60 mg BID resulted in reductions of 80−90% in phosphorylation of AKT, 4EBP1 and S6, and was associated with a 54% reduction in proliferation as assessed by Ki67 in tumor cells. The pattern of inhibition of PI3K pathway phosphoepitopes suggests that XL765 inhibits PI3K and both mTOR/Raptor and mTOR/Rictor in pts.
Conclusions: In this Phase 1 study, XL765 has been generally well tolerated at doses up to 60 mg BID, and further exploration of this dose as well as a QD regimen is in progress. XL765 demonstrates robust pharmacodynamic activity, as assessed by analysis of multiple PI3K and mTOR dependent readouts in surrogate tissues and tumor.

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Pre-clinical evaluation of efficacy and PK/PD biomarkers of GDC-0941, a potent class 1 PI3K inhibitor

S. Patel[1], D. Sampath[2], M. Belvin[2], A. Brown[1], K. Edgar[2], L. Lensun[1], P. Moore[1], L. Salphati[3], H. Stern[4], L. Friedman[2]. *[1]Piramed Pharma, Biology, Berkshire, United Kingdom; [2]Genentech, Cancer Signaling and Translational Oncology, South San Francisco, CA, USA; [3]Genentech, Dmpk, South San Francisco, CA, USA; [4]Genentech, Pathology, South San Francisco, CA, USA*

Background: Constitutive activation of the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway is a frequent event in many types of cancers and results in increased cell growth and survival. We recently discovered GDC-0941, a class 1 PI3K inhibitor currently in phase 1 clinical trials. Pre-clinically, we have demonstrated the potent anti-tumor activity of GDC-0941 *in vitro* and *in vivo*. (Folkes *et al.,* late breaking abstract-146, Friedman *et al.,* late breaking abstract 110, AACR 2008). In this study we evaluate the efficacy, pharmacokinetic (PK) and pharmcaodynamic (PD) markers of GDC-0941 *in vivo*.
Methods: GDC-0941 was dosed up to 200 mg/kg daily by oral gavage in mice bearing breast and prostate cancer xenografts. GDC-0941 plasma PK, tumor PK and tissue PD analysis of downstream PI3K biomarkers (pAKT, PRAS40, pS6, phospho-p70S6K) was evaluated after single dose and multiple daily dose efficacy studies. PD analysis was conducted on *ex vivo* xenograft tumour samples and normal murine tissues using a combination of luminex, mesocale, immunohistochemical and Western blot assays.
Results: GDC-0941 had significant dose dependent *in vivo* efficacy and was well tolerated. In single dose and multiple daily dose efficacy studies GDC-0941 caused rapid downregulation of pAKT, PRAS40, pS6 and phospho-p70S6K in the tumour, consistent with PI3K pathway inhibition. PK analysis of GDC-0941 showed that reduction of pAKT in the tumours was positively correlated to plasma and tumour drug concentrations. There were minimal effects on pAKT in normal murine tissue at 30 min. pAKT knockdown in the tumour was verified and found to be comparable using both luminex and mesocale assays. Imunohistochemical analysis of MDA-MB-361 breast tumours showed heterogenous expression of pAKT and pS6, which was significantly reduced 1hr post dose of GDC-0941. Highly efficacious doses of 75–150 mg/kg GDC-0941 gave prolonged biomarker knockdown, recovering by 24 hrs consistent with the drug exposure.
Conclusions: GDC-0941 had significant anti-tumour activity *in vivo* with concomitant reduction in PI3K signalling in xenograft tumour tissue. These pre-clinical data support the evaluation of pAKT, PRAS40, pS6 and phospho-p70S6K as potential PI3K pathway biomarkers in GDC-0941 clinical studies.

