an EDTA buffer. After overnight incubation, the Alphascreen counts were measured on a Fusion reader. Approximately 65,000 compounds were screened in 384-well format in 17 batches. The kinase inhibitor H8 gave an IC50 of ~30M and was used as a positive control on each plate (43.3 13.1% inhibition at 30M). Across the whole screen Z' factors were 0.830.07. Hits were identified as compounds inhibiting activity by >3SD of the compound wells on each plate. The activity of hits (707) was confirmed on Alphascreen (50) followed by a filter-binding assay. Overall confirmed hit rate was ~0.1%. AlphaScreen false positives included compounds with biotin-like moieties, a surfactant-like compound and two highly coloured azo-dyes, consistent with expected mechanisms of interference with t his assay format. Several hit series were identified including a number of toxoflavins (0.78–15M) and a rhodanine analogue (IC50 26M) that have similarity to previously disclosed PKB/AKT inhibitors and thus confirm the ability of the screen to identify valid hits.

110 POSTER

In vitro screening of anticancer drug penetration into tumour tissue

A.H. Kyle, L.A. Huxham, A.I. Minchinton. *BC Cancer Research Centre, Medical Biophysics, Vancouver, Canada*

The failure of many anticancer drugs to control the growth of solid cancers may stem in part from inadequate delivery to tumour regions distant from vasculature. Unfortunately, extricating the effect of tissue penetration from the many other factors that affect a drug's efficacy in tumours with distance from vasculature is generally not possible. In this study we use a novel, effect-based assay that employs multilayered cell culture (MCC), a tissue-engineered disc grown from tumour cells on a permeable membrane. MCCs are similar to multicellular spheroids but their planar nature permits flux through the cultures to be more easily measured. Like spheroids they exhibit a gradient in proliferation but since MCCs grow as discs, this gradient forms as a mirror image from either side towards the middle. In this study, we exploit this symmetry by exposing MCCs to drugs from one side and then comparing their effect on the exposed side versus the far side of the cultures. This approach circumvents issues that normally arise from the biochemical gradients that occur with distance into tissue (e.g. changing intrinsic sensitivity of cells to drugs with depth into tissue). In this study we have examined representative drugs from a selection of anticancer drug classes, including platinum based agents (cisplatin), microtubule agents (vincristine, vinblastine, vinorelbine and paclitaxel) and topoisomerase II inhibitors (etoposide). The distribution of drug activity within the cultures was assessed 1 to 3 days after exposure via immunodetection of S-phase cells using bromodeoxyuridine. Using an automated computer analysis routine, the effect of the drugs in the first three cell layers located on either edge of the cultures relative to controls was then assessed. Results for penetration of the agents through the MCCs were grouped into three classes: uniform tissue distribution (cisplatin), 1–3 fold decrease in drug exposure to cells on the far side versus the exposed side of the cultures (vincristine, vinblastine and paclitaxel) and greater than 3-fold difference (vinorelbine and etoposide). This model could be applied as a screening system for the discovery of biologically active drugs, which exhibit desirable penetration properties. This research is supported by the National Cancer Institute of Canada with funds from the Canadian Cancer Society, the Michael Smith Foundation For Health Research and the Canadian Institutes for Health Research.

111 POSTER

Development and validation of a mass spectrometric assay to evaluate drug–drug interaction in human liver microsomes. Application to anticancer agents

F.I. Raynaud, J. Moreno-Farre, P. Workman. *The Institute of Cancer Research, Cancer Research UK Centre for Cancer Therapeutics, Sutton, UK*

