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KDL DermPath Update

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KDL Reflex Testing for Metastatic Melanoma: FDA-Approved Companion Diagnostic for BRAF V600E

The U.S. FDA recently approved the use of a new targeted therapeutic agent, vemurafenib (Zelboraf, Roche) for patients with metastatic melanoma or unresectable primary melanoma. Vemurafenib is the first drug specifically targeted for treatment of patients expressing the mutation BRAF V600E. B-Raf is a key component of the KRAS/ERK signaling pathway involved in regulating normal cell growth and survival. The BRAF V600E genotype contains a point mutation that keeps the mutated B-Raf kinase in an active state that may cause excessive signaling, leading to uncontrolled cell growth. BRAF mutations (>90% BRAF V600) are thought to occur in an estimated half of all late-stage melanomas and eight percent of solid tumors.[1]

Vemurafenib acts to specifically block the function of the V600E-mutated B-Raf kinase. BRAF mutations are commonly found in other malignancies including colorectal cancer, non-Hodgkin lymphoma, and non-small cell lung cancer, and treatment of these conditions with targeted EGFR inhibitors following companion diagnostics have been used successfully for some time. Confirmation of the BRAF V600E mutation by an FDA approved diagnostic is required for treatment with vemurafenib. To date, the Cobas® 4800 BRAF V600 Mutation test is the only FDA-approved companion diagnostic for detecting BRAF V600E.

Molecular testing of melanoma for BRAF mutations is offered by a number of laboratories, some of which market directly to clinicians to solicit testing. Much of this marketing fails to communicate how the testing is arranged or the required pathology. The Cobas® BRAF V600E test utilizes a PCR-based technology to identify people whose tumors carry the BRAF V600E mutation. The test is performed from a small amount of formalin fixed paraffin embedded (FFPE) tissue following tumor identification on a microscopic slide by the dermatopathologist; thus, routine histologic processing of the specimen prior to testing is necessary (Fig. 1, 2). The pathologist marked slide and corresponding FFPE tumor block are sent for BRAF analysis. A report documents the presence or absence of the BRAF V600 mutation (Fig. 3).

KDL has partnered with Clarient, a cancer molecular diagnostics laboratory, to offer the Cobas® BRAF V600E Mutation test. KDL will provide reflex testing for BRAF V600E on all new specimens of metastatic melanoma or unresectable primary melanomas. In addition, testing of a primary lesion may be a considered if there is difficulty in obtaining a tissue sample in a patient with metastatic disease. Upon request, BRAF V600 mutation analysis can be arranged on new primary melanoma samples and archival melanoma samples. If vemurafenib becomes available for adjuvant therapy in patients with advanced Stage II or Stage III melanomas, testing of these advanced primary melanomas may be a future consideration.

BRAF V600E mutations occur in both benign melanocytic nevi and melanoma. The presence of BRAF mutations cannot be used to help distinguish between benign and malignant melanocytic

(continued)

neoplasms. Furthermore, the presence of BRAF V600E in primary melanoma may not be predictive of future metastatic behavior. However, in a patient with metastatic melanoma, the presence of BRAF mutations is associated with inferior survival. The presence of BRAF V600E mutation is predictive of response to the BRAF inhibitor vemurafenib (Zelboraf).[1-5] In Phase III studies, patients with advanced metastatic disease treated with vemurafenib demonstrated superior overall and progression-free survival versus patients treated with dacarbazine chemotherapy.[2]

The clinicopathologic features of BRAF V600E-associated melanoma include histopathologic subtype (superficial spreading and nodular melanomas), presence of dermal mitoses, truncal location, single or occult primary melanoma, less evident sun damage, higher total body nevus counts and age at diagnosis of primary tumor (≤ 50 years).[3]

Adverse events reported with vemurafenib (Zelboraf) therapy include rash, arthralgia, fatigue and photosensitivity. Keratoacanthoma, cutaneous squamous cell carcinoma, or both, developed in 18% of patients receiving vemurafenib.[2] Biopsy samples of possible drug reactions and/or neoplasms from patients receiving vemurafenib, or other biologic therapeutics, should be accompanied by pertinent medication history.

As with other FDA-approved diagnostic tests, this test is typically reimbursed by government programs and third party payers. Thus, patients will only be responsible for typical co-pays and deductibles applicable to laboratory charges. KDL believes no patient should be turned away due to high medical or laboratory charges.

Taylor LR, Coleman NM, Googe PB

Please contact a KDL dermatopathologist @ 865.584.1933 to order or further discuss BRAF V600E mutation analysis.

References

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Illustrative Cases of KDL BRAF V600E+ Melanoma

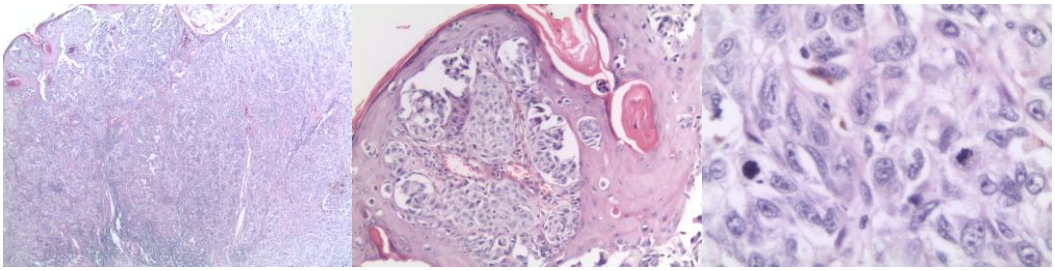


Figure 1. *BRAF V600E positive primary melanoma.* 29 Year old female patient with superficial spreading level III 3.7 mm melanoma with 7 mitoses/sq. mm.

