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## Pharmacokinetics of intrathecal partaject busulfan in a phase I trial for patients with neoplastic meningitis

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We recently reported the results of a phase I study of intrathecally-administered busulfan in adult patients with neoplastic meningitis using a microcrystalline formulation, Partaject(TM) busulfan (Proc Am Soc Clin Oncol 2002;318a). Anti-cancer efficacy was noted, with minimal toxicity encountered to date. We now describe the previously unreported cerebrospinal fluid (CSF) and plasma pharmacokinetics of busulfan in patients treated on the phase I trial. The investigational formulation was administered via injection by the intralumbar or intraventricular route. Adult patients with neoplastic meningitis were enrolled using a limited escalation dose schedule design. Timed, serial plasma and CSF samples (seven of each type per patient) were obtained up to 5 hours following the first injection. Busulfan CSF and plasma concentrations were determined using a validated gas chromatographic assay. Pharmacokinetic parameter estimates were generated via a standard, two-stage, non-compartmental approach. Pharmacokinetic data are available in 23 patients treated on the following dose levels: 2.5, 5, 7.5, 10, 13, 17, 21.25 and 27 mg. The mean values for busulfan CSF clearance and half-life were 2.0 mL/minute and 64 minutes, respectively, each varying over a 10 fold range. No evidence for dose-dependent changes in clearance or half-life were observed. Both the AUC and Cmax values achieved in patients at the 21.25 mg dose level (26 mg/mL*min and 269 mcg/mL, respectively) were approximately 100 fold higher than the typical busulfan plasma AUC and Cmax observed when oral busulfan (1 mg/kg q6h) is used for ablative therapy in stem cell transplantation regimens. Some plasma samples had minimal busulfan concentrations, just above the limit analytic quantitation.

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## Cellular and animal pharmacology of isophosphoramide mustard (IPM)

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Isophosphoramide mustard (IPM) is the active alkylating metabolite of ifosfamide. IPM is being prepared for a Phase I clinical trial - sponsored by DEKK-TEC with NCI support.In the present studies, IPM was administered by infusion once daily $\times$ 3 days to 16 adult beagle dogs with dose escalation between 1 and 100 mg/kg; by one bolus to 2 monkeys (5mg/kg) and to 10 mice (100mg/kg). Blood was drawn after IPM administrations during 24h for PK studies. The samples were extracted, chemically derivatized and assayed by GC-MS with good reproducibility (85%) and a quantification limit of 100 ng/ml. For dogs receiving 5 mg/kg (median) - T1/2 was in the range of 25 min. Plasma clearance was constant between 5 and 10 mg/kg (1.40 L/h/kg) and increased at 100 mg/kg (5.7 L/h/kg). Sparse data for 3 mg/kg prevented interpretations. At 5 mg/kg no IPM was detectable 1.5h after injection; at 10 mg/kg after 2h and at 100 mg/kg after 4h. MTD in dogs was 5 mg/kg. Toxicity noted was death at doses $>$10 mg/kg (renal toxicity) and at 5 mg/kg or less, transient liver and renal, bone marrow, and gastrointestinal dysfunctions although no IPM was detected in plasma. For mice, plasma concentrations decreased in $<$1 hour, with a clearance of 8.44 L/h/kg; T1/2 was 3.42 min (two compartment model). For monkeys mean T1/2a value was 4.86 min and T1/2b was 256 min. Median clearance was 1.65 L/h/kg and no IPM was detected 4h after dosing. No major toxicities were observed at this level. No potential IPM metabolites could be detected. In human blank plasma, 90% of IPM was bound to proteins within 5min of incubation. Human pharmacokinetic parameters were predicted from allometric analysis using the three species. Data predicted an acceptable starting dose of 30 mg/m$^2$ with a clearance of 40.5 L/h, a T1/2a of 10 min and a T1/2b of 1h45 min for a 70 kg patient. This is the proposed starting dose. Cellular studies were conducted with A549 cells (human lung cancer) *in vitro*. Various times of IPM incubation (at various doses) were tested; 10% of IPM was still detected after 24 h incubation at doses of 100, 250 and 500 mg/L. IPM uptake and efflux were studied, IPM was essentially in the nuclear compartment (90%). Effects of IPM on cell cycle was investigated by flow cytometry: S-phase block increased by 30%, as compared to control. The latter studies were attempts to document comparable tissue/IPM interactions that could occur under *in vivo* exposures. Supported by NCI/SBIR grant 44 CA 83552.

