COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Melanoma

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The College of American Pathologists offers these templates to assist pathologists in providing clinically useful and relevant information when reporting results of biomarker testing. The College regards the reporting elements in the templates as important elements of the biomarker test report, but the manner in which these elements are reported is at the discretion of each specific pathologist, taking into account clinician preferences, institutional policies, and individual practice.

The College developed these templates as educational tools to assist pathologists in the useful reporting of relevant information. It did not issue them for use in litigation, reimbursement, or other contexts. Nevertheless, the College recognizes that the templates might be used by hospitals, attorneys, payers, and others. The College cautions that use of the templates other than for their intended educational purpose may involve additional considerations that are beyond the scope of this document.

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CAP Melanoma Biomarkers Template Revision History

Version Code
The definition of the version code can be found at www.cap.org/cancerprotocols.

Version: MelanomaBiomarkers 1.0.0.0

Summary of Changes
This is a new template.

Melanoma Biomarker Reporting Template

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Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team.

MELANOMA

Select a single response unless otherwise indicated.

Note: *Use of this template is optional.*

+ RESULTS

+ BRAF Mutant Protein (by immunohistochemistry) (Note A)
+ ___ Negative
+ ___ Positive
+ ___ Cannot be determined (explain): _____

+ *BRAF* Mutational Analysis (Note A)
+ ___ No mutations detected
+ ___ *BRAF* V600E (c.1799T>A) mutation
+ ___ *BRAF* V600K (c.1798_1799GT>AA) mutation
+ ___ *BRAF* V600R (c.1798_1799GT>AG) mutation
+ ___ *BRAF* V600D (c.1799_1800TG>AT) mutation
+ ___ Other *BRAF* mutation (specify): _____
+ ___ Cannot be determined (explain): _____

+ *NRAS* Mutational Analysis (Note B)
+ ___ No mutations detected
+ ___ *NRAS* Q61R (c.182A>G) mutation
+ ___ *NRAS* Q61K (c.181C>A) mutation
+ ___ *NRAS* Q61L (c.182A>T) mutation
+ ___ *NRAS* Q61H (c.183A>T) mutation
+ ___ *NRAS* G12R (c.34G>C) mutation
+ ___ *NRAS* G12S (c.34G>A) mutation
+ ___ *NRAS* G12D (c.35G>A) mutation
+ ___ *NRAS* G12V (c.35G>T) mutation
+ ___ *NRAS* G13R (c.37G>C) mutation
+ ___ *NRAS* G13S (c.37G>A) mutation
+ ___ Other *NRAS* mutation (specify): _____
+ ___ Cannot be determined (explain): _____

+ *KIT* Mutational Analysis (Note C)
+ ___ No mutations detected
+ ___ *KIT* L576P (c.1727T>C) mutation
+ ___ *KIT* K642E (c.1942A>G) mutation
+ ___ *KIT* V559A (c.1676T>C) mutation
+ ___ *KIT* W557R (c.1669T>A) mutation
+ ___ Other *KIT* mutation (specify): _____

+ ___ Cannot be determined (explain): _____

+ Other Markers Tested (if applicable)
+ Specify marker: _____
+ Specify results: _____

+ METHODS

+ Immunohistochemistry for Mutant BRAF Protein
+ Primary Antibody
+ ___ VE1
+ ___ Other (specify): _____

+ *BRAF* Mutational Analysis Testing Method
+ Assay sensitivity* (specify): _____
+ ___ Cobas 4800 *BRAF* V600 mutation test
+ ___ THxID *BRAF* assay
+ ___ Allele-specific/real time polymerase chain reaction (other platform)
+ ___ Direct (Sanger) sequencing
+ ___ Pyrosequencing
+ ___ SnapShot
+ ___ Mass spectrophotometry genotyping (Sequenom)
+ ___ Next-generation sequencing (specify amplicon vs hybrid capture): _____
+ ___ Other (specify): _____

** Assay sensitivity should be defined as lowest acceptable tumor percentage in a sample according to the pathologist's estimate.*

+ *NRAS* Mutational Analysis Testing Method
+ Assay sensitivity* (specify): _____
+ ___ Direct (Sanger) sequencing
+ ___ Pyrosequencing
+ ___ SnapShot
+ ___ Mass spectrophotometry genotyping (Sequenom)
+ ___ Next-generation sequencing (specify amplicon vs hybrid capture): _____
+ ___ Other (specify): _____

** Assay sensitivity should be defined as lowest acceptable tumor percentage in a sample according to the pathologist's estimate.*

+ *KIT* Mutational Analysis Testing Method
+ Assay sensitivity* (specify): _____
+ ___ Direct (Sanger) sequencing
+ ___ SnapShot
+ ___ Mass spectrophotometry genotyping (Sequenom)
+ ___ Next-generation sequencing (specify amplicon vs hybrid capture): _____
+ ___ Other (specify): _____

** Assay sensitivity should be defined as lowest acceptable tumor percentage in a sample according to the pathologist's estimate.*

+ Testing Method for Other Markers
+ Specify method: _____

+ COMMENT(S)
_____
_____

Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed February 10, 2015).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/; accessed February 10, 2015).

