Marine compounds

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High-dose dexamethasone (Dex) protects against the hepatotoxicity of ET-743 in the female rat

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ET-743, a novel antitumor agent derived from a marine tunicate, is currently in phase II clinical trials. Its activity against sarcomas has been elucidated and studies in breast and ovarian cancer are ongoing. However, hepatotoxicity characterized by reversible transaminitis occurs in most treated patients and subclinical cholangitis in an important part. In the most sensitive animal species, the female rat, toxicity of ET-743 is characterized by hepatic necrosis and bile duct inflammation. Dexamethasone is an anti-inflammatory steroid which induces the activity of the hepatic P450 3A2 subfamily, the isoform primarily responsible for ET-743 metabolism. We investigated the effect of dex on liver damage induced by ET-743 in the female rat. Wistar rats received a single iv dose of ET-743 (40 micrograms/kg). Some rats were pretreated with a single oral dose of dex either at 1, 5, 10 or 20 mg/kg 24 h prior to ET-743 treatment. Liver pathology and plasma concentrations of alkaline phophatase (ALP), aspartate aminotransferase (AST) and total bilirubin (TB) were assessed up to 12 days post ET-743 administration. Conventional histological sections of the livers were examined by light microscopy. At 2 days post ET-743 treatment, livers from rats that had received ET-743 alone showed bile duct inflammation, striking degenerative changes in biliary epithelial cells and zones of hepatic necrosis. Plasma levels of ALP and AST were significantly elevated after 2 days. Cholestasis was reflected by a dramatic increase in plasma TB concentrations, which commenced on day 2 after ET-743. ET-743-induced histopathological changes and elevation of plasma ALP, AST and TB were totally abrogated in rats pre-treated with 10 or 20 mg/kg dex. Whilst dex at 1 mg/kg showed little protection, 5 mg/kg was moderately protective. Microarray analysis of livers from ET-743 treated rats showed a dramatic increase in the expression of ABC transporter genes Abcb1a and Abcb1b, which impart resistance against anticancer drugs, and of Cdc2a and Ccnd1, cell cycle genes. Pre-treatment with dex (10mg/kg) ameliorated these gene changes induced by ET-743. Furthermore, the efficacy of ET-743 against B16 melanoma implanted into mice was not impeded by dexamethasone. These findings suggest that the addition of high-dose dexamethasone to the ET-743 regimen may ameliorate its hepatotoxicity.

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Inhibition of activated DNA transcription by Ecteinascidin 743 (ET-743)

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ET-743, isolated from the marine tunicate Ecteinascidia turbinate, has shown great promise as a chemotherapeutic agent in Phase II trials in Europe and the US. ET-743 exhibits a number of unique properties that suggest that it may be the prototype for a new class of chemotherapeutic agents. One of the most novel properties of ET-743 is its effect on RNA Pol II transcription. Our laboratory has been investigating the role of ET-743 as a transcriptional regulator, initially through analysis of its effect on transcriptional activation of the MDR1 (P-glycoprotein) promoter. These studies were prompted by an earlier report indicating that ET-743 blocked the interaction of the trimeric transcription factor NF-Y with its cognate DNA element *in vitro*. We had previously shown that rapid induction of MDR1 transcription by multiple inducers, including histone deacetylase (HDAC) inhibitors, UV irradiation and the MDR drug doxorubicin, is mediated through an enhancer element that interacts with NF-Y, the GC element binding proteins, Sp1 and Sp3, and histone modifying enzymes (the MDR1 enhancesome). The identification of NF-Y as an integral component in MDR1 activation prompted us to evaluate the effect of ET-743 on NF-Y-mediated activation of the MDR1 promoter. We found that ET-743 blocked activation of MDR1 by all inducers that converged on the MDR1 enhancesome, with little affect on uninduced transcription. In the present study, we show that the effects of ET-743 are not limited to NF-Y-mediated transcription, nor to the MDR1 promoter. Indeed, activation of the p21 promoter, which is regulated by the major-groove binding protein Sp1 and is independent of NF-Y, is also inhibited by ET-743. Moreover, ET-743 blocks induction of Gal4 fusion proteins by TSA, without affecting activation mediated by the fusion proteins in the absence of the inducer. Finally, microarray analysis of SW620 cells treated with TSA and/or ET-743 indicate that activation of all TSA-responsive promoters is blocked by ET-743 with little affect on non-responsive promoters. These results, taken together with previous reports, leads us to suggest a mechanism whereby ET-743 is a novel and general inhibitor of activated, but not uninduced transcription. Moreover, preliminary studies indicate that this mechanism may occur independent of DNA binding.

