administered per os (po) (120mkd × 15d) to SCID mice implanted subcutaneously (sc) with A121 human ovarian tumor xenograft resulted in a 53 day (d) tumor growth delay time versus 45d for po IDN-5109 (120mkd × 4d) and 33d for iv paclitaxel (25mkd × 4d). Using a Pgp expressing, multidrug resistant DLD1 human sc colon tumor xenograft in mice, the therapeutic efficacy of IDN-5390 was decreased (tumor growth delay time of 14d), as was paclitaxel's (16d), as compared to that of IDN-5109 (32d). However, the combination of IDN-5390 with either IDN-5109 or paclitaxel significantly increased antitumor efficacy, as measuerd by longer tumor growth delay times (to 65d and >30d, respectively). The combination of IDN-5390 with IDN-5109 also resulted in a 33% long-term survival rate as compared to no survivors with either drug alone. In a murine angiogenesis assay using bFGF/Matrigel plugs, IDN-5390 was observed by histology and fMRI to inhibit the formation of blood vessels to a greater extent than IDN-5109 or paclitaxel. Based on these findings, metronomic dosing with IDN-5390 appears to result in both excellent antiangiogenic and antitumor activity, which moreover is additive to other taxoids' activities, suggesting new treatment strategies. Preclinical pharmacokinetic, biodistribution and toxicology studies with IDN-5390 in mouse and dog are ongoing, prior to taking this agent into Phase 1 clinical trial. (Partially supported by CA73872, CA16056)

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Suppression of microtubule dynamic instability by discodermolide in living non-small cell lung carcinoma cells and its synergy with paclitaxel

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Suppression of microtubule (MT) dynamics by MT targeting drugs is responsible for their ability to inhibit mitotic progression. However, these drugs differentially affect dynamic instability parameters such as growing and shortening rates, duration of attenuated states or transition frequencies (rescues and catastrophes). Discodermolide is a new MT-stabilizing drug that acts synergistically with paclitaxel to induce cytotoxicity in the human lung cancer cell line A549. In this study, we first analyzed the concentration-dependent effect of discodermolide on microtubule dynamics in living cells as compared with paclitaxel and then we tested the hypothesis that the synergistic action of both drugs on cytotoxicity could be related to a synergistic inhibition of microtubule dynamic instability, induction of aberrant mitosis and apoptosis. We measured the dynamic instability of individual MTs in A549 cells, using microinjection of rhodamine-labeled tubulin and time-lapse fluorescence microscopy. Aberrant mitoses were counted after a drug treatment for 20h by staining DNA with 4,6 diamidino-2-phenylindole. Apoptosis was determined after 72h treatment by double staining of cells (propidium iodide and Annexin V antibody) and flow cytometry. Synergism was defined by a combination index (CI) <1 (Chou and Talalay, 1983). As with paclitaxel, discodermolide inhibits MT dynamicity in a concentration-dependent manner. Overall MT dynamicity was reduced by 50 % with 6 nM paclitaxel or 60 nM discodermolide. Both drugs similarly affect most dynamic instability parameters. However, they differ in their ability to decrease the catastrophe frequency (paclitaxel > discodermolide, $p<0.001$) and to increase the rescue frequency (discodermolide > paclitaxel, $p<0.05$). Moreover, the combination of both drugs acts synergistically on inhibition of MT dynamicity (-71% and -24% for the combination of 2nM paclitaxel and 7 nM discodermolide and each drug alone respectively, CI=0.23) and also causes a synergistic increase in the percentage of aberrant mitoses (26.8 ± 3.2 %, 10.6 ± 1.8 %, 14.1 ± 2.1 % for the combination, discodermolide, and paclitaxel respectively; CI=0.48) and enhanced apoptosis. In conclusion, the combination of the two MT stabilizing drugs at low concentration synergistically inhibited MT dynamic instability and enhanced apoptosis. Thus this combination should be considered for potential clinical use.

