

<https://doi.org/10.7124/bc.000B9A>

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CO-MUTATION ARCHITECTURE OF UNRESECTABLE CUTANEOUS MELANOMA: PAIRWISE NGS ANALYSIS REVEALS BRAF-KRAS EXCLUSIVITY AND FLT3-TP53 CO-OCCURRENCE

Background. Reports on somatic driver mutations in unresectable cutaneous melanoma usually focus on individual genes, leaving patterns of co-occurrence and mutual exclusivity unexplored. This study examined pairwise gene co-mutation patterns in NGS data from 27 Ukrainian cases of unresectable melanoma. **Methods.** We created a binary gene-by-sample alteration matrix using the Custom Ion AmpliSeq Cancer Hotspot Panel for 24 genes found in at least 3 of the 27 samples. We tested 300 gene pairs for co-occurrence and exclusivity using Fisher's exact test and calculated odds ratios (OR). **Results.** The BRAF and KRAS alterations were almost always mutually exclusive: only 1 out of 10 BRAF-positive samples was also KRAS-positive, compared to 8 KRAS-positive samples overall (OR = 0.16, $p = 0.19$). This reflects the known antagonism in the RAS/RAF pathway, though the sample size is small. KRAS-mutant samples did not co-occur with alterations in the PDGFRA, PIK3CA, SMAD4, APC, KDR, or GNAS (0 of 8 pairs), suggesting that the KRAS-driven tumors may be genetically simpler and have fewer additional mutations. In contrast, all FLT3-Mutant samples (6/6) also had TP53 mutations ($p = 0.054$), the strongest co-occurrence observed. This suggests FLT3 alterations tend to appear in tumors that already have TP53 mutations. BRAF and PDGFRA co-occurred more often than expected by chance (5 out of 10 BRAF-positive samples were also PDGFRA-positive, OR = 16, $p = 0.015$), which may indicate a cooperative relationship between these genes. Some gene pairs (the FGFR3-RB1, SMAD4-STK11, SMARCB1-ATM, SMARCB1-KIT, ABL1-NOTCH1) always co-occurred in small groups ($n = 3$ each), but these results are statistically unstable and need further validation. Most pairs involving the TP53, BRAF, or PIK3CA with other altered genes did not show significant associations, which is consistent with the largely independent distribution of passenger-level Tier 2–3 variants in this small group. **Conclusions.** Analyzing gene pairs reveals meaningful patterns that single-gene reports miss, such as BRAF-KRAS mutual exclusivity, FLT3 changes linked to TP53 mutations, and a KRAS-driven group with fewer co-mutations. These findings should be confirmed in larger melanoma studies.

Keywords: melanoma, NGS, co-mutation, mutual exclusivity.

Received: 06.07.2026; Accepted: 08.09.2026; Published: 29.09.2026

Citation: Vladymyrets B.O., Gulkovskyi R.V., Gerashchenko G.V., Bezverkhiy A.M., Melnichuk N.S., Mankovska O.S., Marchyshak T.V., Demydova H.S., Krotevych M.S., Trokhymych S.S., Ruban O.V., Tkachuk Z.Yu., Tukalo M.A., Kashuba V.I. (2026) Co-mutation architecture of unresectable cutaneous melanoma: Pairwise NGS analysis reveals BRAF-KRAS exclusivity and FLT3-TP53 co-occurrence. *Biopolymers & Cell*, Special issue(42), 96. <https://doi.org/10.7124/bc.000B9A>
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