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