Results: 52 patients (31 M, 21 F) median age 60 (range 40-77), median ECOG performance status 1 (range 0-1), prior chemotherapy regimens median 2 (range 1-4) have received 152+ treatments (mean 2.9 cycles, range 1-16+), with dose reductions required in 3% of doses. All patients were resistant to platinum and taxanes, 24% resistant to second line docetaxel, and 55% having failed additional 3rd line salvage therapy including gemcitabine (27%), and EGFR inhibitors (20%). No Grade 4 events were reported. Grade 3 events were infrequent. No myelosuppression, thrombocytopenia or cumulative toxicity was seen. Possibly drug-related toxicities were mild (Grade 1-2) fatigue (38%), nausea (38%), and vomiting (22%). At the interim analysis, 41/52 patients were evaluable for efficacy. Disease stabilization was seen in 21/41 (51%). The median duration of stable disease exceeds 39 weeks. Median survival for both 2nd and 3rd and 4th line patients exceeds 10 months, and requires further patient follow-up to reach median survival. The longest duration of TLK286 therapy was one year. Survival at one year requires further patient follow-up.
Conclusions: TLK286 is well tolerated in this heavily pretreated advanced NSCLC population. Efficacy in this heavily treated population that includes 55% 3rd and 4th line patients is encouraging. Median survival exceeds 10 months and has not yet been reached. Future studies of TLK286 in advanced NSCLC are warranted.

Tubulin interacting agents

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The seco-taxane IDN5390 is able to circumvent paclitaxel resistance in drug-resistant cells with overexpression of class III beta-tubulin

C. Ferlini[1], S. Mozzetti[1], D. Gallo[1], G. Raspaglio[1], P. Apollonio[1], A. Riva[2], P. Morazzoni[2], E. Bombardelli[2], S. Mancuso[1], G. Scambia[1]. *[1]Universita' Cattolica Sacro Cuore, Obstetrics and Gynaecology, Rome, Italy; [2]Indena S.p.A., Milan, Italy*

A primary mechanism of drug resistance to taxanes is the overexpression of class III beta-tubulin isotype. The activity of newly developed taxanes has been assessed against a panel of human cancer cell lines showing inherent or acquired drug resistance and overexpression of such isotype and the seco-taxane IDN5390 has been selected. Levels of beta-tubulin isotypes have been determined by RT-PCR in cells treated with paclitaxel, IDN5390 and with their combination. In wt cells, paclitaxel raised the levels of class III beta-tubulin isotype, whereas IDN5390 induced the opposite effect, and combination of both compounds prevented paclitaxel-dependent class III overexpression. In paclitaxel-resistant cells showing high levels of class III beta-tubulin, paclitaxel treatment did not modulate further class III beta-tubulin, while IDN5390 alone or in combination diminished the expression of the class III isotype. Other beta-tubulin isotypes were unaffected by drug treatments. Starting from these findings, we tested the presence of a potential synergism between paclitaxel and IDN5390. Results indicated a synergism, particularly in class III overexpressing cells. Finally, the synergism has been confirmed in paclitaxel-resistant xenografts transplanted in nude mice: a significant activity was noticed in xenografts treated with combination of paclitaxel and IDN5390 (TWI 52%, LCK to 0.8), whereas as single agents paclitaxel and IDN5390 were devoid of relevant effects (TWI of 29 % LCK of 0.2 and TWI 36% and LCK of 0.4 for paclitaxel and IDN5390, respectively). At the end of the study, we assessed the class III beta-tubulin expression in the xenografts and we found that, in keeping with "*in vitro*" findings, paclitaxel induced the overexpression of class III, while IDN5390 did not and, when combined with paclitaxel, it prevented the class III overexpression. Our data indicates that IDN5390 is able to circumvent paclitaxel-resistance in cellular models with overexpression of class III beta-tubulin and that the combination between seco-analogues and paclitaxel could represent a novel strategy to overcome MDR-independent taxane resistance.

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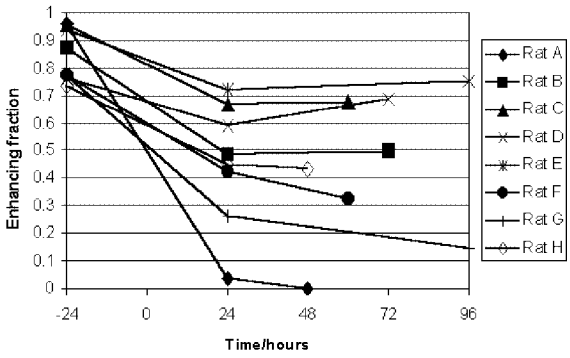
Absence of vascular regrowth at 96hrs in response to the vascular-targeting agent ZD6126 demonstrated by dynamic-contrast enhanced (DCE) MRI

D.J.O. McIntyre[1], S.P. Robinson[1], F.A. Howe[1], J.R. Griffiths[1], A.J. Ryan[2], D.C. Blakey[2], I.S. Peers[2], J.C. Waterton[2]. *[1]St George's Hospital Medical School, Biochemistry and Immunology, London, United Kingdom; [2]AstraZeneca, Macclesfield, United Kingdom*

