had a median of 3 (0–5) prior chemotherapy lines. Colorectal cancer was diagnosed in 4 patients, breast and prostate cancer in 2 patients each, and 10 patients had other solid tumours. The starting dose level was 0.15 mg per day for 5 consecutive days once every 3 weeks, preceded by the same dose as iv infusion 2 weeks before the first oral administration in order to assess the absolute bioavailability of oral diflomotecan. The increases were done in 0.05 mg steps. Up to the dose of 0.35 mg 3 patients per dose level were treated for 1–8 cycles per patient (median 4+). At the maximum tolerated dose (MTD) of 0.35 mg, 2 patients experienced dose limiting toxicities (DLT): one grade 4 neutropenia for more than 7 days, and one cycle delay due to prolonged neutropenia. At the recommended dose (RD) of 0.30 mg, the cohort was extended up to 6 patients and no DLT was observed. One patient treated at the dose of 0.25 mg experienced a toxic death due to infection with grade 4 neutropenia at cycle 2. Five serious adverse events related to diflomotecan were reported in 3 patients: 1 patient had grade 3 fatigue, 1 patient grade 4 infection with neutropenia and 1 patient experienced 3 episodes of anaemia (two grade 2 and one grade 3) In18 patients, grade 3/4 haematotoxicity was reported as follows: neutropenia (8 patients), anaemia (6 patients) and thrombocytopenia (2 patients). Out of 16 patients, study drug related grade 3/4 adverse events were infection with grade 4 neutropenia, vomiting and fatigue (1 patient each). Study drug related grade 1/2 toxicities were fatigue (8 patients), vomiting (7 patients), nausea (6 patients), anorexia and alopecia (5 patients each), infection (4 patients), dypsnea (2 patients), diarrhoea, constipation, abdominal pain, weight loss, depression and cardiac pain (1 patients each)..

Regarding the pharmacokinetic results at the oral RD, the Tmax at day 1 was 1.06 hour and at day 5 1.50. $T_{1/2}$ was around 3 hours at both days, and the AUC was 9.69±3.63 at day 1 and 6.94±3.50 at day 5. Bioavailability was 95% at the RD of 0.30 mg.

One patient with breast cancer treated at RD achieved a partial response, 11 patients had stable disease (5 at RD), and 5 patients had progressive disease after 2 cycles.

These preliminary results are promising, and the second part of the study which investigates food interaction is ongoing. The oral route of diflomotecan administration may be a more convenient way for patients to receive chemotherapy.

504 POSTER

Phase I study of CT-2106 (polyglutamate camptothecin) in patients with advanced malignancies

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Background: CT-2106 is a novel camptothecin (CPT) conjugate in which CPT is bound to a biodegradable water-soluble poly-L-glutamic acid-glycine polymer. CPT-polymer conjugation allows for greater stability of CPT in circulation and enhanced permeability and retention in tumor tissue. CT-2106 has demonstrated anti-tumor activity in several human tumor cell lines in vivo. **Methods**: To determine the maximum tolerated dose (MTD) and evaluate the pharmacokinetics (PK) of CT-2106, 31 pts were treated with a 10-minute IV infusion every 21 days. Toxicity was assessed according to NCI CTC v2. PK samples (cycles 1 and 2) were analyzed for conjugated and unconjugated CPT levels by validated HPLC/FD methods. Cohorts of pts received conjugated CPT doses of 12, 25, 50, 75, 90, or 105 mg/m^2. **Results**: Dose-limiting toxicities (DLTs) included: grade (g) 3/4 neutropenia, thrombocytopenia, and mucositis. One pt experienced a g4 cholinergic reaction and esophageal spasm; this pt had previously experienced a severe reaction to irinotecan. Other related toxicities were g3 increased ALT and ⩽g2 anemia, anorexia, dysgeusia, peripheral sensory neuropathy, fatigue, nausea, diarrhea, vomiting, abdominal pain, alopecia, rash, decreased hemoglobin, and hematuria. No g3/4 hematuria or diarrhea was observed. Using standard response criteria, 1 pt with metastatic pancreatic cancer had a partial response, 2 pts with NSCLC had stable disease (SD) for >35 weeks, and 2 pts with colon cancer had SD for >9 weeks. Preliminary PK parameters calculated from 18 pts treated at 25, 50, 75, or 105 mg/m^2 demonstrated sustained levels of conjugated CPT in systemic circulation, with mean elimination half-life from 16.6 to 50.8 hrs. Cmax and AUC of conjugated CPT increased linearly with dose, suggesting PK linearity. Unconjugated CPT levels suggest that this active form of the compound is generated by a slow, progressive release from the polymer following the distribution of conjugated CPT to tissues. The PK profile of unconjugated CPT is dependent on the disposition profile of the conjugated drug; unconjugated CPT elimination is formation rate limited. Unconjugated CPT half-life ranged from 31.9 to 60.4 hours. Five days after the 1st administration, mean cumulative urinary excretion of conjugated and unconjugated CPT accounted for 27.9% and 5.1% of the administered dose, respectively. A major conjugated CPT species in urine was glu-gly-CPT (6.9% of dose). Accumulation of conjugated or unconjugated CPT was not observed with repeated dose administration. Plasma and urine PK parameters were nearly identical in cycles 1 and 2. The MTD has been established at 75 mg/m^2. **Conclusion**: CT-2106 has been well tolerated with easily manageable toxicities while generating prolonged systemic exposures to free CPT in plasma. Since clinical activity has been observed, phase I/II single-agent and combination trials are planned.

