Review

Take your PIK: phosphatidylinositol 3-kinase inhibitors race through the clinic and toward cancer therapy

Nathan T. Ihle and Garth Powis

M. D. Anderson Cancer Center, Houston, Texas

Abstract

The phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway is currently one of the most exciting drug targets in oncology. However, only a short time ago, the paradigm existed that drugs targeted to the four PI3K class I isoforms would be too toxic for use in cancer therapy due to effects on physiologic signaling. Since that time, studies have delineated the roles of these four isoforms in nonpathologic signaling as well as their roles in cancer. An extensive effort has gone into developing agents that inhibit one or more PI3K isoforms, as well as closely related proteins implicated in cancer. These agents have proved to be tolerable and therapeutically beneficial in animal studies, and a number are in clinical testing. The agents, their properties, and their molecular targets are discussed in this review. [Mol Cancer Ther 2009;8(1):1 – 9]

Phosphatidylinositol 3-Kinase Inhibitors in Cancer

Developing an effective inhibitor to phosphatidylinositol 3-kinase (PI3K)/Akt signaling has become one of the most sought after goals of pharmaceutical companies and academia alike. Such compounds are seen as possessing the potential to have a significant effect on the treatment of human disease, the largest application being in oncology, but certainly encompassing a variety of other pathologic conditions. The field has gone from a handful of ''archetypal'' inhibitors, which dominated the field for upwards of 10 years, to a range of small molecules that are progressing rapidly toward and through early clinical testing. Just a few years ago, the general opinion was that broad-spectrum

inhibitors of the class I PI3Ks would ''almost certainly have unacceptable toxicity if administered continuously'' (1). Eight years later, these inhibitors now fill the oncology landscape, each with their own distinctive profile of inhibition, not only of specific PI3K isoforms but also of the phosphoinositide kinase (PIK) family of proteins to which the PI3Ks belong. Clinical testing of these agents has begun and will serve to define the optimal profile to accomplish the desired therapeutic goals while still maintaining an acceptable therapeutic index.

PIK Family Overview

PI3Ks can be divided in to three classes. Class I PI3Ks exist as heterodimers consisting of one of four p110 catalytic subunits and one of two families of regulatory subunits (Fig. 1). This class acts on phosphatidylinositol 4,5-bisphosphate to produce phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P$_3$; ref. 2], and the process is reversed by the mixed function phosphatase PTEN (Fig. 2; ref. 3). Class II PI3Ks display the ability to phosphorylate phosphatidylinositol and phosphatidylinositol 4-phosphate *in vitro* and are generally resistant to the class I inhibitors. Class III PI3Ks phosphorylate only phosphatidylinositol to generate phosphatidylinositol 3-phosphate. This class has only one member, known as Vps34, which has been shown to play an essential role in trafficking of proteins form the Golgi apparatus in yeast (4). More recently, this class has been linked to autophagy and the activation of mammalian target of rapamycin (mTOR) by amino acids (5). There is also a fourth class of PI3K-related enzymes, which contain a catalytic core similar to the PI3Ks. This class includes enzymes involved in signal transduction and DNA damage response, such as mTOR and DNA-dependent protein kinase (6).

The first class I PI3K identified was an enzyme that copurified with p60v-src, polyoma middle T antigen, and the platelet-derived growth factor receptor. Class I PI3Ks were later subdivided into class Ia, consisting of the α, β, and δ catalytic subunits, and class Ib, consisting solely of the γ catalytic subunit. The class I isoforms are activated under normal physiologic conditions on stimulation by growth factors, either directly by the growth factor receptor or through adaptor proteins. Ligand binding results in tyrosine phosphorylation, which allows docking of the SH2 domain located on the regulatory subunit, leading to activation of the lipid kinase activity of the class Ia enzymes by receptors such as the epithelial growth factor receptor.

Domains:		histone deacetylase		p85 binding	Ras binding	PKC homology	helical	catalytic

