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)

110

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.

111

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.

112

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.

113

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

Oncol 2001, 127:301-313). Since potent anticancer activity of FB642 has been attributed to disruption of microtubule function, the aim of this study was to evaluate if FB642 causes shifts in β-tubulin isotypes in a panel of human pediatric tumor cell lines. While the expression of β_{III} tubulin could be expected in neuroblastoma model, little is known about the expression of β-tubulin isoforms in other pediatric cancers. Human pediatric tumor cell lines CP2C (PNET), Daoy and TE571 (medulloblastoma), IMR-32, CHP-212, SK-N-SH, SK-N-BE(2), and SK-N-DZ (neuroblastoma) were treated with FB642 in a head-to-head comparison with paclitaxel at or below respective drug IC_{50} levels for 5 days. Expression of total β-tubulin and isotypes I/II and III were determined by Western blot and detected by enhanced chemiluminescence. At day 5, all control and drug treatment groups were evaluable except SKN-DZ (the control and two paclitaxel concentrations). The basal level of b-tubulin detected with the pan-b-tubulin antibody was high in all cell lines. The expression of β_{II} tubulin was high in SKN-BE(2), SKN-DZ, SK-N-SH, and TE-571 and this isoform was not detectable in CP2C and Daoy. Heavy expression of bIII tubulin was seen in SKN-BE(2), SKN-DZ and SK-N-SH. Lower levels were detected in TE-571 and no β_{III} tubulin was detected in other cell lines. Treatment of the cells with FB642 or paclitaxel was associated with apparent concentration-dependent downregulation of all tested b-tubulin isoforms in SKN-BE(2) and SKN-SH. Paclitaxel apparently upregulated expression of bII tubulin in CP2C, and both β_{II} tubulin and β_{III} tubulin in TE571. FB642 upregulated pan-β- tubulin and β_{II} tubulin in CP2C. Although FB642 and taxanes likely have different molecular targets, these data show that both drugs may share similar effects on expression of β-tubulin genes. The sensitivity to FB642 varies between pediatric cancer cell lines and warrants further comparisons of FB642 versus paclitaxel in pediatric cancer model to better understand the unique mechanism of action of FB642.

114

Preclinical evaluation of the antitumour activity of the novel vascular targeting agent Oxi 4503

D. Chaplin[1], S. Hill[2]. [1]*Oxigene Inc., Watertown, MA, USA;* [2]*Gray Cancer Institute, Microcirculation Group, Northwood, UK*

Tubulin depolymerizing drugs, which selectively disrupt tumour neovasculature, have recently been identified. The lead drug in this class Combretastatin A4 phosphate (CA4P). has completed Phasel clinical trial.These trials have demonstrated that blood flow shutdown can be induced within solid tumours in humans to a similar extent to that seen in preclinical models thus establishing proof of concept. Encouraged by these results we have continued to synthesise and evaluate a number of Combretastatins with the aim of identifying novel agents with improved therapeutic windows and possessing single agent activity. In the studies presented here we provide data on our lead preclinical compound which has emerged from this work and compare its antivascular and antitumour activity to CA4P in the murine breast adenocarcinoma CaNT. This compound designated Oxi4503 is the diphosphate prodrug form of Combretastatin A1. Our primary comparison was to evaluate vascular function within the tumours before and 24 hours after drug administration.At doses of 1mg/Kg Oxi4503 induced over a 50% reduction in functional vascular volume which increased to over 80% following doses of, 10, 25 and 50mg/kg. In contrast CA4P whilst inducing 50% vascular shutdown at 50mg/Kg caused no significant shutdown at 10mg/Kg. In addition to these vascular effects Oxi4503 at doses of 100, 200 and 400 mg/Kg induced significant retardation of tumour growth of established CaNT tumours. No significant growth retardation was obtained with single doses of CA4P upto 400mg/Kg. In daily times 5 dosing regime where some growth delay was obtained with daily doses of either 50 or 100mg/Kg CA4P a head to head comparison with Oxi 4503 indicated that the latter compound was 10 times more potent. In summary these studies have identified Oxi4503 as a preclinical development candidate with more potent antivascular and antitumour effects as a single agent. The mechanism responsible for this activity is not yet established but since the potency of the parent molecules CA4P and Oxi4503 against the putative target ie tubulin is similar, *in vivo* metabolism and pharmacokinetic mechanisms probably play key roles. Further preclinical evaluation of Oxi4503 is now ongoing with the aim to move the drug towards clinical evaluation.

