Aptamers have shown great potential as novel targeted radiopharmaceutical entities for the diagnosis and imaging of various diseases. They offer reduced immunogenicity, good tumour penetration, rapid uptake and clearance compared to their monoclonal antibody counterparts. In previous work, we have reported on the modification of aptamers against breast cancer related biomarkers and their labelling with radionuclide ligands.

We have conjugated previously selected aptamers against the protein core or the tumour glycosylated MUC1 glycoprotein to different ligands (MAG2 or meso-2,3-Dimercaptosuccinic acid) and labelled them with 99mTc, for the diagnostic imaging of breast cancer. The conjugation is achieved using standard peptide coupling reactions between an amino modification on the aptamer and the carboxylic group on the ligands. An efficient and convenient labelling of the aptamer with short half-life radioisotopes was achieved as the last step of the synthesis (post-conjugation labelling). Labelling with 99mTc has taken place using tin chloride as reducing agent and analysed by HPLC, were both the UV and the gamma emission was monitored. The labelled aptamers were separated from free 99mTc using ultrafiltration and monitored by HPLC at all stages, before being injected and imaged for their pharmacokinetic properties. For the analysis of the pharmacokinetic properties of the aptamer-radionucleotide conjugate, we have used gamma-camera imaging in MCF-7 breast cancer tumour model systems.

The aptamer-chelator conjugates have strong 99mTc binding properties and the resulting complexes are highly stable in vivo, both in terms of nuclease degradation and leaching of the metal. We studied the effect of more than one molecules of aptamer per complex on the binding and pharmacokinetic properties of the radiolabeled products. We checked if different ligands affect the accumulation of the aptamer in different organs, as they alter the lipophilic properties of the conjugate.

These results aim to open new possibilities for the diagnostic imaging and potentially the targeted radiotherapy of breast cancer.

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Gene alteration profile and correlation to sensitivity to small molecule inhibitors in lung cancer cell lines

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The success of targeted therapies in the treatment of cancer relies on the identification of both the key components altered and the markers that can predict response to the treatment.

Here we present a detailed gene alteration profile of a panel of lung cancer cell lines and evaluate the correlation of the genotype with the sensitivity to selected small molecule inhibitors.

The genetic status of several cancer genes was analyzed by direct sequencing and/or real time quantitative PCR in a panel of 13 lung cancer cell lines. Then the effect on kinase activity and cell viability of several drugs was tested.

The genes analyzed were those most commonly altered in lung cancer, namely TP53, P16, KRAS, RB, CMET, EGFR, ERBB2, LKB1, MYC, PTEN, NRAS and PIK3CA. Next, the sensitivity of the cells to treatment with inhibitors of PI3K (LY294002), mTOR (Rapamycin), C-MET (PHA665752) or EGFR (erlotinib) was tested. We used levels of phosphorylation at downstream targets as surrogate markers of the inhibitory ability of the drug in a given cell line and calculated IC50 from dose-response curves. In most cases, an increased sensitivity to a given compound was correlated to a higher ability to reduce the levels of phosphorylation at their downstream targets. Sensitivity to EGFR (erlotinib), PI3K (LY294002) and CMET (PHA665752) inhibitors was evidenced in cells carrying single alterations at ERBB2, PTEN and CMET, respectively as compared to cells carrying simultaneous activation of these pathways.

In summary, we showed the effectiveness of drugs specifically designed against molecules genetically altered in tumour cell lines.

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Expression profiling reveals different targeted genes of the oral platinum(IV) prodrug oxoplatin and its putative activation product cisplatin

