Laboratory Test to Detect Mutations in the *BCR-ABL* fusion Gene Conferring Resistance to Gleevec

The UNC Hospitals Molecular Genetics Laboratory performs reverse transcriptase PCR (RT-PCR) sequencing analysis to detect mutations in the kinase domain of the *BCR-ABL* fusion gene associated with resistance to Gleevec.

Biology of the disease state:

Gleevec® (imatinib mesylate, STI571), a targeted tyrosine kinase inhibitor, is the current therapy of first choice for patients suffering from chronic myelogenous leukemia (CML). The drug works by binding the active site of the ABL kinase, stabilizing the inactive form of BCR-ABL, and inhibiting phosphorylation and downstream activation. Response to Gleevec has been significantly better than with other available therapies, with fewer side effects. Even in the best responders, however, some *BCR-ABL* positive cells usually remain. Many patients who initially respond to Gleevec have been shown to relapse after a period of time due to the development of resistance. The most common mechanism of resistance to Gleevec is due to mutations in the *ABL* kinase domain that affect the ability of Gleevec to bind to the active site. This may occur through steric interference within the site itself or by changing the conformation of the fusion protein so that the binding site is masked.

Clinical utility of testing for Gleevec® resistance mutations:

It is recommended that CML patients on Gleevec be monitored with quantitative RT-PCR and that patients with a log increase in quantitative bcr-abl levels be assayed for the presence of Gleevec-resistance mutations. Depending on the exact mutation present, clinical intervention such as increasing dosage of Gleevec or adding another kinase inhibitor may be effective in controlling the levels of *BCR-ABL*. Some newer alternative therapies have been developed for CML patients with Gleevec resistance. The T315I mutation appears to confer resistance to multiple targeted tyrosine kinase inhibitors, while other mutations may be more responsive to other therapies. Thus, resistance testing may be useful to help guide CML treatment with alternative therapies. The assay cannot detect other mechanisms of drug resistance such as amplification of the BCR-ABL fusion gene.

Laboratory testing for Gleevec resistance mutations:

The preferred sample is EDTA- or ACD-anticoagulated (lavendar or yellow top) blood or bone marrow delivered promptly to the laboratory to prevent RNA degradation. Samples submitted for quantitative *BCR-ABL* analysis can also be used for this testing, provided enough high-quality RNA can be extracted. The RNA is extracted and converted to cDNA using the enzyme reverse transcriptase. The cDNA is subjected to PCR amplification using primers that span the translocation site. The PCR product is then subjected to capillary DNA sequencing of the ABL kinase domain, including the P-loop, catalytic domain, and activation loop. Mutations are compared to a database derived from peer-reviewed reports of mutations causing Gleevec resistance. The limit of detection of the assay allows reporting of mutations in as few as 20% of cells in a background of wild-type *BCR-ABL*.

References:

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3. C Walz and M Sattler. Novel targeted therapies to overcome imatinib mesylate resistance in chronic myeloid leukemia (CML). Crit Rev Onc Hematol 2006;57:145-164.
4. M Talpaz, et al. Dasatinib in Imatinib-resistant Philadelphia chromosome positive leukemias. NEJM 2006; 354:2531-2541.
5. V Nardi, et al. Mechanisms and implications of imatinib resistance mutations in BCR-ABL. Curr Opin Hematol 2003;11:35-43.

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