KRAS Mutational Analysis

Background Information

Certain mutations — such as codons 12 and 13 — in critical areas of the *KRAS* gene are a negative predictor of anti-EGFR (epidermal growth factor receptor) antibodies in colorectal cancer, and result in the expression of a KRAS protein that causes uncontrolled cell growth and proliferation. These oncogenic gene mutations are similarly indicative of resistance to small-molecule tyrosine kinase inhibitors in non-small-cell lung cancer patients.

Colorectal cancer is the second leading cause of cancer-related death in the United States, with approximately 143,000 new cases and 51,000 deaths each year.[1] Up to 50% of patients will suffer from distant metastases during their disease course, usually necessitating systemic chemotherapy. Recently, two 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.

Mutations in KRAS codons 12 and 13 are associated with lack of response to EGFR-targeted therapies in both CRC and NSCLC patients.[3] These results have convinced the American Society of Clinical Oncology (ASCO),[9] the National Comprehensive Cancer Network (NCCN),[10] 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. However, recently published guidelines for NSCLC testing for the Association of Molecular Pathology (AMP), College of American Pathologists (CAP) and the International Association for the Study of Lung Cancer (IASLC) do not mandate KRAS tests as a negative predictor or response.

In July 2012, the FDA approved the real time PCR companion test for *KRAS* mutational analysis implemented by Cleveland Clinic Laboratories, the Therascreen KRAS RGQ PCR Kit (Qiagen; Germany), to aid in identifying CRC patients who would benefit for treatment with cetuximab or panitumumab.

Clinical Indications

This assay is indicated for patients with metastatic CRC or advanced NSCLC who are being considered for treatment with an EGFR antagonist. This test detects mutations in the *KRAS* gene, thereby determining eligibility for therapies targeting epidermal growth factor receptors (EGFR).

Cleveland Clinic tests 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.[11]

The U.S. Food and Drug Administration has approved this test to identify patients with colorectal cancer for treatment with Erbitux® (cetuximab) based on a *KRAS* result of no mutation identified with the Therascreen® KRAS RGQ PCR Kit and Therascreen® KRAS Assay Package Software analysis. This test was validated and its performance characteristics confirmed by the Pathology and Laboratory Medicine Institute at Cleveland Clinic.

Table 1. Impact of *KRAS* Mutations in Patients Treated with an EGFR Antagonist 2-4

Therapy	Treatment Response Rate		Median Patient Survival	
	KRAS Mutation Positive	*KRAS* Mutation Negative	*KRAS* Mutation Positive	*KRAS* Mutation Negative
Metastatic Colorectal Cancer				
Cetuximab	0/36=0%a	34/78=44%a	9 weeks (PFS)	32 weeks (PFS)
Panitumumab	0/84=0%b	21/124=17%b	7 weeks (PFS)	12 weeks (PFS)
Advanced Non-Small-Cell Lung Cancer				
Erlotinib	2/25 = 8%a	27/104 = 26%a	4.4 months (OS)	12.1 months (OS)

PFS, progression-free survival; OS, overall survival.
a Response rate includes partial and complete responders.
b Response rate includes only partial responders.

Methodology

DNA is extracted and purified utilizing the DNA IQ Casework Pro Kit (Promega; Wisconsin) and the Maxwell 16 Instrument (Promega; Wisconsin). The *KRAS* gene is analyzed utilizing real-time qualitative PCR with the Rotor-Gene Q MDx instrument. The IVD therascreen KRAS RGQ PCR Kit detects the seven most common KRAS codon 12/13 mutations when at least 20% of the cells tested harbor the mutant copy. These seven mutations account for >97% of all reported *KRAS* mutations in CRC patients. Based on the predetermined analytical values, the therascreen KRAS software package qualitatively determines and reports the mutation status. In the rare instance of a tumor containing more than one *KRAS* mutation, only one mutation is identified and reported by the software. Results are reported as positive (homozygous or heterozygous for the mutation detected) or negative.

Interpretation

The presence of a *KRAS* mutation in codon 12 or 13 is associated with a high likelihood of resistance to therapies targeting EGFR.[7] Results are reported as "*KRAS* mutation identified" or "No *KRAS* mutation identified." Polymorphisms or mutations at other locations may be associated with drug efficacy or patient outcome will not be detected. Results should be interpreted in conjunction with other laboratory and clinical findings.

Limitations of the Assay

The test only examines codons 12 and 13. Other possible important loci such as KRAS codons 59, 61, 117, 146 or NRAS codons 12, 13, 59, 61, 117, 146 are not tested using this assay. Detection of the mutation is dependent on sample integrity and the amount of amplifiable DNA present in the specimen. The methods used in this assay are highly selected and, depending on the total amount of DNA present, can detect approximately 1% to 5% of mutant DNA in a background of wild-type genomic DNA. In small specimens with wild-type results, a short disclaimer will often be added to suggest that a false negative result is a possibility.

References

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Test Overview

Test Name	*KRAS* Mutational Analysis (by PCR)
Ordering Mnemonic	KRAS
Methodology	Qiagen EGFR RGQ PCR Assay
Specimen Requirements	Tumor sample in paraffin-embedded tissue blocks or cell pellets in PreservCyt or CtyoLyt. Optimal fixation for FFPET is 10% neutral buffered formalin, but some other fixatives (alcohol-based fixatives) may be suitable. Bouin's-containing or decalcifying fixatives are not suitable.
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 >20% tumor cells should be submitted, but smaller, less dense samples can be tested if no better option exists. Cell pellets in PreservCyt or CtyoLyt should be accompanied by the Thinprep slide or a report confirming positive cytology result for malignancy.
Billing Code	88425
CPT Codes	83894; 83898; 83904(x2); 83907; 83909(x2); 83912

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