

KRAS Mutation Analysis

Indication:

KRAS mutation analysis is currently used as a predictive marker of therapy response in the setting of anti EGFR therapy in metastatic colorectal carcinoma and pulmonary adenocarcinoma. Anti EGFR therapy is currently limited to tumors lacking molecular diagnostic evidence for *KRAS* or BRAF mutations.

Background and Rationale:

KRAS (Kirsten rat sarcoma virus) gene, mapped to 12p12.1, is a member of the *RAS* gene family that also includes *HRAS* and *NRAS*. *RAS* genes encode GDP/GTP-binding proteins involved in signal transduction during cellular proliferation, differentiation and senescence.

Activation of *KRAS*, its upstream regulators (eg. EGFR) or downstream pathway members (eg. RAF/MAP) are common events during oncogenesis. Several members of the *KRAS* signal transduction pathway are attractive targets of therapy in a variety of solid tumors¹⁻³. It is in the setting of targeted therapy using tyrosine kinase inhibitors that *KRAS* mutational status has acquired a role as a predictive marker of therapeutic response.

It is estimated that 17 to 25% of all human tumors harbor an activating *KRAS* mutation⁴. Although pancreatic carcinomas carry the highest frequency of *KRAS* mutations (over 90%), *KRAS* mutations are also frequently encountered in pulmonary non-small cell and colorectal carcinomas (approximately 40% each). Critical regions of oncogenic *KRAS* activating mutations include codons 12, 13, 59, 61, and 63⁵⁻⁷. Such mutations cause *KRAS* protein to accumulate in its active GTP-bound state⁸ leading to constitutive activation of downstream signaling. Such activating mutations provide a mechanism to bypass the antitumor effect of therapeutic strategies directed to upstream receptor tyrosine kinase regulators such as EGFR. Indeed, several clinical trials have now demonstrated lack of efficacy of anti EGFR agents such as Erlotinib (small molecule agent) and Cetuximab or Panitumumab (anti EGFR monoclonal antibodies) in colorectal and non-small cell lung carcinoma patients^{5, 6, 9-13}.

Variant Mutations:

In colorectal cancers, somatic *KRAS* mutations predominantly involve codons 12 and 13 of exon 1. Among the seven most commonly encountered mutations, six involve codon 12 and one mutation affects codon 13. The next most frequent mutation is in codon 61; however, most assays currently in use do not target this mutation. Geographical variations in the incidence of the different mutations are reported and are thought to be potentially attributed to geographic variations in carcinogen exposure.

In pulmonary adenocarcinoma, somatic *KRAS* mutations are far more frequent in tumors that are associated with a history of cigarette smoking¹⁴. The G12C mutation in codon 12 is by far the most frequent mutation in these tumors.

Although primary tumor samples were used in the original clinical trials that unveiled the predictive role of *KRAS* mutational analysis, both metastatic and primary tumor specimens are currently used in clinical practice for mutational analysis¹⁵. Several studies have indicated a reliable correlation of *KRAS* mutation status in primary and metastatic tumor specimens^{16, 17}.

Methodology:

Several molecular methodology platforms are currently in use for the detection of *KRAS* mutations in formalin fixed paraffin embedded (FFPE) tumor samples (table). Usually, manual microdissection is necessary to increase the fraction of tumor cells in the sample. Intratumoral heterogeneity, the presence of wild type alleles in the tumor cells, and dilution by stromal and inflammatory cells pose additional detection and sensitivity challenges. Therefore, a highly sensitive method is required for the detection of *KRAS* mutations. Methods currently in use are sensitive to about 10% mutant alleles in a sample, or tissue with at least 20% tumor containing a mutation of *KRAS* in one allele.

Allele specific amplification and detection systems include the amplification refractory mutation system (ARMS)/ real-time PCR detection method,¹⁸ single nucleotide extension, the oligonucleotide ligation assay, and short oligonucleotide mass analysis (SOMA). These methods will only detect the specific mutations covered by the assay. Additional mutations can be detected by mutation screening methods, such as high resolution melting analysis¹⁹, single stranded conformation polymorphism (SSCP) analysis, and restriction fragment length polymorphism (RFLP) analysis.

Sequencing techniques can detect all possible mutations in the targeted region. Traditional Sanger sequencing is not in general use for *KRAS* mutation detection due to its low sensitivity for minor alleles in a heterogeneous mixture of tumor and inflammatory / stromal tissue. Pyrosequencing is as sensitive as allele specific amplification methods.

Hybridization-based detection systems, including chip-based and reverse dot-blot strip assays, permit simultaneous screening for multiple mutations in one assay.

There is increasing interest in non-invasive techniques to detect *KRAS* mutations. Among these methods is magnetic capture and separation of tumor cells in peripheral blood, followed by nested allele-specific amplification of *KRAS*. The clinical utility of such alternate methods is yet to be proven.

TABLE

DIAGNOSTIC METHOD	ADVANTAGES	LIMITATIONS
Sanger sequencing	All possible mutations may be detected in target region	Low sensitivity for minor alleles (20%); labor intensive
Pyrosequencing ²⁰	High sensitivity; can customize assay design	Requires specialized equipment
Allele-specific real time PCR (ARMS/ Scorpions) ¹⁸	High sensitivity; kit based	Expensive; limited to 7 common mutations
Single nucleotide extension	High sensitivity; can customize assay design	Multiplexing necessary
Allele specific PCR and high resolution melting analysis ²¹	High sensitivity; can customize assay design	May detect minority populations (?mutations not biologically relevant)
Short oligonucleotide mass analysis (SOMA) ²²	High sensitivity; can customize assay design	Requires specialized equipment
Chip based assays	Ease of multiplexing	Requires specialized equipment
Strip Assay	Kit based	Limited to 10 common mutations

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