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## Pharmacokinetics of D709119 (DRH-417), a DNA minor groove-binding pyrrolobenzodiazepine monomer with a novel mechanism of action

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D709119 is a DNA minor groove-binding pyrrolobenzodiazepine derivative that shows significant *in vitro* cytotoxicity towards a select number of cell lines in the 60-cell line NCI screen. This activity translates into human tumour xenograft models where *in vivo* antitumour activity has been demonstrated for a number of models including melanoma, renal and ovarian. On the basis of this *in vivo* activity, together with the novel mechanism of action of D709119 as suggested by findings from an NCI COMPARE analysis, the compound is now in pre-clinical development with the EORTC. The aim of this study was to develop a highly selective LC/MS analytical method in order to investigate the pre-clinical pharmacokinetics of D709119 in mice. A further goal was to measure tumour concentrations of the drug, and also brain concentrations in order to assess the degree of CNS penetration. An LC/MS-based method has been developed using an acetonitrile/ammonium formate mobile phase with a Hypersil phenyl reversed-phase HPLC column. Selective detection is achieved using electrospray MS analysis with a SIR of m/z 368.4 to detect the parent ion, the limit of detection is 50 nM. All *in vivo* studies have been approved by the UK Home Office. Pharmacokinetic studies have been carried out in NMRI mice bearing either MAC 29 or MAC15A murine colon tumours. Although an extraction efficiency of *50 % was achieved from plasma and brain homogenates, only $\sim$15 % extraction was achieved from spiked tumour homogenate suggesting extensive binding to tumour proteins. After i.p. administration at the MTD of 0.5 mg/kg, D709119 was easily detectible in mouse plasma up to 4 h post-dose, with peak concentrations of 171 nM after 30 min and a t1/2 of 2.3 h. The AUC was calculated to be 0.54 uM h. D709119 levels were below the limit of detection in both brain and tumour homogenates. An $IC_{50}$ value for D709119 was determined experimentally in the human ovarian adenocarcinoma cell line SK-OV-3 to be 2.75 nM. These studies suggest that D709119 is bioavailable following i.p. injection at plasma concentrations well in excess of those necessary to achieve *in vitro* cytotoxicity. Further studies are now underway to probe the extensive binding of D709119 in tumour tissue.

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## Intracellular and *in vivo* distribution of the pyrrolobenzodiazepine dimer SJG-136, a novel sequence-selective DNA minor groove cross-linking agent

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The pyrrolobenzodiazepine dimer SJG-136 is a novel sequence-selective DNA minor groove cross-linking agent with potent DNA stabilising activity and remarkable *in vitro* cytotoxicity (e.g. $IC_{50}$ value of 23 pM in A2780 cells). The aim of this study was to characterise the *in vitro* cellular distribution of the agent and to establish its *in vivo* preclinical pharmacokinetic properties. SJG-136 is highly fluorescent and this enables its *in vitro* visualisation. Cell lines selected from the NCI's 60-cell line panel were treated with SJG-136 and fluorescence microscopy was used to monitor cellular uptake. It was demonstrated to produce a high level of nuclear-specific staining in HCT-116, UACC62, SK-MEL-2, SK-OV-3 and M14 cell lines, indicating both cellular penetration of the drug and localisation by covalent fixation within the nucleus, the proposed site of drug action. A sensitive and reproducible HPLC-based analytical method has been developed for SJG-136 that will be used to obtain pharmacokinetic data in forthcoming clinical trials. Using