Tumour neovasculature is structurally distinct from normal vasculature and is therefore an attractive therapeutic target. The novel vascular-targeting agent ZD6126 is metabolised to the tubulin-binding agent ZD6126 phenol *in vivo*. Its action leads to the selective disruption of the cytoskeleton of newly divided endothelial cells, occlusion of tumour blood vessels and haemorrhagic tumour necrosis. We have previously shown the antivascular effect of 50 mg/kg ZD6126 on rat GH3 prolactinomas to be profound 24 h after administration [1]. This was consistent with the induction of massive central tumour necrosis with a residual viable rim of tumour cells, a common feature of the response to this agent. Tumour regrowth has been previously shown to occur from this viable rim after treatment with ZD6126 [2]. In this study we used DCE-MRI to assess regrowth of the tumour tissue up to 96 h post-treatment with ZD6126. GH3 prolactinomas were grown in the flanks of 8 Wistar Furth rats. DCE-MRI data were obtained 24 h pre-treatment using a 4.7T Varian Unity Inova. MRI was repeated 24 h post-treatment with 50 mg/kg ZD6126, followed by a final scan at 48, 60, 72 or 96 h post-treatment. Multislice dynamic data were obtained using a spin-echo sequence (TR = 120, TE = 10) for 10 min post gadopentetate injection. The gadopentetate concentration was calculated voxelwise and integrated over the first 10 images to give an IAUC. Tumour data were normalised to the median IAUC of muscle. Tumour IAUC values greater than the muscle median were defined as highly-enhancing. After the final scan tumours were excised and scored for necrosis. Post-treatment, all tumours showed a significant reduction (between 20-80%) in highly-enhancing voxels. Images of tumour IAUC implied that ZD6126 reduced the IAUC close to zero in certain areas, typically in the centre of the tumour. The fraction of highly-enhancing voxels at the final time point (48 - 96 h) remained similar to that at 24 h post-treatment for all tumours, independent of the time elapsed to the final time point.



Analysis of tumour necrosis supported this finding, indicating that, notably, there was no significant tumour regrowth up to 96 h post ZD6126 treatment in this model.
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IDN-5390, an orally active, antiangiogenic taxoid with low toxicity, ideally suited for metronomic dosing

R. Bernacki[1], M. Vredenburg[1], P. Kanter[1], L. Pendyala[1], N. Chaudhary[1], R. Mazurchuk[1], I. Ojima[2], P. Morazzoni[3], A. Riva[3], E. Bombardelli[3]. *[1]Roswell Park Cancer Institute, Pharmacology & Therpeutics, Buffalo, USA; [2]State University of New York at Buffalo, Stony Brook, USA; [3]Indena SpA, Milan, Italy*

Our goal is the development of new semisynthetic taxoids that both overcome Pgp-based multidrug resistance and are less toxic to the host. Use of metronomic dosing (i.e., long-term, low dosing) to lower host toxicity, may be feasible with a taxoid that targets both tumor and growth of tumor vasculature. Previous studies demonstrated that IDN-5390 actively inhibits endothelial cell migration, suggesting antiangiogenic specificity (Taraboletti et al., Cancer Res. 8: 1182, 2002). However, in these studies both MCF7 human breast tumor cells and human umbilical vein endothelial cells (HUVEC) were growth inhibited by IDN-5390 *in vitro* at similar concentrations (IC_{50} 15nM). In addition, IDN-5390 was found to be less potent than paclitaxel (2nM) and the taxoid IDN-5109 (0.4nM) selected for its ability to overcome Pgp multidrug resistance. Interestingly, IDN-5390 had a lower fold resistance (121x) than paclitaxel (647x) in Pgp positive multidrug resistant MCF7/Adr cells but higher than that (45x) for IDN-5109. *In vivo*, IDN-5390

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

S. Honore[1], K. Kamath[2], D. Braguer[1], L. Wilson[2], C. Briand[1], M. Jordan[2]. *[1]UMR-CNRS 6032, University of "la mediterranee", Marseille, France; [2]MCDB, University of California, Santa Barbara, CA, USA*

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

W.J. Loos[1], S.D. Baker[2], J. Verweij[1], J.G. Boonstra[3], A. Sparreboom[1]. *[1]Erasmus MC - Daniel den Hoed, Medical Oncology, Rotterdam, The Netherlands; [2]The Sidney Kimmel Comprehensive Cancer Center at Jo, Experimental Therapeutics, Baltimore, USA; [3]Erasmus MC - Daniel den Hoed, Clinical Chemistry, Rotterdam, The Netherlands*

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

S. Baasner[1], P. Emig[1], M. Gerlach[1], G. Mueller[1], K. Paulini[1], P. Schmidt[1], A.M. Burger[2], H.-H. Fiebig[2], E.G. Guenther[1]. *[1]Zentaris AG, Drug Discovery, Frankfurt am Main, Germany; [2]Oncotest GmbH, Institute for Experimental Oncology, Freiburg, Germany*

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*

E. Izbicka, G. Carrizales, S. Rani, G. Piazza. *CTRC Institute for Drug Development, Dept. of Molecular Targets, San Antonio, USA*

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