Materials and Methods: Epo-B was given as a 60-min infusion, 30 mins prior to a 30-min carboplatin infusion on day 1 q 21 days. Pharmacokinetic samples were taken on the first cycle. 25 patients were treated on this schedule at 4 dose levels (Epo-B doses of 30 + 40 mg/m2 + carboplatin AUC 5 + 6). Dose limiting toxicity (DLT) occurred at Epo-B 40mg/m2 + carboplatin AUC6 on this schedule with myelosuppression as the major toxicity. A further 14 patients were recruited onto an amended schedule with Epo-B doses split on days 1 and 8 q 21 days, carboplatin being given on day 1. DLT on this schedule was at carboplatin AUC 6 with Epo-B 25 mg/m2 d1 + 8. The preceding dose level (carboplatin AUC 6 + Epo-B 20 mg/m2 d1 + 8) was expanded and a total of 8 patients were treated.

Results: 39 patients (18 male/19 female/2 missing) were treated over 7 dose levels; 92% were WHO performance status 0 or 1 at entry; mean age 55 years (range 31-74). 56% patients had received prior chemotherapy (3 or fewer regimens) and 51% prior radiotherapy. The major toxicities were myelosuppression, myalgia and peripheral neuropathy. 73% of patients developed CTC grade 3/4 neutropenia during the course of their treatment, this was generally short lived and well tolerated, febrile neutropenia being reported in only 15% of cycles. 14% of patients developed grade 3/4 thrombocytopenia, despite using clinically active doses of carboplatin. Cumulative sensory neuropathy was observed, CTC grade 2 occurring in 23% of all patients and grade 3 in 18%. 11 patients withdrew from the study because of study drug toxicity, peripheral neuropathy was reported in all these patients. The regimen was active: partial responses were reported in 5 patients (2 breast, neuro-endocrine, unknown primary carcinomas and mesothelioma), 48% of patients showed disease stabilisation for more than 2 months.

Analysis of Epo-B plasma levels show that Cmax and AUC increase with dose, the volume of distribution is high and half-life is ~ 30 hours.

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Phase I studies with CERA (Continuous Erythropoiesis Receptor Activator), an innovative erythropoietic agent

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Background: CERA, an innovative erythropoietic agent with an extended serum half-life, has demonstrated a greater erythropoietic response than epoetin beta in animal models. Phase I studies were employed to investigate the pharmacokinetic and pharmacodynamic properties of CERA.

Materials and methods: Two single ascending dose (SAD) and two multiple ascending dose (MAD) studies were conducted in healthy volunteers (18-60 years). In the SAD studies, subjects were randomised to receive 1) single intravenous (IV) doses of CERA 0.4, 0.8, 1.6 or 3.2 μg/kg or placebo (n=38), or 2) single subcutaneous (SC) doses of CERA 0.1, 0.2, 0.4, 0.8, 1.6, 2.4 or 3.2 μg/kg or placebo (n=70). In the MAD studies, subjects were randomised to receive CERA 0.4, 0.8, 1.6 or 3.2 μ g/kg or placebo as 1) three doses IV at 3-week intervals (n=61), or 2) four doses SC at 2-week intervals (n=48).

Results: In the SAD studies, a potent dose-dependent erythropoietic response was observed with both IV and SC administration. Response to CERA was rapid; peak increases in reticulocytes occurred within 10 days and returned to baseline after 20 days. Mean reticulocyte response to CERA 0.4 μg/kg IV was increased by 119% vs baseline, suggesting that the minimum threshold for stimulation of erythropoiesis was less than the lowest dose used in the study. The highest study dose of 3.2 μg/kg IV produced a mean reticulocyte increase of 334%. The minimum threshold dose for stimulation of erythropoiesis after SC administration of CERA was 0.8 μg/kg, with a mean increase in reticulocytes of 262% at the highest dose. Soluble transferrin receptor levels also increased in a dose-dependent manner after both IV or SC injection, while serum ferritin and serum iron levels decreased. In the MAD studies, dose-dependent increases in reticulocyte response were also seen. Peak increases in reticulocytes occurred within 7 and 10 days for IV and SC dosing, respectively, and returned to baseline after 20 days. Repeated dosing did not appear to have a clinically relevant effect on the pharmacokinetics of CERA. CERA was generally well tolerated following both routes of administration in all studies.

