Blood (ASH Annual Meeting Abstracts) 2011 118: Abstract 1687

632. Chronic Myeloid Leukemia - Therapy: Poster I

Chronic Myeloid Leukemia: Molecular Monitoring of Residual Disease by Genomic DNA Compared to Conventional mRNA Analysis in Follow-Ups up to 8 Years

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Background: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder resulting from the t(9;22)(q34;q11) balanced reciprocal translocation within a pluripotent stem cell (SC). The resulting Philadelphia (Ph) chromosome produces BCR-ABL1 fusion gene coding for a deregulated Abl tyrosine-kinase with constitutive and tumorigenic activity. The first line therapy of CML is imatinib mesylate, which targets Bcr-Abl protein, inhibiting proliferation pathways. Complete cytogenetic response can be achieved in 95% of patients treated in the early chronic phase (CP)[1]. Molecular monitoring of minimal residual disease is crucial to detect poor responses to imatinib and optimizing treatment with second generation tyrosine-kinase inhibitors or allogeneic stem cell transplantation. Residual leukemia is assessed by a quantitative manner evaluating levels of BCR-ABL1 transcripts by real-time reverse transcriptase PCR (qRT-PCR). Although qRT-PCR detects mRNA levels in a very sensitive manner, the negative result is difficult to interpret, because undetectable levels of chimeric transcript can reflect either an effective elimination of leukemia cells, or the presence of a quiescent leukemia SC transcriptionally silent.

Methods: We developed a novel highly sensitive method to identify quiescent leukemic cells through quantitative real-time PCR (Q-PCR) targeting the genomic breakpoint sequence[1]. In CML each patient shows a unique genomic fusion sequence[1], that need to be characterized in order to design a specific genomic assay. We selected 14 patients with CML diagnosed in the early CP. We identified junctions sequences by long-range PCR[2]. We carried out Q-PCR assay using common primer forward and probe in BCR, and 2 different primers reverse, in ABL or BCR, used as control[1]. The percentage of leukemic cells (LCs) was calculated using the derivation of the δCt formula[1]: LC= [100*(2/2$^{\delta Ct+1}$)]/n], where δCt is the difference between amplification cycles of BCR-ABL1 and BCR reactions, and n is the number of experimental replicates. We tested the sensitivity and the efficiency of the method on K562 cell line. According to the human C value, K562 were diluted in normal commercial genomic DNA until 10^{-4} dilutions. Eight CML patients in early CP were the object of this study. A patient specific Q-PCR assay was performed on DNA obtained at diagnosis and subsequently applied to monitor minimal residual disease during Imatinib treatment for up to 8 years, for a total of 61 samples. In parallel the same peripheral blood samples were tested by standard qRT-PCR, and the percentage of residual disease (international scale) measured by mRNA was compared with DNA analysis.

Results: Positive levels of mRNA were obtained in 79% of samples analyzed by qRT-PCR,while we detected Ph-positive cells in 92% of samples. In all positive samples for chimeric transcript we measured positive levels of corresponding genomic DNA, confirming the sensitivity of the Q-PCR method. In 13% of samples, with undetectable levels of mRNA, we observed the persistence of quiescent leukemic cells, transcriptionally silent like shown by patient 2 in figure 1. This could probably indicate the presence of pluripotent LSCs or progenitor cells, that does not respond to imatinib treatment. Finally undetectable levels of mRNA were confirmed by a correspondent DNA negativity in 8,2% of the samples. This datum should be investigated further in order to establish if the disease was been eradicated. Patients negative by mRNA detection in several consecutive follow-ups could be candidates to stop imatinib therapy. The development of

a DNA base technique could be a powerful tool to evaluate the effective presence/absence of leukemic cells. Patient 8 resulted negative at 70 months monitored by RNA and DNA technique could be a candidate to stop the therapy (figure 2).

Conclusion: Although the initial characterization of genomic breakpoint sequence is still time consuming, it may provide a patient-specific DNA biomarker that can be used to detect the presence of quiescent leukemic cells otherwise undetectable using a conventional qRT-PCR. The DNA genomic Q-PCR could be a very sensitive and direct technique to detect minimal residual disease in CML patients treated with tyrosine-kinase inhibitors and allogeneic transplantation.

We thank AIL Varese and Bergamo.

Disclosures: No relevant conflicts of interest to declare.

Footnotes

* Asterisk with author names denotes non-ASH members.