chloride as reducing agent. Radiolabelled conjugates were separated from free ^{99m}Tc using microcon filters. Pharmacokinetic and biodistribution studies were performed in immunocompromised mice bearing MCF-7 breast tumours. The difference between pegylated and non-pegylated aptamers was assessed in Wistar rats.

Results: The PEGylation of the aptamer using various PEGs was achieved in high yield without a significant effect on the aptamer binding to MUC1, for PEGs up to 40KDa. The aptamer conjugated to PEG and MAG2 demonstrated interesting pharmacokinetic and biodistribution properties. The PEGylated aptamers had improved circulation time, making them more suitable for therapeutic applications, whilst they maintained their ability to bind to MUC1. Furthermore, the presence of the PEG minimised the effect of chelators on the pharmacokinetic properties and biodistribution of the aptamer.

Conclusions: PEGylated MUC-1 aptamers of varying molecular weights have been produced successfully, maintaining their ability to bind to their target *in vitro* and *in vivo*. The bi-functionalised aptamer, conjugated on the amino modified 5′ end to MAG2, a strong ligand for ^{99m}Tc, and on the thiol modified 3′ end to different PEGs has been found to have improved pharmacokinetic properties and the ability to localise in the tumour and remain in the system longer, when compared to non-PEGylated aptamers, and counteract some of the effect the chelators have on the *in vivo* aptamer properties. These results can lead to the development of aptamers as a novel targeted radiotherapy for breast cancer.

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228 Renal cancer histone acetylation and protein ubiquitination enhanced synergistically by bortezomib and suberoylanilide hydroxamic acid

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Background: There is no curative systemic therapy for advanced renal cancer. Inhibiting histone deacetylase (HDAC) and proteasome activity acts against malignacies cooperatively, but this treatment strategy has not been tested against renal cancer. In the present study we combined the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) with the protease inhibitor bortezomib and found that they inhibited renal cancer growth by enhancing histone acetylation and protein ubiquitination synergistically.

Material and Methods: The viability and clonogenicity of renal cancer cells (769-P, A498, ACHN, Caki-1) treated with SAHA (1−5 μM) and/or bortezomib (5–100 nM) were assessed by MTS assay and colony formation assay, and the in vivo efficacy of the combination was evaluated using a murine subcutaneous tumour model. The expression of acetylated histone, phosphorylated retinoblastoma protein (Rb), cyclin D1, cyclin-dependent kinase (CDK) 4 and HDACs 1, 2, 3, and 6 was assessed using western blot analysis. Apoptosis was assayed using flow cytometry and detecting active caspase 3 and cleaved poly (ADP-ribose) polymerase (PARP). Protein ubiquitination was evaluated by western blot analysis.

Results: The combination of SAHA and bortezomib induced apoptosis and inhibited cancer cell proliferation and colony formation synergistically. In murine subcutaneous tumour models using Caki-1 cells, SAHA and bortezomib in combination inhibited tumour cell growth significantly more than did each agent alone. SAHA alone induced histone acetylation and Rb dephosphorylation, and bortezomib enhanced this acetylation and dephosphorylation by inhibiting the expression of HDACs 3 and 6, cyclin D1 and CDK4. Bortezomib alone increased protein ubiquitination in a dose-dependent fashion, and SAHA enhanced this protein ubiquitination.

Conclusions: SAHA in combination with bortezomib inhibited the proliferation of renal cancer cells in vitro and in vivo, and the effectiveness of the combination is due to their enhancing histone acetylation and protein ubiquitination synergistically.

229 Effects of CYP2C19 genotype on tamoxifen and estrogen metabolism

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Background: Cytochrome P450 2C19 (CYP2C19) is an important drug-metabolizing enzyme with variability determined by genetic polymorphism. The CYP2C19*2 and *3 variant alleles result in reduced enzymatic activity. In contrast, CYP2C19*17 is associated with ultrarapid CYP2C19 activity and identifies patients likely to benefit from tamoxifen treatment [4] and may lead to decreased estrogen levels and therefore reduces breast cancer risk [3]. Since tamoxifen and estrogens are also partly metabolized by these enzymes, these enzymes may influence tamoxifen and estrogen metabolism which may in turn affect treatment outcome. In the present study we examined the influence of CYP2C19 genotype on serum concentrations of estrogens as well as the levels of tamoxifen and its metabolites.

Methods: Tamoxifen and its metabolites were measured by using liquid chromatography-tandem mass spectrometry in samples from 90 post-menopausal breast cancer patients during steady state tamoxifen treatment [2]. Estrogen levels were determined using a sensitive radioimmunoassay [1].

Results: We observed negative associations between increased CYP2C19-predicted enzymatic activity and the serum concentrations of tamoxifen metabolites and estrogens. Lower serum levels of tamoxifen metabolites N-dedimethyltamoxifen (NDDtam) and tamoxifen-N-oxide (tamNox) ($p = 0.043$ and $p = 0.031$, respectively) and estrogens estrone (E1) and estradiol (E2) ($p = 0.008$ and $p = 0.045$, respectively) were observed in subjects carrying CYP2C19*17 allele when compared with patients being heterozygous or homozygous for the CYP2C19*2 and *3 alleles.

Subjects carrying CYP2C19*17 allele encoding for ultrarapid enzyme activity showed a significant higher 4-hydroxytamoxifen (4OHtam) to tamoxifen ratio ($p = 0.008$) and lower tamNox to tamoxifen ($p = 0.03$) and NDDtam to N-demethyltamoxifen (NDtam) ($p = 0.027$) compared with subjects heterozygous and homozygous for the variant allele CYP2C19*2 and wild type allele. No difference was observed between CYP2C19 genotypes and metabolic ratio of 4-hydroxy-N-demethyltaoxifen (4OHNDtam) to NDtam.

Conclusion: The observed benefit of tamoxifen treatment in patients carrying CYPC19*17 can be explained by increased hydroxylation of the potent hydroxylated metabolite 4OHtam and reduced circulating estrogen levels.

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230 Glutathione modulation reverses the growth-promoting effect of growth factors, improving the SN-38 antitumour response in WiDr colon cancer cells

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Background: Several growth factors (GF) are involved in the paracrine growth mechanisms of colon cancer cells and have therefore been identified for their potential to modulate the sensitivity of tumour cells to chemotherapeutic agents, such as SN-38. Since glutathione (GSH) plays an important role in the growth-promoting effect of GFs and it is also involved in the protection against cellular injury caused by various anticancer agents, modulating cellular susceptibility to chemotherapy, manipulation of GSH levels might yield a therapeutic gain for chemotherapy in the presence of GFs.

Materials and Methods: The effect of GSH modulation on SN-38 activity on the WiDr colon cancer cell line was studied. Cell proliferation and GSH content were assessed. Cells were exposed to the GSH modulators, L-buthionine-SR-sulfoximine (BSO) or L-2-oxothiazolidine-4-carboxylate (OTZ), before treatment with SN-38 in the presence of hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) or epidermal growth factor (EGF).

Results: Exposure to GFs significantly increased GSH levels and induced a pro-tumour effect, producing a near 20% reduction in SN-38 antitumour activity after 48 h of incubation. Treatment with OTZ and BSO abrogated the growth-promoting effects of GFs. Moreover, the addition of OTZ to SN-38 resulted in a synergistic effect at 24 h and produced a nearly 70% increase in the cytotoxic activity of SN-38 in the presence of GF at 72 h. Similarly, the combination of BSO and SN-38 produced a significantly greater antitumour effect than SN-38 alone, leading to an approximately 50% increase in the cytotoxic activity of SN-38 in the presence of GF at 72 h.