 4.5-fold at a value representing 0.01% and more than 2-fold for values above 0.1%. Our paper emphasized that a more than 2-fold rise occurring between values equating to 0.01% and 0.1% may fall within the coefficient of variance (CV) of the assay and would not then have biologic significance. Our more recent paper also suggested that to maximize reproducibility and to enhance detection of low BCR-ABL transcript levels, the ideal would be to divide the sample into 2 aliquots and assay both samples separately. Each analysis would include both the reverse transcriptase (RT) and the quantitative polymerase chain reaction (PCR) stages. Therefore, our recommended procedure already does take into account the possible impact of interassay variability that Hertzberg and McDonald address.

The suggestion from Hertzberg and McDonald that including the previous RNA sample for each patient will mitigate the effect of interassay variability is of interest. One must remember, however, that thawing and retesting RNA open the door to degradation during storage and during thawing. Degradation may introduce additional variability of the result since BCR-ABL levels will be lowered in this situation. The authors need to demonstrate that their suggested method is more reliable for identifying a true rise rather than merely to use appropriate criteria based on the CV of the assay, which reflects the varying measurement reliability across the dynamic range. Thus, the Hertzberg and McDonald suggestion may prove valuable, but before it is accepted as a major advance it
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