them with penetratin allowing them to enter the cells. Here, we studied the effect of penetratin-peptidimer on HER2 signaling and its synergistic effect with "Docetaxel (Taxotere(R))" on HER2-overexpressing cancer cells in vitro and in vivo. Transformed NIH3T3/HER2 cells and SKBr3 (a human breast cancer line overexpressing HER2) cells were seeded on 6-wells plates and the penetratin-peptidimer was added to the culture medium to test its anti-tumor potential in a clonogenic assay. Treated cell colonies were stained and counted 2 to 3 weeks later. The penetratin-peptidimer inhibited the colony formation with an IC50 of 0.5 μM and 0.05 μM for SKBr3 cells and NIH3T3/HER2 cells, respectively. The levels of phosphorylated AKT and ERK proteins were assessed in order to determine the peptidimer effects in the HER2-dependant signaling pathway. Cells treated with the peptidimer showed a reduction in phosphorylated AKT but ERK phosphorylation remained unchanged. Docetaxel treatment induces overexpression of HER2, in a human prostate adenocarcinoma xenograft established in nude mice. "Trastuzumab (Herceptin(R))", a humanized recombinant monoclonal antibody directed against HER2 was shown to synergize the Docetaxel induced effects. We observed an increased HER2 expression in the two cell lines following Docetaxel administration. The peptidimer significantly enhanced sensitivity to Docetaxel in both NIH3T3/HER2 and SKBr3 cells. This combination was tested in an independent hormone xenograft model, using nude mice, of human prostate cancer overexpressing HER2. A synergistic effect of the peptidimer and Docetaxel was also obtained. These results suggest that the SH3-Grb2 inhibitor has an anti-tumor activity and enhanced cytotoxicity when combined with Docetaxel in HER2-expressing breast cancer cells and in the prostate xenograft.

**318** POSTER

**Dihydropyrrolopyrazoles as TGF-beta receptor kinase inhibitors for cancer therapy**

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TGF-β is a cytokine with diverse biological activities. TGF-β can mediate diametrically opposed activities depending upon the physiological state of a cell. Perhaps the most dramatic example of this phenomenon is TGF-β 's role as a tumor suppressor and tumor promoter. In many cells, TGF-β mediates a growth inhibitory signal via a heteromeric receptor complex composed of two transmembrane serine/threonine kinase receptors, the type I and type II receptors. However, once this inhibitory pathway is disrupted in tumor cells, TGF-β becomes a potent tumor promoter that can be secreted at high levels from the resistant tumor cells. This increased TGF-β expression modulates the extracellular matrix and has angiogenic and immunosuppressive activities. In addition, TGF-β contributes to the epithelial-to-mesenchymal transition of tumor cells, thus creating a more invasive and metastatic phenotype. These tumor-promoting activities of TGF-β provide rationale to target this pathway for therapeutic intervention. A series of orally bioavailable, small molecule kinase inhibitors that are potent and selective for the TGF-β receptors has been identified and characterized in *in vitro* kinase and cell based assays. Direct measures of target modulation in cells involved evaluation of P-Smad2 inhibition. A hallmark of our discovery program has been the characterization of *in vivo* target modulation using a subcutaneous xenograft tumor model to define PK/PD relationships for target modulation in animals. Evaluation of the dihydropyrrolopyrazole SAR *in vivo* yielded compounds with $IC_{50}$ values ranging from 0.020 to 2 μM. In time course experiments, target modulation paralleled plasma exposure with 8–12 hr of measurable activity that necessitated an oral BID schedule in the subsequent anti-tumor efficacy models. A pan-TGF-β neutralizing antibody was used to validate the involvement of TGF-β in the growth of MX1 breast cancer xenografts. Evaluation of dihydropyrrolopyrazole compounds in the MX1 model showed a statistically significant decrease in tumor growth. Anti-tumor efficacy has also been observed for this series of compounds in the Calu6 NSCLC xenograft model. A series of microarray experiments in the Calu6 model has been conducted to evaluate the biological effects of TGF-β in this system *in vitro* and to evaluate the differential sensitivity of TGF-β regulated genes to type I receptor selective or dual type I/type II receptor inhibitors. Extension of this approach to the Calu6 xenograft model will assist in the identification of potential biomarkers for evaluation of on-target compound activity in future clinical trials.

