promoter activity from constructs bearing these same mutations. We hypothesize that the ability of TMPyP4 to stabilize the chair-type quadruplex in the NHE III1 prohibits normal binding of this protein, and results in the decrease in c-MYC expression we have seen previously. A model is proposed, which explains how quadruplex formation occurs normally in the c-MYC promoter and regulates expression through the relative ability/inability of this protein to bind to the DNA.

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## Additive/Synergistic interaction between MKP-1 inhibitors and anti-cancer drugs in human non-small-cell lung cancer cell H292

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A group of novel MKP-1 inhibitors developed in MAXIA has been shown to suppress tumor growth both in *in vitro* and *in vivo* by activation of JNK and subsequent induction of apoptosis (see abstract by Allan Kaspar, et. al.). The JNK pathway has been reported to be targeted by several marketed anti-cancer drugs such as cisplatin (CDDP), paclitaxel and adriamycin for their apoptosis induction. Therefore, it is rationale to hypothesize that inhibition of MKP-1, a downregulator of JNK activity, may enhance the chemotherapeutic effects of those antineoplastic agents that depend on the JNK pathway. This study investigated the combination therapy of MAXIA's MKP-1 inhibitor, MX7091, with cisplatin, paclitaxol and adriamycin. Apoptosis induction and general cytotoxic activity were evaluated in the non-small-cell lung cancer (NSCLC) cell line H292 by the cell death ELISA assay and the 3-4,5-dimethylthiazol-2-yl-2,5-diphenyl-tetrazolium bromide (MTT) assay, respectively. Concurrent exposure of H292 cells overnight with MX7091 (1 uM) and cisplatin (10 uM) led to an 11 fold increase of apoptosis whereas MX7091 alone only induced six-fold increase of apoptosis and cisplatin (10 uM) alone did not significantly affect cell viability (1.4 fold). MTT assays revealed the similar observation in cell viability in both H292 cells and BxPC-3 cells (a pancreatic cancer cell line). Western blot analysis showed that JNK and c-Jun phosphorylation was synergistically enhanced by combined use of MX7091 and cisplatin as compared with each agent alone. These results suggest a synergistic interaction between MX7091 and cisplatin and are in agreement with a previous report that overexpression of MKP-1 can block the JNK activation and apoptosis by cisplatin. Paclitaxel (0.5 uM) or adriamycin (0.5 uM) alone induced apoptosis at 4.2 and 8.2 folds, respectively. Combination of MX7091 with paclitaxel or adriamycin resulted in 13.4 and 11.9 fold increase in cell apoptosis, respectively, indicating additive/synergistic interactions between MX7091 and those two drugs. More experiments are being performed to study the combination index (CI) of these drug combinations in different cancer cells. Animal studies are also being conducted to explore the *in vivo* synergism of the MKP-1 inhibitors and cisplatin and other anti-tumor agents.

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## New differentiation-involution inducing agents for the treatment of breast cancer

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Breast cancer displays many properties that are exhibited during the developmental cycle of the mammary gland. The mammary gland comprises stromal and epithelial cells that communicate through an extracellular matrix (ECM) to control the function of the gland. The stromal cells control the ECM composition and its network structure in the mammary gland. Upon hormonal stimulation during pregnancy and lactation, stromal adipocyte dedifferentiate into preadipocyte causing a change in the ECM content and signal the ductal epithelial cells to proliferate. Once lactation seizes, stromal preadipocytes redifferentiate into adipocytes and change the ECM content such that the proliferating epithelial cells to undergo apoptosis and the mammary gland to remodel back to its resting or lead to adult nulliparouse state. This process is called involution. An imbalance or incomplete involution can lead to breast cancer. Existing breast cancer drugs such as anti-hormone, and apoptosis inducing agents, act mainly on epithelial cells. MAXIA developed a new class of anti-breast cancer agents that mimic involution and function through stromal fat cells and the ECM. These compounds induce differentiation of preadipocytes into adipocytes and downregulate integrins, cadherins and Wnts. The changes caused by our compounds induce growth arrest and apoptosis of breast cancer cells and lead to tumor regression and prevention *in vivo*. Western blot analysis revealed that cylin D1, a down stream target of the integrin, cadhedrin and Wnt signaling pathways, is also down regulated. The compounds showed additive/synergistic activity in the *in vivo* model with the anti-estrogen Tamoxifen. Thus, we have discovered a new class of differentiation/involution inducers that promise a new effective treatment for breast cancer when used alone or in combination with anti-hormonal therapies.