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Influence of polysorbate 80 on unbound fractions of anticancer agents

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One of the major problems in drug development is the water insolubility of potentially new drugs. For clinical use, these drugs are frequently dissolved in solutions containing polysorbate 80 (PS-80). Little is known about the influence of this delivery vehicle on the binding of drugs to blood components, while knowledge of the extent of binding of anticancer agents to blood compartments is of importance for understanding the clinical pharmacological behavior of the drug. Here, we studied the pharmacokinetics of the model drug docetaxel, by using a newly developed equilibrium dialysis method, in the presence and absence of its delivery vehicle PS-80 and in 23 cancer patients treated with docetaxel as a 1-hour i.v. infusion. In the absence of PS-80, binding of docetaxel *in vitro* to plasma obtained from healthy volunteers was ~93% (fraction unbound (fu), 7.01 ± 0.487%). PS-80, added at clinically relevant concentrations (up to 5.0 μL/mL), caused a profound increase (44%) in fu ($P < 0.0001$) This effect is consistent with the hypothesis of esterase-mediated release of oleic acid from PS-80 and displacement of docetaxel from protein binding sites by the fatty acid. The pharmacokinetics of unbound docetaxel in cancer patients were characterized by a fast clearance of 315 ± 71.4 L/h/m^2 and a terminal half-life of 12.0 hours. Of various serum proteins, only a1-acid glycoprotein was significantly related to fu ($P < 0.0018$), with higher fu in the presence of lower protein levels. Total docetaxel clearance was related to a1-acid glycoprotein ($R2 = 0.13$), although it did not reach a level of significance, and was significantly related to fu(pre-treatment) ($R2 = 0.15$, $P = 0.039$) and the area under the plasma concentration-time curve ratio of unbound to total drug ($R2 = 0.29$, $P = 0.0048$). These data indicate, that the fraction unbound docetaxel in human plasma samples is significantly influenced by the presence of PS-80, which effect was shown to be concentration-dependent, leading to changes in the pharmacokinetic behavior of docetaxel. In view of the use of PS-80 as a drug delivery vehicle for various current and future anticancer agents, measurement of unbound concentrations is considered essential during (pre)clinical drug development. Currently we are investigating the mechanism for the decreased drug plasma binding in the presence of PS-80.

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D-82318 - a new, synthetic, low molecular weight tubulin inhibitor with potent *in vivo* antitumor activity

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4-Phenyl-1-piperazinyl-carbonyl-substituted nitrogen containing heterocycles were discovered as a new class of potent, synthetic, small molecule tubulin inhibitors. The potential development candidate D-82318 has shown potent *in vitro* antiproliferative activity against a panel of more than 35 established human tumor cell lines including multidrug resistant (MDR1) phenotypes. Mode-of-action studies revealed that our compounds are competing with [3H] colchicine for tubulin binding, and are effectively inhibiting microtubule formation. Dividing cells were arrested in the G2/M phase of the cell cycle and were subsequently undergoing apoptosis. For further characterization, tumor cell growth inhibition was assessed using a long term soft agar colony formation assay by seeding xenograft derived single cell suspensions in a semisolid agar layer. D-82318 showed a markedly differential sensitivity profile in a panel of 14 human tumor xenografts in this clonogenic assay, with IC70 values ranging from 20 nM to 1000 nM. The antiproliferative activity (mean IC70 = 199 nM) was comparable to the potency of vindesine (mean IC70 141 = nM) and significantly higher than the activity of paclitaxel (mean IC70 = 1170 nM). For testing of *in vivo* activity, tumor bearing nude mice were treated with D-82318 in a broad range of doses and schedules. A maximal tolerated dose was found at 24 mg/kg/d for i.p. (Q3dx6) and 50 mg/kg/d for p.o. (Qdx5) administration. Xenografts were originally established by serial passage of fragments from patient tumor explants. Tumors likely to respond were selected from the clonogenic assay tumor panel profile. D-82318 proved to be a potent inhibitor of *in vivo* tumor growth in different xenograft models including mammary and renal cancers.

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Analysis of biomarkers in response to FB642 in human neuroblastoma cells *in vitro*

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FB642 (methyl-2-benzimidazolecarbamate, carbendazim), a systemic fungicide from the benzimidazole family with antitumor activity against a broad spectrum of tumors both *in vitro* and *in vivo*, is being evaluated in clinical trials. The drug is effective in p53 deficient cells, and exhibits antitumor activity in drug- and multidrug resistant cell lines (J Cancer Res Clin