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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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.

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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.

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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 ± 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.