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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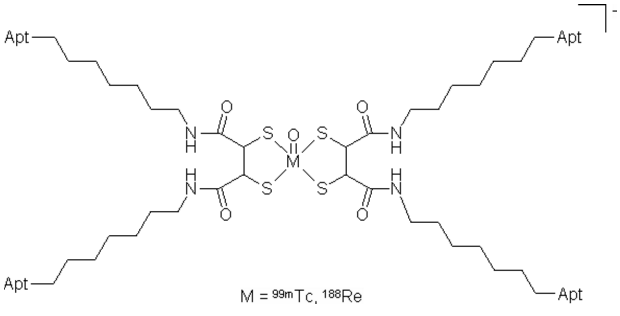
Aptamers are novel oligonucleotide-based recognition molecules that can bind to almost any target, including extracellular receptor proteins, antibodies, peptides and small molecules. Aptamers can be rapidly generated and offer reduced immunogenicity, good tumour penetration, rapid uptake and clearance, 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.

We have previously reported the generation of high affinity and specificity DNA aptamers against the protein core of the MUC1 glycoprotein as a tumour marker on epithelial cancer cells, with the aim to develop them into targeted radiopharmaceuticals.
We now report the coupling of the aptamer with the highest affinity for the MUC1 glycoprotein to *meso*-2,3-Dimercaptosuccinic acid (dmsa), a commercially available chelator. The aptamer was synthesised using solid phase synthesis and HPLC purified. The conjugation was achieved using standard peptide coupling reactions between an amino modification on the aptamer and the carboxylic groups of dmsa, after protection of its sulphur groups.
Aptamers have been coupled to dmsa to generate a multi-aptamer radiolabeled complex. It is possible to have an efficient and convenient labeling of the aptamer with short half-life radioisotopes (^{99m}Tc and ^{188}Re) as the last step of the synthesis (post-conjugation labeling) leading to the product shown in the figure, which has been subsequently tested for activity.



The $[MO(dmsa)_2]^-$ (M = ^{99m}Tc or ^{188}Re) core is proven to be highly stable *in vivo* and the presence of more than one molecules of aptamer enhances the binding properties of the radiolabeled bioconjugate to the target and modifies its pharmacokinetic properties.
Aptamers have shown great potential for tumour imaging and targeted radiotherapy in experimental models and are currently under development as novel targeted radiopharmaceuticals.

212 POSTER

A Phase I study of huC242-DM4 to assess the safety and pharmacokinetics of huC242-DM4 administered as a single intravenous infusion once every three weeks to subjects with solid tumors

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Background: huC242-DM4 is a novel, targeted anti-cancer agent for the treatment of CanAg-expressing tumors such as carcinomas of the colon and pancreas as well as other gastrointestinal tumors. This agent is formed by the conjugation of the potent cytotoxic maytansinoid drug, DM4, with the humanized monoclonal antibody, huC242, and is a structural analog of the previously evaluated antibody-drug conjugate, cantuzumab mertansine (huC242-DM1). Pre-clinical studies reveal that huC242-DM4 has about a two-fold increase in $t_{1/2}$ and has markedly increased activity in human tumor xenografts in immunodeficient mice compared with the previous huC242-DM1. These findings, coupled with the clinical activity observed with cantuzumab mertansine in phase I studies in patients, provide a compelling rationale for the current Phase I trial.
Methods: Subjects were enrolled with metastatic or inoperable colorectal, pancreatic, and other CanAg expressing tumors who have failed standard therapy.
Results: Twenty subjects have been treated with huC242-DM4, receiving a single intravenous (IV) infusion once every three weeks. Cohorts of 3 subjects initially were enrolled on each dose level. Subjects have received huC242-DM4 at 18, 36, 60, 90, 126, and 168 mg/m^2. Enrollment at the 168 mg/m^2 dose level is ongoing. At present, no dose limiting toxicity has been observed. A patient treated at 168 mg/m^2 had an asymptomatic grade 3 elevation in lipase which was not considered clinically significant. One patient had a drug related serious adverse event. The latter patient was treated at 168 mg/m^2 and experienced grade 2 diarrhea, grade 2 creatinine elevation associated with dehydration that improved with IV fluids. This cohort is being expanded to 6 patients. One patient treated at 126 mg/m^2 had a mild hypersensitivity reaction that improved with brief interruption of infusion, diphenhydramine and steroid administration, and subsequently tolerated restarting the infusion. At present, there has been no clinically significant myelosuppression and no evidence of formation of antibody to humanized antibody (HAHA) or of antibody formation to drug (HADA) as evaluated by ELISA methods. Current data suggest that the half-life of huC242-DM4 is longer when compared to huC242-DM1 as demonstrated in preclinical studies.
Conclusions: This phase I study provides evidence of safety of huC242-DM4 given on this schedule. The MTD is not yet defined and enrollment of patients is ongoing.

