Dermatology Reports

Primary malignant melanoma with pseudovascular morphology: a diagnostic pitfall

Monia Di Prete,[1] Paola Pasquini,[1] Elisa Melucci,[2] Viviana Lora,[3] Carlo Cota[1]

[1]Dermatopathology Research Unit, San Gallicano Dermatological Institute IRCCS, Rome; [2]Department of Pathology, Regina Elena National Cancer Institute IRCCS, Rome; [3]Clinical Dermatology, San Gallicano Dermatological Institute IRCCS, Rome, Italy

doi: 10.4081/dr.2025.10415

Supplementary Material: description of the analytical procedures and the gene panel used in the molecular analysis of this case of melanoma, with pseudovascular morphology.

Genomic DNA was extracted on the QIAcube® platform using the QIAamp DNA FFPE tissue kit (Qiagen) according to the manufacturer's instructions. Automated library preparation was performed on 10 ng of DNA (range: 1-20 ng) using the Ion Chef System (Thermo Fisher Scientific) with the Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHPV2) (Thermo Fisher Scientific). CHPV2 generates 207 amplicons covering approximately 2,800 Catalog of Somatic Mutations in Cancer (COSMIC) mutations in 50 different oncogenes and tumour suppressor genes: *ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAS, GNAQ, HNF1A, HRAS, IDH1, JAK2, JAK3, IDH2, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NMP1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53*, and *VHL*. Sequencing was performed on the Ion S5 system (Thermo Fisher Scientific), with the Ion 530 chips. Analysis was carried out using Ion Torrent Suite™ Software version 5.4 and Ion Reporter™ version 5.4. The Torrent Suite™ Software was used to perform initial quality control, including chip loading density, median read length, and number of mapped reads. The Coverage Analysis plugin was applied to all data and used to assess

amplicon coverage for regions of interest. Variants were identified by the Ion Reporter filter with a detection threshold of 5% variants. A cut-off of 500X coverage was applied to all analyses. Only single-nucleotide variants (SNVs) resulting in a nonsynonymous amino acid change, or a premature stop codon, and all short indels resulting in either a frameshift or insertion/deletion of amino acids were selected. All variants were manually reviewed with Integrative Genomics Viewer (IGV v.2.8.0, Broad Institute, Cambridge, Massachusetts, USA) and with the support of publicly available datasets reporting on their established or predicted oncogenicity (*i.e.*, COSMIC, cBioPortal, Clinical Trials, ClinVar, dbSNP, dbVar, Catalog of somatic mutations in cancer, My Cancer genome, personalized cancer therapy, NCBI genome, RefSeqGene, and Locus reference Genomic). For further examination of NGS data, in order to prioritize variants and find the relevant cancer drivers, we used the genomic analysis software "Oncomine Reporter". NGS analysis revealed the *NRAS* Q61R (c.182A>G) mutation with a variant allele frequency (VAF) of 30%. Other mutations with >5% VAF were not detected.