Conclusions: In phase I studies, CERA demonstrated potent, prolonged dose-dependent erythropoietic activity when administered both IV and SC. Phase II studies of CERA administered in several extended dosing intervals to patients with cancer are ongoing.

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A phase I and pharmacokinetic study of oral administration of SU5416 in patients with advanced solid tumors.

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SU5416 is a small molecule inhibitor of VEGF-mediated signaling through Flk-1/KDR, a receptor tyrosine kinase expressed on endothelial cells. SU5416 suppresses tumor growth when administered intraperitoneally (25 mg/kg/day) or orally (100 mg/kg/day) in xenograft models. We conducted a study to assess the safety and bioavailability of oral SU5416 in patients with advanced malignancies. The oral formulation consisted of a Nanocrystal Colloidal Dispersion (NCD), the IV administration was a Cremophor-based liquid formulation. The recommended IV dose is 145 mg/m^2. Fourteen patients (8M/6F;median age 51) with advanced and extensively pretreated solid organ tumors were enrolled. All patients received a single dose of 75mg IV on Day 1 and oral SU5416 under one of four different treatment schedules (single, weekly, twice weekly, daily) starting on Day 8.

Thirteen patients (93%) experienced treatment-related adverse events; the most common side effects were vomiting (43%), nausea, injection site pain (29%), abdominal pain, injection site burning and pain NOS (21%). Four patients (29%) experienced adverse events with intensity severe or greater; one patient died during the study due to fatal asphyxia. No clinically significant laboratory abnormalities were observed.

Following IV administration of SU5416, peak SU5416 concentrations were generally observed 15 minutes after the beginning of the infusion. Thereafter, the concentration declined with a mean half-life of 43.5 minutes. The IV pharmacokinetics (PK) was very similar to those observed in previous studies. Oral SU5416 revealed generally lower plasma concentrations during Days 15, 21/22 as compared to Day 8. By Day 15, a number of patients SU5416 concentrations were below the level of detection. Mean concentrations (C) of oral SU5416 were approximately 12-fold lower than those observed following IV infusion. C following a single oral dose of NCD SU5416 were seen at 67 minutes. The mean bioavailability for a single dose and weekly, twice weekly and daily dosing of NCD SU5416 on Day 8 were 18.9%, 20.6%, 20.7% and 36.8%, respectively. However, there was a large interpatient variability ranging from 1.3% to 68%.

Further development of oral NCD SU5416 was not pursued, because it was unlikely that effective plasma concentrations could be achieved with it. This could most likely be due to clearance induction of SU5416. The study is part of the basis for the decision not to pursue the development of oral SU5416. It confirmed the difficulty to achieve constant plasma concentrations with this drug. The study revealed important information for the future development of this class of compounds.

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A phase I study to determine the safety and pharmacokinetics of intravenous administration of SB715992 on a once weekly for three consecutive weeks schedule in patients with refractory solid tumors.

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Mitotic kinesins play exclusive and essential roles in assembly and function of the mitotic spindle and represent novel targets for development of therapeutics against cancer. SB-715992 is the first mitotic KSP inhibitor to enter clinical trials. SB-715992 is a unique and potent inhibitor of KSP (HsEg5), a mitotic kinesin that is essential for assembly of a functional mitotic spindle and is preferentially overexpressed in malignant cells. SB-715992 is 70,000-fold more selective for KSP than other members of the kinesin family, and disrupts the assembly of functional mitotic spindles, thereby causing cell cycle arrest in mitosis and subsequent cell death. Since KSP functions exclusively in mitosis and is not expressed in terminally differentiated neurons, SB-715992 is not expected to produce neurotoxicity. However, these preclinical findings need to be confirmed in clinical studies. In preclinical efficacy studies in a broad range of human tumor xenografts, doses of SB-715992 substantially below the maximum tolerated dose produce prominent growth inhibition, tumor regression, and cures. In the current study, toxicity, feasibility and pharmacology of SB-715992 administered as an IV infusion once a week for 3 consecutive weeks is being evaluated in pts with advanced solid malignancies. At least 2 pts are being treated at each dose level, and doses are escalated from the

