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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

overexpression and/or mutations have also been reported in acute myeloid leukemia, lung, prostate, breast and colon carcinomas.
Here we describe preclinical studies performed with NMS-P626, an orally available, highly potent and selective small-molecule inhibitor of TrkA.
Proliferation profiling of NMS-P626 against an extended panel revealed that, amongst other lines previously described as being sensitive to TrkA inhibition, the human colorectal cancer cell line KM12 is highly sensitive, suggesting dependence on TrkA signaling: 72 hour proliferation of KM12 was inhibited with an IC50 of 19 nM. Western blot analysis of KM12 cell lysates revealed the presence of a phospho-TrkA immunoreactive band with a molecular weight of ca.70 kDa, consistent with Tropomyosin3 (TPM3)-TrkA, the product of a 1q21/23 inversion previously described as a recurrent chromosomal aberration in papillary thyroid carcinoma and in a single case of colorectal cancer. cDNA sequencing and biochemical analyses confirmed expression in KM12 cells of a TPM3-TrkA fusion protein identical to the previously identified form, in which an N-terminal portion of the TPM3 protein is fused to the kinase domain of TrkA, resulting in constitutive kinase activation. RNA silencing of TrkA confirmed that knockdown of TPM3-TrkA leads to cell growth arrest and inhibition of AKT and MAPK pathways in KM12 cells. Likewise, phospho-TrkA, phospho-AKT and phospho-MAPK signals were inhibited in KM12 treated with NMS-P626. When administered orally to nude mice bearing KM12 tumor xenografts, NMS-P626 induced tumor stabilisation (>90% TGI), with ex vivo analysis confirming sustained target modulation.
Together, these data demonstrate that activated TrkA is a driving mutation in the KM12 colon carcinoma cell line, and that pharmacological modulation with NMS-P626, a selective TrkA inhibitor with an excellent preclinical profile, yields significant therapeutic benefit in this tumor model.

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Combination treatment of targeting Stat3 and HIF-1alpha is a potent strategy for prostate cancer therapy

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Background: Two pathways which are upregulated in prostate cancer are the signal transducer and activator of transcription 3 (Stat3) pathway and the hypoxia sensing pathway. Stat3 was identified as an important target for cancer therapy since it participates in oncogenesis through the upregulation of genes encoding apoptosis inhibitors (Bcl-xL, Bcl-2, Mcl-1, and survivin), cell-cycle regulators (cyclin D1 and c-*myc*), and inducers of angiogenesis (VEGF). Stat3 is constitutively activated in 80% of prostate cancer. HIF-1alpha (HIF-1a) and HIF-2alpha (HIF-2a), which mediate the cellular response to hypoxia, activate the transcription of many genes crucial for cancer progression, including angiogenesis, cell survival, glucose metabolism, invasion and metastasis. Overexpression of HIF-1a in human cancers associates with poor prognosis and treatment failure in a number of cancers. Moreover, prolonged use of a target drug can result in drug resistance and reducing drug responsibility. Here we developed a combination treatment with targeting both phospho-Stat3 and HIF-1a to increase tumor response and reduce drug resistance and treatment failure.
Methods: We employed western blots, cell cycle analyses, immunohistochemistry, TUNEL and xenograft models to determine the drug efficacy and mechanism of the combination treatment.
Results: We combined two anti-cancer agents: T40214 (a phospho-Stat3 inhibitor) (Jing et al. 2004; PMID:15374974) and JG244 (a HIF-1a inhibitor) (Guan et al, 2010; PMID:19755960) together to evaluate the drug efficacy of the combination treatment in mice bearing human (or murine) prostate tumors. Our results demonstrated that (1) after treatments the mean tumor volumes in mice xenografts treated by placebo, T40214 and JG244 alone were increased 5.8, 3.1 and 2.5 folds, respectively. The mean tumor volume in mice treated by JG244 and T40214 combination was only increased 1.5 ($P < 0.002$) folds. (2) The drug efficacy in inmmuno-competent mice (C57BL/6) bearing murine prostate tumors (TRAMP-C2) showed that comparing with the tumors treated by placebo and T40214 alone, the combination treatment with mixing T40214 and JG244 together significantly suppressed the growth of murine prostate tumors. (3) The mechanism studies indicated that this combination treatment dramatically increased apoptosis of prostate cancer cells in tumor and significantly suppressed prostate tumor growth as well.
Conclusion: Our results provided solid evidence that compared with each agent used alone, the combination treatments dramatically increased apoptosis in tumors and promoted drug efficacy, suggesting that combination treatment including a HIF-1a/2a inhibitor not only has therapeutic efficacy in targeting HIF-1a/2a, but also could reduce the hypoxia-induced drug resistance to other therapies (e.g. T40214) and enhance drug efficacy. This approach could make prostate cancer treatments more effective and improve survival even in patients with metastatic disease.