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A phase I dose-escalation study of the safety, pharmacokinetics and pharmacodynamics of XL147, a novel PI3K inhibitor administered orally to patients with advanced solid tumors

E. Calvo[1], G. Edelman[2], J. Baselga[1], E. Kwak[3], C. Scheffold[4], L. Nguyen[5], G.I. Shapiro[6]. *[1]Hospital Universitario Vall d'Hebron, Oncology, Barcelona, Spain; [2]Texas Onc, Medical Oncology, Irving, USA; [3]Massachusetts General Hospital, Medical Oncology, Boston, USA; [4]Exelixis Inc., Clinical Development, South San Francisco, CA, USA; [5]Exelixis Inc., Non-Clinical Development, South San Francisco, CA, USA; [6]Dana Farber Cancer Institute, Medical Oncology/Molecular and Cellular, Boston, USA*

Background: XL147 is a potent, selective, oral inhibitor of Class I PI3 kinases. Administration of XL147 leads to tumor growth inhibition or regression in preclinical cancer models and has been shown to enhance the anti-tumor activity of EGFR-targeted agents and cytotoxic drugs.
Methods: XL147–001 is a Phase 1, non-randomized, open label, dose escalation, safety and pharmacokinetics (PK) study. Patients (pts) with advanced solid tumors are enrolled in successive cohorts to receive XL147 daily on Days 1−21 of 28-day cycles (21on/7off schedule). In Cycle 1, clinical and laboratory data are obtained to assess safety and pharmacodynamics. Tumor response is evaluated by RECIST criteria every 8 wks.
Results: To date, 19 pts have been treated with XL147 across six dose levels from 30 mg to 600 mg daily. At 600 mg daily a DLT of Grade 3 skin rash was observed. At 400 mg daily, one Grade 1 gastritis, and one Grade 1 skin rash were reported possibly related to study drug. There were no SAEs related to XL147. Plasma PK analysis indicated exposure increased with dose. The mean terminal plasma half-life at steady state ranged from 3−6 days, with steady state plasma concentrations achieved between Days 15 and 20. Accumulation was evident, as plasma exposures were 5 to 10-fold higher on Day 21 than on Day 1. XL147 transiently augmented food-induced changes in plasma insulin with a trend suggesting dose- and exposure-dependence. Inhibition of PI3K pathway signaling was demonstrated in pts by reductions in phosphorylation of PI3K pathway components including AKT, PRAS40, and 4EBP1 in PBMCs and hair bulbs. In one example, administration of 120 mg daily resulted in reduced phosphorylation of AKT (32%), PRAS40 (74%), 4EBP1 (46%) and S6 (57%) in hair bulbs. Similar analyses are ongoing in skin and tumor samples from multiple pts. As of May 2008, one pt with basal cell carcinoma has remained on study >10 cycles; three pts with NSCLC and one pt with NHL remained on study >8 cycles; an additional two pts remained on study >4 cycles.
Conclusions: XL147 has been generally well tolerated at doses up to 400 mg daily on the 21on/7off dosing schedule. Inhibition of PI3K signaling has been demonstrated and there are preliminary signs of clinical benefit as assessed by time on study. Cohort expansion at the 600 mg dose level is ongoing to determine the MTD for the 21/7 dosing regimen. In parallel, pts are being enrolled on a continuous daily dosing schedule.

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NVP-BEZ235, a dual pan-PI3K/mTOR kinase inhibitor, is effective in human lung cancer models harboring EGFR mutations

C.R. Schnell[1], J. Vaxelaire[1], M. Vogelsanger[1], M. Hattenberger[1], F. Stauffer[1], S. Arnal[1], C. Garcia-Echeverria[1], S.M. Maira[1]. *[1]Novartis Pharma AG, Oncology, Basel, Switzerland*

