The gene set was able to prospectively predict the outcome of 8/9 of the models.
Conclusion: These data suggest panitumumab can inhibit the growth of different tumor xenografts and that the tissue type has more influence on the clustering of the models than the responsiveness (or lack of) to panitumumab. Using a supervised analysis, gene lists can be generated from microarray data that can prospectively predict response in xenograft models. This approach may aid in the selection of genes that could stratify patients that respond to panitumumab.

303 POSTER

Peptide vectors for the intracellular delivery of 125I-anti-carcinoembryonic antigen (CEA) antibodies as the first step towards auger electron radioimmunotherapy

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Background: Carcinoembryonic antigen (CEA) is the reference antigen for immunotargeting of gastrointestinal tumors due to an over-expression in almost all colorectal tumors (>95%), a high antigenic density expression (up to $1x10^6$ CEA molecules per cell) and a very long residence time at the cell surface. However, in radioimmunotherapy (RIT), the non-internalization of CEA rules out the use of low range radioisotopes such as Auger emitters which are attractive for the treatment of very small tumor nodules. In order to overcome this limitation, we used peptide vectors (DPV) to induce internalization of the anti-CEA MAb 35A7 and analyze the potential of ^{125}I-35A7-DPV conjugates for Auger electron RIT.
Material and Methods: Three different peptides selected for their nuclear tropism were used to prepare, using the SMCC technique, MAb-DPV conjugates containing 3 to 5 peptides molecules per MAb molecule (DPV10: VKRGLKLRHVRPRVTRMDV; DPV10: SRRARRSPRHLGSG; DPV15: 16 AA un-published sequence patent application pending). Internalization in LS174T human colon carcinoma cells was analyzed using immunofluorescence microscopy. Cytotoxicity was measured in a clonogenic assay. An irrelevant MAb, PX, was used as control in all the experiments.
Results: Immunofluorescence analysis demonstrated that all 35A7-DPV conjugates internalized in LS174T cells although native 35A7 did not. In the clonogenic assay, ^{125}I-35A7-DVP conjugates demonstrated a cytotoxicity dependent on the peptide: ^{125}I-35A7-DVP15 > ^{125}I-35A7-DVP10 > ^{125}I-35A7-DVP1047. Non-radiolabeled 35A7 and 35A7-DPV conjugates as well as ^{125}I-35A7 did not show any cytotoxicity. The irrelevant conjugate, ^{125}I-PX-DPV15, exhibited a limited cytotoxicity as compared with ^{125}I-35A7-DVP15 demonstrating the need of a specific MAb to eradicate all the LS174T cells.
Conclusions: These *in vitro* studies demonstrate that the therapeutic effect of ^{125}I-MAb is dependent on internalization due to the very short particle range of the Auger electron. ^{125}I-anti-CEA MAb derived with DPV are potential candidates for Auger electron radioimmunotherapy in digestive cancers.

304 POSTER

Pharmacokinetics of CNTO 95, a fully human MAB to human integrin receptors following single or multiple IV injections to cynomolgus monkeys

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Background: CNTO 95 is a fully human monoclonal antibody (mAb) that binds with high affinity and specificity to the human integrin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Results from animal studies demonstrate that CNTO 95 can inhibit tumor growth and angiogenesis. This poster summarizes the pharmacokinetics of CNTO 95 in cynomolgus monkeys following single IV injection or weekly IV injections for up to two months.
Material and Methods: Cynomolgus monkeys, a total of 9, 30 and 24 males and females, were used in the single IV injection (2, 10 and 50 mg/kg dose), one month and two month weekly IV injections (10 and 50 mg/kg dose), respectively. Pharmacokinetic calculations were conducted using WinNonlin. Dose proportionality was evaluated following single and multiple dose administrations.
Results: The single dose PK analysis following 2, 10 and 50 mg/kg IV injection indicated that all of the PK parameter estimates were dose-dependant and could be characterized by a Michaelis-Menten elimination model with the half-life ranging from 0.69–3.11 days. The Cmax and AUC(0–72h) after the first dose of the one month or two month weekly 10 and 50 mg/kg IV injections indicated a greater than dose proportional increase. Steady state was reached around 43–50 days after the first injection (6–7 doses) and the approximate dose proportionality was observed at steady state. The half-life after one month of weekly injections at 10 or 50 mg/kg injections was approximately 9 days. No significant gender effect was observed in studies.
Conclusion: These studies indicate that CNTO 95 undergoes absolute tissue binding which could be characterized by a Michaelis-Menten elimination model. At low doses following single administration, the drug is rapidly cleared from the serum; however, as the binding sites for the drug become saturated, the pharmacokinetics change from a less than dose proportional to a dose proportional relationship. These PK studies could be useful in optimizing dosing regimen to maintain complete integrin receptors saturation in vivo.