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Plasma metabolomic analysis of genetic and pharmacological manipulation of PI 3-kinase pathway activation in mice using liquid chromatography coupled to mass spectrometry (LC-MS)

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Background: This study evaluated the plasma metabolome of mice in which the PI3K pathway in the host (or an implanted tumour) was activated by loss of the upstream suppressor, PTEN. Plasma samples were collected from PTEN knockout (+/−) mice and their wild-type littermates as well as from normal athymic mice and those bearing PTEN null human tumour xenografts (U87MG glioblastoma or PC3 prostate adenocarcinoma). The effects of the PI3K inhibitor GDC-0941 were also evaluated in mice bearing U87MG xenografts and compared with the effects of the cytotoxic agent BCNU.
Materials and Methods: Protein was removed from plasma samples using Whatman protein precipitation plates. The extracted plasma samples were analysed on an LC-MS system with chromatographic separation achieved on a 1.8 μm particle column with a 13 minute water/acetonitrile gradient containing 0.1% formic acid.
Results: Seventeen plasma metabolites were significantly different in *PTEN* KO (+/−) mice compared with their wild-type littermates. These metabolites included amino acids (proline, citrulline, tyrosine and tryptophan), glycerophospholipids (glycerophosphocholine and ethanolamines), acylcarnitines (palmitoylcarnitine, linoleyl carnitine and stearoylcarnitine) and osmoregulators (proline betaine). Similar changes were identified in animals bearing PTEN null tumours: proline betaine, m/z 160.13, carnitine and indoxyl sulphate were increased in the case of U87MG tumour and m/z 160.13 and indoxyl sulphate in PC3 tumour-bearing animals. A single treatment of the pan-class I PI3K inhibitor GDC-0941 gave opposite effects to that observed in PTEN KO mice with changes observed in six metabolites including proline, proline betaine, *m/z* 160.13, carnitine, tyrosine and glycerophosphocholine. Chronic GDC-0941 treatment affected proline betaine, acetylcarnitine, citrulline and carnitine in a dose-dependent manner. The metabolomic signature following cytotoxic treatment of U87MG tumour bearing animals with BCNU showed different changes in several metabolites when compared with GDC-0941 treatment including proline betaine, *m/z* 160.13, phenylalanine, carnitine, glycerophosphoethanolamine.
Conclusions: LC-MS based metabolomics has successfully identified distinct exo- metabolomic signatures in *PTEN* KO mice and in PTEN null human tumour xenograft models following PI3K inhibitor and BCNU treatment.

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BIIB024, a potent pan-Raf kinase inhibitor for melanoma and solid tumors

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The Raf kinases (A-Raf, B-Raf and C-Raf) are key regulators of cell proliferation and survival that control signaling through the MAPK pathway, composed of Ras, Raf, MEK and ERK. This pathway is frequently deregulated in cancer by mutations, leading to increased cancer cell proliferation and survival. In particular, Ras oncogenes are mutated in 25% of all cancers and B-Raf is mutated in 7% of all cancers, including 60% of melanomas. B-Raf is an attractive therapeutic target because most tumors with B-Raf mutations and some tumors with Ras mutations are sensitive to inhibition of Raf or MEK in pre-clinical models. In addition, clinical efficacy has been observed in B-Raf mutant melanomas with the PLX4032 and GSK2118436 B-Raf inhibitors. BIIB024 is a potent, oral pan-Raf kinase inhibitor that is being developed for the treatment of melanoma and solid tumors. BIIB024 potently inhibits oncogenic B-Raf^{V600E} mutant kinase and the wild-type B- and C-Raf kinases in biochemical assays. In a large biochemical kinase screening panel containing 222 unique human kinases, BIIB024 inhibited a small subset of kinases in a similar potency range as Raf kinases. To determine which cancer cell types are sensitive to BIIB024, in vitro pERK signaling and proliferation assays were conducted