Material and Methods: The IMMU-110 conjugate was prepared as described in the above reference, and tested in a newly developed model of disseminated MM. The MC/CAR human MM cell line was maintained in tissue culture in RPMI 1640 media supplemented with fetal bovine serum, penicillin/streptomycin (1%), and glutamine (2%). Cells were split the day before injection to ensure log-phase growth. C.B-17 FOX CHASE SCID^{TM} mice were pretreated with Fludara (0.4 mg/mouse) and Neosar (2 mg/mouse) 5 days prior to an *i.v.*-injection of 10^7 MC/CAR cells, and thereafter monitored daily for signs of hind-leg paralysis, at which point they were sacrificed for humane reasons. Survival studies were analyzed by Kaplan-Meier plots (log-rank analysis) using the GraphPad Prism software package.
Results: SCID mice, left untreated, succumbed to paralysis, due to disseminated disease, at a median of 32 days post-tumor cell injection. A single injection of 350 μg of IMMU-110 in MC/CAR-bearing SCID mice resulted in 6/10 survivors at > 175 days post-tumor cell challenge. Unconjugated hLL1 alone, at 350 μg, showed considerable efficacy, with an increase in median life extension to ca. 60 days, while an equivalent mixture of hLL1 and free doxorubicin demonstrated an increase in median life extension to 68 days. In a second experiment, delaying treatment to 5, 10 and 14 days post-MC/CAR cell injection resulted as follows: in the day-5 group, 3/10 animals survived to 150 days, and in the day 10 group, 5/10 animals survived at 150 days. Animals with advanced disease treated at 14 days post-tumor cell challenge succumbed, but with a statistically significant improvement in survival time from 28 to 35 days ($p < 0.002$), compared to matched, untreated control animals. In ongoing dose-finding experiments, the maximum tolerated single dose of IMMU-110 has not been reached at 2.5 mg/mouse, while early efficacy is being seen at single doses ranging from 35–2000 μg/mouse.
Conclusion: IMMU-110 is a doxorubicin-anti-CD74 conjugate that can cure some mice treated with only a single injection of conjugate, at an essentially non-toxic dose, and therefore exhibits significant potential as a new therapeutic agent for the treatment of MM. Future studies will examine multiple dosing of the IMMU-110.

300 POSTER

Cetuximab/irinotecan/HD-FU/LV in first line therapy of metastatic colorectal cancer (CRC)

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Cetuximab (C225) has be shown to be active in patients with CRC failing irinotecan (Cunningham, ASCO 2003).
We performed a phase I/IIa study to evaluate toxicity, efficacy and PK of C225 combined with CPT-11, infusional 5-FU and in the first line therapy of patients with EGFR+ metastatic colorectal cancer.
Pts. received 250 mg/m2 cetuximab weekly after a loading dose of 400 mg/m2.
Chemotherapy consisting of 80 mg/m2/1h CPT-11, 500 mg/m2/2h LV, and infusional 5-FU (24h) in dose levels of 1.500 mg/m2 and 2.000 mg/m2 was administered weekly × 6, q d50.
Dose limiting toxicities (DLT) were defined as neutropenia or skin toxicity > grade 3, any febrile neutropenia/leukopenia, or thrombopenia, diarrhea, mucositis, hepatic toxicity > grade 2 and other relevant organ toxicity > grade 1, each in the first cycle.
After inclusion of 6 patients at the dose level of 1.500 mg/m2 5-FU without occurrence of DLTs, 15 pts. were enrolled at the dose level of 2.000 mg/m2. At this dose level, 3 DLTs were observed (2 pts. diarrhea grade 3, 1 pt. diarrhea grade 4). Skin toxicity grade 3/4 occurred in 3/19 pts during the first cycle.
Dose modification of chemotherapy during the first cycle was necessary in 2/6 and 7/13 pts. in the dose level of 1.500 and 2.000 mg/m2, respectively. Therefore, we recommend 1.500 mg/m2 5-FU for the phase II trials.
Pharmacokinetics of cetuximab was not influenced by the different 5-FU dose levels.
14 out of 19 evaluable patients achieved objective response (74%, 95% CI 51–88%; 2 pts. CR, 12 pts. PR). Secondary resection of liver metastases was performed in 4/19 patients (21%).
The combination of cetuximab with irinotecan/inf. 5-FU/LV has a promising activity. Final data of this phase I/IIa study will be presented at the meeting.

