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.

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

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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.

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

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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*.

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

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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

the number of cells with DNA values greater than in G2M compartment of cell cycle, while CDDP reduced number of cells in G1 phase of cell cycle, slowed down the passage of cells through S phase with a block in late S phase. We propose that the observed increase in antitumour effectiveness is mainly due to higher platinum accumulation in tumour cells, which we unambiguously demonstrated by measurement of platinum content in the tumour cells, leading to increased cytotoxicity as well as to cell cycle dependent effects of VLB and CDDP.

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Apoptotic pathways and novel activity of the epothilone B analog bms-310705 in human non-small cell lung carcinoma (NSCLC)

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Novel semisythetic analogs of epothilone B (EPO-B) are potential chemotherapeutic agents for human NSCLC, due to their activity in paclitaxel (PCT) refractory tumors overexpressing P-glycoprotein or harboring tubulin mutations. In the present study, we have determined cell death mechanisms induced by the novel water-soluble semisynthetic analog of EPO-B, BMS-310705.The models used were derived from a patient with a primary lung lesion (NSCLC-3), and from a metastatic lymph node lesion (NSCLC-7) in a patient previously treated with chemotherapy and radiation. NSCLC-3 and NSCLC-7 were treated with BMS-310705 (0.01 -0.5 μM) for 1h, and evaluated for apoptosis and/or caspase activity. Apoptosis was detected by fluorescent microscopy after staining with Hoechst 33342 and propidium iodide. Caspase activity was determined by fluorimetric assay using target peptide substrates. In NSCLC-3 cells, BMS-310705- induced apoptosis (15 -70%) was dose dependent and was detectable as early as 24h and attained maximal values by 72h. In NSCLC-7 cells (10-fold resistant to PCT compared to NSCLC-3 cells) apoptosis was also detected, albeit lower (35% in NSCLC-7 versus 70% in NSCLC-3) at equimolar concentrations. Since the anti-apoptotic role of the transcription factor NF-kappa B may be involved in chemotherapy resistance, we investigated the apoptotic response in NSCLC-3 or NSCLC-7 cells transfected with pUSEamp/neo (control vector) or pUSEamp/ml kappa B alpha(S32A/S36A) dominant negative mutant. In stable transfectants of NSCLC-3 or NSCLC-7 cells, apoptosis was comparable in the neo or ml kappa B alpha(dominant negative mutant) cells. Apoptosis was initiated via the mitochondrial pathway based on release of cytochrome c and significant activation of the initiator caspase 9. Increased activity of the initiator and executioner, caspase 9 and caspase 3 respectively, were observed at 24 h. Our studies demonstrating the rapid and significant induction of apoptosis by BMS-310705, especially in NSCLC-7 resistant to PCT, is of considerable interest in view of results from our ongoing Phase I trial of BMS-310705, wherein partial responses were observed in a PCT pretreated ovarian cancer patient and in a patient with NSCLC who failed first-line platinum based therapy. Apoptosis induced by BMS-310705 is via the mitochondrial pathway and is unaffected by inhibition of NF-kappa B. In summary, BMS-310705 is a promising chemotherapeutic agent with activity in tumor models and patients refractory to PCT.

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Vascular-targeting activity of ZD6126 against primary pancreatic tumour growth and lymph node metastasis following orthotopic tumour cell injection in a nude mouse model

