

Review

PI3K and Akt as molecular targets for cancer therapy: current clinical outcomes

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The PI3K-Akt pathway is a vital regulator of cell proliferation and survival. Alterations in the *PIK3CA* gene that lead to enhanced PI3K kinase activity have been reported in many human cancer types, including cancers of the colon, breast, brain, liver, stomach and lung. Deregulation of PI3K causes aberrant Akt activity. Therefore targeting this pathway could have implications for cancer treatment. The first generation PI3K-Akt inhibitors were proven to be highly effective with a low IC_{50} , but later, they were shown to have toxic side effects and poor pharmacological properties and selectivity. Thus, these inhibitors were only effective in preclinical models. However, derivatives of these first generation inhibitors are much more selective and are quite effective in targeting the PI3K-Akt pathway, either alone or in combination. These second-generation inhibitors are essentially a specific chemical moiety that helps to form a strong hydrogen bond interaction with the PI3K/Akt molecule. The goal of this review is to delineate the current efforts that have been undertaken to inhibit the various components of the PI3K and Akt pathway in different types of cancer both *in vitro* and *in vivo*. Our focus here is on these novel therapies and their inhibitory effects that depend upon their chemical nature, as well as their development towards clinical trials.

Keywords: anticancer drug; kinase inhibitors; PI3K; Akt; clinical trials

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Introduction

The phosphoinositol-3-kinase (PI3K) pathway was discovered in human cancers over 20 years ago as an enzymatic activity associated with a viral oncoprotein. This pathway has received much attention in the study of human cancer because it is important for the cell cycle, proliferation, growth, survival, protein synthesis and glucose metabolism^[1]. PI3K pathway deregulation is the driver in approximately 30% of all cancers. This deregulation occurs via various genetic and epigenetic mechanisms and leads to a wide range of tumor types.

PI3K is a heterodimer with p85 regulatory and p110 catalytic subunits. The class IA PI3K consists of a p85 α regulatory subunit and its truncated splice variants p50 α and p55 α , as well as p85 β and p55 γ and one of several p110 catalytic subunits (α , β , or δ)^[2,3]. The regulatory subunits p85 α , p50 α , and p55 α are encoded by the *PIK3R1* gene. Evidence suggests that p85 α is the most abundantly expressed regulatory isoform of PI3K, and p55 α and p50 α are two minor alternative splicing isoforms^[4,5]. The p110 subunit is encoded by the *PIK3CA* gene and has three isoforms: α , β , and δ . The p110 α isoform is the

most common and important subunit in PI3K and the components of p110 include an N-terminal p85-binding domain (p85BD), a RAS binding domain (RBD), a protein-kinase-C homology-2 (C2) domain, a helical domain, and a C-terminal kinase domain. Class IB PI3K comprises p101 regulatory and p110 γ catalytic subunits, which feature similar activity^[6]. These two types of enzymes catalyze the phosphorylation of lipid substrate phosphatidylinositols such as PI(4)P and PI(4,5)P₂ at their D3 position and the resulting product is PI-3,4,5-P₃, which activates downstream signaling pathways through the phosphorylation of several kinases. These kinases include Akt and 3-phosphoinositide-dependent kinase (PDK) and appear to be involved in the regulation of cellular responses varying with the cell types and stimuli studied^[7].

The role of receptors in PI3K deregulation

The class I PI3K is activated by receptor tyrosine kinases (RTK). RTK activation results in the association of PI3K with the receptor through one or two SH2 domains in the adaptor unit binding to phosphotyrosine consensus motifs. Previously published data suggest that allosteric activation of the catalytic subunit of PI3K in this manner leads to PI-3,4,5-P₃ production within a few seconds. The effect of polyphosphoinositide on cells is mediated through specific binding to at least two dis-

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tinct protein-lipid binding domains, such as Fab-1, YGL023, Vps27, and EEA1 domain (FYVE) and pleckstrin homology (PH) domains. Proteins containing the latter domain are critical mediators for PI3K class IA-induced signaling. The protein serine/threonine kinase 30-phosphoinositide-dependent kinase1 (PDK1) and Akt/PKB are both critical for the transforming effects of deregulated PI3K activity. Ligand-dependent activation of protein tyrosine kinase receptors and receptors coupled with either G-proteins or integrins results in PI3K activation. Such activation may also occur independently of the receptor, as is the case in cells expressing constitutively active Ras. PI3K activation can also occur in the presence of some selected receptor families (*eg*, EGF and FGF) in cases where they are missing the consensus sequence of the p85 regulatory subunit, necessary for PI3K activation^[8].

