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The antiproliferative activity of ENMD-1420, a diaryl inhibitor of tubulin polymerization, is selective for the Z isomer

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Background: ENMD-1420 (previously CC-5079) is a synthetic diaryl compound that inhibits tubulin polymerization and TNF-α production with in vitro and in vivo antitumor activity. One strategy to optimize the effects of these compounds has included defining molecular stereoselectivity for activity.
Material and Methods: ENMD-1420 was synthesized at EntreMed by a Wittig reaction to give an E/Z mixture which was purified by chromatography to give individual isomers. Alternatively, pure E and Z isomers were prepared directly though a Heck coupling reaction. Cell proliferation was assessed in cancer cell lines and endothelial cells (HUVEC) using the WST-1 reagent. The in vivo antitumor activity of ENMD-1420 was evaluated in the Lewis lung carcinoma (LLC) experimental pulmonary metastatic model.
Results: For the 1:1 E/Z isomer mixture, the IC50 values of ENMD-1420 were determined to be 21, 16 and 12 nM against MDA-MB-231, Lewis lung carcinoma and HUVEC, respectively. The Z isomer (ENMD-1427) was statistically more potent than the mixture against these cell lines (IC50 = 6, 6 and 4 nM against MDA-MB-231, LLC, and HUVEC, respectively, $p < 0.01$). The IC50 values of the E isomer (ENMD-1916) were statistically less potent against MDA-MB-231, LLC and HUVEC (IC50 = 64, 73 and 63 nM, $p < 0.01$). The increased potency observed with the Z isomer of ENMD-1420 was also observed with 5 additional analogs tested. Treatment of B6 mice bearing metastatic pulmonary Lewis lung carcinoma with the E/Z isomer mixture of ENMD-1420 (25 mg/kg qd × 5) resulted in an 84% inhibition of tumor growth with minimal body weight loss. As a single administered dose of 25 mg/kg, the pharmacokinetics of the E/Z isomer of ENMD-1420 gave a Cmax of 151 ng/mL and an AUC of 445 ng/mL*h. The in vivo antitumor activity of 5 analogs have also been assessed in this model with tumor growth inhibition ranging from 78−96% The impact of the Z isomer of ENMD-1420 and analogs on in vivo tolerability and antitumor activity is now being evaluated and will be reported.
Conclusions: The stereoselectivity of ENMD-1420 and analogs for in vitro antiproliferative activity against tumor and endothelial cells resides in the Z isomer which indicates that a synthetic strategy targeting this particular configuration should be used to optimize antitumor activity and lead compound identification.

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Paclitaxel poliglumex cellular uptake by normal tissues and human tumor xenograft: an IHC study in nude mice

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Background: Paclitaxel poliglumex (PPX, XYOTAX™) is a novel chemotherapeutic that links paclitaxel to a biodegradable polymer, poly-L-glutamic acid. Biodistribution studies demonstrate that PPX accumulates in reticuloendothelial organs and in syngeneic or xenogeneic tumor tissues. The aim of this study was to investigate PPX localization at the cellular level using immunohistochemistry (IHC) techniques in xenograft tumor bearing mice.
Methods: Female nude mice were subcutaneously implanted with NCI-H460 (NSCLC) human tumor fragments. Animals were administered PPX (90 mg/kg i.v. paclitaxel equivalents) and sacrificed 24 hours post-treatment. Lung, liver, spleen and tumor were collected, formalin fixed and paraffin embedded. IHC staining of tissue sections was performed with an anti-PPX monoclonal antibody (CT-2D5) which recognizes full length PPX but not PPX fragments, poly-L-glutamic acid or free paclitaxel.
Results: Positive staining for CT-2D5 was found in the cytoplasm of cells in all collected organs, with the ratio of positive cells higher in liver and spleen compared to tumor and lung. Morphology of positive cells was consistent with that of liver macrophages (Kupffer cells), spleen resident macrophages, type II pneumocytes and tumor-associated macrophages (TAMs). Positive staining for CD45 (common leukocyte antigen) and F4/80 (specific macrophage antigen) in the same organs confirmed that CT-2D5 positive cells were of macrophage lineage. CT-2D5 positive cells were mainly localized in the tumor capsule and F4/80 staining confirmed that TAMs infiltration was limited to that area. Strong CT-2D5 positive staining, mainly extracellular but in some cases intracellular, was observed in perinecrotic areas. The few positive cells were positive for CD45, but negative for F4/80. Morphologic analysis and specific staining (Myeloperoxidase) suggests that they are infiltrating polymorphonuclear leukocytes (PMN) and intracellular PPX is due to their phagocitic activity in the necrotic area.
Conclusions: Our results confirm that PPX is largely taken up by macrophages in the reticulo-endothelial organs and TAMs. In this tumor model, the role of TAMs in the delivery of PPX in areas different from the capsule was not elucidated. However, the presence of extracellular PPX in the perinecrotic areas suggests that other mechanisms of PPX penetration, such as the vascular leak and the consequent enhanced permeability and retention (EPR) of macromolecules might be involved.

