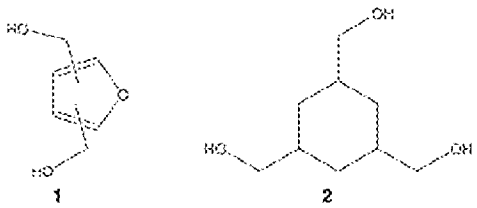

1 2

biotin. Reaction with avidin-FITC revealed discrete staining patterns on epithelial tumour cell lines, while normal human fibroblasts showed no staining. The influence of the synthetic carbohydrates upon the adhesion of B16F1 and B16F10 mouse melanoma cells to different extracellular matrix proteins (ECM) was then investigated. The adhesion of the highly metastatic B16F10 cells to fibronectin, vitronectin or laminin was inhibited by the galactosides, while the low metastatic B16F1 cells showed only marginal sensitivity. The greatest effects were 80% inhibition by 1,3 (Gal)2-TMH on fibronectin and 70% by 3,4 (Gal)2-furan on laminin at 40 mM each. Lower concentrations enhanced binding of both cell lines to ECM. Zymography of serum free conditioned medium showed reduced expression and activation of matrix metalloproteases in B16F10 cells incubated with 3,4 (Gal)2-furan. The consequence of this is apparent in a reduced migration of these cells through matrigel. These synthetic di- and triantennary carbohydrate mimics are therefore versatile tools which can be modified to influence processes of adhesion and migration and in addition can be derivatised with fluorescent labels for diagnostic purposes.

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## Endothelin a receptor blockade with atrasentan does not change PSA secretion in prostate cancer cell lines

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In the United States, it is estimated prostate cancer will account for 30% (189,000) of new cancer cases in men, with 30,200 deaths from the disease this year. Worldwide, it is estimated 204,000 men die each year of prostate cancer. Endothelin-1 (ET-1) has been identified as a potentially important factor in the pathophysiology of advanced prostate cancer. The effects of ET-1 appear to be mediated primarily through the endothelin-A (ETA) receptor, as the ETB receptor gene, EDNRB, is frequently hypermethylated. Atrasentan (ABT-627), developed by Abbott Laboratories, is a potent, ETA-selective receptor antagonist. In phase II clinical trials of men with advanced, hormone-refractory disease, atrasentan treatment significantly delayed time to disease progression in evaluable men, and delayed time to prostate specific antigen (PSA) progression. In some men, there was a drop in PSA following exposure to atrasentan. It is our hypothesis that the decline in PSA is evidence of anti-tumoral activity rather than a direct effect of atrasentan on PSA secretion by prostate cancer cells. To test this hypothesis *in vitro*, two human, PSA-secreting, androgen sensitive prostate cancer cell lines, LAPC-4 and LNCaP, were studied. In LNCaP cells, ETA receptor expression is very low, and the ETB receptor gene is hypermethylated. In LAPC-4 cells, saturation by 125[I]-ET-1 demonstrates high affinity binding (Kd 1.3 nM, 1200 binding sites/cell). LAPC-4 also has hypermethylation of EDNRB. Both LNCaP and LAPC-4 cells were exposed to atrasentan, or an ETB receptor antagonist (A192621) across a wide range of concentrations (10-6-10-10 M) for 24 hours. Neither atrasentan nor an ETB antagonist changed PSA concentrations in the media of LNCaP or LAPC-4, while addition of dihydrotestosterone, a positive control, produced a significant increase in PSA. These data suggest that changes in serum PSA in men treated with atrasentan are not due to a direct effect of that agent on the secretion of PSA from prostate cancer cells, but may result from an antitumoral activity.

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## Image guided proteomics for molecular target discovery: a human giloma study

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Gliomas are the most common type of brain cancer and glioblastoma multiforme (GBM) is the most malignant form. Classic MRI appearance of GBM reflects the tumor's heterogeneous pattern of hypercellularity, necrosis, neovascularity, and invasion. Image guided proteomics may help elucidate mechanisms responsible for the imaging patterns and pathologic variability seen in GBM and offers a novel technique for discerning new molecular targets. In this study, image-guided protein expression profiling was performed on human GBM specimens. Samples were taken from MRI Gd-enhanced (CE) and non-enhanced (NE) regions of each tumor during surgical resection. Proteins were isolated from the fresh-frozen tissue samples followed by proteomic mass spectral analysis. Protein expression profiles of enhanced regions differ from non-enhanced regions. The NE regions across the four tumors and from different NE regions within the same tumor are markedly homogeneous. In contrast, no common protein profile can be determined from the CE regions. Protein expression profiles were further characterized by 2D-gel electrophoresis for mass spectrometric protein identification. This first use of MRI-guided proteomics in human tumors identified a protein fingerprint that correlates with spatial contrast enhancement patterns in solid tumors. Specific proteins identified by this method to account for the different patterns may prove useful as diagnostic markers and/or therapeutic targets.