PIK3CA (p110α)
124k Da

PIK3CB (p110β)
123 kDa

PIK3CD (p110δ)
126 kDa

PIK3CG (p110γ)
119 kDa

P101 Binding

G protein binding sites
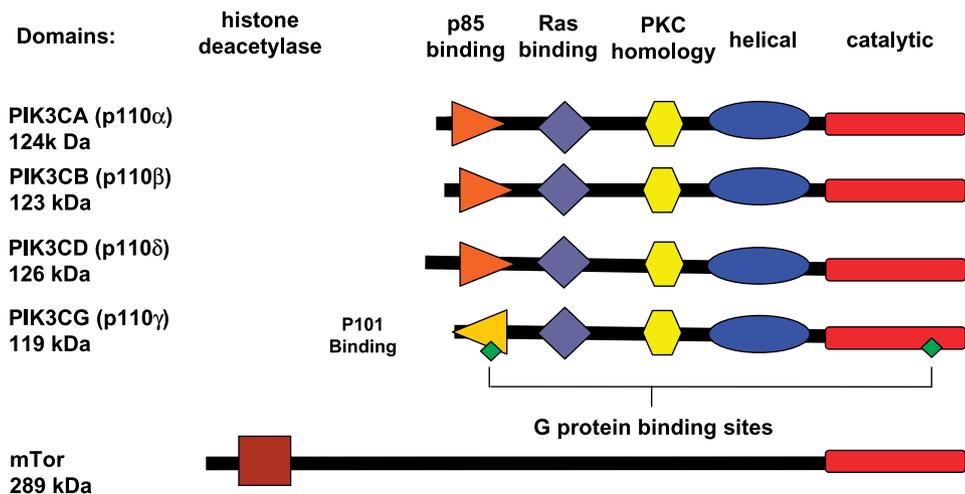
mTor
289 kDa

Figure 1. Domain structure and amino acid homology of the class I PI3K catalytic subunits and mTOR. The regulatory subunits are not shown. Also shown is a cladogram showing evolutionary relationships.

Cladogram

Amino Acid Homology

	alpha	beta	delta	gamma	mTor
alpha	100	39	40	31	12
beta	39	100	57	29	12
delta	40	57	100	29	13
gamma	31	29	29	100	12
mTor	12	12	13	12	100

mTor gamma delta beta alpha
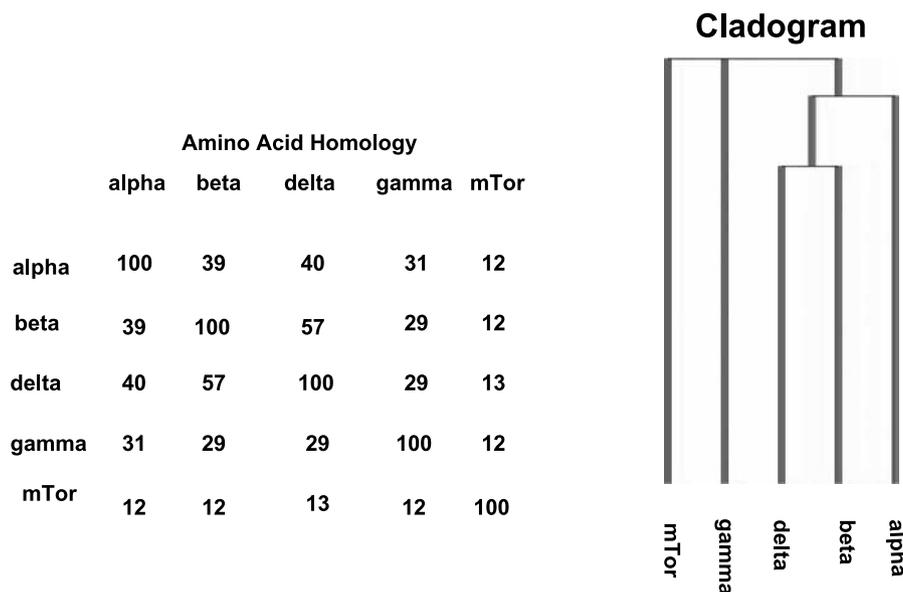
Additionally, active Ras has been shown to have the ability to activate class I enzymes (7). The generation of PI(3,4,5)P$_3$ by PI3Ks allows for the recruitment to the plasma membrane of proteins containing a pleckstrin homology (PH) domain (8). Among the best characterized of these proteins is Akt, which when recruited to the plasma membrane is phosphorylated on threonine 308 by another PH domain–containing protein, phosphoinositide-dependent protein kinase 1, and on serine 473 by phosphoinositide-dependent protein kinase 2, which has been identified as potentially one of at least 10 proteins including DNA-dependent protein kinase and the rictor-mTOR complex (Fig. 2; ref. 9). It should be noted that there are inhibitors of Akt itself in development that act through inhibition of kinase activity or PH domain–dependent translocation (10). How these inhibitors will compare clinically to the emerging PI3K inhibitors will be of great interest. Examples of the multitude of targets phosphorylated by activated

