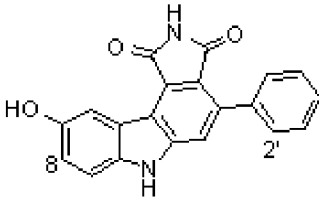
toxicity of MEGC-PEG-rMETase in *Macaca fascicularis* monkeys using an escalating-dose strategy.
Results: Dose ranging at 1000, 4000 and 8000 U/kg i.v. determined that a single dose of 4000 U/kg was sufficient to reduce plasma methionine to less than 5 μM for 12 hours. Pharmacokinetic analysis with the 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.