# Practicalities of Drugging the Phosphatidylinositol-3-Kinase/Akt Cell Survival Signaling Pathway

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## Background

The phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) signaling pathway is the most frequently mutated or overexpressed signaling abnormality in human cancer. PI3K is activated by a number of mechanisms, including growth factor receptors, integrins, and Ras, converting phosphatidylinositols to phosphatidylinositol-3-phosphates (Fig. 1). This action of PI3K is antagonized by the tumor suppressor phosphatase and tensin homologue. Akt (thymoma in AKR mouse) binds through its pleckstrin homology domain to phosphatidylinositol-3-phosphate. Akt is phosphorylated and activated by phosphoinositide-dependent protein kinase I and mammalian target of rapamycin/rictor. Heat shock protein 90 also binds to Akt to regulate Akt levels. Akt phosphorylates multiple downstream targets to modulate several cell functions.

Activation of the PI3K pathway confers resistance to cell death induced by a wide variety of stress inputs, including chemotherapy and radiation. There have been several recent excellent reviews of the details of PI3K/Akt signaling that will not be repeated here (1, 2). This review will be concerned with practical issues of developing PI3K inhibitors as anticancer drugs. Some PI3K inhibitors in late preclinical development are wortmannin analogues (Wyeth), BEC235 (Novartis), PX-866 (ProIX), ZSTK474 (Riken), SF1126 (Semafore), and fused heteroaryl and imidazopyridine-based inhibitors (Genentech/Piramed). The PI3K/Akt pathway is context dependent, and the effects of inhibiting the pathway depend on the cellular and physiologic environment in which it is active. Importantly, stress is required for the full effects of inhibition of the PI3K/Akt pathway to become apparent. For example, complete inhibition of PI3K can be achieved in cells at low nanomolar concentrations of wortmannin, but micromolar concentrations are required to inhibit cell proliferation (3), which is likely to be due to inhibition of targets other than PI3K (4). Low concentrations of wortmannin potentiate the antiproliferative effects of stress stimuli, such as serum deprivation (5) or cancer drugs (6). Tumors *in vivo* are naturally stressed with insufficient oxygen and nutrients because of the abnormal tumor vasculature (7). For this reason, inhibition of PI3K in tumors can be sufficient to inhibit growth (8). This is not inconsequential because it distinguishes PI3K from signaling targets whose inhibition alone does not lead to antitumor activity. Evidence of activity in phase I testing is often considered necessary justify further clinical development of an agent (9); therefore, the single-agent activity of PI3K inhibitors could be crucial.

## Clinical Translational Advances

***Inhibiting the pathway: upstream or downstream?*** The PI3-K/Akt pathway can be activated by multiple inputs, including cytokines, growth factors, and mutant Ras. Once activated, however, there are an increasing number of outputs as the signal moves down the pathway. Inhibiting PI3K should give maximum inhibition of all the outputs, whereas inhibiting downstream targets, such as Akt, and even further downstream targets, such as mammalian target of rapamycin, will give progressively more selective output inhibition (Fig. 1). The effects of upper pathway inhibition may have to be balanced against the possibility of increased toxicity compared with downstream inhibition. Without hard data, however, we can only speculate on the relative therapeutic indices for different targets. Animal data suggest that inhibiting PI3K signaling may not be particularly toxic (10). Off-target toxicities could play the greatest role in determining the therapeutic window. This has been seen with Akt inhibitors that may inhibit both Akt and related kinases, such as protein kinase A (11). Inhibitors of Akt that block the lipid-binding pleckstrin homology domain, such as perifosine (12) or PX-316 (13), seem much less toxic. Clinical evidence obtained with the mammalian target of rapamycin inhibitor rapamycin indicates that hyperglycemia and hypertriglyceridemia caused by perturbation of insulin-signaling may be important toxicities (14).

***Expected unexpected toxicities.*** The multiple actions of PI3K/Akt signaling mean that on-target toxicities are always likely to be reflected in the toxicity profile of inhibitors. In particular, the well-known role of PI3K/Akt signaling in mediating the effects of insulin suggests that hypergylycemia may be a particularly common, if not a hallmark toxicity. The broad nonselective Akt inhibitor UCN-01 causes insulin insensitivity (15) and has produced severe hyperglycemia requiring prolonged insulin infusion in a patient who received the drug in clinical trial. Prolonged administration of the experimental PI3K inhibitor PX-866 to mice either i.v. or orally increases blood glucose. There is decreased glucose tolerance and increased plasma insulin, suggesting insulin insensitivity similar to that of type II diabetes (10). Importantly, the decreased glucose tolerance can be prevented by the clinically used antidiabetic peroxisome proliferator-activated receptor γ agonist pioglitazone without affecting the antitumor activity of PX-866 (16).