**319** POSTER

**Factors that govern the cell death response induced by inhibition of the molecular chaperone heat shock protein 90**

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The molecular chaperone heat shock protein 90 (Hsp90) has emerged as an exciting anticancer drug target due to its role in maintaining the conformation and stability of key oncogenic client proteins. The Hsp90 inhibitor; 17-allylamino, 17-demethoxy geldanamycin (17AAG) binds and inhibits the intrinsic ATPase which is essential for Hsp90 function, and is the first in-class Hsp90 inhibitor to enter and complete a phase 1 clinical trial. *In vitro* 17AAG treatment induces both cytostasis and apoptosis, the extent of which is cell line dependent. The aim of this study was to identify factors that may influence the cell death response following 17AAG treatment. Our laboratory has previously hypothesised that the apoptotic response to 17AAG operates via a Bax-dependent mechanism, based on the absence of apoptosis in KM12 cells that lack Bax, after exposure to 17AAG. This possibility has been explored using an isogenic pair of the human colon cancer cell line HCT116, which differ only in their expression of Bax. We demonstrate that Bax expression is required for apoptosis induced by 17AAG treatment and in its absence necrosis becomes the predominant mechanism of cell death. We also demonstrate that the apoptotic response to Hsp90 inhibition could be further influenced by increased expression of the Hsp70 family, which are inhibitors of the apoptotic pathway. We and others have previously shown that the constitutive (Hsc70), mitochondrial (Mortalin) and inducible (Hsp72) isoforms of Hsp70 are induced in response to 17AAG treatment. Here we use an siRNA approach to show that selectively repressing the induction of Hsp72 in response to 17AAG treatment increased cell death in HCT116 cells after only 24 hours exposure to 17AAG, which is earlier than normally associated with the cell death response in this cell line. The influence of Hsc70 and Mortalin on the cell death response to 17AAG treatment has also been explored using siRNA. In summary, these findings suggest that 17AAG induces apoptotic cell death via the intrinsic pathway mediated by Bax, the degree of which may be impaired by the induction of the anti-apoptotic Hsp70 family. However when Bax is not present 17AAG causes cell death to a lesser extent and via a necrotic mechanism. The anti-apoptotic effect of Hsp72 may begin to explain the predominance of tumour cytostasis versus cytotoxicity, as observed in human tumour xenografts and some patients treated with 17AAG during phase 1 clinical trial.

**320** POSTER

**Identification of potent, selective, soluble and permeable small molecule PI3 kinase inhibitors for the treatment of cancer**

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Phosphatidylinositol-3-kinases (PI3K) are lipid kinases that mediate cell signalling pathways controlling growth, proliferation, survival and motility. There is significant evidence suggesting that deregulation of the PI3K/c-Akt pathway is important in tumour progression, including loss of function of the tumour suppressor PTEN, the phosphatase that counteracts PI3K, and high frequency of mutation of the PI3K p110α isoform in human malignancies.[1]

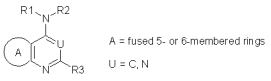


Fig. 1: Generic structure of fused heterocyclic compounds.

Table 1. *In vitro* biochemical and physicochemical properties of PI3K inhibitors

| | $IC_{50}$ (μM) | | PI3K, SPA | | Solubility (μM) | Mouse Microsome Stability[a] |
|---|---|---|---|---|---|---|
| | p110 α | p110 β | p110 δ | p110 γ | | |
| **PI103** | 0.0015 | 0.003 | 0.003 | 0.015 | 3.5 | 13 |
| PI509 | 0.0045 | 0.037 | 0.019 | 0.112 | 20 | 5 |
| PI516 | 0.004 | 0.045 | 0.006 | 0.063 | >100 | 11 |
| PI540 | 0.010 | 0.044 | 0.009 | 0.321 | >100 | 91 |

[a] % compound remaining after 30 min.

