Published in final edited form as:

Mol Aspects Med. 2010 April ; 31(2): 135–144. doi:10.1016/j.mam.2010.02.003.

Inhibitors of phosphatidylinositol-3-kinase in cancer therapy

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Abstract

The phosphatidylinositol-3-kinase (PI3K) signaling pathway is implicated in multiple aspects of tumorigenesis and tumor maintenance, and recent years have seen significant efforts towards developing agents to inhibit the pathway. However, the development of such agents raises issues such as what specific member or members in the PI3K family should be inhibited to achieve maximal therapeutic benefit, and can specific inhibitors be developed with the necessary pharmacologic properties to allow them to proceed to clinical trials? The number of PI3K inhibitors has gone from a handful of archetypal inhibitors which largely determined how the pathway was initially defined through their inhibition of PI3K, but also due to their off target properties, to a much larger number of inhibitors of not only PI3K but also other members of the PI3K family. The question remains to be answered whether greater therapeutic efficacy will be obtained through the use of inhibitors with increased specificity, or through inhibitors that target a spectrum of targets within the pathway. This review will cover the development of agents targeting the pathway, and will discuss current issues surrounding the development of such agents.

Keywords

PI3K inhibitors; PI3K; antitumor activity

Oncology and PI3K inhibitors

PI3K plays a large role in the growth and survival of many, perhaps a majority of mammalian cancers (Hennessy et al., 2005)). Early work in the field of PI3K signaling was dominated by a select group of archetypal inhibitors which provided the primary means of manipulating PI3K activity for upwards of ten years. They became the generally accepted means for proving varying functions of the pathway, but they also had questionable specificity and undesirable pharmacologic profiles. The properties of these inhibitors, as well as embryonic lethality seen in PI3K knockout mice, lead to the assumption that inhibition of the pathway would be toxic to humans unless specific components could be selectively targeted. This paradigm was ultimately disproven with the advent of broad spectrum inhibitors and even inhibitors targeting both PI3K and other members family, that can be safely administered to patients.

