Conclusions: We employed *in vivo* phage display in human prostate tumor-bearing mice to identify peptides that extravasate the vasculature and specifically target the tumor cells, not just the surrounding vasculature. This finding is significant because previous *in vivo* selections preferentially retrieved phage that bound vascular components (Arap, Nat Med 8:121). Thus, our studies may facilitate the development of a range of cancer cell-surface or internalizing molecules not previously realized.

49 POSTER

DNA aptamers that recognize the MUC1 tumour marker

C. Fereira, S. Missailidis. *The Open University, Chemistry, Milton Keynes, UK*

Mucins are high molecular weight glycoproteins that provide a protective layer on epithelial surfaces and are involved in cell-cell interactions, signalling, and metastasis. The membrane-bound MUC1 mucin is expressed in normal mucosas and the aberrant expression of its under-glycosylated forms has been reported in various carcinomas of the epithelium. MUC1 is a human tumour antigen expressed in breast, pancreatic and ovarian cancers. Agents able to bind tightly and specifically to the surface of malignant cells would greatly benefit cancer diagnosis and treatment, whereas the targeting of cell surface receptors would have significant implications on inflammation and immunity. While antibodies have the ability to specifically recognise some tumour cell markers, their large size and own immunogenicity markedly limit their pharmacological value. The development of nuclease resistant DNA molecules, termed *aptamers*, has provided a new alternative to antibodies. Using the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) methodology, one can generate vast libraries of oligonucleotide ligands (DNA, RNA, or unnatural products), screened rapidly for specific sequences that have appropriate binding affinities and specificities to the clinically relevant marker.
We have identified synthetic DNA oligonucleotide aptamers that bind to the MUC1 tumour marker with low nanomolar affinity via the 20 tandem repeat sequence of the MUC1. These specific aptamers were selected from an initial library that contained a degenerate region of 25 bases to result in 4^{25} random-sequence DNA molecules. Ten rounds of in vitro selection and amplification were performed, to confer affinity maturation of aptamers for MUC1. Selected aptamers were cloned, sequenced and found to be sharing some unique consensus sequences. The affinity of each aptamer for MUC1 was studied by qualitative and quantitated methods such as ELISA, BIAcore, and EMSA. Affinities on the nanomolar range have been identified and confirmed.
Efforts in our laboratory are now focusing on optimization of their delivery, functionality and structural properties. Fluorescent labelled aptamers have been successfully used in the identification of MUC1 expressing tumour cell lines such as the MCF7 breast tumour cell line and can be used in future diagnostic assays, whereas radiolabelled aptamers can be used clinically to enable imaging and therapy of the tumour marker bearing cancer cells.

50 POSTER

Inhibition of histone deacetylase 2 increases apoptosis and P21 expression

S.C. Hooi, M.L. Laban, B.H. Huang, L. Lee, G.C. Raju, C.K. Lee, M. Salto-Tellez. *National University of Singapore, Physiology, Singapore, Singapore*

Background: Histone deacetylases (HDACs)1 and 2 share a high degree of homology and are known to co-exist within the same protein complexes containing other transcriptional co-repressors. Despite their close association, studies have shown that each possesses unique functions, which cannot be compensated by the other. This study describes the regulation of HDACs1 and 2 in colorectal and cervical cancers and the function of HDAC2 in apoptosis.
Materials and Methods: A combination of quantitative RT-PCR and tissue array was used to determine the expression of HDAC1 and HDAC2 in colorectal cancer and matched normal mucosal samples. Immunohistochemistry was used to determine the expression of HDACs1 and 2 in cervical cancers and dysplasias. HDAC2 expression in HeLa cells were knocked-down by specific siRNAs designed against HDAC2 (Dharmacon Inc). The efficacy of knock-down was confirmed by Western blot analysis. Apoptosis was assayed by annexin V-FLUOS staining and flow cytometry. P21 expression was determined by Western blot analysis using a specific antibody (Snata Cruz Biotechnologies).
Results: Both HDACs1 and 2 are upregulated at the mRNA (n=16) and protein (n=45) levels in colorectal cancer. The upregulation of HDAC2 was more robust and occurred more frequently in the samples. It also occurred early in the carcinogenic process, with 4 of the 5 polyps showing upregulation of HDAC2 compared to normal mucosa. In cervical carcinoma (n=9), the expression of both HDACs1 and 2 was correlated with the severity of cervical dysplasias and invasive carcinomas of the cervix. However, HDAC2 expression showed a clear demarcation of higher intensity staining at the transition region of dysplasia. Further, more cells were stained for HDAC2 than HDAC1 in cervical dysplasia. The functional significance of HDAC2 upregulation was determined by knocking down the expression of HDAC2 with HDAC2-specific siRNA. Cells displayed an increased number of cellular extensions reminiscent of cell differentiation after HDAC2 knockdown. There was also an increase in apoptosis, associated with an increase in P21 expression.
Conclusion: The results suggest that histone deacetylases, especially HDAC2, are important enzymes involved in the early events of carcinogenesis, making them candidate markers for tumor progression and targets for cancer therapy.

