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)

T. Mekhail[1], N. Takigawa[2], F. Lee[3], R. Peck[3], J. Skillings[3], D. Lebwohl[3], R. Bukowski[2], R. Ganapathi[2]. [1]*Hematology and Medical Oncology,* [2]*Cancer Center, The Cleveland Clinic Foundation, Cleveland;* [3]*Bristol Myers Squibb, Princeton, NJ, USA*

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

C.J. Bruns[1], G. Kohl[1], A. Kleespies[1], M. Friedrich[1], A. Ryan[2], A. Barge[2], K.-W. Jauch[1]. [1]*University of Regensburg, Department of Surgery, Regensburg, Germany;* [2]*AstraZeneca, Alderley Park, Macclesfield, UK*

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

D.W. Siemann[1], S. Lepler[1], C. Pampo[1], A.M. Rojiani[2]. [1]*Shands Cancer Centre, University of Florida, Department of Radiation Oncology;* [2]*H. Lee Moffitt Cancer Centre, University of South Florida, Departments of Interdisciplinary Oncology and Pathology, USA*

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

E. Izbicka[1], G. Carrizales[1], S. Kadapakkam[1], C. Scott[1], G. Piazza[1], M. Salunek M[2], R. Getts[2]. [1]*CTRC Institute for Drug Development, Molecular Targets, San Antonio, USA;* [2]*Genisphere Inc., Microarray Laboratory, Hatfield, USA*

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

expression were evaluated in drug-sensitive (A2780) and drug-resistant (A2780cp) ovarian carcinoma cells *in vitro* in total RNA extracted from the cells using Operon's Human Cancer OpArrays cDNA microarrays with optimized 70mers representing 1,154 known human genes. Data mining of gene expression in drug-treated versus untreated ovarian carcinoma cells has provided a database of genes induced and repressed by HMN-176. In A2780, HMN-176 had no significant effect on gene expression at 5 and 8 h. At 16 h, six genes including BTG family member 2, copine V, $p21^{waf}$, prostate differentiation factor were upregulated and four genes including survivin, lactate dehydrogenase A, TIMP1, and topoisomerase II were downregulated. In A2780cp, no genes were significantly affected at 8 h, and two genes (ras analog and telomerase) were downregulated at 16 h. By 24 h, three genes (including connective tissue growth factor and TIMP1) were upregulated. At this time point, 18 genes were downregulated, including prostate differentiation factor, cyclin G2, MAPK 9, and VEGF. Effects of HMN-176 on cyclin G2, topoisomerase II, and $p21^{waf}$ are consistent with a known mechanism of action of the drug. Upregulation of TIMP1 by HMN-176 in A2780 and A2780cp suggests that this gene may be a novel marker of drug response. Significant downregulation of cancer genes by HMN-176 in A2780cp suggests that HMN-176 could potentially overcome tumor drug resistance. Low numbers of genes were significantly affected by HMN-176, consistent with a specificity of the drug action. It is expected that upon further validation using *in vivo* human xenografts models, some of the molecular targets could serve as surrogate endpoints in ongoing clinical trials of HMN-176. Supported by Nippon Shinyaku and Cancer Center Council.

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Development of a sensitive and reliable LC-MS-MS assay to quantitate dimethyl benzoylphenylurea (BPU) in human plasma

M.A. Rudek[1], M. Zhao[1], Y. Zabelina[1], A.C. Wolff[1], S.D. Baker[2]. [1]*Medical Oncology,* [2]*Experimental Therapeutics, Kimmel Cancer Center at Johns Hopkins, Baltimore, USA*

