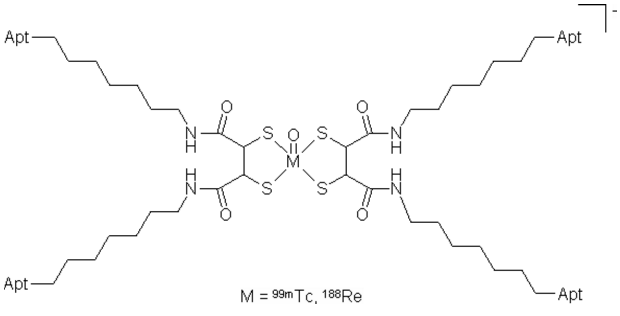
We have previously reported the generation of high affinity and specificity DNA aptamers against the protein core of the MUC1 glycoprotein as a tumour marker on epithelial cancer cells, with the aim to develop them into targeted radiopharmaceuticals.
We now report the coupling of the aptamer with the 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

activity. One of the lead antibodies termed R1507 potently inhibits IGF-1R signaling. R1507 binds with low nanomolar affinity to the human IGF-1R as measured by BIAcore. It does not show detectable binding to the closely related human Insulin Receptor (IR). In cell-based assays the huMab competes both with IGF-1 and IGF-2 for receptor binding and inhibits IGF-1R autophosphorylation and subsequent signal transduction. Furthermore, it inhibits ligand-induced proliferation of human tumor cells in vitro. Incubation with huMAb R1507 leads to a rapid downregulation of IGF-1R from the cell surface. In vivo testing of the antibody in a recombinant model (3T3 murine fibroblasts transfected with the huIGF-1R) and in several human xenograft models (including NCI-H322M and Colo205) demonstrated dose-dependent single agent activity against established tumors which was correlated with the downmodulation of IGF-1R in the tumor tissue. Taken together, the in vitro and in vivo data make R1507 a promising molecule for further evaluation in clinical trials and development of a novel immunotherapeutic approach for treatment of IGF-1R expressing cancers.

215 POSTER

HER2/Neu-Herceptin biomarker development for theranostic management of breast cancer patients

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Background: Approximately 25% of breast cancers over-express the HER2/Neu gene, measured by Immunohistochemistry (IHC) staining or FISH probe counts. A new antibody-based therapy (Herceptin™) is highly effective in these cases. Current methods of HER2/Neu evaluation are neither cost-effective nor highly accurate; therefore it is desirable to find more practical and effective predictive biomarkers.
Materials and Methods: We developed techniques for automated objective analysis of IHC-labeled tissue sections and microarrays utilizing laser scanning cytometry. Standardized CAP survey arrays stained for HER2/Neu were evaluated and compared with results of traditional methods. Excellent correlation between the automated results, the pathologist's evaluation and FISH probe spot counts was achieved.
Herceptin is humanized hybrid antibody containing human Fc fragment and mouse variable region. It would be reasonable to assume that the actual therapeutic Ab Herceptin may be a better primary antibody in theranostic IHC tests for Herceptin therapy patient selection. We developed a novel method of staining breast tissue with Herceptin, overcoming a major challenge of human Fc fragment IHC staining.
Results: Serial breast tumor TMA sections were stained for HER2/Neu and Herceptin. Most HER2/Neu-positive core elements also showed Herceptin expression. Conversely, 9% of tissue core elements were labeled with polyclonal anti-HER2/Neu but not with Herceptin. The discordance suggests that binding specificity of Herceptin differs from that of the xeno-antibodies. In 3% of cases, tissue was reactive to Herceptin but not to polyclonal anti-HER2/Neu. The latter result could not be explained by existing knowledge of polyclonal and monoclonal antibodies specificity.
We performed double staining of IHC tissue sections and TMAs (HER2/Neu and Herceptin (DAB and BCIP/NBT)) to clarify this phenomenon. Some tissues exhibit mosaic staining patterns, with various cells positive for both markers and neighboring cells positive for only one marker. Automated analysis methods allowed objective evaluation of the degree of mosaicism in the tumor tissues. Demonstrated mosaic staining of tumor tissues may identify additional candidate patients for use of anti-HER2/Neu therapeutic antibodies different from Herceptin-target peptide areas. Current studies are underway to investigate mosaicism in tissues from patients undergoing Herceptin therapy.
Conclusions: We demonstrated that Herceptin can effectively replace xeno-antibodies for IHC-based patient selection for breast cancer therapy. Our data suggests that Herceptin binds to a different epitope than traditional HER2/Neu Abs, perhaps resulting in a more relevant specificity. Automated laser scanning cytometry analysis was proven to be an invaluable tool in objective tissue characterization.

