the N-terminal region of ASK-1. When activated in response to various cytotoxic stresses (e.g. $H_2O_2$, TNF-α, UV light, heat shock) ASK-1, a MAPKKK, can activate both the c-Jun N-terminal kinase (JNK) and p38 MAP kinase pathways leading to a variety of cellular responses including apoptosis. Immunoprecipitation of Trx from lysates of control or treated HCT116 cells has shown that PMX 464 triggers dissociation of ASK-1 from Trx. Futhermore, by using antibodies specific for their phophorylated forms, JNK and P38 activation, are observed.
In order to investigate *in vitro* binding of cellular proteins, a carboxylate analog was immobilized to a solid media leading to identification of peroxiredoxin (Prx) as a molecular target. Prxs act as antioxidants and also regulate $H_2O_2$-mediated signal transduction, possessing a strictly conserved catalytic cysteine-SH (thiol). Overexpresion of Prx, detected in several cancers correlates with resistance to apoptosis induced by radiation or cisplatin.
In conclusion, perturbation of events downstream of Trx inhibition by PMX 464 has been detected. PMX 464 is not an indiscriminate thiol inhibitor, however, additional molecular targets such as Prx, involved in redox regulation have been identified.

**432** POSTER

**Phase I study of Amplimexon™ (imexon, inj.) in patients with advanced solid tumors and lymphomas: final report**

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**Background:** Amplimexon (AMP) is an iminopyrrolidone agent, which causes cancer cell kill by inducing mitochondrial injury, cytochrome C leakage and apoptosis. We report here the final results from the phase I trial of Amplimexon in patients with advanced solid tumors and lymphomas.
**Methods:** The purpose of this trial was to establish safety and maximally tolerated dose of AMP and to investigate pharmacokinetic (PK) and pharmacodynamic (PD) parameters on this schedule. AMP was administered as a 30 min IV infusion, daily X 5, every 14 days. The dose was escalated from 20 up to 1000 mg/m$^2$.
**Results:** A total of 49 patients were treated. The MTD was established as 875 mg/m$^2$ dose. Dose limiting toxicities at 1000 mg/m$^2$ included grade 3 abdominal pain and grade 4 neutropenia. Common grade 1–2 toxicities included constipation, nausea, fatigue, anemia and anorexia. The systemic clearance of AMP averaged 160mL/min/m$^2$ at the MTD of 875 mg/m$^2$. The plasma half life was 95 minutes and the Cmax was 53 ug/mL. This yielded an AUC of 5517 min ug/mL and a Vd ss of 19.1 L/m$^2$. There were no differences in clearance on day 1 versus day 5, and for the different dose levels of imexon. Pharmacodynamic studies showed that plasma cystine, the Cys-Cys dimer, decreased in a dose-dependant fashion at doses $\geqslant$750 mg/m$^2$, with a 30% decrease noted 8 hours after the 875 mg/m$^2$ infusion ended. Other plasma thiols were unchanged by AMP. A patient with a refractory follicular Non-Hodgkin lymphoma achieved a partial response and 10 patients with other solid tumors achieved stable disease for >3 months.
**Conclusions:** AMP could be safely administered at 875 mg/m$^2$/d dose and has demonstrated encouraging antitumor activity in this phase I study. Phase II studies of AMP in patients with both epithelial and lymphoid malignancies are warranted.