Dimethyl benzoylphenylurea (BPU), a poorly water-soluble benzoylphenylurea derivative, inhibits tubulin polymerization *in vitro* with activity against solid tumors. BPU is currently being tested in Phase I clinical trials in the United States. There are no published methods to quantitate BPU in human plasma. A sensitive and specific method using LC-MS-MS has been developed for the quantitation of BPU in human plasma to perform pharmacokinetic (PK) and pharmacodynamic (PD) studies of BPU administered orally once a week. BPU is extracted from plasma into acetonitrile-n-butylchloride (1:4, v/v) and separated on a Waters X-TerraTM MS C18 (50 $\times$ 2.1 mm, 3.5 mm) column with 0.1% formic acid in acetonitrile/ 0.1% formic acid in water mobile phase (80:20, v/v) using isocratic flow at 0.15 mL/min for 5 min. The analyte of interest was monitored by tandem-mass spectrometry with electrospray positive ionization with a cone voltage 15 V for BPU and 30 V for the internal standard (IS, paclitaxel). The detector settings allowed the monitoring of the [MH]+ ion of BPU (m/z 470.3) and that of the paclitaxel (m/z 854.5), with subsequent monitoring of the daughter ions of BPU (m/z 148.0) and paclitaxel (m/z 286.1). Calibration curves were generated over the range of 0.05 to 10 ng/mL with values for coefficient of determination of $>$ 0.99. The values for precision ($<$20%) and accuracy ($<$15%) were well within the generally accepted criteria for analytical methods. Following administration of BPU 5 mg/m^2 as a weekly oral dose to a patient with advanced solid tumor malignancies, the maximum plasma concentration was 6.5 ng/mL and were quantifiable to 173 hours after administration. The LLOQ is 0.05 ng/mL and allows for successful measurement of plasma concentrations of BPU in patients receiving therapy with BPU as a once weekly oral dose.

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Final results of the phase I study of the novel epothilone BMS-247550 administered weekly in patients (pts) with advanced solid tumors

A. Awada[1], S. Jones[2], M. Piccart[1], S. Calvert[2], D. Crabeels[3], S. Mc Cabe[1], C. Holtkamp[3], D. Lebwohl[3], M. Voi[3], H. Burris[2]. [1]*Jules Bordet Institute, Chemotherapy Unit, Brussels, Belgium;* [2]*Sarah Cannon Cancer Center, Nashville, USA;* [3]*Bristol-Myers Squibb, Waterloo/Wallingford, Belgium/USA*

BMS-247550 (BMS) is a novel derivative of epothilone B which induces tubulin polymerisation and G2M arrest leading to apoptosis in cancer cells. BMS has preclinical activity in taxane-sensitive and resistant tumors. BMS was administered at doses of 1, 2.5, 5, 10, 20, 25 and 30 mg/m^2.

Results: During the dose escalation phase, 33 pts received 250 doses of BMS (median 6, range 1-31) administered as a 30-min infusion on a continuous weekly schedule. Grade 3 fatigue was the DLT at 30 mg/m^2. The 25 mg/m^2 dose level was subsequently expanded to include a total of 12 pts. Grade 3 toxicities at this dose level included fatigue (3 pts), and myalgia / arthralgia (2 pts). Cumulative sensory neuropathy/neuropathic pain (Grade 2: 4 pts / Grade 3: 1 pt) were also observed. In order to reduce neuropathy, the study was amended to explore a 1-hour infusion given weekly for 3 weeks followed by a one-week break. Forty pts are enrolled to date on this schedule: 26 pts at 25 mg/m^2, 10 pts at 20 mg/m^2, and 4 pts at 15 mg/m^2. At this time, pts treated at 25 and 20 mg/m^2 are evaluable. The toxicity pattern is similar to that of pts treated with 30-min infusion but more patients have been able to continue on therapy and to receive BMS cycles as scheduled with no missed doses or delayed cycle (92% and 78% for 20 mg/m^2 and 25 mg/m^2 respectively). Promising anti-tumor activity has been observed at both 20 mg/m^2 (3 pts with breast and 1 pt with ovarian cancer had $>$ 50% decrease in tumor markers) and 25 mg/m^2 (3 partial responses in patients with ovarian, colorectal and head and neck cancers). BMS administered over 60 min results in halving of Cmax as compared to 30-min, with comparable AUCs. An increase from baseline of median tubulin polymerisation in PBMC was observed. Accrual is ongoing at 15 mg/m^2 for a total of 8 pts in order to more fully explore BMS cumulative toxicity. Full clinical, pharmacokinetic and pharmacodynamic analysis will be available at the time of presentation.

