BCR-ABL1 Mutation Analysis for Tyrosine Kinase Inhibitor Resistance

Indications for Ordering

Detect mutations that may impart tyrosine kinase inhibitor (TKI) resistance in either chronic myelogenous leukemia (CML) or Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL)

Test Description

Next generation sequencing (NGS)

- RNA extracted from whole blood or bone marrow aspirate
- PCR amplification of *BCR-ABL1* SH2, SH3, and kinase domains
- Mutations identified by massively parallel sequencing

Test to Consider

BCR-ABL1 Mutation Analysis for Tyrosine Kinase Inhibitor Resistance by Next Generation Sequencing 2008420

- Order only for patients with an established diagnosis of a *BCR-ABL1*-positive leukemia
- Use to determine if a mutation is present that would interfere with response to TKI therapy in Ph+ ALL or CML
  - Detects all common mutations, including T315I
  - Higher sensitivity than traditional Sanger sequencing techniques
  - Offers coverage of SH2, SH3, and kinase domain

Disease Overview

Treatment issues

CML

- CML is characterized by *BCR-ABL1* translocations
- Initial treatment protocol is TKI therapy
  - Imatinib (Gleevec) – inhibits tyrosine kinase activity caused by the *BCR-ABL1* gene fusion
  - Dasatinib (Sprycel) – dual specific SRC/ABL inhibitor
  - Nilotinib (Tasigna) – imatinib derivative with 30-fold potency compared to imatinib
- Resistance to TKI therapy may result from
  - Acquired point mutations within the ABL kinase domain
  - *BCR-ABL1* amplification
  - Low bioavailability
  - Quiescent CML stem cells
- Resistance may be overcome with
  - Dose adjustments
  - Change in therapy
- Newer drugs may be useful when resistance develops
  - Bosutinib (Bosulif) – dual SRC/ABL inhibitor active in low nanomolar range
  - Ponatinib (Iclusig) – pan *BCR-ABL1* inhibitor (includes T315I mutant inhibition)
- Use of NGS improves ability to detect low-level clones across larger sections of the gene

ALL

- *BCR-ABL1* mutation is present in a subset of ALL patients
  - More common in adults than children
- Detection of mutations in *BCR-ABL1* is important in helping to determine potential response to TKI therapy

Genetics

Gene fusion – *BCR-ABL1*

Mutations

- Four regions tested
  - Adenosine triphosphate binding-loop (P-loop) mutations
  - Drug-binding sites
  - Catalytic domain
  - Activation loop
- >130 mutations currently identified

Test Interpretation

Results

- Detected – mutation detected in the SH2, SH3, or kinase domain (ABL1 amino acid residues 46-542)
- Not amplified – multiple attempts to amplify the *BCR-ABL1* translocation were unsuccessful by PCR
- Not detected – no mutation detected

Limitations

- Negative result does not exclude mutations below the level of detection or outside the sequenced region
  - Sensitivity of this assay may be limited
  - Sequencing may not be possible in patient samples containing low tumor burden (ie, low levels of *BCR-ABL1* fusion transcript by International Scale [IS]% or normalized copy number [NCN])
- This assay is not intended to be used for detection or quantification of *BCR-ABL1* fusion transcripts

Analytical Sensitivity

Variant class	No. Variants Tested	Positive Percent Agreement (PPA)	PPA, 95% Tolerance at 95% Reliability
Single nucleotide variant (SNV)	396	96.5%	94.3-98.0%

Related Tests

BCR-ABL1, Qualitative with Reflex to *BCR-ABL1* Quantitative 2005010

- Recommended when submitting initial diagnostic specimen for CML or Ph+ ALL (no previous *BCR-ABL1* testing)
  - When qualitative test is positive, the appropriate corresponding quantitative test is performed

BCR-ABL1, Major (p210), Quantitative 2005017

- Aids in diagnosis and monitoring of individuals with CML and a subset of individuals with Ph+ ALL who have e13a2 or e14a2 transcripts (p210)

BCR-ABL1, Minor (p190), Quantitative 2005016

- Aids in diagnosis and monitoring of individuals with Ph+ ALL who have e1a2 transcripts (p190)

Acute Lymphocytic Leukemia (ALL) Panel by FISH, Pediatric 2002719

- Recommended FISH panel for children with newly diagnosed ALL

Chromosome FISH, Interphase 2002298

- Use to order individual or multiple FISH probes when standard FISH panels are not desired
  - Specific FISH probe for t(9;22); *BCR-ABL1* must be requested

Acute Lymphocytic Leukemia (ALL) Panel by FISH, Adult 2002647

- Recommended panel for adults with newly diagnosed ALL