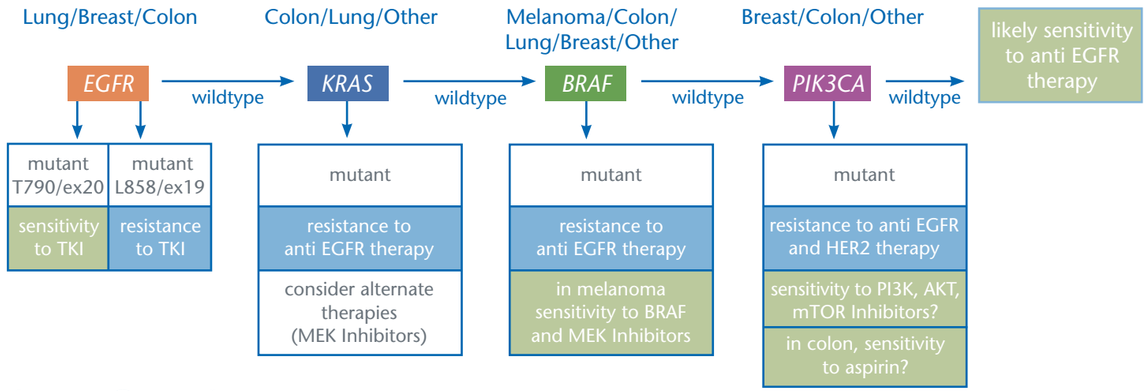
Treatment Indications



Assay Overview

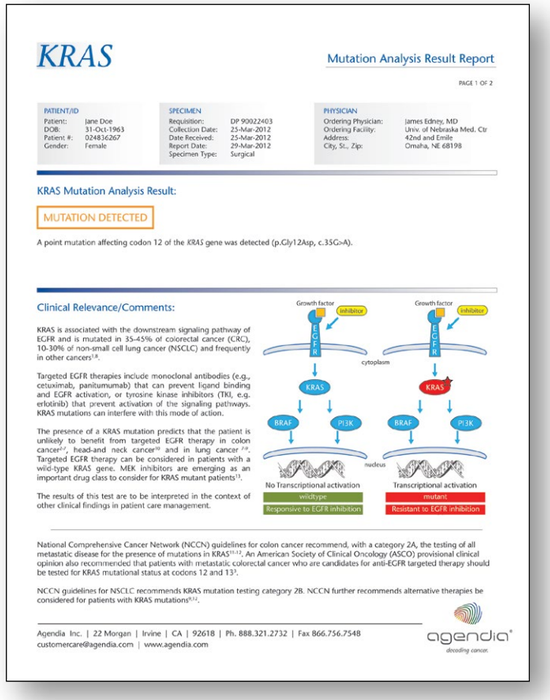
Mutation analysis tests detect somatic variants for:	
EGFR	29 mutations, insertions and deletions at codons 790, 858, 861, 768, 719 and exons 19 and 20
KRAS	7 mutations at codons 12 and 13
BRAF	5 mutations at codon 600
PIK3CA	4 mutations at codons 542, 545 and 1047

Limitations

The tumor load in the specimens should be above 30%. The highly sensitive assays are capable of reproducibly detecting mutations in samples with as little as 1 to 5% mutated DNA in a background of wildtype DNA. Primers were designed so that known variants do not interfere with mutation detection; however, the presence of previously unknown variants might interfere with assay performance on rare occasions. Poor DNA quality from FFPE specimens may limit analysis.

Methodology

Amplification refractory mutation system (ARMS) and real-time polymerase chain reaction using Scorpions™ technology.



KRAS

Mutation Analysis Result Report

PAGE 1 OF 2

PATIENT/ID
Patient: Jane Doe
DOB: 31-Oct-1963
Patient #: 024836267
Gender: Female

SPECIMEN
Requisition: DP 90022403
Collection Date: 25-Mar-2012
Date Received: 25-Mar-2012
Report Date: 29-Mar-2012
Specimen Type: Surgical

PHYSICIAN
Ordering Physician: James Edney, MD
Ordering Facility: Univ. of Nebraska Med. Ctr
Address: 42nd and Emile
City, St., Zip: Omaha, NE 68198

KRAS Mutation Analysis Result:

MUTATION DETECTED

A point mutation affecting codon 12 of the *KRAS* gene was detected (p.Gly12Asp, c.35G>A).

Clinical Relevance/Comments:

KRAS is associated with the downstream signaling pathway of EGFR and is mutated in 35-45% of colorectal cancer (CRC), 10-30% of non-small cell lung cancer (NSCLC) and frequently in other cancers[1,8].

Targeted EGFR therapies include monoclonal antibodies (e.g., cetuximab, panitumumab) that can prevent ligand binding and EGFR activation, or tyrosine kinase inhibitors (TKI, e.g. erlotinib) that prevent activation of the signaling pathways. *KRAS* mutations can interfere with this mode of action.

