Abstract

The BCR-ABL fusion gene has a strong causal association with chronic myeloid leukemia (CML). This justifies the interest in understanding the origin of the BCR-ABL rearrangement, and in using BCR-ABL to measure residual disease in CML patients. BCR-ABL mRNA is present in the peripheral blood of a significant proportion of healthy individuals, which raises questions relating to 1) the development of CML; and 2) the interpretation of the low-level positivity for BCR-ABL mRNA that is occasionally seen in patients in long-term remission (LTR) post-hematopoietic stem cell transplantation (HSCT), most of which will not experience disease relapse and are functionally cured. With imatinib, some patient become negative for BCR-ABL transcripts, achieving what has been termed a complete molecular response (CMR). It is not clear whether this is an attainable ultimate goal of therapy for the majority of patients, given that transcripts are detectable in healthy individuals and in patients in LTR post-HSCT. Most imatinib-treated patients in CMR relapse within months if therapy is stopped; it is not known whether CMR equates with eradication of the CML clone in those patients that remain relapse-free without imatinib.

We initially wanted to investigate whether the transcript-positivity of healthy individuals originates from immature hematopoietic progenitor cells (CD34+), a task that is complicated by their paucity. The experimental system we used to expand CD34+ cells in vitro produced significant proliferation, which was associated with myeloid differentiation and decreased BCR-ABL expression. We did not pursue this approach further since we could not demonstrate that it would increase the sensitivity of detection of putative rare CD34+, BCR-ABL-positive cells.

Unlike BCR-ABL transcripts, the genomic BCR-ABL fusion (gBCR-ABL) is virtually unique to each individual CML case. We sequenced gBCR-ABL from CML patients in order to design patient-specific, highly sensitive, DNA quantitative polymerase chain reactions (PCR) to test follow-up samples from patients in LTR post-HSCT and in CMR on imatinib for the presence of the original leukemic clone. The gBCR-ABL sequences were also used to try to understand the genesis of the rearrangement. Analysis of breakpoints from p210 CML and p190 and p210 acute lymphoblastic leukemia cases found no evidence for significant clustering and no association with sequence motifs, except for a breakpoint deficit in repeat regions.
within *BCR* for p210 cases; it suggests that p190 and p210 rearrangements are formed by different mechanisms.

Returning to our main objective, we sought to elucidate the quality of the molecular response in imatinib-treated patients in CMR and in patients in LTR post-HSCT using a genomic DNA based PCR. In 5 imatinib-treated patients in CMR, \(gBCR-ABL\) was detected in transcript-negative samples; 4 patients became \(gBCR-ABL\) negative with continuing imatinib therapy. In contrast, of 9 patients in LTR (13-27 years) post-HSCT, \(gBCR-ABL\) was detected in only 1, despite occasional transcript-positive samples in 8 of them.

We concluded that, in imatinib-treated patients, absence of transcripts should not be interpreted as absence of the leukemic clone, although continuing imatinib after achievement of CMR may lead to further reduction of residual disease. Post-HSCT, we found little evidence that the transcripts occasionally detected originate from the leukemic clone.