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