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## The cyclin-dependent kinase inhibitor cyc202 is effective in human leiomyosarcoma (LMS) cell lines in combination with doxorubicin

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The cyclin-dependent kinase (CDK) inhibitor CYC202 (R-roscovitine) is believed to exert its anticancer effects by inhibition of CDK types 1 and 2 and by inhibition of phosphorylation of pRb (retinoblastoma protein), thus preventing cell cycle progression. For this study 2 human LMS cell line models have been used: SK-UT-1 and SK-LMS-1. Both cell lines are mutant for p53, SK-UT-1 is pRb deficient, whilst SK-LMS-1 is pRb wild-type. Flow cytometric analysis for DNA cell cycle measurements using propidium iodide showed that when both cell lines were treated with DOX for 24h a G2M cell cycle arrest was induced. Treatment with CYC202 (20uM) in combination with DOX showed complete abrogation of the G2M arrest at 24h, with CYC202 treatment alone showing a G1 arrest for both cell lines. When SK-LMS-1 cells were treated with CYC202 a decrease in pRb phosphorylation was apparent, an effect that was further enhanced when CYC202 was combined with DOX, seen at 48h. For SK-UT-1 cells no p53 induction was seen following treatment with CYC202, DOX or the drugs in combination at 48h. SK-LMS-1 cells showed induction of p53 following treatment with DOX and in combination with CYC202 in the same experiments. In both cell lines there was no induction of either p21waf-1 or $p27^{kip-1}$ following treatment with DOX, CYC202 singly or in combination. The SK-UT-1 cell line shows relatively high levels of cyclins D and E which, following treatment with CYC202 remained unaltered after 6h. Cytotoxicity testing (MTT assay) showed an overall additive effect between DOX and CYC202 (used simultaneously) in SK-LMS-1 cells, but there was a suggestion of some synergy seen for the SK-UT-1 cells. CYC202 is effective in tumours showing a variety of molecular abnormalities such as human LMS which are very chemorefractory in nature. It may also be a useful agent when used in combination with chemotherapy.

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## Cytochrome P450 1B1 (CYP1B1) is expressed in human colon adenocarcinomas, but its expression is not limited to the malignant epithelial cells

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Cytochrome P450 monooxygenases (CYPs) are the protein products of a superfamily of genes. CYPs have great diversity of expression and activity. They are involved in activation and detoxification of endogenous and exogenous compounds. CYP1B1 has been reported as present in humans only in tumours, being absent from the corresponding normal tissues; thus, CYP1B1 may represent a target for selective anticancer drug development. This study aimed to validate CYP1B1 as a target by looking at its expression in human colon cancer biopsy material. Immunohistochemical methods were used to investigate the expression pattern of CYP1B1 in human colon adenocarcinomas, in order to determine its potential role in drug metabolism. Sections (5um) of formalin-fixed and paraffin-embedded malignant human colon adenocarcinomas from fifty patients were used. These originated from tumours representing a range of stages, grades, and pa-

tient gender and age. Consent to use these specimens in the current study was obtained from the relevant Local Research Ethics Committee. The sections were dewaxed and rehydrated, then probed for CYP1B1 using a commercially available antibody. Bound antibody was detected using a peroxidase/diaminobenzidine (DAB) visualising system. Antibody validity was confirmed by western blots using the same antibody, and by limited replication of the immunohistochemistry using a privately supplied antibody. Malignant epithelial cells were positive for CYP1B1 in over 75% of cases, as was normal colon epithelium. However, results showed that the staining was not confined to the malignant epithelium within tumours. The tumour-associated smooth muscle and blood vessel pericytes were positive in all our specimens. Macrophages were also identified as positive for CYP1B1. A number of colon samples, removed some distance from the tumour, reveal that expression of CYP1B1 in pericytes and colon epithelium is decreased. These preliminary results indicate upregulation of CYP1B1 in tumour-associated blood vessels. CYP1B1 is not confined to malignant epithelium. These data confirm that CYP1B1 is expressed in cells within colon tumours, particularly in pericytes surrounding tumour vasculature. The protein is almost undetectable in pericytes some distance from the tumour. Expression of this drug metabolising enzyme in tumours may have implications for carcinogenesis studies, as well as the metabolism of current and future chemotherapeutics.

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## Elucidation of the molecular mechanism underlying the anti-invasive activity of a novel proteasome inhibitor (Bz-Leu-Leu-Leu-COCHO)

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The ubiquitin/proteasome pathway is an attractive chemotherapeutic target because of its involvement in fundamental cell processes such as cell cycle progression and transcriptional regulation. Particular interest in the proteasome has arisen since it was discovered to play a major role in controlling the activity of the oncogenic transcription factor NF-kB. We have synthesized a peptidyl alpha–keto aldehyde and shown it to be a reversible inhibitor of the chymotrypsin-like proteolytic activity of the proteasome (Ki=3.7nM). Interleukin-1 (IL-1) is a known activator of NF-kB and it stimulates the proteasomal degradation of the inhibitor of NF-kB, IkB, so that NF-kB can translocate into the nucleus and exert its effects. Assays looking at the ability of RKO and HT29 colorectal carcinoma cell lines to invade through an extra-cellular matrix mimic revealed that IL-1 (10 ng/ml) induced a 2-fold increase in the invasive capacity of these cell lines. In the presence of our proteasome inhibitor this could be reversed to levels below basal invasion in a dose dependent manner (100 nM- 10 uM). In HT29 cells the compound also inhibited unstimulated invasion of the cells but no effect on this basal invasion was observed in the RKO cell line. MMP-9 in conditioned medium from the two cell lines was detected by an enzyme linked immunsorbent assay (ELISA). Stimulation of the cells with IL-1 (10 ng/ml) increased the secretion of MMP-9 by approximately 2-fold and this could be reduced to sub-basal levels by treatment with our proteasome inhibitor. This effect on MMP-9 activity is in keeping with a previous report that has demonstrated the presence of a NF-kB response element in the gene of this protease. Luciferase assays have confirmed that IL-1 stimulates transcription of MMP-9 and further experiments are underway to determine whether the proteasome inhibitor reduces MMP-9 secretion at the transcriptional level. In summary these results provide strong evidence that inhibition of the ubiquitin/proteasome pathway represents a potential target for anti-cancer therapy. This compound represents a useful lead compound for this approach and the full role of NF-kB in this anti-invasive activity is under further investigation.