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.

507 POSTER

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.

508 POSTER

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

509 POSTER

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

and tunel assays; in vivo antitumour efficacy in CD1 nu/nu male mice bearing SK-N-DZ xenografts where gimatecan was administered orally at 0.2mg/Kg/d and 0.3 mg/Kg/d doses and q4dx3 schedule.
Results: Gimatecan was about 1.4–4 times and up to 40-fold more cytotoxic than SN38 and topotecan respectively. All analogues induced a dose-dependent arrest in G2-M phase of the cell cycle after 1h incubation and 24/48/72 hours of recovery in drug-free medium. Gimatecan was more efficient than SN38 and topotecan in inducing caspase-3 dependent apoptosis and DNA strand breaks. DNA damage was dose-dependent and was up to 4-fold higher with gimatecan at 10xIC50 dose. The acellular Comet assay showed that gimatecan was the most efficient DNA-damage inducer also in nude nuclei. Repair/reversal of the drug-mediated DNA damage was similar for all analogs and was almost complete by four hours from drug removal. In the in vivo study, gimatecan showed a complete tumour regression in 100% of mice at both doses used. Toxicity was negligible with no toxic deaths and less that 10% in weight loss. **Conclusions:** Taken as a whole, our findings show that gimatecan induces higher DNA strand breaks and apoptosis in neuroblastoma where it appears very active with limited toxicity. The striking antitumour activity of gimatecan observed at preclinical level justifies clinical investigation in neuroblastoma.
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510 POSTER

Pharmacokinetics (PK) and effects on irinotecan (CPT-11) disposition of selenium (Se) during a phase I study of CPT-11 in combination with selenomethionine (SLM) in patients with advanced solid tumors

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Background: SLM increases the cure rates of nude mice with human tumor xenografts treated with CPT-11 and protects them from toxicity and lethality (Cao *et al*., Clin. Cancer Res., 10:2561–2569, 2004). A phase I clinical trial of the combination of SLM and CPT-11 based on these findings is ongoing at RPCI (Fakih *et al*., this meeting). PK of Se, CPT-11, SN-38 and SN-38G are being studied during this trial.
Materials and Methods: SLM (2200 μg Se) is given orally daily, starting on day 1 and continuing while the patient remains on study. CPT-11 (125 or 160 mg/m^2/weekly) is given weekly × 4, q 6 weeks (wks) starting on day 8. Blood for PK determinations is drawn on days 1, 2, 8, 15, 29 and 50 (and later where possible). Multiple samples are drawn on days 8 and 29 for complete PK studies. Se is measured by Atomic Absorption Spectrophotometry and CPT-11 and its metabolites by HPLC. PK parameters are determined by fitting a 2-compartment model with a lag-time (SLM) to the data, or by non-compartmental analysis (CPT-11 and metabolites) using WINNONLIN.
Results: In 9 patients evaluated to date, Se absorption was variable and trough levels on day 8 ranged from 363 to 985 ng/ml (median, 544). The day 8 PK data indicate a t_{max} between 2 and 8 h (median 3 h) and C_{max} between 457 and 1107 ng/ml (median 726 ng/ml). The mean (SD) serum half-life was 183 (94) h, and CLt/F 0.10 (0.04) L/h. Modeling of data suggests steady state attainment after ~30 days in the average patient, with a median steady state level of 844 (range 585–1300) ng/ml. PK of CPT-11, SN-38 and SN-38G were available for 9 patients for wk 1 and 4 patients for wks 1 and 4. The mean±SD of half-life and CLt for CPT-11 in the 9 patients on wk 1 were 10.2 (4.5) h and 13.5 (2.8) L/h/m^2 respectively; for SN-38 they were 16.8 (4.0) h and CLt/Fm 137.9 (55) L/h/m^2 and for SN-38G, 15.5 (8.1) h and CLt/Fm 52.5 (24) L/h/m^2. From wk 1 to wk 4 the AUC for SN-38 declined significantly in 2/4 patients, in one with a concomitant increase in SN-38G and in another with a significant increase in another metabolite. The biliary index as expressed by $AUC_{CPT11} \cdot AUC_{SN38}/AUC_{SN38G}$ is reduced in 2/4 by 59% & 69% respectively.
Conclusions: The plasma levels of selenium attained by day 8 when CPT-11 treatment starts are well below the 15 μM (~1200ng/ml) level shown to be protective in animal models (Azrak *et al*., this meeting), which may account for the inability to dose escalate CPT-11 in this trial (Fakih *et al*., this meeting). Future clinical trials of Se and CPT-11 at RPCI will include an appropriate loading dose and maintenance dose of SLM to reach and maintain the target level Se (⩾ 1200 ng/ml) early in the course of therapy. Potential modulation of CPT-11 metabolism by Se requires further studies.

