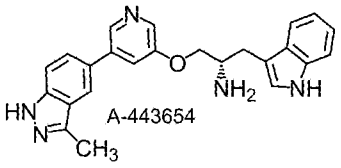
Akt. These compounds displayed significant antitumor activity in multiple tumor models (xenograft, syngeneic, orthotopic, flank). Tumor growth was almost completely inhibited during compound administration, with tumor regrowth occurring upon cessation of dosing. These effects were dose dependent. The compounds were not only efficacious as monotherapy, but also when combined with other anti-tumor agents. Pharmacodynamic and pharmacokinetic studies demonstrate that Akt was inhibited within tumors at concentrations achieved during dosing. Although increased insulin secretion is observed concomitant with administration of these pan-Akt inhibitors, no significant changes were observed in blood glucose concentrations, whether measured randomly, or in oral glucose tolerance tests. In mouse tumor models, significant weight loss occurred at super-therapeutic doses (>MTD), and this was the dose limiting toxicity.



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Silencing of survivin gene by small interfering RNAs (siRNAs) induces apoptosis in human prostate cancer cells and increases their sensitivity to 17-allylamino-17-demethoxygeldanamycin (17AAG)

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Background: Survivin is an anti-apoptotic gene overexpressed in most human tumors and also involved in mitotic checkpoint control. Recent evidence points to a role for HSP90 in survivin function regulation. While the survivin-HSP90 association may help tumor cells to increase their anti-apoptotic threshold and promote their growth, it may also suggest new opportunities for the design of rational anti-cancer strategies. In this context, we evaluated the effects of the inhibition of survivin expression, accomplished through RNA interference technology, on the proliferative potentials of human prostate cancer cells and their sensitivity profile to the HSP-90 inhibitor 17AAG.
Material and Methods: Five 21-mer double-stranded siRNAs directed against different portions of survivin mRNA were designed. DU145 human androgen-independent prostate cancer cells were transfected with each siRNA (100 nM) using Lipofectamine-2000 for 8 h. At different intervals after transfection, cells were collected and analyzed for survivin mRNA and protein expression, cell growth rate, ability to undergo apoptosis, and sensitivity to a 72-h exposure to 10–100 nM 17AAG.
Results: Transfection of DU145 cells with siRNAs induced a variable extent of inhibition of survivin mRNA levels, ranging from −35% to −85%, compared to Lipofectamine-treated samples, as a function of the different oligomers. Such an inhibition was paralleled by a reduction in the abundance of survivin protein, ranging from −30% to −72% of controls. The three siRNAs able to reduce survivin expression (mRNA and protein) by more than 50% also caused a time-dependent inhibition of DU145 cell growth and enhanced the rate of spontaneous apoptosis from 5% of the overall cell population detected in control samples to 25–40%, with a concomitant 2–3 fold increase in the catalytic activity of caspase-9 and caspase-3. Sequential treatment of DU145 cells with siRNA and 17AAG induced supra-additive growth suppression and enhanced caspase-9-dependent apoptotic response.
Conclusions: These findings suggest that strategies aimed at interfering with the survivin-HSP90 connection, which couples apoptosis resistance to the cellular stress response, may provide novel approaches for treatment of androgen-independent prostate cancer.

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Antitumor activity, pharmacodynamics and toxicity of PX-866 a novel inhibitor of phosphoinositide-3-kinase