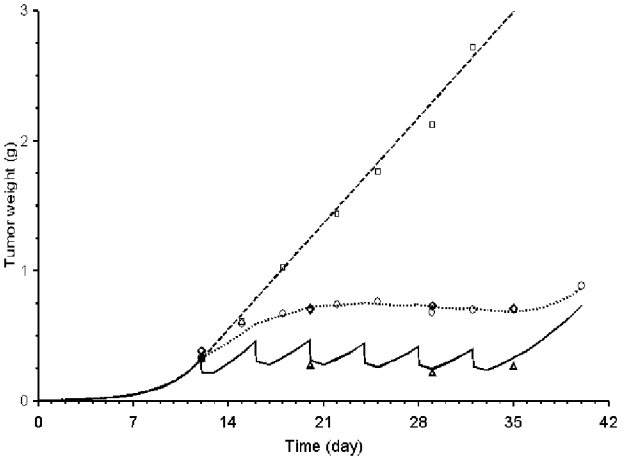
Most anticancer agents are administered in combination with other anticancer drugs or alternative therapies. New drugs that affect molecular targets are likely to be prescribed chronically i.e orally thereby increasing the risk of interactions with other drugs. This can potentially lead to toxicity and it is therefore important to predict possible drug-drug interactions with novel compounds. We present an in vitro assay in human liver microsomes, which screens for potential inhibition of CYP1A2, 2A6, 2C9, 2C19, 2D6, 2E1 and 3A4 in a single microsomal incubation. A mixture of probe substrates (phenacetin at 10 μM, coumarin at 5 μM, tolbutamide at 60 μM, amitriptyline at 5 μM, cloroxazone at 50 μM, bufarolol at 5 μM and midazolam at 3 μM) are incubated in human microsomes (0.4 mg of protein in 200 μL) for 30 min followed by protein precipitation with methanol. The metabolites are measured by LCMSMS. 6-hydroxychloroxazone is detected by selected reaction monitoring in negative ionisation mode following separation on a Synergi hydro-RP (50mmx4.6mm, 4 μm) column with a gradient of 10mM ammonium acetate and methanol whilst all other metabolites are monitored in positive mode following elution from the same column with 0.1% formic acid and methanol. The total run time is 11 minutes. Validation of the assay, performed by measurement of the K_m for each substrate showed values similar to those reported in the literature. Studies with known inhibitors 7-ethoxyresorufin (10 μM), 8-methoxypsoralen (10 μM), omeprazole (50 μM), sulfaphenazole (5 μM), quinidine (2 μM), diethyl-dithiocarbamate (50 μM), testosterone (50 μM), ketoconazole (1 μM), confirmed the ability of this method to identify specific inhibitors of all the enzymes probed for. Currently used drugs such as doxorubicin, daunorubicin, carboplatin, 5-fluorouracil, mitomycin C, etoposide, tamoxifen, vincristine, vinblastin have also been evaluated in this drug-drug interaction screen. Novel agents such as CYC202, SR4554, 17-AAG have also been tested. This validated technique is currently routinely used in our drug development programs.
This work was supported by Cancer Research UK.

112 POSTER

Pharmacokinetic-pharmacodynamic (PK-PD) modeling of tumor growth inhibition in mice: a magnetic resonance imaging study

M. Rocchetti[1], A. De Grassi[1], M. Simeoni[2], E. Pesenti[1], M. Russo[1], I. Poggesi[1]. [1]*Nerviano Medical Science, Pharmacokinetics, Dynamics and Metabolism, Nerviano, Italy;* [2]*University of Pavia, Dipartimento di Informatica e Sistemistica, Pavia, Italy*

A PK-PD model, based on a system of differential equations, has been developed linking the dosing regimen to the tumor growth in xenograft models (Simeoni et al, Cancer Res. 64:1094; 2004). In this model it is assumed that tumor cells hit by an anticancer drug stop proliferating and go to death through a transit compartmental system, which represents the hypothetical progressive degrees of damage of tumor cells. The model is therefore able to predict the mass of proliferating and damaged cells fitting the total tumor mass vs. time curves. This communication describes the results of a study in which total and viable tumor mass was monitored using magnetic resonance imaging (MRI).



Ø: controls measured by caliper, dashed line is model-fitted controls; ○: total tumor mass measured by caliper, ♦: total tumor mass measured by MRI, dotted line is model-fitted total tumor mass; Δ: viable tumor mass measured by MRI, solid line is model-predicted mass of proliferating cells.

HCT 116 tumor bearing nude mice were given placebo or irinotecan (50 mg/kg, q4dx6). MRI assessment was performed using T2-weighted images on a Bruker Pharmascan equipment. Total tumor mass and viable tumor mass were evaluated using the multislice procedure. Total tumor mass were also evaluated using timely standard caliper measurements. Plasma pharmacokinetics of irinotecan were obtained from a previous assessment. Simultaneous fitting of the average total tumor mass in control and treated animals was performed using non-linear regression (Winnonlin 3.1, Pharsight).
Model fittings were good and provided reliable parameter estimates. Pharmacodynamic parameters were consistent with those previously published for irinotecan. The MRI-viable tumor cells were in agreement with the mass of cycling cells (i.e., not damaged) predicted by the model. This reinforces the biological relevance of this PK-PD model.