511 POSTER

Potentiation of cell sensitivity to the DNA topoisomerase I inhibitor gimatecan by TRAIL in prostate carcinoma cells

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Since hormone-refractory prostate cancer is a chemotherapy-resistant disease, we explored the possibility of modulating TRAIL-induced apoptosis by exposure to DNA topoisomerase I inhibitors in two cell systems (DU-145 and PC-3 cell lines) which express TRAIL receptors. In the present study, the novel 7-substituted analog of camptothecin (gimatecan), currently undergoing clinical development, was used. The employed cell lines exhibited low susceptibility to TRAIL-induced apoptosis as shown by annexin V-binding assay. Flow cytometry analysis of antibody-stained cells indicated that exposure to gimatecan resulted in up-regulation of the expression on TRAIL-R1 and -R2 receptors in both cell systems. An increased susceptibility to TRAIL-mediated apoptosis was also observed. In DU-145 cells, enhancement of drug-induced apoptosis was achieved by lower TRAIL concentrations as compared with those required in PC-3 cells. The different cell response to the combination was not closely related to the level of up-regulation of TRAIL receptors. Moreover, susceptibility to apoptosis following combined treatment was higher in DU-145 cells than in PC-3 cells, in which camptothecins slightly induced Bcl-2 expression. The observed sensitivity to apoptosis was also in relation with differential activation of caspases (i.e caspase 8 and 9) by treatment, as evidenced by Western blotting. Indeed, activation of caspase 8 required a higher TRAIL concentration in PC-3 than in DU-145 cells, and caspase 9 was activated only in DU-145 cells. Our results support that synergistic interaction between gimatecan and TRAIL is dependent not only on TRAIL receptor expression, but involves differential activation of apoptosis-related factors and apoptotic pathway efficiency.

512 POSTER

BN80927: a novel homocamptothecin that inhibits proliferation of human tumor cells in vitro and in vivo

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BN80927 belongs to a novel family of camptothecin analogues, the homocamptotecins, developed on the concept of topoisomerase (Topo) I inhibition and characterized by a stable 7-membered β-hydroxylactone ring. Preclinical data reported here show that BN80927 retains Topo I poisoning activity in cell-free assay (DNA relaxation) as well as in living cells, where *in vivo* complexes of topoisomerases experiments (ICT) and quantification of DNA-Protein-Complexes (DPC) stabilization, have confirmed the higher potency of BN80927 as compared to the Topo-I inhibitor SN38. In addition, BN80927 inhibits Topo II-mediated DNA relaxation *in vitro* but without cleavable-complexe stabilization, thus indicating catalytic inhibition. Moreover, a Topo I altered-cell line (KBSTP2) resistant to SN38, remains sensitive to BN80927, suggesting that part of the antiproliferative effects of BN80927 are mediated by a Topo I independent pathway. This hypothesis is also supported by *in vitro* data showing an antiproliferative activity of BN80927 on a model of resistance related to the non-cycling state of cells (G0/G1 synchronized).
In cell growth assays BN80927 is a very potent antiproliferative agent as shown by IC_{50s} consistently lower than those of SN38 in tumor cell lines as well as in their related drug resistant lines. BN80927 shows high efficiency in vivo in tumor xenograft studies using human androgen independent prostate tumors PC3 and DU145. Altogether, these data strongly support the clinical development of BN80927.

513 POSTER

Design of the selective DNA topoisomerase I poison, NU:UB 235

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The recent clinical introduction of the camptothecins topotecan and irinotecan, has further validated DNA topoisomerase I (topo I) as a target in cancer therapy, however, usefulness is limited by the inherent structural lability of this class of compounds. Furthermore, the stability and persistence of the drug-stabilised DNA-topo I cleavable complex (poisoning action) is directly related to efficacy, which for the camptothecins often reverse within minutes of removal of the drug, resulting in the need for