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The PI3K Family

The PI3K family is divided into three classes. Class I PI3Ks exist as a heterodimer of one of two regulatory subunits and one of four p110 catalytic subunits that act on PI(4,5)P₂ to produce PI(3,4,5)P₃ (Vanhaesebroeck and Waterfield, 1999) in a process that is reversed by the mixed phosphatase PTEN (Parsons, 1998). Class II PI3Ks display the ability to phosphorylate PI and PI-4-P. Class III PI3Ks, whose only member is Vps34, phosphorylate PI to produce PI-3-P. Vps34 has been shown to play an essential role in trafficking of proteins from the Golgi apparatus in yeast (Fruman et al., 1998). Vps34 has additionally been linked to autophagy. There is a fourth class of PI3K related enzymes which contain a catalytic core similar to the PI3Ks. This class includes enzymes involved in signal transduction, such as mTor, and DNA damage response, such as Ataxia telangiectasia mutated (ATM). The Class I PI3Ks are the most implicated in cancer and will be the focus of this review. Class I PI3Ks are subdivided into Class Ia consisting of the α , β and δ catalytic subunits and Class Ib consisting of the γ catalytic subunit. The Class I PI3K's were first identified in a complex co-purifying with p60v-src, polyoma middle T antigen and the PDGF receptor. Activation of Class I PI3Ks under normal physiologic conditions is mediated by ligand activated growth factor receptors such as the insulin-like growth factor receptor (IGFR) and the epidermal growth factor receptor (EGFR). Ligand binding to the receptor results in tyrosine phosphorylation of the Class I PI3Ks and docking of a regulatory subunit SH2 domain leading to activation of the PI3K's lipid kinase activity. This activation may occur by direct binding to the receptor, or through an adaptor protein which links the receptor to PI3K activation. Additionally, active Ras has been shown to activate the p110 α and p110 γ (Domin and Waterfield, 1997). Activation of PI3K results in conversion of PI(4,5)P₂ to PI(3,4,5)P₃ which then recruits proteins containing a pleckstrin homology (PH) domain to the plasma membrane (Toker, 2002). Such proteins include Bruton's tyrosine kinase, a member of the Tec family of non-receptor tyrosine kinases, and the most studied PH domain containing protein, the serine threonine kinase Akt. Akt is recruited to the plasma membrane by PIP3 and phosphorylated by another PH domain containing protein, PDK1, on its threonine 308 site. Akt's serine 473 is phosphorylated by PDK2 whose identity is potentially one of at least ten proteins including DNA-PK and the rictor-mTor complex, with phosphorylation of both sites resulting in Akt activation (Dong and Liu, 2005) (Figure 1). Inhibitors of Akt are being developed that either compete at the ATP binding site or that inhibit PH domain-dependent translocation. Examples of the many targets phosphorylated by activated Akt are AS160 which regulates translocation of Glut-4 to the plasma membrane, thus, impacting glucose uptake, MDM2 a negative regulator of cell growth and survival through interactions with p53, and inhibition of Bad, a promoter of apoptosis (Manning and Cantley, 2007). TSC2 (tuberin) is another widely studied substrate of Akt that when phosphorylated by Akt disassociates from its partner TSC1 (hamartin) leading to its degradation and the conversion of Rheb to a GTP bound state. It is important to note, however, that the TSC complex can be influenced by multiple inputs including growth factor signaling through the MEK-ERK pathway, low energy response through the LKB1- AMPK pathway, and hypoxia through the HIF1 α -REDD1 pathway (Engelman, 2009). The activation of Rheb allows mTor to be activated through association with raptor and other TORC1 complex members, stimulating TOR dependent mRNA translation through p70S6Kinase, and cap-dependent translation thorough inhibition of the eIF4e repressor, 4E-BP (Huang and Manning, 2008). The first inhibitors of this pathway approved for clinical use were rapamycin derivatives, so called rapalogs, which specifically inhibit the raptor-mTor complex (Abraham and Eng, 2008). A limitation of rapalog inhibitors is 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 that may serve to phosphorylate Akt at the serine 473 site. This Akt activation has been seen in both cell models and clinical tumor samples (O'Reilly et al. 2006) and can be abrogated through the use of inhibitors of mTor kinase activity, as opposed to inhibitors of raptor-mTor (Abraham and Eng, 2008).

Aberrant PI3K signaling plays an important role in multiple aspects of tumorigenesis including uncontrolled proliferation, resistance to apoptosis, angiogenesis and metastasis. This aberrant signaling can occur through dysfunction of pathways upstream of the PI3K Class I isoforms, such as mutational activation or overexpression of growth factor receptors, mutant Ras, or activation of the pathway itself (Luo et al., 2003). It has been proposed that the efficacy of inhibition of growth factor receptors can be determined by whether Akt activation is attenuated (Rexer et al. 2009). Loss or inactivation of the tumor suppressor PTEN which occurs at high frequency in multiple tumor types was first mechanism discovered by which the PI3K/Akt pathway is directly activated. Many cellular pathways influence PTEN and new mechanisms by which cancer cells alter PTEN function or expression continue to be found (Sansal and Sellers, 2004). Most recently mutations in the PH domain of Akt1 which causes electrostatic alterations leading to increased binding of the Akt PH domain to PI(3,4,5)P₃ have been found to lead to aberrant activation of the pathway. Thus far, the 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 (Carpten et al., 2007). Additionally mutations in AKT3 have been observed in melanoma lines (Davies et al., 2008). Larger studies to precisely determine the frequency and tumor type specificity of this mutation remain to be conducted.

Class I isoforms and the oncogenic process

The PI3K isoforms have been found to have overlapping and unique roles in physiology and tumor development. Since all four isoforms perform the same function of converting PIP₂ to PIP₃ determining how each isoform might contribute a unique biological activity has been a challenge. Various models have been proposed to explain isoform specific functions such as differential tissue expression, dependence of the membrane concentration of PIP₂, and different downstream effectors (Meier et al., 2004). Activation of all four isoforms has been shown to be capable of inducing transformation in experimental models with PI3K α and δ causing transformation by themselves, while PI3K β and γ require input from Ras (Kang et al., 2006). This suggests that each isoform is capable of regulating multiple overlapping cellular functions (Denley et al., 2008). Both genetic manipulation and pharmacological inhibitors have proven invaluable in understanding the roles individual PI3K isoforms, revealing distinct kinase functions as well as kinase independent functions.

