to be considered rather as a sialic acid-specific than a galactose-specific type II ribosome-inactivating protein, and neolacto-series gangliosides with Neu5Acalpha2-6Galbeta1-4GlcNAc-terminus are true functional and physiologically relevant rViscumin receptors.

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Effect of NK1 and NK2 tachykinin receptor antagonists on the growth of human breast carcinoma cell line, MDA-MB-231

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Several evidences suggest a role for tachykinins and their receptors on human cancer progression. Neuropeptides have been implicated in the growth of many tumours, including non-small cell lung cancers (NSCLC), central nervous system (CNS) and also breast cancers. The oestrogen receptor negative (ER -) human breast carcinoma cell line MDA-MB-231 expresses, as demonstrated by PCR analysis, both the NK1 and the NK2 receptors. In the present study, we demonstrate that neurokinin A (NKA) and substance P (SP) play a role in proliferation of this tumour cell line. In *in vitro* experiments, the specific receptor antagonists MEN11467 (NK1) and MEN 11420 (NK2) inhibited tumour cell proliferation and blocked the stimulatory effect of SP and NKA. Antitumoural activity of NK1 and NK2 receptor antagonists was tested in nude mice, measuring growth inhibition of MDA-MB-231 tumour cells xenografted s.c. and by using the hollow fiber assay. In both systems a significant inhibition was found for both compounds although more evident for NK1 antagonist MEN 11467, when administered at 5 mg/Kg i.v. every day for two weeks. Results obtained from both these models suggest that the *in vivo* activity of NK1 and NK2 antagonist may be a result of cytostatic effect rather than a cytotoxic one. Beside these considerations, the control of breast carcinoma (ER-) growth by tachykinin receptor antagonists may become a new form of targeted therapy for these important human tumours.

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Phase I and pharmacokinetic (PK) Trial of 3'-C-ethylnylcytidine (TAS-106) in solid tumors

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TAS-106 is a novel nucleoside that inhibits RNA synthesis by blocking RNA polymerases I, II, and III. TAS-106 is phosphorylated by cytidine/uridine kinase, which is preferentially distributed in malignant cells rather than normal cells. The active metabolite of TAS-106, the analog triphosphate (ECTP), is retained in animal tissues for protracted periods following brief TAS-106 exposure. Preclinically TAS-106 demonstrated broad and potent antitumor activity in human cancer xenografts including lung (LX-1, LC-11, Lu-61) and pancreatic cancer (PAN-4, PAN-12, H-48). Schedule dependency was not demonstrated for antitumor activity in animal models; thus, a feasibility study administering TAS-106 as a brief IV infusion every 21 days for enhanced convenience, was initiated in patients with solid neoplasms. To date 30 patients (median age 60; range 40-88) have been treated with 65 courses (median 2, range 1-5) at 0.67, 1.0, 1.5, 2.25, 2.81, 4.21, 6.31, and 9.46 mg/m^2. Dose-limiting toxicity consisted of a grade 3 cumulative sensory peripheral neuropathy (PN) starting after cycle 4 in 2 patients treated at the 6.3 mg/m^2 dose level. The majority of patients (67%) who experienced PN also had skin toxicity consisting of peeling over digits and 1 episode of erythema and peeling of palmar/plantar surfaces. Other observed TAS-106 related non-hematologic toxicities included fatigue, nausea, vomiting, transaminitis, and diarrhea. One grade 3 cardiac event attributed to thyroid dysfunction was also observed. Myelosuppression was rare with only 2 pts experiencing grade 3 neutropenia during cycle 1. No antitumor activity was observed, other than stable disease in 2 patients, in this predominantly colon cancer population (27/30 pts; 90%). Pharmacokinetic data from blood and urine analyses will be presented. The MTD and recommended phase II dose on this schedule is 4.21 mg/m^2. Due to PN, further exploration of TAS-106 dosing will proceed on a more prolonged infusion schedule.