The presence of a KRAS mutation predicts that the patient is unlikely to benefit from targeted EGFR therapy in colon cancer[2-5], head-and neck cancer[10] and in lung cancer [7,9]. Targeted EGFR therapy can be considered in patients with a wild-type KRAS gene. MEK inhibitors are emerging as an important drug class to consider for KRAS mutant patients[11].

The results of this test are to be interpreted in the context of other clinical findings in patient care management.

National Comprehensive Cancer Network (NCCN) guidelines for colon cancer recommend, with a category 2A, the testing of all metastatic disease for the presence of mutations in KRAS[11-12]. An American Society of Clinical Oncology (ASCO) provisional clinical opinion also recommended that patients with metastatic colorectal cancer who are candidates for anti-EGFR targeted therapy should be tested for KRAS mutational status at codons 12 and 13[1].

NCCN guidelines for NSCLC recommends KRAS mutation testing category 2B. NCCN further recommends alternative therapies be considered for patients with KRAS mutations[9,12].

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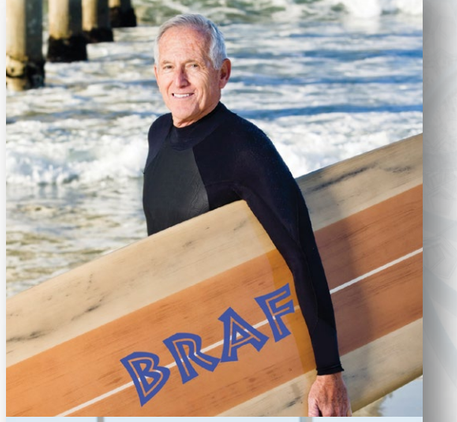


Mutation Analysis Guides Therapy Evaluation for Cancer Patients

EGFR • KRAS • BRAF • PIK3CA

agendia®
decoding cancer

Improving Response and Outcomes with Targeted Therapies

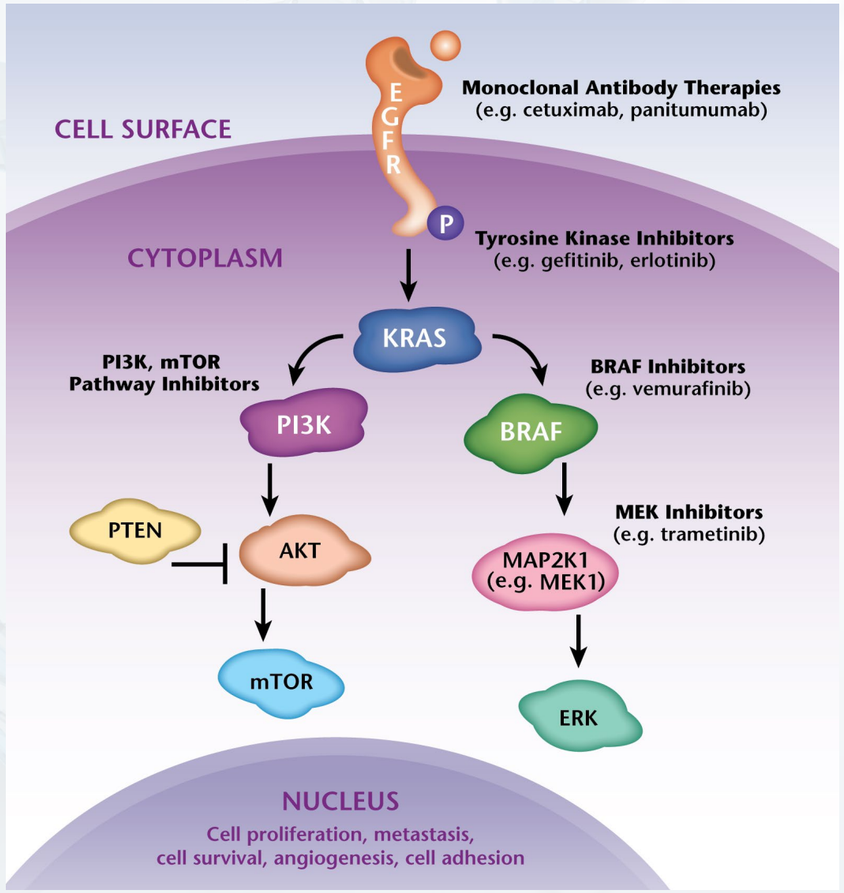
Therapies targeting key cell signaling pathways, such as those influenced by epidermal growth factor receptor (EGFR), can improve response and outcome in patients with metastatic cancers.

Biomarkers play an important role in identifying patients who may benefit from specific targeted therapies such as monoclonal antibodies (moAbs) and tyrosine kinase inhibitors (TKIs). To aid physicians in their treatment decisions, Agendia offers mutation analysis of key signaling genes.

By focusing on key genes for which mutations have a clearly understood effect, Agendia is able to provide physicians with important clinical information relevant to their treatment decisions. Assays are available for:

EGFR • KRAS • BRAF • PIK3CA

Each gene is analyzed for multiple mutations.