P110 α (PIK3CA)

Early studies revealed that knockout of the PI3K α isoform resulted in embryonic lethality (Bi et al., 1999) which was subsequently determined to be most likely due to deficient migration of endothelial cells resulting in a loss of angiogenic activity (Graupera et al., 2008). A conditional knockout of PI3K α in adult mice resulted in impaired insulin-induced glucose uptake (Foukas et al., 2006) similar to that seen in Akt2 knockout mice (Garofalo et al., 2003). Similar impaired insulin-induced glucose uptake has been seen in cultured muscle cells treated with PI3K α specific inhibitors (Knight et al., 2006). PI3K α has been implicated in cancer cell proliferation and tumor angiogenesis (Yuan et al., 2008) and has been shown to assist in Ras induced transformation and to be necessary for tumor formation in a mouse model of Ras induced oncogenesis (Gupta et al., 2007). More recently, activating mutations in both the helical and kinase domains of PI3K α have been identified, particularly in breast and colon tumors, occurring frequently in similar locations within the protein known as “hotspots”. The most common sites for these hotspots are around amino acid 1047 in the kinase domain, and amino acid 545 in the helical domain (Samuels et al., 2004) that promote PI3K activation through distinct mechanisms. Under normal physiological conditions p85 represses the activation of the p110 kinase domain when the p85 SH2 domain is not in contact with an activated tyrosine receptor. Mutations found at or close to amino acid 545 abrogate this p85 induced repression, allowing PI3K activation independent of upstream activation. In contrast mutations occurring at or near the amino acid 1047 are located near the activation loop and

appear to work through changes in the way p110 α interacts with the membrane (Mandelker et al., 2009). It has recently been shown that helical mutations are not oncogenic without input from Ras, while kinase domain mutants are oncogenic even with their Ras binding domain deleted (Engelman, 2009). Notably, both hot spot mutations are found exclusively in the PI3K α isoform, and mutations induced at the same locations in PI3K β do not having similar effects in stimulating PI3K activity (Zhao et al., 2005). When a colon cancer line, HCT-116, heterozygous for the PI3K α hotspot mutation had its wild type allele deleted, enhanced survival was seen under stress conditions together with increased metastasis (Samuels et al., 2005). In cancer cells with a mutant K-Ras, inhibition of p110 α prevents the formation of tumors, however once these tumors are established, they are able to maintain themselves even when p110 α is inhibited. Furthermore, while tumor xenografts with an independent p110 α mutation display dramatic growth reduction by PI3K inhibition, xenografts with both p110 α mutation and a mutant K-Ras showed resistance to pan PI3K inhibition (Ihle et al., 2009). Discordant results have been found whether over-expression of PI3K α is capable of causing transformation. Some studies have found that PI3K α over-expression is sufficient (Okudela et al., 2007), while others have found that only constitutive activation by membrane localization or activating mutation is capable of causing transformation while over-expression is not (Kang et al., 2006). One study found that constitutive activation by mutation was incapable of transforming cells unless another oncogenic lesion was present (Oda et al., 2005). The reason for these disparate results is unknown but could be due in part to the use of different model systems.

P110 β (PIK3CB)

Mice deficient in the PI3K β isoform show embryonic lethality (Bi et al., 2002) while conditional knockout of PI3K β in adult mice resulted in mice similar to PI3K α knockouts in that they exhibited impaired insulin signaling, although this effect was found to be independent of Akt signaling. Additionally PI3K β knockout mice were found to be deficient in lyophosphatidic acid signaling (Jia et al., 2008). Significantly, the PI3K β isoform has been implicated as necessary for transformation induced by the loss or inactivation of the PTEN tumor suppressor both *in vitro* (Torbett et al., 2008) and *in vivo* (Zhao et al., 2005).

p110 δ (PIK3CD)