51 POSTER

HSV-tk gene transduction enhances proliferation rate and COX-2 expression in rat gliosarcoma cells

K. Al-Athamen, A. Konson, G. Rimon, A. Danon, R. Agbaria. *Ben-Gurion University of the Negev, Department of Clinical Pharmacology, Beer-Sheva, Israel*

Background: Transduction of tumor cells with the Herpes Simplex Virus thymidine kinase (HSV-tk) gene and consequent treatment with ganciclovir is widely used for suicide gene therapy of brain tumors. Recently we observed that HSV-tk gene transduction of rat gliosarcoma (9L) cells enhances the expression of cyclooxygenase-2 (COX-2) and the release of prostaglandin E_2 (PGE_2). It is well established that COX-2 overexpression with increased production of COX-2-derived prostaglandins are associated with multiple aspects of carcinogenesis, including the control of cell proliferation and increased resistance to apoptosis and chemotherapy.
Aims: In the current work we aimed to: a) determine the effect of HSV-tk gene transduction on proliferation rate of 9L cells; b) evaluate the effect of nimesulide (NIM), a selective COX-2 inhibitor, on proliferation rate of wild-type (9L) and HSV-tk transduced (9L/HSV-tk) 9L cells.
Results: Western blot analysis of COX-2 protein expression in 9L and 9L/HSV-tk cells showed that COX-2 is overexpressed in HSV-tk transduced cells, while wild-type cells did not express COX-2 at detectable levels. COX-2 overexpression in HSV-tk transduced cells was accompanied by increased release of PGE_2, assessed by radioummunoassay, into the culture medium ($2.5\pm$ 0.2 *vs* 102.3 ± 9.4 ng/10^6 cells for 9L and 9L/HSV-tk cells, respectively). In order to determine the effect of HSV-tk gene transduction on cell proliferation rate, 9L and 9L/HSV-tk cells were incubated for 120 hrs in 24-well culture plates and the number of attached cells was counted every 24 hrs. We found that proliferation rate of 9L/HSV-tk cells was 2 to 3-fold higher, compared to that of wild-type 9L cells. To evaluate whether increased release of PGE_2 accounts for the observed enhancement in proliferation rate in the 9L/HSV-tk cells, we investigated the effect of NIM on proliferation rate of both wild-type and HSV-tk transduced cells. Incubation of 9L and 9L/HSV-tk cells with NIM at a concentration which inhibits COX-2 activity, completely abolished PGE_2 release in both wild-type and HSV-tk transduced cells for as long as 96 hrs. However, at this concentration, NIM failed to affect proliferation rate of 9L and 9L/HSV-tk cells.
Conclusions: Taken together, we demonstrate herein that HSV-tk gene transduction enhances proliferation rate and COX-2 protein expression and activity in rat gliosarcoma cells. Additionally, the enhanced proliferation rate of HSV-tk transduced cells appears to be independent on prostaglandins overproduction since treatment with the selective COX-2 inhibitor failed to abolish the enhancement of the proliferation rate.

52 POSTER

Attenuated immunogenicity and toxicity of PEGylated recombinant methioninase (PEG-rMETase) in primates

Z. Yang[1], J. Wang[2], Y. Kobayashi[3], C. Lian[4], S. Li[1], X. Sun[1], Y. Tan[1], S. Yagi[1], E.P. Frenkel[5], R.M. Hoffman[1]. *[1]AntiCancer, Inc., San Diego, CA, USA; [2]Jiangsu Kinglsey Pharma. Co. Ltd., Nanjing, P.R. China; [3]Shionogi and Co. Ltd., Osaka, Japan; [4]Suzhou West Hill Exp. Animal Co., Suzhou, P.R. China; [5]Univ. of Texas, Southwestern Med. School, Dept. of Internal Medicine, Dallas, TX, USA*

Background: Methionine depletion by recombinant methioninase (rMETase) has been demonstrated to be an effective antitumor regimen in tumor-bearing mouse models. However, the therapeutic potential of rMETase has been limited by its short plasma half-life and immunological effects, including high antibody production in mice and monkeys and anaphylactic reactions in monkeys.
Materials and Methods: In order to improve the therapeutic potential of rMETase, a PEG-rMETase conjugate has been developed by coupling the enzyme to methoxypolyethylene glycol succinimidyl glutarate (MEGC-PEG-5000). In this study, we evaluated the pharmacokinetics, antigenicity and

