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