chromatography and characterized by a variety of techniques, including SE-HPLC, SDS-PAGE, in vitro transcription translation (IVTT) assay using luciferase reporter system, and competition ELISA to measure the binding affinity for CD74. The in vitro cytotoxicity was determined in a B-cell lymphoma cell line (Daudi) and a multiple myeloma cell line (MC/CAR), using the MTS tetrazolium dye reduction assay or a BrdU colorimetric assay.

Results: The purified protein was shown to be a single peak by SE-HPLC and its MW determined by MALDI-TOF to be 177,150, which is in agreement with the MW of one IgG (150,000) plus two rpRNase molecules (24,000). Reducing-SDS-PAGE revealed the presence of 3 bands, one corresponding to the heavy chain and the other two appearing to be derived from the rpRNAse-fused light chains (38,526 and 36,700 by MS). Occurrence of the 2 light chains was due to uneven glycosylation of rpRNase, since the two light chain bands converged to a single band after treatment with N-glycosidase. The EC_{50} of RNAse activity, as measured by the IVTT assay, was 300 pM for rpRNAse-hLL1 and 30 pM for free rpRNAse. The binding affinity of rpRNAse-hLL1 for CD74 was indistinguishable from that of hLL1. rpRNAse-hLL1 was significantly more cytotoxic to Daudi (EC_{50} 280pM (than MC/CAR (EC_{50} 50nM). Free rpRNAse did not demonstrate significant cytotoxicity at the concentrations tested.

Conclusion: A novel immunotoxin was expressed in a mammalian system. About 60% of the rpRNAse was found to be glycosylated. The fusion protein retained activity of rpRNAse and the binding affinity of hLL1 antibody, and demonstrated potent toxicity to CD74+ cells.

307 POSTER

Therapeutic implications of an antibody to the human macrophage-stimulating protein receptor tyrosine kinase (RON)

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The Macrophage-Stimulating Protein receptor aka. MSP-R or RON belongs to the c-MET family of receptor tyrosine kinases. The ligand for c-MET – Hepatocyte Growth Factor (HGF) as well as RON's ligand, MSP are members of the kringle-domain plasminogen-related protein family. As its name implies, MSP was originally found to stimulate macrophages by a variety of means. For example, addition of MSP to certain RON-expressing macrophages induced shape changes, chemotaxis, macropinocytosis and phagocytosis. RON was also found to be expressed in epithelial cells such as keratinocytes where MSP was shown to phosphorylate RON and activate a number of signaling pathways that elicited cell adhesion/motility, anti-apoptotic and proliferative responses. Within the last few years, however, over-expression of RON has been observed in several epithelial tumors and cell lines (ex. colon, breast and lung). In addition, the oncogenic potential of RON was recently demonstrated when lung tumors developed in transgenic mice engineered to over-express RON in their lungs. Although these data suggest a link between RON expression and cancer, studies to address whether inhibition of RON could abrogate tumor or cancer cell line growth have not been reported. Through the screening of a Fab phage display library, we have developed IMC-41A10, a monoclonal antibody that binds to human RON with an affinity of ~1.5 nM and inhibits MSP binding to RON with an IC_{50} of ~2 nM. IMC-41A10 demonstrated significant inhibition of the proliferation of RON-expressing HT-29 colon cancer cells grown adherently or as colonies in soft agar. Moreover, IMC-41A10 showed a 50–60% inhibition of tumor volumes when HT-29 cells were grown subcutaneously in nude mice. To our knowledge, this is the first demonstration that inhibition of the RON receptor tyrosine kinase negatively influences the proliferation of colon cancer cells *in vitro* and *in vivo*. In addition, it underscores the potential therapeutic utility of inhibiting RON in colon and possibly other cancers.

308 POSTER

Inhibition of FLT3-expressing leukemia cells by a monoclonal antibody-auristatin conjugate

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The receptor tyrosine kinase FLT3 is overexpressed in blasts of ~90% of acute myelogenous leukemia (AML) and the majority of B-lymphoid leukemia patients. Internal tandem duplications (ITDs) in the juxtamembrane region and point mutations in the kinase domain of FLT3 are found in ~37% of AML patients and are associated with a poor prognosis. We have recently developed a fully human monoclonal antibody (IMC-EB10) which binds with high affinity to FLT3 receptor on human leukemia cells. In the present study, a novel auristatin conjugate of the anti-FLT3 antibody (EB10-MMAF) was prepared using a dipeptide linker that allows for drug release inside the lysosomes of antigen-positive cells. The MMAF conjugates were stable in buffers and plasma. EB10-MMAF (drug/antibody raito=7.6) was highly potent, and selectively inhibited the growth of FLT3-expressing leukemia cells with an IC50 of 0.19 nM and 0.08 nM for MV4;11 and BaF3-ITD cells (both positive for FLT3-ITD), 1.11 nM, 6.18 nM and 1.82 nM for REH, EOL-1, EM3 cells (all three positive for wild-type FLT3), and 135 nM for JM1 (negative for FLT3). An MMAF conjugate with a control antibody was not active in these cell lines (IC50s > 5.9 uM). Flow cytometric analysis with annexin V indicated that EB10-MMAF treatment induced apoptosis of leukemia cells *in vitro*. The *in vivo* efficacy of the conjugate is being investigated in several FLT3-positive human xenograft leukemia models in NOD-SCID mice.

