

BRAF Mutation Test in Colorectal Cancer

The UNC Hospitals Molecular Genetics Laboratory performs an assay to detect the most common *BRAF* mutation, V600E, in colorectal cancer tissue.

Pathobiology: The *BRAF* gene encodes a serine-threonine kinase in the RAS/RAF/MAPK signaling pathway. Upon activation by RAS, BRAF sequentially phosphorylates and activates MEK and ERK. This signaling is unchecked in the setting of an activating *BRAF* gene mutation. The *BRAF* gene is composed of 18 exons, and the most common activating mutation is found in exon 15 at nucleotide position 1799. This *BRAF* c.1799T>A [p.Val600Glu] mutation is colloquially termed *BRAF* V600E, and it constitutes about 90% of known activating *BRAF* mutations. The encoded protein has over 10 times more kinase activity than its normal counterpart. In colorectal adenocarcinomas and in certain other cancers, *BRAF* V600E acts as an oncogene driving tumor cell proliferation. Cappuzzo et al showed that patients whose colon cancer harbored *BRAF* mutation had poor response to anti-EGFR therapy (e.g. panitumumab or cetuximab). *BRAF* mutation is found in about 14% of *KRAS* wild type cancers. (See separate flyer on use of *KRAS* testing to predict response to anti-EGFR therapy.) Patients whose colon cancer harbors *BRAF* mutation also have a worse prognosis based on time to progression and survival data, especially when the cancer is microsatellite stable (MSS).

Another well established indication for *BRAF* mutation testing is in the workup of Lynch Syndrome. In colon cancers with microsatellite instability or with loss of MLH1 protein expression, *BRAF* mutation implies that the patient is unlikely to have Lynch syndrome, whereas lack of *BRAF* mutation suggests a need for further evaluation of possible Lynch Syndrome using tests such as CpG island methylator phenotyping (CIMP), a panel of immunohistochemical assay for mismatch repair protein expression, or sequencing of the relevant mismatch repair genes.

Finally, *BRAF* mutation subclassifies certain types of cancers such as melanoma and papillary thyroid carcinoma.

Clinical Indications: 1. Colorectal adenocarcinoma in a patient who is a candidate for anti-EGFR therapy, when *KRAS* mutation is absent. 2. Work-up of Lynch syndrome in colorectal carcinomas having microsatellite instability (MSI-H) or loss of MLH1 protein expression. 3. Subclassification of cancer when traditional histopathology is inadequate (melanoma, papillary thyroid carcinoma).

Laboratory testing: The preferred specimen is a paraffin block containing at least 50% malignant cells, or ten 5-10um thick unstained paraffin sections on plain glass slides plus an H&E stained slide. A copy of the surgical pathology or cytopathology report is requested. Tumor cells are enriched by macrodissection if needed, and extracted DNA is PCR-amplified and then pyrosequenced. Results are interpreted by a pathologist.

References:

1. Cappuzzo F, Varella-Garcia M, et al. Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. *Br J Cancer* 99:83-89, 2008.
2. Xing M, Clark D, et al. BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. *J Clin Oncol* 27:2977-82, 2009.
3. Smalley KS, Nathanson KL, Flaherty KT. Genetic subgrouping of melanoma reveals new opportunities for targeted therapy. *Cancer Res* 69:3241-4, 2009.

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http://labs.unchealthcare.org/directory/molecular_pathology/index_html