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Combined inhibition of EGFR and protein kinase CK2 synergistically blocks phosphorylation of ribosomal protein S6, induces apoptosis in cancer cells and displays enhanced antitumor activity in xenograft models

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Background: Deregulated EGFR is known to play a prominent role in tumorigenesis by hyperactivating multiple pro-survival/pro-proliferation signaling pathways including MEK/ERK, PI3K/AKT and STATs. Similarly, protein kinase CK2 modulates multiple pro-proliferative and pro-survival signals through many of these same signaling pathways. Although CK2 was identified more than 55 years ago, an understanding of the extensive contribution of this kinase to maintenance of the tumor phenotype and drug resistance is just beginning to emerge. Several small molecule and biologic agents that target EGFR have been approved for the treatment of cancer, while many more are at various stages of development. In contrast, CK2 is an unexploited oncology target with only one selective small molecule inhibitor, CX-4945, currently under evaluation in a phase I clinical trial. Overexpression of EGFR and CK2 have been frequently observed in solid tumors. EGFR has been proposed to regulate CK2 through ERK, while CK2 may control EGFR signaling through cdc37/Hsp90 machinery and/or direct phosphorylation of EGFR signaling mediators. Considering the apparent self-perpetuating interplay between EGFR and CK2 signaling, we examined the effects of combining CX-4945 with EGFR inhibitors *in vitro* and *in vivo* in cancer models with various genetic backgrounds.
Methods: Cancer cells with amplified wild-type EGFR, EGFR activating mutations, EGFR inhibitor-resistance mutation (T790M), amplified HER2 and cells with compensatory mutations known to abrogate the effects of EGFR targeted therapies, such as mutant KRAS and PIK3CA were used for antiproliferative assays, PhosphoScan, western blot analysis and xenograft studies.
Results: Combination of CX-4945 with erlotinib further inhibited phosphorylation of AKT at S473 and T308, as well as ribosomal protein S6 at S235/S236, when compared to either agent alone. In addition, Mcl-1 levels were significantly reduced. Combined inhibition of CK2 and EGFR enhanced induction of apoptosis and resulted in synergistic killing of cancer cells. Furthermore, synergistic tumor growth inhibition in xenograft models of human cancers was observed when CX-4945 was combined with EGFR targeting agents.
Conclusions: Inhibition of CK2 by CX-4945 augments the anticancer activity of EGFR targeting agents and provides a compelling scientific rationale for combining these molecular targeted agents in the clinic to improve therapeutic outcomes in patients with EGFR and CK2-driven malignancies.

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Inhibition of PDGFRalpha in tumor stroma with MEDI-575 enhances activity of carboplatin/paclitaxel and delays tumor regrowth in a NSCLC xenograft model

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Background: Platelet-derived growth factor receptor alpha (PDGFRα) is a receptor tyrosine kinase that regulates proliferation and survival of cancer cells and mediates stromal support of tumor progression. While expression of PDGFRα on epithelial tumors is low, the stroma has emerged as a tumor component where PDGFRα is expressed. We explored whether inhibition of human PDGFRα expressed in a transgenic mouse enhanced the activity of carboplatin/paclitaxel or gemcitabine/cisplatin using a non-small cell lung cancer (NSCLC) xenograft model.
Material and Methods: MEDI-575 is a fully human IgG2 monoclonal antibody that targets human PDGFRα and does not bind to mouse PDGFRα. Calu-6 cells (human NSCLC) that do not express PDGFRα were grown as xenografts in KI/KO SCID mice in which the murine PDGFRα gene had been replaced with the human PDGFRα gene. Efficacy was measured by tumor growth inhibition (dTGI) and by delay in tumor regrowth after cessation of treatments. Human tumor microarrays were stained for PDGFRα protein expression to determine which indications benefit from targeting the tumor stroma with MEDI-575.
Results: Dosing with MEDI-575 at 10 mg/kg (2×/wk) resulted in 48% dTGI using the Calu-6/hPDGFRα KI/KO model. This effect correlated with a decrease in phosphorylated PDGFRα expressed in the tumor stroma. Carboplatin (25 mg/kg, Q4DX3) and paclitaxel (10 mg/kg, Q2DX5) were efficacious when given as a doublet (72% dTGI) and Calu-6 tumors grew back rapidly following cessation of treatments. The triple combination with MEDI-575, carboplatin, and paclitaxel blocked Calu-6 xenograft growth (102% dTGI) and tumor regrowth was delayed compared to treatment with carboplatin/paclitaxel. No weight loss was observed. Gemcitabine (50 mg/kg, Q4DX3) combined with cisplatin (4 mg/kg, Q4DX3) showed efficacy (119% dTGI) and addition of MEDI-575 did not enhance the anti-tumor benefit of the doublet; possibly because gemcitabine/cisplatin was already effective in this model. Preliminary findings indicated that treatment of mice with gemcitabine/cisplatin/MEDI-575 was not well tolerated. Human tumor microarray analysis demonstrated PDGFRα expression in the stroma of lung, breast, colon and ovarian cancer patient samples while direct tumoral expression of PDGFRα was shown in <25% of NSCLC tissue samples.
Conclusions: Enhanced anti-tumor efficacy and tolerability of MEDI-575 with carboplatin/paclitaxel provide a framework for testing the PDGFRα stromal hypothesis in NSCLC patients during clinical development of MEDI-575.