***Isoform selectivity.*** There is no consensus on whether isoform inhibitors of PI3K or Akt offer advantages over pan

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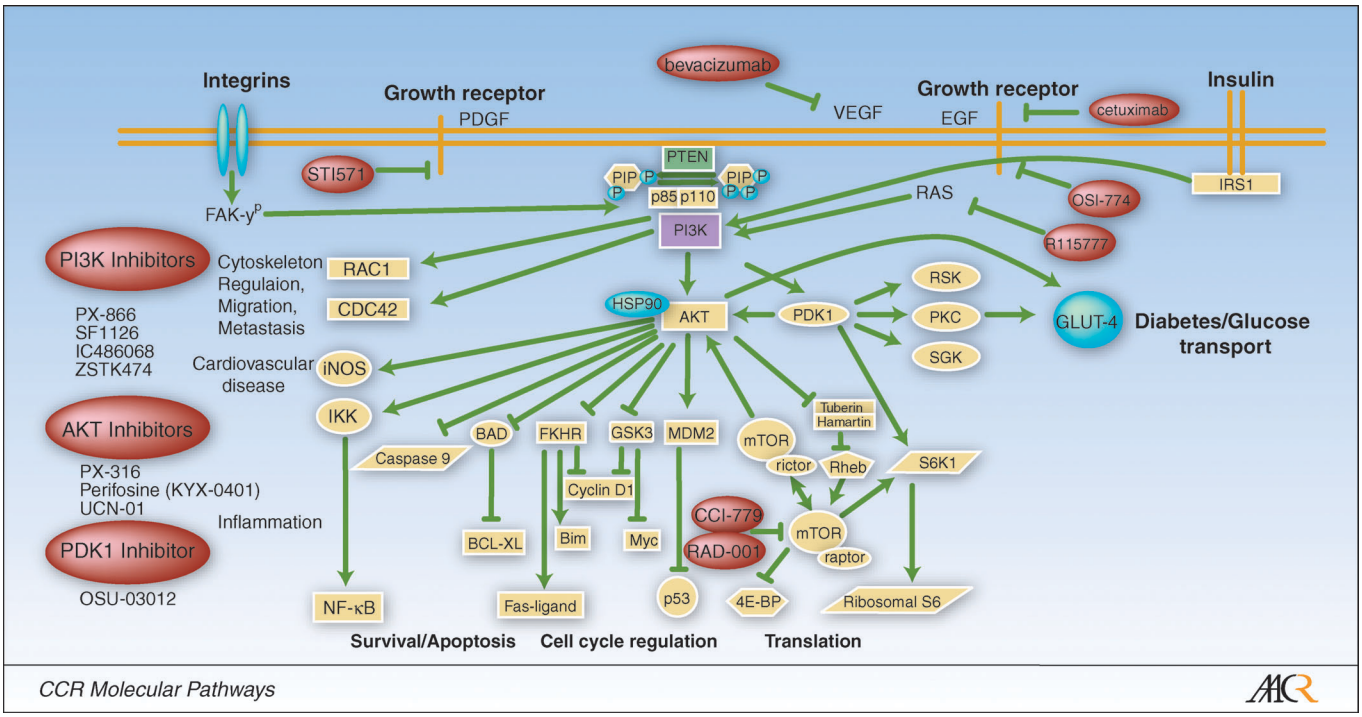


**Fig. 1.** PI3-K/Akt signaling showing multiple inputs, outputs, and crosstalk. PI3K is activated by a number of mechanisms, including growth factor receptors, integrins, and Ras, converting phosphatidylinositols (*PIP*) to phosphatidylinositol-3-phosphates. This action of PI3K is antagonized by the tumor suppressor phosphatase and tensin homologue (*PTEN*). AKT (thymoma in AKR mouse/protein kinase B) binds through its pleckstrin homology domain to phosphatidylinositol-3-phosphates and is activated by phosphorylation by phosphoinositide-dependent protein kinase 1 (*PDK1*) and mammalian target of rapamycin (*mTOR*)/rictor. Heat shock protein 90 (*HSP90*) binds to Akt to regulate Akt levels. Akt phosphorylates multiple downstream targets, including focal adhesion kinase (*FAK*), inducible nitric oxide synthase (*iNOS*), and Iκ kinase (*IKK*), thus regulating nuclear factor-κB (NF-κB), BAD, and Bcl-xl (B-cell lymphoma mutant, extra long), forkhead transcription factor (*FKHR*), glycogen synthase kinase-3 (*GSK-3*), murine double minute 2 (*MDM2*), mammalian target of rapamycin, and thus eukaryotic initiation factor 4E (eIF4E) binding protein (*4E-BP*) and ribosomal S6 protein kinase (*S6K*), Ras homologue enriched in brain (Rheb). RSK, ribosomal S6 kinase; PKC, protein kinase c; SGK, serum and glucocorticoid induced kinase; Glut-4, glucose transporter-4; RAC1, Ras-related C3 botulinum toxin substrate 1; CDC42, cell division cycle 42. The cell functions modulated by the different signaling pathways are indicated. There are a number of PI3K and Akt signaling inhibitors in development (*red ovals*) and cancer drugs with which they might be combined.

