Response:

Conventional Western blotting techniques will not reliably quantify p210 BCR-ABL

We welcome the opportunity to respond to the comments of Patel et al. We were aware of the selective rapid degradation of p210BCR-ABL and c-ABL after lysis of total mononuclear cells (MNCs), which has been reported previously1 and makes assessment of p210BCR-ABL and c-ABL protein levels in mature cell compartments difficult. In order to overcome this, we used multiple techniques to assess BCR-ABL expression in chronic myeloid leukemia (CML) cells.

First, we measured BCR-ABL transcripts using real-time quantitative reverse transcriptase–polymerase chain reaction (RT-PCR). This showed that, compared with total MNCs, BCR-ABL transcripts were significantly increased in both CD34+ and CD34+/CD38− populations (P = .016 and P = .031, respectively). We then developed a novel flow cytometry assay to measure CrKL phosphorylation (P-CrKL), as a marker of BCR-ABL activity in CML cells.2 This confirmed that P-CrKL was significantly increased in CD34+/CD38− populations compared with total CD34+ populations. The P-CrKL assay was then validated by Western blotting using a method previously described3 and comparing MNCs, CD34+ cells, and CD34+/CD38− cells. Once again the levels were lowest in MNCs—we are not aware that the degradative activity should affect P-CrKL. This Western blotting technique has also been used for the detection of BCR-ABL, c-ABL, and P-Tyr in BCR-ABL–transduced CD34+ cord blood samples cultured for up to 12 days (Ravi Bhatia, City of Hope National Medical Center, Duarte, CA; personal communication, October 15, 2006). By 12 days, these cultures contain differentiated cells equivalent to an MNC preparation, and we therefore felt confident using this technique for assessment of BCR-ABL and P-Tyr in our experiments. In addition, another group has also successfully developed a flow cytometry assay for measuring total P-Tyr in MNCs and CD34+ CML cells to predict response to imatinib treatment.4,5

Based on the combination of techniques used, we do believe that BCR-ABL levels are very significantly lower in MNCs than in CD34+ and CD34+/CD38− populations. However, we would disagree with the statement that BCR-ABL levels would be expected to be lower in the CD34+ compared with CD34+/CD38− population due to degradative activity. First, all the CD34+ selected CML samples we used were more than 95% CD34+ after magnetic-activated cell sorting using the CLINIMACS system (Miltenyi Biotec, Bisley, United Kingdom). This was confirmed by flow cytometry after cell sorting, and in some cases the samples were more than 98% purified for CD34+ cells. Therefore, we believe that the degradative activity from contaminating mature myeloid cells in these samples would be negligible. Further, in the report by Maxwell et al.,1 they found that enriching blast cells by Ficoll-Hypaque gradient centrifugation in blast crisis CML samples was sufficient to detect p210BCR-ABL kinase activity.

We would be concerned by the results of Patel et al.,6 who failed to detect BCR-ABL protein expression in CD34+ CML cells. This has never been an issue in our experience. While we find their description of a novel method to overcome the degradative activity of total MNCs using high pH to inhibit an, as yet, unidentified acid-dependent hydrolase interesting, because of the increased degradative activity in total MNCs, we would wish to see it validated in total MNCs as well as CD34+ cells.

We would like to thank Patel et al for highlighting the importance of the degradative activity of total MNCs on BCR-ABL, c-ABL, and P-Tyr levels and will keep this in mind for future Western blotting experiments.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


To the editor:

Update on factor V Leiden association with venous thromboembolism in the LITE Study

In a previous article,1 we reported population-based findings from the Longitudinal Investigation of Thromboembolism Etiology (LITE) on factor V Leiden and risk of venous thromboembolism (VTE). We used a nested case-control design (301 new VTE cases and 630 controls through 1998) from the prospective Atherosclerosis Risk in Communities (ARIC) Study and Cardiovascular Health Study (CHS). The odds ratio, overall, for risk of VTE for carriers of factor V Leiden was 3.67 (95% CI, 2.20-6.12).1

We recently extended LITE to additional cases and controls through 2002. In the process, we discovered and corrected an error in the original selection of controls for ARIC that had caused us to oversample participants who had died into the control group for our earlier report. Compared with our published report, the updated sample of 502 cases and 1021 controls yielded an almost identical odds ratio for factor V Leiden of 3.46 (95% CI, 2.20-5.43). Genotype distributions and other odds ratios for factor V Leiden
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