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

cohorts to date in the US study and 4 patients have been enrolled in the UK study. GDC-0941 has been well tolerated clinically to date. At least one patient has been on study for at least 92 days. Preliminary PK data demonstrate dose proportional increases in Cmax and AUC in fasting conditions. PD assays, including assessment of pAKT levels in platelet rich plasma coinciding with PK evaluation, FDG-PET scans, and evaluation of tumor biopsies for marker modulation, are underway. Preliminary data show decreases in pAKT levels in platelet rich plasma in patients in cohort 2 that are reflective of drug levels in plasma.
Conclusions: GDC-0941 is a potent pan-PI3K inhibitor with promising preclinical activity. Ongoing Phase I studies have indicated favorable preliminary PK and safety profiles in the first few cohorts. Updated data including clinical PK and PD will be presented.

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Assessment of the antitumor activity of NVP-BEZ235 in experimental renal cell carcinoma models

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Background: Renal cell carcinoma (RCC) is among the ten leading causes of cancer-related deaths worldwide. Over the past few years, the understanding of cellular and molecular processes underlying the tumor biology of RCC has made substantial progress. A genetic change involved in RCC tumorigenesis (75% of cases) is inactivation of the von Hippel-Lindau (VHL) gene leading to constitutive induction of hypoxya-inducible factor (HIF). This and other genetic alterations often found in RCC (PTEN deletions or silencing) result in activation of PI3K and downstream effectors.
Materials and Methods: To study the potential use of the dual PI3K/mTor inhibitor NVP-BEZ235, which is currently in Phase I clinical trials, in the treatment of RCC, the compound was tested *in vitro* and *in vivo* in several genetically characterized human RCC models. As part of its *in vivo* evaluation, head-to-head studies were performed with RAD001, an allosteric mTORC1 kinase inhibitor that has been shown promise in the treatment of RCC.
Results: NVP-BEZ235 significantly inhibited the proliferation (GI_{50} <50 nM) of a panel of human RCC cell lines −A498, RENCA; SK-RC-01, 786−0, ACHN, Caki1, Caki2, and Sk-RC-02- by specifically blocking the phosphorylation and activation of Akt. No correlation was found between specific genetic alternations and tumor cell line sensitivity. A similar experimental observation was obtained when the compound was tested under anchorage independent conditions. To confirm these cellular findings, several *in vivo* efficacy studies were conducted using human tumor RCC models with different genetic alterations. When administered orally at 30 mg/kg/day, a statistically significant antitumor activity (R 25%) was obtained in the 786−0 tumor model (PTEN deletion, VHL mutant). Under the same experimental conditions, RAD001, which was administered orally at 10 mg/kg/day, displayed a similar antitumor effect. Different results were obtained when NVP-BEZ235 was tested in the Caki-1 RCC model (VHL wild-type). Altough efficient blockade of pAkt and pS6 was confirmed upon *ex-vivo* analysis of tumor tissues at the end of the efficacy study, NVP-BEZ235 did not show a significant antitumor effect in this model.
Conclusions: Our results suggest that concomitant blockade of PI3K and mTOR by low molecular mass kinases inhibitors like NVP-BEZ235 may represent an effective therapeutic strategy for patients with RCC, in particular tumors that harbor VHL mutations.

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Evaluation of antitumor activity of a novel PI3K inhibitor ZSTK474 by various human cancer xenograft models

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Phosphatidylinositol 3-kinase (PI3K) is a key enzyme playing important roles in various cellular functions such as cell growth, survival and metabolism. The dysregulation of PI3K is known to associate with carcinogenesis and cancer progression. Therefore, PI3K is a potential molecular target. We recently developed a novel PI3K inhibitor ZSTK474, 2-(2-difluoromethylbenzimidazol-l-yl)-4, 6-dimorpholino-1,3,5-triazine, and demonstrated its therapeutic efficacy in some in vivo models (Yaguchi S. et al. J Natl Cancer Inst 2006; 98: 545−56). ZSTK474 is a pan-PI3K inhibitor and inhibits PI3K in a ATP-competitive manner (Kong D. et al. Cancer Sci 2007; 98: 1638−42). The purpose of this study was to further evaluate its efficacy in various types of human cancer xenograft. We subcutaneously transplanted 24 human cancer xenografts derived from 9 different organs into nude mice. When the tumor volume reached approximately 100 mg (Day 0), oral administration of ZSTK474 (200 or 400 mg/kg/day) was started and continued for 14 days. The tumor size was measured, and the tumor growth inhibition (T/C %) was examined. A wide antitumor spectrum across the 24 xenografts was observed without severe toxicity, which demonstrated again the in vivo efficacy of ZSTK474. However, the values of T/C varied from 10% to 70% depending on the xenograft type. These results suggested that the sensitivities of human cancers to ZSTK474 are diverse. Therefore, identification of biomarkers predicting its efficacy would be necessary.