inhibitors. Most attention has focused on the PI3Kα isoform, particularly with the identification of activating mutations that cause a constitutive signal to Akt. It has been reported that wild-type PI3Kβ, PI3Kγ, and PI3Kδ have oncogenic potential, whereas PI3Kα is only oncogenic when mutated (17). PI3Kδ has the ability to activate Akt, whereas the PI3Kβ and PI3Kγ rely on upstream factors. If these findings are consistent in more complex models, it could explain the lack of mutations in PI3Kβ, PI3Kγ, and PI3Kδ seen in human cancers, while still implicating them as important activators of downstream prosurvival pathways. This would suggest that inhibition of multiple isoforms of PI3K may be essential for disrupting the prosurvival pathway in a greater variety of cancers. The PI3K inhibitors in development, besides pegylated wortmannin and SF1126, a prodrug form of LY294002, all seem to have some selectivity, including pure p110α inhibitors (18), PX-866, a PI3Kα, PI3Kγ, and PI3Kδ inhibitor, showing relatively little PI3Kβ inhibition (10) and IC486068, which is selective for PI3Kδ (19).

***Combination therapy.*** With many targeted agents in clinical development and the currently approved cancer drugs, the design of trials to evaluate the potential of PI3K inhibitors in combination may be daunting. An example is that of bevacizumab (Avastin), which has been involved with >60 completed or ongoing combination clinical trial studies with cytotoxic agents alone worldwide, and additional studies are under way with approved signaling anticancer drugs (20). Ultimately, it would be beneficial to have exquisitely designed animal studies to suggest not only the combinations to study and the dose range but also the schedule providing optimum inhibition of the targets, in such a way as to provide the greatest therapeutic benefit. There is also the need to select cancers against which to initiate trials based on the drugs where combination activity has been seen and the best markers by which to evaluate the agent's target inhibition. Evidence is growing to suggest that the overexpression of PI3K in lung, ovarian, and breast cancers and glioblastoma (21–23) would provide good cancers to evaluate PI3K inhibitors with phospho-Akt as a surrogate marker of activity (24).

Preclinical studies suggest that combinations of PI3K inhibitors with both cytotoxic and molecularly targeted agents would result in additive to greater-than-additive benefit. Combination of PI3K inhibitors with gemcitabine, cisplatin, bevacizumab and heat shock protein 90, mammalian target of rapamycin, and tumor necrosis factor inhibitors has been shown to be greater than additive (8, 25, 26). Gefitinib, which inhibits epidermal growth factor receptor signaling, in a treatment schedule showing little effect against A-549 non–small cell lung tumor xenografts when combined with a PI3K inhibitor, provided complete stasis of growth until the dosing of both agents was stopped (10). A practical issue is that it is difficult to consider developing an experimental agent in

combination with a second experimental agent, and in the near future, most clinical work is likely to be with a PI3K inhibitor and currently approved agents. A challenge is to design trials that exploit to the full the properties of the PI3K inhibitor and the other drug(s) in combination. Because it is unlikely that the delivery schedule of the marketed agent will change, it is crucial to determine when and how to administer the PI3K inhibitor on that agent's possesive schedule. That determination will only be accomplished by using biomarkers in the early trial to know at what dose and timeline the PI3K pathway has been inhibited. At this point, it is unclear as to the ultimate specificity of any of the PI3K inhibitors under development; however, if the target has been inhibited without undue toxicity, the delivery of the agent should be deemed optimum.

## Conclusion

The introduction of inhibitors of PI3K/Akt signaling inhibitors into clinical trials has been long in arriving and awaited with considerable anticipation in the belief that inhibiting such a major signaling abnormality that controls cancer cell survival must offer some therapeutic benefit. As with all drugs, it may take time to understand how best to use the inhibitors. It is already apparent that the importance of the PI3K/Akt pathway to survival is context dependent. Simply put, it seems that the more stress the cell is under, the greater the effect of pathway inhibitor will be. A growing tumor experiences intrinsic stress generated by the continuing need to increase the supply of oxygen and nutrients so that stand-alone activity of the agents should be expected. It is likely, however, that the greatest benefit of PI3K/Akt signaling inhibitors will be in combination with other drugs. It will take insight and perseverance to discover out the best combinations. Dealing with the toxicities of the inhibitors, particularly the on-target toxicities, will also require knowledge of the pathways being affected. Finally and of critical importance, will be to identify patients most likely to respond to PI3-K/Akt signaling inhibitors or combinations of agents, to achieve the goal of personalized medicine.

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