Targeting BRAF Fusions with Pan-RAF Inhibitors Highlights Fusion Type-Associated Heterogeneity in Drug Responses and the Need for Combination Therapy with MEK/ERK Inhibitors

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Background. *BRAF* fusions are oncogenic drivers in multiple tumor types. They are present *de novo* in <1% of non-small cell lung cancer (NSCLC) or as an acquired resistance mechanism in ~1-3% of *EGFR* mutant NSCLC after EGFR targeted therapy. However, no therapy has been approved for patients with tumors driven by *BRAF* fusions. Here, we aimed to develop tractable preclinical patient-derived and isogenic disease models and explore therapeutic strategies using clinically active pan-RAF, MEK1/2, and ERK1/2 inhibitors.

Methods. Tumor samples collected from patients undergoing routine care at MSKCC were implanted into immunodeficient mice (NSG strain) to generate patient-derived xenograft (PDX) and cell line models. DNA or RNA from patient samples were profiled by MSK-IMPACT or MSK-Fusion panels, respectively. *BRAF* fusions were modelled by cDNA overexpression or CRISPR-Cas9-mediated gene editing in human bronchiolar epithelial cells (HBECs). CRISPR-engineered monoclonal populations were isolated. Transforming capacity and efficacy studies were conducted in subcutaneous xenograft models. Synergy studies were conducted using a concentration matrix approach.

Results. We generated seven PDXs, including four NSCLC (one with both *EGFR*ex19del and *PJA2::BRAF*, MSK-138), one dedifferentiated liposarcoma, one rectal adenocarcinoma, and one undifferentiated pleomorphic sarcoma. Seven *BRAF* fusions consisting of partners joined to different *BRAF* exons that were identified in patient samples were generated by cDNA overexpression (*KIAA1549::BRAF*ex9-18, *MKRN1::BRAF*ex9-18, *EML4::BRAF*ex10-18) or CRISPR-Cas9 (*AGK::BRAFe*x8-18, *AGAP3::BRAFe*x9-18, *TRIM24::BRAFe*x10-18, *SND1::BRAFe*x11-18). All BRAF fusion-positive HBEC cell lines were tumorigenic. These cell lines were also resistant to the pan-RAS inhibitor RMC-6236 and showed differential sensitivity to pan-RAF inhibitors, with BRAF ex8/9 fusions being the most responsive compared to ex10/11: specifically, average IC_{50} values for growth inhibition by exarafenib were AGK::BRAFex8: 66nM; AGAP3::BRAFex9: 60nM; *TRIM24::BRAFe*x10: 321nM; *SND1::BRAFe*x11: 1921nM. Similar patterns of sensitivity were observed with other pan-RAF inhibitors (LY3009120, belvarefinib, encorafenib). All CRISPR lines were exquisitely sensitive to MEK1/2 (binimetinib, cobimetinib) and ERK1/2 (ulixertinib) inhibitors, compared to isogenic control cells. Combinations of ulixertinib+exarafenib, binimetinib+exarafenib, and binimetinib+ulixertinib were synergistic in HBEC-AGK::BRAF cells. Exarafenib and LY3009120 treatment led to dose-dependent blockage of downstream signals in HBEC-AGK::BRAF cells.

Exarafenib (30mg/kg QD) and LY3009120 (15mg/kg BID) reduced the growth of HBEC-EML4-BRAF xenograft tumors by 55±3% and 49±6%, respectively. In MSK-138pdx (*EGFR*ex19del+*PJA2::BRAF*), osimertinib (2mg/kg QD) combined with exarafenib (30mg/kg QD) was much more effective than monotherapy, leading to ~40% tumor shrinkage compared to vehicle treatment.

Conclusion. *BRAF* fusion-driven cancers are susceptible to targeted inhibition of the RAF-MEK-MAPK pathway. While *in vitro* and *in vivo* results support increased sensitivity to pan-RAF, MEK1/2, and ERK1/2 inhibitors, we observed differences in pan-RAF drug sensitivities associated with BRAF fusion structure. How the partner gene or BRAF domains included in the fusion determines this differential sensitivity remains to be determined. Combining exarafenib with MEK1/2 or ERK1/2 inhibitors may be a promising therapeutic strategy covering a broader patient population, regardless of fusion structure. We also found that the combination of exarafenib and osimertinib is effective *in vivo* for *EGFR*-mutated NSCLC with acquired *BRAF* fusion. Exarafenib is currently in clinical trials for *BRAF*- and *NRAS*-mutant solid tumors (NCT04913285).