Figure 2. *BRAF V600E detected in metastatic melanoma.*

Male patient with level III 1.5 mm superficial spreading melanoma on R. arm in 1999. Developed subcutaneous metastases on chest and right axilla in 2010 and 2011, respectively. BRAF mutation detected in subcutaneous metastasis R axilla.

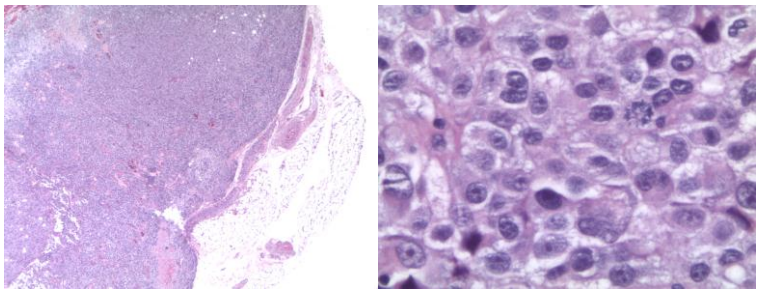


Figure 3. *KDL/Clarient BRAF V600E report.*

CLARIENT

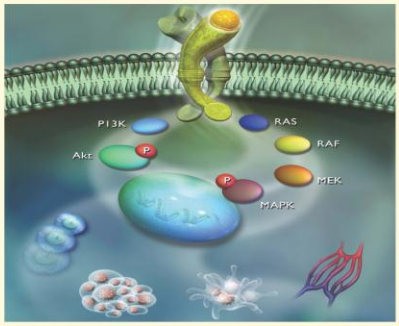
Kenneth J. Bloom, M.D.
Laboratory Director
CLIA I.D. #05D1021650

Patient Name		**Client Name**	Knoxville Dermatopathology Lab
Date of Birth		**Client ID#**	
Gender	M	**Ordering Physician**	Dr. Paul Googe
Medical Record #		**Treating Physician**	
Master Accession		**Date/Time Collected**	8/4/2011
Case No		**Date/Time Received**	8/12/2011 1:32:00 AM
Concurrent Cases			

KDL PATHOLOGY

BRAF V600 Mutation - Melanoma

Left Axilla Tissue 940312/D113738-E : BRAF Mutation Detected



Genotype Result:
Mutation V600E

Reference Range
Mutation Detected: V600E Mutation Detected in the BRAF codon 600 site in exon 15
Mutation Not Detected*: V600E Mutation Not Detected in the BRAF codon 600 site in exon 15

Methodology: This test was performed using the cobas® 4800 BRAF V600 Mutation Test. Tumor areas were identified, selectively microdissected, and lysed and DNA extracted. Real-time PCR using a single primer set was used to amplify the region of the BRAF gene which contains the mutation site. Two fluorogenically-labeled probes were used to specifically detect the wild-type and V600E mutant sequences.

Intended Use: The cobas® 4800 BRAF V600 Mutation Test is an in vitro diagnostic device intended for the qualitative detection of BRAF V600E mutation in DNA extracted from formalin-fixed, paraffin-embedded human melanoma tissue. The cobas® 4800 BRAF V600 Mutation Test is a real-time PCR test on the cobas® 4800 system, and is intended to be used as an aid in selecting melanoma patients whose tumors carry the BRAF V600E mutation for treatment with vemurafenib.

* A "Mutation Not Detected" result does not preclude the presence of a mutation in the BRAF codon 600 site since results depend on percent mutant sequences, adequate specimen integrity, absence of inhibitors, and sufficient DNA to be detected.

Electronically Signed By John Glassco, M.D., Pathologist on 8/26/2011 4:43:54 PM

John Glassco, M.D., Pathologist

This test is used for clinical purposes. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing. The results of this study are to be interpreted in the context of all other clinical findings.

KDL Pathology

Dedicated to Providing Superior Diagnostics

KDL is dedicated to providing quality surgical pathology services to physicians treating skin related diseases.

Diagnostic interpretations are performed by fellowship-trained dermatopathologists, certified by the American Boards of Dermatology and Pathology.

Services:

- **Evaluation of skin biopsies/excisions**
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- **Regional couriers & overnight mail kits**
- **Immunofluorescence**
- **Immunohistochemistry**
- **Expert consultation on problem cases**
- **Web reporting/available interface with EMR**

CAP ACCREDITED

Paul B. Googe, M.D., Founder and Laboratory Director of KDL, is board certified in dermatopathology and anatomic pathology. A native of Knoxville, TN, he completed his post-graduate training in pathology and dermatopathology at Massachusetts General Hospital in Boston, MA following medical school and an internal medicine internship at The University of Tennessee College of Medicine. Dr. Googe has practiced dermatopathology for over 20 years and holds volunteer faculty appointments as Clinical Professor of Pathology at The University of Tennessee Graduate School of Medicine and Vanderbilt University.

KDL Pathology

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