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Phase 1 trial of a novel epothilone, KOS-1584, using a weekly dosing schedule

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Background: KOS-1584 (9,10-didehydroepothilone D) was discovered as part of a screening program to develop a new generation of epothilones with higher potency and an improved pharmacologic/pharmacokinetic (PK) profile. Epothilones stabilize microtubule polymerization, inducing rapid G2/M arrest and apoptosis. Antitumor activity of KOS-1584 (Chou *et al* 2003) is approximately 3–12 fold more potent compared to the structurally-related Epothilone D. KOS-1584 demonstrates enhanced tumor tissue penetration and reduced exposure to selected tissues (including CNS). This dose-escalation trial explores a weekly administration schedule of KOS-1584.
Methods: Define MTD, toxicity profile and PK of KOS-1584 administered to patients with advanced solid malignancies via 1-hour infusion on Days 1, 8 and 15 every 4 weeks. Pharmacodynamics are assessed by serial sampling of PBMCs for soluble and polymerized microtubules by immunoblot.
Results: 27 pts (17 F; median age 57; median ECOG PS 1; median prior regimens 4, range 1–13) enrolled in 9 dose levels (0.8, 1.5, 2.5, 5.0, 7.5, 10, 13, 16 and 20 mg/m^2). To date, no Cycle 1 DLT has been seen; one Grade 3 episode of arthritis occurred in Cycle 2. Drug-related toxicities, all Grade 1 or 2 severity (n = 24): nausea (n = 9), fatigue (n = 8), diarrhea/vomiting/constipation/anorexia/neuropathy (all n = 6). Neutropenia is more commonly observed, starting at 16 mg/m^2. Neurotoxicity is not a notable toxicity (no observations greater than grade 1 severity). PK/parent (n = 24): t1/2 23.3±7.2 h, Vz 557±217 L and CL 17.4±6.3 L/h with no evidence of dose dependency. 16.0 mg/m^2 Cmax 567±206 ng/mL; AUCtot 3108±2248 ng/mL*h. Dose proportional increase in exposure and Cmax was observed over the range tested to date. Metabolite (Seco-D KOS-1584) AUCtot was 8.9±3.2% of parent. Comparison between 1-h and 3-h infusions shows slightly slower clearance for the shorter infusion (17.4 versus 26.6 L/h) and similar Vz. Dose-dependent increases in polymerized microtubules were observed, with maximal effect at the end of infusion. Antitumor activity: patient with ovarian cancer (40% decrease in CA125), NSCLC (minor response), and stable disease (patients with head & neck and breast cancer of 5 and 4 months, respectively).
Conclusions: Accrual is continuing in order to define the optimal dose on this regimen. Exposure and Cmax remain linear within this dose range; slower systemic clearance is observed for the same dose administered over 1 vs 3 h.

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RTA 301 (peloruside): a novel microtubule stabilizer with potent in vivo activity against lung cancer and resistant breast cancer

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RTA 301 (peloruside) is a novel microtubule stabilizer isolated from a marine sponge. It potently inhibits the growth of many human tumor cell lines, including those that express high levels of P-glycoprotein (P-gp) or have mutations in β-tubulin. It is much more water soluble than standard taxanes and binds to a different site on β-tubulin than the taxanes and epothilones. Although the activity of RTA 301 has been characterized *in vitro*, it has never before been tested *in vivo*. We performed three xenograft studies in athymic *nu/nu* mice to assess the efficacy of RTA 301 compared to standard anticancer agents. RTA 301 was administered by intraperitoneal (i.p.) injection. The first study examined the effect of two doses of RTA 301 compared to docetaxel and paclitaxel on the growth of NCI-H460 non small cell lung carcinoma (NSCLC) tumors. RTA 301 was administered at 5 and 10 mg/kg every day for five days (QDx5), docetaxel was administered at 6.25 mg/kg i.v. every other day for a total