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Novel ceramide analogues display selective cytotoxicity for drug-resistant breast tumor cells over normal breast epithelial cells

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The sphingolipid ceramide is involved in diverse cell signaling pathways involving cell proliferation and differentiation. Elevated ceramide also triggers apoptosis. Synthetic ceramide derivatives have been shown to be cytotoxic to tumors. Naturally occurring ceramides differ according to acyl chain length. Chemical modifications of the structure are often designed to increase water solubility or alter metabolism of the compound. The synthesis of the aromatic ceramide analog,D-erythro-benzene-C4-ceramide, has been described (Chun et al., J. Org. Chem. 65: 7634-7640, 2000). A C8-ceramide was prepared containing a trans double bond between C3 and C4 with a C5 hydroxy group in the R conformation(HB214T). We have evaluated the cytotoxic potency of these compounds in the metastatic drug-resistant breast tumor cell lines, SKBr3 and MCF-7/Adr-, compared to normal breast epithelial cells. Both tumor cell lines possess p53 mutations. MCF-7/Adr- overexpresses the drug transporter p-glycoprotein (MDR-1), while SKBr3 overexpresses Her2/neu. Cytotoxicity was assessed by quantifying the release of lactate dehydrogenase into the culture medium. The EC50 values (uM) at 24 hr for the designated compounds in SKBr3, MCF-7/Adr-, and normal breast epithelial cells, respectively, are as follows: D-erythro-benzene-C4-ceramide, 19.6 $\pm$ 7.1, 16.32 $\pm$ 2.4, $>$ 100; 5-OH-D3-ceramide-T (HB214T), 18.5 $\pm$ 5.6, 11.9 $\pm$ 5.1, 46.4 $\pm$ 11.7; and for adamantyl ceramide 12.5 $\pm$ 2.7, 15.3 $\pm$ 1.8, $>$100. For tumor cell lines, maximal cytotoxicity was observed between 30-100 uM at 24 hr, where 70-100% cell kill was observed between experiments. At a dose of 30 uM, the fold-increase in %cytotoxicty in tumor cells over normal breast cells was as follows: D-erythro-benzene-C4-ceramide 79.3, 77.2; HB214T 23.7, 19; and for adamantyl ceramide 11.2, 10.3, in SKBr2 and MCF-7/Adr- respectively. The relatively selective toxicity of these compounds in drug-resistant, metastatic breast tumor cell lines, which may result from differential cell uptake or metabolism, supports further research into their utility as therapeutic agents.

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The angiogenic factor CYR61, a downstream effector of heregulin, protects breast cancer cells from paclitaxel-induced cell death through integrin alphav beta3

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We recently established that the angiogenic factor CYR61 is a downstream effector of heregulin-induced breast cancer chemomigration and metastasis, probably through interactions with the integrin alphav beta3. Both heregulin (HRG) and CYR61 enhance tumor neovascularization, and this up-regulation of angiogenesis may contribute to a more aggressive disease. Chemotherapy effectiveness could be compromised by the high concentrations of pro-angiogenic survival/growth factors present in the tumor microenvironment. Since we previously demonstrated that HRG expression is related to doxorubicin (DOX) efficacy we envisioned that, in addition to their role as pro-angiogenic factors, HRG and/or CYR61 can also act as survival factors modifying breast cancer chemosensitivity. To address this question, we first evaluated the impact of HRG expression in modulating breast cancer response to anticancer drugs such as cisplatin (CDDP), 5-Fluorouracil (5-FU), and paclitaxel (PTX). MCF-7 cells transfected with the full-length HRG cDNA (MCF-7/HRG cells) were significantly more resistant to CDDP as compared to control cells. A weaker but significant increase in 5-FU resistance was observed in MCF-7/HRG cells. Also, MCF-7/HRG became more resistant to PTX. Next, HRG-negative MCF-7 cells engineered to overexpress CYR61 gene were assessed for chemotherapy effectiveness. MCF-7/CYR61 transfectants and control cells were equisensitive to DOX, CDDP and 5-FU. However, CYR61 overexpression resulted in PTX resistance levels similar to those found in MCF-7/HRG cells. Functional blocking of the integrin alphav beta3 induced a profound inhibitory effect on the cell growth of MCF-7/HRG and MCF-7/CYR61 cells. Of note, RGD peptidomimetics directed against integrin alphav beta3 -and based on CYR61 protein structure- synergistically reversed the PTX-resistance in MCF-7/HRG and MCF-7/CYR61 cells. PTX resistance in MCF-7/CYR61 cells was also reversed by wortmannin, a pharmacological inhibitor of the

