to assess for pharmacokinetic (PK) interactions when DX-8951f is given concurrently with gemcitabine, (4) to identifiy, preliminarily, evidence of antitumor activity, and specifically in a cohort of patients with pancreatic cancer. Between 5/00 and 10/01, 70 patients were enrolled at 3 academic centers. 36 men (51%), 34 women (49%). Median age, 58 years, range 26-81, Median ECOG 1, range 0-2. 67% of patients had no or up to one prior chemotherapy regimen (minimally pretreated, MP). 33% were deemed to be heavily pretreated (HP), having had significant prior chemotherapy or radiation exposure. Patients were accrued seperately to MP and HP cohorts. 39 (56%) had pancreatic cancer, 11 (16%) colon cancer, and the remainder a variety of other solid tumors. In the MP cohort, dose-limiting toxicity was identified at the 2.5 mg/m^2 DX-8951f and gemcitabine 1,000 mg/m^2 cohort. Myelosuppression,(neutropenia more than thrombocytopenia), was the principal toxicity. There were 10 episodes of fever and neutropenia. There was one treatment-related death. Grade 3-4 non-hematologic toxicities were uncommon, while the Grade 1-2 included fatigue, nausea, and vomiting. In general, the combination was well-tolerated and both drugs could be given at full dose intensity. The recommended combination doses are 2.0 mg/m^2 DX-8951f and gemcitabine 1,000 mg/m^2. Significant antitumor activity was seen at all dose levels, including, partial and complete responses in pancreatic cancer and heavily pretreated ovarian and GE junction cancers. In the pancreatic cohort (N=39), there were 2 CR's (5%), 4 PR's (10%), 2 MR's (5%) and 19 (49%) with stable disease. In 27 patients with previously untreated pancreatic cancer, the median time to progression and median survival time were 6.3 and 8.4 months respectively. The combination of DX-8951f is active, safe and very tolerable. Complete PK, toxicity and response data will be presented. An ongoing phase III trial is comparing the combination (DX-8951f 2.0 mg/m^2, gemcitabine 1,000 mg/m^2) to gemcitabine alone in advanced pancreatic cancer.

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Open label phase II study on RFS 2000 in advanced/metastatic urothelial tract tumors

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Background: RFS 2000 is a direct, highly potent inhibitor of the DNA-religating activity of topoisomerase-I. The aims of this study were to evaluate the activity and safety profile of RFS 2000 as a second line chemotherapy in patients with advanced /metastatic urothelial tract tumors.
Patients and methods: Eligible patients were to have failed first line treatment for advanced/metastatic disease (MVAC or other treatment). Patients received RFS 2000 as one daily oral intake at the dose of 1.5 mg/m^2/day according to a 5 days on/2 days off schedule continuously. One cycle was arbitrarily defined as a 3 week period. Patients were required to have adequate oral hydration to prevent cystitis previously described in phase I trials with RFS2000. Gehan design was used for sample size determination. Drug activity was evaluated according to the RECIST criteria and toxicity according to CTC version 2.
Results: A total of 21 patients (pts) were entered from June 2000 to September 2001, and all pts received treatment. Male/female/no data: 14/5/2 with median age 67 years (range: 43-78). 8, 11, and 2 patients had performance status 0, 1, 2 respectively. Data are validated for 19 patients who received 50 cycles (median 2; range 1-8). Safety profile: 8 patients developed neutropenia Grade (G) 3-4 which was complicated with febrile neutropenia in 3 patients and lead to treatment interruption in 2 of them, 4 thrombocytopenia G3-G4 and 4 anemia G3-4. Other G 3-4 adverse reactions were: 4 fatigue, 5 diarrhea, 2 nausea and 2 vomiting. 1 hematuria G3 was considered partially related to tumor progression and to RFS 2000 toxicity. To date, among 19 patients documented for activity data, 1 confirmed partial remission (5.2%, 95%CI: [0.1-26]%) and 6 stable disease were reported. 16 patients stopped treatment for progressive disease and 3 patients for hematological toxicity.
Conclusion: RFS 2000 administered orally as a 5 days on/2 days off schedule continuously shows modest activity in patients with advanced/metastatic urothelial tract tumors who failed first line therapy. Hematological toxicity requires careful monitoring and the occurrence of diarrhea G3-4 confirms similar observations in other studies. This trial is now closed and full data analysis will be available at the time of presentation.

