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.

333 POSTER

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.

335 POSTER

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

in a murine renal tumor model (Renca). Despite its lack of effects on Renca cell growth in culture, SSG as a single agent in Balb/c mice induced 61% growth inhibition of Renca tumors coincident with increase (2 folds) of tumor-infiltrating macrophages (Mϕ). SSG/IL-2 combination induced 91% growth inhibition of Renca tumors in Balb/c mice accompanied by 4-fold increase in tumor infiltrating Mϕ. IL-2 by itself failed to inhibit Renca tumor growth as reported previously and did not induce tumor-infiltrating Mϕ. The safety of SSG and SSG/IL-2 combination was indicated by the tolerance of the treatments in these mice. Although SSG/IL-2 treatment did not increase tumor-infiltrating T cells in association with Renca tumor growth inhibition in the Balb/c mice, its lack of anti-Renca tumor activity in athymic nude mice indicated a T-cell-dependent immune action mechanism of the combination. Involvement of the tumor-infiltrating Mϕ in the action was supported by the capacity of SSG to increase cytotoxic activity (4 folds) of Raw 264.7 Mϕ against Renca cells in vitro. Evidence of more marked Mϕ increases in spleen in the mice treated with SSG (3 folds) or SSG/IL-2 combination (6 folds) indicated a systemic Mϕ expansion, which recapitulates a prominent feature of murine genetic SHP-1 deficiency and is consistent with in vivo inhibition of SHP-1 by SSG in these mice. These results together demonstrated an anti-Renca tumor activity of SSG that interacts with IL-2 via a T-cell-dependent immune action mechanism in connection with expansion/activation of Mϕ. They provided evidences suggesting a therapeutic potential of SSG in anti-RCC immune therapy and indicating SHP-1as a target molecule for augmenting anti-tumor immunity and improving the efficacy of immune therapies.

336 POSTER

De novo resistance to epidermal growth factor receptor blockade by gefitinib in colorectal cancer cells involves increased insulin receptor isoform A signalling

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Background: Gefitinib (Iressa®) is an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) that has antitumour activity in a range of cancer types. It has become apparent, however, that high EGFR expression and activity do not always predict for response and clinical data have indicated the existence of both *de novo* and acquired resistance to gefitinib in tumours reported to be EGFR positive. In this study, the possible involvement of the insulin receptor isoform A (InsR-A) in *de novo* gefitinib resistance has been investigated in LoVo colorectal human cancer cells.
Materials and Methods: LoVo cells were exposed to 1 μM gefitinib for 7 days. Cell proliferation was determined by evaluating anchorage-dependent growth. Alterations in signalling pathways were assessed by immunocytochemical, Western blotting and/or reverse transcription polymerase chain reaction techniques. Cell sensitivity to the InsR/insulin-like growth factor (IGF)-1 receptor inhibitor ABDP (AstraZeneca) was also measured.
Results: The highly EGFR-positive LoVo cells demonstrated negligible growth inhibition (<15%) after exposure to 1 μM gefitinib. Compared with controls, the gefitinib-treated cells showed a reduction (45%) in phospho-EGFR (Tyr-845) activity, the tyrosine site located in the kinase domain of the receptor. However, levels of activity of the downstream receptor tyrosine sites Tyr-1068 and Tyr-1173 were unchanged in the presence of 1 μM gefitinib, as was the activity of phospho-ERK1/2. Compared with a panel of various cancer cell line types (A549, DU145 and MCF-7), it was noted that the LoVo cells produced markedly higher levels (9-fold) of IGF-II mRNA, unprocessed (~230 kD) inactive pro-IGF-1R protein, mature InsR protein (~130 kD α-subunit) and substantially elevated (10-fold) amounts of InsR-A mRNA, with minimal detection of InsR-B mRNA. Exposure of LoVo cells to insulin and IGF-II resulted in growth promotion (20% and 40%, respectively) and increased phospho-EGFR (Tyr-1068) and EGFR (Tyr-1173) activity over 60 min. Furthermore, challenge with the InsR/IGF-1 receptor inhibitor ABDP resulted in a dose-dependent decrease in basal growth (IC_{50}=0.25 μM). Interestingly, both phospho-InsR and phospho-Akt levels increased (2-fold and 5-fold, respectively) after treatment with gefitinib.
Conclusions: We propose that *de novo* resistance to gefitinib in LoVo colorectal cancer cells may, in part, be directed through the InsR-A, utilising IGF-II as a ligand. The interactions between InsR and EGFR are currently being further assessed. Evidence suggests that InsR-A overexpression is important in the promotion of cancer growth in IGF-II-producing tumours (Sciacca *et al*. Oncogene 2002;21:8240–50). Co-targeting cells with gefitinib and an InsR/IGF-1R inhibitor may prove more effective than gefitinib alone.
Iressa is a registered trademark of the AstraZeneca group of companies

