and time-dependent manner. EC in log phase growth were found to be particularly sensitive to the growth inhibitory effects of 17AAG, with IC50's in the low nanomolar range, however, quiescent endothelial cells were relatively resistant. We then tested EC migration using a "haptotaxis" (wounded monolayer) assay and a real-time fluorescence based chemotaxis assay. 17AAG inhibited both EC haptotaxis and chemomigration towards FCS, VEGF, HGF, bFGF and EGF at concentrations below those required to inhibit proliferation. Invasion of Matrigel-coated filters was more potently inhibited than migration, suggesting possible additional effects on matrix proteolysis. We found no effects on MMP-2 activity, but 17AAG inhibited uPA production, as shown previously for tumour cells. 17AAG also significantly reduced EC tubule differentiation on Matrigel. In addition, in several human tumour cell lines, 17AAG inhibited the upregulation of VEGF mRNAs and proteins induced by ligand activation of c-*erb*B oncogenes or hypoxia. *In vivo* we found that murine endothelial cell client proteins were downregulated by 17AAG and growth inhibition of human tumour xenografts was associated with reduced microvessel density.
These results identify HSP90 as an important protein chaperone in tumour cell production of, and functional responses to VEGF and other EC activators. HSP90 inhibitors may have a useful role in cancer therapy not only by directly inhibiting tumour cell proliferation but also via interference with several distinct rate-limiting steps in the angiogenic cascade. The fact that rapidly proliferating EC are more sensitive to Hsp90 inhibitors than quiescent EC suggests that normal vasculature may be spared relative to "angiogenic" vasculature.

332 POSTER

Fragment-based and structure based optimisation of potent PKB/AKT inhibitors

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The serine/threonine kinase PKB/AKT is a major downstream target of PI3K. Extensive studies of this protein kinase show that it plays a key role in promoting cancer cell proliferation and survival via inhibition of apoptosis. Growth factor over-expression, mutations in the ras genes, overexpression of the lipid kinase PI3 kinase and loss of the lipid phosphatase tumour suppressor gene PTEN lead to activation of PKB and have been identified in multiple forms of cancer, implicating this kinase pathway in tumour development. The identification of small molecule inhibitors were sought in order to develop molecules useful for the treatment of cancer.
An integrated fragment-based approach utilizing virtual screening, X-ray crystallography and NMR was applied to identify novel leads for PKB. From a library of ~300,000 fragments including our drug fragment set and kinase biased set, 8 key fragments were identified and validated by structural studies. The fragment hits had a spread of potency in-vitro (16uM-1mM), low molecular weights and were considered to have drug-like properties.
Further structure-based design identified 2 lead series with single digit nano-molar potencies, whilst maintaining drug-like properties and low molecular weights (<400). Furthermore, using SBD, we took one of our original hits (80uM) and identified a 30nM lead compound from the synthesis of only 14 analogues.
In summary, we have identified a number of novel, potent and drug like inhibitors of PKB using fragment-based discovery.. We will present our approach in detail and the associated biological data for the lead compounds.

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Akt pathway siRNA screening using automated fluorescence imaging

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Background: The Akt-family of kinases constitutes a major node of a signaling pathway that regulates cell-growth and apoptosis and is implicated in the process of tumorigenesis. We have combined RNAi gene knockdown techniques and automated fluorescence imaging of multiple nodes of the Akt pathway to investigate the interplay of various pathway proteins and to facilitate a screen for novel components of the pathway.
Material and Methods: siRNA transfections were carried out using 25nM/well (96-well plate) of siRNA using oligofectamine. Cells were fixed in formaldehyde 72 hours post transfection and prepared for immunofluorescence staining of various phospho-epitopes of proteins downstream of Akt signaling. Samples were analyzed using automated fluorescence imaging on a Cellomics ArrayScan II measuring the distribution of fluorescence stain within different components of the cell.
Results: RNAi of Akt2, mTOR or p70S6Kinase had the expected result reducing phosphorylation levels of their substrates and inhibiting signaling events lower in the pathway. Unexpectedly RNAi knockdown of certain downstream components of Akt signaling pathways such as mTOR, p70S6Kinase and EIF-4E-BP1 also modulated the phosphorylation of proteins higher in the classic Akt pathway.
Conclusions: Our data suggest that the various signaling pathways downstream of Akt are not simple linear pathways but involve feedback loops and cross-talk that complicate the positional interpretation of novel components of these pathways.

