between antitumor effects (T/C%) and biomarker measurements made on tumor samples collected prior to treatment. Although biomarker data is still accumulating, significant correlations have been detected for monotherapies, but not with the cocktail due to its more consistent antitumor effects. Among these correlations is reduced efficacy of DC101 in xenograft models with higher human VEGF expression evaluated by ELISA ($r^2 = 0.46$, $p = 0.03$). Related to this, cetuximab significantly reduced tumor HIF-1 activity and VEGF concentration. Moreover when given in combination with DC101, cetuximab prevented the increase in tumor HIF-1 activity and VEGF production induced by DC101 monotherapy in multiple xenograft models. Cetuximab, therefore, prevented HIF-1 activity and VEGF production from overcoming or weakening the effects of VEGFR2 targeted therapy. Thus in the preclinical models tested, inhibition of pathways including HIF-1, results in antitumor effects of combination targeted therapy that are more consistent than monotherapy effects. This point is further illustrated in an orthotopic HT-29 colon cancer model using in vivo imaging, where DC101 monotherapy only inhibited primary tumor growth, cetuximab monotherapy only inhibited lymph node metastasis, and the cocktail treatment inhibited both. In conclusion, combination targeted inhibition of EGFR, VEGFR2, and IGF-IR, and in particular EGFR and VEGFR2, results in greater and more consistent tumor growth inhibition than monotherapies in preclinical cancer models, demonstrating the potential of this strategy in multiple cancer indications.

208 POSTER

Pharmacokinetic (PK), pharmacodynamic (PD) modeling and simulation analysis of PRO132365, a HER2 antibody-drug conjugate

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Objectives: PRO132365 is an antibody-drug conjugate targeting the HER2/neu receptor. Modeling and simulation approaches were used to integrate mouse xenograft model exposure-anti-tumor activity relationships and cynomolgus monkey pharmacokinetics to determine an efficacious dosing regimen for PRO132365 in the clinic.
Methods: Doses identified to provide maximum anti-tumor activity using a Q3W dosing schedule in an athymic mouse xenograft model were subsequently fractionated and tested using Q1W and Q2W regimens. A population tumor-kill PK/PD model was developed from the composite individual animal data using NONMEM. A transit compartment model with a non-linear tumor-cell kill function, which is dependent on PRO132365 serum concentrations, was implemented to describe the PK/PD relationship. A two-compartment model from separate PK studies was used as a forcing function for modeling these tumor-volume data. PRO132365 exposure-anti-tumor clinical projections were enabled by first predicting human PRO132365 PK disposition from monkey data (PRO132365 has been shown to bind the human and cynomolgus monkey HER2 receptor, but does not cross-react with the corresponding rodent receptor neu), and subsequently utilizing the predicted human PK to simulate the predicted PRO132365 anti-tumor activity derived from the tumor-kill model. The optimal regimen was determined as the dose and dose regimen providing a probability of achieving a target treatment outcome defined as a $\geqslant$30% reduction in tumor volume from baseline in the majority of simulated subjects.
Results: Classification and regression tree analysis (CART) demonstrates that the probability of predicting successful treatment outcome is greatly increased by achieving an exposure/minimum tumoricidal concentration (AUC/MTC) ratio $\geqslant$40 [days mg/L]/1 [mg/L] in an individual subject. This AUC/MTC ratio is predicted to be achieved in the majority of subjects with a dose schedule of $\geqslant$10 mg/kg PRO132365 dosed once every 3 weeks.
Conclusions: For the antibody-drug conjugate PRO132365, population modeling and simulation methodologies were employed to achieve the integration of preclinical PK and efficacy data with desired clinical outcome. This allowed the estimation of an optimal clinical dose and dose regimen that was useful in guiding decision-making as this novel therapeutic enters clinical trials for the treatment of HER2/neu positive breast cancer.