Background: Mutations in the intracellular kinase domain of the epidermal growth factor receptor (EGFR) are often found in a subset of patients with lung cancer and correlate with therapeutic response to EGFR kinase modulators. Resistance to these targeted anticancer agents invariable develops, and current treatments have limited long-term antitumor efficacy. In this study, we evaluated the effectiveness of a dual PI3K/mTOR kinase inhibitor, NVP-BEZ235, in the treatment of human lung cancer models harboring EGFR mutations.
Materials and Methods: To study the potential use of NVP-BEZ235, which is currently in Phase I clinical trials, in the treatment of lung adenocarcinomas, the compound was tested *in vitro* and *in vivo* in several human lung models that require a functional EGFR for tumor maintenance.
Results: NVP-BEZ235 significantly inhibits the proliferation (GI_{50} <50 nM) of lung tumor cell lines harboring EGFR-activating mutations as well as EGFR tyrosine kinase resistance somatic mutations by specifically blocking the biological function of PI3K signaling components. In animal models, oral treatment with NVP-BEZ235 (35 mg/kg qd) caused tumor stasis ($T/C = 0.04$, $p < 0.05$) in NCI-H1975 xenografts, an EGFR mutant model (Leu858Arg and Thr790Met), which has shown high levels of EGFR tyrosine kinase inhibitor resistance. Moreover, ex-vivo analysis of tumor samples treated with NVP-BEZ235 revealed significant inhibition of pS473-Akt and downstream targets with no inhibition of YP/TP-ERK1/2 at 1 h post last dose. NVP-BEZ235 was well tolerated at the efficacious doses when compared with vehicle treated animals, with no significant difference seen in the body weight. Significant antitumor activity was also observed in a mechanistic c-MET amplified gefitinib resistant model (Hcc827GR), and in an EGFR mutant model sensitive to EGFR kinase inhibitors (Hcc827, ex 19 del).
Conclusions: These preclinical evaluations suggest that NVP-BEZ235 may represent an effective therapeutic strategy for patients with lung cancers harboring drug-sensitive EGFR mutations or those with the novo and acquired resistance to EGFR tyrosine kinase inhibitors.

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Combination of class I PI3K inhibitor, GDC-0941, with standard of care therapeutics results in enhanced anti-tumor responses in human cancer models in vitro and in vivo

D. Sampath[1], M. Belvin[1], J. Guan[1], K. Edgar[1], J. Wallin[1], W.W. Prior[1], R. Kassees[1], P. Moore[2], S. Patel[2], L. Friedman[1]. *[1]Genentech Bio Oncology Inc., Cancer Signaling and Translational Oncology, South San Francisco, CA, USA; [2]Piramed Pharma, Biology, Slough, United Kingdom*

Background: Phosphoinositide 3-kinases (PI3Ks) are lipid kinases that regulate tumor cell growth, migration and survival. We previously reported

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.

221 POSTER

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

K.R. Auger[1], L. Luo[2], S. Knight[3], G. Van Aller[2], P.J. Tummino[2], R.A. Copeland[2], M. Diamond[1], D. Sutton[1], J.R. Jackson[1], D. Dhanak[3]. *[1]GlaxoSmithKline, Biology, Collegeville, USA; [2]GlaxoSmithKline, Enzymology and Mechanistic Pharmacology, Collegeville, USA; [3]GlaxoSmithKline, Medicinal Chemistry, Collegeville, USA*

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

B.T. Chua[1], A. Ullrich[1], D. Gallego-Ortega[2], A. Ramirez de Molina[2], J.C. Lacal[2], J. Downward[3]. *[1]Institute of Medical Biology, Singapore OncoGenome Project, SIngapore, Singapore; [2]La Paz Centro Nacional de Biotecnología, Translational Oncology Unit, Madrid, Spain; [3]Signal Transduction Laboratory, Cancer Research UK, United Kingdom*

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

P. LoRusso[1], D. Sarker[2], D. Von Hoff[3], R. Tibes[3], M.K. Derynck[4], J.A. Ware[4], Y. Yan[4], G.D. Demetri[5], J.S. de Bono[2], A.J. Wagner[5]. *[1]Karmanos Cancer Institute, Phase I, Detroit, MI, USA; [2]Royal Marsden Hospital, Institute of Cancer Research, London, United Kingdom; [3]TGen Scottsdale Healthcare, Oncology, Scottsdale, AZ, USA; [4]Genentech Inc., Oncology, South San Francisco, CA, USA; [5]Dana Farber Cancer Institute, Hematology/Oncology, Boston, USA*

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