penetration of panitumumab and decreased staining for Ki67 and pMAPK in tumor tissue. In a parallel study, panitumumab treatment of established A431 xenograft tumors resulted in statistically significant dose-dependent partial regressions and complete regressions remaining free of disease for greater than 10 months off treatment. Treatment of established HT-29 xenograft tumors also resulted in a significant dose-dependent regressions. Panitumumab and irinotecan combination therapy resulted in greater tumor regression compared to either treatment alone.
Conclusions: Panitumumab inhibited ligand-induced EGFR autophosphorylation *in vitro* and *in vivo* in A431 epidermoid and HT29 colon carcinoma model systems. Immunohistochemistry demonstrated that Panitumumab is present in the tumor tissues and correlates with a reduction in Ki67 and pMAPK. Panitumumab monotherapy demonstrated dose-dependent regressions and eradications in A431 xenografts and significant regressions in HT-29 xenografts. Combination therapy with panitumumab and irinotecan in a model of colon cancer resulted in significant tumor regressions compared to either alone. These data provide preclinical evidence for the clinical application of panitumumab for treatment of colorectal cancer.

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Anti-tumor activity of a novel, human anti-epidermal growth factor receptor (EGFR) monoclonal antibody (IMC-11F8) in human colon carcinoma xenograft models with enhanced activity in combination with CPT-11

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Molecular inhibition of epidermal growth factor receptor (EGFR) function is a promising approach to cancer therapy. In this report, we describe the *in vivo* activity of a novel human anti-EGFR monoclonal antibody, designated IMC-11F8. Anti-tumor activity of IMC-11F8 was evaluated in DLD-1, HT-29 and GEO models of colon carcinoma in athymic mice. Dose-dependent inhibition of tumor growth in all models was observed in mice treated with IMC-11F8 monotherapy (1mg or 0.3mg; 3x/week) with T/C values ranging from 3% to 17% for the 1mg dose and from 38% to 81% for the 0.3mg dose. IMC-11F8 and CPT-11 (irinotecan; 100mg/kg, q7d) combination therapy experiments were also performed. Treatment with combination therapy significantly inhibited the growth of these tumors compared to IMC-11F8 or CPT-11 monotherapy with a greater-than-additive effect. Combination therapy with the high dose of IMC-11F8 and CPT-11 resulted in a synergistic anti-tumor effect in all three tumor models with T/C% values of 8%, 3%, and 10% for DLD-1, GEO and HT-29, respectively. Combination therapy with IMC-11F8 and CPT-11 produced tumor regressions in 50% of the DLD-1 and HT-29 animals and in 90% of the GEO tumors. Histological examination of residual tumors after combination treatment showed an increase in pyknotic nuclei and a decrease in mitotic figures; this resulted in a substantial decrease in viable tumor compartment with near elimination of neoplastic cells. Decreased pMAPK was observed in GEO tumors treated with IMC-11F8, suggesting an inhibitory effect on the expression of MAPK-related signaling events. The present study shows that IMC-11F8 may be an effective therapy in the treatment of EGFR-positive tumors and warrants clinical evaluation of this agent.

Signal transduction modulators

315 POSTER

Molecular signature of the PTEN tumor suppressor-identification of IGFBP2 as a surrogate marker for PTEN/Akt signaling