213 POSTER

Enhanced antitumour effect by combination of HER2-targeting antibodies with bevacizumab in a human breast cancer xenograft model

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Over-expression of HER2 correlates with poor prognosis in breast cancer. Trastuzumab, a recombinant humanized monoclonal antibody (mab) binding to the extra-cellular domain of HER2 has become standard of care in the treatment of HER2-positive breast cancer. Another HER2-targeting humanized mab, pertuzumab, specifically binds to an epitope different from the trastuzumab binding site and thereby inhibits homodimerisation of HER2 as well as its heterodimerisation with other HER-family members that are activated by their respective ligands. Bevacizumab is a mab binding to human VEGF. In the present study, we used the HER2-positive human breast cancer cell line KPL-4 in order to address the following questions: (i) Is it possible to enhance antitumour activity of HER2-targeting antibodies by modulation of vascular growth and development through concomitant administration of bevacizumab? (ii) Can progressive tumour growth during bevacizumab monotherapy be stopped by combination therapy? As the KPL-4 xenograft model forms metastases in lung and liver, we investigated the effect of the various treatment regimens not only by measuring primary tumour size, but also by quantification of human Alu-sequences in the DNA of explanted murine lung and liver tissue by PCR technology.
KPL-4 cells were injected orthotopically into the mammary fat pad of female SCID beige mice. Trastuzumab and pertuzumab were administered once weekly at 15 mg/kg i.p. following a 2-fold loading dose. Bevacizumab was injected i.p. twice weekly at a dosage of 5 mg/kg. Monotherapy with either of the two HER2-targeting antibodies delayed tumour growth by about one week compared to the control group. Treatment with bevacizumab alone delayed tumour growth by about three weeks. However, the combination of bevacizumab with either trastuzumab or pertuzumab produced tumour stasis over the whole treatment period of 11 weeks, with partial tumour regression in the bevacizumab plus trastuzumab combination group. Finally, we found that tumours progressing after bevacizumab monotherapy were actually shrinking as soon as trastuzumab was added to continued bevacizumab treatment. Quantification of human Alu-sequences in the DNA-extracts from organs indicated that the formation of lung and liver metastases was significantly suppressed by all of the antibody combination regimens, and to varying degrees by the respective monotherapies.
In conclusion, the addition of bevacizumab to HER2-targeting antibodies trastuzumab or pertuzumab can significantly enhance antitumour activity. Furthermore, tumour remission can be induced by trastuzumab after progression during prolonged bevacizumab monotherapy. This finding is surprising as the KPL-4 tumour xenograft model is not efficiently inhibited by trastuzumab alone.

214 POSTER

Characterization of a recombinant, fully human monoclonal antibody directed against the human insulin-like growth factor-1 receptor

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The Insulin-like Growth factor-1 Receptor (IGF-1R) regulates important cellular activities involving cellular proliferation, differentiation and apoptosis. In vitro and in vivo studies have shown that the IGF-1R pathway plays an important role in the development and progression of cancers including breast, prostate, lung and colon making it a potential target for therapeutic intervention. Recently, several approaches to inhibit IGF-1R signaling which interfere with the growth of tumor cells both in vitro and in vivo have been described including application of antisense nucleic acids, use of inhibitory IGF-binding proteins, neutralizing antibodies and low molecular weight (receptor kinase) inhibitors. By immunizing human antibody transgenic mice, we have generated a panel of fully human monoclonal antibodies (huMAb) (IgG1, κ) recognizing different epitopes on human IGF-1R. Detailed profiling of these antibodies revealed that they differ in their functional properties including inhibitory and stimulatory