DecisionDx-CMSeq Report

3 Gene Sequencing Panel, Cutaneous Melanoma, Tumor
BRAF (V600, NonV600), ***NRAS*** (G12, G13, Q61) ***KIT*** (hotspots)

FINAL REPORT

Patient:
Sex:
DOB:
Client:
Clinician:

Tumor Type:
Tumor Site:
Specimen ID:
Provided Diagnosis:
Collected:
Received:
Reported:

RESULTS SUMMARY

Clinically significant alterations were identified in the following genes:

Mutations in BRAF occur frequently in cutaneous melanoma (~40-50%) and typically result in kinase and subsequent downstream pathway activation (PMID 12068308, 28467829, 26091043). BRAF is the most commonly mutated gene in melanoma (26091043) with certain mutations having strong evidence as clinically significant and actionable.

Mutations in NRAS occur frequently in cutaneous melanoma (~15-20%) and typically result in active state conformations and downstream pathway activation (PMID 28467829, 26091043). NRAS is the second most commonly mutated gene in melanoma (PMID 26091043).

RESULTS

BRAF NRAS ~~KIT~~

Gene	Variant (DNA)	Variant (Protein)	Variant Type	Observed Variant Allele Frequency
BRAF	c.1799T>A	p.V600E	Missense Mutation	0.41
NRAS	c.181delCinsA	p.Q61K	Missense_Variant	0.43

RESULTS INTERPRETATION

BRAF

BRAF c.1799T>A (p.V600E)
The test identified a missense mutation (c.1799T>A) in exon 15 of BRAF, which results in an amino acid change from valine (V) to glutamic acid (E) (p.V600E). This mutation results in activation of kinase function and downstream pathways (PMID 24957944). Patients with this mutation may be eligible for mutation-specific therapies and/or clinical trials (PMIDs 29100459, 29061773, 28648698, 27480103, 28891408, 22554099, 29061773). This mutation is considered to be Tier I (a variant of strong clinical significance) with Level A Evidence (FDA-approved options for late stage melanoma and included in NCCN guidelines) (PMID 29148538, NCCN Melanoma Guidelines Version 1.2018).

NRAS
The test identified a substitution (c.181C>A) in exon 3 of NRAS, which results in an amino acid change from glutamine (Q) to lysine (K)(p.Q61K). This mutation occurs within Switch II of the G domain resulting in decreased GTP hydrolysis and increased time in an active state (PMID 27160069, 17384584, 23069660). While the prognostic value of NRAS mutations in melanoma is still debated, some reports suggest NRAS mutant melanomas are agressive with poor prognosis (PMID 24985059, 21615881, 22180178). Clinical trials, case reports and in vitro studies indicate that Q61 NRAS mutations are potentially significant as they are often activating and may contribute to therapy response (PMID 28284557, 18390968, 21576590, 23414587, 29061773, 22983396, 11960693, 25736262). Patients with melanoma harboring this mutation may eligible for clinical trials (PMID 25180764). This mutation is considered to be Tier II (a variant of potential clinical significance) with Level C Evidence (biomarkers that serve as inclusion criteria for clinical trials) (PMID 29148538).

METHODOLOGY

Genomic DNA extracted from the submitted tissue sample was subjected to targeted amplification of specific genomic regions using the Ion Ampliseq Cancer Hotspot Panel v2 and sequenced on an Ion GeneStudio S5 Prime instrument. These regions include ***BRAF*** (V600, NonV600), ***NRAS*** (G12, G13, Q61) and ***KIT*** (hotspots). Non-hotspot mutations within those regions may be detected. Reads are aligned to the human reference sequence (GRCh37) using TMAP (Torrent Suite (5.8)) and variants are detected and annotated with Ion Reporter (5.10). Observed variants within the reportable range are interpreted in the context of a single clinically relevant transcript, indicated below. Unless otherwise indicated, all reportable regions are sequenced at a minimum 200X coverage, with an average overall depth of ≥500x. This test has an analytic sensitivity of >99% and specificity of >99%. Variants indicated by <5.0% of aligned sequence reads are not detected. Frequent reportable variants have been validated according to New York State guidelines. Rare or new reportable variants are confirmed using an orthogonal technology. Variants classified as benign or likely benign are not validated or reported, but are available upon request. Variants are classified following the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer (PMID 27993330) and ACMG/AMP's Standards and Guidelines for the Interpretation of Sequence Variants (PMID 25741868). Each PubMed ID (PMID) referenced herein is annotated to a specific, scientific publication accessible at http:///www.ncbi.nlm.nih.gov/pubmed by searching with the PMID number.

Interpretation of variants and assignment of significance, Tier, and Level of Evidence are performed using literature searches and several databases, including ExAC, ClinVar, and COSMIC. The most recently updated version of each database available at the time of reporting is used.

Gene	Transcript ID	Genomic position (start-end)	Region tested (specific variant if hotspot)
BRAF	NM_004333.4	chr7:140453132-140453146	codons 594-601 including p.V600
NRAS	NM_002524.4	chr1:115258743-115258748	p.G12 and p.G13
NRAS	NM_002524.4	chr1:115256528-115256530	p.Q61
KIT	NM_000222.2	chr4: 55561654-55561784	
KIT	NM_000222.2	chr4:55592157-55592246	
KIT	NM_000222.2	chr4:55593417-55593513	
KIT*	NM_000222.2	chr4:55593575-55593695	p.L576P, p.V559A, p.W557R, p.V560D
KIT*	NM_000222.2	chr4:55594170-55594279	p.K642E
KIT	NM_000222.2	chr4:55595496-55595562	
KIT	NM_000222.2	chr4:55597436-55597524	
KIT*	NM_000222.2	chr4:55599280-55599358	p. D820Y, p.D816H, p.N822K

(*) the most relevant hotspot KIT mutations for CM are listed, however other hotspots within KIT exist and will be reported if found

TESTING LIMITATIONS

Although not validated for this assay, likely pathogenic mutations outside the reportable range or small insertions or deletions (<40bp as limited by the Ion software) may be detected and reported if verified by an orthogonal method. Sequence properties in some targets may disrupt the detection of some classes of mutations and yield sub-optimal data. This assay does not detect copy number changes. This report reflects the analysis of extracted DNA from a provided tissue sample that contains a minimum of 40% tumor tissue. Some studies have reported discordance in mutations found in matched primary and metastatic melanoma tissue, therefore it is possible that the mutations (or lack thereof) reported in primary tumor tissue may not be conserved in metastatic tissue. Due to intratumoral heterogeneity, regions of the primary tumor not submitted for testing may harbor mutations not present in the tested tissue. In addition, a negative (wild type) result does not rule out the presence of a mutation that may be present, but below the limits of detection of this assay. These results and interpretations are made within the limits of sample collection, methodology and current knowledge. They should be correlated by the referring physician with respect to the ongoing clinical situation of the patient.

For more information, please visit the Castle Biosciences Skin Melanoma website www.SkinMelanoma.com.

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

Castle Biosciences, Inc. | Sherri Borman, PhD, HCLD, Laboratory Director