Explanatory Notes

Background

The incidence of melanoma has increased 2% per year over the last decade with a concomitant 1% increase per year in mortality in the same period.[1] Melanoma is unique among human tumors in its ability to give rise to metastatic disease even when only a few millimeters in size or at low primary stage.[2] Historically, there were few effective therapies for metastatic melanoma; however, recent breakthroughs in immunotherapy (eg, checkpoint inhibition) and targeted therapies against commonly activated oncogenes have led to improvements in response rates and survival. In most melanomas, oncogenic growth/proliferation signaling appears to be driven by alterations in the RAS/RAF/MAPK and PI3K pathways, with 70% to 80% of cutaneous melanomas containing somatic oncogenic mutations in 1 of 3 oncogenes and 2 tumor suppressors—*BRAF*, *NRAS*, *KIT*, *PTEN*, *NF1*—highlighting the importance of the ERK and AKT pathways in this disease.[3] Only BRAF activating mutations are currently validated for use in clinical practice as a predictive marker of response for approved BRAF-mutant directed therapies, but this field is rapidly evolving.

A. *BRAF* Mutational Analysis

BRAF mutations occur in up to 50% of melanomas. Of these mutations, 95% occur at amino acid 600, most commonly as Val600Glu (V600E) or sometimes Val600Lys (V600K), and lead to constitutive MAPK pathway activation.[4] A randomized phase III trial of a targeted inhibitor of V600E mutated BRAF, vemurafenib, was first published in 2011. This trial was limited to BRAF V600-mutated melanomas and demonstrated a significant improvement in overall survival at 6 months in patients treated with vemurafenib as compared to dacarbazine, the only chemotherapeutic agent approved for treatment of metastatic melanoma at the time.[5] Treating a patient lacking a BRAF V600 mutation with a BRAF inhibitor may not just result in lack of benefit, but could actually accelerate disease progression. Approximately 50% of patients in this trial demonstrated a rapid objective response to therapy (as compared to 5% in the dacarbazine arm); however, subsequent trials with longer follow-up demonstrated a median duration of response of less than 7 months.[6] Similar results have been reported for a randomized phase III trial of the BRAF inhibitor dabrafenib.[7] In the majority of cases, tissues taken at relapse show increased ERK activation via phosphorylation; genomic profiling at relapse has demonstrated acquired mutations in *MEK1* and *NRAS* in a subset of cases, though additional biochemical adaptations in signaling have also been noted.[8] MEK inhibition with trametinib has also shown a significant benefit in BRAF-mutant melanoma as compared to chemotherapy in a randomized phase III trial that included patients with either BRAF V600E or V600K-mutant melanoma.[9] Trials combining BRAF inhibitors with MEK and other pathway inhibitors are ongoing. Trials combining MEK and BRAF inhibitors may result in superior disease control compared with single use of either agent as measured by percent response and progression free survival of the cohorts.[10,11] The majority of patients enrolled in these trials had tumors harboring the BRAF V600E mutation; however a small number of patients had V600K-mutant tumors, which can also respond to BRAF and MEK inhibitors. There are limited case reports of patients with V600R mutated tumors showing objective responses to BRAF inhibitors.[12] Several clinical trials of combination therapy with both targeted and immune therapies are available for patients with BRAF-mutant melanoma. Much less commonly encountered are non-BRAF V600 cases that include exon 15 mutations in codons surrounding V600 and additional mutations in exon 11. Many of these are weaker activators of the MEK/ERK pathway than are the V600 mutants. Responses of these cases to BRAF and MEK inhibitors are an active area of investigation, and in many cases their responses are less impressive than those in the V600-mutated cases.

There are now a large number of publications demonstrating excellent correlation between BRAFV600E (VE1)-mutation specific immunohistochemistry and molecular-based analysis.[4] However in the absence of established proficiency testing or clear regulatory guidelines, laboratories utilizing this immunohistochemistry assay should perform rigorous validation and have available confirmatory molecular testing.

B. *NRAS* Mutational Analysis

NRAS is mutated in approximately 20% of melanomas, with ~80% of mutations occurring in exon 3 at codons 60 and 61 and ~20% in exon 2 at codons 12 and 13.[13] To date, direct inhibitors of NRAS have not demonstrated significant clinical activity. In untreated tumors, *NRAS* and *BRAF* V600 mutations generally occur in a mutually exclusive fashion. Clinical trials of single-agent targeted therapies and combinations are an active area of clinical investigation for patients with NRAS-mutant melanoma.

C. *KIT* Mutational Analysis

KIT is a receptor tyrosine kinase expressed at the cell surface that binds stem cell factor (SCF) and triggers downstream MAPK, PI3K, JNK, and JAK/STAT pathways leading to cell growth, proliferation, migration, and differentiation.[14] Overall, *KIT* is mutated in fewer than 5% of melanomas and most frequently occurs in melanomas arising in mucosal, acral, and chronically sun-damaged skin. These mutations are scattered throughout the kinase domain in a pattern similar to that described in gastrointestinal stromal tumors (GIST), except that missense mutations are predominant and deletions and insertion/duplications are rare. In addition, the mutations are more commonly seen in exons KIT 13 and 17 than in GIST. The most common alterations occur in exons 11 and 13, with L576P and K642E accounting for close to 50% of melanoma-specific mutations in this gene.[4] Small insertions and deletions in exon 11 are rare in melanoma. Targeted inhibitors of KIT and related tyrosine kinase receptors have demonstrated some efficacy in KIT-mutated but not in KIT-wild type melanomas in case reports and clinical trials, with best response documented most consistently in patients with tumors harboring mutations in the L576 and K642 hotspots. *KIT* copy number gain, including gene amplification alone, does not appear to independently predict response to KIT inhibitors in clinical trials.[15,16] No KIT inhibitors are currently approved for melanoma; clinical trials are ongoing for patients with KIT-mutant melanoma.

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