Abstract: PB2602

Title: ALLELE-SPECIFIC PCR FOR THE MAIN BCR::ABL KINASE DOMAIN MUTATIONS CONFERING RESISTANCE TO TYROSINE KINASE INHIBITORS IN CHRONIC MYELOID LEUKEMIA

Abstract Type: Publication Only

Topic: Chronic myeloid leukemia - Clinical

Background:

The main cause of resistance to tyrosine kinase inhibitor (TKI) treatment in patients with chronic myeloid leukemia (CML) is due to the occurrence of mutations in the ABL domain of the *BCR::ABL1* fusion gene. The emergence of certain mutations can affect the choice of subsequent treatment. E255K (11-21%), F359V (11%) and M244V (10%) are the most common mutations in patients with imatinib-resistant CML for which second-generation TKIs are effective. The T315I "panresistant" mutation occurs in 7-15% of patients and confers resistance to both imatinib and second-generation TKIs. Once detected, it requires the prescription of third-generation TKIs such as ponatinib or asciminib. We propose the use of allele-specific polymerase chain reaction (AS-PCR) as a sensitive, specific, and rapid method for the detection of the main clinically significant mutations within the DNA extracted from the blood or bone marrow samples of patients. This approach can help to identify the specific mutations responsible for resistance to TKI therapy, allowing for more effective and personalized treatment options.

Aims:

To evaluate the AS-PCR approach for monitoring T315I, E255K, F359V and M244V mutations in the*BCR::ABL1* gene using DNA isolated from blood and bone marrow samples. To validate the results using high-throughput sequencing (NGS) techniques.

Methods:

DNA samples from 15 CML patients admitted to the National Research Center for Hematology (Moscow, Russia) in 2020-2023 with the history of the resistance to certain TKIs were incuded in the study. Custom allele-specific primers and probes (see Table 1) to detect T315I, E255K, F359V, M244V mutations were ordered from Syntol (Russia). Chimeric BCR::ABL1 transcript was monitored using AmpliSense Leukemia Quant M-bcr-FRT kit (Interlabservice, Russia). NGS targeted to ABL gene kinase domain was performed on MiSeq apparatus (Illumina, USA).

Results:

15 DNA samples isolated from peripheral blood samples of patients with TKI resistance were analyzed by AS-PCR for the above mutations. BCR::ABL1 transcript levels ranged from 2.18% to 87.70% at the time of analysis. T315I mutation was detected in 8 samples out of 15, E255K in 1 sample, F359V in 2 samples, and M244V in 1 sample. No mutations were detected in 5 samples. However, two patients were found to have combined mutations: one had M244V and F359V, and the other had T315I, F359V, and E255K. All 15 DNA samples were analyzed by the NGS to detect mutations in the tyrosine kinase domain of the chimeric BCR::ABL1 gene. T315I mutation was detected in the same 8 samples as shown by AS-PCR; E255K mutation - in 2 samples, F359V in 2 of 15 samples, and M244V - in 1. Comparison of the results obtained by the two methods shows them to be almost identical, except single case with E255K mutation detected by NGS and not detected by the AS-PCR. This discrepancy requires further verification.

Summary/Conclusion: The developed AC-PCR approach for detecting main TKI resistance mutations (T315I, E255K, F359V, and M244V) in the BCR::ABL fusion gene, provides reliable results that are consistent with those obtained by next-generation sequencing (NGS). However, the AC-PCR method is faster, less time-consuming, less laborious than NGS, and can also be used to analyze

Table 1 Mutation points, primers, and fluorescent Taqman probes.

Target Gene	Amino Acid Substitution	Primer/ Probe	Sequence (5′ to 3′)
Chimeric gene BCR-ABL1	p.Thr315Ile	Forward w Forward mt Reverse com Forward probe	5'-TCCCGTAGGTCATGAACTCTG-3' 5'-TCCCGTAGGTCATGAACTCTA -3' 5'-GACAGTTGTTTGTTCAGTTGGGA-3' 5'-Cy5-CAACAAGACAACGAGGACTTCAACACGTG-RTQ2-3'
	p.Met244Val	Reverse w Reverse mt Forward com Reverse probe	5'-CGCCCAGCTTGTGCTTCTT-3' 5'-CGCCCAGCTTGTGCTTCTC-3' 5'-CTATGGTGTGTCCCCCAACT-3' 5'-Cy5-GTCCGTGCGTTCCATCTCCCACTTG-RTQ2-3'
	p.Glu255Lys	Reverse w Reverse mt Forward com Reverse probe	5'-ACACGCCCTCGTACACCAC-3' 5'-ACACGCCCTCGTACACCAT-3' 5'-CTATGGTGTGTCCCCCAACT-3' 5'-Cy5-GTCCGTGCGTTCCATCTCCCACTTG-RTQ2-3'
	p.Phe359Val	Forward w Forward mt Reverse com Forward probe	5'-GGAGTACCTGGAGAAGAAAAACAT-3' 5'-GGAGTACCTGGAGAAGAAAAACAV -3' 5'-CCTGAGACCTCCTAGGCTG-3' 5'-Cy5-CAGCCTGCGCCATGGAGTCACAG-RTQ2-3'

Keywords: Chronic myeloid leukemia, Tyrosine kinase inhibitor, Philadelphia chromosome, Drug resistance