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ONCOLOGY: *Molecular Biomarkers in Cutaneous Melanoma*

Samples to Test: Primary or recurrent tumors; formalin-fixed paraffin embedded tissue (FFPE), fresh, fresh frozen.

Biomarker	Specific alterations Alternative terms	Type of Melanoma	Indications	Result Interpretation Significance	Assays Techniques
BRAF	Mutations at codon 600 (e.g., V600E, V600K, V600R/M/G)[1,9]	Low-CSD/SSM (>50%)[1]	Therapeutic	Associated with sensitivity to BRAF and/or MEK inhibitors. – Clinical trials have shown that the combination of BRAF and MEK inhibitors are superior to either agent alone in patients with BRAF V600 mutations.	NGS, pyrosequencing, Sanger sequencing, genotyping, PCR-based assays. IHC may be used to screen; confirmatory *BRAF* molecular testing is encouraged if negative IHC.
BRAF	Non codon 600 mutations (e.g., V599E, V599D) [1,2,9]	High-CSD/LMM		Mutations in codons near V600 in exon 15 (specifically BRAF L597 and BRAF K601) have shown response to MEK inhibitors and BRAF and MEK inhibitor combinations. Fusions in BRAF have also shown responses to MEK inhibitors and non-specific RAF inhibitors (eg, sorafenib). Mutations in other codons in exon 11 or exon 15 have not demonstrated response to either BRAF or MEK inhibitors.	NGS, pyrosequencing, Sanger sequencing, genotyping
KIT	Mutations in exon 11 and 13 (eg, W557R, V559D, L576P, K642E), mutations in exon 17 (e.g., D816H); and amplification	High-CSD/ LMM (28%)[2] Acral/mucosal melanomas (15%-40%)	Therapeutic	Exon 11 and 13 mutations are associated with sensitivity to KIT inhibitors. D816H mutation is associated with resistance to KIT inhibitors. KIT amplifications appear to have minimal or no sensitivity to KIT inhibitors.	NGS, pyrosequencing, Sanger sequencing, PCR-based assays, microarray
NRAS	Mutations in codon 12, 13, 61 (e.g., Q61R)	High-CSD/LMM, DM. Acral/mucosal melanomas (15%)[3]	Prognosis Therapeutic	Associated with poor survival. May be associated with response to MEK inhibitors in some patients.	NGS, pyrosequencing, Sanger sequencing, PCR-based assays
KRAS[1,2]	Mutations in codon 12, 13 and 61	Non-CSD/Acral/mucosal melanomas	Prognosis Therapeutic	Associated with poor survival. May be associated with response to MEK inhibitors in some patients.	NGS, pyrosequencing, Sanger sequencing, PCR-based assays
PTEN[6]	Loss of function mutation	Low-CSD/SSM High-CSD/LMM (30%-40%)	Prognosis	Associated with a highly aggressive phenotype and resistance to targeted- and immuno-therapy.	NGS, pyrosequencing, Sanger sequencing
NF1[4]	Nonsense, frameshift, or splice-site mutations, (e.g., Q1188*, A656fs)	DM (55%) High-CSD/LMM	Potential therapeutic	Novel NF1 binding partner: Calpain1 (CAPN1).[5]	NGS, pyrosequencing, Sanger sequencing.

CDKN2A[2]	Deletion of 9p21	Acral/mucosal melanomas, Malignant Spitz tumor, Low-CSD/SSM		Familial cases often show mutations of CDKN2A gene on chromosome 9p21[3]	FISH, NGS
TERT promoter[7]	Most common mutations are −57A/C, −124C/T, −146C/T, upstream the *TERT* gene ATG	Many melanomas[3]		Encodes for telomerase; mutations may lead to increased transcriptional activity and immortalization of tumor cells. Also identified in some atypical/malignant spitzoid tumors; reportedly associated with worse prognosis.	NGS, pyrosequencing, Sanger sequencing
ALK[8]	Rearrangement with various fusion partners: *DCTN1, TPM3, NPM1, TPR, GTF3C2, and CLIP1*	Malignant Spitz tumor	Therapeutic Diagnosis	Fusion-directed therapy	FISH, NGS
ROS1	Fusions		Therapeutic	Fusion-directed therapy	FISH, NGS
GNAQ[1–3] ***GNA11***	E.g., *GNAQ R183Q, GNA11 R183C*	Melanoma in blue nevus (90%), uveal melanoma (50%)	Diagnosis		NGS, pyrosequencing, Sanger sequencing
CCND1[1]	Amplification	Acral/mucosal melanomas (24%)			FISH, NGS
BAP1[1]	Loss of function mutation	Melanoma in blue nevus	Diagnosis	Described in some familial melanoma kindreds and sporadic tumors. Often associated with BRAF-V600E mutations.	NGS, pyrosequencing, Sanger sequencing
NTRK1, NTRK2, NTK3	Fusions		Therapeutic	Fusion-directed therapy	NGS

Abbreviations:

NGS: Next-Generation Sequencing, IHC: immunohistochemistry, **CSD:** Cumulative sun damage, **LMM:** Lentigo maligna melanoma, **SSM:** Superficial spreading melanoma, **DM:** Desmoplastic melanoma.

Where to test: Testing should be performed in the laboratories that are certified under clinical laboratory improvement amendments of 1988 (CLIA-88) as qualified to perform high complexity molecular pathology testing.

References:

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