Cleveland Clinic Laboratories

Technical Brief

KRAS Mutation Testing in Colorectal Cancer

Background Information

Colorectal cancer is the second leading cause of cancer-related death in the United States, with approximately 150,000 new cases and 50,000 deaths each year.[1] Up to 50% of patients will suffer from distant metastases during their disease course, usually necessitating systemic chemotherapy. Recently, two epidermal growth factor receptor (EGFR) inhibitors, cetuximab and panitumumab have been FDA-approved for the treatment of metastatic colorectal carcinoma. Unfortunately, only a small minority of patients will respond to these expensive and potentially harmful therapies.

Numerous retrospective and lookback analyses of prospective studies have found that patients with metastatic colorectal cancer whose tumors harbor a *KRAS* mutation in codons 12/13 almost invariably do not respond to anti-EGFR targeted therapies[2-7]; therefore, patients with *KRAS* codon 12/13 mutations do not derive benefit from anti-EGFR therapy. These results have convinced the American Society of Clinical Oncology (ASCO)[8], the National Comprehensive Cancer Network (NCCN)[9], and the Food and Drug Administration (FDA) to recommend KRAS mutation testing prior to receiving treatment with anti-EGFR monoclonal antibodies. Since *KRAS* mutations occur in about 30-40% of colorectal cancer, *KRAS* mutation testing has tremendous cost/healthcare resource saving potential.

Clinical Indications

Cleveland Clinic tests all Stage 4 (distant metastasis) colorectal cancers for *KRAS* mutations in codons 12/13.

Excellent concordance has been demonstrated between primary and metastatic samples such that either primary or metastasis may be tested.[10]

Interpretation

Electropherograms are examined to identify the position of codons 12 and 13, corresponding to a forward sequence of GGTGGT, encoding two glycine residues. Mutations do not occur in the third position of either codon due to wobble redundancy. The forward and reverse sequencing reactions are analyzed to detect mutant alleles at these positions, in addition to neighboring sites in the DNA sequence.

Results are reported as "KRAS mutation identified" or "No KRAS mutation identified." Mutations in codons 12 and 13 of the *KRAS* gene are almost exclusively point mutations, and in accordance with College of American Pathologist (CAP) reporting guidelines, point mutations are recorded with the coding DNA mutation position, wild-type nucleotide, and mutant nucleotide (e.g., c.34G>T signifies a G-to-T mutation at position 34 in codon 12.

Although treatment decisions are not made based upon the particular mutation site, reporting of the particular *KRAS* mutation remains important for possible future impact. For instance, the c.38G>A mutation was recently reported to show improved survival in cetuximab-treated patients compared to other *KRAS* mutations in retrospective analyses.[11]

Methodology

1. The *KRAS* gene is examined by performing PCR for a 263 base pair amplicon that includes the most common mutation sites (codons 12 and 13).
2. Cycle sequencing is performed for the forward and reverse strands, using the BigDye Terminator kit (Applied Biosystems; Foster City, Calif.).
3. The sequence is analyzed by capillary electrophoresis.

Limitations of the Assay

Traditional Sanger sequencing is considered to have about a 25% analytical sensitivity, meaning samples containing less than 25% tumor cells may show false wild-type results. Review of the H&E with meticulous selection of the area containing the most dense tumor cell concentration, followed by careful microdissection are critical steps in the process of minimizing the possibility of false negative results. In small specimens with wild-type results, a short disclaimer will often be added to suggest that a false negative result is a possibility.

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Test Overview

Test Name	PCR for KRAS mutation
Specimen Requirements	Tumor sample in paraffin-embedded tissue blocks. Optimal fixation: 10% neutral buffered formalin, but some other fixatives (alcohol-based fixatives) may be suitable. Bouin's-containing or decalcifying fixatives are not suitable.
Test Ordering Information	KRAS
Special Notations	Tumor should be viable, and blocks should be selected in which the tumor is maximally dense and contaminating normal stroma, necrosis, blood, and inflammatory cells are minimized. A minimum of 0.5 cm2 of tumor with > 50% tumor cells should be submitted, but smaller, less dense samples can be tested if no better option exists.
Billing Code	83968
CPT Codes	83894; 83898; 83904(x2); 83907; 83909(x2); 83912

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