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Human carboxylesterase isoform 2 (hCE2) mRNA expression in peripheral blood lymphocytes as a predictive marker of irinotecan activation rate in vivo

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Background: Irinotecan {7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyl-oxycamptothecin} is a pro-drug used in cancer therapy as topoisomerase I inhibitor. Its activation occurs mainly by the action of the human carboxylesterase–converting enzyme isoform 2 hCE2 that cleaves the bulky piperidino side chain and generate the metabolite SN38 which is the biologically active molecule responsible for the therapeutic effect as well as for the toxic reactions associated with the drug. The pharmacological inter-patient variability of irinotecan gives rise to unpredictable toxicity in certain individuals. This could be due also to the highly variable extent of irinotecan activation found among patients. In an attempt to identify a marker to predict irinotecan activation in cancer patients we consider the hCE2 mRNA expression in lymphocytes to correlate it with in vivo activation rate of irinotecan to SN38. hCE2 was the isoform considered in this study since it shows the higher affinity for irinotecan among the human hCEs.

Materials and methods: Twenty-one gastro-intestinal cancer patients treated with irinotecan including schemes have been analysed for hCE2 mRNA expression. Total RNA was extracted from peripheral lymphocytes. hCE2 mRNA was relatively quantified using specific primers by RT-PCR associated with the Real Time technology and the SYBR Green chemistry. Irinotecan pharmacokinetic analysis was performed in each single patient. Irinotecan, SN38 and SN38-glucuronide plasmatic concentrations were determined by HPLC at 2, 6, 10 and 50 hours after the beginning of drug infusion.

Results: A high inter-individual variability was found in terms of mRNA expression. The median value of expression in relative units is 1.235 (range: 0.01–14.07). The activation rate was described as the concentration ratio of total SN38 (free and glucuronide) to irinotecan. The median values found among the patients are: 0.048 (0.013–0.126) at 2 hours, 0.100 (0.031–0.294) at 6 hours, 0.136 (0.047–1.774) at 10 hours and 0.544 (0.257–2.303) at 50 hours. A significant correlation was found between the relative hCE2 mRNA expression and the irinotecan activation rate at 2 (R=0.631, p=0.0022), 6 (R=0.553, p=0.0093) and 50 hours (R=0.591, p=0.0048) by the linear regression analysis.

Conclusion: Though these results should be confirmed by further investigation in a larger population the preliminary data support a predictive power of hCE2 mRNA expression in peripheral lymphocytes for the activation

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Durable disease stabilization and antitumor activity with rubitecan, an orally administered topoisomerase I (topo-I) inhibitor, in combination with gemcitabine: a phase I and pharmacokinetic study in patients with advanced cancer

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Background: Rubitecan, an oral camptothecin analogue and potent inhibitor of topo-I, has demonstrated clinical activity in gemcitabine-sensitive malignancies, such as pancreas, breast, ovarian and urothelial tumors, as well as gemcitabine-resistant cancers. Preclinical synergism between topo-I inhibitors and gemcitabine as well as the presence of nonoverlapping toxicities, provide a sound rationale for their evaluation in combination.