216 POSTER

The cytotoxicity of antibody-drug conjugates to bystander cells

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One approach to limit toxic effects of a chemotherapeutic drug to tumor tissues is to target the drug to the tumor with the help of a tumor-selective monoclonal antibody. Several antibody-drug conjugates have been developed that specifically target and kill antigen-positive cells. However, solid tumors often contain both antigen-positive and antigen-negative cells. Therapeutic agents that kill not only antigen-presenting cancer cells, but also the adjacent antigen-negative cancer cells, may be more effective in eradicating such tumors. We have previously reported that a conjugate of the microtubule polymerization inhibitor, maytansine, attached via a disulfide linker to the anti-CanAg antibody, huC242, is cytotoxic to both the target cells and proximally located antigen-negative cells in culture and in mouse xenograft models (1). The maytansinoid species produced by the target cells following their exposure to the conjugate were also identified (2). We performed similar studies with antibody-drug conjugates containing various cytotoxic drugs, including several new maytansinoids, taxol analogues and analogues of the DNA alkylator, CC1065, and found that these conjugates can generate the bystander effect. The disulfide linkage between the antibody and the drug was a prerequisite for the bystander cytotoxicity of the antibody-drug conjugates. To further investigate the role of the linker cleavage in the bystander cytotoxicity, a series of huC242-maytansinoid conjugates with various disulfide-containing linkers were constructed. These linkers differed in the degree of hindrance around the disulfide bond, and in the rate of cleavage by cytoplasmic thiols within the target cells. The bystander potencies of these conjugates correlated with the cleavage rates of their disulfide linkers; faster disulfide bond cleavage resulted in stronger bystander cytotoxicity. Analysis of the metabolism of these conjugates in CanAg-positive target cells revealed that the nature of the linker affected the composition of metabolites. The accumulation of a stable metabolite, *S*-methyl-maytansinoid, correlated with the bystander potency of a conjugate. This metabolite is a hydrophobic molecule that can diffuse out of the antigen-positive target cells and kill proximally located dividing cells. This study defines requirements for effective antibody-drug conjugates.

References

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217 POSTER

Circulating levels of ligand as a potential biomarker for optimal dosing of targeted antibody drugs to the epidermal growth factor receptor

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Background: A lack of biomarkers that are predictive of the optimal biologic dose (OBD) is a major hurdle for the successful clinical translation of many targeted therapies. We have recently reported such a marker for a targeted anti-angiogenic drug called DC101 – a monoclonal antibody which blocks the mouse endothelial cell receptor tyrosine kinase for vascular endothelial growth factor (VEGF), known as VEGFR-2 (flk-1 in mice, KDR in humans; *Bocci et al Cancer Research 2004; 64:6616–6625*). Administration of DC101 to both normal and tumor-bearing mice leads to a rapid and remarkable increase in the plasma levels of circulating VEGF, which parallels anti-tumor activity. In contrast, small molecule VEGFR-2 antagonists did not cause a similar effect in normal mice. This discovery has lead to the examination of antibodies against human EGFR to determine whether this surrogate marker paradigm may extend to this biological system.
Materials and Methods: Human tumor cell lines that co-express EGFR and one or more of its ligands were grown in culture in the presence of Cetuximab (Erbitux), Nimotuzumab (TheraCIM/h-R3), Erlotinib (Tarceva), or Gefitinib (Iressa). TGFa and EGF were subsequently measured in conditioned media using ELISA. Similar experiments were conducted *in vivo* utilizing the HT29 cell line injected into the peritoneum of nude mice and treated with Cetuximab.
Results: *In vitro* experiments utilizing numerous human cancer cell lines showed a rapid elevation in human TGFa in the conditioned media (within 15 minutes) after treatment with antibody drugs that target the external ligand-binding domain of EGFR, but not when small molecule inhibitors were used. The elevation showed a dose-response effect and plateau at higher drug concentrations. Factors that appear to influence the nature of this result include antibody affinity, EGFR expression level, and endogenous ligand production. Human TGFa/EGF elevations were also demonstrated in the ascites fluid of mice injected with HT29 after a single Cetuximab injection.
Conclusions: These results suggest that the ligand elevations observed with DC101 and mouse VEGF extend to antibodies aimed against human EGFR, and should be explored as potential biomarkers to aid