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Oxoplatin (cis,trans,cis-diammine-dihydroxo-dichloro-platinum(IV)), presently under investigation as an oral antitumor agent, was screened in vitro using MTT proliferation assays in a panel of 38 diverse human tumor cell lines. With exception of renal cell and ovarian cancer, cell lines sensitive to oxoplatin were detected for all other tumor entities. Using flow cytometry, the drug was shown to induce cell cycle arrest (propidium iodide staining), reactive oxygen species (ROS; dihydroethidium fluorescence) and cell death (annexinV / propidium iodide staining). Generally, the mean IC50 value for oxoplatin (sensitive cell lines: 7.6 ± 5.8 µg/ml; n = 27) was ~ 2.5-fold higher than for cisplatin. However, the small cell lung cancer (SCLC) cell line H526 showed similar sensitivity to oxoplatin and cisplatin and was used for genome wide expression profiling (Applied Biosystems Human Genome Survey Microarray V2.0) following application of the two compounds. Only 35 % of the downregulated and 10 % of the upregulated genes were identical in response to cisplatin or oxoplatin, respectively. Diametrically regulated genes included ribosomal proteins, DEAD box polypeptide 1, tubulins, pyruvate kinase, stathmin and high mobility group nucleosomal BD2 (HMGN2), indicating that oxoplatin is not a simple prodrug of cisplatin and that these two platinum compounds comprise distinct mechanisms of action. Since untreated H526 cells exhibited marked acidification of the medium to pH = 6.5 rapidly, effects of acids on oxoplatin were investigated. While addition of 50 – 800 µg/ml lactate had no effect on the cytotoxic activity of oxoplatin against COLO 205 cells, pretreatment of the compound with 5 mM ascorbic acid yielded enhanced activity (~ 2.5-fold), and thiols activated oxoplatin to a minor extent (< 10%). Following a 15 min exposure of oxoplatin to 0.1 M HCl representing gastric acid, the resulting platinum species cis-diammine-tetrachloro-platinum(IV) revealed a more than twofold increase in cytotoxicity against various cell lines. In conclusion, oxoplatin constitutes an effective oral anticancer agent that can be converted from its relatively inert native form into more active platinum intermediates different from cisplatin by acidic conditions in the stomach or tumor regions, depending on its pharmaceutical formulation. Platinum(IV) compounds are expected to have a higher probability than platinum(II) drugs to reach the malignant tissue intact and to be activated eventually at the intended target site.

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Increasing doxorubicin antitumor activity through COX-2 inhibition without increasing cardiotoxicity

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The clinical use of doxorubicin (DXR) and other anticancer anthracyclines is limited by a severe cardiotoxicity upon chronic administration. DXR induces cardiotoxicity by acting unchanged or after reductive metabolisation to toxic species. We recently discovered that anthracyclines can also undergo an oxidative degradation to non-toxic compounds. In the heart, anthracycline degradation is mediated by myoglobin and diminishes cardiotoxicity. In tumors, anthracycline degradation should be mediated by peroxidases with a consequent loss of antitumor activity. We analyzed the effect of COX-2 as an important catalyst of DXR oxidative degradation in tumors and we investigated if by impairing COX-2 activity it is possible to increase DXR activity in the tumor without increasing its toxicity in the heart.

COX-2 over-expressing (MDA-MB-231, SkOv3) and low-expressing human cancer cell lines (MDA-MB-468, T47D) were treated with DXR in the absence or presence of celecoxib, a specific COX-2 inhibitor. In both types of COX-2 over-expressing cells DXR and celecoxib had greater than addictive effects, whereas in COX-2 low-expressing cells celecoxib did not synergize with DXR. We next sought to establish whether celecoxib synergized with DXR through suppressing its degradation by the peroxidase component of COX-2. To this end we measured the cellular levels of DXR, its reduced metabolites, and the products of partial or complete DXR degradation. Celecoxib increased the levels of DXR and its reduced active metabolites in SkOv3 cells, but not in MDA-MB-468 cells, as one would expect if celecoxib inhibited DXR degradation that was specifically mediated by COX-2. Conversely, but consistently, DXR oxidative degradation products were detected only in SkOv3, and their levels decreased upon co-treatment with celecoxib. The effect of COX-2 was quite specific to DXR as Celecoxib did not synergize with non-anthracycline test compounds. Studies with isolated cardiomyocytes also showed that celecoxib had marginal or no effects on DXR toxicity; this latter finding was in keeping with the notion that in cardiomyocytes DXR would be degraded primarily by myoglobin.

Collectively, these experiments show that celecoxib increased the antitumor activity of DXR, but not its cardiotoxicity, by blocking its COX-2 dependent degradation in tumors. Improving the therapeutic index of DXR through the co-administration of coxibs is an hypothesis worth of testing in preclinical translation models.