309 POSTER

Therapeutic efficacy of the Y-90 labeled antibody 19G9, targeting a novel protein RG-1, expressed in metastatic prostate cancer

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RG-1 (a human mindin homologue) is expressed selectively in prostate tissues in the human male. We have shown by analysis of RG-1 mRNA and protein levels that its expression is high in prostate tumors but low in most other tissues. We have extended this analysis to include prostate tumor metastases to soft tissues and bone and found that RG-1 expression is maintained in greater than 75% of metastatic bone tumors, 70% of lymph node metastases, and 85% of locally recurrent tumors in androgen unresponsive patients. Fully human antibodies, 19G9 and 34E1, have been generated against the RG-1 protein and have been shown to accumulate at high abundance in LNCaP tumor xenografts. This has been observed with hybridoma generated antibodies and antibodies expressed in CHO cells. Conjugates of these antibodies with CHX-A"-DTPA have been generated and radiolabeled with either Y-90 or Y-86. MicroPET imaging with the Y-86 radiolabeled 19G9 antibody demonstrated very specific accumulation of the antibody in LNCaP tumor xenografts with clear tumor delineation apparent at 4 hours and exceptional tumor to background contrast at 72 hours. The therapeutic efficacy of Y-90-CHX-A"-19G9 was evaluated in mice bearing LNCaP xenografts. An MTD study identified a non-toxic therapeutic dose to be 75–100 μCi. Significant anti-tumor efficacy of the Y-90 antibody conjugate was seen with a single administration of radiolabeled antibody to animals bearing 200–400 mm^3 tumors. Inhibition of tumor growth was seen in all treated animals over a 45-day period. At 49 days post treatment, slow tumor growth recurred but this regrowth could be prevented for an additional 40-day period by a second administration of a 75 μCi dose on day 49. We conclude that Y-90-CHX-A"-19G9 is a novel human antibody conjugate that has considerable promise for the effective therapy of metastatic prostate cancer in androgen unresponsive patients.

310 POSTER

Enhanced apoptosis and tumor regression induced by a direct agonist antibody to TRAIL-R2

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Substantial evidence indicates that supraoligomerization of the death receptors for Fas ligand (FasL) and Tumor necrosis factor-related apoptosis-Inducing ligand (TRAIL) is necessary for efficient activation of the apoptotic pathway. Oligomerization of Fas or TRAIL receptors with bivalent IgG antibodies can mimic the natural ligands, but only after these antibodies are further oligomerized by the addition of secondary crosslinking reagents. We report here a novel fully human IgG antibody to TRAIL-R2 (KMTR2, also known as HGS-TR2J) that directly activates tumor cell apoptosis in vitro without the requirement of crosslinking IgG. Size-exclusion chromatography demonstrated the apoptosis activity co-eluted with monomeric IgG and was effective independent of the

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.

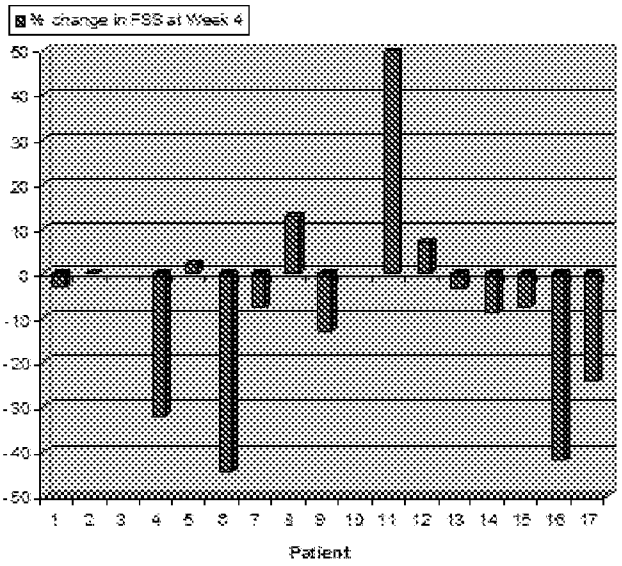

Figure 1: Percentage change in FSS 4 weeks after first treatment.

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