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A phase I clinical and pharmacokinetic (PK) study with KahalalideF (KF) in patients (pts) with advanced solid tumors (AST) with a continuous weekly (W) 1-hour iv infusion schedule

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KF is a COMPARE negative (US-NCI), marine derived compound proposed to target the erb-2 pathway. Morphological studies indicate that lysosomes might be the intracellular target for KF. *In vitro* and *in vivo* activity in experimental models has been noted in different tumor types. In this study 25 pts bearing Advanced Solid Tumors, median (m) age=55,median number of previous chemotherapy lines: 2(0-7), have been entered across 6 dose levels (DL) ranging from 266 mcg/m^2/w -1200 mcg/m^2/w with 163 infusions (inf) given. 1200 mcg/m^2/w represents the maximal tolerated dose (MTD). The Dose Limiting Toxicity has been early onset, asymptomatic g4 elevation of transaminases not-reversible by day 7. Pruritus on hands was a very common toxicity in all levels. As anticipated in experimental models bone marrow toxicity has not been observed. Clinical benefit has been noted in 3 pretreated progressive pts: hepatocarcinoma at 400 mcg/m^2/w (24 inf), 1 squamous ca cavum at 400 mcg/m^2/w (9 inf) and in one patient with NSCLC at 530 mcg/m^2 (16 inf). All patients are sampled for PK analysis: the PK profile fits with linearity up to 800 mcg/m^2/w. The median (m) 1/2 life=28' the m Vss=3.1L/m, the m CL=74ml/min*m^2) and the m AUC at the MTD=533.5 h*ng/ml. The plasma levels achieved seem to compare well with the *in vitro* active concentrations. The data indicates that KF is feasible in adult pts with Advanced Solid Tumors suggesting a positive therapeutic index.

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Cell cycle perturbations and apoptosis induced by the novel marine compound variolin B

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Variolin B is a novel marine natural product which was isolated from the sponge Kirkpatrickia varialosa from Antarctic. This compound has shown potent cytostatic and cytotoxic effects against different human leukemia cell lines, K-562, U-937, MOLT-4 and Jurkatt, human ovarian carcinoma cell lines, OVCAR-3, SKOV-3 and Igrov-1 and human intestinal carcinoma LoVo cell line. Variolin B was found to be equally effective against LoVo carcinoma cell line and its multidrug resistant variant LoVo/Dx overexpressing Pgp. By using biparametric BrdU/DNA flow cytometric analysis it was found that in LoVo cells, Variolin B concentrations in the nM range for 1 h cause an arrest of cells in G1 and a decrease of the rate of progression of S-phase cells to G2, whereas at concentrations in the mM range, even for a short time, the compound induced a blockade in G2 phase. In both leukemic and epithelial cancer cells Variolin B was found to be a strong activator of apoptosis, assessed by morphological and biochemical methods. Apoptosis occurred very rapidly, within 6-8 h following a short drug exposure. Variolin B induced apoptosis also in K562 erythroleukemia andepithelial ovarian cancer cell lines which do not activate apoptosis after treatment with conventional anticancer drugs. While the *in vivo* antitumor activity of Variolin B is under investigation, studies have been initiated to elucidate the molecular mechanisms underlying the interesting biological activities of this new compound.