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Conserved mechanism of CDC45 function in protecting yeast and human cells from DNA damage

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Camptothecin (CPT) reversibly stabilizes a covalent intermediate formed between DNA topoisomerase I (Top1) and a 3' phosphoryl end of DNA. Replication fork collisions with CPT-Top1-DNA complexes produce the DNA lesions that induce cell cycle arrest and cell death. As cellular pathways that recognize and repair Top1-DNA lesions are poorly understood, a yeast genetic screen was designed to isolate temperature sensitive mutants with enhanced sensitivity to a self-poisoning Top1T722A mutant that mimics the cytotoxic mechanism of CPT (Reid R et al, 1999 PNAS 96:11440-5). A novel set of TAH genes were defined that function to protect yeast cells from CPT-induced lethality, including the essential CDC45 gene whose function is required for the initiation of DNA replication. The cdc45-10 mutant exhibits a partial loss of function- DNA replication initiation appears to be unaffected, but processive replication is delayed in early S-phase. Viability is unaffected in the absence of DNA damage; however, cdc45-10 mutant cells exhibit enhanced sensitivity to hydroxyurea and UV (independent of Top1) and to CPT (in a Top1-dependent manner). Thus, alterations in processive DNA replication potentiate Top1 poison toxicity. To address the conservation of Cdc45 function, human hCDC45 was PCR amplified from untransformed human IMR90 fibroblast cDNA and expressed in yeast. Constitutive expression of hCDC45 failed to complement cdc45-10 cell hypersensitivity to DNA damaging agents, yet galactose-induced overexpression of hCDC45 restored cdc45 deletion cell viability. This suggests that the essential function of Cdc45 in replication initiation is distinct from the defect in cdc45-10 cells that enhance CPT toxicity. hCDC45 function in modulating cell sensitivity to Top1 poisons was addressed using siRNA to down regulate hCDC45 levels in IMR90 cells. At sub lethal topotecan concentrations, CDC45 siRNA induced a dramatic S-phase arrest and a decrease in cell number. No alterations in cell cycle distribution or cell number were observed in drug-treated cells without siRNA. This mirrors the response of yeast cdc45-10 cells, supporting a conserved mechanism of CDC45 protection against CPT-induced DNA lesions. As deletion of the RAD9 DNA damage checkpoint exacerbates the cdc45-10 mutant phenotype, future studies will focus on hCDC45 down-regulation in the absence of the p53 checkpoint in isogenic IMR90 cell lines. This work is supported by NIH grant CA70406 and ALSAC.

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Antiangiogenic potential of the novel camptothecin ST1481

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Camptothecins are potent cytotoxic agents and their antitumor activity has been ascribed to stabilization of a covalent DNA-topoisomerase I complex. In addition to the direct cytotoxic effect, camptothecins have been described for possessing antiangiogenic effects. ST1481 is a new lipophilic camptothecin active by oral route, which, based on its favorable preclinical profile, is currently in clinical development. Aim of the study was to investigate the antiangiogenic effects of ST1481, delivered by oral route, in a subcutis growing human tumor xenograft, the A549 NSCLC, which is only moderately sensitive to the agent delivered by an intermittent schedule (q4dx4, MTD 2 mg/kg), but sensitive to a prolonged daily low-dose (0.5 mg/kg) treatment. A tumor volume inhibition of 67 and 83% was achieved by ST1481 administered q4dx4 or daily, respectively ($p < 0.05$, by Student's t test). Immunohistochemical analysis showed a reduced number of microvessels (CD31+ cells) in tumors of both treated groups versus controls, and a significantly higher reduction in the daily versus the q4dx4-treated tumors ($P < 0.0001$, by Student's t test). In our experimental model, microvessel density and tumor volumes were related ($r = 0.738$, by Spearman rank test). ST1481 resulted more potent than topotecan in inhibiting endothelial cells growing *in vitro*. In conclusion, the study showed that ST1481 has a significant antiangiogenic potential in *in vivo* systems and that such effect is enhanced with a daily prolonged administration of low drug doses.