



KRAS Testing Fact Sheet

WHAT IS KRAS?

KRAS is a gene that codes for a protein involved in the Epidermal Growth Factor Receptor (EGFR) signalling cascade which is important in cell proliferation, angiogenesis, migration, cell survival and cell adhesion. When KRAS is 'turned on' by a mutation, it leads to uncontrolled cell growth and division, resulting in cancer.

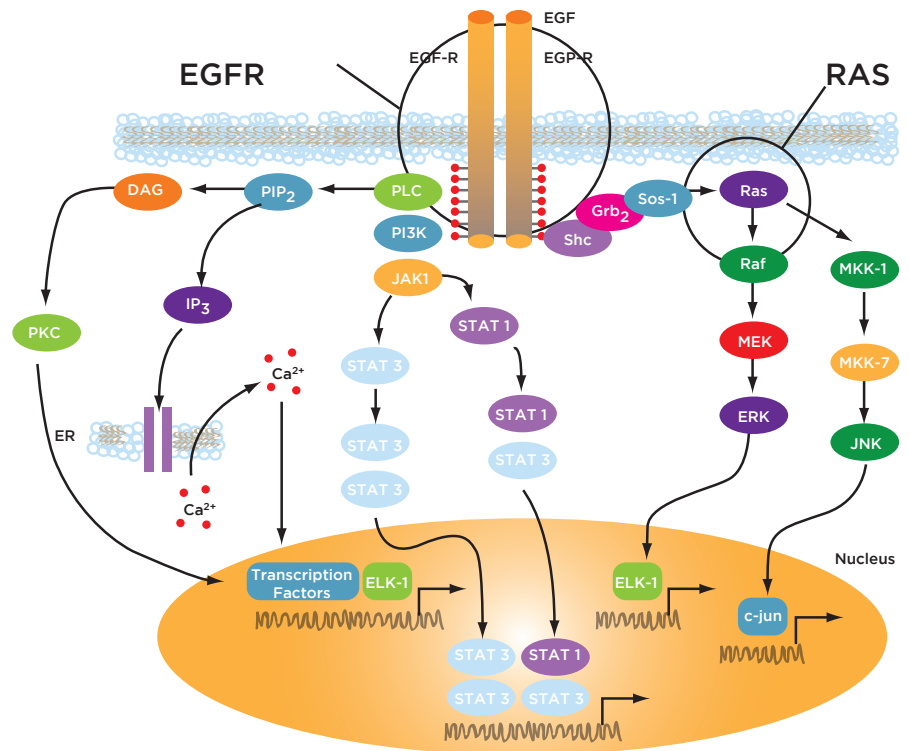
KRAS AND THE EGFR PATHWAY

EGFR is activated when the naturally occurring ligands, such as Epidermal Growth Factor (EGF), binds to the extracellular domain. This triggers internal cellular signals that stimulate cell growth. One route for the signal from the EGFR to be transmitted to the nucleus is via the KRAS-BRAF-MEK-ERK pathway (See Figure 1).

KRAS MUTATIONS IN COLORECTAL CANCER AND NON SMALL CELL LUNG CANCER (NSCLC)

KRAS mutations are identified in 30-40% of colorectal carcinomas and in 15-30% of NSCLCs.² In these tumours, the activated KRAS gene contributes significantly to several aspects of the tumour growth, including tumour survival, angiogenesis, proliferation and metastasis.¹ Patients with KRAS-mutant tumours do not benefit from EGFR inhibition and should not be treated with anti-EGFR therapies.

KRAS testing represents a step towards personalised medicine. The goal is to select the best treatment for each patient based on the unique features of each person's tumour.



EGF RECEPTOR SIGNAL TRANSDUCTION PATHWAY

Figure 1: The EGFR signalling cascade.

The KRAS protein regulates other proteins, downstream in the EGFR signalling cascade, that are associated with tumour survival, angiogenesis, proliferation and metastasis.¹

Mutation in KRAS results in continuous activation of the downstream pathways, regardless of whether the EGFR is activated or pharmacologically blocked. The association between KRAS mutations and a failure to respond to anti-EGFR therapies has been observed for both monoclonal antibodies and small molecule Tyrosine Kinase Inhibitors.²

TARGETED THERAPIES

Colorectal Cancer

As KRAS plays an important role in EGFR signalling, recent studies have investigated the use of KRAS mutational status as a predictor of response to EGFR inhibitors. The most striking results have come from studies of patients with metastatic Colorectal Cancer (mCRC) treated with Erbitux® (Cetuximab) or Vectibix® (Panitumumab). The EGFR gene is over-expressed in colorectal cancer and Vectibix® or Erbitux® bind to EGFR and inhibit the growth and survival of cells expressing EGFR. They competitively inhibit the binding of ligands to EGFR, which in turn prevents signal transduction leading to reduced cell growth and apoptosis. The effect of the KRAS mutation is to lock the KRAS gene product permanently in the 'on' position. It can be seen from the diagram that as KRAS is downstream of EGFR, inhibiting EGFR will have little effect on tumours where growth is being driven by KRAS gene mutations.