phosphatidylinositol 3-kinase (PI3 kinase) activity. DNA from CYR61 transfectants treated with PTX displayed no signs of the classical DNA laddering pattern of apoptotic death. Inhibition of PTX-induced apoptosis in CYR61 transfectants was also demonstrated by TUNEL assay. Moreover, MCF-7/CYR61 cells were unable to induce p53 expression in response to PTX-induced damage. It is tempting to postulate that the angiogenic factor CYR61 -a downstream effector of HRG- might protect breast cancer cells from PTX-induced apoptosis by enhancing alphav beta3-PI3 kinase pro-survival signaling and inhibiting p53 pro-apoptotic functions. We suggest that new anti-HRG, anti-CYR61, and/or anti-integrin alphav beta3 strategies may prevent vessel growth simultaneously rendering tumor cells more sensitive to PTX-based chemotherapy in breast cancer.

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NFkappaB repression by sulfasalazine sensitizes pancreatic carcinoma cells to cytostatic drugs *in vivo*: a new concept of combined chemotherapy

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The pancreatic carcinoma is still characterized by a poor prognosis and ranks 5th among malignancy-associated deaths. Surgical resection is feasible only in a minority of patients and other therapy options based on chemotherapy are only palliative. One reason for this might be the resistance of tumor cells towards cytostatic drugs. To improve the therapy of pancreatic cancer it will be important to understand the mechanisms how tumor cells achieve chemoresistance and to define molecular targets for new therapeutic strategies. Some pancreatic carcinomas are characterized by a constitutively elevated NF-kappaB activity accounting for chemoresistance. In order to elucidate whether blockade of NF-kappaB activity with the anti-inflammatory drug sulfasalazine is suitable for overcoming this chemoresistance *in vivo*, we employed a mouse model with subcutaneously xenotransplanted human Capan-1 pancreatic carcinoma cells. Fourteen days upon tumor inoculation, animals were randomized in six groups, receiving no treatment, treatment with gemcitabine (2.5 mg/kg day, intraperitoneal), treatment with etoposide (6.5 mg/kg day, intraperitoneal), either alone, or in combination with sulfasalazine (70 mg/kg day, oral), or with sulfasalazine alone. Upon treatment with etoposide or gemcitabine alone, tumor sizes were moderately reduced to 50 % and 60-70 %, respectively, as compared to untreated tumors. The corresponding combination groups (etoposide: 20-25 %, gemcitabine: 45-50 %) showed significantly higher reduction in tumor sizes. TUNEL-staining revealed higher numbers of apoptotic cells in tumors from combination groups, and proliferation as indicated by Ki67 staining was strongly reduced. Furthermore, combined treatment of sulfasalazine with the cytostatic drugs led to a decreased blood vessel density. Immunohistochemical staining of the activated p65 subunit revealed that sulfasalazine treatment abolished the basal NF-kappaB activity in tumor cells. These data imply that NF-kappaB inhibition sensitizes pancreatic carcinoma cells to cytostatic drugs *in vivo*. In particular, a combined chemotherapy with the well established anti-inflammatory drug sulfasalazine offers great potential for improved anti-tumor responses in pancreatic carcinomas.

