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.

572 POSTER

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