ErbB receptor family and their role in PI3K/Akt activation

Among the surface receptor tyrosine kinases, the epidermal growth factor receptor (ErbB) family can also cause the deregulation of the PI3K pathway. The ErbB receptor family consists of the following four members: ErbB1/Her1, ErbB2/Neu/Her2, ErbB3/Her3, and ErbB4/Her4. ErbB ligand (EGF related peptide growth factor) binding leads to receptor dimerization followed by the activation of an intrinsic tyrosine residue that provides docking sites for PI3K. One unique characteristic of the ErbB receptor family is the ability to form both homodimers and heterodimers. Heterodimer formation leads to signal amplification as well as signal diversification. Interestingly, ErbB2 has the efficiency to form dimers independently of ligand availability, which attributes an important role in tumorigenesis in a large number of breast and ovarian cancers. The synergistic ability of ErbB2 to form dimers with most commonly ErbB3, as well as other ErbB receptors, has clear impact on abnormal cell proliferation as well as tumor progression. It has been shown that ErbB2-ErbB3 dimers are activators of the PI3K-Akt pathway^[9]. Studies in breast cancer cells, primary breast tumors, and transgenic mice all indicate that ErbB2, when overexpressed, is constitutively associated with ErbB3^[10]. ErbB3 possesses seven phosphorylatable tyrosine residues that act as binding sites for the SH2 domains of the p85 regulatory subunit of PI3K. Tumor cells overexpressing ErbB2 shows constitutive Akt activity^[11].

Insulin receptors and their role in PI3K/Akt activation

The insulin receptor (IR) and the type 1 insulin-like growth factor receptor (IGFR) evolved from a single ancestral receptor protein and have 70% homology with respect to protein structure. Both proteins are receptor tyrosine kinase that consist of two half-receptors, each comprising one extracellular alpha-subunit and one transmembrane beta-subunit that possess tyrosine kinase activity^[12].

Current data suggest that insulin resistance has a great impact on cancer progression. Insulin resistance that develops in cancer patients leads to increased levels of circulating insulin combined with IR overexpression. This phenomenon results in abnormal stimulation of the nonmetabolic effects

of IR, such as cell survival, proliferation and migration. IR is activated by insulin, which is generally secreted from the pancreas.

IR has two isoforms: IR-A and IR-B. Although IR-B has a greater specificity and affinity for insulin, it plays a less important role in cancer progression than IR-A. Current evidence suggests that IR-A is overexpressed in many malignancies and signaling through IR-A results in more mitogenic effects than signaling through IR-B, which activates the metabolic signaling pathway. Recently, it was found that IR can activate metabolic pathways independently of IGF binding. The main activation mechanism of IR is via the RAS/Raf/MEK/MAPK pathway. The PI3K pathway is the second most common option for IR activation, which occurs via the activation of Akt and mTOR/p70S6K. After insulin binding to IR and subsequent activation of IRS1, activated PI3K catalyzes the production of PI-3,4,5-P3, which results in the phosphorylation of PDK1 and several downstream enzymes, including Akt and PKC. Based on cellular context, Akt can activate or inhibit several proteins involved in glucose and lipid metabolism (*ie*, GLUT4, PDE3B, Foxa2, GSK3, and AMPK), as well as in cell growth, division, and survival. IR-A has a greater affinity towards the IGFs, especially for IGF2, which is a primary cause of tumorigenesis in many cancer discussed below^[13].