Akt are AS160, which regulates translocation of Glut-4 to the plasma membrane, thus affecting glucose uptake; nuclear p27, a negative regulator of cell growth, thus allowing cell proliferation; and inhibition of Bad, a promoter of apoptosis (11). Another downstream target of Akt is tuberous sclerosis complex 2 (tuberin), which when phosphorylated by Akt disassociates from its partner tuberous sclerosis complex 1 (hamartin), leading to its degradation and loss of its GTP activation activity against the small G protein Rheb, which serves as a negative regulator of the PIK family member mTOR. With this negative regulation of Rheb, the mTOR protein becomes active through association with raptor and other factors, stimulating TOP-dependent mRNA translation through p70S6 kinase and cap-dependent translation through inhibition of the eiF4e repressor, 4E-BP, completing the signaling cascade known as the PI3K/Akt/mTOR axis (12). Notably, inhibitors of the raptor-mTOR complex

including rapamycin derivatives, or rapalogues, are now approved for clinical use as antitumor agents (13). However, these inhibitors have also revealed that, in some cases, inhibition of mTOR has the ability to activate PI3K signaling either by feedback to growth factor receptors or by promoting the formation of an alternative mTOR complex with rictor, which may serve to phosphorylate Akt, seen in both cell models and clinical samples (14). This potentially undesirable effect may be nullified through the use of direct inhibitors of mTOR as opposed to inhibitors of raptor-mTOR (13).

Aberrant PI3K signaling has been found to play an important role in multiple aspects of tumorigenesis including uncontrolled proliferation, resistance to apoptosis, angiogenesis, and metastatic capability. This aberrant signaling may occur through dysfunction of pathways upstream of the PI3K class I isoforms, such as mutationally activated growth factor receptors, Ras, or activation of the pathway itself (15). The first mechanism discovered by which the PI3K/Akt pathway is directly activated was the loss or inactivation of PTEN, identified as a tumor suppressor. The inactivation is found at a high frequency in multiple tumor types, and new mechanisms by which cancer cells can alter the function of PTEN continue to be found (16). Most recently, mutations in the PH domain of Akt1, which causes electrostatic alterations leading to increased binding of the Akt PH domain with PI(3,4,5)P₃, have been found to aberrantly activate the pathway. Thus far, the initial mutation found at amino acid 17 of the Akt PH domain has been identified in 8% of the breast tumors studied, 6% of colorectal tumors, and 2% of ovarian cancers. Larger studies to precisely determine the frequency and tumor type specificity of this mutation remain to be conducted (17).
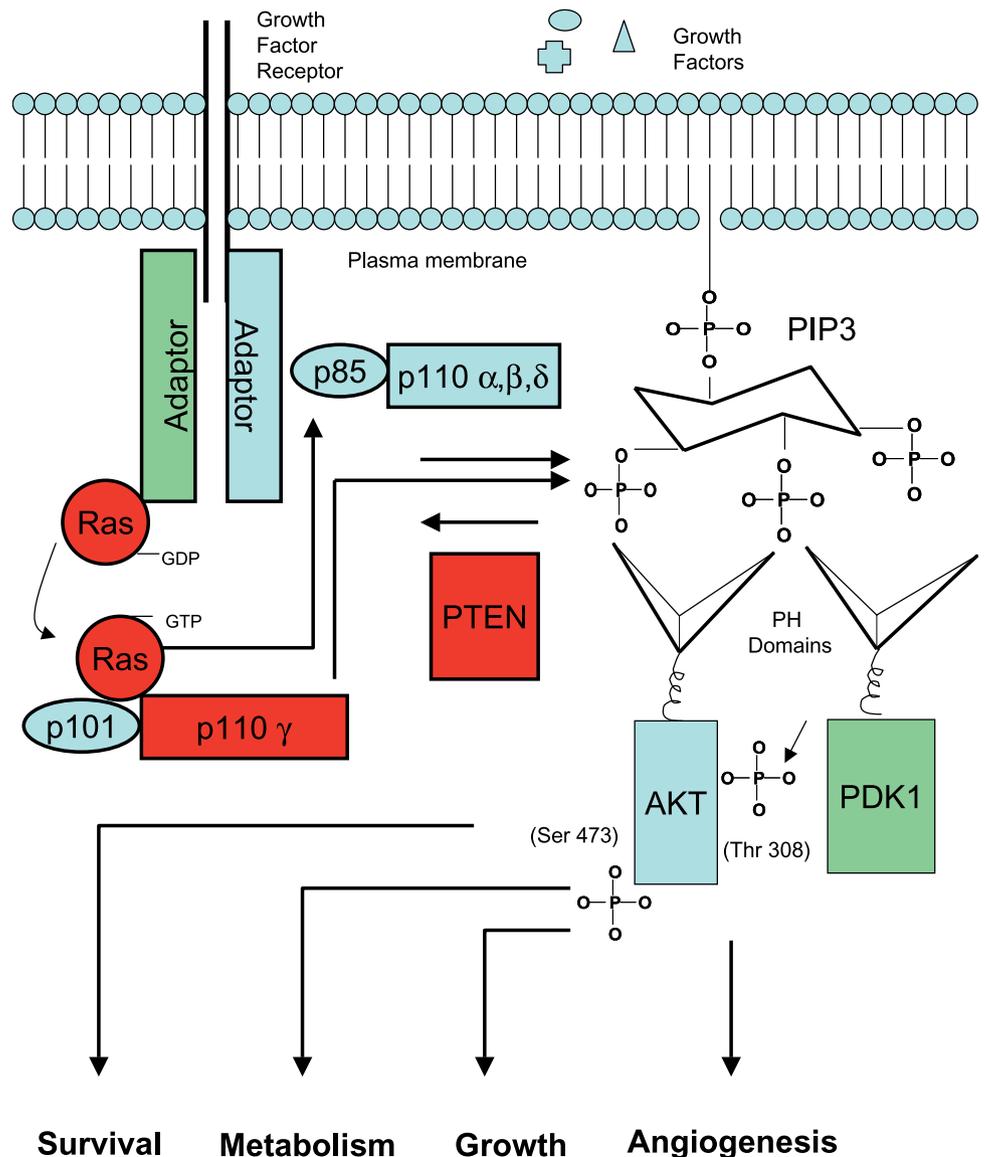
Figure 2. PI3K/Akt signaling in cells. For explanation, see text.