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Phase I dose-escalating trial of KOS-862 (epothilone D) in patients with advanced malignancies

L. Rosen[1], F. Kabbinavar[1], P. Rosen[1], J.R. Hecht[1], M. Parson[1], G. Cropp[3], H. McDaid[2], J. Han[1], R. Johnson[3], A. Hannah[3]. [1]*UCLA Jonsson Comprehensive Cancer Center, Division of Hematology-Oncology, Los Angeles;* [2]*Albert Einstein College of Medicine, Molecular Pharmacology, Bronx;* [3]*Kosan Biosciences, Clinical Research, Hayward, San Francisco, USA*

KOS-862 (epothilone D) is one of a class of naturally occurring cytotoxic macrolides that stabilize microtubules and induce mitotic arrest. Epothilones bind to the same site as paclitaxel (PXL) in 1:1 stoichiometric ratio of a,b-tubulin heterodimers. KOS-862 was potent in PXL-sensitive lines (mean *in vitro* cytotoxicity IC_{50} 9-36 nM) and significantly more potent than PXL in multidrug resistant cell lines that overexpress P-glycoprotein (Chou et al, PNAS 2001). Target organ in toxicology studies (rat and dog) was bone marrow, with reversible neutropenia/anemia. Starting dose for first use in humans was 9 mg/m^2 (1/10 LD10 in the rat). Protocol objectives are to determine the toxicity and PK of escalating doses of KOS-862 administered every 3 weeks via IV infusion (150 cc/hour) to patients with advanced solid tumors. Groups of 3 patients were enrolled with doses doubled until Grade 2 drug-related toxicities, and a Fibonacci-based scheme thereafter. Plasma specimens (to 48 hours post-dose) were analyzed using LC/MS/MS (2-498 ng/mL quantification). Tubulin polymerization in PBMCs was evaluated (to 24-hours post-dose; Cycle 1&2) by immunohistochemistry. To date, 21 patients are enrolled (7 colon, 3 prostate, 3 sarcoma, 8 other) in 7 dose levels (9 - 150 mg/m^2); dose escalation continues. No dose limiting toxicity has been observed. Mild peripheral neuropathy (tingling/numbness; sometimes impaired gait) was observed in 1-2 patients per dose level (n=8 total). All episodes were Grade 1; some were self-limited, others persisted as Grade 1. Other potentially drug-related toxicities (mild-to-moderate severity) include: N/V (n=6); fatigue (n=5); rash (n=2); and alopecia (n=1). No neutropenia or thrombocytopenia has been observed; Grade1-2 anemia (n=8; only one new onset Grade 2) was seen. Preliminary PK results indicate linear, first order kinetics. PK parameters (n=21; mean$\pm$SD): clearance 13.4 $\pm$ 5.7 L/hour; elimination half-life 9.95 $\pm$ 2.6 hours; Vz =184 $\pm$75 L; $AUC_{0\rightarrow\infty}$ increased linearly with dose (r2=0.83). No dose dependence was observed. Between 9-120 mg/m^2, %maximal microtubule bundle formation vs. KOS-102 plasma concentration at end-of-infusion was linear (r^2=0.89). A patient with testicular cancer (10 cycles; escalated from 9 - 120 mg/m^2) showed a rapid decrease in AFP (83 to 22 ng/mL) and decrease in longest diameter of paraaortic lymph nodes (65 to 47 mm). KOS-862 is a promising novel epothilone; Phase 1 trials with alternate schedules are ongoing.