As discussed above, increased levels of insulin in the portal circulatory system cause an increase in the expression of the growth receptor and an increase in IGF-I production.

Earlier evidence showed that higher level of IGF-I has been observed in colorectal, pre-menopausal breast cancer, and prostate cancer. *In vivo* studies using p53-deficient mice and LID mice with colon adenocarcinomas demonstrated that IGF-I deficiency reduces tumor growth. In addition to the growth-promoting effects of insulin and IGF-I, IGF-II overexpression has also been observed to play a role in tumor development. IGF-II is produced in the liver and many other tissues in adult humans. In contrast, in rats IGF-II expression decreases in postnatal life and is only expressed to a significant degree in the adult rat brain. In normal conditions, IGF-II expression is controlled by the paternal chromosome under the control of the differentially methylated region (DMR) associated with the H19 gene located upstream on chromosome 11. A loss of imprinting resulting from the methylation of the DMR on the maternal allele leads to overexpression of IGF-II, which has been observed in many tumor types^[14].

Insulin-like growth factor binding proteins (IGFBPs) play an important role in the stabilization of IGF-I and -II. Among the 6 IGFBP proteins, named IGFBP-1 to IGFBP-6, IGFBP-3 is the most predominant. These binding of proteins increase the circulating half-lives of IGF-I and IGF-II and protect them from further degradation. The main disadvantage of the IGFBPs is that although they increase the stability of IGF, they in turn reduce the availability of IGF for receptor binding^[14].

IGFR consists of two isoforms: IGF-1R and IGF-2R. These two isoforms, along with the insulin receptors, form the hybrid receptors IGF-1R/IR-A and IGF-1R/IR-B. All of the IGF receptors have significant homology, thus resulting in

structural similarity and the possibility of signaling crosstalk. IGF-1R is a tetrameric receptor consisting of two α -subunits and two transmembrane β -subunits that are linked by disulfide bonds. The α -subunits are extracellular and bind IGF. Each transmembrane β -subunit contains an intracellular tyrosine kinase domain. Binding of IGF-I or IGF-II ligand to IGF-1R leads to the phosphorylation of three key tyrosine residues in the kinase domain, leading to the phosphorylation of downstream substrates. In addition, the phosphorylation of additional tyrosine residues in other areas of the β -subunit provide "docking sites" that allow for the recruitment of adaptor proteins. Insulin receptor substrate family members are some of the many adaptor proteins that are known to have an important role in IGF-1R signaling^[15].

Ligand binding to IGF-1R causes its autophosphorylation and the tyrosine phosphorylation of IGF-1R substrates, especially the IR substrate 1 (IRS-1) and the Src and collagen-homology (SHC) protein. Tyrosine-phosphorylated IRS-1 and SHC bind different effector proteins (enzymes and/or adaptors), inducing multiple signaling cascades, including several interconnecting pathways that control cell survival and proliferation. The critical survival pathway is activated by IGF-I stems from IRS-1. IRS-1 recruits and stimulates PI3K due to the presence of the same homology domain, which then transmits the signal to Akt^[12, 15, 16].

PDGF receptors and their role in PI3K/Akt activation

The PDGF family of signaling molecules regulates the growth and motility of connective tissue cells. PDGF is a dimer that consists of disulphide bonded homologous A and B-polypeptide chains. These A and B-polypeptides arrange themselves to form homodimers (PDGF-AA/BB) and heterodimers (PDGF-AB). PDGF isoforms act upon their target cells by binding to two structurally related protein tyrosine kinase receptors. PDGF receptors have two isoforms, α and β . The α -receptor binds both the A- and B-chains of PDGF, whereas the β -receptor binds only the B-chain. Due to these properties, the PDGF-AA and PDGF-BB homodimers typically tend to bind with the PDGF- $\alpha\alpha$ and - $\beta\beta$ receptors, respectively. However, PDGF-AB binds only the β receptors. The α - and β -receptors for PDGF each contain five extracellular immunoglobulin domains and one intracellular tyrosine kinase domain with a characteristic inserted sequence^[17].

