presence secondary antibody or FcR-expressing effector cells. The KMTR2 antibody formed supracomplexes with soluble recombinant and membrane-anchored TRAIL-R2 and enhanced clustering of TRAIL-R2 on the surface of cell without crosslinking. The KMTR2 antibody was dramatically efficacious in reducing establish human xenograft tumors in vivo when compared to other anti-TRAIL-R2 antibodies of similar isotype and affinity suggesting the agonistic anti-tumor activity is independent of host effector function. These results indicate that this monoclonal agonist antibody can direct antibody-dependent oligomerization of TRAIL-R2 and initiates efficient apoptotic signaling and tumor regression.

311 POSTER

Anti-cancer efficacy of a functional monoclonal antibody targeting melanoma-associated chondroitin sulfate proteoglycan

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Melanoma-associated chondroitin sulfate proteoglycan (MCSP) is a glycoprotein-proteoglycan complex present on the surface of melanoma cells, and some other cancers, either as a free glycoprotein or modified by the addition of chondroitin sulfate. MCSP has been purported to have a role in cancer progression by enhancing adhesion and invasion of melanoma cells through multiple mechanisms. AR11BD-2E11–2 is a functional monoclonal antibody that targets MCSP, which was discovered using the ARIUS FunctionFIRST™ platform. Mice were immunized with human breast cancer cells. The functional screening process identified a hybridoma that produces an antibody that is cytotoxic to breast and ovarian cancer cells but not to normal cells. AR11BD-2E11–2 was evaluated *in vivo* in order to further examine its anti-cancer effects. In a xenograft MCF-7 breast cancer model, AR11BD-2E11–2 suppressed tumor volume by 80% compared to isotype control-treated mice, and conferred a significant survival benefit. In a second xenograft model, the increase in body weight due to ascites was used as a marker of OVCAR-3 ovarian cancer progression. The mice in the control-treated group showed a 60% tumor-related weight gain, while the AR11BD-2E11–2 treated mice showed a significantly lower weight gain of 40%, and had a significantly longer mean survival time. Antigen characterization was carried out using immunoprecipitation followed by mass spectrometry. The identity of the target antigen for AR11BD-2E11–2 was determined to be MCSP. The IHC staining pattern of the epitope recognized by AR11BD-2E11–2 on frozen human breast cancer sections was found to be highly specific for malignant cells. On a panel of frozen human normal tissues, staining with AR11BD-2E11–2 was generally restricted to the smooth muscle fibers of blood vessels. The generation of a functional anti-cancer antibody that recognizes MCSP has confirmed the relevance of this antigen as a target for cancer therapy, and has demonstrated its potential as a target in ovarian and breast cancer.

312 POSTER

Pilot study of the use of Infliximab for fatigue in advanced cancer

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Background: Many patients with advanced cancer experience fatigue, some with cachexia. There is evidence that the pro-inflammatory cytokine Tumour Necrosis Factor alpha (TNF-α) may be a mediator[1].
Infliximab (Remicade®) is a chimeric monoclonal antibody to TNF-α licensed for the treatment of Crohn's disease and rheumatoid arthritis. We investigated whether Infliximab used in advanced cancer would improve measurable fatigue.
Method: Seventeen patients with advanced cancer (various solid tumours, age range 42–82 years) scoring over the threshold on the Fatigue Severity Scale (FSS) (Stone et al[2]) were recruited from a Specialist Palliative Care Unit in London (patients with specific risk factors were excluded[3]). Subjects received 5mg/kg Infliximab intravenously, repeated at 4 weekly intervals so long as there was clinical improvement. On each visit measures of fatigue, appetite, body mass, performance status, quality of life, depression, pain, serum TNF-α and leptin levels were recorded. Serum will be analyzed for the presence of TNF-α gene promoter polymorphisms. Treatment with Infliximab was discontinued if any intolerable adverse effects were reported or when clinical benefit ceased.
Results: Six patients reported subjective clinical benefit. Four patients showed greater than 20% reduction in fatigue severity score (primary outcome) 4 weeks after first treatment [Figure 1].
Four patients died during the study, 1 due to disease progression, 1 possibly due to adverse effects of treatment (acute infection) and 2 from causes probably unrelated to treatment (cerebral infarct and myocardial infarction). 8 treated patients died from disease progression after completing the study. 5 treated patients remain alive.
Secondary outcome measures (change in appetite, body mass, mood, pain, QOL, serum TNF-α and leptin) showed no emerging pattern.