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Anti-angiogenic activity of a novel PI3K inhibitor, ZSTK474

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PI3 Kinase (PI3K) dysregulations are known to be involved in tumor initiation and progression. It was recently reported that PI3K promotes angiogenesis via inducing expressions of HIF-1a and vascular endothelial growth factor (VEGF), which are well known to play important roles in angiogenesis. ZSTK474, 2-(2-difluoromethylbenzimidazol-l-yl)-4, 6-dimorpholino-1,3,5-triazine, is a novel ATP-competitive pan-PI3K inhibitor that we developed as an anticancer drug in recent years. (Kong D., et al. Cancer Sci 2007, 98: 1638−42) ZSTK474 showed favorable in vivo antitumor effect on human cancer xenografts without obvious toxicity observed. (Yaguchi S., et al. J Natl Cancer Inst 2006; 98: 545–556) The purpose of the present study is to examine the anti-angiogenic effect of ZSTK474. WST-8 assay was utilized to evaluate the growth inhibition activity of ZSTK474 against human umbilical vein endothelial cells (HUVEC). ZSTK474 dose-dependently inhibited HUVEC growth at submicromolar concentrations. Furthermore, Matrigel capillary-like tube formation assay indicated that ZSTK474 blocked in vitro tube formation by HUVEC; and Boyden chamber assay showed that ZSTK474 inhibited VEGF-induced migration of HUVEC with a comparable activity with SU5416, a well-known VEGF-R inhibitor. On the other hand, ZSTK474 treatment inhibited expressions of p-Akt, HIF-1a and VEGF in RXF-631L renal cancer cells. Finally, immunohistochemical staining with anti-CD31 antibody of tumor tissues from RXF-631L mouse xenograft models showed the significant reduction of the microvessels in ZSTK474-treated mice compared to vehicle group. These results indicated that ZSTK474 had anti-angiogenic activity which might be attributed to inhibition of the expression of HIF-1a and VEGF via PI3K-Akt pathway.

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Anti-angiogenic effects of PI3K/Akt/mTOR pathway inhibitors

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Background: The PI3K signaling pathway is important for VEGF mediated angiogenesis. We utilized selective small molecule inhibitors of PI3K alpha/delta, pan-PI3K (GDC-0941), Akt, TORC1, and mTOR/PI3K to investigate the signaling and mechanistic effects of inhibition at various nodes in the pathway.
Methods: HUVEC sprouting and migration assays were carried out in vitro. In vivo experiments for compound effects on neovascularization were performed in mice. Endothelial specific proliferation in xenograft tumors was assessed utilizing FFPE sections immunostained with MECA32 and Ki67. Combination efficacy studies of various pathway inhibitors with the anti-VEGF antibody B20 were carried out in xenograft tumor models.
Results: Inhibition of PI3K, Akt, or PI3K/mTOR using small molecule inhibitors all caused a decrease in VEGF expression in cultured tumor cells. While PI3K and PI3K/mTOR inhibitors robustly diminished HUVEC sprouting and migration in a dose-dependent manner, Akt inhibition was less effective. In HUVEC migration the TORC1 inhibitor had a broad effective range, although the maximum effect was 50% in VEGF-induced response. Dual mTOR/PI3K inhibitors produced a significant decrease in the microvascular density in the pancreas, smooth muscle, and trachea of perinatal mice. Xenograft tumor studies indicate inhibitors of the PI3K pathway have roles in targeting the tumor cells directly as well as tumor vasculature. Dual mTOR/PI3K inhibitors produced a greater decrease, compared with selective PI3K inhibitors, in the number of proliferating endothelial cells in xenografts. Combination with anti-VEGF antibody resulted in enhanced efficacy for the Akt inhibitor and PI3K inhibitors.
Conclusions: Dual PI3K/mTOR inhibition had significant effects on endothelial cell sprouting and migration in culture, and in vivo treatment resulted in decreased microvascular density, decreased tumor angiogenesis, and decreased endothelial cell survival. Effects of other inhibitors of the pathway were less robust.