The main event of PDGF activation is receptor dimerization. The close interaction between the kinase domains of the receptors causes phosphorylation *in trans* between the receptors, which is called autophosphorylation. The main purpose of this autophosphorylation is to regulate the catalytic activity of the kinase and provide a docking site for further downstream target molecule regulation. It has been observed that the tyrosine residues play the major role in the autophosphorylation process. In the case of the β -receptor, the phosphorylation site is Tyr857, which is located inside the kinase domain. This tyrosine is conserved in the α -receptor (Tyr849) and in almost all other tyrosine kinase receptors. Mutational analysis studies demonstrated that replacing the tyrosine with phe-

nylalanine caused lower kinase activity. The involvement of various domains helps to a great extent in an intracellular system. These include the Src homology 2 (SH2) domains and the phosphotyrosine binding (PTB) domains that recognize phosphorylated tyrosine residues in specific environments, the SH3 domains that recognize proline-rich regions, the pleckstrin homology (PH) domains that recognize membrane phospholipids and the PDZ domains that recognize a C-terminal valine residue^[18]. Current data suggest that more than 10 different SH2-domain-containing molecules tend to bind to different autophosphorylation sites in the PDGF α - and β -receptors. There are two types of molecules that bind to the SH2 domains of PDGF receptors: enzymes that have catalytic activity and adaptor molecules that help to bind other molecules. Phosphatidylinositol 3'-kinase is one such molecule that is activated by PDGF^[19].

PI3K activates PDGF by interacting with the SH2 domain of the p85 subunits. Two tyrosines with a methionine at the +3 position are present in the PDGF β -receptor (*ie*, Tyr740 and Tyr751) in the autophosphorylation sites, which helps to bind PI3K. Both of these tyrosines are conserved in the α -receptor, where they are also important for the binding of PI3K. The p85 subunit of PI3K has been shown to be phosphorylated at Tyr508 after binding to the PDGF β -receptor, but it is not clear if this has any functional relevance. However, the binding of p85 to a phosphorylated region of a protein has been shown to lead to a conformational change and increased enzymatic activity of the associated catalytic subunit^[19].

VEGF and its role in PI3K/Akt activation

Vascular endothelial growth factor (VEGF) is an endothelial cell (EC)-specific mitogen and chemotactic agent that is involved in wound repair, angiogenesis of ischemic tissue, tumor growth, microvascular permeability, vascular protection, and hemostasis. VEGF predominantly known as VEGF-A, belongs to the VEGF-PDGF supergene family. The other family members of the VEGF family include VEGF-B, VEGF-C, VEGF-D, and VEGF-E, which all show varying degrees of homology with VEGF-A. Alternative splicing of VEGF results in four isoforms containing 121, 165, 189, or 206 amino acids. The dominant form of VEGF 165 is a 45 kDa homodimeric glycoprotein that exists in a partly matrix and partly membrane bound form. The VEGF isoforms 189 and 206 are basic and mainly stay in the extracellular matrix. The VEGF 121 isoform is acidic and is secreted^[20].

The VEGF family of proteins binds to three receptor-type tyrosine kinases: Flt-1 (VEGF receptor-1), KDR/Flk-1 (VEGF receptor-2), and VEGFR-3. VEGFR-1 and -2 are normally expressed in vascular ECs, whereas VEGFR-3 is expressed in the lymphatic endothelium. Of the VEGF receptors, KDR/Flk-1 is believed to play the most important role in mediating EC proliferation, migration, and permeability^[21]. VEGFR-1 is able to bind VEGF, VEGF-B, and placental growth factor. VEGFR-2 is activated primarily by VEGF, but proteolytically cleaved forms of VEGF-C and VEGF-D may also activate this receptor. VEGFR-3 is activated only by VEGF-C and

VEGF-D^[22].