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ZD6126 is a novel vascular-targeting agent that acts by disrupting the tubulin cytoskeleton of endothelial cells. In immature endothelium, resultant morphological changes lead to the selective occlusion of tumour blood vessels and subsequent tumour necrosis. The anti-tumour effects of ZD6126 have been evaluated further in a mouse model of metastatic pancreatic cancer. Nude mice (n=3/group) were injected with 1×10^6 L3.6pl human pancreatic cancer cells into the pancreas. The mice received one of three treatment regimes 14 days post-injection: a single dose of ZD6126 (150mg/kg i.p.) or the cytotoxic agent gemcitabine (GEM: 100mg/kg i.p.) or a combination of both agents. The animals were sacrificed 24h post-treatment. H&E staining revealed extensive central necrosis in 2/3 pancreatic tumour samples following treatment with ZD6126 or combination therapy but not with GEM alone. In a longer-term experiment, nude mice (n=8 to 10/group) were treated 9 days after injection of 1×10^6 L3.6pl cells into the pancreas, with GEM alone (100mg/kg i.p. twice weekly), ZD6126 alone (75mg/kg i.p. 5 days per week), or a combination of both agents. Animals were sacrificed 21 days after the start of treatment. Compared with the average weight of control tumours (1320mg), tumours in treated animals reached an average weight of 687 (GEM), 541 (ZD6126) and 443mg (GEM + ZD6126). While lymph node metastases were present in 10/10 control and GEM treated animals, only 2/8 and 3/8 animals on ZD6126 or combination treatment displayed lymph node metastases, respectively. No significant differences in body weight, incidence of liver metastasis and wound tumours were seen between the groups. In the proliferating areas at the periphery of the tumour, microvessel density, as measured by CD31 staining and proliferation index (Ki67), were significantly reduced in primary pancreatic tumours treated with ZD6126 and combination therapy compared with controls or tumours treated with GEM alone. These data confirm previous observations of the anti-tumour effect of a single dose of ZD6126, resulting in necrosis of established tumours. Longer-term therapy with ZD6126 appeared to be well tolerated and resulted in a decrease in primary pancreatic tumour growth when compared with GEM alone. The effect was, however, more pronounced with combination treatment. Furthermore, ZD6126 induced a significant reduction of lymph node metastasis compared with control animals or animals treated with GEM alone.

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The novel vascular-targeting agent ZD6126 shows enhanced anti-tumour efficacy in large, bulky tumours

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The efficacy of the vascular-targeting agent ZD6126 was examined in rodent and human tumour models ranging in size from 0.1-2.0 g. Mice were injected i.p. with a 150 mg/kg dose of ZD6126 and response was assessed by morphologic and morphometric means as well as an *in vivo* to *in vitro* clonogenic cell survival assay. Both the extent of vascular shutdown and percentage of tumour necrosis induced were strongly dependent on the size of the tumours at the time of treatment, with larger tumours showing the most extensive effects. For example, the reduction in patent tumour blood vessels in KHT sarcomas following ZD6126 treatment was 10-20% in small (0.1-0.2 g) versus > 90% in large (> 1.0 g) tumours. Histological evaluation revealed that the extent of central tumour necrosis following ZD6126 treatment, while minimal in small KHT sarcomas, became more extensive as the tumour size increased. Clonogenic cell survival assessments made 24 h after ZD6126 exposure indicated increased tumour cell death, presumably as a result of prolonged ischaemia. This was quantifiable as a decrease in tumour surviving fraction from $\sim 3 \times 10^{-1}$ to 1×10^{-4} with increasing tumour size. Two other rodent tumour models (SCCVII, RIF-1) and three human tumour xenografts (Caki-1, KSY-1, SKBR3) showed a similar strong correlation between increasing tumour size and treatment effect. Since large bulky neoplastic disease is typically the most difficult to manage and ZD6126 previously has been shown in preclinical models to enhance the efficacy of both radiotherapy and cytotoxic drugs[1,2], these findings provide further support for the potential utility of ZD6126 as a tumour vascular targeted approach to cancer therapy.

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Biomarkers of *in vitro* response to HMN-176 in human ovarian cell lines

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HMN-176 is a novel drug from the stilbazole family, whose antitumor activity has been demonstrated in a broad spectrum of tumors in preclinical studies. HMN-176 rapidly induces microtubule polymerization in mitotic cells and increases the amount of cyclins. Its *in vitro* potency is comparable to that of cisplatin, doxorubicin, and etoposide. To evaluate drug effects on tumor biomarkers at the gene level, effects of HMN-176 on differential gene