The p110 δ isoform is primarily associated with immune system function and knockout of p110 δ gives viable mice displaying deficient antigen receptor signaling in both B and T cells (Okkenhaug et al., 2002). The PI3K δ isoform most prominently expressed in myeloid cells, has been shown to play an essential role in cell proliferation in acute myeloid leukemia (Sujobert et al., 2005). In a different model, p110 δ has been implicated in tumor angiogenesis, particularly in the context of repair after destruction of tumor blood vessels with radiation (Geng et al., 2004).

p110 γ (PIK3CG)

Similar to p110 β , p110 γ can be activated by G coupled protein receptors, and as with p110 α it can be activated by Ras. Mice with a deletion of the p110 γ isoform show decreased thymus size and defective thymocyte survival. p110 γ deficient mice also exhibit an inability to active T cells, but unlike knockout of p110 δ , no effect was seen on B cells. The p110 γ isoform has been found to be utilized by the BCR-ABL fusion oncogene, implicated in chronic myeloid leukemia, for proliferation and drug resistance (Hickey and Cotter, 2006), and is also known to be a Ras effector (Pacold et al., 2000).

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. Furthermore, as isoform specific inhibitors become

available it may be possible to match these inhibitors to specific oncogenic conditions in which isoforms play a specific role. However, the weight of current evidence suggests that redundancy of signaling among the PI3K isoforms may in fact nullify the effects of isoform specific inhibition (Ghaussade et al., 2007).

Early PI3K inhibitors and continued development

LY294002

Quercetin was the first compound discovered which had inhibitory activity against PI3K. (Matter et al., 1992). From this compound LY294002 was developed with increased specificity towards PI3K and an IC_{50} for PI3K in the 1–20 μ M concentration range (Vlahos et al., 1994). This concentration was later found to directly overlap the range necessary to inhibit other members of the PIK family such as mTor and DNA-PK. LY294002 has also been found to inhibit other kinases such as casein kinase 2 and Pim, and to have PI3K independent effects such as the inhibition of calcium signaling. Recent studies looking in more details at the activity of LY294002 both in enzymatic assays and in cells, have shown that its affinity for some targets, such as casein kinase 2, is higher than its affinity for the Class I PI3Ks (Gharbi et al., 2007), leading to the conclusion that its use as a tool to study PI3K signaling should be discontinued (Bain et al., 2007). LY294002's off target effects its non drug like properties including insolubility and a short half life in animals have precluded its use as a therapeutic agent. However, attempts to harness the antiproliferative effects of LY294002 have led to the creation SF1126, consisting of LY294002 linked to a RDGS integrin binding element designed to target the compound to the tumor and tumor vasculature. SF1126 has shown antitumor effects against tumor xenografts (Garlich et al., 2008) and is currently in early clinical development. Additionally, derivatives of LY294002 have been reported to display isoform selectivity among the Class I PI3K enzymes, although this has not been translated to a cellular level (Knight et al., 2004). LY294002 has been the most utilized PI3K inhibitor in laboratory studies and has helped validate pathway inhibition. However, the use of LY294002 also set back the development of PI3K inhibitors because of its associated toxicities resulting from off-target effects which has not been fully appreciated until recently (Gharbi et al., 2007).

Wortmannin

The next PI3K inhibitor identified was wortmannin (Arcaro and Wymann, 2004) which had previously been identified as an inhibitor of myosin light chain kinase (MLK) (Nakanishi et al., 1992). Wortmannin is a member of a class of steroidal furanoids derived from viridin. Structural studies have shown that wortmannin binds in an irreversible fashion through an electrophilic site at the C-20 position of the furan ring directly to the ATP catalytic site of PI3K at lysine 802 (Walker et al., 2000). Modifications negating the electrophilicity in the furan ring render the compound inactive, while minor modifications of other parts of the structure of wortmannin show only modest effects on the *in vitro* efficacy (Norman et al., 1996). Wortmannin inhibits 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-PK at higher concentrations of 250 and 16 nM respectively, and unrelated enzymes such as polo like kinase (PLK) and MLK with IC_{50} 's of 24 nM and 170 nM, respectively (Wipf and Halter, 2005).