From gene targeting studies in mice, it has been proven that VEGF and VEGFR have remarkable effects on the development of the vascular system. *Vegfr2*^{-/-} mice die between embryonic day 8.5 and 9.5 due to defects in the development of hematopoietic and endothelial cells, resulting in impaired vasculogenesis^[23].

The mechanism of VEGF activation involves receptor dimerization followed by autophosphorylation on several tyrosine residues and initiation of intracellular signaling pathways. These pathways are mediated by several effectors, which consist of docking sites composed of the phosphorylated tyrosine residues of the activated receptors. These interactions are mediated by Src homology 2 domains, phosphotyrosine-binding domains and other domains of the signaling proteins. Recent data demonstrate that VEGFR-2 is a major component of the VEGF mediated response in endothelial cells and it plays a critical role in signal transduction in both physiologic and pathologic angiogenesis.

Tyrosine 1175 in human VEGFR-2 has been identified as a major autophosphorylation site following VEGF binding. It serves as a docking site for phospholipase C- γ ^[24], which indirectly mediates the activation of the mitogen activated protein kinase pathway that regulates cell proliferation. After VEGF stimulation, VEGFR-2 is phosphorylated at Tyr1175 and Src associates with the receptor, which results in the binding of the SH2 domain of Shb to the phosphorylated Tyr1175 in the C-terminal tail of VEGFR-2. Src phosphorylates Shb, which allows the subsequent activation of PI3K^[25]. Previous studies demonstrated that Akt is required for the VEGF-mediated survival signal^[26]. Thus, VEGF-mediated signaling acts through the PI3K-Akt pathway^[27].

The role of G-protein coupled receptors in the activation of PI3K class IA and IB

In previous sections of this review, it was discussed that the class I PI3K is activated by RTKs. However, recent data indicate that most class I PI3K subunits might be activated by G protein-coupled receptors (GPCRs), either directly through G $\beta\gamma$ protein subunits (in the case of p110 β and p110 γ) or indirectly, for example through Ras. The class IB regulatory subunits (p84 and p101) do not have SH2 domains and they link the single class IB PI3K catalytic subunit (p110 γ) to GPCRs.

GPCRs are a superfamily of seven transmembrane spanning proteins that consist of G α and G $\beta\gamma$ subunits. The activation of GPCRs involves the coupling of agonist-activated GPCRs to a wide variety of effector systems via their interaction with heterotrimeric guanine nucleotide binding proteins (G proteins). The binding of an agonist to a GPCR selects for a receptor conformation state that promotes the exchange of GDP for GTP on the G protein α -subunit, which is presumed to allow the dissociation of the G protein G α - and G $\beta\gamma$ -subunits. Subsequently, the activated G α - and G $\beta\gamma$ -subunits can either positively or negatively regulate the activities of effector enzymes and ion channels^[28].

Earlier it was discussed that class IB consists of p101 regula-

tory and p110 γ catalytic subunits. Due to the absence of the p85 subunit, the p110 γ catalytic subunit of the class IB PI3K does not form a complex with p85 but is instead activated directly by G $\beta\gamma$ ($\beta\gamma$ subunits of GTP-binding protein). The tightly associated regulatory p101 subunit is not essential for G $\beta\gamma$ -induced activation of p110 γ but rather plays a role in recruiting p110 γ to the plasma membrane and determining substrate specificity. The non-catalytic p101 subunit is also known to determine the substrate specificity of p110 γ . The molecular mechanisms of the G $\beta\gamma$ -induced activation of p110 γ have been shown to involve the direct interaction of G $\beta\gamma$ with both the NH₂- and COOH-terminal of p110 γ ^[29]. These data suggest that the major isoform of PI3K that is transmitting the signal from GPCRs is p110 γ . On the other hand, data suggest that GPCRs cause the activation of class IA PI3K in many cell lines, including hematopoietic cells. One of the possible mechanisms for this activation is that p110 β , one of the catalytic subunits of class IA PI3Ks, is activated by G $\beta\gamma$ in cell-free systems. Other members of the class IA PI3Ks, p110 α and p110 δ , show no sensitivity to G $\beta\gamma$. However, the structural basis for the G $\beta\gamma$ sensitivity of p110 β has not been examined^[30].