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PTEN is an important tumor suppressor associated with many cancers including glioblastoma and prostate cancer. The well established function of PTEN is its lipid phosphatase activity, which antagonizes PI3K function and reduces the activation of Akt, a kinase involved in many cellular processes including survival, growth, and metabolism. Using expression profiling of prostate cancer xenografts and glioblastoma tissue samples, of which 11 tissues samples have the wild-type PTEN gene and 14 have mutated PTEN gene, we have identified a molecular signature for the PTEN tumor suppressor. The molecular signature consists of a minimum of 12 genes, several of which are involved in different pathways that were implicated in tumor formation. The identified molecular signature is able to predict the PTEN status of all tumors in the training set in different algorithms, including Random Forest analysis, multidimensional scaling analysis, and hierarchical clustering, using standard leave-one-out and/or permutation analysis for statistical validation. Validation studies using an independent set of tumors are ongoing. Among 12559 genes in the microarray analysis, an increase in IGFBP-2 mRNA was the most consistent change associated with PTEN mutations. The consistent upregulation of IGFBP-2 was confirmed at the protein level by western blot and immunohistochemical analysis, and was extended to samples not included in the microarray analysis. Using syngenic mouse embryonic fibroblasts, pharmacological and molecular biological manipulations, we found that IGFBP-2 expression is negatively regulated by PTEN, and positively regulated by PI3K and Akt activation. In addition, we established that IGFBP-2 plays a functional role in PTEN tumor suppressor function by manipulation of PTEN and IGFBP-2 expression levels. Furthermore, we showed that IGFBP-2 is required for Akt transformation by using IGFBP-2 knockout MEFs. Currently we are working to determine how IGFBP-2 is involved in PTEN tumor suppressor function.

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Cetuximab-induced clearance of the epidermal growth factor receptor (EGFR) overcomes resistance of cancer cells to EGFR tyrosine kinase (TK) inhibitors

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Analysis of global gene expression profiles of cancer cell lines exposed for 24 hours to Erlotinib (E), a quinazoline derivative that reversibly inhibits the EGFR TK, showed a marked increase in expression of the EGFR mRNA in resistant cell lines but not in susceptible ones. Because Cetuximab (C), a quimeric MAb that binds the EGFR in its extracellular domain is known to induce EGFR downregulation, we explored the hypothesis that combined treatment with both agents results in augmented antitumor effects. HuCCT1 cells were treated (growth media, E [5 μM], C [50 nM], and E+C) and harvested at different time points (baseline, 1, 6, 12, 24, and 48 hours of treatment). Four groups of 10 nude athymic mice were injected with $5x10^6$ cells, and treated during 14 days (vehicle, E [50 mg/kg], C [50 mg/kg], and E+C); tumors were extracted at baseline, 1, 14, and 28 days after therapy started. EGFR mRNA and protein levels *in vitro* and *in vivo* were analyzed. HuCCT1 cells were resistant to E *in vitro*, and showed a modest growth arrest when C was added, either as single agent or in combination with E. None of the agents induced a significant tumor regression *in vivo*, but C-treated mice showed a growth arrest that lasted 4 weeks after completion of therapy. Mice allocated to E received C after completion of E, and a significant growth arrest was observed. E induced EGFR mRNA synthesis *in vitro*, whereas the addition of growth media or C to serum starved cells inhibited EGFR mRNA production. EGFR mRNA upregulation induced an increase in total EGFR levels *in vitro*. An increase in total EGFR levels was demonstrated after E, as opposed to a decrease in EGFR levels after C, both *in vitro* and *in vivo* (and both as primary therapy, or after failure of E). Downstream pathway analysis showed that EGFR activation status is unrelated to response in HuCCT1 xenografts, whereas MAPK activation status is related to tumor growth. Further analysis using small interfering RNA against the EGFR mRNA, and an E-acquired resistance model are underway to further validate this novel mechanism of resistance. In summary, E induces an EGFR mRNA and protein upregulation that could be in part responsible for the observed resistance of HuCCT1 to this agent. mRNA upregulation is closely followed by an increase in protein synthesis *in vitro* and *in vivo*. C induces tumor growth arrest, prompts a decrement in EGFR levels, and is able to abrogate E-induced EGFR upregulation, both *in vitro* and *in vivo*.

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SH3-Grb2 inhibitors inactivate HER2 signaling and enhance the anti-tumor effects of Docetaxel

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Expression of HER2 has been reported in approximately 30% of human breast cancers and has been correlated with a poor prognosis of this particular type of cancer. HER2 protein exhibits tyrosine kinase activity and plays an important role in human malignancies by activating the Ras signaling pathway. In this pathway, Grb2, a small adaptor protein, interacts with HER2 through its SH2 domain. Via its SH3 domains, it interacts with the proline-rich motives of Sos, the exchange factor of Ras, mediating Ras activation. To interfere in this pathway, we have already designed ligands called "peptidimers", targeting both SH3 domains of Grb2, and conjugated