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

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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

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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

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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.