EGFR

- EGFR is one member of a family of cell surface membrane receptors. Activation of EGFR leads to tyrosine kinase activation and subsequent signaling events resulting in cell proliferation, discohesion, adhesion, inhibition of apoptosis, increased angiogenesis and resistance to chemotherapy.[1]
- Monoclonal antibodies (moAbs) such as cetuximab and panitumumab bind the extracellular receptor and can block downstream signaling. MoAbs can be effective single agents or be used in combination with chemotherapy agents when treating metastatic colorectal cancer (mCRC), but only 10-20% of patients benefit clinically due to changes in downstream effectors such as KRAS and BRAF.[2]
- Tyrosine kinase inhibitors (e.g. gefitinib and erlotinib) are a second type of agent used to modulate EGFR signaling. However, EGFR mutation status is associated with changes in sensitivity to treatment with an EGFR-TKI in patients with NSCLC.[3,4]
  - Common activating mutations exon 19 deletions, p.Leu858Arg, and less frequent p.Leu861Gln and p.Gly719Ala/Ser/Cys are associated with response to EGFR TKIs.
  - Mutations such as p.Thr790Met and exon 20 insertions are associated with resistance to TKIs.
- In NSCLC, patients harboring sensitizing EGFR mutations should be considered for first line treatment with erlotinib or gefitinib.[5-8]

NCCN Guidelines for NSCLC recommend testing recurrent or metastatic adenocarcinoma of the lung for EGFR mutation (category 1) and a combined level of evidence score of 1A owing to multiple randomized clinical trials.[4]

KRAS

- Mutations in EGFR's downstream signaling partners (KRAS, BRAF, PIK3CA) can also change the signaling status and override modulation by anti-EGFR therapy.
- KRAS is mutated in 35-45% of colorectal cancer (CRC), 10-30% of non-small cell lung cancer (NSCLC) and frequently in other cancers.[6,9]
- The presence of a KRAS mutation predicts that the patient is unlikely to benefit from targeted EGFR therapy. Targeted EGFR therapy can be considered in patients with a wild-type KRAS gene.
- MEK inhibitors are an important emerging drug class to consider for KRAS mutant patients.[12]

NCCN guidelines for colon cancer recommend testing of all metastatic disease for the presence of mutations in KRAS (category of evidence 2A).[10,11] ASCO provisional clinical opinion also recommended that patients with metastatic colorectal cancer who are candidates for anti-EGFR targeted therapy should be tested for KRAS mutational status at codons 12 and 13.

NCCN guidelines for NSCLC recommend KRAS mutation testing (category of evidence 2B). NCCN further recommends alternative therapies be considered for patients with KRAS mutations.

BRAF

- BRAF is mutated in over 60% of melanomas and in a smaller percentage of other cancers, including but not limited to papillary thyroid cancer, colorectal cancer (CRC), non-small-cell lung cancer (NSCLC), breast cancer and ovarian cancer.
- The most common mutations occur at position V600 (p.Val600).
- In colon cancer, BRAF mutations at codon 600 are an indicator of poor prognosis.[13] The common p.Val600Glu mutation is predictive of negative response to EGFR targeted therapy.[11] KRAS and BRAF mutations are typically not present in the same tumor.
- In melanoma, the presence of p.Val600Glu or p.Val600Lys mutation is associated with response to specific BRAF and MEK kinase inhibitors like vemurafenib or trametinib.[14,15] BRAF V600 mutations might also sensitize other cancers to BRAF and MEK inhibitors.[16]

The NCCN Guidelines for Colon Cancer recommend that BRAF testing be considered in the metastatic setting if the KRAS gene is found to be wildtype (category of evidence 2A).

Per FDA guidelines vemurafenib (ZELBORAF™) is indicated for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test. ZELBORAF is not recommended for use in patients with wildtype BRAF melanoma.[17]

PIK3CA

- PIK3CA mutations are frequent in lobular breast carcinoma (~40%), luminal breast cancer (~30%), endometrial cancer (~23%), ovarian cancer (~11%), colorectal cancer (~13%), cancer of the urinary tract (~19%), cervical cancer (~11%), and squamous cell cancer of the head and neck (~9%).[18]
- In colon cancer, evidence suggests that PIK3CA wildtype tumors that are also negative for KRAS and BRAF mutations are more likely to respond to anti-EGFR monoclonal antibody therapy.[19] It remains controversial if presence of one of the common mutations in either exon 9 or exon 20 is sufficient as an independent prognostic factor, or predictive for response to anti-EGFR targeted therapy.
- Evidence suggests a more negative prognosis for patients with an exon 9 mutation plus a second exon 20 mutation.[20] Recent data indicates the benefit of aspirin treatment in PIK3CA mutation positive colon cancers.[21]
- Clinical data in diverse solid tumors also indicate that patients with PIK3CA mutations treated with PI3K/AKT/mTOR pathway inhibitors have significantly higher response rates than patients without documented mutations.[22] Concomitant KRAS or BRAF mutations may mediate resistance.
- Multiple clinical trials are currently assessing the benefit of PI3K-inhibitors in breast, ovarian and endometrial cancers.