New combination therapies for melanoma

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BRAF inhibitors such as vemurafenib (VEM) are only effective as single agent mealnoma therapy in BRAF-mutant melanomas and resistance to the treatment develops within 6 to 12 months. We investigated whether targeting VEGF receptors could increase the efficacy of the BRAF inhibition therapy. We measured levels of VEGF-A secretion from our unique NZM panel of melanoma cell lines. Variations of the VEGF pathways in these cells were analysed by exome sequencing, RNASeq and western blotting. Xenograft and syngeneic models were used to investigate efficacy and safety of a combination of VEM and the VEGFR2 inhibitor axitinib (AXI) *in vivo*. Species specific tumour RNA sequencing was performed to identify pathways uniquely affected by the drug combination in tumour cells and host stroma. RNAscope and immunohistochemistry were used to further analyse effects of the drugs in the tumours.

V600E-mutant melanoma cell lines secreted VEGF at significantly higher levels compared to lines with RAS mutations or nonBRAF/nonRAS lines. VEM downregulated VEGF secretion in V600E-mutant cell lines but not in RAS-mutant or nonBRAF/nonRAS cell lines. We found that the VEM + AXI combination synergistically inhibited the tumor growth. Interestingly, the combination also inhibited the growth of BRAF-wildtype xenografts and syngeneic B16 tumors. When AXI was replaced with our in-house VEGFR2 inhibitor SN35332, the combination also provided synergistic effect, suggesting the combined effects were likely pathway specific. Pathway-related synthetic lethality was identified in EMT, p53, TGF-beta, and angiogenesis hallmark pathways. Finally, we developed a cell line resistant to vemurafenib and showed that the combination of VEM + AXI resensitized the tumors to BRAF inhibition therapy.

Together, this study provides an important link between the VEGF axis and BRAF signalling in melanoma biology and co-targeting those two axes could enhance the efficacy of BRAF inhibition therapy not only in BRAF-mutant but also in BRAF-wild type tumours.