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Inside This Issue

BRAF V600E Mutation Analysis of Tumor Tissue

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SUMMARY

Beginning August 1, 2012, Marshfield Labs will begin testing for V600E mutations in the *BRAF* gene (V-raf murine sarcoma viral oncogene homolog B1) that encodes the serine/threonine-protein kinase B-Raf protein. Marshfield Labs will use the Roche cobas® 4800 BRAF V600 Mutation Test which is an in vitro diagnostic device intended for the qualitative detection of BRAF V600E mutation (1799T>A) in DNA extracted from formalin-fixed, paraffin-embedded human melanoma tissue. The BRAFMEL test is intended to be used as an aid in selecting melanoma patients whose tumors carry the BRAF V600E mutation for treatment with vemurafenib. The BRAFOTH test is used to detect the BRAF V600E mutation from samples of other tumor types.

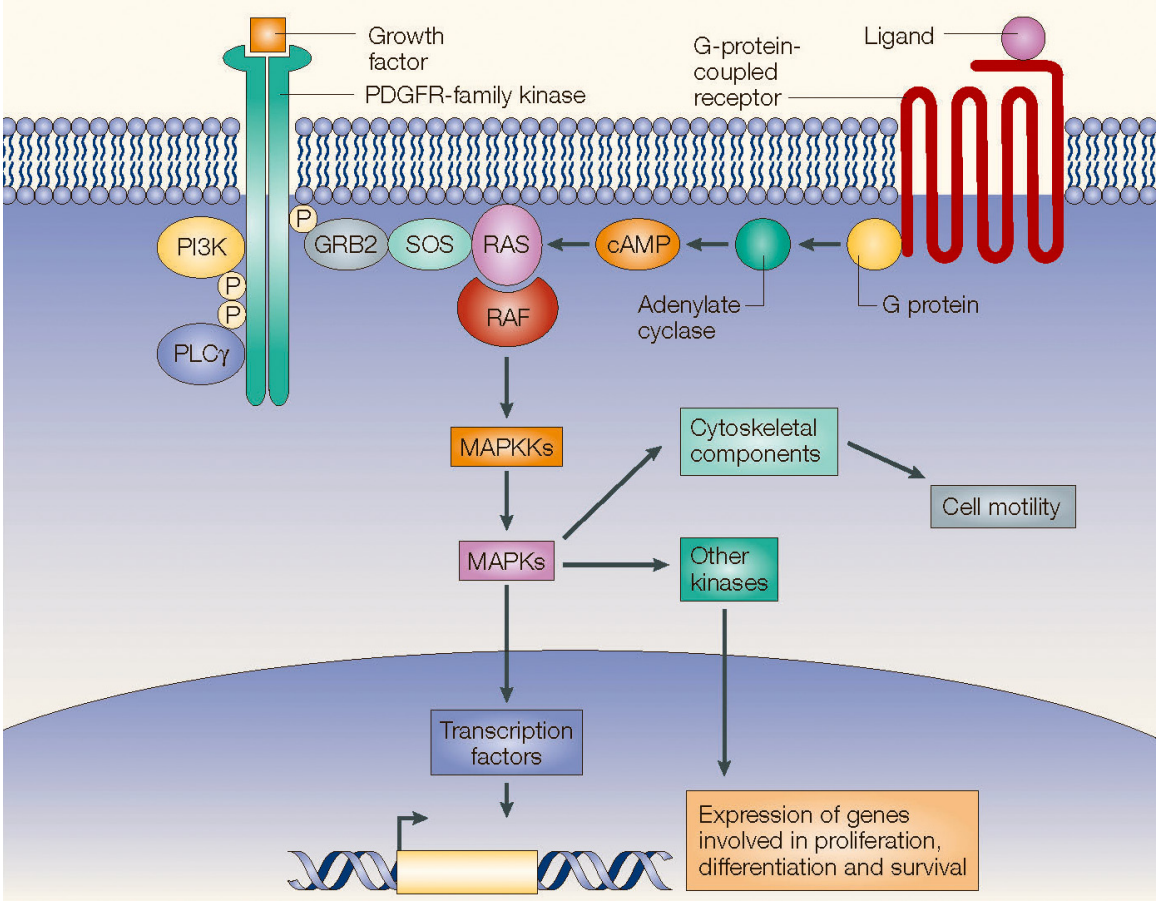
The lab test codes will be **BRAFMEL, BRAFOTH**.
These will replace the BRAFSO and BRMELSO tests.

BACKGROUND

B-Raf is a kinase protein that plays a role in regulating the MAP kinase/ERKs signaling pathway affecting cell division, differentiation, and secretion. Missense B-Raf kinase domain mutations are frequently observed in hairy cell leukemia (~100%), melanoma (60-80%), papillary thyroid cancer (~45%), colon cancer (5%), and less frequently in lung, and other cancer types[1]. The vast majority (>90%)

continued on page 2

involve a thymine to adenine mutation at base pair 1799 of the mRNA generating a glutamic acid for valine substitution at codon 600 (V600E), which results in elevated B-Raf kinase activity. Activating mutations such as V600E lead to constitutive activation of the pathway, resulting in abnormal cell growth.



