

# Increased TGF alpha as a mechanism of acquired resistance to the anti-EGFR inhibitor cetuximab through EGFR-MET interaction and activation of MET signaling in colon cancer cells

E.A.V. Ferraiolo<sup>1</sup>, L.P. Ciuffreda<sup>1</sup>, T. Troiani<sup>2</sup>, E. Martinelli<sup>2</sup>, R. De Palma<sup>3</sup>, F. Ciardiello<sup>2</sup>, L. Berrino<sup>1</sup>

<sup>1</sup>Seconda Università di Napoli, Dipartimento di Medicina Sperimentale, Sezione di Farmacologia Via Costantinopoli, 16 - 80138 Napoli

<sup>2</sup>Seconda Università di Napoli, Dipartimento Medico-Chirurgico di Internistica Clinica e Sperimentale "F. Magrassi - A. Lanzara, Via Pansini, 5 - 80131 Napoli

<sup>3</sup>Seconda Università di Napoli, Dipartimento di Internistica Clinica e Sperimentale 'F. Magrassi - A. Lanzara', Via Pansini, 5 - 80131 Napoli

**PURPOSE:** Although cetuximab, an anti-EGF receptor (EGFR) monoclonal antibody, is an effective treatment for KRAS wild type metastatic colorectal cancer (mCRC) patients, its clinical use is limited by onset of resistance.

**EXPERIMENTAL DESIGN:** We characterized a CRC model to study the mechanisms of acquired resistance to cetuximab.

**RESULTS:** Following chronic treatment of nude mice bearing cetuximab-sensitive human GEO colon xenografts, cetuximab-resistant GEO cells (GEO-CR) were obtained. In GEO-CR cells proliferation and survival signals were constitutively active despite of EGFR inhibition by cetuximab treatment. Whole gene expression profiling identified a series of genes involved in the hepatocyte growth factor HGF-MET-dependent pathways, that were up-regulated in cetuximab-resistant GEO-CR cells. Further, activated, phosphorylated MET was detected in GEO-CR cells. Inhibition of MET expression by siRNA restored cetuximab sensitivity in GEO-CR cells, whereas exogenous activation of MET by HGF stimulation in cetuximab-sensitive GEO cells induced resistance to cetuximab. Treatment of GEO-CR cells with PHA665752, a selective MET inhibitor, inhibited cell growth, proliferation and survival signals and impaired cancer cell migration. Overexpression of transforming growth factor alpha (TGF alpha) a specific EGFR ligand, TGF alpha was involved in the acquisition of cetuximab resistance in GEO-CR cells. In fact, TGF alpha overexpression induced the formation of EGFR-MET heterodimers, with subsequent MET phosphorylation and activation of MET down-stream effectors in GEO-CR cells.

**CONCLUSIONS:** These results suggest that overexpression of TGF alpha through induction of EGFR-MET interaction contributes to cetuximab resistance in CRC cells. The combined inhibition of EGFR and MET receptor could represent a strategy for preventing and/or overcoming cetuximab resistance in CRC patients.

1. Malvezzi M et al. European cancer mortality predictions for the year 2011. *Ann Oncol* 2011. 22: 947-956.
2. Galizia G et al. Cetuximab, a chimeric human mouse anti-epidermal growth factor receptor monoclonal antibody, in the treatment of human colorectal cancer. *Oncogene* 2007. 26: 3654-3660.
3. Ciardiello F et al. EGFR antagonists in cancer treatment. *N Engl J Med* 2008. 358:1160-1174.
4. Normanno N et al. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat Rev Clin Oncol* 2009. 6:519-27
5. Kimura H et al. Antibody-dependent cellular cytotoxicity of cetuximab against tumor cells with wild-type or mutant epidermal growth factor receptor. *Cancer Sci* 2007. 98: 1275-1280.
6. Di Nicolantonio F et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008. 26: 5705-5712.
7. De Roock W et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010. 11: 753-762.
8. Karapetis CS et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; 359: 1757-65.
9. Van Cutsem E et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; 29: 2011-9.
10. Bokemeyer C et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009; 27: 663-71.
11. Misale S et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012; 486: 532-36.