**433** POSTER

**A population pharmacokinetic/pharmacodynamic model for the hematological effects of BI 2536 in cancer patients**

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**Background:** Myelosuppression is usually one of the principal dose-limiting toxicities observed in patients treated with anticancer drugs. In this context, the population pharmacokinetic/pharmacodynamic (PK/PD) modelling approach has been shown to be an excellent tool to explore the drug response behaviour under a variety of dosing regimens, making the dose selection process less empirical. BI 2536 is a novel highly potent and specific inhibitor of the serine-threonine Polo-like kinase 1 (Plk1), a key regulator of cell cycle progression. Neutropenia as a mechanism-related toxicity indicates target inhibition in vivo and was the dose limiting toxicity observed in advanced cancer patients. The objective of the population pharmacokinetic/pharmacodynamic analysis was to develop a model that describes the haematological effects of BI 2536 and can serve as a tool to predict the influence of dose and schedule on hematotoxicity.
**Methods:** BI 2536 was administered as a 60 min intravenous infusion on day 1 of a 21 day treatment cycle (Dose levels 25–250 mg). Blood samples to determine the drug plasma concentration and the neutrophil count were taken at different time points during the 21 day treatment cycle. A semi-mechanistic model of chemotherapy-induced myelosupression (Friberg et al. J Clin Oncol 2002; 20: 4713−21) was used to describe the data. The analysis was performed using NONMEM, version V.
**Results**: BI 2536 BS plasma concentrations could best be described by a linear three compartment model. A moderate interindividual variability was established on clearance.
The neutrophil counts were adequately described using the semi-mechanistic model. This model allows the discrimination between system and drug related parameters. The estimates of the system related parameters obtained during analysis were similar to those reported previously for other compounds (Friberg et al).
**Conclusion:** The pharmacokinetics of BI 2536 were best described using a linear three compartment pharmacokinetic model. The time course of the hematological toxicity induced by BI 2536 and measured by the neutrophil cell counts was adequately described using a semi-mechanistic model developed and recently published (Friberg et al). The model developed will serve as a tool to predict hematologic side effects of further dosing schedules of BI 2536 given as a single agent or in combination with other modalities.

**434** POSTER

**Preclinical pharmacokinetic and comparative biodistribution studies of PX-866, a broad spectrum phosphatidylinositol-3-kinase (PI-3K) inhibitor, in F344 rats**

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PX-866, a semisynthetic inhibitor of PI-3K, has antitumor activity as a single agent and in combination with inhibitors of EGF and VEGF signal transduction, and is in late stage pre-clinical development. Constitutive PI-3K activity is found in small cell lung cancer and in 40% of ovarian, head and neck, urinary tract, and cervical cancers. PX-866 is the result of a nucleophilic modification of the furan ring in wortmannin, conferring chemical stability and reduced toxicity. We investigated the plasma pharmacokinetics and tissue distribution in F344 rats following a large single dose of 12.5 mg/kg given IV or PO (0.5 mL of 4 mg/mL PX-866 in NS:DMA, 80:20, v:v). Following sacrifice, plasma and tissue samples were collected (5 animals/timepoint) over a span of 5 minutes to 72 hours following drug administration and immediately processed for analysis. PX-866 was extracted from plasma and tissues using either protein precipitation or tissue disruption followed by liquid-liquid extraction. Samples were quantified using LC/MS/MS in ESI+ mode (LLOQ = 0.1 ng/mL). PK parameters for PX-866 were determined fitting both two-compartment ($r^2 > 0.99$) and non-compartmental ($r^2 = 0.88$) models to the mean measured plasma concentration vs. time data. PX-866 given IV rapidly distributed with a peak plasma concentration of 12.1 ug/mL at 5 minutes and could be measured to 4 hours. Tissue distribution of PX-866 IV bolus was rapid and significant, achieving concentrations 4 times greater than concurrently measured in the plasma in highly perfused organs over the first 30 minutes following injection. IV PK parameters $C_{max}$, AUC, $V_d$, Cl, $t_{1/2}$, and MRT were 12.1 ug/mL, 1166.4 ng hr/mL, 3.2 L/kg, 10.7 L/hr/kg, 0.27 hrs, and 0.29 hrs, compared to PO values of 81.7 ng/mL, 19.2 ng hr/mL, 146.4 L/kg, 636.1 L/hr/kg, 0.16 hrs, and 0.25 hrs, respectively. Oral bioavailability of parent PX-866 was 1.64%, similar to the previously reported value of 1.05% in mice. In conclusion, PX-866 biodistribution is extensive, with rapid clearance from plasma and most major organs in F344 rats following 12.5 mg/kg IV bolus delivery. Further investigations of single and multiple dose of PX-866 in these and other species will be conducted to ascertain the drugs behavior in both rodent and non-rodent species.

