

## **KRAS Mutations Conferring Drug Resistance**

The UNC Hospitals Molecular Genetics Laboratory performs DNA pyrosequencing to detect mutations in *KRAS* codons 12, 13, and 61 associated with resistance to EGFR inhibitors such as Erbitux (cetuximab) and Vectibix (panitumumab).

**Biology of the process:** *KRAS* is one of many molecules downstream in the Epidermal Growth Factor Receptor (EGFR) signaling pathway that is responsible for controlling critical cell functions such as proliferation, differentiation, and apoptosis. A wide range of cancers have mutations in *KRAS* that seem to render the *KRAS* protein constitutively active regardless of whether the EGFR cell surface receptor is stimulated or inhibited. Drugs that function as EGFR inhibitors have been shown to be less effective against *KRAS*-mutated colorectal or lung cancer (non-small cell type, NSCLC). The mutations that are demonstrated to be relevant are those in codons 12, 13, and 61 of *KRAS*, and research is underway to study other mutations and other cancer types for which *KRAS* mutation testing is predictive of outcome in response to EGFR-inhibitors. To date, the drugs whose effectiveness is known to be influenced by *KRAS* mutation status are Erbitux (cetuximab) and Vectibix (panitumumab). *KRAS* mutations are found in about 40% of colorectal adenocarcinoma and 20% of lung adenocarcinoma (especially in smokers). Of note, *KRAS* and *EGFR* or *BRAF* mutations appear to be mutually exclusive, which makes sense given that all three factors function in the same EGFR-RAS-RAF-MAPK and PI3K/PTEN/AKT signaling pathways. *KRAS* mutation is associated with limited clinical response to EGFR inhibitors.

**Clinical Indications for *KRAS* mutation testing:** Patients with colon or lung adenocarcinoma who are candidates for Erbitux (cetuximab) or Vectibix (panitumumab).

**Laboratory testing for *KRAS* mutations:** The preferred sample is a paraffin block containing at least 50% malignant cells representing either primary or metastatic colorectal or lung adenocarcinoma, or five 10um unstained paraffin sections on plain glass slides plus an H&E-stained slide. Other specimens such as fine needle aspirates will be considered if the pathology report demonstrates a high proportion of malignant cells in fresh, stabilized (e.g. PreservCyt), or cell block form. A copy of the surgical pathology or cytology report is requested. Tumor cells are enriched by macrodissection if needed, and extracted DNA is PCR-amplified followed by DNA pyrosequencing to identify mutations in codons 12, 13, or 61 of the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene. The limit of detection is 5%, implying that a mutated allele must comprise at least 5% of *KRAS* alleles. Results are interpreted by a pathologist.

### References:

1. Amado RG et al: Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol.* 26:1626-1634, 2008.
2. De Roock W et al: *KRAS* wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 19:508-515, 2008.
3. Lièvre A et al: RAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 26:374-379, 2008
4. Miller VA et al: Molecular characteristics of bronchioloalveolar carcinoma and adenocarcinoma, bronchioloalveolar carcinoma subtype, predict response to erlotinib. *J Clin Oncol.* 26:1472—1478, 2008.
5. Walther A et al: Genetic prognostic and predictive markers in colorectal cancer. *Nature Rev Cancer* 9:489, 2009.

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