NSCLC

Tyrosine Kinase Inhibitor therapies (TKI), Tarceva® (Erlotinib) and Iressa® (Gefitinib), are used in treating advanced NSCLC. These drugs inhibit EGFR tyrosine kinase by binding to the ATP-binding site of the enzyme. The binding of this site blocks the activation of the KRAS signal transduction cascade and inhibits cell cycle progression. The effect of the KRAS mutation is to lock the KRAS gene product permanently in the 'on' position. As KRAS is downstream of EGFR, inhibiting EGFR will have little effect on tumours where growth is being driven by KRAS gene mutations.

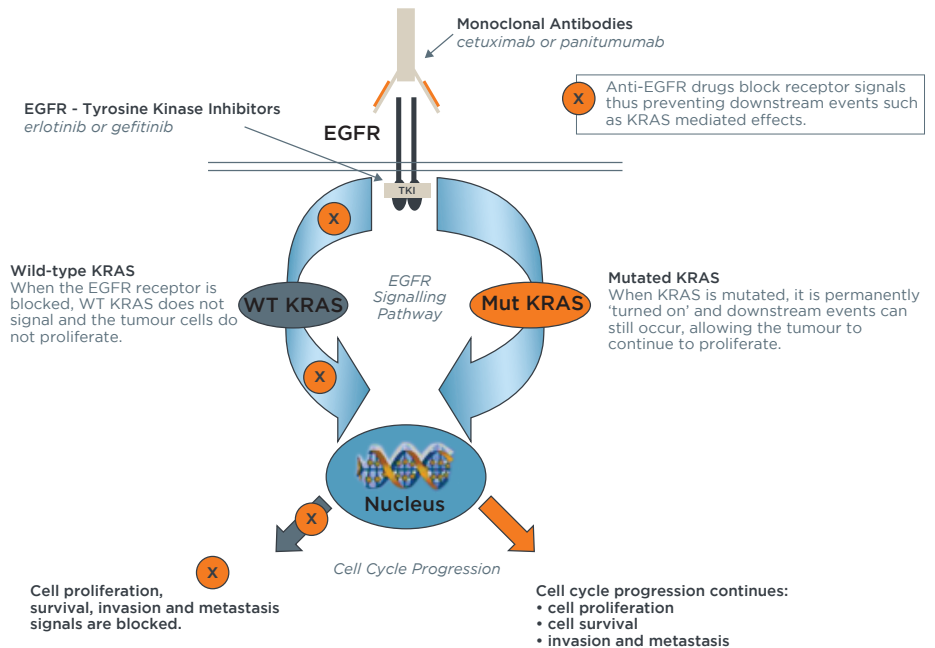
IMPORTANCE OF TESTING FOR KRAS MUTATIONS

KRAS gene is mutated in about 40% of colorectal cancers and 30% of NSCLCs. When this happens, anti-EGFR therapies do not work. By testing for KRAS mutations, doctors can give their patients the most effective treatment and not expose patients to unnecessary side effects.

REFERENCES

1. Benvenuti S. et al. (2007) Oncogenic Activation of the RAS / RAF Signalling Pathway Impairs the Response of Metastatic Colorectal Cancers to Anti-Epidermal Growth Factor Receptor Antibody Therapies. *Cancer Res* 67: 2643-2648.
2. Raponi M. et al. (2008) KRAS Mutations Predict Response to EGFR Inhibitors. *Curr Opin Pharmacol* 8(4): 413-418.
3. Di Nicolantonio F. et al (2008) Wild-type BRAF is Required for Response to Panitumumab or Cetuximab in Metastatic Colorectal Cancer. *J Clin Oncol* 26: 5705-5712.

ANTI-EGFR THERAPY AND KRAS MUTATIONS



BENEFITS OF TESTING FOR BOTH KRAS AND BRAF IN COLORECTAL CANCER

- BRAF is a gene that codes for a protein involved in the Epidermal Growth Factor Receptor (EGFR) signalling cascade. It is a downstream molecule from KRAS and studies have shown that patients with mutated BRAF do not respond to anti-EGFR treatment. KRAS mutations account for about 30-40% of the patients who are non-responsive to Vectibix® or Erbitux®. BRAF mutations account for another 10% of the non-responsive patients. BRAF and KRAS mutations are mutually exclusive and therefore are not found in the same tumours.
- However, when BRAF testing is performed on patients whose tumours are demonstrated to be wild-type (normal) KRAS, approximately 50% of patients who will be non-responsive to anti-EGFR treatment are identified.³ Healthscope Advanced Pathology offers testing for both KRAS and BRAF mutations.

KRAS TESTING AT HEALTHSCOPE ADVANCED PATHOLOGY

- Formalin-fixed paraffin embedded tumour sample or seven unstained slides and one H & E stained section are required for mutation analysis.
- All the logistics in getting the tumour block to our laboratory for analysis is handled by Healthscope Advanced Pathology staff.
- Each sample undergoes a pathologist review to ensure that tumour cells are present and a macrodissection to enrich for tumour cells that are used for analysis.
- To detect KRAS mutations, a highly sensitive nested PCR for codons 12 and 13 of exon two of the KRAS gene is performed. This is followed by a single base extension assay.
- Results are available within 5-7 working days from sample receipt in the laboratory.
- Currently this test is not Medicare rebated but we are optimistic about reimbursement as the market becomes more aware of the value of such testing.