BCR/ABL1 Kinase Domain Mutation Analysis

Background

The t(9;22)(q34;q11) translocation, which results in the *BCR/ABL1* fusion gene, is found in all cases of chronic myeloid leukemia (CML) and in a subset of acute lymphoblastic leukemia (i.e., Philadelphia-chromosome positive ALL). Tyrosine kinase inhibitors, which target the activity of the *ABL* kinase domain, are central to current treatment of CML and Philadelphia-chromosome positive ALL.[1-7] A variety of tyrosine kinase inhibitors are currently in clinical use, including imatinib, dasatinib, nilotinib and ponatinib.

During the course of therapy with a tyrosine kinase inhibitor, point mutations in the kinase domain may be acquired that impart clinical resistance. Most mutations described to date have been associated with resistance to imatinib therapy.[2-5] Some mutations (F317L and V299L) are reported to be resistant to dasatinib while others (Y253H, E255K/V, and F359V/C) are reported to be resistant to nilotinib. One mutation, T315I, imparts resistance to all currently used tyrosine kinase inhibitors except ponatinib.[2-5]

Identification of an *ABL1* kinase domain mutation therefore can be of assistance as an appropriate therapeutic strategy for patients with CML and Philadelphia-chromosome positive ALL.[6-7] In order to detect the wide spectrum of mutations that may occur, a reverse-transcription polymerase chain reaction (RT-PCR) Sanger sequencing strategy is employed.

Clinical Indications

This assay is intended for detection of mutations in the kinase domain of p210 or p190 *BCR/ABL1* transcripts in patients with CML and Philadelphia-chromosome positive ALL. Current National Comprehensive Cancer Network (NCCN) guidelines recommend kinase domain mutation analysis in chronic phase CML patients with an inadequate initial response or loss of response to tyrosine kinase inhibitors, in progression of CML to accelerated or blast phase, and in relapsed/refractory Philadelphia-chromosome positive ALL.[6-7]

Interpretation

Results of the RT-PCR step are reported as positive for p190 and/or p210 *BCR/ABL1* transcripts, or as "*BCR/ABL1* transcripts not detected." When *BCR/ABL1* transcripts are detected, Sanger sequencing is performed. Sequencing results are reported using Human Genome Variation Society (HGVS) nomenclature and an interpretation is provided.

Limitations of the Assay

This assay is expected to detect >99% of reported mutations in the *BCR/ABL* kinase domain, provided that the mutated transcripts represent more than approximately 20% of all *BCR/ABL1* transcripts. Lower level (<20%) mutations may not be detected. Nucleotide changes that may have occurred in the other exons of *BCR/ABL1* or in its intronic sequences are not detected. Common germline polymorphisms are considered to represent wild type sequence and are not included in this report. The presence of nucleotide polymorphisms or variants at the annealing sites of the primers used in reverse transcription and sequencing may result in false negative results. This assay is not intended for detection of minimal residual disease. The limit of detection of the RT-PCR step of this assay corresponds to a percent ratio value of approximately 1% (*BCRABL/ABL1*). For more sensitive detection of p190 and/or p210 *BCR/ABL1* transcripts, please order the corresponding quantitative RT-PCR assay. Rare variant *BCR/ABL1* isoforms, including the e19a2 (p230) variant, are not detected by this assay.

Methodology

RNA is extracted from the sample, and cDNA is prepared by reverse transcription. PCR is performed using primers specific for the p190 (e1a2) and p210 (b2a2 and b3a2) transcripts of *BCR/ABL1*. An additional reaction using primers for a housekeeping gene is also performed in order to ensure adequate amplification. PCR products are visualized by gel electrophoresis. When *BCR/ABL1* transcripts are detected, the kinase domain (ABL1 exons 4-10, amino acid residues

200-520) is subjected to bidirectional cycle sequencing utilizing the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) on the ABI 3730 Genetic Analyzer (Applied Biosystems). Results are aligned to a wild type sequence (GenBank NM_005157) and analyzed for the presence of mutations.

References

1. Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, Taylor K, Herrmann R, Seymour JF, Arthur C, Joske D, Lynch K, Hughes T. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood*. 2003;102:276-283.
2. Jones D, Kamel-Reid S, Bahler D, Dong H, Elenitoba-Johnson K, Press R, Quigley N, Rothberg P, Sabath D, Viswanatha D, Weck K, Zehnder J. Laboratory Practice guidelines for detecting and reporting BCR-ABL drug resistance mutations in chronic myelogenous leukemia and acute lymphoblastic leukemia. *J Mol Diagn*. 2009;11(1):4-11.
3. Chien JH, Tang JL, Chen RL, Li CC, Lee CP. Detection of BCR-ABL gene mutations in Philadelphia chromosome positive leukemia patients resistant to STI-571 cancer therapy. Leuk Res. 2008;32(11):1724-34.
4. Branford S, Hughes T. Detection of BCR-ABL Mutations and Resistance to Imatinib Mesylate. *Methods Mol Med*. 2006;125:93-106.
5. Akahane D, Tauchi T, Okabe S, Nunoda K, Ohyashiki K. Activity of a novel Aurora kinase inhibitor against the T315I mutant form of BCR-ABL: In vitro and in vivo studies. *Cancer Sci*. 2008;99(6):1251-7.
6. National Comprehensive Cancer Network. Chronic Myeloid Leukemia (Version 2.2014). Accessed 1/7/2014. URL: www.nccn.org.
7. National Comprehensive Cancer Network. Acute Lymphoblastic Leukemia (Version 2.2013). Accessed 1/7/2014. URL: www.nccn.org.

Test Overview

Test Name	*BCR/ABL1* Kinase Domain Mutation Analysis
Reference Range	Mutations not detected
Specimen Requirements	10 mL Whole blood EDTA (Lavender). Place specimen on ice after draw. Specimen must be delivered to testing lab by 2 pm on Fridays.
Minumum Specimen Requirement	5 mL Whole blood EDTA (Lavender). Place specimen on ice after draw. Specimen must be delivered to testing lab by 2 pm on Fridays.
Alternate Specimen Requirement	5 mL Bone marrow EDTA (Lavender). Place specimen on ice after draw. Specimen must be delivered to testing lab by 2 pm on Fridays.
Billing Code	87994
CPT Codes	81403, G0452

Technical Information Contact:

Lisa Buhrow, MT(ASCP)
216.444.8034
buhrowl@ccf.org

Scientific Information Contact:

James R. Cook, MD, PhD
216.444.4435
cookj2@ccf.org