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Validation of reliable assay methods for glutathione quantitation and glutathione s-transferase activity in cancer patients

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Glutathione s-transferases (GST) are members of a superfamily of enzymes that catalyze the reaction of electrophilic compounds with glutathione (GSH) to form inactive conjugates. These enzymes are of great importance in cancer biology since their levels have been correlated with resistance to cytotoxic drugs such as alkylating agents, platinum compounds and anthracyclines. Conversely, recently has been published that a DNA minor groove binder is activated by the GST/GSH systems (Geroni et al, Cancer Res. 2002). GST distribution in cancer patients has been extensively investigated but mixed data have been reported. The objectives of this study were primarily to validate robust and reliable assays for GSH/GST detection, suitable for routine clinical use and to explore the correlation between blood and tissue levels for both. Matched blood and tissue samples (normal and malignant) from 52 cancer patients (NSCLC and SCCHN) were investigated. GSH concentration and GST activity were measured by an enzymatic assay. GST content was also analysed by HPLC. Moreover, since the existence of regions of tissue heterogeneity is well documented within the tumor, multiple samples from seven cancer specimens have been analysed. Data were evaluated for either intra- and inter-patient variability to verify whether GSH/GST exhibit heterogeneity in samples from different areas of the same specimen. Both GST activity and GSH levels were higher in cancer than in normal tissue. The difference was statistically significant in NSCLC ($p=0.0004$ and $p=0.0002$, respectively for GSH and GST) and borderline in SCCHN ($p=0.03$ and $p=0.02$, respectively for GSH and GST). Moreover GSH levels in whole blood showed a highly significant correlation with GST activity in matched cancer samples in both malignancies ($p=0.003$, $r=0.53$ in NSCLC, $p<0.0001$, $r=0.89$ in SCCHN). The strong correlation found between GST activity in cancer tissue and GSH level in whole blood indicates that GSH could have a clinical relevance as a surrogate marker of GST activity in tumor tissue and should be further investigated. However, tissue heterogeneity analysis suggested that GSH and GST levels could be linked to tissue variability in both normal and tumour tissues. Since statistical analysis indicates that heterogeneity is a true biological fact rather than an analytical artefact, it is recommended to have a larger sample of tumor tissue for GSH and GST biochemical analysis to confirm the validity of GSH as biomarker.

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Activation of wt p53 protein in normal and tumor cells by a novel anti-cancer drug CHS 828

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CHS 828, a novel cyanoguanidine, represents a new class of drugs for cancer treatment, with an unknown primary mechanism of action. It is known that anti-cancer drugs induce p53 response thereby triggering the cell cycle arrest or apoptosis We investigated the effect of anti-cancer drug CHS 828 on p53 induction in normal and tumor cells. We observed dose-dependent upregulation of wt p53 by CHS 828 in MCF-7 cells as well as in human and mouse fibroblasts. The drug, however, failed to induce p53 protein in PARP-1 deficient cells even at a 30-fold higher dose and after prolonged treatment. Combined treatment of PARP-1 -/- cells by CHS 828 and MDR modulators did not alter p53 expression. CHS 828 inhibited cell proliferation and DNA replication in tested cells. Interestingly, the DNA synthesis as well as proliferation of PARP-1 -/- cells was inhibited by about three-fold lower drug concentration then their normal counterparts. The treatment of mouse cells by CHS 828 for 48h impaired the integrity of plasma membrane in PARP-1 deficient mouse cells as evidenced by Trypan blue dye exclusion test. The effect of CHS 828 on p53 in normal cells seems to be cell cycle dependent. Treatment of quiescent cells resulted in downregulation of p53 protein. These results show that the drug is able to activate p53 response depending on cell cycle and PARP-1 functional status. The inactivation of PARP-1 sensitizes cells to the novel anti-cancer drug CHS 828.

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PPARgamma ligands modulate PPARgamma and RARbeta expression in human glioblastoma cell lines

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A recent approach to cancer therapy is the control of cell growth and induction of apoptosis via ligands of nuclear hormone receptors (NHR). Among NHR, much attention has been focused on peroxisome proliferator-activated receptor gamma (PPARgamma), that has been involved in the control of growth and/or differentiation of several malignant cell types. Currently, we are evaluating whether PPARgamma may be a molecular target for novel therapies in glioblastoma. We have already reported that PPARgamma is expressed in glioblastoma tumors and cell lines (A172, U87-MG,