PTU-106 ★ **CETUXIMAB COMBINED WITH MULTI-TARGETED SIRNA AGAINST HSP90, UBCH5, AND SRC CIRCUMVENTED ONCOGENE ADDICTION, TRANSACTIVATION, AND ACQUIRED RESISTANCE AS A RESULT OF EGFR UBIQUITINATION, AND MUTATIONS/DELETIONS IN THE KINASE DOMAIN OF EGFR IN COLORECTAL CA**

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Introduction Colorectal carcinoma (CRC) cells develop resistance to cetuximab through insertion mutations at exon 20, ubiquitination and c-Src which activates EGFR in the absence of ligand despite treatment with cetuximab. We aim to circumvent this acquired resistance.

Methods CRC cells were obtained from metastatic patients resistant to cetuximab due to insertion mutations at exon 20, ubiquitination and overexpressed c-Src. Orthotopic mouse CRC models generated from our patients' tumour cells were injected with multitargeted siRNA against HSP90, UbcH5 and c-Src.

Results Multi-targeted siRNA inhibited expression of the E2 enzyme UbcH5, blocking the covalent attachment of ubiquitin to target protein EGFR, and neutralizing the multi-enzyme cascade. E1 deactivated ubiquitin, blocking transfer to the cysteine residue of E2 ubiquitin conjugating enzyme (UbcH5). This inhibited the E2 ligation of ubiquitin via its carboxy terminus to lysine residues of the protein substrate EGFR. Multi-targeted siRNA inhibited expression of HSP90 resulting in degradation of EGFR with kinase domain deletion type mutations in exon 19, substitutions in exon 21, and resistant insertion mutations at exon 20. Inhibition of c-Src circumvented transactivation and inhibited EGFR signalling, inhibiting tumour proliferation, and metastasis to the liver, and peritoneum. Addiction to EGFR was circumvented. Inhibition of EGFR blocked the activation of downstream mediators including STAT3, AKT, Erk/MAPK and PI3K, while IRF-1 was upregulated. There was enhanced cell to cell adhesion and membrane localisation of b-catenin, while MMP-9 invasive activity was blocked. HIF-1a/Met pathway was blocked downregulating CAIX. VEGFR-2 and VEGFR-3 were blocked inhibiting vascularisation and lymphangiogenesis, respectively. We observed type I, II and III PCD in tumour cells.

Conclusion These results indicate that systemic treatment of multitargeted siRNA against UbcH5, c-Src and HSP90 circumvented resistance to cetuximab suppressing tumour growth and metastasis in orthotopic mouse mCRC model.

Competing interests None.
Keywords cetuximab, siRNA, HSP90, UbcH5, c-Src, EGFR, ubiquitination, mCRC.