PI3K deregulation by mutational approach

Deregulation of PI3K is most commonly due to the amplification of the *PIK3CA* gene. It has also been suggested that mutations in the *PIK3R1* gene, which encodes the p85 subunit, lead to the activation of p110^[29]. Activating somatic mutations in the p85 α regulatory subunit of PI3K (PIK3R1) are prevalent in primary ovarian, colon, and breast cancer^[31]. Interestingly, more than 80% of the mutations in the *PIK3CA* cluster have been found in two small conserved regions within the helical and kinase domains^[32]. These include three hot spots that result in single amino acid substitutions. The first two hotspots are in exon eight and lead to E545K and E542K mutations in the helical domain. The third hotspot is located in exon twenty and produces a H1047R mutation in the kinase domain by replacing arginine. These first two mutations act cooperatively and relieve the inhibitory effect of p85 on p110 α , and the third mutation allows for easier access to p110 α in the membrane. Therefore, these mutations lead to increased kinase activity. The non-random distribution of these changes in highly conserved regions results in a series of cellular responses, including increased enzymatic function, enhanced downstream signaling elements, including Akt, and enhanced oncogenic transformation^[33,34].

Although *PIK3CA* mutations and ErbB overexpression are critical drivers of tumor development, mutations in the PTEN lipid phosphatase are also found to be major causes for PI3K activation in human cancer. The main substrate of PTEN is PIP3. PTEN dephosphorylates PIP3 at the D3 position and acts as a negative regulator of PI3K signaling. In addition, PTEN also acts as tumor suppressor gene. Mutation of PTEN leads to the formation of sporadic cancers, including glioblastoma, endometrial carcinoma, melanoma and bladder, lung and lymphatic cancer^[35].

The mechanisms of Akt activation, deregulation and oncogenesis

Akt is one of the downstream targets of PI3K. It is a serine/threonine kinase that is activated due to the formation of PIP3 by PI3K. PIP3 binds the PH domains of Akt and aids in the recruitment of Akt to the plasma membrane, which alters the conformation of Akt to allow for subsequent phosphorylation by PDK1^[36]. 3-Phosphoinositides help to increase the phosphorylation of this residue to achieve Akt activation. Akt is activated through the phosphorylation of Thr308, but phosphorylation of the Ser473 residue located at the hydrophobic C-terminal region is also required for full activation of the kinase^[8]. Phosphorylation of Thr308 helps to increase the activation of Akt approximately 100-fold. However, full activation requires the phosphorylation of the Ser473 residue. A different hypothesis regarding Ser473 activation has been proposed for decades. However, recent data have shown that the kinases that aid in this activation are members of the PIKK (PI3 kinase-related kinase) superfamily (mammalian target of rapamycin complex 2 (mTORC2) and DNA-PK)^[37].

mTORC2 is composed of mTOR, rictor, mSIN1, and mLST8. The most important function of mTORC2 is the phosphorylation of Akt on Ser473. Studies suggest that targeting TSC2 deficient cells with a myristoylation sequence (myr-Akt) that is independent of the PI3K pathway and *Sin1*^{-/-} MEFs, which completely lack mTORC2 activity, show a higher level of Ser473 phosphorylation. From this study, it can be concluded that recruiting Akt to the plasma membrane through either increased PI3K activity or membrane-targeting sequences is sufficient to stimulate phosphorylation at Ser473 under conditions of low or even no mTORC2 activity^[38].

The contribution of Ser473 phosphorylation in the regulation of Thr308 phosphorylation, Akt activity, and phosphorylation of downstream substrates is not yet fully understood. Initial studies using alanine mutants shows that Akt Thr308 and Ser473 can be phosphorylated independent of each other. Recent evidences confirm that mice deficient in the mTORC2 components rictor, mSIN1 or mLST8 have selective inhibition of Ser473 phosphorylation, leaving the majority of Thr308 phosphorylation intact. In contrast, RNA interference of rictor, which disrupts the mTORC2 complex, and small molecule inhibitors of mTOR decrease both Ser473 and Thr308 phosphorylation, suggesting that Ser473 phosphorylation can facilitate Thr308 phosphorylation^[39].