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Identification of EGFR regulated genes in cetuximab resistant tumor cell models

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The epidermal growth factor receptor (EGFR) is a central regulator of proliferation and progression in many human epithelial cancers including head and neck squamous cell carcinoma (HNSCC), non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and brain cancer. Cetuximab (Erbitux®) is an EGFR-blocking antibody that is FDA approved for use in patients with metastatic CRC and HNSCC. EGFR inhibition has demonstrated major tumor regressions in approximately 10−20% of advanced cancer patients. However, many tumors do not show response to EGFR inhibition and the majority of responders eventually develop resistance to treatment. Therefore, elucidation of molecular mechanisms that underlie the development of acquired resistance to cetuximab therapy is essential for the success of this promising molecular targeting agent. We established and investigated mechanisms of acquired resistance to cetuximab using a NSCLC model with the NCI-H226 tumor cell line. We found that cetuximab-resistant NCI-H226 clones have nuclear EGFR (nEGFR). nEGFR has been shown to transcriptionally regulate several key genes involved in G1/S progression. In addition, our laboratory has shown that nEGFR contribute to resistance to cetuximab therapy. To better understand the function of nEGFR in cells with acquired resistance to cetuximab, we propose to identify additional genes that are transcriptionally regulated by nEGFR. We utilized three NCI-H226 clones (HC1, HC4, HC8) that acquired resistance to cetuximab and parental control cells (HP) for this study. Immunofluorescence and Western blot analysis of subcellular fractions confirmed that all cetuximab-resistant clones have nEGFR. Next we performed chromatin immunoprecipitation (ChIP) using an anti-EGFR antibody and determine the size distribution and relative yield of EGFR-DNA ChIP products between cetuximab-resistant cells and cetuximab-sensitive cells by Agilent Bioanalyzer. Cetuximab-resistant cells yielded 3-fold more EGFR-DNA complexes than parental control cells. By quantitative real-time PCR, nEGFR complexes were enriched 5- to 9-fold for Cyclin D1, B-Myb, Aurora-A and iNOS promoter sequences in cetuximab-resistant clones compared to parental control. Current studies are utilizing ChIP-on-Chip (3 × 720K Refseq Promoter Array, NimbleGen) and ChIP-sequencing analyses (Roche/454) to identify other gene promoters regulated by nEGFR, which may play a critical role in cancer progression and/or cetuximab resistance.

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Identification and preclinical characterization of NMS-P626, a potent, selective and orally bioavailable TrkA inhibitor with anti-tumor activity in a TrkA-dependent colorectal cancer

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The TrkA receptor tyrosine kinase is a high affinity receptor for NGF and belongs to the neurotrophin receptor family that includes also TrkB and TrkC. In adults TrkA is expressed in the CNS and in sympathetic neurons. Several chromosomal rearrangements involving TrkA have been described in human papillary thyroid carcinoma, where TrkA was demonstrated to be the driving force for neoplastic transformation and tumor progression. TrkA