Dissection of PI3K Class I Isoform Signaling in Normal Physiologic Signaling and the Oncogenic Process

Both genetic manipulation and pharmacologic inhibitors have proved valuable in distinguishing the activities of each of the PI3K isoforms in normal cellular signaling. Early studies revealed that knockout of the PI3Kα isoform resulted in embryonic lethality (18), later determined to be due to deficient migration of endothelial cells resulting in a loss of angiogenic activity (19). A conditional knockout of PI3Kα in developed mice resulted in impaired insulin-induced glucose uptake (20) similar to that seen in Akt2 knockout mice (21). Similar results were found in cultured muscle cells treated with PI3Kα-specific inhibitors (22). Mice deficient in the PI3Kβ isoform also showed embryonic lethality (23). Conditional knockout of PI3Kβ in developed mice resulted in mice that were similar to PI3Kα knockouts in that they exhibited impaired insulin signaling, although this effect was found not to be dependent on Akt signaling. Additionally, these PI3Kβ knockout mice were deficient in lyophosphatidic acid signaling (24). Knockout of either the γ or δ isoforms of PI3K resulted in viable mice displaying alteration in immune function. Mice deficient in PI3Kδ showed deficient antigen receptor signaling in both B and T cells, as well as inflammatory bowel disease (25). Mice with a deletion of the PI3Kγ isoform had defective thymocyte survival and decreased thymus size and showed an inability to activate T cells, but unlike the knockout of PI3Kδ, no effect was seen on B cells. Additionally, PI3Kγ knockout mice showed a loss of the migratory ability of neutrophils, which were unable to generate the respiratory burst generated by GTP-coupled protein receptor signaling (26).

In the context of oncogenesis, the PI3K isoforms have been found to have overlapping and unique roles. Overexpression of all four isoforms has been shown to be capable of inducing transformation in experimental models, PI3Kα and PI3Kδ, independently and PI3Kβ and PI3Kγ with input from Ras (27). PI3Kα has been implicated in cancer cell proliferation and tumor angiogenesis (28). Additionally, this isoform has been shown to assist in Ras-induced transformation and to be necessary for tumor formation in a mouse model of Ras-induced oncogenesis (29). More recently, activating mutations in both the helical and kinase domains of PI3Kα have been identified particularly in breast and colon tumors, occurring frequently in similar locations, known as "hotspots," within the protein. The most common site for these hotspots is around amino acid 1047 in the kinase domain and amino acid 545 in the helical domain (30). Notably, these mutations have been found exclusively in the PI3Kα isoform to date, and mutations induced at the same location as the hotspots in PI3Kα did not have similar effects in activating PI3Kβ (31). When a colon line, HCT-116, heterogenous for the PI3Kα hotspot mutation, was given the homozygous mutation, enhanced survival was seen under stress conditions together with increased metastasis (32). The PI3Kβ isoform has been implicated as necessary for transformation induced by the loss or inactivation of the PTEN tumor suppressor both *in vitro* (33) and *in vivo* (31). The PI3Kδ isoform, most prominently expressed in myeloid cells, has been shown to play an essential role in cell proliferation in acute myeloid leukemia (34) and has also been implicated in tumor angiogenesis, particularly in the context of repair after destruction of tumor blood vessels with radiation (35). The PI3Kγ isoform seems to function largely in the context of the immune system and has been found to be used by the BCR-ABL fusion oncogene, implicated in chronic myeloid leukemia, for proliferation and drug resistance (36), and is also known to be a Ras effector (37). By determining the specific functions of each isoform in both normal physiology and the pathology of cancer, it may be possible to predict on-target effects resulting from patient treatment with pan class I PI3K inhibitors. Additionally, as isoform-specific inhibitors become available, the possibility of matching these inhibitors to specific conditions of oncogenesis is an attractive concept. However, current evidence indicates that, in many cases, redundancy of signaling among the PI3K isoforms may make this goal unobtainable (38).

