BRAF Codon 600 Mutation Analysis

Background Information

The BRAF protein plays a central role in cancer cell growth and survival. BRAF is a downstream effector of the RAS/RAF/MAPK/MEK signaling pathway. Signal transduction often is initiated via ligand binding of transmembrane receptors such as epidermal growth factor receptor (EGFR) or platelet-derived growth factor receptor (PDGFR), but there are many upstream activators of the RAS/RAF/MAPK/MEK pathway. The T1799A point mutation causes the V600E transversion in exon 15 of *BRAF* gene located at the 7q34 locus. This valine to glutamine mutation accounts for the vast majority of oncogenic *BRAF* mutations, and *BRAF* mutational analysis serves several different purposes in clinical practice.

Melanoma

BRAF mutations occur in 40-70% of cutaneous melanomas, with V600E mutations accounting for >90% of mutations. *BRAF* mutations seem to predict clinical response to either BRAF or MEK inhibitors in melanoma and other tumors. Vemurafenib (Zelboraf®) and dabrafenib (Tafinlar®) have been approved by the U.S. Food & Drug Administration for the treatment of inoperable or metastatic melanoma that is positive for the *BRAF* V600E mutation. Trametinilib (Mekinist®) is approved to treat patients whose tumors express the *BRAF* V600E or 600K gene mutations.

Although BRAF V600E accounts for the vast majority of V600 mutations, other activating mutations have been reported. The most common of which is the *BRAF* V600K mutation, which, based on limited data, seems to have a response rate similar to V600E mutant melanomas after undergoing targeted therapy. Other mutations in codon 600 (e.g., V600M) may also respond to BRAF or MEK inhibitor therapy, but actual data are quite limited.

Lynch Syndrome

Lynch syndrome or hereditary non-polyposis colorectal cancer (HNPCC) patients have up to an 80% lifetime incidence of colorectal cancer in addition to increased risk for endometrial, skin, urinary tract, ovarian, small intestinal, biliary and gastric cancers, among others. High microsatellite instability (MSI-H) characterizes >90% of colorectal carcinomas occurring in the setting of Lynch syndrome and serves as a surrogate marker of DNA mismatch repair deficiency. The DNA mismatch repair machinery is composed of a number of genes, with *MLH1, MSH2, MSH6,* and *PMS2,* as well as the recently identified *EPCAM*, accounting for about 95% of the identifiable causative mutations in Lynch syndrome. About 10-15% of sporadic colorectal carcinomas also demonstrate MSI-H phenotype. Both sporadic and HNPCC-associated MSI-H colorectal carcinomas can demonstrate defects in MLH1 expression by immunohistochemistry, whereas loss of expression of MSH2, MSH6 or isolated PMS2 are more commonly associated with Lynch syndrome/HNPCC than sporadic cancers.

Gene sequencing all carcinomas with MLH1 loss would be unnecessarily laborious and expensive, and *BRAF* mutation testing can help exclude about half of these patients. *BRAF* V600E mutations occur in up to 75% of sporadic MSI-H colorectal carcinomas, whereas, until recently, these mutations were never reported in HNPCC-associated carcinomas. There are rare reports of Lynch syndrome-associated mutations (1-2%; reported in PMS2, MLH1 and MSH2 mutation carriers) with concomitant *BRAF* V600E mutation, so the discovery of a *BRAF* mutation does not absolutely exclude Lynch syndrome (*J Med Genet.* 2012 Mar;49(3):151-7).

Prognostic/predictive marker in metastatic colorectal carcinoma

Several retrospective studies have found that tumors harboring *BRAF* V600E mutations do not respond to anti-EGFR monoclonal antibody therapy, similar to *KRAS* codon 12/13 mutations. Anti-EGFR therapies are extremely expensive, have side effects and may delay the use of other, more effective treatments in these patients. However, this predictive role of *BRAF* mutation recently has come under question following publication of the first prospective data examining *BRAF* mutation status and outcome. KRAS/BRAF wild type tumors had a significantly reduced risk of disease progression and significantly increased odds of response when treated with conventional chemotherapy and an anti-EGFR monoclonal antibody compared with those who received conventional chemotherapy alone. However, there was no difference in overall survival in these groups. Accordingly, the National Comprehensive Cancer Network views *BRAF* mutation testing in metastatic colorectal cancer as optional.

BRAF V600E also has prognostic value in colorectal cancer. Patients with metastatic colorectal carcinoma or microsatellite stable (MSS) non-metastatic colorectal carcinoma harboring *BRAF* V600E mutations have been found to have significantly worse overall survival, progression-free survival and response rates to conventional chemotherapy.

Thyroid carcinoma

BRAF is the most commonly mutated gene in papillary thyroid carcinoma (PTC), occurring in approximately 45% of tumors. Greater than 95% of *BRAF* mutations in PTC are the V600E transversion. *BRAF* mutations usually are encountered in PTC with conventional or tall cell histology, whereas *BRAF* mutation in the follicular variant of PTC is uncommon. *BRAF* mutations are not seen in follicular neoplasms, making *BRAF* mutations a good marker of PTC. In addition, *BRAF* V600E mutations have been correlated with aggressive histologic features in PTC, poor treatment outcomes, tumor recurrence and tumor-related death.

Clinical Indications

BRAF Codon 600 mutation testing is reflexively performed on all MSI-H colorectal neoplasms that demonstrate loss of MLH1 immunohistochemical protein expression. For the remaining numerous possible indications, the test is performed on a case-by-case basis.

Interpretation

Results are reported as “No BRAF mutation detected,” or “BRAF p.V600E (c.1799T>A) mutation detected.”

If a mutation other than *BRAF* V600E is detected, the particular mutation will be reported in a similar manner to V600E.

Results are confirmed using both forward and reverse sequences.

Limitations of the Assay

Tissue may be fresh-frozen or, most commonly, formalin-fixed and paraffin-embedded. If paraffin-embedded, a pathologist will review a representative H&E-stained slide and select the most appropriate region for microdissection; microdissection is routinely performed to increase tumor DNA yield. The lower limit of reliable mutation detection is 25% tumor cells, and mutations may not be detected in samples with abundant dilution by non-tumor DNA. This is particularly relevant in the post-adjuvant therapy setting.

Methodology

BRAF mutation testing is performed by DNA sequencing on the ABI 3730 Genetic Analyzer. Following PCR amplification DNA with primers flanking codon 600 within exon 15, the purified PCR product is subjected to cycle sequencing using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, Calif.). Forward and reverse strands are sequenced. Cycle sequencing products are purified using the BigDye XTerminator® Purification Kit (Applied Biosystems) and loaded on the ABI 3730 Genetic Analyzer (Applied

Biosystems). Sequences are aligned with wild type sequences and assessed for the V600E point mutation or any other mutations in the sequenced DNA.

Suggested Reading

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10. Loupakis F, Ruzzo A, Cremolini C, *et al*. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer*. 2009;101:715-21.
11. Nikiforova MN, Nikiforov YE. Molecular diagnostics and predictors in thyroid cancer. *Thyroid*. 2009;19:1351-61.
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Test Overview

Test Name	BRAF V600E Sequencing
Methodology	Polymerase Chain Reaction (PCR); Capillary Electrophoresis (CE); Sequencing
Specimen Requirements	Block, formalin-fixed paraffin or 10 unstained slides. Slides must include 1-2 cm^2 of total tumor area from formalin-fixed, paraffin-embedded tissue
Billing Code	87800
CPT Codes	83891; 83894; 83898; 83904 (x2); 83909 (x2); 83912; 88381

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