Patients (pts) and Methods: Escalating oral doses of rubitecan from a starting dose level of 1.0 mg/m^2/day × 5 days every 7 days × 3 weeks, every 28 days, with a fixed dose of gemcitabine 1000 mg/m^2 IV on days 1, 8 and 15, every 28 days, were administered to patients (pts) with advanced solid malignancies who had received minimal prior myelotoxic therapy.

Results: To date, 31 pts (median age 57, [30–75]; 17 male/14 female; pancreas/biliary tract [15/5], colon [3], esophagus [2], NSCLC/SCLC [2/1], other [3]) have received 137 courses (range 1–22) at rubitecan dose levels of 1.0 mg/m^2/day (13 pts), 1.25 mg/m^2/day (8 pts), and 1.5 mg/m^2/day (10 pts) with full doses of gemcitabine. First cycle DLTs have been uncomplicated gr 4 neutropenia >5 days (1 pt) at 1.0 mg/m^2/day; gr 3 vomiting (1 pt), gr 3/4 thrombocytopenia (2 pts) at 1.25 mg/m^2/day; and febrile neutropenia (1pt), gr 3 transaminase elevation (1 pt) at 1.5 mg/m^2/day. Other toxicities are mostly mild to moderate, and also include non dose-limiting gr 4 neutropenia (6 pts), gr 4 thrombocytopenia (2 pt), gr 3 transaminase elevations (5 pts), gr 3 diarrhea (2 pts), and gr 3 vomiting, fatigue, cystitis, weight loss, and epistaxis (each 1 pt). Patient accrual continues at 1.0 mg/m^2 of rubitecan, which is the recommended phase II dose, in combination with 1000 mg/m^2 of gemcitabine. 9-NC and gemcitabine AUCs (n=23 and 14 pts, respectively) increased with increasing dose levels. No drug-drug interactions were identified. Overall clearance of 9-NC and Gemcitabine were 7555.86±12901.74 mL/hr and 314.3±1133.5 mL/hr, respectively. Other PK parameters (9-NC and Gemcitabine, respectively) were: $T_{1/2}$, 12.9±6.5 h and 10.2±13.6 h; AUC, 800.5±635.9 h*ng/mL and 254,779±227,986 h*ng/mL; and Vd, 92.3±97.6 L and 1071.3±3247.3 mL. A partial response has been observed in 4 of 16 pts with evaluable pancreas/biliary tract cancer (25%; 95%CI, 7.3% to 52.4%), of whom 2 were gemcitabine-refractory, and also in 1 pt with esophagus cancer. Additionally, 11 of 24 pts evaluable for tumor response have shown stable disease lasting 3+–22+ months (pancreas, 4 pts; biliary tract, 4 pts; colon, lung, and H&N, 1 pt each). Remarkably, 12 of 16 patients with evaluable pancreatic or biliary tumors had a partial response or durable stable disease as best response.

Conclusions: Disease-directed evaluations of this safe and feasible regimen are planned in pancreatic and biliary tumors, where impressive preliminary activity has been observed.

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Role of topoisomerase I inhibition in the cytotoxic action of synthetic derivatives of the anticancer marine alkaloid lamellarin D

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We have recently identified the marine alkaloid Lamellarin D (Lam-D) as a novel potent inhibitor of human topoisomerase I with an efficacy comparable to that of the reference drug camptothecin (*Cancer Res.* 2003, *63*, 7392–7399). This natural product is highly cytotoxic and insensitive to P glycoprotein-mediated drug efflux; its cytotoxicity is dependent, at least in part to its capacity to promote DNA cleavage by topoisomerase I. In the present work, we have analyzed the topoisomerase I inhibitory properties of 8 lamellarin derivatives diversely substituted on the benzopyranopyrroloisoquinolinone B-F pentacyclic planar chromophore or the orthogonal phenol A-ring (*J. Nat. Prod.* 2002, *65*, 500–504). Stabilization of topoisomerase I-DNA covalent complexes was studied using complementary electrophoretic methods with supercoiled plasmid and radiolabeled DNA restriction fragments. The cytotoxicity of the test compounds was evaluated by a conventional tetrazolium-based assay using a pair of cell lines expressing a normal or mutated topoisomerase I gene. Human CEM leukemia cells are highly sensitive to Lam-D whereas the CEM/C2 cells resistant to camptothecin are cross-resistant to Lam-D. The mutation of the Asn722 to a Ser residue adjacent to the active site Tyr723 residue of the human topoisomerase I enzyme considerably decreases the cytotoxicity of Lam-D and its analog FI-02 lacking a methoxy group on the F-ring. In contrast the deletion of the adjacent hydroxy group considerably reduces the cytotoxicity of the compound and almost abolishes its ability to interfere with topoisomerase I. The hydroxyl group on the phenol A ring is also a crucial element both for cytotoxicity and topoisomerase I inhibition. This study (i) reveals a solid correlation between the cytotoxic potential of the 8 lamellarin derivatives tested and their ability to inhibit topoisomerase I, and (ii) provides important structure-activity relationships to guide the development of antitumor agents in this chemical series.