Early PI3K Inhibitors: Classic and Modern Twists

The earliest report of a compound that showed an inhibitory effect on PI3K was the nonspecific kinase inhibitor quercetin (39). The next inhibitor identified was wortmannin (40), already known at the time as an inhibitor of myosin light chain kinase (41). Shortly thereafter, a quercetin analogue, LY294002, was developed with increased specificity toward PI3K (42). Wortmannin and LY294002 were both evaluated as potential agents for clinical development but quickly found to be unsuitable candidates (42).

Wortmannin is a member of a class of steroidal furanoids, which includes viridin. Extensive structural studies have been done and wortmannin has been found to bind in an irreversible fashion through an electrophilic site at the C-20 position of the furan ring to lysine 802 in the ATP catalytic site of PI3K (43). Minor modifications to the structure of wortmannin had only slight effects on the *in vitro* efficacy, whereas modifications negating the electrophilicity in the furan ring rendered the compound inactive (44). Wortmannin has been found to have equally potent activity against all the class I PI3K enzymes with IC_{50}s in the single digit nanomolar concentration range, while inhibiting other members of the PIK family, such as mTOR and DNA-dependent protein kinase, at higher concentrations of 250 and 16 nmol/L, respectively, and unrelated enzymes such as polo-like kinase and myosin light chain kinase with IC_{50}s of 24 and 170 nmol/L, respectively (45).

LY294002 has a significantly lower potency for the class I PI3Ks than does wortmannin, having an IC_{50} in the 1 to 20 μmol/L concentration range (42). This was later found to directly overlap the range necessary to inhibit other members of the PIK family such as mTOR and DNA-dependent protein kinase. LY294002 has been found to

inhibit additional kinases, such as casein kinase 2 and Pim, and to have other PI3K-independent effects such as the inhibition of calcium signaling. Additionally, LY294002 had unfavorable pharmacologic properties of insolubility and a poor half-life in animals. Recent studies looking in more detail at the activity of LY294002 both in enzymatic assays and in cells have shown that its affinity for some targets is higher than its affinity for the class I PI3Ks (46), leading one study to conclude that its use as a tool to study PI3K signaling should be discontinued (47). Despite these inadequacies, both wortmannin and LY294002 proved to be valuable tools for the early study of PI3K inhibition, most importantly showing that shutting down class I PI3K signaling was not instantly toxic to cells or to animals and, thus, might have a therapeutic benefit in cancer. On the other hand, they also set back the development of PI3K inhibitors because of associated toxicities that resulted from off-target effects, which would not be fully defined until recently (46).

Prodrugs of wortmannin have been developed in attempts to extend its half-life in biological systems, and analogues created, which improve its pharmacologic properties, such as extending its half-life and favorably altering its selectively profile. Attempts to directly use the antiproliferative effects of wortmannin have used wortmannin conjugated to polyethylene glycol to delay its breakdown in biological systems (48). Additionally, modifications of wortmannin at its active C20 position through opening of its furan ring have yielded compounds that not only extend its half-life but also have increased its selectively for particular PI3K isoforms. Such a compound is PX-866, which was found to have selectivity for the α, δ, and γ class I PI3K isoforms while inhibiting the β isoform at higher concentrations and showing decreased selectivity for mTOR (Fig. 3; refs. 49, 50). PX-866 is currently in early clinical testing by Oncothyreon. PX-866 is the only irreversible PI3K inhibitor currently being developed clinically and has sustained oral activity against PI3K at low oral doses. Derivative compounds have been synthesized, including WAY-266176 and WAY-266175, which have a modification at the C-20 position in 17-hydroxywortmannin, a wortmannin derivative (51). The irreversible PI3K inhibitors may display a unique advantage as their *in vitro* enzymatic and cellular activity translates closely to their *in vivo* activity, likely due to their irreversible inhibition of the enzyme.

