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.

314 POSTER

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.

316 POSTER

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*.

317 POSTER

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

them with penetratin allowing them to enter the cells. Here, we studied the effect of penetratin-peptidimer on HER2 signaling and its synergistic effect with "Docetaxel (Taxotere(R))" on HER2-overexpressing cancer cells in vitro and in vivo. Transformed NIH3T3/HER2 cells and SKBr3 (a human breast cancer line overexpressing HER2) cells were seeded on 6-wells plates and the penetratin-peptidimer was added to the culture medium to test its anti-tumor potential in a clonogenic assay. Treated cell colonies were stained and counted 2 to 3 weeks later. The penetratin-peptidimer inhibited the colony formation with an IC50 of 0.5 μM and 0.05 μM for SKBr3 cells and NIH3T3/HER2 cells, respectively. The levels of phosphorylated AKT and ERK proteins were assessed in order to determine the peptidimer effects in the HER2-dependant signaling pathway. Cells treated with the peptidimer showed a reduction in phosphorylated AKT but ERK phosphorylation remained unchanged. Docetaxel treatment induces overexpression of HER2, in a human prostate adenocarcinoma xenograft established in nude mice. "Trastuzumab (Herceptin(R))", a humanized recombinant monoclonal antibody directed against HER2 was shown to synergize the Docetaxel induced effects. We observed an increased HER2 expression in the two cell lines following Docetaxel administration. The peptidimer significantly enhanced sensitivity to Docetaxel in both NIH3T3/HER2 and SKBr3 cells. This combination was tested in an independent hormone xenograft model, using nude mice, of human prostate cancer overexpressing HER2. A synergistic effect of the peptidimer and Docetaxel was also obtained. These results suggest that the SH3-Grb2 inhibitor has an anti-tumor activity and enhanced cytotoxicity when combined with Docetaxel in HER2-expressing breast cancer cells and in the prostate xenograft.

318 POSTER

Dihydropyrrolopyrazoles as TGF-beta receptor kinase inhibitors for cancer therapy

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TGF-β is a cytokine with diverse biological activities. TGF-β can mediate diametrically opposed activities depending upon the physiological state of a cell. Perhaps the most dramatic example of this phenomenon is TGF-β 's role as a tumor suppressor and tumor promoter. In many cells, TGF-β mediates a growth inhibitory signal via a heteromeric receptor complex composed of two transmembrane serine/threonine kinase receptors, the type I and type II receptors. However, once this inhibitory pathway is disrupted in tumor cells, TGF-β becomes a potent tumor promoter that can be secreted at high levels from the resistant tumor cells. This increased TGF-β expression modulates the extracellular matrix and has angiogenic and immunosuppressive activities. In addition, TGF-β contributes to the epithelial-to-mesenchymal transition of tumor cells, thus creating a more invasive and metastatic phenotype. These tumor-promoting activities of TGF-β provide rationale to target this pathway for therapeutic intervention. A series of orally bioavailable, small molecule kinase inhibitors that are potent and selective for the TGF-β receptors has been identified and characterized in *in vitro* kinase and cell based assays. Direct measures of target modulation in cells involved evaluation of P-Smad2 inhibition. A hallmark of our discovery program has been the characterization of *in vivo* target modulation using a subcutaneous xenograft tumor model to define PK/PD relationships for target modulation in animals. Evaluation of the dihydropyrrolopyrazole SAR *in vivo* yielded compounds with IC_{50} values ranging from 0.020 to 2 μM. In time course experiments, target modulation paralleled plasma exposure with 8–12 hr of measurable activity that necessitated an oral BID schedule in the subsequent anti-tumor efficacy models. A pan-TGF-β neutralizing antibody was used to validate the involvement of TGF-β in the growth of MX1 breast cancer xenografts. Evaluation of dihydropyrrolopyrazole compounds in the MX1 model showed a statistically significant decrease in tumor growth. Anti-tumor efficacy has also been observed for this series of compounds in the Calu6 NSCLC xenograft model. A series of microarray experiments in the Calu6 model has been conducted to evaluate the biological effects of TGF-β in this system *in vitro* and to evaluate the differential sensitivity of TGF-β regulated genes to type I receptor selective or dual type I/type II receptor inhibitors. Extension of this approach to the Calu6 xenograft model will assist in the identification of potential biomarkers for evaluation of on-target compound activity in future clinical trials.