We have previously reported that PI103, a potent and selective PI3K inhibitor with established *in vivo* efficacy in xenograft models, had been identified as a starting point for the development of a series of novel small molecule therapeutics for the treatment of cancer (Figure 1).[2] However, several features of PI103, including its low aqueous solubility at physiological pH, were subsequently identified as areas for lead optimisation. A medicinal chemistry effort at PIramed has resulted in the discovery of a second generation of PI3K inhibitors with promising biochemical affinity and functional activity, and with improved physicochemical properties. Three such compounds, PI509, PI516, and

PI540, displayed low nanomolar potency for the PI3K target family in an isolated enzyme assay, with improved selectivity for class Ia PI3 kinases over class Ib compared with PI103 (Table 1). Additionally, PI509 was seen to be highly selective for PI3 kinases when evaluated in a kinase profiling screen[3] and did not inhibit any of 72 serine/threonine and tyrosine protein kinases when tested at 0.5 μM.

The compounds exhibited potent *in vitro* anti-proliferative effects in a range of tumour cell lines, and a concomitant decrease in phospho-AKT ($Ser^{473}$) was also detected by Western blotting in treated cells. In addition to exhibiting promising *in vitro* efficacy, PI509, PI516 and PI540 displayed improved physicochemical properties that offered significant advantages for *in vivo* efficacy; these include increased solubility at pH 7.4 enabling improved dissolution rate, and high permeability levels, as measured in Caco-2 cells, anticipated to confer excellent cellular permeation and gastrointestinal absorption (Table 1). The compounds displayed no activity against the major isoforms of the cytochrome P450 enzyme family, and did not block the hERG channel, as measured in a rubidium efflux assay. Further, PI540 showed significantly increased metabolic stability over PI103 when incubated with mouse and human microsomes. Computational analysis confirmed high predicted human intestinal absorption of both PI509, PI516 and PI540.[4] Furthermore, PI509, a more lipophilic compound, was determined to have high predicted CNS permeation potential, whereas PI516, a derivative containing a hydrophilic residue, was estimated to have lower blood-brain barrier permeation.[4] This suggests that specific compounds from this series may have central and peripheral modes of action, which may have a valuable bearing on the potential utility of compounds from this class in the treatment of specific tumour types. In conclusion, the discovery of PI509, PI516 and PI540 represents a significant advancement in lead optimisation for this series of small molecule PI3K inhibitors; these compounds are currently under further investigation in xenograft models.

**References**

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[4] As determined using Discovery Studio© from Accelrys Inc.

**322** POSTER

**Novel Isoquinoline-5-sulfonamides as biochemical and cellular inhibitors of PKB/AKt**

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Protein Kinase B (PKB), also known as AKT, is an important regulator of both cell proliferation and survival via inhibition of apoptosis. Both mutation and activation of PKB have been identified in multiple forms of cancer, implicating this kinase in tumour development. We have therefore undertaken to generate small molecule inhibitors of PKB as a therapeutic strategy for the treatment of cancer.

Complementary chemical starting points for this strategy resulted in two main chemical series, one of which has been the isoquinoline-5-sulfonamide class of kinase inhibitors. Medicinal chemistry investigations, aided by x-ray crystallography, have led to a series of compounds that have been shown to occupy the ATP pocket and have sub-micromolar biochemical inhibitory activity towards PKB together with selectivity over PDK1 and PKC.

Further analysis on the cellular activity of selected compounds has shown that they have growth inhibitory activity concomitant with their ability to inhibit PKB in an in vitro kinase assay. These compounds also inhibit phosphorylation of the PKB substrate, GSK-3beta, as detected by western blot. We have also developed and implemented a high throughput (96 well format) cellular readout for GSK3-beta phosphorylation, which allows us to generate cellular substrate IC50 data that can then be correlated with both enzyme and growth inhibitory IC50 data.

In conclusion, we have shown that sub-micromolar potency PKB inhibitors have the anticipated effect on the PKB signalling pathway in cells, supportive of this protein as a target for drug development. We will also present data on the effect of these compounds on other PKB substrate markers and on cell survival. We are also in the process of investigating anti-tumour effects in animals on these series using validated PD markers.