301 POSTER

Genomic discovery, characterization and validation of a transmembrane protein overexpressed in human ovarian and pancreatic cancers: a promising new target for therapeutic monoclonal antibodies

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We utilized comprehensive data mining and subtractive library evaluation to identify >30,000 sequences differentially regulated in cancer. Genes encoding secreted and transmembrane proteins showing upregulated mRNA expression by microarray and quantitative PCR with 208 human tumor and normal tissues were selected for progression as diagnostic and therapeutic antibody targets, respectively. One gene, dDx115o, encodes a transmembrane serine protease. QPCR showed that dDx115o mRNA is overexpressed in human ovarian and pancreatic cancer tissue with little or no expression in any normal tissues. Recombinant proteins were used to raise a series of monoclonal antibodies that recognize dDx115o. We demonstrated that dDx115o is a glycoprotein which can be specifically identified by western blot analysis using extracts of human tumor cell lines and ovarian tumors but not other normal tissues tested. dDx115o protein was localized to the membrane of dDx115o-expressing tumor cell lines by FACS and immunofluorescence of live cells. Immunohistochemical studies using monoclonal antibodies against dDx115o revealed strong cell surface staining in sections of human ovarian and pancreatic cancers. In functional validation experiments overexpression of dDx115o, but not a dDx115o mutant lacking protease activity, induced growth of test cells in soft agar as well as induced tumor growth in SCID mouse xenograft studies. Furthermore, siRNA-mediated knockdown of dDx115o expression in cultured tumor cells led to apoptosis and increased caspase activity. Monoclonal antibodies able to bind live cells demonstrated an ability to inhibit tumor cell proliferation in culture. The restricted nature of dDx115o over-expression and the demonstrated functional role in promoting phenotypes of malignant transformation makes this cell surface antigen an ideal target for a monoclonal antibody therapeutic strategy. Mouse xenograft efficacy studies are in progress.

302 POSTER

Patterns of gene expression can prospectively predict Panitumumab (ABX-EGF) monotherapy responsiveness in xenograft models

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Background: Epidermal growth factor receptor (EGFr) is a transmembrane tyrosine kinase expressed on many different tumor types. There is increasing preclinical and clinical evidence suggesting that blocking the EGFR signaling pathway can provide clinical benefit to patients whose tumors express EGFR. Panitumumab, a fully human antibody, binds to the EGFR with high affinity ($5x10^{-11}$ M) preventing ligand-induced activation resulting in arrest of tumor cell proliferation and apoptosis in some cases [1,2]. The objective of this study was to determine a gene array profile that could predict responsiveness to panitumumab monotherapy.
Methods: Responsiveness to panitumumab in ten xenograft models was determined. Animals were treated twice per week with 20, 100, 200, and 500 μg/mouse per dose and response was determined as a 40% reduction of tumor volume (versus control). To determine a set of genes that could potentially help prospectively stratify patients based on responsiveness, untreated xenograft samples, with known responsiveness to panitumumab, were arrayed on the Affymetrix human U133 gene chip. Supervised ANOVA, univariate and multivariate analysis were performed to determine transcripts that predict responsiveness to panitumumab.
Results: Panitumumab treatment of mice bearing 300 mm^3 established xenografts determined A431, PC-3, MIAPaCa and HT-29 models were responsive and NIH H1299, SK MES PD, MCF-7, U87, ZR75–1 and Colo 205 models were non-responsive. An initial unsupervised cluster analysis demonstrated that the tissue type had greater influence on the clustering of genes than the responsiveness to panitumumab. A two-way analysis of variance that modeled tissue affect and drug responsiveness revealed 2156 genes that were differentially expressed in responders and non-responders (FDR corrected p-value <0.05). Concurrently, a supervised univariate and multivariate classification technique was used to identify 11 genes in a training set of 10 responsive/ non-responsive xenograft models. The gene set was used to prospectively determine the outcome on 9 more xenograft models for which the response to panitumumab was previously unknown.

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