5PD The acquired resistance to the combination of the anti-EGFR cetuximab and the MEK-inhibitor refametinib in KRAS mutated colorectal cancer cell lines depends on PI3K-signalling

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Background: Previous studies showed that the combination of an anti-epidermal growth factor (EGFR) and a selective MEK-inhibitor displays a significant anti-tumour activity in RAS-wild type colorectal cancers (CRCs), while the same combination partially reverts anti-EGFR primary resistance in KRAS mutated colorectal cancer cell lines. However, mechanisms of resistance to this combination are still unexplored.

Methods: We generated KRAS mutated CRC cell lines (HCT15 and HCT116) resistant to a combination of cetuximab (an anti-EGFR antibody) and BAY86-9766 (refametinib, a selective MEK1/2-inhibitor) after continuous exposure to increasing concentration of the drugs for 8 months. Resistant clones had an IC_{50} 20-100-fold higher than the parental cells. We evaluated by Western Blot (WB) analysis and quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) the expression and activation status of a panel of receptor tyrosine kinases (RTKs) and intracellular transducers. We further analysed by MTT assay the sensitivity of these cetuximab-MEKi resistant (CM-res) cell lines to GDC-0941 (pictilisib, a selective PI3Kα inhibitor) and afatinib (BIBW 2992, an irreversible pan-HER inhibitor) either used alone or in combination.

Results: We found consistent hyperactivation of the PI3K-AKT pathway and concurrent inactivation of the MAPK pathway, coupled to the activation of multiple RTKs of the HER family such as HER2 and HER3 in resistant cells when compared to parental cells. Treatment with GDC-0941 was able to partially restore the sensitivity to the drug combination, suggesting a central role for this pathway in mediating resistance in this setting, while afatinib was not capable of reverting the resistant phenotype when used alone but showed synergistic activity when combined to GDC-0941.

Conclusions: These preliminary results suggest that PI3K activation plays a central role in the acquired resistance to the combination of anti-EGFR and MEK-i. PI3K activation depends at least in part by the activation of the HER family of RTK, but it can also be activated by other receptors. In vivo experiments on mice are currently ongoing.

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