Attempts to harness the antiproliferative effects of LY294002 have also led to the creation of a prodrug, SF1126, which consists of LY294002 linked to a RDGS integrin binding element designed to target the compound to the tumor and tumor vasculature and has shown antitumor effects on tumor xenografts (52). SF1126 is currently in early clinical development. Additionally, derivatives of LY294002 have been identified, which are reported to display isoform selectivity among the class I PI3K enzymes (53). However, given the extreme nonspecificity of the parent compound for different molecular targets, it is difficult to envision LY294002 derivatives offering a truly selective approach to PI3K inhibition.

Recently Developed Inhibitors: Reflecting Divergent Paradigms

There has been a recent a flood of PI3K inhibitors from academia and industry, reflecting an intense effect of making agents with increased specificity for desired class I PI3Ks. The goal in this effort has been to maximize the therapeutic effects of the inhibitors against the effects of deregulated isoforms specific to particular cancers, thus hopefully minimizing their total effect and increasing their therapeutic index. Many compounds have been developed with varying specificity for PI3K isoforms and other PIK family members, and their selectivity profiles determined through extensive profiling (22). Despite this, few compounds have been deemed to exhibit pharmacologic profiles suitable for advancement beyond preclinical testing. A concern is that although active at low nanomolar concentrations against purified PI3Ks, the compounds exhibit only high nanomolar activity in cells often well in excess of their isoform selective range (Fig. 3) Furthermore, although these compounds distinguish between isoforms, often at single digit nanomolar concentrations, the threshold at which they exert this distinction in cells and *in vivo* is unknown. Despite these concerns, an early success with this strategy came with the development of a specific inhibitor of the PI3Kδ isoform, CAL-101, now in early clinical trial for hematologic malignancies. IC87114, the preclinical inhibitor, was found to inhibit acute myelogenous leukemia proliferation and augmented the effects of a topoisomerase-2 inhibitor by the specific inhibition of the PI3Kδ isoform, with proof of selectivity established in cells as well as against the enzyme (54). This compound is simultaneously entering the clinic for nononcology applications. To date, it is the only inhibitor in clinical trials that distinguishes between class I isoforms. The newly developed inhibitors GDC-0941 and PX-866 are reported to have selectivity for the class I isoforms, with varying profiles (50, 55). Which selectivity is optimal and whether the specificity seen in preclinical testing will carry into the clinic will have to be proved.

Another widely studied compound in recent years has been PI-103. The introduction of this compound brought a new paradigm of the development of PI3K inhibitors. PI-103 was found to have increased efficacy in inhibiting the growth of glioma cells due to its activity against both the class I PI3Ks and the PIK family member mTOR (56). It is also notable that this compound had activity against DNA-dependent protein kinase (22). This proved a contrary perspective to the long-held goal of achieving increased specificity against particular class I PI3K family members, in that perhaps with a less specific inhibitor greater antitumor effects could be achieved. There was also the observation that combined inhibition of the class I PI3Ks and mTOR eliminated the increased Akt signaling that an mTOR inhibitor alone often caused (57). However, PI-103 was found to have pharmacologic properties unsuitable for clinical development, leaving untested the concept of inhibiting multiple points in the PI3K signaling cascade for increased efficacy. This concept has been subsequently

	XL147	XL765	GDC- 0941	BEZ235	PX-866	IC87114 (CAL-101)	SF1126 (LY294002))
PI3Kα (nM)	39	39	3	4	5	200,000	1000-3000
PI3Kβ (nM)	383	113	33	75	> 300	16000	1000-3000
PI3Kδ (nM)	36	43	3	7	3	130	N.R.
PI3Kγ (nM)	23	9	75	5	9	61000	2000
mTor (nM)	>1500	157	580	N.R.	900	N.R.	3000
cellular p-Akt (nM)	400-942	68-709	28-46	250	25-50	200	10000
antitumor (xenograft) (mg/kg daily)	100	30 x 2	75	25 x 2	2.5	N.R.	20 x 3 /week
structure	1	2	3	4	5	6	7
reference	58	59	54	57	49	73	73

Figure 3. Profiles of PI3K inhibitors in clinical trial. The table shows the different PI3K inhibitors currently in clinical trial together with their reported activities against the PI3K α, β, γ, and δ isoforms and mTOR; the concentration reported to inhibit cellular PI3K signaling measured by phospo-Akt inhibition; and the doses given to tumored animals to obtain antitumor activity, whether once or twice daily or thrice per week. *NR,* no report. *Bottom,* structures of the compounds.

