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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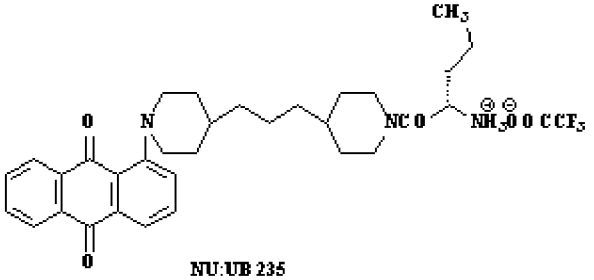
The recent clinical introduction of the camptothecins topotecan and irinotecan, has further validated DNA topoisomerase I (topo I) as a target in cancer therapy, however, usefulness is limited by the inherent structural lability of this class of compounds. Furthermore, the stability and persistence of 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

long infusion times for patients. Strong motivation thus exists for the design of new anti-topo I agents that stabilise cleavable complexes without the drawback of hydrolytic lability problems.
NU:UB 235, a spacer-linked, conformationally restricted anthracenedione conjugate of the unnatural amino acid norvaline, is representative of a new rationally designed series of topoisomerase I inhibitors.
NU:UB 235 has broad spectrum cytotoxic potency against a panel of human and animal tumour cell lines, with mean GI_{50} value ~1 μM in the NCI 60 cell line panel and transplantable, refractory MAC15A adenocarcinoma of the colon.
Enzyme-mediated relaxation of supercoiled pBR322 plasmid by either topo I or the α or β-isoforms of human topo II at concentrations up to 100 μM was not observed with NU:UB 235, compared to standard agents. However, the norvaline conjugate stabilised cleavable complex formation *in vitro* and reproducibly gave mean increases of 170% in nicked plasmid formation compared to 80% with camptothecin at equimolar concentrations (50 μM). This approximately 2-fold increase over camptothecin was observed also with the alternative ϕ X174RF plasmid.



NU:UB 235

Additionally, NU:UB 235 stabilised cleavable complex formation with topo I in intact HL60 leukaemic cells [following 45 minute exposure at 200 μM]. At equimolar concentrations, immunoband band depletion, by NU:UB 235, of the topo I Western blot signal in HL60 extracts exceeded (by 5-fold) that produced by camptothecin. The level of DNA damage induced by topo I poisoning is consistent with the non-intercalating, DNA groove-binding properties (Q_{50} value 0.9 μM for Hoechst 33258 displacement) of NU:UB 235, measured by fluorescence quenching.
Continuing pre-clinical development is warranted, given the demonstration that structurally stable NU:UB 235 is an effective non-camptothecin topo I poison.

514 POSTER

Pharmacokinetic study of the distribution, metabolism and excretion of non-radiolabeled DX 8951f following repeated intravenous administration to patients with solid tumors