305 POSTER

In vitro evaluation of a doxorubicin-antibody conjugate, on non-Hodgkin's lymphoma and multiple myeloma cell lines

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Background: Antibody-targeted selective delivery of anticancer drugs against antigens expressed on cancer cells can potentially improve the therapeutic index of anticancer drugs. We have developed an immuconjugate, IMMU-110, comprised of doxorubicin (DOX) conjugated to the humanized form of the anti-CD74 monoclonal antibody (mAb), hLL1, at 8 drug molecules per antibody molecule. CD74 is a rapidly internalizing type-II transmembrane chaperone molecule associated with HLA-DR, and has high expression on human non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) clinical specimens and cell lines. Here, we investigated the in vitro efficacy of IMMU-110 on CD74+ cell lines of NHL (Daudi, Raji) and MM (MC/CAR).
Methods: Cell binding of IMMU-110 to antigen-positive cells was determined by an indirect cell surface binding ELISA assay. Internalization of Alexa 488 labeled IMMU-110 was evaluated using fluorescence microscopy. In vitro cytotoxicity of IMMU-110 was determined using a tetrazolium assay (MTS dye reduction assay).
Results: Cell binding of IMMU-110 with the CD74+ cells was significantly higher than that of isotype-matched mAb-DOX conjugate (DOX conjugated to a mAb against epithelial glycoprotein-1; DOX-hRS7), and was similar to that of naked hLL1. Both IMMU-110 and naked hLL1 bound CD74 with subnanomolar affinity. Following binding, IMMU-110 internalized inside the cells, unlike DOX-hRS7. The in vitro cytotoxicity of IMMU-110 was higher than DOX-hRS7 by 40-fold in MC/CAR cells, by 23-fold in Daudi cells, and by 160-fold in Raji cells. The cytotoxicity of IMMU-110 approached that of free DOX in all the three-cell lines. In CD74-cells lines (ARD, OPM-6), IMMU-110 was significantly less toxic than free DOX, having similar cytotoxicity to DOX-hRS7.
Conclusions: IMMU-110 specifically associates with and is cytotoxic against CD74+ NHL and MM cells. IMMU-110 is being further developed as a potential therapeutic agent for the treatment of CD74+ tumors.

306 POSTER

Construction and characterization of a novel immunotoxin consisting of two ranpirnase (rpRNAse) molecules fused to an anti-CD74 humanized IgG4 antibody

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Background: rpRNAse is a monomeric protein (MW 11800) isolated from *Rana pipiens* eggs that specifically degrades RNAs upon internalization. Previous studies indicated that cytotoxicity of rpRNase can be enhanced more than 1,000-fold when the enzyme is chemically conjugated to an internalizing antibody. Here we describe the construction, characterization, and *in vitro* cytotoxicity of a novel immunotoxin fusion protein, 2L-rpRNAse-hLL1-g4P, composed of two rpRNase molecules fused to the internalizing anti-CD74 humanized IgG1 antibody, hLL1. To reduce the potential cytotoxicity to non-target cells, the constant region of hLL1 was replaced with an IgG4 constant region that contains a proline mutation in the hinge region.
Methods: The rpRNAse gene was inserted at the N-terminus of the light chain in the expression vector of hLL1. The constant region of IgG1 was replaced with IgG4 and a serine residue in the hinge region of IgG4 was substituted with proline to prevent the formation of half-molecules. NSO mouse myeloma cells were transfected, and positive clones were identified by ELISA screening. The fusion protein was purified by protein A column

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