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.

363

## 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.

364

## 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.

365

## 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.

366

## 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