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.

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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