Molecular Biology for BCR-ABL1 Quantification for Chronic Myeloid Leukemia Monitorization and Evaluation

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Chronic Myeloid Leukemia (CML) is a clonal disorder originated by a pluripotent hematopoietic stem cell, which presents the translocation t(9;22) (q34;q11) in 90% of the cases. This genetic abnormality is a balanced translocation between Abelson Murine Leukemia (ABL) located in chromosome 9 with the Breakpoint Cluster Region (BCR) gene at chromosome 22, generating Philadelphia chromosome (Ph) or BCR-ABL1, which codes an oncoprotein of 210 kDa [1-4]. This alteration represents a hallmark in oncology and for CML research, diagnosis, and prognosis. The standard treatment of CML patients is with Tyrosine Kinase Inhibitors (TKIs), and the first line is Imatinib [5]. This drug was developed in 1990s by Brian Druker and Nicholas Lydon and in 1998 a phase 1 clinical trial was conducted, causing cancer regression in most patients with CML in chronic phase. Five years later, 98% of patients from this trial were still in remission. In 2001, this drug was approved by FDA, revolutionizing the treatment of CML. Imatinib is quite selective for BCR-ABL1 oncoprotein, so it does not inhibit other tyrosine kinase enzymes, so far representing one of the pioneers in oncological targeted therapy [6].

The detection of the BCR-ABL1 translocation is an important method for CML diagnosis and can be also used to monitor the disease. Some approaches can be used to detect BCR-ABL1 translocation, such as RT-qPCR, bone marrow karyotyping, Fluorescence in Situ Hybridization (FISH), flow cytometry, anchored ChromPET, and Digital PCR. The quantification of BCR-ABL1 expression quantification by molecular techniques in CML patients is a mandatory and crucial process to monitor clinical management and TKIs treatment effectiveness. In this scenario, the most common and standardized method is RT-qPCR, which has been extensively validated and is based on IRIS studies [7-9], NCCN and ELN guidelines [5,10]. The standardization of BCR-ABL1 monitoring is based on the use of an international scale (IS), which is a numeric scale anchored to the standardized baseline established in the IRIS trial (100%IS) with a 3-log reduction from baseline defined as a major molecular response (MMR; MR3; 0.1%IS). This method relies on the quantification of the expression of BCR-ABL1 and a reference transcript (usually ABL1, BCR or GUSB), followed by the generation of a ratio between BCR-ABL1/Reference (in logarithmic result). Ratios of 10%, 1%, 0.1%, 0.01%, 0.0032%, and 0.001% correspond to 1, 2, 3, 4, 4.5, and 5 logs reduction, respectively [11-13]. BCR-ABL1 ≤ 1% is denoted as Complete Cytogenetic Remission (CcR), while BCR-ABL1 ≤ 0.1% is defined as major molecular response (MMR) or MR3. A molecular response 4 (MR4) occurs when the levels of BCR-ABL1 transcripts are ≤ 0.01% or undetectable with >10,000 ABL1 transcripts. A MR4,5 is defined by ≤ 0.0032% BCR-ABL1 transcript levels or by undetectable with >32,000 ABL1 transcripts. Finally, the last molecular response is MR5, reached when BCR-ABL1 transcript levels are ≤ 0.001% and with >100,000 ABL1 transcripts [5,14]. The monitoring of BCR-ABL1 transcript levels by the IS must be performed at 3, 6, and 12 months, to determine whether the current treatment should be continued (optimal response), changed (failure/resistance), or carefully considered for continuation or change, depending on patients’ characteristics, comorbidities and tolerance (warning) [5].

Treatment of CML patients with TKIs is efficient in about 95% of cases, but before considering drug discontinuation, patients must be monitored at a deeper molecular level (MR4 or deeper) for long periods. So, monitoring has an important role in managing the CML discontinuation or in identifying patients at risk of progression. In this context,
droplet digital PCR (dd-PCR) is becoming a prominent and important player once because of it presents advantages over RT-qPCR. For example, this method relies on amplification performed independently in each droplet, eliminating the need of internal references and standard curves, so that dd-PCR can be considered a direct absolute quantification. Also, it has been proved that dd-PCR is more sensitive to detect targets at low copy numbers than the RT-qPCR [15], showing the higher sensitivity and accuracy of this method. The estimated absolute quantification in dd-PCR is derived from the count of the proportion of positive droplets relative to the total number of droplets, using Poisson distribution [16].

In a comparative study performed with K562 cell line suspension dilutions (10^6-10^-6), the minimum detectable concentration of BCR-ABL1 was 10^-6 in dd-PCR, while in RT-qPCR it was 10^-5 [17]. This same study showed that, from 61 CML negative patients by RT-qPCR, 11 were positive by dd-PCR. Also, BCR-ABL1 transcripts were detected earlier by dd-PCR in 4 out of ten patients with CML (MR4.5 ≤ 6 months). The results indicated the higher sensitivity of dd-PCR compared to RT-qPCR in CML quantification, showing its amazing applicability.

Other study that compared dd-PCR with RT-qPCR used cDNA of 230 CML patients and showed some shifts between MR classes. The use of dd-PCR assay resulted in a shift to lower molecular response classes compared to RT-qPCR aligned to International Scale. By RT-qPCR, 39 patients (17%) were >MR3, 91 patients (40%) were scored MR3, 33 patients (14%) MR4, 44 patients (19%) MR4.5, and 23 pts (10%) MR5. In contrast, by dd-PCR 53 patients (23%) did not achieve MR3, 100 pts (43%) were in MR3, 35 patients (15%) in MR4, 20 patients (9%) in MR4.5, and 22 (10%) in MR5 (P=0.02). Comparatively, fewer patients (n=77) achieved MR4 or better by dd-PCR as compared to RT-qPCR (n=100, P=0.035, Chi-Square-Test). Considering individual samples, 134 patients (58%) were classified in the same MR category by both methods, and twenty-five patients (11%) were scored into deeper molecular response categories and 71 patients (31%) into a less deep molecular response group by dd-PCR [18].

In an analytical study, Chung and collaborators [19], evaluated the performance of the QXDx BCR-ABL1 %IS (Bio-Rad, Hercules, CA, USA) droplet digital PCR (dd-PCR) and correlated with BCR-ABL1 Mbcr IS-MMR (Qiagen, Hilden, Germany) real-time quantitative PCR assay, resulting in a r2=0.996 (using a regression analysis according to the CLSI guidelines EP09-A3 [16], showing a very strong correlation between these two diagnostic products based on different techniques. For this reason, the QXDx BCR-ABL1 %IS dd-PCR is a promising tool for MRD monitoring in CML patients. To evaluate the QXDx BCR-ABL1 %IS performance with clinical samples, an international inter laboratory study was performed [17] with 10 positive samples that were tested in 23 laboratories. These positive samples with ranging levels between MR1≤0.1- MR5≤1 were also by RT-qPCR, showing similar LOD, linearity and reduced inter laboratory variation.

So far, dd-PCR does not require standard curves or certified reference material, as it is an absolute quantification and uses the ratio of positive droplets to the total droplets to quantify the BCR-ABL1 translocation. The increased sensitivity makes dd-PCR more indicated for drug discontinuation studies. Moreover, this method has higher sensitivity, accuracy and robustness when compared to RT-qPCR. Considering the statements above this method can be considered in future clinical diagnosis of CML, as well as other genetic diseases.

References