337 POSTER

Velcade, an NF-kb inhibitor, enhances tamoxifen sensitivity in Akt-induced resistant breast cancer xenograft tumors

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Tamoxifen resistance is the underlying cause of treatment failure in a significant number of patients with breast cancer. Only approximately 50% of estrogen receptor α-positive tumors will initially respond to hormonal therapy, and of those tumors that do initially respond, a refractory phenotype will develop in a majority of them within 5 years. Activation of Akt, a downstream mediator in the PI3K signaling pathway has been implicated as one of the mechanisms involved in tamoxifen resistance. Breast cancers with heightened Akt activity are frequently associated with an aggressive disease and resistance to chemo- and hormone-therapy induced apoptosis. In previous studies we have demonstrated that MCF-7 breast cancer cell lines expressing a constitutively active Akt (myrAkt1) are resistant to tamoxifen, both *in vitro* as well as *in vivo* in xenograft models. One potential mechanism is through activation of NF-κB, a known regulator of pro-survival genes. In our model, Akt activity correlated with increased inhibition of IκB (the negative regulator of NF-κB), and increased NF-κB DNA binding. We found that co-treatment with an NF-κB inhibitor or transfection with a non-degradable IκB restored tamoxifen sensitivity to our refractory myrAkt1 MCF-7 cells. Velcade, a specific and reversible inhibitor of the proteasome that prevents NF-κB activation in cancer cells, is currently in clinical development for the treatment of several types of carcinomas, and recently received FDA approval for the treatment of multiple myeloma. We hypothesized that treatment with Velcade would increase the responsiveness of our Akt-induced resistant breast cancer cells to tamoxifen. Nude mice were injected contralaterally with either the myrAkt1 or Control MCF-7 cells, and tumors were allowed to develop to a size of 200 mm^3. Mice were then separated into four (4) treatment groups: Placebo, Velcade as a single agent, tamoxifen as a single agent, or the combination of Velcade with tamoxifen. We found that while Velcade as a single agent had no significant inhibitory effects on our myrAkt1 tumors, tumor growth inhibition was 73% greater with the combination of Velcade and tamoxifen compared to tamoxifen as a single agent. These findings suggest that Velcade as a co-treatment for breast cancer tumors with high levels of Akt activity could be an effective approach for overcoming growth-factor induced resistance and may have implications for future clinical studies of Velcade in breast cancer.

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Computer-aided immunohistochemical analysis of EGFR signaling in paired colorectal cancer and normal colon tissue samples

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Background: The EGFR plays a role in multiple tumor cell processes including proliferation, survival, and susceptibility to chemotherapy. EGFR signaling is complex and multilayered, and appears to have several downstream effector pathways including PI3K-Akt and Ras-Raf-MAPK. The present study aimed to validate and apply a novel computer-aided immunohistochemical (IHC) technique to characterize the status of EGFR signaling in matched colorectal tumor and normal colon tissue samples. We previously presented results in which paired samples were poorly correlated, likely due to differing amounts of confounding stromal tissue in the sample. We present here a re-analysis using a "graphic microdissection" technique in which cell clusters of interest are analyzed separately in an effort to improve correlation between paired samples.
Materials and Methods: Tissue Microarrays (TMA) were made using samples from both cancerous and normal colorectal tissue in 18 patients and stained with antibodies against EGFR, phospho-EGFR (pEGFR), Akt, pAKT, MAPK, and pMAPK. TMA's were quantitatively scored using the Automated Cellular Imaging System (ACIS II, Chromavision, Inc), which couples a computer-controlled brightfield microscope to a camera with imaging analysis software. Prior to image analysis, cell clusters of interest were chosen under the supervision of a gastrointestinal pathologist and circled using the computer mouse. For validation, cell line pellets were analyzed using ACIS II and compared against Western blotting (A431 cells) and ELISA (8 head and neck cancers). Xenograft experiments were also performed in which A431 cells were implanted subcutaneously in nude mice treated with erlotinib versus control vehicle.
Results: ACIS analysis was highly reproducible and results were well correlated with Western blotting and ELISA. The "graphic microdessection" technique of analyzing heterogeneous human samples showed good corre-