209 POSTER

Cancer therapy with antibodies conjugated to radionuclides emitting low-energy electrons

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Background: To kill a cell with radioactive decays on the cell surface or in the cytoplasm, the optimal electron energy is 20–25 keV. In contrast, the beta-particles generally used for radioimmunotherapy have tissue path lengths at least 50 times longer than a cell diameter. These studies were intended to evaluate the potential of radionuclides emitting low-energy electrons (LEEs) for single-cell kill in vitro and for tumor therapy in vivo. LEEs include both Auger and conversion electrons, which are emitted by most photon-emitters and by other radionuclides.
Materials and Methods: Antibodies (Abs) were conjugated to 111-In using the chelator benzyl-DTPA, to a specific activity of 40–80 mCi/mg. The Abs tested included Abs to CD20, CD74 and HLA-DR, for B-lymphoma target cells; and Abs to EGFr and HER-2, for carcinomas. In vitro, cells were incubated with the Abs for 2 days in vitro, then evaluated in clonogenic assays. Immunodeficient mice, both nude and scid, bearing human tumor xenografts were treated with radiolabeled Abs, injected i.v., at various times after tumor inoculation. Non-reactive control Abs labeled in the same way were tested similarly.
Results: Tumor cells were killed effectively and specifically with these Ab conjugates. Essentially 100% kill could be obtained (>5 logs). The radiation dose delivered to the nucleus was estimated from subcellular S values (for decays occurring on the cell surface or in the cytoplasm), and was consistent with the level of toxicity observed. In vivo, therapy of microscopic tumors was effective, with many cures, but effective therapy of macroscopic tumors has not yet been achieved.
Conclusions: For high-density antigens, which allow the delivery of large amounts of radioactivity per cell, these conjugates are potent and specific toxic agents. They are effective from the cell surface or the endosomal/lysosomal compartment, and do not require delivery to the cytosol or nucleus, as do drug- or toxin-Ab conjugates. Although treating macroscopic tumors is more difficult, this approach was effective in therapy of micrometastases, and thus is applicable to patients with minimal residual disease.

210 POSTER

Development of drug-conjugated monoclonal antibodies against MUC16 for the treatment of epithelial ovarian cancers

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The MUC16 glycoprotein is highly expressed on the surface of epithelial ovarian cancer cells, particularly of the serous subtype, and the shed extracellular sequence (CA125) is widely used as a marker for disease progression and response to therapy. We have generated antibodies against the extracellular mucin repeats of MUC16, such that each copy of the protein is bound simultaneously by multiple antibodies. As expected, these antibodies generate larger flow cytometry shifts on human ovarian cancer cell lines as compared with antibodies that recognize unique sites on MUC16. The repeat-binding antibodies are specific for MUC16 and do not bind to cells or tissues that lack MUC16 expression. One such antibody (Ab1) was conjugated to cytotoxic small molecules of the auristatin class using stable and labile linkers. The antibody-drug conjugates are potent anti-proliferative agents in vitro (IC50 < 10 ng/mL) and in vivo against human ovarian cancer models. For example, a single dose of one such conjugate at 6 mg/kg was sufficient to eliminate established OVCAR-3 mammary fat pad tumors in 8/10 mice. While in vitro activities were comparable among conjugates, in vivo studies revealed differences in efficacy and safety depending on the cytotoxin and linker. Importantly, efficacious doses of the conjugated antibodies do not elicit significant toxicity in rats or cynomolgous monkeys, including rats bearing xenograft tumors that express the target antigen and carry the MUC16 extracellular domain (CA125) in circulation. We believe that these drug-conjugated antibodies are promising therapeutics for ovarian cancer.

211 POSTER

Design of an anti MUC1 DNA aptamer as novel radiopharmaceutical for the diagnostic imaging and targeted radiotherapy of tumours

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Aptamers are novel oligonucleotide-based recognition molecules that can bind to almost any target, including extracellular receptor proteins, antibodies, peptides and small molecules. Aptamers can be rapidly generated and offer reduced immunogenicity, good tumour penetration, rapid uptake and clearance, which favour their application as effective vehicles for cytotoxic agents or radioisotopes. Thus, these molecules can be used as alternatives to monoclonal antibodies in molecular targeted radiotherapy and diagnostic imaging applications and overcome some of the problems associated with the latter.