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.

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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

platelets 1.04 [IC 95% (0.47-2.32)], for AST 0.85 [IC 95% (0.61-1.17)], for ALT 0.63 [IC 95% (0.51-0.77)] and for AP 1.57 [IC 95% (0.19-13.2)]. The RR of developing g1-4 nausea was 2.41 [IC 95% (1.61-3.62)], vomiting 1.78 [IC 95% (1.11-2.86)], fatigue 1.81 [IC 95% (1.27-2.60)] and febrile neutropenia 1.45[IC 95% (0.32-6.54)]

Conclusions: The toxicity profile of ET-743 1.3 mg/m^2 over 3 h plus the administration of corticosteroid treatment day -1 to day +2 seems safe, being neutropenia, thrombopenia and reversible and not cumulative aminotransferases increase the principal laboratory toxicities.

574 POSTER

Pharmacokinetics and metabolism of CPT-11 (Campto®) combined with capecitabine (Xeloda®) in patients with advanced colorectal cancer: altered disposition of the metabolites SN-38 and SN-38 glucuronide?

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The plasma concentrations of irinotecan (CPT-11) and its metabolites SN-38 and SN-38gluc were evaluated in 10 patients who were treated with a regimen of Camptoő / Xelodaő against advanced colorectal cancer (paired cross over, Camptoő monotherapy versus Camptoő + Xelodaő schedule). Both cytostatics are prodrugs which have to be bioactivated by liver carboxyesterase to the cytotoxic agents.

Samples were first analysed under acidic conditions to calculate the total amount of **CPT-11** and its metabolites in blood and second under neutral conditions for information of the carboxylate lactone equilibrium of CPT-11 and SN-38. Capecitabine (CCB) did not alter the mean CPT-11 plasma concentrations, only small differences ranging from 4% to + 16% could be found ($p < 0.55$). Noncompartment pharmacokinetic analysis confirmed the results of plasma data: no statistically significant change of cmax, AUC, Vd, Cl and MRT could be observed in the combination schedule.

Contrary to CPT-11 disposition, mean **SN-38** plasma concentrations seemed to be altered in the CCB group of the study. Differences of SN-38 concentrations in the combination treatment (compared to the control arm) were strongly time - dependent: per cent difference increased from -53% at 15 min to + 23% at 300 min after start of infusion ($p < 0.005$, corr = 0.981). For SN-38gluc, a very similar effect was evaluable: from - 39% at 15 min to + 6% at 300 min ($p < 0.027$, corr = 0,959). Analysis of lactone versus carboxylate forms revealed that this effect might base on lower lactone concentrations of SN-38, when CCB was coadministered.

After acidic analytical conditions, PHK parameters of **SN-38gluc** seemed to be unaffected by CCB (noncompartment PHK model). But the apparent formation - rate of SN-38gluc was delayed by CCB significantly: t1/2 appin = 25.0 ± 9.6 min versus 42.3 ± 12.5 min in the CCB group ($p < 0.004$). Accordingly, tmax occurred later: 108.0 ± 32.2 min versus 150.0 ± 31.6 min ($p < 0.016$); cmax was slightly lower (not significant). From our in-vitro results we know that there exists a certain potential of drug-interaction between CPT-11 and CCB. Even small changes in the disposition of SN-38 (activation pathway) and SN-38gluc (detoxification pathway) may have pharmacological consequences in CPT-11 chemo-therapy.

Detailed **in-vivo and in-vitro results** (including drugs of premedication as tropisetrone and dexamethasone) are presented and discussed (studies ongoing).

575 POSTER

Dose finding study of oral vinorelbine (VRL) in combination with capecitabine (CAP) in patients with metastatic breast cancer (MBC)

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Background: VRL and CAP are active in MBC and are often combined with various cytotoxics. Both are orally available, have different mechanisms of action and involve carboxylesterases in their metabolism.

Material and methods: We aimed at determining the maximum tolerated dose (MTD) and the recommended dose (RD) of oral VRL given on days (D) 1 and 8 at 60 or 80 mg/m^2 and CAP from D1 to D14 at doses ranging from 1650 to 2500 mg/m^2/d every 3 weeks. At the RD, a weekly schedule of oral VRL is to be evaluated. Pharmacokinetics of VRL, CAP and metabolites are determined on D1 and 7 of cycle 1 to study putative mutual interaction. Dose limiting toxicities (DLTs) are defined during the first cycle as grade (gr) 4 neutropenia for 7 days, gr 3 thrombocytopenia, febrile neutropenia, neutropenic infection, one week-delay in starting cycle 2 due to toxicity, gr 3/4 non-haematological toxicity except asthenia, inadequately treated nausea/vomiting or diarrhoea. Cohorts of 3 to 6 patients are treated per dose level (DL). DLT in 2 pts in any DL determines the MTD.

