Letters to the Editor

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References


5. Abl RNA transcripts are suitable molecular markers for minimal residual disease (MRD) analysis in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) and may be used to guide therapy. However, MRD analysis by real time polymerase chain reaction (RT-PCR) may be affected by intra- and inter-assay variability as well as potentially by changes in Bcr-Abl copies per blast and differences between bone marrow (BM) and peripheral blood (PB).

PB and BM samples were obtained from 56 patients with relapsed or refractory Ph+ ALL who were treated in phase II studies with imatinib (CIST/51 109 and CIST/51 114) as described previously.3,4 Total RNA was extracted from mononuclear cells (Ambion) and cDNA synthesized from 1–5 µg RNA (RNA at 70°C for 10 min, 4°C until addition of core mix including 500 U SuperScript II RTase II (Invitrogen), 50 U RNAguard (Amersham), 6.25 µM dNTPs (Amersham), 1 µM dNTP and 10 µM DTT in 50 µL; then thermocycled at 25°C for 10 min, 42°C for 45 min and 99°C for 3 min) before undergoing column purification (Qiagen). Ten-fold plasmid standard dilutions with 10 to 10³ copies per blast and differences between bone marrow (BM) and peripheral blood (PB).

Bcr-Abl RNA transcripts are suitable molecular markers for minimal residual disease (MRD) analysis in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) and may be used to guide therapy. However, MRD analysis by real time polymerase chain reaction (RT-PCR) may be affected by intra- and inter-assay variability as well as potentially by changes in Bcr-Abl copies per blast and differences between bone marrow (BM) and peripheral blood (PB).

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The correlation coefficient of paired PB and BM levels of e1a2, b2a2 or b3a2 was high (0.9, 0.85 and 0.93, respectively). Levels of Bcr-Abl were higher in BM than in PB: by 0.8 log for e1a2 (p<0.001), 0.3 log for b2a2 (p=0.20) and 0.7 log for b3a2 (p=0.004). Since Bcr-Abl levels normalized to 100%
blasts did not differ significantly between BM (–1.31) and PB (–1.10), differences in Bcr-Abl levels between PB and BM in individuals appear to reflect different proportions of leukemic blast cells.

The numbers of Bcr-Abl transcripts per blast in 25 sequential BM and 8 sequential PB sample pairs did not change significantly during evolution of Ph+ ALL. Furthermore, intra-individual variance did not differ from technical variance. The mean number of p210 copies per blast was more than 1.1 log higher than that for p190 (p=0.0006) (Figure 2).

We found a sensitivity between 10^{-5} and 10^{-6} with patients’ blasts, which is similar to the 10^{-5} sensitivity in other reports.5 The technical variability increased with decreasing MRD levels. At high (>10^{-4}), low (<10^{-4} to >10^{-6}) and very low (<10^{-6}) Bcr-Abl/GAPDH levels, changes by more than 0.6 log, 1.0 log and 1.5 log, respectively, are considered significant. However, clinical therapeutic decisions should be based on more than one sample.

Our data confirm previous reports that BM samples offer a greater sensitivity in B-lineage ALL monitoring. 5-8 However, the degree of divergence between PB and BM levels may vary according to treatment phase.3

The fact that we found higher copy numbers of p210Bcr-Abl per blast than p190Bcr-Abl, confirming observations of others who found a 6- to 7-fold difference,5 suggests that specific prognostic criteria may be required for these subgroups.

Figure 1. Technical inter-assay variability of Bcr-Abl quantification in aliquots (n=5) of one patient’s sample is depicted for transcripts e1a2 (a), b2a2 (b) and b3a2 (c) at various MRD levels. Technical variability reflects inter-assay variability of RNA extraction, cDNA synthesis and Taqman PCR for Bcr-Abl and GAPDH. Logarithmic values of Bcr-Abl/GAPDH ratios are shown with means and calculated range including 90% of the Gaussian distribution. A change of the Bcr-Abl level to a value outside this range in a subsequent sample would indicate an actual biological increase or decrease of Bcr-Abl expression (5% probability of error).

Figure 2. Logarithmic Bcr-Abl/GAPDH values normalized to 100% leukemic blasts in patients with p210^{Bcr-Ab} (b2a2 or b3a2) were 1.1 log higher than those in patients with p190^{Bcr-Ab} (e1a2) (p=0.0006). Individual statistical sub-testing revealed a difference between both e1a2 and b2a2 (p=0.014) as well as between e1a2 and b3a2 (p=0.008), but not between b2a2 and b3a2 (p=0.98). Within a group of patients with the same fusion transcript, inter-individual variance did not exceed intra-individual variance significantly.

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Key words: acute lymphoblastic leukemia (ALL), Philadelphia chromosome, Bcr-Abl tyrosine kinase, real-time PCR, minimal residual disease (MRD).

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The Philadelphia chromosome (Ph) is the cytogenetic hallmark of chronic myeloid leukemia (CML) and is present in approximately 90–95% of patients. The loss of genomic sequences on der(9) chromosome has been described in approximately 9% of CML patients. These deletions identify a sub-group with a worse prognosis. Albano et al. recently reported the occurrence of deletions on the third chromosome involved in variant complex t(9;22) translocations. In the present paper, we report the molecular cytogenetic characterization of a Ph+ chromosome/Bcr-Abl positive acute lymphoblastic leukemia with the Abl-tyrosine kinase inhibitor imatinib (STI571). Blood 2003;101:85-90.

Figure 1. A) FISH co-hybridization with clones specific for ABL (red) and BCR (green) genes showed a fusion signal on both Ph and der(9) chromosomes. A faint BCR signal was observed on the der(9) chromosme, suggesting a partial deletion. B) WCP#14 (red) and WCP#15 (green) revealed the t(14;15) translocation. C) FISH experiments with clones RP11-796G6 (red) and RP11-356L8 (green) allowed us to map the breakpoint in 14q32.31. D) Clones RP11-350L3 (red) and RP11-114H15 (green), both mapping in 14q32.31, gave hybridization signals only on the normal 14 chromosome, indicating deletion of the chromosomal region encompassed by the two clones.

A novel translocation t(14;15)(q32;q24) bearing deletion on der(14) in Philadelphia-positive chronic myeloid leukemia

We report the molecular cytogenetic characterization of a case of Philadelphia positive chronic myeloid leukemia (CML) with a t(14;15)(q32;q24) at onset, showing deletions on der(14) in addition to the loss of chromosome 22 sequences on der(9). To our knowledge, the presence of deletions on chromosomes involved in distinct and concomitant rearrangements other than t(9;22) has never been observed in CML patients.

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References