ROUTINE KRAS MUTATIONS ANALYSIS METHODS

	DIRECT SEQUENCING	PYROSEQUENCING	DHPLC	HRM High Resolution Melting	Real time PCR	LCD array
SENSITIVITY (MT/WT, %)	15-20 %	5 %	2-5 %	2-5 %	1-2 %	<1 %
ADVANTAGES	▪ Detects all genetic variations in the sequenced amplicons	▪ Sensitive ▪ Detects only specific mutations tested	▪ Sensitive ▪ Detects all genetic variations in the amplicon tested	▪ Sensitive ▪ Detects all genetic variations in the amplicon tested	▪ Sensitive ▪ Rapid TAT (1 week) ▪ Detects only specific mutations tested	▪ Sensitive ▪ Rapid TAT (1 week) ▪ Doesn't require any technical background ▪ Only 1 PCR to identify all the mutations tested ▪ Presence of Wild Type suppressor sequence (WSC) to enhance sensitivity of the mutation detection ▪ Detects only specific mutations tested
DISADVANTAGES	▪ Lack of sensitivity ▪ Technical background required ▪ Extended TAT (4 days to 2 weeks)	▪ Technical background required ▪ Extended TAT (4 days to 2 weeks)	▪ High technical background required ▪ Sequencing confirmation required ▪ Extended TAT (4 days to 2 weeks)	▪ High technical background required ▪ Sequencing confirmation required ▪ Extended TAT (4 days to 2 weeks)	▪ Not convenient: detects only a single specific mutation per reaction ▪ Expensive (2 primers and 2 probes for each specific mutation in each PCR reaction)	

References

Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR.Heinemann V et al - Cancer Treat Rev. 2009 May;35(3):262-71

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Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. Eberhard DA et al - J Clin Oncol. 2005 Sep 1;23(25):5900-9.

Ras oncogenes in human cancer: a review. Bos JL. Cancer Res. 1989 Sep 1;49(17):4682-9.

Sample preparation

PRE-ANALYTICAL REQUIREMENT

Molecular biology for KRAS status is available on:

Paraffin embedded tumoral tissue

- Formalin - not Bouin
- Tumor sample is returned after analysis

or

Unstained slides (10-20 µm thick)

- Paraffin embedded (or cryo) mass tumor : 5 unstained slides
- Paraffin embedded (or cryo) biopsy : 10 unstained slides

Histology report is mandatory.

TAT: 10 days

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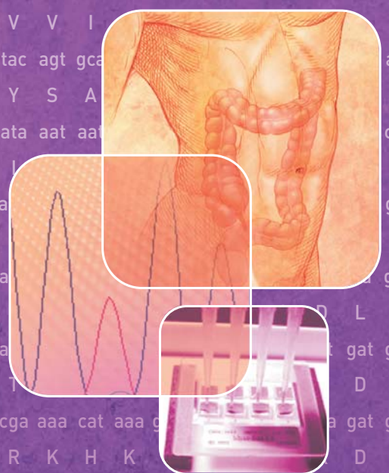
Focus on...



KRAS mutation status

A Biomarker to establish the clinical outcome in Metastatic Colorectal Cancer

APRIL 2009

biomnis
SPECIALISED MEDICAL PATHOLOGY

Clinical use

Colorectal cancer (CRC) is the second most commonly diagnosed cancer. CRC represents the second most common cause of deaths world wide. Approximately 30 % of patients with CRC have metastatic disease at the time of diagnosis (mCRC).
KRAS mutations screening is performed in cases of metastatic colorectal cancer (mCRC) for therapeutic decision. Several studies showed the presence of a hotspot mutational site in codons 12, 13 , 61 and 63 in *KRAS* oncogene. *KRAS* mutation status allows for the identification of patients who might benefice from anti-EGFR therapies and avoid a costly and potentially toxic administration of this treatment in non responsive patients.

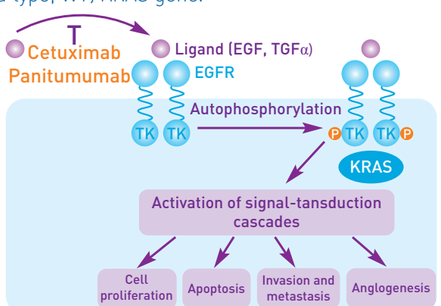
Wild-type *KRAS* status :
Responsive to anti-EGFR therapies
Mutated *KRAS* :
Non responsive to anti-EGFR therapies

Scientific background

Somatic mutations in the *RAS* oncogene family (*HRAS*, *KRAS* and *NRAS*) are observed in a variety of various malignancies, including colorectal cancer (33~53 %), pancreatic cancer (~80 %), lung adenocarcinoma (~30 %), ovarian and endometrial cancer, gall bladder cancer, bile duct cancer (~45 %), thyroid cancer (~55 %) and hematological malignancies.
The *KRAS* gene is located on chromosome 12 and encodes for a G protein involved in colorectal carcinogenesis. The KRAS protein plays a central role in tumor development, regulating downstream proteins involved in proliferation, survival, metastasis and angiogenesis *via* the EGFR signalling pathway.
The KRAS protein regulates PI3K/AKT and RAS/MEK/ERK signalling pathways located downstream of many growth factor receptors, including EGFR. When bound to it's ligand, EGFR stimulates tyrosine kinase activity leading to activation of KRAS and signalling pathways.
Genetic alterations of the intracellular effectors involved in EGFR-related signalling pathways may have an effect on response to this targeted therapy. The presence of an activating mutation in codons 12 and 13, the KRAS protein is permanently turned on, even without being triggered by EGFR mediated signalling and the therapies targeting EGFR are ineffective.

Specific target-directed therapies

3 monoclonal antibodies have been approved for colorectal cancer therapy including monoclonal antibodies against epidermal growth factor receptor (EGFR) and vascular endothelial growth facter (VEGF).
The new therapies targeting EGFR are cetuximab (Erbitux®; Merck Serono) and panitumumab (Vectibix®; Amgen). The action of cetuximab or panitumumab is the blockage of ligand binding receptor and thereby causing the inhibition of ligand mediated pathway.
The European Commission has granted a market authorization for cetuximab and panitumumab for the treatment of patients whose tumors harbor normal, non-mutated (wild type; WT) *KRAS* gene.



KRAS mutations in exon 2

Kras mutations Distribution in metastatic colorectal cancer		
p.G12V; c.35G > T	21,7 - 28,1 %	Codon 12 80 %
p.G12A; c.35G > C	6,4 - 8,2 %	
p.G12D; c.35G > A	35,7 - 38 %	
p.G12S; c.34G > A	7,6 - 9,9 %	
p.G12C; c.34G > T	5,3 - 7,6 %	
p.G12R; c.34G > C	1,2 - 1,6 %	
p.G13D; c.38G > A	11,7 - 15,8 %	Codon 13 15 %
p.G13C; c.37G > A	0,6 %	
Others (codon 61 and 63)		< 5 %

Additional advances in bio marker use for direct target therapies

BRAF and *PI3K* mutations are being explored and seem to give promising data for a more accurate therapeutic approach.

HOW TO MANAGE *KRAS* MUTATION SCREENING? BIOMNIS' STRATEGY