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A National Comprehensive Cancer Network phase II study of gemcitabine and irinotecan in metastatic breast cancer: can topoisomerase I localization predict response to irinotecan?

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Background: Gemcitabine, a nucleoside analogue, and irinotecan, a topoisomerase I (topo I) inhibitor, have both demonstrated efficacy as single agents in patients with metastatic breast cancer and preclinical data indicate that the incorporation of gemcitabine into DNA enhances cleavage complexes *in vitro* when combined with a topo I inhibitor. Since topo I requires nuclear localization to exert its activity, predominate localization of topo I within the cytoplasm may predict for drug resistance.

Methods: After obtaining informed consent, 16 patients received therapy with gemcitabine at 1000 mg/m^2 and irinotecan at 100 mg/m^2 on days 1 and 8 of a 21 day cycle. Tumors from 5 patients were biopsied by fine needle aspiration (FNA) prior to initiation of therapy. 2×10^5 cells were used to create cytospin slides for immunofluorescence staining of topo I. A monoclonal antibody against histone was used to identify nuclei and function as an internal control for sample variation. Topo I was detected using the C-21 murine monoclonal IgM antibody directed against an epitope in the C-terminal 67 kDa. Immunofluorescence was observed with a Leitz Orthoplan 2 microscope and images were captured by a CCD-camera with Smart Capture program. Quantification of topo I was performed on 50 randomly selected tumor cells/sample with measurements confirmed by Adobe Photoshop 7.0. Each cellular compartment was quantified separately in pixels and nuclear/cytoplasmic ratios were calculated individually for each cell with the mean value for each variable listed in the table below. The ratios were plotted on scattergrams and the mean values and standard deviations were calculated with GraphPad 4.0 software.

Results: Of the 16 patients enrolled, 14 have been evaluated for response with an overall response rate of 36% (CR=0, PR=5, SD=3, PD=6). The results of the five patients who had tissue biopsies to assess for topo I are listed in the table.

Conclusion: Preliminary results indicate that gemcitabine and irinotecan is an active combination for metastatic breast cancer and that topo I localization can be measured in breast cancer patients using immunofluorescence in tumor samples obtained by FNA. In this limited data set, the tumor sample with the highest nuclear/cytoplasmic ratio of topo I was associated with a partial response while the lowest ratio was associated with progression of disease.

Patient no.	Pixel density		Nuclear/cytoplasmic ratio*	Clinical response
	Nuclear Topo I	Cytoplasmic Topo I		
003	76646	58633	1.5	PD
004	74707	90233	0.91	PR
006	150114	149439	1.23	SD
010	53411	6531	13.5	PR
013	79158	184587	0.5	PD

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Antitumour activity of the novel 7-substituted camptothecin ST1481 (Gimatecan) in human neuroblastoma

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Background: Gimatecan (ST1481, 7-tert-Butoxyiminomethylcamptothecin), is a novel lipophilic camptothecin analog showing a better pharmacological profile and a lack of cross-resistance to topotecan and irinotecan. Gimatecan is currently under evaluation in Phase I/II clinical trials administered by oral route. In the present study we compared the in vitro antitumour activity of gimatecan, SN38 (the active metabolite of irinotecan) and topotecan in neuroblastoma.

Methods: Cytotoxicity was evaluated by growth inhibition assay and clonogenic survival in a panel on neuroblastoma cell lines (SK-N-DZ; BE(2)M17; LAN-1; RNGA and BE(2)c). From these studies SK-N-DZ cells were selected for further evaluation of cell cycle distribution by flow cytometry; induction of DNA strand-breaks induction by alkaline Comet assay; induction of apoptosis through the hypoploid peak, active caspase-3