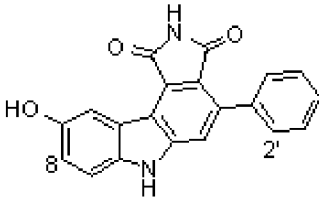
toxicity of MEGC-PEG-rMETase in *Macaca fascicularis* monkeys using an escalating-dose strategy.
Results: Dose ranging at 1000, 4000 and 8000 U/kg i.v. determined that a single dose of 4000 U/kg was sufficient to reduce plasma methionine to less than 5 μM for 12 hours. Pharmacokinetic analysis with the single 4000 U/kg dose showed that MEGC-PEG-rMETase holoenzyme activity was eliminated with a $T_{1/2}$ of 1.3 hours and the MEGC-PEG-rMEtase apoenzyme was eliminated with a $T_{1/2}$ of 90 hours. A seven-day i.v. administration of 4000 U/kg every 12 hours resulted in a steady-state depletion of plasma methionine to less than 5 μM. The only manifest toxicity was decreased food intake and slight weight loss. Red-cell values and Hgb declined transiently during treatment, but recovered after cessation of treatment. Subsequent challenges on days 29, 50 and 71 did not result in any immunologic reactions. Anti-MEGC-PEG-rMETase antibodies (at 10^{-2}) were found on day 29, and these increased to 10^{-3}–10^{-4} on day 71, 100–1,000-fold less than antibodies elicited by naked rMETase. Although anti-MEGC-PEG-rMETase antibodies were produced, no neutralizing antibody was identified and each challenge dose was effective in depleting plasma methionine levels.
Conclusions: The results suggested that PEGylation greatly prolonged serum half-life of rMETase apoenzyme. Results from studies of PEG-rMETase in mice suggest that co-infusion of the cogactor for PEG-rMETase, pyridoxal-5′-phosphate well greatly prolong holoenzyme half-life as well in primates. Anaphylactic reactions were eliminated. The results of the present primate study present a safety profile with respect to toxicity and antigenicity that suggest clinical potential of MEGC-PEG-rMETase.

53 POSTER

Pyrrolo[3,4-c]carbazole-1,3-dione inhibitors of the G2/M checkpoint kinase wee1

B.D. Palmer[1], R.J. Booth[2], W.A. Denny[1], K. Hook[2], A.J. Kraker[2], H.H. Lee[1], D. Ortwine[2], J.B. Smaill[1], C. Squire[3], A.M. Thompson[1].
[1]Auckland Cancer Society Research Centre, University of Auckland School of Medicine, Auckland, New Zealand; [2]Pfizer Global Research and Development, Ann Arbor, USA; [3]School of Biological Sciences, University of Auckland, Auckland, New Zealand

The kinase enzyme wee1 is involved in regulation of the G2/M checkpoint in the eukaryotic cell cycle, through its inhibitory phosphorylation of Cdc2 on tyrosine 15. Many cancer cells lack a functional p53 gene, which means that their G1/S checkpoint is not controlled. Inhibitors of wee1 would abrogate the G2/M checkpoint, and should preferentially enhance the cytotoxic effects of DNA damaging agents on p53-negative cells, by allowing them to bypass both of the checkpoints where damaged cells normally arrest to allow time for DNA repair. High throughput screening identified (**1**) as a novel potent (IC_{50}=95 nM) and selective inhibitor of wee1. An X-ray structure of a co-crystal of (**1**) and the enzyme revealed that the inhibitor was bound at the ATP site of the kinase, and identified key features of the mode of binding.



(**1**)

A large number of derivatives of (**1**) were prepared, guided in part by molecular modelling and X-ray co-crystallography of key compounds, seeking to improve potency, selectivity and physical properties. Introduction of lipophilic functionality at the 2′-position was found to increase potency and selectivity, leading to low nanomolar inhibitors, while improvements in physical properties were best achieved by attaching solubilizing groups at the 8-position. The results of a comprehensive SAR study for this series will be presented, together with *in vitro* evidence that co-administration of a wee1 inhibitor with DNA-damaging agents does lead to enhanced cytotoxicity compared with the cytotoxin alone.