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Background: The novel camptothecin derivative, DX 8951f (exetecan; D), is a potent topoisomerase I inhibitor with activity in the treatment of solid tumors. A pharmacokinetic (PK) study was designed to characterize the distribution, metabolism and excretion of *non-radiolabeled D* administered intravenously over 30-minutes daily for 5-days every 3 weeks by determining: the plasma PK, urinary and fecal excretion of D, D lactone and the two known metabolites of D, UM1 and UM2. Concentrations of D in saliva and *in vivo* plasma protein binding were also determined.
Methods: Patients were stratified into minimally (MP) and heavily pretreated (HP) groups based on prior treatment. Starting doses of D for MP and HP were 0.5 mg/m^2/day and 0.3 mg/m^2/day, respectively. Since D is predominantly metabolized by CYP3A4 and CYP1A2 enzymes, the enzyme activity was assessed in each patient pre-treatment by erythromycin breath test and caffeine urinary test, respectively. Limited blood samples were collected post-treatment, from days 1–6. Urine was collected continuously for 10-days and feces for 10–14 days during course 1. Saliva was collected at baseline and concomitantly with plasma samples, on day 1. Patients were hospitalized for up to 14 days of course 1. A recovery of 70% of D and its metabolites in all bodily secretions was defined as the apriori mass balance target.
Results: Twelve patients; 5♂:7♀; median age 57 (range 20–66); ECOG PS 0–1 (n=10), 2 (n=2) received 29 courses (median 2.5; range 1–5) of D. Tumor histology were pancreas (2), hepatocellular (2), renal/adrenal (2), gall bladder (1), esophageal (1) and others (4). Grade 4 neutropenia and thrombocytopenia were the predominant hematologic toxicities. Non-hematologic toxicities included nausea, emesis, fatigue and abdominal cramps. Overall, the total drug recovery ranged from 21.4 to 57.7% of the total administered dose of D. About 10% of D was eliminated unchanged in urine. UM1 and UM2 metabolite appeared to account for up to 25% and 15% of the total D administered, respectively. The PK parameters Clearance, half-life and Vss estimated noncompartmentally for D were within 2 standard errors of the values previously reported. The plasma exposure to UM-1 and UM-2 was approximately 4.3% and 1.3% of the parent compound.
Conclusions: Up to 57.7% of the administered dose of D is recovered in urine, feces and emesis fluid. Because less than 70% of the administered dose is accounted for by the parent drug and the two major metabolites, additional as yet unrecognized excreted metabolites may be present.

Topoisomerase II inhibitors

515 POSTER

Induction of unique structural changes in guanine-rich DNA regions by the triazoloacridone C-1305, a topoisomerase II inhibitor with potent activity toward solid tumors

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C-1305 is a triazoloacridone with excellent activity in lung and colon cancer models. We have recently reported that C-1305 is a topoisomerase II poison which is able to induce topoisomerase II-mediated cleavable complexes *in vitro* as well as in living cells. An unusual feature of C-1305 is the induction of low levels of very toxic cleavable complexes in tumor cells. To explore the molecular mechanisms underlying this phenomenon, we have investigated the *in vitro* sequence specificity of DNA binding for C-1305 and other triazoloacridones in comparison with classical topoisomerase II inhibitors and other DNA binders.
A 3′-end labelled 176 bp DNA fragment from pBluescript plasmid was used for studies on drug-DNA interactions. To determine the sequence specificity of DNA binding of triazoloacridone derivatives, DNA footprinting, chemical probing of DNA with DEPC and osmium teraoxide were used. Surface plasmon resonance (SPR) experiments were undertaken to determine the DNA binding affinity of C-1305 and C-1533 compounds. For determination of structure-activity relationship, thermal denaturation and chemical probing with DEPC was performed for a series of triazoloacridone.
Compound C-1305 shows almost 10 times higher preference for GC rather than AT DNA sequences as revealed by SPR ($K=3.0\times10^5$ M^{-1} for GC-rich DNA compared to 4.9×10^4 M^{-1} for AT-rich DNA). Chemical probing with DEPC showed that C-1305 induced structural perturbations in DNA regions with at least three consecutive guanine residues. This effect was detectable already at nanomolar concentrations of C-1305 and was highly specific for the C-1305 derivative, since none of the 22 other DNA-interacting drugs tested were able to induce similar structural changes in DNA. Structure-activity relationship studies with a series of triazoloacridone derivatives representing different chemical structures indicated that a hydroxyl group in the 8 position of the triazoloacridone ring as well as an aminoalkyl side chain containing three methylene groups are crucial for the unusual interaction of C-1305 with guanine-rich DNA regions.
We here show that the topoisomerase II inhibitor triazoloacridone C-1305 binds strongly to DNA at guanine-rich regions resulting in unique conformational alterations. Our results suggest that C-1305 might specifically influence the expression of genes that are regulated by guanine-rich elements in the promoter regions.

516 POSTER

Disruption of PKCzeta elicits hypersensitivity to submicromolar amounts of etoposide independently of the non-homologous end-joining pathway

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Background: To investigate the mechanisms by which wortmannin, a phosphatidylinositol 3-kinase (PI3K) inhibitor known to inactivate ATM