Results: To date 24 pts were included in 5 DLs. Age ranged from 31 to 66 years. Main metastatic sites were visceral (83%) and bone (38%). Fourteen pts had 2 or more organ involved. DL1 (60 VRL + 2000 CAP) was well tolerated without gr 3/4 event in 3 pts and 13 cycles. MTD was reached at DL3 (60 VRL + 2500 CAP) and DL4 (80 VRL + 1650 CAP): DLTs consisted in persisting neutropenia which resulted in delay in starting cycle 2 for 5 pts (gr 2, gr 3 and gr 4 neutropenia each in 1 pt at DL3, and, at DL4, gr 2 neutropenia in 2 pts) and febrile neutropenia in 1 pt. DL2 (60 VRL + 2250 CAP) was a RD. As per protocol a weekly schedule of 60 VRL + 2250 CAP was tested and MTD (gr 2 neutropenia on D21 in 1 pt, gr 3 thrombocytopenia concomitant with gr 3 neutropenia in another pt) was reached.

Gr 3/4 toxicities among 31 cycles at DL2 were one episode of gr 3 diarrhoea (2 pts) and, in 1 pt each, gr 4 bilirubin, gr 3 nausea and gr 3 wound infection while haematological events consisted in gr 4 neutropenia (2 pts, 2 cycles) and gr 3 leucopenia once in 1 pt. To date, 1 CR and 3 PRs are confirmed in the study population. Drug-drug interaction has not been suspected up to now.

Conclusions: Oral VRL 60 mg/m^2 on D1 and 8 and CAP 2250 mg/m^2 from D1 to D14 every 3 weeks is currently the RD. The weekly administration of oral VRL 60 mg/m^2 and CAP 2000 mg/m^2 every 3 weeks and a 4-week regimen are now being investigated.

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Phase I dose-finding study of the combination of alimta (pemetrexed) and paclitaxel in patients with solid tumors

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Background: Pemetrexed, a novel multitargeted antifolate, has activity in mesothelioma, NSCLC, breast and colon cancers. Paclitaxel exerts its anti-neoplastic effect via disruption of microtubule assembly and also has activity in a variety of solid tumors. This reports on one schedule of 3 different sequences evaluated in a phase I study of pemetrexed in combination with paclitaxel in patients with advanced malignancies.

Patients and methods: The primary study objective was to determine the maximum tolerated dose (MTD) of the combination; secondary objectives included determination of dose-limiting toxicity (DLT) and recommended doses for phase II study. DLT was defined as the occurrence of * 1 of the following during cycle 1: Grade (G) 4 neutropenia lasting * 5 days (d), febrile neutropenia, G4 thrombocytopenia, or G3 non-hematologic toxicity (except G3 nausea, vomiting, and transaminase elevation). Paclitaxel was infused over 3 hours on d1 and d8 of a 21d cycle; standard taxane premedications were also administered. P emetrexed was infused over 10 minutes on d8 prior to paclitaxel; oral folic acid and parenteral vitamin B_{12} were also administered to reduce pemetrexed toxicity.

Results: Twenty-one patients (15 men, 6 women) with a median age of 59 (range, 34-77) and a WHO performance status 0/1 (90%) were enrolled and treated as described below. Tumor types represented in this study include: pancreas (4), esophagus (3), colorectal (3), lung (3), liver (2), head and neck (1), melanoma (1), and other (4). 12/21 patients had received prior chemotherapy. 71 cycles were administered with a median 3 cycles (range, 1-10). There were no dose reductions or omissions. 17/25 dose delays were due to scheduling conflicts; myelosuppression (5), nasopharyngitis

Pemetrexed/ paclitaxel (mg/m^2)	Cohort (#pts)	DLT (# pts)	Other Clinically Significant Toxicity (#pts)
400/30	1 (6)	G3 bilirubin (1)	G4 neutropenia (1) G3 anemia (1)
500/30	2 (6)	G4 thrombocytopenia + + G4 febrile neutropenia + G3 edema (1)	G3 asthenia (1) G3 bilirubin (1)
500/40	3 (6)	G3 bilirubin + G3 alkaline phosphatase (1)	G3 hyperglycemia (1) G3 thrombocytopenia (1) G4 hemorrhagic ulcer (1)
500/50	4 (3)	None	G3 fatigue (1) G3 nausea (1) G3 transaminitis (1)