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 $\beta_{III}$ 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 $\beta_{II}$ 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 $\beta_{III}$ 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 $\beta_{II}$ tubulin and $\beta_{III}$ tubulin in TE571. FB642 upregulated pan-β- tubulin and $\beta_{II}$ 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