starting dose of 1 mg/m^2 (1/6th of the canine TDL) in increments as high as 100% using an accelerated titration design. Thus far, 5 pts (median age 61.5 yrs, range 60-70 yrs; tumor types: 2 breast, 1 gastric, 1 mesothelioma, 1 RCC) have received 6 courses at 1 and 2 mg/m^2. No clinically significant toxicities have been noted to date. Preliminary results of pharmacokinetic studies indicate AUC(0-inf) values of 302.7 ng.hr/ml (n=1), Cmax values of 28-39 ng/ml (56-78 nM) at 1 mg/m^2 (n = 3) and a half-life value of 43 hr at 1 mg/m^2 (n = 1). Updated experience on safety, tolerability and pharmacokinetics will be presented.

571 POSTER

Novel mechanisms of bisdioxopiperazine resistance

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The DNA topoisomerases are essential nuclear enzymes capable of modulating DNA tertiary structure. The bisdioxopiperazines with their highly specific mechanism of catalytic inhibition of topoisomerase II (topoII) have been the subject of much interest since they were shown in 1991 to target this enzyme through sequestring topoII to DNA in a closed clamp formation. Five newly selected SCLC (small-cell lung cancer) subcultures resistant to ICRF-187 were established in our laboratory, of which three contained mutations in the Walker A motif of topoIIa. The remaining two subcultures, NYH/187/pp-1 and NYH/187/pp-2 present with no detectable mutations in their topoIIa cDNA, protein levels of topoIIa are unchanged and no cross resistance to other drugs is observed, indicating specific mechanisms of resistance towards bisdioxopiperazines. Also, drug accumulation levels are unaltered. Remarkably, both resistant cell lines are characterized by DNA polyploidization, and cell volumes are twice that of the parental cell line. Thus, alternative mechanisms of bisdioxopiperazine resistance may be in force in these subcultures. Cellular DNA content analysed by flow cytometry reveals a 50% increase in DNA for pp-1. pp-2 appears to be composed of two subpopulations, of which one takes over as resistance is lost with time, when grown in the absence of drug. This non-resistant passage of pp-2 resembles resistant pp-1 in DNA content, hence the aneuploid pattern in itself seems not to be functionally linked to resistance. Recent studies demonstrate that deficiency in G2 or postmitotic checkpoint responses can cause resistance to bisdioxopiperazines, which normally arrest cells in G2 due to activation of the recently described decatenation checkpoint. The possibility of changes in cell cycle checkpoint control being responsible for acquired bisdioxopiperazine resistance in pp-1 and pp-2 cell lines were therefore investigated. Flow cytometric analysis indeed reveals the absence of a bisdioxopiperazine induced G2 arrest in pp-1, but not in pp-2. However, expression levels of proteins involved in the decatenation checkpoint are unchanged. On the contrary, the checkpoint kinases (Chk1 and Chk2) inherently involved in DNA damage and replication checkpoints are found to be constitutively activated by phosphorylation at ser345 and thr68 respectively, as shown by western blotting using phospho-specific antibodies. This however causes no G2 arrest in either cell line, as both proliferate at normal rates in the absence of drug and Chk is constitutively activated in both cell cycle deficient and proficient cells. Alternatively, other downstream responses to Chk activation may be operating to enhance cellular survival of pp-1 and/or pp-2. Indeed, Chk phosphorylation vanishes as pp-2 looses resistance, indicating a functional involvement of the phosphorylation observed in this cell line. Also, as pp-2 looses drug resistance over time it maintains a polyploid DNA pattern, which suggests that Chk activation is not merely the cellular response to the altered DNA constitution. As pp-1 was found to be refractory to ICRF-187 mediated inhibition of decatenation activity, there might be no signal for a G2 arrest in this cell line. Thus, drug resistance in these cell two cell lines appears to be due to alterations in signals involving the checkpoint kinases rather than changes in bisdioxopiperazine sensitive G2 checkpoint responses.

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Kahalalide F (KF), a new marine compound, in vitro radiosensitizes human tumoral cell lines.

