آغا خان یونیورسٹی ہسپتال
The Aga Khan University Hospital

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Clinical LaboratoriesUpdate

DNA Sequence Analysis of *BCR-ABL1* Kinase Domain for Mutations Associated with Resistance to Tyrosine Kinase Inhibitors

Introduction

Treatment of chronic myeloid leukemia (CML) and Philadelphia chromosome (Ph)+ acute lymphoblastic leukemia (ALL) with tyrosine kinase inhibitors represents a model for targeted cancer therapy. However, the emergence of resistant leukemia clones bearing mutations in the *BCR-ABL1* kinase domain (KD) represents an important mechanism of disease recurrence. It requires a change in therapy, often to another tyrosine kinase inhibitor (TKI) that differs in pharmacokinetics and kinase inhibitory properties.

Among patients with chronic phase CML who develop (secondary) resistance to imatinib, 30% to 50% demonstrate the presence of one or more *BCR-ABL1* KD mutations. In addition, mutation frequencies are higher in those with accelerated or blast phases of the disease. In ALL, *BCR-ABL1* KD mutations occur much more frequently (80% to 90% of cases) at the time of relapse in Ph+ ALL and patients treated with TKIs as initial or maintenance therapy.

More than 70 mutations involving 57 different amino acids have so far been reported in the *BCR-ABL1* kinase domain region. A total of 15 amino acid substitutions account for 80% to 90% of all the reported imatinib-resistant mutations, and seven mutated codons (G250, Y253, E255, T315, M351, F359, and H396) account for a cumulative 60% to 70%. Moreover, specific mutations have been reported in patients treated with second and third generation TKIs, such as dasatinib and nilotinib.

BCR-ABL1 KD mutation screening in chronic phase CML is recommended for patients with an inadequate initial response to TKIs or those with evidence of loss of response. Mutation screening is also recommended at the time of progression to accelerated or
blast phase CML.

Principle of the assay

Total RNA extracted from blood or bone marrow aspirate of *BCR-ABL1* positive patients is reverse transcribed to cDNA, followed by PCR amplification and Sanger Sequencing. Interpretation of sequence data is performed using Mutation Surveyor software.

Specimen Collection

03 ml whole blood is required in RNA Tempus Tube

Limitations

- It is a qualitative assay and is performed on blood specimens positive for *BCR-ABL1* fusion mRNA.
- Low *BCR/ABL1* mRNA transcript (below 0.01% normalized *BCR/ABL1* on the International Scale: IS) may not be efficiently amplified for ABL Kinase analysis.
- A negative *BCR-ABL1* KD mutation report does not rule out acquired drug resistance due to less common resistance mechanisms such as *BCR-ABL1* gene amplification, *BCR-ABL1* overexpression, alterations in drug efflux kinetics, upregulation of different kinase pathways, and rare *BCR-ABL1* mutations outside of the KD.

Reporting Schedule:

Test performed on Every Monday, Report issued on following Friday