a potent Class I PI3K inhibitor, GDC-0941, that is orally bioavailable and demonstrates excellent single agent anti-tumor activity in multiple human cancer models. The purpose of these studies was to determine if combination of GDC-0941 could enhance the anti-tumor activity of approved chemotherapeutic agents such as docetaxel (Taxotere®) or gemcitabine (Gemzar®) in human cancer models *in vitro* and *in vivo*.
Materials and Methods: Combination studies of GDC-0941 and chemotherapeutics were accomplished *in vitro* using the Chou and Talalay method of Combination Index. Tumor cell lines were treated either with GDC-0941, with the chemotherapeutic, or simultaneously with a constant ratio of GDC-0941 and the chemotherapeutic, and assayed after 4 days for viability. For the *in vivo* studies, tumor cell lines were implanted subcutaneously in the hind flank of female nu/nu mice and dosed orally for 14 or 21 continuous days with GDC-0941. Docetaxel was dosed intravenously 3 times every 4 days while gemcitabine was dosed intraperitoneally 4 times every 3 days.
Results: GDC-0941 combines with docetaxel and gemcitabine to produce low Combination Index (C.I.) scores that indicate synergy in >20 tumor cell lines that represent breast, prostate, ovarian and other cancers. The *in vitro* synergism corresponds to increased apoptosis as measured by annexin V, cleaved PARP, and propidium iodide FACS. Moreover, we discovered that low C.I. correlates with the ability of the chemotherapeutic to induce an increase in phosphoAKT levels. The combination effects of GDC-0941 with chemotherapeutics *in vitro* were recapitulated *in vivo* as enhanced anti-tumor responses were observed in multiple human tumor xenograft models (n = 7). At the doses tested, all *in vivo* combinations were tolerated as measured by animal body weights and morbidity indices. Biomarkers of combination treatment responses are presently being investigated.
Conclusion: Combination therapy of the Class I PI3K inhibitor, GDC-0941, augments the efficacy of chemotherapeutics in the treatment of human cancers models *in vitro* and *in vivo*. This enhanced response in combination may be due to the chemotherapeutic reliance on the PI3K/Akt pathway for survival.

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A novel inhibitor of phosphoinositide 3-kinase for the treatment of cancer

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Background: The phosphoinositide 3-kinase (PI3K) signaling pathway is activated in a broad spectrum of human cancer. Activation of this pathway often occurs indirectly by the activation of receptor tyrosine kinases or the inactivation of the PTEN tumor suppressor. Recently, direct activation of PI3K has been demonstrated with the discovery of several activating mutations in the PIK3CA gene itself, the gene that encodes the p110α catalytic subunit of PI3Kα. Several of the mutations found in PIK3CA have been shown to increase the lipid kinase activity of PI3Kα, induce activation of signaling pathways, and promote transformation cells in culture.
Methods and Results: We disclose herein the structure and activities of GSK615, a novel thiazolidinedione inhibitor of the class I family of PI3K enzymes. In biochemical studies, GSK615 is a highly potent (app. Ki = 0.42 nM), ATP-competitive small molecule inhibitor that unlike wortmannin, does not irreversibly inactivate PI3K. The biochemical inhibition of enzyme activity translates effectively to activity in cellular assays. GSK615 inhibits AKT phosphorylation in a variety of human tumor cell lines including the T-47D breast ductal carcinoma cell line (IC_{50} = 34 nM). Signal transduction downstream of AKT is also attenuated as indicated by inhibition of p70S6K phosphorylation and translocation of the FOXO transcription factor. Inhibition of PI3K with GSK615 leads to cell cycle arrest and inhibition of cell growth (T-47D gIC_{50} = 196 nM). GSK615 is also active at inhibiting cell growth and inducing cell death in a larger panel of tumor cell lines where these effects are both time and compound concentration dependent. Increased levels of caspase 3/7 activity measured in lysates after treating cells with GSK615 suggest that cell death is mediated by the induction of apoptosis. GSK615 has tumor growth inhibitory activity vs. human tumor cells grown in mouse xenograft models. Oral dosing with either once or twice daily regimes decreases tumor pAKT levels and inhibits the growth of breast and lung carcinoma tumors without significant overt toxicity or body weight loss.
Conclusion: GSK615 has an attractive biological profile and is progressing toward Phase 1 human clinical trials.

