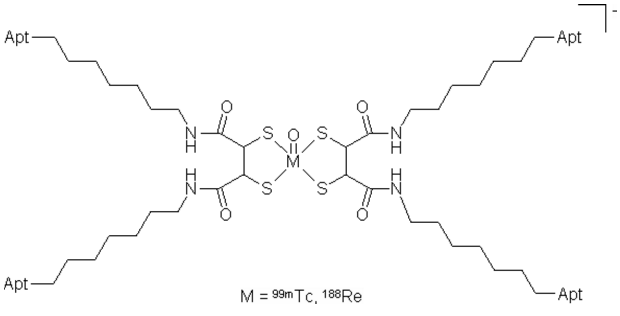
We have previously reported the generation of high affinity and specificity DNA aptamers against the protein core of the MUC1 glycoprotein as a tumour marker on epithelial cancer cells, with the aim to develop them into targeted radiopharmaceuticals.
We now report the coupling of the aptamer with the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

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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