54 POSTER

In vitro, in vivo and in silico examination of the activity of antitumor 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazoles

T. Bradshaw[1], C. Leong[1], M. Suggitt[2], D. Swaine[2], M. Bibby[2], M. Stevens[1]. *[1]University of Nottingham, School of Pharmacy, Nottingham, UK; [2]University of Bradford, School of Life Sciences, Bradford, UK*

The dihydrochloride salt of the lysylamide prodrug of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (Phortress) is a potent and selective experimental antitumor agent undergoing Phase I clinical evaluation. Its novel mechanism of action involves induction of CYP1A1-catalyzed metabolism of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203) by sensitive tumor cells to generate electrophilic species, which exact lethal damage to DNA of sensitive tumor cells only. The exquisitely selective antitumor activity *in vitro* has been observed *in vivo*. Moreover, the perceived mechanism of action *in vitro* has been validated in xenograft models: selective induction by 2-(4-aminophenyl)benzothiazoles of CYP1A1 protein, and subsequent generation of DNA adducts *in vitro* and *in vivo* have been reported. We report the effects of adduct formation upon progression through cell cycle and cellular DNA integrity in sensitive and inherently resistant tumor cells by single cell gel electrophoresis (SCGE; comet assay); and discuss whether this sensitive and relevant pharmacodynamic (PD) endpoint may be exploited to a) probe the clinical mechanism of action of Phortress and b) predict tumor response.
Human-derived tumor cells were cultured *in vitro*, or *in vivo* in polyvinylidine fluoride (PVDF) hollow fibers implanted at subcutaneous (s.c.) and intraperitoneal (i.p.) sites, or as s.c. xenograft implants in the flanks of pure strain NMRI female mice. SCGE demonstrated dose and time-dependent single and double strand breaks exclusively in DNA of sensitive cells following treatment with 5F 203 *in vitro* (10 nM-10 μM; 24–72 h). The comet assay also afforded a reliable method to determine DNA damage encountered by MCF-7 tumors *in vivo*, following treatment of mice with a clinically efficacious concentration of Phortress (20 mg/kg, i.p.). Moreover, by SCGE, we were able to distinguish clearly between sensitive (MCF-7) and inherently resistant (MDA-MB-435) tumor cells grown in hollow fibers at s.c. and i.p. sites.
In view of the CYP1A1-mediated generation of DNA damage, it may be argued that Phortress represents a P450-activated cytotoxic class of agent comparable to oxazaphosphorine anticancer prodrugs, however, examination of the mechanism of action *in silico*, by self organized map (SOM) cluster analyses led to speculation that the aminophenylbenzothiazole class of antitumor agents may modulate phosphatases or kinases associated with cell cycle regulation; indeed, this hypothesis is supported experimentally by the selective perturbation of the cell cycle by 5F 203 in sensitive tumor cells only.

55 POSTER

A phase I study of SB-715992, a novel kinesin spindle protein (KSP) inhibitor: pharmacokinetic (PK)/pharmacodynamic (PD) modeling of absolute neutrophil counts (ANC)

D. Williams[1], S. Kathman[1], Q. Chu[2], K. Holen[3], E. Rowinsky[2], G. Wilding[3], P. Mudd[1], J. Herendeen[1], J. Orr[1], L. Pandite[1].
[1]GlaxoSmithKline in collaboration with Cytokinetics Inc., Clinical Pharmacology, Research Triangle Park, NC, USA; [2]CTRC, San Antonio, TX, USA; [3]Comp Cancer Center, Madison, WI, USA

Objective and Background: SB-715992 acts by a novel mechanism of action, namely inhibition of KSP, which is critical for centrosome separation, and formation of a bipolar spindle during mitosis. Neutropenia was the dose limiting toxicity in the first clinical trial of SB-715992 when administered on a once every 21-day schedule. An analysis was initiated to assess the impact of the PK of SB-715992 and demographic variables on the ANC.
Methods: Cycle 1 data were collected from 42 solid tumor patients. SB-715992 was administered as a 1-hour I.V. infusion once every 21 days in a Phase I open-label, non-randomized dose-escalation study at doses ranging from 1–21 mg/m^2. Neutrophil counts were followed weekly on Days 1, 8 and 15 of a 21-day cycle. Two models were developed to analyze the PK/PD relationship: an Emax model for % decrease from baseline ANC, and an ordinal model for 3 categories of neutropenia (NCI CTC Version 2) Gr 0, Gr 1–3 and Gr 4. These models were used to examine the contribution of the following independent variables: dose (mg/m^2), total dose (mg), AUC0-∞ (log transformed), Cmax (log transformed), time above concentration threshold, and the following demographic data: baseline ANC, Body Surface Area (BSA), gender, and extent of previous treatment. **Results.** In the Emax and ordinal model, the most predictive independent variables were dose and total dose, followed by AUC (log transformed) or Cmax (log transformed) when evaluated separately. None of the demographic data contributed significantly to the ANC decline.