**323** POSTER

**Targeted use of combination of erbB targeted therapy**

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**Background:** Over-expression of erbB receptors is associated with aggressive cancers. Therapeutic strategies targeting these oncoproteins are in clinical trials. One approach is the use of monoclonal antibodies (Mab) to erbB1 and erbB2 such as Herceptin and C225. Another is the use of tyrosine kinase inhibitors (TKIs) that block the nucleotide-binding site of the erbB kinases (such as GW572016 and ZD1839). However, each approach has different results. Mab downregulates the receptors – however receptors can still transmit downstream signals – whereas TKI do not change receptor expression but do inhibit downstream signals.

**Methods:** We used cancer cell lines as well as tissue biopsies from patients before and after treatments to understand the mechanism associated with response to targeted erbB treatments. The cancer cells and biopsies were immunostained for all erbB members, their phosphorylated forms, phosphorylated ERK and AKT, IGFR, pS6, and their ligands TGF alpha and Heregulin. Levels and localization were analyzed using immunohistochemistry, quantitated by image analysis and confocal microscopy (antibodies were purchased from Cell Signaling and Dako). Treatment included GW572016, ZD1839 and multiple antibodies to erbB1 and erbB2 as well as polyclonal antibodies.

**Results:** Response to antibodies based therapies in cells showed downregulation of receptors expression; cells and patients who responded to TKI therapies exhibited downregulation and translocation of pAKT and pERK from nuclear component to cytoplasmic component in cancer cells. Best response to TKI was obtained in inflammatory breast cancers where pERK and pAKT were localized in the cytoplasm at treatment initiation. Response was confirmed using biological biomarkers and objective clinical response. Disease progression was associated with persistent high levels of pAKT and pERK and nuclear localization. None of the ErbB targeted treatments was effective when IGFR pathway was highly activated. Finally, optimized treatment results in cell lines were accomplished using multiple antibodies in combination with various TKI.

**Conclusions:** Our results indicate that using combination therapy may lead to therapeutic strategies to selectively abrogate oncogenic-related signaling.

**324** POSTER

**High field MRI characterization of tumor growth kinetics, vascularity and cellularity in a PDGF-driven tv-a mouse model of glioma**

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**Introduction:** RCAS/*tv-a* transgenic technology constitutes a promising new platform for mouse tumor models that are both tissue and oncogenic pathway-specific, especially suited to development of target-based treatments. The technology relies on somatic gene transfer through infection by RCAS viral vectors derived from the avian retrovirus (ALV-A) in mice expressing the gene for the RCAS receptor (*tv-a*). Using high field MRI, we characterized growth, cellularity and vascularity in PDGF-driven glioblastoma multiforme in a mouse that expresses *tv-a* under the control of the nestin promoter expressed in glial-progenitors (*Ntv-a* mouse).

**Methods:** *Ntv-a* mice that had developed tumors following intracranial injection with ALV virus encoding PDGF underwent weekly brain MRI to characterize tumor growth and development, until they reached a moribund state and were sacrificed. T2-weighted fast spin-echo MRI was used (3 minute images). At multiple time points, tumors were also evaluated using T1-weighted spin-echo MRI (3 minute images), pre- and post-contrast agent injection, to delineate regions of dense and/or 'leaky' microvasculature. Tumor cellularity was also evaluated during the course of the study by diffusion-MRI measurement of the apparent diffusion coefficient (ADC). When signs of illness were apparent, animals were sacrificed, and the brains harvested for histology.

**Results and Discussion:** The mean time for tumor appearance was $3.3 \pm 0.4$ weeks, with tumors appearing in 75% of injected mice. After appearance, the tumors grew rapidly and invasively. Tumor cellularity was higher at the outer margins, with ADC similar to that which has been measured in implanted glioma xenografts (~100–120 $cm^2/s$). Enhancing regions were evident in gadolinium contrast images. Histologic sections correlated well with T2-weighted contrast, gadolinium contrast and ADC maps, confirming the presence of high grade glioma.