an acetonitrile/ ammonium formate gradient with a reversed-phase phenyl column and fluorescence detection, a limit of detection for SJG-136 of 1 nM in serum has been achieved. Extraction efficiencies from serum were >60% across a range of concentrations (1-100 nM). All *in vivo* studies have been approved by the UK Home Office. In pilot pharmacokinetic studies where SJG-136 was administered i.p. to NMRI mice at the MTD of 0.2 mg/kg, the drug could be observed at detectible levels with a Cmax of 336 nM after 30 min in mouse plasma. A calculated terminal t1/2 of 0.98 h and an AUC of 0.34 uM h resulted in a clearance of 17.72 ml / min kg. Preliminary plasma protein-binding studies demonstrate that the agent is poorly bound to proteins (≤20 %), suggesting that SJG-136 is readily bioavailable in the blood with peak plasma concentrations substantially higher than those needed for *in vitro* cytotoxicity. Studies are currently in progress to establish the levels of SJG-136 that can be achieved in tumours.

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## Brostallicin potentates the antitumor activity of other cytotoxic antineoplastic agents in experimental tumor models

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Brostallicin (PNU-166196) is a synthetic a-bromoacrylic, second generation DNA minor groove binder, currently in Phase II clinical evaluation. Unlike other cytotoxics, its antitumor activity is increased both in the presence of high levels of glutathione (GSH) and glutathione S-transferases (GST) and the GSH/GST system is involved in its mechanism of DNA interaction (Geroni C. Cancer Res.; 62:2332, 2002). Moreover, brostallicin is fully active against DNA-mismatch repair deficient tumor cells and circumvents resistance to akylating agents and camptothecins. Multiple combinations of brostallicin with compounds belonging to major classes of antitumor agents have been studied on the basis of brostallicin's newly determined mode of action and ability to overcome drug-resistance. In nude mice bearing the human colon carcinoma HCT-116 model, the sequential combination of cisplatin (day1) and brostallicin (day3) yields a delay in tumor growth (14 days) that is significantly superior (p=0.02) to the best delays caused by either drug alone (2 and 3 days, respectively). In terms of toxicity, the maximum tolerated dose of each agent could be administered without additional toxicity. Synergism with doxorubicin (DX) is observed on a murine leukemia model (L1210) when DX treatment is given 24h before brostallicin. Both brostallicin and DX administered as a single agent shows 33% increase in life span (ILS); conversely, in combination the antitumor activity is significantly higher (100%ILS). An increase in toxicity is observed when DX and brostallicin are administered simultaneously. Supraadditive antitumor effect is shown when brostallicin is tested in combination (simultaneous, single i.v. treatment) with gemcitabine on L1210 leukemia (58,50,117 %ILS for brostallicin and gemcitabine alone and in combination, respectively). The antitumor activity of simultaneous administration of brostallicin and taxotere has been tested on human NSCLC xenograft model (A549). Clear additivity is shown, both in terms of % of tumor regression and tumor growth delay at all tested doses, without any additive toxicity. Further combination studies are ongoing. Although the precise mechanism of interaction has not yet been identified, a clear therapeutic gain is observed in preclinical models when brostallicin is combined with other anticancer agents. These results indicate the value of brostallicin in cancer combination treatment protocols.

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## Structure-activity relationships of oxalatoplatinum(II) complexes: identification of oxaliplatin derivatives with improved activity