115

Characterisation of the hollow fibre assay for the determination of tubulin interaction *in vivo*

M. Suggitt[1], D.J. Swaine[1], G.R. Pettit[2], M.C. Bibby[1]. [1]*Univ. of Bradford, Cancer Research Unit, Tom Connors Cancer Research;* [2]*Arizona State Univ., Cancer Research Institute and Dept. of Chemistry, AZ, USA*

The hollow fibre assay (HFA) is used routinely as a screening model for anticancer drug discovery by the National Cancer Institute (NCI). This study investigates whether the HFA can be used as a short term *in vivo* assay to demonstrate pharmacodynamic endpoints. In this instance interaction with tubulin and subsequent effects on cell cycle kinetics have been selected for study. A549 lung carcinoma cells were seeded into hollow fibres and implanted into NMRI mice for 5 days. Home Office guidelines for the welfare of animals were adhered to throughout the study. Paclitaxel (taxol) was administered intraperitoneally (i.p.) (20mg/kg) on day 4 post implantation. A pure population of A549 cells was retrieved from hollow fibres at 24 hours and analysed using flow cytometry. Results revealed taxol-treated cells to have a mean G2/M phase population of 48.% (i.p.) and 15.5% (s.c.) compared to untreated controls (6.8% and 5.4% respectively). These differences were statistically significant for both i.p. and s.c. sites ($p = <0.001$). Combretastatin A4 phosphate is showing interesting activity in early clinical trials. Here we have investigated a new analogue combretastatin A1 phosphate (CA1P). CA1P binds tubulin *in vitro*. CA1P was administered i.p. (150mg/kg), a previously determined effective dose, to mice bearing hollow fibres. CA1P-treated cells had a mean G2/M phase population of 36.3% (i.p.) and 29.4% (s.c.) compared to untreated controls (5.6% and 5.5% respectively). These differences were statistically significant for both i.p. and s.c. sites ($p = <0.001$). Additionally cells were retrieved from fibres and observed for disruption of microtubules using fluorescence and laser confocal microscopy. Paclitaxel (20mg/kg) induced the formation of spindle asters, a known hallmark of paclitaxel-induced tubulin damage, compared to untreated controls. CA1P was shown to block cells in mitosis compared to untreated controls. These data indicate that both taxol and CA1P induce a G2/M block in the A549 cell line when treated at their respective effective doses using the hollow fibre assay *in vivo*. Supportive evidence was provided from microscopy studies of tubulin morphology. In conclusion these data demonstrate that the HFA can be used as an *in vivo* tool for studying the effects of both standard and novel compounds on tubulin. This suggests that the hollow fibre assay can be utilised to demonstrate specific drug/molecular target interactions *in vivo*.

116

Interactions between vinblastine and cisplatin in EAT tumours in mice: schedule dependency

M. Cemazar[1], M. Auersperg[1], J. Scancar[2], I. Srebotnik Kirbis[1], A. Pogacnik[1], G. Sersa[1]. [1]*Institute of Oncology, Department of Tumor Biology, Ljubljana, Slovenia;* [2]*Jozef Stefan Institute, Ljubljana, Slovenia*

Combined chemotherapy schedules including cisplatin (CDDP) and various tubulin-binding agents are well-established chemotherapy combinations and are used for treatment of various malignancies. However, little attention is paid to timing of drugs or possible interaction of drugs in a particular combined schedule. Both these factors could be crucial for the clinical effect of chemotherapy. The increasing knowledge and understanding of molecular mechanisms of drug-induced cytotoxicity forms the basis for rational planning of clinical chemotherapy. Information on the *in vivo* antitumour efficiency of the combination of vinca alkaloids in animal tumour models, especially vinblastine (VLB) with CDDP is very limited. Therefore, the aim of our study was to explore whether antitumour schedule-dependency exists for the combination of CDDP and VLB on intraperitoneal (i.p.) Ehrlich ascites tumours in mice. Animals were treated three days after tumour transplantation with low doses of VLB (0.006 mg/kg) or CDDP (0.05 mg/kg) alone, VLB followed by CDDP and CDDP followed by VLB. The time interval between i.p. injections of the drugs was 24 h. Effects of therapies were evaluated 24 h after the second drug injection. Cell number was measured by counting viable cells using Trypan Blue exclusion assay, cell platinum content by electrothermal atomic absorption spectrometry, DNA distribution pattern using flow cytometry, apoptosis by flow cytometric TUNEL assay and cell morphology. Combination of CDDP and VLB resulted in additive interaction when VLB preceded CDDP as determined from cell survival data 24 h after completion of the therapy and in increased platinum content (2-times) compared to the same combination in a reverse schedule (CDDP given before VLB), which resulted in antagonism. None of the treatment combinations induced apoptosis. Both, CDDP and VLB caused marked changes in cell cycle distribution 24 h after the treatment. VLB increased