The structure of wortmannin has been optimized in attempts to increase its biological stability and to improve its pharmacologic properties such as extended half life and selectively profile. One approach has been to conjugate wortmannin to polyethylene glycol to delay its breakdown in biological systems (Zhu et al., 2006). Modifications of wortmannin through the opening of its furan ring at its active C20 position have yielded compounds which not only extend its half life but also have increased the selectivity for particular PI3K isoforms. PX-866 is an example

of a C-20 modified wortmannin which has been found to have selectivity for the α , δ and χ Class I PI3K isoforms while inhibiting the β isoform at higher concentrations, and showing decreased selectivity for mTor (Ihle et al., 2004, 2005) (Figure 2). PX-866 is the only irreversible PI3K inhibitor currently in clinical trials and has shown to be tolerated in human subjects. Wyeth has synthesized similar wortmannin analogues compounds including WAY-266176 and WAY-266175 which have a modification to the C-20 position in 17-hydroxywortmannin, a related viridin (Yu et al., 2008).

Recent inhibitors

With the discovery that PI3K inhibitors can be tolerated, a number of pharmaceutical companies as well as academic groups have developed inhibitors to PI3K. The result has been compounds with varying specificities for PI3K isoforms and other PIK family members, showing often unique individual selectivity profiles (Knight et al., 2006). However despite a large effort and many compounds generated, only a handful have been found to exhibit pharmacological profiles suitable for advancement beyond preclinical testing and are now in clinical testing

Isoform specific inhibitors

The early paradigm that pan inhibition of the PI3K isoforms would be poorly tolerated in human subjects resulted in extensive efforts to develop inhibitors with specificity towards individual isoforms. Despite these efforts, and reports of compounds displaying specific isoform inhibition, specificity has not been demonstrated to translate to the cellular level, and only one compound has been shown to have isoform selectivity in human subjects. An issue is that while the reported level of selectivity between isoforms occurs at single digit nanomolar concentrations, it is unknown whether specificity is maintained in cells when often several fold higher concentrations of compound have to be used, and even more so in animals when often large doses are employed (Ihle and Powis, 2009). The exception has been the development a specific inhibitor of the PI3K δ isoform, CAL-101, now in early clinical trial for hematological malignancies, and simultaneously for management of allergic response. IC87114, a preclinical precursor, found to inhibit AML proliferation and to augment the effects of traditional chemotherapy. In these studies, proof of selectivity against the p110 δ isoform was established in cells. (Billottet et al., 2006). To date CAL-101 is the only inhibitor in clinical trial that specifically inhibits an individual Class I isoform for oncology applications. Recent structural studies of the common PI3K α mutations in cancer have led to the suggestion that it may be possible to develop inhibitors with an increased selectivity for not only the p110 α isoform but also its mutant forms (Amzel et al., 2008). Such specificity has been achieved with another mutated kinase, B-Raf (Tsai et al., 2008).

Pan inhibitors: Specificity versus broad enzyme inhibition

With studies revealing that PI3K inhibition could be tolerated *in vivo*, a new generation of inhibitors was developed to Class I isoforms. The newly developed inhibitors GDC-0941 and PX-866 are reported to have varying profiles of selectivity for the Class I isoforms. Both compounds have shown antitumor activity in multiple tumor types as well as synergy with both traditional and targeted therapies (Ihle et al., 2005; Friedman et al., 2008). With the new generation of inhibitors also came PI-103, which ushered in a new way of thinking about what constitutes the optimal specificity for PI3K inhibitors. In a study in glioma cells PI-103 was found to inhibit growth and to have activity against both the Class I PI3Ks and the PIK family member mTor (Fan et al., 2007). It was observed that combined inhibition of the Class I PI3Ks and mTor eliminated the increased Akt signaling seen with mTor-ricor inhibitors (Vogt and Kang, 2006). Additionally, PI-103 has been found to exhibit activity against DNA-PK in cells (Knight et al., 2006). While PI-103 showed activity against tumor xenografts and an acceptable

toxicity profile its pharmacological properties were less than ideal and it has not been developed as a clinical candidate.