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RNAi screen for Akt regulator

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The serine/threonine kinase PKB/Akt controls various cellular processes such as cell growth and proliferation, metabolism and cell survival. Growth signals are transduced from the extracellular environment through the growth factor receptors into the cell via the PI3K/Akt pathway. The importance of the Akt pathway is highlighted by the mutation of various components of the pathway in human cancers such as the PTEN and PI3-kinase (P110?). In recent years, much has been invested in the search for other Akt substrates in the hope of understanding the different cellular processes controlled by Akt. To date over fifty Akt substrates have been identified.
In this project, we employed an RNA interference library consisting of synthetic oligonucleotides targeting all human protein kinases to screen for kinases involved in the regulation of Akt activation. Akt is fully activated upon phosphorylation at Threonine 308 by PDK1 and Serine 473 by "PDK2", whose identity remains controversial, but may include the mTor/Rictor complex. In this screen, we transfected MDA-468 breast cell line with the siRNA library and measured Akt activation using antibody specific for phosphoserine 473. The initial screen data suggested that phosphorylation of Akt at Ser473 can be regulated by about 30 kinases.
Importantly, Akt phosphorylation can be drastically reduced by silencing of Choline kinase. Choline uptake into the cells are phosphorylated by Choline kinase. Phosphorylcholine is then utilised for the synthesis of phosphatidylcholine, one of the major component of the plasma membrane, in the Kennedy pathway. Interestingly, high Choline kinase expression and activity have been implicated in tumor development and metastasis, and knock down of kinase promotes differentiation of breast carcinoma cells. The mechanism by which Choline kinase is involved in tumor formation is not clear. Currently, work is underway to investigate if Choline kinase acts through Akt to promote cell survival and proliferation

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Pharmacokinetics and pharmacodynamic biomarkers for the pan-PI3K inhibitor GDC-0941: Initial Phase I evaluation

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Background: The phosphoinositide-3 kinase (PI3K)/AKT signaling pathway is deregulated in a wide variety of cancers. GDC-0941 is a potent and selective ATP competitive inhibitor of the class I PI3K with 3 nM IC50 for the p110 alpha subunit and 28 nM IC50 in a cell based pAKT assay. GDC-0941 demonstrates broad preclinical activity in xenograft models of glioblastoma, breast, lung, and prostate cancer.
Materials and Methods: The relationship of pharmacokinetic (PK) and pharmacodynamic (PD) biomarkers of the pan-PI3K inhibitor GDC-0941 was evaluated in preclinical models to support clinical evaluation in phase I studies. Phase I dose escalation studies using a 3+3 design were initiated in patients with solid tumors that had progressed on or were intolerant of standard therapy. An initial dose of GDC-0941 was administered followed by a one week washout to characterize single dose PK and PD. GDC-0941 was then administered once daily on a 3 week on, 1 week off schedule. PK and PD were also evaluated after one week of continuous dosing of GDC-0941. In the absence of significant toxicity or disease progression, patients were eligible to continue dosing in 28 day cycles.
Results: Preclinical studies have explored several pharmacodynamic (PD) readouts. PD decreases in downstream markers of pathway activity including pAKT and pS6 were demonstrated in xenograft tumor lysates from mice dosed with GDC-0941. Continuous PD knockdown was not observed at doses consistent with efficacy, suggesting that continuous pathway inhibition is not required for single agent activity. IHC assays for PD marker evaluation have been developed for clinical tumor biopsies. In addition, a surrogate PD marker for pAKT in platelet rich plasma has been developed and demonstrates ex-vivo knockdown in human blood samples. Based on non-clinical studies demonstrating good oral bioavailability and linear PK characteristics, two ongoing phase I studies were initiated, in the U.S and in the U.K. Ten patients have been enrolled in 3 successive