Signal Transduction by PDGFR-family Kinases and BRAF

NATURE REVIEWS | CANCER

TESTING INDICATIONS

Melanoma [BRAFMEL]:

Vemurafenib (marketed as Zelboraf™) is a B-Raf enzyme inhibitor that received FDA approval for the treatment of late-stage melanoma in August 2011. Vemurafenib has been shown to cause programmed cell death in melanoma cells through interruption of the B-Raf/MEK pathway in tumors with a V600E BRAF mutation. In an open-label, multicenter, international, randomized Phase III study in previously untreated patients with BRAF V600 mutation-positive unresectable or metastatic melanoma, 675 patients were randomized 1:1 to treatment with Zelboraf™ (960 mg twice daily) or dacarbazine (1,000 mg/m^2 every 3 weeks)[2]. Statistically significant and clinically meaningful improvements were observed in overall survival and progression-free survival. Overall survival was longer with Zelboraf™ compared to dacarbazine with a hazard ratio of 0.37 representing a 63% decrease in the hazard of death with Zelboraf™ compared to dacarbazine. Progression-free survival was improved with Zelboraf™ compared to dacarbazine with a hazard ratio for progression or death of 0.26 representing a 74% decrease in the hazard of progression or death for Zelboraf™ compared to dacarbazine. Patients carrying the V600K mutation have also shown response

continued on page 3

to vemurafenib[2], and pre-clinical evidence demonstrates effects against the V600D mutation as well[3]. Melanoma cells without these activating mutations are not inhibited by vemurafenib and the drug paradoxically stimulates normal B-Raf which may promote tumor growth in such cases.

Metastatic Colorectal Carcinoma [BRAFOTH]:
Similar to KRAS mutations (see Marshfield Labs Laboratory Newsletter Vol. 34., No. 2, Feb. 18, 2011) BRAF V600E mutations have been associated with poor prognosis and are predictive of limited clinical response to cancer therapies such as those targeted to the EGFR pathway. Recent studies have demonstrated approximately 5-8% of colorectal carcinoma contain the BRAF V600E-activating mutation. The presence of this mutation correlates with resistance to treatment with cetuximab and panitumumab and is mutually exclusive with KRAS activation mutations. Marshfield Labs will reflexively order BRAF mutation analysis (BRAFOTH) on all KRAS mutation-negative colorectal carcinoma samples.

Other Tumor Types [BRAFOTH]:
The BRAF V600E mutation has also been identified in thyroid[4,5], lung[6], hairy cell leukemia[5] and other cancers. Testing for the BRAF V600E mutation may be appropriate in many of these tumor types.

QUALITATIVE INTERPRETATION

Sample Positive Report
Positive: V600E Mutation Detected in the BRAF codon 600 site in exon 15. *BRAFMEL reports will include this additional sentence:* The FDA has approved vemurafenib (Zelboraf™) for the treatment of metastatic melanoma harboring a BRAF V600E mutation.

Sample Negative Report
Negative: V600E Mutation Not Detected in the BRAF codon 600 site in exon 15. *BRAFMEL reports will include this additional sentence:* Vemurafenib (Zelboraf™) treatment of metastatic melanoma is not recommended in tumors without a BRAF V600E mutation.

Sample Insufficient Tumor Report
The specimen was examined microscopically, and the proportion of tumor to normal cells was determined to be insufficient for further analysis.

Sample Indeterminate Report
DNA sample extensively degraded; analysis inconclusive due to poor DNA integrity.

How To Order This Test: *BRAF* Melanoma V600E Analysis or *BRAF* Other V600E Analysis

Lab Test Code:
BRAFMEL or BRAFOTH

Clinic (Clinical Order Manager):
BRAFMEL, BRAFOTH

HOSPITAL (Centricity): *BRAF* Melanoma V600E Analysis, *BRAF* Other V600E Analysis. Downtime: Write-In (Form I)

Specimen Requirements:
Local – Fresh tissue (Marshfield Clinic Center ONLY)
10% neutral buffered formalin fixed, paraffin embedded tumor block with an H and E if possible or cytology paraffin-embedded cell block from fine needle aspirate. Please send a copy of the pathology report with the sample. Fresh and formalin fixed samples should be clearly labeled indicating BRAF testing.

Outreach - 10% neutral buffered formalin fixed, paraffin embedded tumor block with an H and E if possible or cytology paraffin-embedded cell block from fine needle aspirate. Please send a copy of the pathology report with the sample.

Minimum:
Microscopic slide shows 2mm area containing >50% tumor cells.

Rejection Criteria:
Microscopic slide shows 2mm area containing <50% tumor cells.

Storage:
Room temperature.

Available:
Test is set up one time per week; analytic time of 10-12 days.

CPT Codes:
Commercial: 81210, 88363(if needed).

Medicare/Medicaid: 83891, 83896x2, 83898x2, 83907, 83912, 88363(if needed), 88381(if needed).

continued on page 4

Both the negative and positive reports will include the following information:
Microscopy: Microscopic analysis of the sample showed XX% tumor cells. Sample is suitable for BRAF mutation analysis. This test can detect the BRAF V600E mutation at ≥5% mutation level using the standard input of 125 ng at a concentration of 5ng/μL among a 95% wild-type sequence background. A negative test does not exclude the presence of a mutation.

Methodology: Pathology tissue sample is micro dissected to ensure sufficient tumor is present. The tumor sample is deparaffinized and DNA is isolated after proteinase K digestion. PCR amplification and detection target DNA is accomplished using a complementary primer pair and two oligonucleotide probes labeled with different fluorescent dyes. One probe is designed to detect the wild-type BRAF V600 sequence and one is designed to detect the V600E mutation sequence. This test methodology also shows limited sensitivity for other BRAF activating mutations such as V600K, V600D, and V600E2 but will not discriminate between these and the V600E mutation. Tumors containing these activating mutations may also respond favorably to vemurafenib.

For questions and additional information, please contact:
Dr. Uphoff, Dr. Krawisz, Dr. Sitwala, or Dr. Resnick at 800-222-5835.

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