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## Novel inhibitors of MKP-1 have potent anti-cancer activity *in vivo*

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The mitogen-activated protein kinase phosphatases (MKPs) are a subfamily of dual-specificity phosphatases that are capable of dephosphorylating and inactivating members of the mitogen-activated protein kinase (MAPK) family. Activation of c-Jun N-terminal kinase (JNK), a member of the MAPK family, has been shown to be involved in mediating apoptotic cell death. JNK activity is negatively regulated by MKP-1, which has been shown to dephosphorylate JNK and to protect cells from certain apoptotic stimuli. Additionally, MKP-1 overexpression has been observed in patients with prostate, ovarian, and lung cancers. MAXIA has developed small molecule inhibitors of the phosphatase MKP-1, MX7091 and related analogues, which induces JNK-activation and apoptotic death of tumor cells. *In vitro* phosphatase assays show that MX7091 and related compounds selectively inhibit MKP-1 activity. Treatment of cancer cell lines with nanomolar concentrations of MX7091 results in JNK activation, caspase activation, and apoptotic cell death. Additionally, MX7091 synergizes with the chemotherapeutic cisplatin to induce apoptosis of tumor cells. MX7091 efficacy correlates with overexpression of MKP-1 in tumor cells, consistent with MKP-1's role in maintaining cell survival. MX7091 significantly reduces tumor size and induces tumor remission, while increasing animal survival time in *in vivo* models of pancreatic, colon, and non-small cell lung cancer. These studies demonstrate that MAXIA's MKP-1 inhibitors represent a novel class of anti-tumor agents with potential clinical utility.

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## alpha2-6-sialylated neolacto-series gangliosides serve as receptors for the anticancer drug rViscumin

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rViscumin is a heterodimeric cytotoxic plant protein currently in clinical phase 1 trials. Like ricin and other type II ribosome inactivating proteins (RIP) rViscumin consists of an enzymatically active A-chain being responsible for the toxicity towards tumor cells through inactivation of the translational machinery of the cell and a B-chain with carbohydrate binding activity. The B-chain is responsible for binding to the surface of the target cells, subsequently leading to internalisation. In this study we set out to investigate potential differences in the carbohydrate specificity of rViscumin and ricin which could explain the good tolerability and efficacy of rViscumin during preclinical and clinical development. In recent literature rViscumin as well as ricin are described as galactose-specific carbohydrate binding proteins. Employing solid phase binding assays rViscumin was shown to preferentially bind to terminally alpha2-6-sialylated neolacto-series gangliosides IV6Neu5Ac-nLc4Cer, VI6Neu5Ac-nLc6Cer, and VIII6Neu5Ac-nLc8Cer. Only marginal binding of rViscumin to galactose-terminated neutral glycosphingolipids was determined, whereas reinvestigation of ricin specificity demonstrated this type II RIP as galactose-binding protein. In cytotoxicity assays with human promyelotic HL-60 cells and human bladder carcinoma 5637 cells $IC_{50}$ values of 1.16 pM and of 12.1 pM rViscumin were determined, respectively. CHO-K1 cells were resistant to rViscumin treatment up to a concentration of 5.26 nM tested. A direct correlation of rViscumin cytotoxicity and the expression of the receptor ganglioside IV6Neu5Ac-nLc4Cer was shown by means of a specific anti-Neu5Acalpha2-6Galbeta1-4GlcNAc-R antibody. The data revealed 3.7x106 and 1.5x106 receptor molecules per HL-60 and 5637 cell, respectively. CHO-K1 cells were negative, lacking alpha2-6-sialylated gangliosides. Moreover, CHO-K1 cells were rendered susceptible towards rViscumin cytotoxicity after exogenous application of human granulocyte gangliosides (HGG) which contain predominantly alpha2-6-sialylated gangliosides. From these data we conclude that rViscumin in contrast to ricin has

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