319 POSTER

Factors that govern the cell death response induced by inhibition of the molecular chaperone heat shock protein 90

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The molecular chaperone heat shock protein 90 (Hsp90) has emerged as an exciting anticancer drug target due to its role in maintaining the conformation and stability of key oncogenic client proteins. The Hsp90 inhibitor; 17-allylamino, 17-demethoxy geldanamycin (17AAG) binds and inhibits the intrinsic ATPase which is essential for Hsp90 function, and is the first in-class Hsp90 inhibitor to enter and complete a phase 1 clinical trial. *In vitro* 17AAG treatment induces both cytostasis and apoptosis, the extent of which is cell line dependent. The aim of this study was to identify factors that may influence the cell death response following 17AAG treatment. Our laboratory has previously hypothesised that the apoptotic response to 17AAG operates via a Bax-dependent mechanism, based on the absence of apoptosis in KM12 cells that lack Bax, after exposure to 17AAG. This possibility has been explored using an isogenic pair of the human colon cancer cell line HCT116, which differ only in their expression of Bax. We demonstrate that Bax expression is required for apoptosis induced by 17AAG treatment and in its absence necrosis becomes the predominant mechanism of cell death. We also demonstrate that the apoptotic response to Hsp90 inhibition could be further influenced by increased expression of the Hsp70 family, which are inhibitors of the apoptotic pathway. We and others have previously shown that the constitutive (Hsc70), mitochondrial (Mortalin) and inducible (Hsp72) isoforms of Hsp70 are induced in response to 17AAG treatment. Here we use an siRNA approach to show that selectively repressing the induction of Hsp72 in response to 17AAG treatment increased cell death in HCT116 cells after only 24 hours exposure to 17AAG, which is earlier than normally associated with the cell death response in this cell line. The influence of Hsc70 and Mortalin on the cell death response to 17AAG treatment has also been explored using siRNA. In summary, these findings suggest that 17AAG induces apoptotic cell death via the intrinsic pathway mediated by Bax, the degree of which may be impaired by the induction of the anti-apoptotic Hsp70 family. However when Bax is not present 17AAG causes cell death to a lesser extent and via a necrotic mechanism. The anti-apoptotic effect of Hsp72 may begin to explain the predominance of tumour cytostasis versus cytotoxicity, as observed in human tumour xenografts and some patients treated with 17AAG during phase 1 clinical trial.

320 POSTER

Identification of potent, selective, soluble and permeable small molecule PI3 kinase inhibitors for the treatment of cancer

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Phosphatidylinositol-3-kinases (PI3K) are lipid kinases that mediate cell signalling pathways controlling growth, proliferation, survival and motility. There is significant evidence suggesting that deregulation of the PI3K/c-Akt pathway is important in tumour progression, including loss of function of the tumour suppressor PTEN, the phosphatase that counteracts PI3K, and high frequency of mutation of the PI3K p110α isoform in human malignancies.[1]

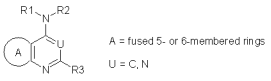

Fig. 1: Generic structure of fused heterocyclic compounds.

Table 1. *In vitro* biochemical and physicochemical properties of PI3K inhibitors

	IC_{50} (μM)		PI3K, SPA		Solubility (μM)	Mouse Microsome Stability[a]
	p110 α	p110 β	p110 δ	p110 γ		
PI103	0.0015	0.003	0.003	0.015	3.5	13
PI509	0.0045	0.037	0.019	0.112	20	5
PI516	0.004	0.045	0.006	0.063	>100	11
PI540	0.010	0.044	0.009	0.321	>100	91

[a] % compound remaining after 30 min.

We have previously reported that PI103, a potent and selective PI3K inhibitor with established *in vivo* efficacy in xenograft models, had been identified as a starting point for the development of a series of novel small molecule therapeutics for the treatment of cancer (Figure 1).[2] However, several features of PI103, including its low aqueous solubility at physiological pH, were subsequently identified as areas for lead optimisation. A medicinal chemistry effort at PIramed has resulted in the discovery of a second generation of PI3K inhibitors with promising biochemical affinity and functional activity, and with improved physicochemical properties. Three such compounds, PI509, PI516, and