* with targeted modality
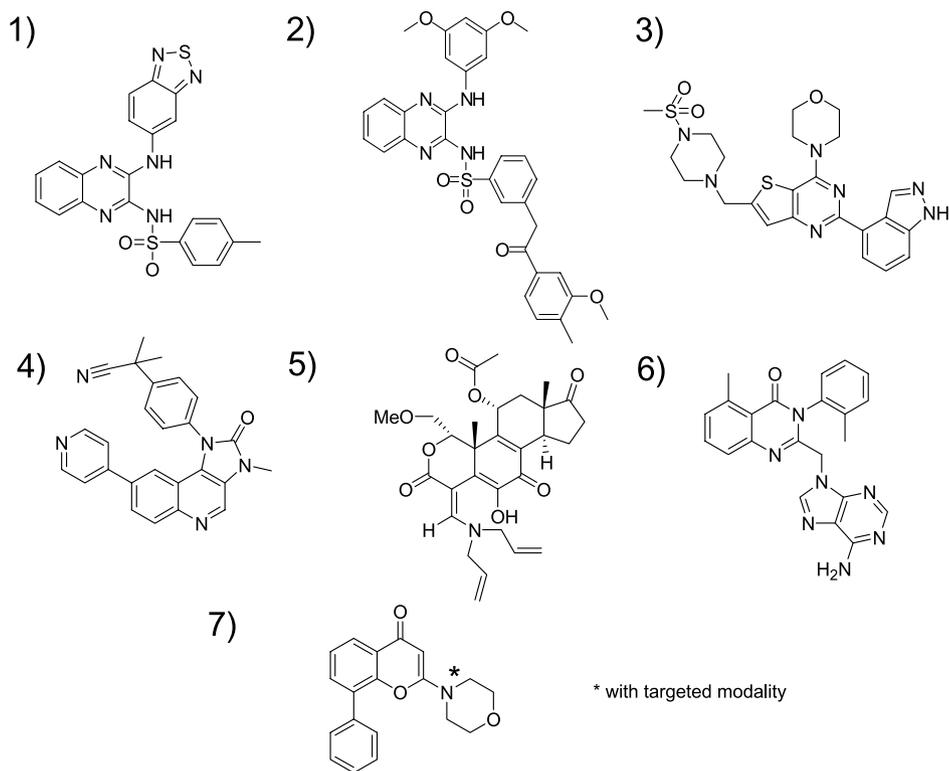
used by Novartis in their selection of BEZ235 as a lead compound now in clinical trial, which was found to have activity against both the class I PI3K isoforms and mTOR (58). Exelixis has advanced two compounds as potential leads: XL147, which targets only the class I PI3Ks, and Xl765, which was found to have activity against the class I PI3Ks as well as mTOR (59, 60). Whether this nonspecific approach will translate to clinical agents with an acceptable therapeutic index is unknown. Although other classes of kinase inhibitors have capitalized on unexpected activity against other targets, which has proved useful in certain tumor types (61), this is unknown for the PI3K inhibitors. Activity against mTOR may reflect broad-spectrum activity against a number of additional PIK family members and unrelated targets resulting in unpredictable toxicities, which could include the cardiac toxicity seen with many other current kinase inhibitors (62).

Unanswered Questions

As the PI3K inhibitors move into the clinic, answers to many important concepts coming from the preclinical models are beginning to take shape. Preclinical models provide strong evidence about what may occur with this class of inhibitors, but despite this, for proof-of-principle, these concepts must be shown in multiple clinical trials

with an inhibitor deemed to be effective to become validated, which may then provide a guide for future prospective clinical trials. Some of the unanswered questions and the emerging results from the clinical use of PI3K inhibitors are as follows.

The first question is whether, as originally suggested, inhibition of such a ubiquitously used pathway will prove too toxic to achieve therapeutic benefit. The expected undesirable effects associated with inhibiting this pathway, most notably metabolic disturbance and increased blood glucose, are being seen but have been reported to be mild or treatable, at least preclinically (50), and in early clinical evaluation, have been manifest only as an increase in insulin levels (63). It is also notable that a metabolic disturbance most likely arises as a result of inhibiting the PI3Kα isoform (20), and this is also the isoform that to some presents the most attractive target in the broadest range of cancers. Thus, a more specific inhibitor of this isoform is unlikely to eliminate the metabolic on-target toxic effect. It is also likely that every PI3K inhibitor in the clinic will present a subset of unique toxicities, due not only to its PI3K inhibition profile but also to its individual off-target effects.