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Oxaliplatin, (trans-R,R-1,2-diaminocyclohexane)oxalatoplatinum(II), has been the first representative of the extensively studied class of diaminocyclohexane(DACH)-containing platinum complexes to become established in clinical practice. This class of compounds is known to display activity profiles different from those of cis/carboplatin, which has been confirmed by the clinical activity of oxaliplatin in colorectal cancer. Structure-activity relationships are well-explored with respect to the leaving group and the stereoisomers of DACH. However, despite the key role of the stable amine ligand for the altered activity profile no attempts have been made to improve the pharmacological properties by structural modifications of DACH so far. Ligands derived from DACH by stepwise substitution of cyclohexane have been used to prepare new oxalatoplatinum(II) derivatives in order to define the structural requirements essential for the oxaliplatin-like activity and to explore possibilities of improving this activity. Results obtained from cytotoxicity assays in human colon (SW480) and ovarian (CH1) cancer cell lines demonstrate that increasing the steric demand by introduction of substituents to cyclohexane is a promising strategy to this end. Racemic mixtures of (trans-1,2-diamino-4-alkylcyclohexane)oxalatoplatinum(II) (alkyl = methyl, ethyl) show equivalent to slightly higher potency compared to the enantiomerically pure oxaliplatin. Since trans-R,R-1,2-DACH-containing platinum complexes generally display a superior activity compared to their trans-S,S-1,2-DACH-containing congeners, we expect activity to be further improved by use of the more active enantiomer. Evaluation of the pure enantiomers and of further derivatives *in vitro* and *in vivo* will be presented.

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## Growth arrest, apoptosis and potentiation of 5-fluorouracil and Raltitrexed cytotoxic effect induced by histone deacetylase inhibitor SAHA in colorectal cancer cells

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Histone deacetylase (HDAC) inhibitors have been recently shown to induce growth arrest and apoptosis, in a variety of human cancer cells by mechanism that cannot be solely attributed to the level of histone acetylation. Suberoylanilide hydroxamic acid (SAHA), an orally active HDAC inhibitor, has shown promising preclinical effect in human cancer cells and phase I clinical studies have been recently completed. To determine if SAHA has potential clinical applications as antitumor agent for patients with colorectal cancer we analyzed the effect of SAHA on growth, apoptosis and cell cycle regulation in four human colorectal cancer cells. SAHA induced growth inhibition in a time and dose-dependent manner in all cells with $IC_{50}$ values ranging from 0.5uM to 10 uM independently of p53 status. Cell cycle analysis revealed an increased percentage of cells in G1 after 24 h and up to 72 h. Moreover SAHA induced time and dose-dependent apoptotic cell death beginning after 24 h of incubation. To investigate the mechanism of SAHA induced growth arrest and cell cycle perturbation we examined the expression of p27 and p21 cyclin-dependent kinase inhibitors in untreated and treated mut-p53 HT29 and wt-p53 LoVo colon cancer cells. In both cell lines SAHA induced time dependent upregulation of both of p27 and p21, beginning after 12 h of treatment with a peak between 24 and 48 h. Interestingly, SAHA treatment led to reduced expression of mut-p53 in HT29 and to upregulation of wt-p53 in LOVO cells. In addition protein expression of Thymidilate Synthase, a critical target for chemotherapeutic agents active in colorectal cancer such as 5-fluorouracil (5FU) and Raltitrexed, was downmodulated by SAHA treatment. On the basis of this observations, we have investigated if the combination of SAHA and Raltitrexed or 5FU enhanced cell growth inhibition compared to single drug schedule in HT29 and LoVo cell lines.Preliminary results show that simultaneous exposure to SAHA and either Raltitrexed or 5FU produced a supra-additive to additive antiproliferative effect, as demonstrated by median drug effect analysis calculating a combination index. Overall these results demonstrated that SAHA has antiproliferative and proapototic activity in human colorectal cancer derived cells. Moreover, SAHA can be combined with cytotoxic drugs currently used for colorectal cancer treatment and it should be further investigated for therapeutic use in patients with this malignancy.

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## Structure-activity relationships of platinum(II) phosphonate compounds for the treatment of bone malignancies

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In order to produce platinum complexes with selective activity in primary and secondary bone tumors aminobis/trismethylenephosphonates with high affinity for the mineral bone matrix have been used as ligands for platinum(II). Previously, accumulation in bone tissue has been confirmed by autoradiography and therapeutic activity superior to cisplatin has been found in an orthotopically transplanted rat osteosarcoma model which disseminates to the lung producing lethal osteoid-forming metastases. Current attempts to optimize the pharmacological ef-