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Background: Phosphoinositide-3-kinase (PI-3-K) activates an important cell survival signaling pathway and constitutive activation is seen in ovarian, head and neck, urinary tract, cervical and small cell lung cancer. PI-3-K signaling is attenuated by the phosphatase activity of the tumor suppressor PTEN that is absent in a number of human cancers. Inhibiting PI-3-K presents the opportunity to inhibit a major cancer cell survival signaling pathway and to overcome the action of an important deleted tumor suppressor, providing antitumor activity and increased sensitivity of tumors to a wide variety of cancer drugs. PX-866 was identified as a pan-PI-3-K inhibitor from a library of synthetic viridins and inhbits PI-3-K with IC_{50} of 0.1 nM and cancer cell PI-3-K, measured by phosphoSer473-Akt (pAkt), with an IC_{50} of 20 nM.
Methods: The study evaluated the antitumor activity of PX-866 against human tumor xenografts in scid mice, alone or in combination with taxol, gemcitabine, cisplatin, or Iressa. The studies were accompanied by the evaluation of toxicity and pharmacodynamic activity of PX-866.
Results: *Antitumor activity* PX-866 as a single agent administered Q2D×5 intravenously (iv) at 12 mg/kg provided OvCar-3 ovarian tumor growth inhibition (TGI = 100 − T/C%) of 58%, and at 4 mg/kg orally (po) of 53%. When given on same schedule with taxol 12 mg/kg ip, PX-866 iv 4 hr before, gave a TGI of 83% versus 58% for taxol alone. PX-866 po with taxol produced a TGI of 88%. Similar potentiation by PX-866 was observed for the antitumor activity of gemcitabine in Panc-1 pancreatic cancer and cisplatin in A-549 lung cancer. Against large (0.5 g) A-549 xenografts in combination with EGFR kinase inhibitor Iressa (75 mg/kg po 3 times a week × 5) PX-866 (12 mg/kg iv; 4 hr before) gave a TGI of 84% versus Iressa alone 46% or iv PX-866 alone 40%. The combination with po PX-866, 4 mg/kg, gave a TGI of 76% versus po PX-866 alone of 56%. There were no significant changes in blood WBC, NE, RBC, platelets, ALT, AST or glucose by PX-866 at doses that increased Iressa antitumor activity. *Pharmacodynmic activity*: PX-866 iv or po as a single dose inhibited tumor pAkt for up to 48 hr. Tumor pAkt was not inhibited by Iressa, while pEGFR was inhibited by Iressa but not by PX-866. Iressa and PX-866 combined inhibited pAkt and pEGFR.
Conclusions: The PI-3-K inhibitor PX-866 inhibits p-Akt survival signaling in human tumor xenografts, has antitumor activity as a single agent and potentiates the antitumor activity of a variety of cancer drugs including an EGFR-kinase inhibitor.

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Novel small molecule inhibitors of 3′-phosphoinositide-dependent kinase-1 (PDK-1)

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The PI-3 kinase/PDK-1/Akt pathway is highly activated in many cancers and plays a key role in regulating tumor cell survival, growth, metabolism and angiogenesis. From chemical screens using a coupled assay measuring PDK-1-activated AKT2 activity, we identified and optimized potent inhibitors of PDK-1 that display selectivity against a panel of related kinases. Here, we describe the biological characterization of three PDK-1 inhibitors (Compound 1, (*N*-(3-((5-iodo-4-((3-((2-thienylcarbonyl)-amino)propyl)amino)-2-pyrimidinyl)-amino)phenyl)-1-pyrrolidinecarboxamide); Compound 2, (*N*-(3-((5-bromo-4-((2-(*1H*-imidazol-4-yl)ethyl)amino)-2-pyrimidinyl)amino)phenyl)-1-pyrrolidinecarboxamide); and Compound 3, (N-[3-[[5-bromo-2-[[3-[(1-pyrrolidinylcarbonyl) amino] phenyl] amino]-4-pyrimidinyl] amino] propyl]-2,2-dimethyl-propanediamide). The compounds block PDK-1 and Akt signaling in PC-3 prostate cancer cells. Compound 1 (IC_{50}=6 nM) and Compound 3 (IC_{50}=22 nM) blocked both Thr308-Akt and Thr389-S6K1 phosphorylation with IC_{50} values of 0.3 μM and 1–3 μM, respectively. The compounds also blocked colony formation in soft agar by tumor cell lines. For example, Compound 3 displayed an average IC_{50} of 0.4 μM for inhibition of 9 tumor cell lines tested. Furthermore, PDK-1 inhibitors induced massive apoptosis by MDA-468 cells cultured on plastic. Other cells tested showed less dramatic or no apparent apoptotic response when cultured on plastic, although a large proportion of cell lines were effectively growth-inhibited by compounds (IC_{50} < 2 μM). After 18 h of treatment, compounds were found to delay in the G2/M phase of the cell cycle. In contrast, the PDK-1 inhibitors weakly inhibited growth of normal human primary mammary and prostate epithelial cells, thus showing selectivity for tumor cells. A number of cell lines displaying high levels of Akt activity (e.g., MDA-453, U87-MG and PC-3) were markedly more sensitive (>30 fold) to growth inhibition by Compound 3 in soft agar than in culture on plastic. These data suggest that tumor cells undergoing metastasis may be particularly vulnerable to PDK-1 inhibitors. Treatment of mice with Compound 3 inhibited a blood borne metastasis model in which tumor burden in the lung was measured 4 weeks subsequent to tail-vein injection of LOX melanoma cells. In conclusion, we report on the development of potent inhibitors of PDK-1 in the aminopyrimidine class that support further development of PDK-1 inhibitors as anticancer agents.