



CML Disease Management

Introducing Gleevec® Mutation Analysis

Overview

Chronic Myelogenous Leukemia (CML) is a clonal expansion of transformed hematopoietic progenitor cells, and accounts for 15% of leukemias in adults.¹ Many patients are asymptomatic at the time of diagnosis, but can present with a variety of signs and symptoms including fatigue, weight loss, bleeding, purpura, abdominal fullness, anemia, thrombocytosis and splenomegaly.¹

The molecular hallmark of CML is the Philadelphia (Ph) chromosome, a shortened version of chromosome 22 that is the result of a translocation between chromosomes 9 and 22 that is found in 95% of CML patients.^{1,2}

The Ph translocation results in segments from the *BCR* and *ABL* genes combining to create a hybrid *BCR-ABL* gene.² This hybrid gene is transcribed to produce *BCR-ABL* mRNA, which can be detected and quantitated by various molecular-based methods, including RT-PCR.³

Traditional treatments include stem-cell transplantation and interferon-alpha (IFN- α).³ Imatinib mesylate (Gleevec® or Glivec, in Europe), an FDA-approved drug for Ph chromosome positive CML patients after IFN- α therapy failure, has shown great promise in recent trials; however, some patients develop genetic resistance during the course of imatinib treatment.⁴ Various treatment options are now emerging based on the specific mutations causative of imatinib resistance.

Diagnosis

Cytogenetic karyotyping studies, the standard diagnostic test for CML, reveal the presence of the Ph chromosome in ~90% of CML patients.¹ Karyotyping can not only identify the Ph chromosome in most CML patients, but can also detect the presence of other clinically significant chromosomal abnormalities.

#5800 Hematological Disorders, Acquired

In the 5% of CML patients without a detectable Ph chromosome, the pathogenetic translocation may be identified by the following molecular tests:

#5834 BCR-ABL Gene Rearrangement (Philadelphia Chromosome) [FISH]

#5342 BCR-ABL UltraQuant® Major 210 kd Transcript Bone Marrow [RT-PCR]

#5352 BCR-ABL UltraQuant® Major 210 kd Transcript Whole Blood [RT-PCR]

In addition to the above studies, bone marrow examination is usually performed on all patients suspected of having CML, to determine the percentage of blasts in the bone marrow, which is important for staging. Bone marrow examination is also performed intermittently during the course of therapy, or whenever new clinical symptoms arise.

Immunophenotyping of blood or bone marrow by flow cytometry is a useful adjunct to morphologic examination in accurately assessing blast percentage and differentiating among hematopoietic cell populations.

#1680 Leukemia/Lymphoma Analysis

Treatment Monitoring

Treatment effectiveness and therapeutic response are assessed by monitoring residual disease as reflected by quantitative measurement of *BCR-ABL* mRNA.³ Sensitive quantitation is important when establishing an accurate pre-treatment baseline, as well as monitoring therapeutic efficacy.^{3,5} An enhanced RT-PCR, incorporating Minor Groove Binder probes,⁷ is now available exclusively through *Specialty* for quantitative monitoring of *BCR-ABL* concentrations.

The enhanced sensitivity of *Specialty's* BCR-ABL UltraQuant® assays improves the physician's ability to monitor changes that can indicate drug resistance and early signs of relapse. These assays are useful in: **monitoring therapeutic efficacy** of the anti-leukemia drug imatinib (Gleevec®) and establishing **pre-treatment baseline** BCR-ABL mRNA concentrations.

Gleevec® Resistance Testing

Imatinib mesylate (Gleevec®) has proven to be initially effective in many patients. However, a number of patients have begun to relapse, due to Gleevec® resistance.^{4,7} Mutations affecting the binding of the drug to the target site are reported, and can cause drug resistance. One of the most common mutations, T315I, is associated with absolute resistance to Gleevec®.^{4,7} As additional mutations are discovered and new treatment options emerge, *Specialty* will expand its mutation analysis to include those mutations important to patient management.

#5361 Gleevec® Resistance Mutation Analysis

Ordering Information & Specimen Requirements

Test Code	Test Name	Specimen Requirements
5800	Hematological Disorders, Acquired	3 (1) mL Bone Marrow Heparinized or 5 (2) mL Whole Blood Heparinized; Ambient. Ship immediately by overnight courier.
1680	Leukemia/Lymphoma Evaluation Panel	5 (3) mL Whole Blood or Bone Marrow ACD/Heparin/EDTA; Ambient. Ship immediately by overnight courier to arrive within 24 hours of collection. Submit with smear, if available.
5834	BCR-ABL Gene Rearrangement [FISH]	3 (1) mL Bone Marrow Sodium Heparin or 3 (1) mL Whole Blood Sodium Heparin; AMBIENT only. Ship immediately by overnight courier.
5352	BCR-ABL UltraQuant [®] Major 210 KD Transcript, Whole Blood	8 (5) mL Whole Blood EDTA only; REFRIGERATED. Ship on cold pack by overnight courier to arrive at <i>Specialty</i> within 24 hours of collection. DO NOT FREEZE.
5342	BCR-ABL UltraQuant [®] Major 210 KD Transcript, Bone Marrow	2 (1) mL Bone Marrow EDTA only; REFRIGERATED. Ship on cold pack by overnight courier to arrive at <i>Specialty</i> within 24 hours of collection. DO NOT FREEZE.
5361	Gleevec [®] Resistance Mutation Analysis T3151 mutation	8 (5) mL Whole Blood EDTA only or 1.5 (0.5) mL Bone Marrow EDTA only; REFRIGERATED. Ship on cold pack by overnight courier to arrive at Specialty within 24 hours of collection. DO NOT FREEZE.

Methodology

Karyotyping

Fluorescent *In Situ* Hybridization (FISH)

Flow Cytometry (FC)

Minor Groove Binder (MGB) with Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Reverse Transcriptase-Polymerase Chain Reaction with Matrix-Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (RT-PCR MALDI-TOF-MS)

MGB technology is performed pursuant to an agreement with Epoch Pharmaceuticals, Inc.

PCR tests are performed pursuant to a license agreement with Roche Molecular Systems, Inc.

References

1. Faderl S, Talpaz M, Estrov Z, et al. The biology of chronic myeloid leukemia. *N Engl J Med* 1999;341:164-72.
2. Sawyers C. Chronic myeloid leukemia. *N Engl J Med* 1999;340:1330-40.
3. Bagg A. Chronic myeloid leukemia: a minimalistic view of post-therapeutic monitoring. *J Mol Diagn* 2002;4:1-10.
4. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by *BCR-ABL* gene mutation or amplification. *Science* 2001;293:876-80.
5. Moravcova J, Nadvornikova S, Lukasova M, Klamova H. Polymerase chain reaction analyses should be used as a basis for clinical decision making in patients with chronic myelogenous leukemia [Letter]. *Blood* 1999;94:3609-11.
6. Kutyavin IV, Afonina IA, Mills A, et al. 3'-Minor groove binder-DNA probes increase sequence specificity at PCR extension temperatures. *Nucleic Acid Res* 2000;28:655-61.
7. von Bubnoff N, Schneller F, Peschel C, Duyster J. *BCR-ABL* gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet* 2002;359:487-91.