The concept was subsequently utilized by Novartis and Exelixis in their selection of BEZ235 and XL765 as lead compound PI3K inhibitors, both of which are now in clinical trial. Both compounds have activity against Class I PI3K isoforms and mTor. The companies also simultaneously introduced into clinical trial compounds specific for Class I PI3K isoforms over mTor, Exelixis with XL147 and Novartis with BGT226 (Liu et al., 2009). This strategy likely reflects the uncertainty as to which approach will ultimately prove the most effective. Those compounds which display specificity for the Class I isoforms may have compromised efficacy due to the activation of feedback loops within the PI3K pathway, or due to redundant pathways. On the other hand, activity against mTor may reflect broad spectrum activity against a number of additional PIK family members and unrelated targets producing off target effects which are difficult predicted. Despite these concerns, it should be noted that there are notable examples of other classes of kinase inhibitors which have capitalized on unexpected activity against other targets and have proved useful in certain tumor types (Tuveson et al., 2001).

Current Clinical Considerations

Preclinical models have provided strong evidence that PI3K inhibition holds the promise of a cancer therapy with an acceptable therapeutic index. However, proof-of-principle validation will have to await the results of clinical trials. Practical issues will also have to be addressed. The first is whether the agents are hitting their desired targets in patient's tumors. 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. Second, tumor biopsies necessary to demonstrate target inhibition are often difficult to obtain and great care has to be taken how they are handled since delayed or improper processing may distort important biomarkers of activation, such as phosphorylated proteins (Williams et al., 2006). More easily collected surrogate normal tissues are sometimes used to assess target inhibition. Surrogate normal tissues have already been utilized clinically with the EGFR targeting agents such as erlotinib in multiple tumor types (Baselga, 2003). Preclinical studies utilizing mouse and human hair follicles have shown the PI3K inhibitor, PX-866, causes a significant decrease in phosphorylated Akt (Williams et al., 2006). Phase I studies with the PI-3-K inhibitor XL765 put this concept into practice in patients (Markman et al., 2008). Phase I studies with the irreversible PI3K inhibitor PX-866 have utilized patient peripheral blood mononuclear cells to monitor PI3K inhibition.

The largest concern whether PI3K inhibitors would be tolerable in patients came from the role PI3K plays in signaling between the insulin receptor and glucose uptake. In preclinical models, inhibition of this pathway resulted in a dramatic increase in glucose and insulin levels (Ihle et al., 2005). However, in early clinical evaluation of the inhibitors the only effect manifest has been a rise in insulin levels (Shipiro et al., 2007). This may indicate that in humans redundancy allows for disruption of the PI3K pathway without a serious disruption in glucose homeostasis. Another hypothesis suggests that in humans insulin glucose regulation comes through the liver and because insulin induces levels of PIP3 in the liver, down regulation of the PI3K by these inhibitors may not be sufficient to affect metabolism. Studies have suggested that Akt activation in the liver may not be impaired unless PI3K is inhibited 95% or more (Engelman, 2009). Additionally liver specific p110 α may be may resistant to inhibition perhaps due to its association with p50 α as opposed to p85 α , a complex which has shown resistance to wortmannin (Inukai et al., 1997).

Future Clinical Considerations

With many of the PI3K inhibitors showing target inhibition with acceptable therapeutic indices additional questions will need to be addressed as the compounds move to more advanced testing. One concept that will be tested is whether oncogenic alterations in the PI3K pathway will serve as a guide for patient selection for treatment with PI3K inhibitors. Preclinical studies indicate that patient selection is possible, however there is discord how best to determine the optimal population. Some studies have found maximal effects of PI3K inhibitors in cell types with mutations in PI3K α (Samuels et al., 2005), while 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 PI3K α (Stemke-Hale et al., 2008). Some of the discrepancy may come from differing model systems. Lines cultured in 2-dimensional cell culture has been observed to show different sensitivity to targeted therapies to 3-dimensional cell culture or xenograft models which serve to more accurately reflect the tumor microenvironment. These differences have been noted with PX-866 in sensitivity between *in vitro* effects of PI3K inhibition on cell growth between 2 and 3 dimensional cell culture, as well as on cell migration (Howes et al., 2007).