**435** POSTER

**Modulation of the activity of tumour associated carbonic anhydrases for therapeutic benefit**

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Carbonic anhydrases (CAs) are metalloenzymes involved in the reversible hydration of carbon dioxide to bicarbonate and various physiological

processes that involve ion exchange. Of the 13 α-CA isozymes, the membrane bound CA IX and CA XII are regulated by the hypoxia inducible transcription factor (HIF). CA IX is over-expressed in a number of solid tumours and its expression has been linked to poor prognosis and is implicated in tumorigenesis.
CA IX is involved in the acidification of extracellular pH (pHe), and several selective CA IX inhibitors are able to reduce extracellular acidification in MDCK-CA IX cells under hypoxic conditions. Recent studies have suggested that CA IX selective inhibitors may enhance the action of some chemotherapeutic agents (e.g. Doxorubicin) when used in a combination therapy.
HT-29 colon carcinoma cells express high basal CA activity, which is induced under hypoxic conditions (5-fold). Using a novel series of selective CA IX inhibitors and the clinically used broad range CA inhibitor acetazolomide (AZM) the effects of modulating CA activity in HT-29 cells was evaluated. The ability of selective CA inhibitors to enhance the uptake and cytotoxicity of the anticancer agent doxorubicin (DOX) was also investigated. The clonogenic assay was used to assess changes in cytotoxicity and FACS to investigate DOX uptake under aerobic and hypoxic conditions.
When used in combination with DOX (5 μM) at a non-toxic concentration of 100 μM, the CA IX selective inhibitor WRM-34 was able to enhance the cytotoxicity of DOX under hypoxic conditions (2.3-fold). A 2-fold increase in DOX (5 μM) uptake was observed in HT-29 cells when combined with WRM-34 (100 μM) under hypoxic conditions. No enhancement was seen under aerobic conditions.
The non-selective CA inhibitor AZM (100 μM) was able to enhance the cytotoxicity of DOX (5 μM) (1.8-fold) and uptake (1.4-fold) when used in combination under hypoxic conditions, with no enhancement observed under aerobic conditions.
The combination of a potent and specific CA IX inhibitor increased the cytotoxicity and uptake of the anticancer agent DOX in HT-29 cells under hypoxic conditions. This suggests that CA IX selective inhibitors may be used to enhance the uptake and efficacy of weakly basic chemotherapeutic agents, possibly via a mechanism, which decreases the pHe microenvironment surrounding tumour cells. In conclusion, selective CA IX inhibitors may be useful diagnostics and therapeutic agents in the treatment of solid tumours.

**436** POSTER

**Single nucleotide polymorphisms of Akt1 in colorectal cancer**

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**Background:** Various molecular pathways are altered by cancer cells to confer a survival advantage. By molecularly characterizing prosurvival and apoptotic pathways, more effective pharmacologic interventions can be used to target cancer cells. For example, the presence of a tumor-specific mutation at a known drug target site may better predict positive response to therapy than an alternative drug target site. The three major signal transduction pathways that have critical roles in sensing and integrating important signals in the cell are the p53 pathway, the Akt pathway, and the mTOR pathway. There is extensive cross-talk between these pathways in the regulation of cellular responses. In addition, p53, PTEN, PI3K, Akt1, and mTOR are some the most frequently altered tumor suppressor genes and oncogenes in cancers. Akt1 is an important prosurvival protein functionally involved in antiapoptosis in various cancers. Single nucleotide polymorphisms (SNP) are single base changes in the gene that are stable, heritable and occurs in greater than 1% of the population. Furthermore, approximately 30 million SNPs occur in the human genome. SNPs found in intron 3 (C/T) and exon 9 (A/G) of Akt1 affect apoptosis with the homozygous SNP haplotype (CC/GG) having decreased apoptosis.
**Materials and Methods:** The Trizol protocol was used for DNA extraction from HT-29 cells. Primers designed for Akt1 were used to produce amplicons that were then sequenced using the CEQ DNA Analyzer.
**Results:** The HT-29 colorectal cancer cell line was used to determine whether SNP 3 and SNP 4 in Akt1 can be detected. Both SNPs were detected in HT-29 cells. The haplotype predominant in the HT-29 cell line is the (CC/GG) haplotype which confers decrease apoptotic signals.
**Conclusions:** By characterizing cell lines in a pathway specific manner, a genetic profile for colorectal cancer can be constructed to make drug discovery more efficient. The data obtained indicate at least one molecular event conferring a survival advantage to cancer cells in HT-29 cell line, the (CC/GG) haplotype in Akt1. Finally, with the ability to identify molecular biomarkers predictive of drug response will help clinicians to better select the combinations of cancer treatments that maximize benefits for their patients and minimize their side effects.