The second question is whether oncogenic alterations in the PI3K pathway will serve as a guide for patient selection for treatment with PI3K inhibitors. Many preclinical studies indicate that patient selection is possible, with at least one inhibitor going into a breast cancer, which one could speculate was chosen due to its high rate of PI3K mutations (64). However, there seems to be a discord with some studies finding maximal effects of PI3K inhibitors in cell types with mutations in PI3Kα (32), whereas others have found PI3K inhibitors to have maximal effect in lines with an inactive PTEN and modest or unpredictable activity in lines with a mutated PI3Kα (65). Some of this discrepancy may come from the use of two-dimensional cell culture to elucidate sensitivity, as opposed to three-dimensional cell culture or xenograft models, which would serve to more accurately reflect the tumor microenvironment. At least one study has observed discrepancies in sensitivity to *in vitro* effects of PI3K inhibition on cell growth between two- and three-dimensional cell cultures, as well as on cell migration, using a PI3K inhibitor currently in clinical development (66). Furthermore, it is becoming increasingly apparent that additional mutations activating redundant pathways, such as an oncogenic Ras, can confound this analysis of activity (33, 51).

A potential limitation of reversible PI3K inhibitors is that although they display potent activity against purified PI3K enzymes, they are considerably less active against cells, and their *in vivo* administration requires large doses, often multiple times daily, to achieve antitumor efficacy. This may be due to significantly higher levels of ATP with which they have to compete in biological systems than in the enzymatic assays or to cellular binding and metabolism. Thus, a practical question arises whether the large doses will be acceptable to patients on long-term therapy or whether irreversible inhibitors requiring smaller and, perhaps, less frequent dosing will provide a better alternative.

Finally, there remains the question of which current chemotherapies will be best to combine with PI3K inhibitors, once acceptable candidates are identified. PI3K inhibitors have direct antitumor activity through their antiproliferative and antiangiogenic effects (15). Preclinical models have validated that PI3K inhibitors can enhance the effects of conventional cytotoxics and radiation (49, 59). PI3K signaling inhibits apoptosis and stimulates cell survival (11), which can allow cancer cell survival under periods in which the tumor is stressed. Thus, PI3K inhibitors may also have a role in combination therapy by facilitating apoptosis in tumors treated with cytotoxic agents or radiation (67). Whether this will have unacceptable adverse effects on the therapeutic window of these agents remains to be determined and may place limitations on this practice. Additionally, the concept of combining these agents with other targeted agents is proving promising. Resistance to both antibodies and small molecules targeting growth factor receptors has been shown to occur not only through oncogenic Ras, which lies upstream of PI3K and other pathways, but also through direct alterations to the PI3K/Akt pathway itself, both through a suppression of PTEN and an activation of PI3Kα (68, 69). Preclinical data have provided strong evidence that resistance to inhibitors of growth factor receptors can be overcome with PI3K inhibitors. Additionally, because growth factor receptors and oncogenic Ras activate both the PI3K and Raf signaling cascades, in certain circumstances it may be beneficial to combine PI3K inhibitors with inhibitors already in development to various points in the Raf cascade (51). Whereas it is well established that these pathways have redundant functions in cells (70), the increased efficacy may be offset by an increase in undesirable effects that may come with inhibiting these pathways simultaneously.

Current Status and Future Directions

Several inhibitors of PI3K have moved through preclinical studies and into phase I and II clinical trials. These range from inhibitors reported to act on a single class I PI3K such CAL-101; to inhibitors of multiple class I PI3K isoforms such as PX-866, XL-147, and GDC-0941; and to inhibitors acting on multiple class I isoforms and other PIK family members such as BEZ235 and XL765. Efforts to make more selective PI3K inhibitors to various PI3K isoforms have been aided by the recent identification through structural studies of the mechanism of inhibitors already known to be selective (70). Additionally, more detailed analysis of structural differences between the class I PI3K isoforms has recently been published (71). This information should allow for the development of compounds with a larger differential for inhibition of class I isoforms. Structural studies of the common PI3Kα mutations in cancer have also led to the concept that it may be possible to develop inhibitors with an increased selectivity for the mutant forms of the kinase (72), as has been achieved with another mutated kinase, B-Raf (73).

The ultimate answers to these questions will only come with time and more preclinical and clinical experience but will certainly provide insights for discussion and further drug development for some time to come.

Disclosure of Potential Conflicts of Interest

G. Powis: consultant with ownership interest in Oncothyreon. No other potential conflicts of interest were disclosed.

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