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Purpose: To evaluate in vitro radiosensitizing properties, cell cycle changes and apoptosis induced by KF in a panel of tumoral cell lines.

Method and Materials: We used 5 tumoral cell lines: DU145, HeLa, HT29, HN30 and HOP62. In vitro chemosensitivity was assayed by crystal violet method. The IC10 and IC50 were calculated for 1 h, 24 h and 7 days (continuous exposure). Radiosensitization was evaluated by conventional colony assay and the sensitizing enhancement ratio at 2 Gy (SER) was calculated. BrdUrd DNA-labelling and flow cytometry were used to analyze cell cycle distribution. The amount of apoptosis was calculated by annexyn-V labelling.

Results: Mean IC50 were 3.4 microM (0.78-4.8), 1.7 microM (0.48-4.1) and 1.8 microM (0.4-4.9) for 1 h, 24 h and 7 days, respectively. Most sensitive cells were HT29 (IC50: 0.5 microM at 24 h) and HN30 (IC50: 0.48 microM at 24 h). In the time-course experiment there were no benefits of continuous exposure beyond 24 h. A dose-dependent radiosensitization was observed in all cell lines with a SER of 1.4, 1.87, 1.3, 2.7 and 1.6 at IC50 of continuos exposure doses for DU145, HeLa, HN30, HOP62 and HT29, respectively. A low level of apoptosis was observed in HeLa and DU145 cells, presenting after 48 h of drug exposure. After treatment with KF cells cumulated in G0-G1 phase in all cell lines.

Conclusions: KF is a promising radiosensitizing drug whose potential use should be further investigated in the experimental and clinical setting.

573 POSTER

Safety profile for yondelis (ET-743) 1.3 mg/m^2 over 3 hours (h)

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Aim: ET-743 has shown activity against STS and ovarian cancer. ET-743 1.5 mg/m^2 over 24 h has been extensively investigated and its safety profile characterized. In the present study, we describe the safety profile of ET-743 1.3 mg/m^2 (3 h) given as an initial dose, which is the recommended dose. In addition, we compare the risk of developing most frequent toxicities with 24 h vs 3 h infusion.

Material and Methods: Sixty-six patients were treated with ET-743 1.3 mg/m^{2}over 3 h in four phase II clinical trials addressed to Soft Tissue Sarcoma, Ovary and Non Small Cell Lung Cancer. 184 cycles have been evaluated. Patients received corticosteroid treatment day 1 to day +2. This data was compared to a cohort of 205 patients and 788 cycles from 9 different phase II clinical trials using ET-743 at 1.5 mg/m^2. Relative risk (RR) of developing grade (g) 3-4 neutropenia, thrombopenia, AST, ALT and Alkaline Phosphatase (AP) as well as g1-4 nausea, vomiting, fatigue or febrile neutropenia was calculated for ET-743 1.5 mg/m^2 (24h) vs ET-743 at 1.3 mg/m^2 (3 h).

Results: see table.

	Per patient		Per cycle	
	G3	G4	G3	G4
Neutrophils	9 (13.6%)	9 (13.6%)	16 (8.9%)	10 (5.6%)
Platelets	5 (7.7%)	2 (3.1%)	5 (2.8%)	2 (1.1%)
Hemoglobin	3 (4.6%)	1 (1.5%)	4 (2.2%)	1 (0.6%)
AST	28 (43.1%)	1 (1.5%)	35 (19.6%)	1 (0.6%)
ALT	40 (61.5%)	9 (13.8%)	65 (36.3%)	11 (6.1%)
Creatinine	0	2 (3.1%)	0	0
CK	0	0	0	0
Bilirubin	2 (3.1%)	0	2 (1.1%)	0
Alk Phosphatase	1 (1.5%)	0	1 (0.6%)	0

Other adverse events were: grade 3-4 vomiting 4.5%, g1-2 fatigue 28.8%, g3 fatigue 4.5% and febrile neutropenia3%. One (1.5%) drug-related death occurred. RR per patient of ET-743 1.5 mg/m^2 (24 h) vs ET-743 (3 h) for developing grade 3-4 neutropenia was 1.86 [IC 95% (1.23-2.82)], for