**437** POSTER

**In vivo evaluation of sorafenib (Nexavar®) and sunitinib (Sutent®) alone and in combination with Rapamycin in two human renal tumor xenograft models**

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Sorafenib (Nexavar®) and sunitinib (Sutent®) are recently approved anticancer agents targeting vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Rapamycin is an immunosuppressive agent found to demonstrate potent anticancer activity by modifying cellular signaling through specific pathways, resulting in apoptosis. Recent preclinical and clinical studies have demonstrated some single agent treatment benefit; however, whether sorafenib or sunitinib ± rapamycin would demonstrate additive or synergistic combination effects was unclear. To test this hypothesis, we examined tumor growth inhibition (TGI) potential of these agents both alone and in combination in two human renal tumor xenograft models, G401 and Caki-1; sorafenib was dosed per os (PO) via oral gavage at 60 mg/kg (QD × 10), sunitinib PO at 40 mg/kg (QDx21), and rapamycin PO at 8 mg/kg (QDx14). Significant endpoints included tumor growth inhibition (TGI), tumor regression, weight loss, and agent toxicity. Differential single agent activity was noted in these studies, with rapamycin demonstrating significant TGI in G401 ($p < 0.05$) compared with sorafenib and sunitinib alone. Some benefit was also reported in combination groups compared with control in G401 and some selective activity was noted between sorafenib and sunitinib in these studies. Overall, these results demonstrate single agent activity of rapamycin towards renal cancer and suggest a benefit with combination therapy.

**438** POSTER

**Organic and inorganic arsenics operate by different biochemical pathways to induce apoptosis in cancer cells**

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**Background:** Arsenics are potent anti-cancer drugs. ZIO-101 (S-dimethyl-arsino-glutathione), a new organic arsenic, is active against diverse cancers in experimental models in vitro and in vivo. ZIO-101 is also unassociated with many of the toxicities of inorganic arsenics, like arsenic trioxide ($As_2O_3$). At equimolar extra-cellular arsenic concentrations, ZIO-101 is 5–10-fold more efficient than $As_2O_3$ in entering cancer cells resulting in more mitochondrial damage and apoptosis than $As_2O_3$. Furthermore, ZIO-101 induces apoptosis in leukemia cells from subjects resistant to therapy with $As_2O_3$.
**Methods:** NB4 (leukemia) and U937 (lymphoma) cells were cultured alone or with microM of $As_2O_3$ or ZIO-101 for 24 h. Aliquots of treated cells were tested in parallel for viability (MTT) and gene-expression profile using the Affymetrix U133_Plus_2 arrays. Differentially expressed genes were identified by fitting a linear model for each gene, making contrast for each of the comparisons based on linear model estimates, using a Beta-Uniform Mixture model (BUM) to analyze p-values of linear model F-test and t-test of each contrast and estimating cutoffs of p-values of F-test and t-test by setting FDR 0.05 for BUM results to identify differentially expressed⩽ genes.
**Results:** Data are summarized in the Table.

| Comparison | Cutoff for F-test | Cutoff for t-test | N differential genes |
|---|---|---|---|
| U937 + $As_2O_3$ vs U937 + control | P < 0.03 | P < 6.5e-011 | 0 |
| U937 + ZIO 101 vs U937 + control | P < 0.03 | P < 0.006 | 2534 |
| NB4 + $As_2O_3$ vs NB4 + control | P < 0.03 | P < 0.0005 | 190 |
| NB4 + ZIO-101 vs NB4 + control | P < 0.03 | P < 0.00008 | 14 |

**Conclusions:** These data indicate the biochemical pathways by which ZIO-101 and $As_2O_3$ induce apoptosis in cancer cells differ. This is consistent with discordant effects of modulators of reactive oxygen species (ROS) on apoptosis-induction and by the differential patterns of gene-expression in cancer cells treated with these drugs. The data support the notion that ZIO-101 may be active in cancer cells resistant to $As_2O_3$.