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First-in-human study of the safety, tolerability, pharmacokinetics, and pharmacodynamics of oral cp-724, 714, a selective, small molecule inhibitor of her2 in patients with advanced cancer

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Background: HER2 is expressed in a variety of tumor types and plays an important role in oncogenic signaling. HER2 inhibitors have demonstrated benefits in patients with advanced HER2-overexpressing cancers. CP-724,714 is a reversible, highly selective, small-molecule HER2 tyrosine kinase inhibitor currently in clinical development.
Methods: The safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of oral CP-724,714 (250 mg qd, 250 mg bid, 250 mg tid, and 400 mg bid) administered in 3-week cycles were assessed in patients with HER2 positive advanced solid tumors using a dose-escalation design. Safety assessments included adverse events (AEs), clinical laboratories, ECG, and MUGA scans. Blood sampling for PK was performed for up to 48 and 12 hours after the first dose in cycles 1 and 2, respectively. Serum CP-724,714 concentrations were measured by LC/MS/MS following solid phase extraction with PK parameter values estimated using noncompartmental techniques. PD measures included serial assessment of HER2-related signaling pathways via immunohistochemistry analyses of tumor biopsies and ELISA of serum HER2 extracellular domain (ECD) concentrations.
Results: To date, 23 pts have been enrolled, with data available on 17 pts [median (range) age 50.5 (37–71); PS (%) (0 (41.2); 1 (58.8))]. HER2 FISH status evaluations of pretreatment archival tissue: amplified (n=7), non-amplified (n=5), and not reported (n=5). The median number of cycles started was 2 (range 1–5). The most common treatment-related AEs were mild nausea (58.5%), fatigue (35.3%) and hyperbilirubinemia (29.4%). Dose-limiting reversible, grade 3 conjugated hyperbilirubinemia and grade 3 elevated ALT/AST/GGT were noted in 1 patient each in Cycle 1 in the 400 mg bid dose group. No treatment-related cardiomyopathy has been reported. The mean (SD) PK parameter values are AUC 8460 (5230) and 11600 (5900) ng?h/mL, Cmax 3170 (2060) and 3980 (2150) ng/mL and median Tmax 1.5 and 1.6 h, respectively, for a single dose of 250 mg and 400 mg. Systemic exposure steady state in both the 250 and 400 mg dose cohorts exceed the predicted efficacious exposures based on preclinical efficacy experiments. PK/PD analyses using tumor biopsy and serum HER2 ECD data are ongoing. To date, no objective responses have been reported in this population of 16/17 trastuzumab-pretreated patients.
Conclusions: Daily administration of CP-724,714 (250 mg qd and 250 mg bid) appears safe and well tolerated. DLTs, observed at 400 mg bid, are reversible hyperbilirubinemia (1/5) and elevated ALT/AST/GGT (1/5). Systemic exposure exceeds the threshold for efficacy as predicted from preclinical studies. Enrollment is continuing at 250 mg tid.

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SHP-1 protein tyrosine phosphatase as a target molecule in anti-tumor immune therapies: SHP-1 inhibitor SSG interacts withIL-2 to increase anti-murine renal tumor immunity

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SHP-1 is a key negative regulator of cytokine signaling and immune cell activation. This functional role of the protein tyrosine phosphatase suggests that it may have potential as a molecular target for augmenting anti-tumor immunity induced by cytokine- and immune cell-therapies. IL-2 therapy induces responses in advanced renal cell carcinoma (RCC) in connection with its ability to expand and activate immune cells. Based on our recent finding of sodium stibogluconate (SSG) as a SHP-1 inhibitor, the potential of SSG to interact with IL-2 and augment anti-RCC immunity was investigated