An additional question is which of the current chemotherapies will be best to combine with PI3K inhibitor. Preclinical models have shown that PI3K inhibitors augment conventional cytotoxics, radiation or other targeted therapies. For cytotoxic chemotherapy or radiation this has been proposed to occur through a block of the anti-apoptotic effects of PI3K or through modulation of the ability of tumors to modulate DNA repair through both Akt dependent and independent pathways (LoPiccolo et al., 2008). Resistance to antibodies and small molecules targeting growth factor receptors has been shown to occur through direct alterations to the PI3K/Akt pathway itself, both through a suppression of PTEN and an activation of PI3K α (Baselga and Rosen, 2008; Bems et al., 2007). Preclinical data from several groups has provided strong evidence that resistance to inhibitors of growth factor receptors can be overcome with PI3K inhibitors in these cases. Additionally growth factor receptors other than those being inhibited, or oncogenic Ras which lies upstream of PI3K, can be a cause of resistance to many conventional and targeted therapies. Growth factor receptors and oncogenic Ras activate both the PI3K and Raf signaling cascades, which indicates it may be beneficial to combine PI3K inhibitors with agents already in development that inhibit various points in the Raf cascade, a concept validated in K-Ras initiated lung tumors in mice. (Yu et al., 2008). While it is well established that these pathways have redundant functions in cells (She et al., 2005), the increased efficacy may be offset by an increase in undesirable side effects as the price to pay for inhibiting these pathways simultaneously, although model organisms provide suggest the combination is tolerable. Additionally, it should be noted that in the case of K-Ras mutants, the combination of PI3K inhibitors with radiation was found to be effective (Kostantinidou et al., 2009).

Conclusion

PI3K signaling clearly plays a role in the growth and survival of many, and perhaps a majority of mammalian cancers. The Class I PI3Ks are most implicated in cancer and PI3K isoforms have been found to have overlapping and unique roles in physiology and tumor development. Since all four isoforms perform the same function of converting PIP2 to PIP3 understanding how each isoform contributes a unique biological activity has been a challenge. The results obtained to date suggest that each isoform is capable of regulating multiple cellular functions but with significant redundancy. Although there was initially a concern from the properties of first generation inhibitors that PI3K inhibition might lead to unacceptable patient toxicity it is now clear from the use of second generation inhibitors that this is not the case. Third generation inhibitors are already beginning to explore the advantages of isoform selectivity. Preclinical

models have provided strong evidence that PI3K inhibition holds the promise of a cancer therapy with an acceptable therapeutic index and proof-of-principle validation is awaited from ongoing clinical trials. The next important questions to be addressed will be how to select those patients whose tumors are most likely to respond to PI3K inhibitor therapy, which biomarkers or biomarker profiles should be used, and then how these new agents perhaps combined with other signaling agents, can be integrated into conventional cytotoxic therapy? This is an exciting time for this new class of signaling inhibitors with early indications of clinical tolerability, and the hope that we will soon be able to move on to important questions of patient selection and integration with other therapies.

List of abbreviations

PI3K	phosphatidylinositol-3-kinase
mTor	mammalian target of rapamycin
ATM	Ataxia telangiectasia mutated
PTEN	phosphatase and tensin homolog
IGFR	insulin-like growth factor receptor
EGFR	epidermal growth factor receptor
PDK	phosphatidylinositol-dependent kinase 1
DNA-PK	DNA-dependent protein kinase
MDM2	murine double minute 2
TSC2	tuberin
TSC1	hamartin
Rheb	Ras homolog enriched in brain
MEK	MAP kinase kinase
ERK	extracellular signal-regulated kinase
AMPK	AMP-activated protein kinase
HIF-1	hypoxia inducible factor-1
REDD1	regulated in development and DNA damage responses
TORC1	TOR complex 1
PIP	Phosphatidylinositol-bisphosphate

Acknowledgments

Supported by NIH grants CA52995, CA17094, CA95060 and CA610159.

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Figure 1. PI3K/Akt signaling in cells
For an explanation see the text.

Pan PI3K Inhibitors in oncology

Reversible

XL147

GDC-0941

Irreversible

PX-866

BKM226: structure not revealed

Pan PI3K/ mTor Inhibitors in oncology

BEZ235

XL765

SF1126

* with targeted modality

Isoform Specific Inhibitors

IC87114
(Cal-101)

Figure 2. Profiles of PI3K inhibitors in clinical trial

The table shows the different PI3K inhibitors currently in clinical trial.