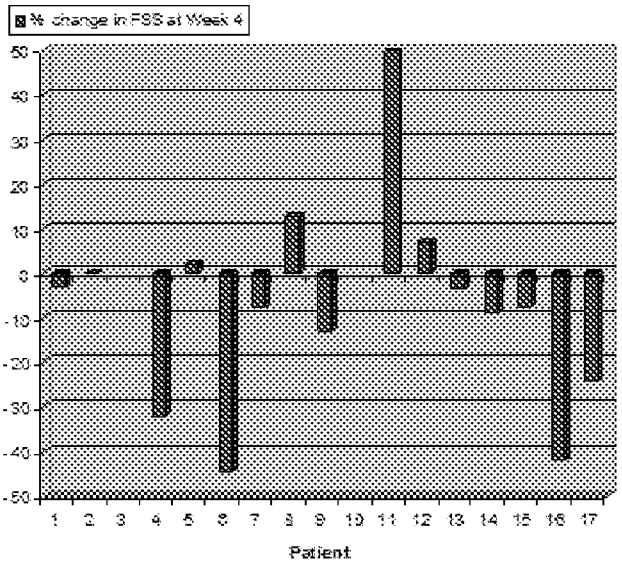

Figure 1: Percentage change in FSS 4 weeks after first treatment.

Note: a negative change in FSS score indicates decreasing fatigue
Conclusions: Numbers in this pilot study are small and the results therefore are descriptive. A few patients showed clinical benefit but initial data are inconclusive. There may be an improvement in fatigue in a selected group of patients with advanced cancer. It is hoped that further data analysis may determine future research questions in this area.

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313 POSTER

Mono- and combination-therapeutic activity of panitumumab (ABX-EGF) on human A431 epidermoid and HT-29 colon carcinoma xenografts: correlation with pharmacodynamic parameters

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Background: Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor. Overexpression has been correlated with aggressiveness and poor prognosis in many tumor types including colon. Panitumumab, a fully human antibody, binds to the EGFR with high affinity ($5x10^{-11}$ M) preventing ligand-induced autophosphorylation resulting in arrest of tumor cell proliferation and increased apoptosis in some cases [1,2]. The purpose of this study was to examine the effects of panitumumab as a monotherapy and in combination with irinotecan in the HT-29 xenograft tumor model of colon cancer.
Methods: Inhibition of ligand-induced autophosphorylation was determined *in vitro* and *in vivo*. *In vitro*, A431 and HT-29 cells were treated with 0.5, 2, and 10 μg/ml of panitumumab for one hour prior to 100 ng/ml EGF stimulation. *In vivo*, tumor-bearing mice were treated with 100 ng of *rh*EGF 30 minutes prior to removing the tumor and measuring the phosphorylation of EGFR. To measure efficacy, tumor bearing were treated twice per week with panitumumab at 100, 200 or 500 μg/mouse, or panitumumab in combination with 100 mg/kg irinotecan once per week. Immunohistochemistry was performed to evaluate the extent of panitumumab penetration into tumors and changes in pMAPK and Ki67 staining as a result of panitumumab administration.
Results: *In vitro,* panitumumab treatment resulted in a dose-dependent cytostatic effect in both A431 and HT-29 carcinoma cells and a concomitant reduction in ligand-induced phosphorylation of EGFR both *in vitro* and *in vivo*. Immunohistochemistry demonstrated dose-dependent tumor

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