Akt activity is controlled by several molecules that help to dephosphorylate Akt. Among these negative regulators, PP2A (protein phosphatase 2A) regulates Akt activity by inducing Akt Thr308 dephosphorylation. The PH domain leucine-rich repeat protein phosphatase (PHLPP) suppresses Akt activity by dephosphorylating Akt at Ser473. FKBP51 (FK506 binding protein 51) helps maintain the interaction between PHLPP and Akt, serves as a scaffold for Akt and PHLPP and negatively regulates Akt phosphorylation at Ser473. Akt regulates itself like an autocrine system because its activity is controlled by phosphorylation and dephosphorylation cycles within the Akt molecules. Akt phosphorylation and activation is also

regulated by promyelocytic leukemia protein (PML) tumor suppressor. PML forms a complex with PP2A and Akt and facilitates PP2A-mediated Akt dephosphorylation at Thr308. PML cooperates with the PTEN tumor suppressor to restrict prostate cancer development in an animal model of the disease^[40].

Akt activation due to cellular stress

Akt activation often occurs due to the cellular stress response, which is independent of the PI3K pathway. Interestingly, each Akt subtype responds to different stimuli because the response of Akt to cellular stress differs slightly among the family. Stimulation of cells with H₂O₂, CdCl₂, and NaAsO₂ activates different Akt subtypes that show distinct responses. This cellular stress-mediated Akt activation occurs due to the association of Akt with Hsp27. By binding Akt, Hsp27 helps to alter the conformation of Akt, which is required for phosphorylation^[41]. Recent data have shown that oxidative stress induced Akt activation, which leads to apoptosis through the increased expression of FOXO3a. This increased FOXO3a expression in turn increases the expression of its transcriptional targets Bim and p27kip1^[42].

Deregulation of the Akt pathway occurs due to the genetic amplification of the *Akt* gene. The three isoforms of Akt that have been identified are Akt1, Akt2, and Akt3. These three isoforms are the protein products of the *Akt1*, *Akt2*, and *Akt3* genes, respectively. It has been found that ovarian, breast, pancreatic, and gastric cancer can be caused by Akt gene amplification^[43, 44]. Akt activates a cascade of reactions through the phosphorylation of several molecular targets. Akt activates mTORC-1, a complex also modulated by ERK, which leads to the stimulation of protein synthesis and cell growth by regulating both the ribosomal p70S6 kinase (S6K) and the eukaryotic translation initiation factor 4E (eIF4E)^[45]. Akt regulates cell cycle progression and events associated with them by modulating cell cycle inhibitors, such as p21, p27kip1, and GSK3, and cell cycle stimulators, such as c-myc and cyclin D1, by phosphorylation^[36]. Akt also regulates apoptosis by inhibiting the translocation of the forkhead family of proteins from the cytoplasm to the nucleus. Inhibiting this translocation blocks transcription of the death genes FASL and BIM^[46, 47], increases the transcription of survival genes by NF-κB and CREB transcription factor activation^[48, 49], phosphorylates and inactivates the proapoptotic protein BAD^[50, 51], and maintains mitochondrial integrity by activating hexokinase (Figure 1). Akt regulates many cellular processes, including differentiation, proliferation and transformation by phosphorylating GSK-3^[52, 53]. Through the upregulation of the transcription repressor Snail, Akt modulates the induction of the epithelial-mesenchymal transition and tumor cell invasion. Akt/mTORC1 induces the expression of HIF-1 and VEGF, which are key elements involved in angiogenesis. Feedback inhibition mechanisms have also been identified in this complex pathway. One of the most notable examples that has clinical relevance is the ability of S6K to phosphorylate and inhibit IRS-1, an upstream member of the PI3K/Akt pathway^[54].

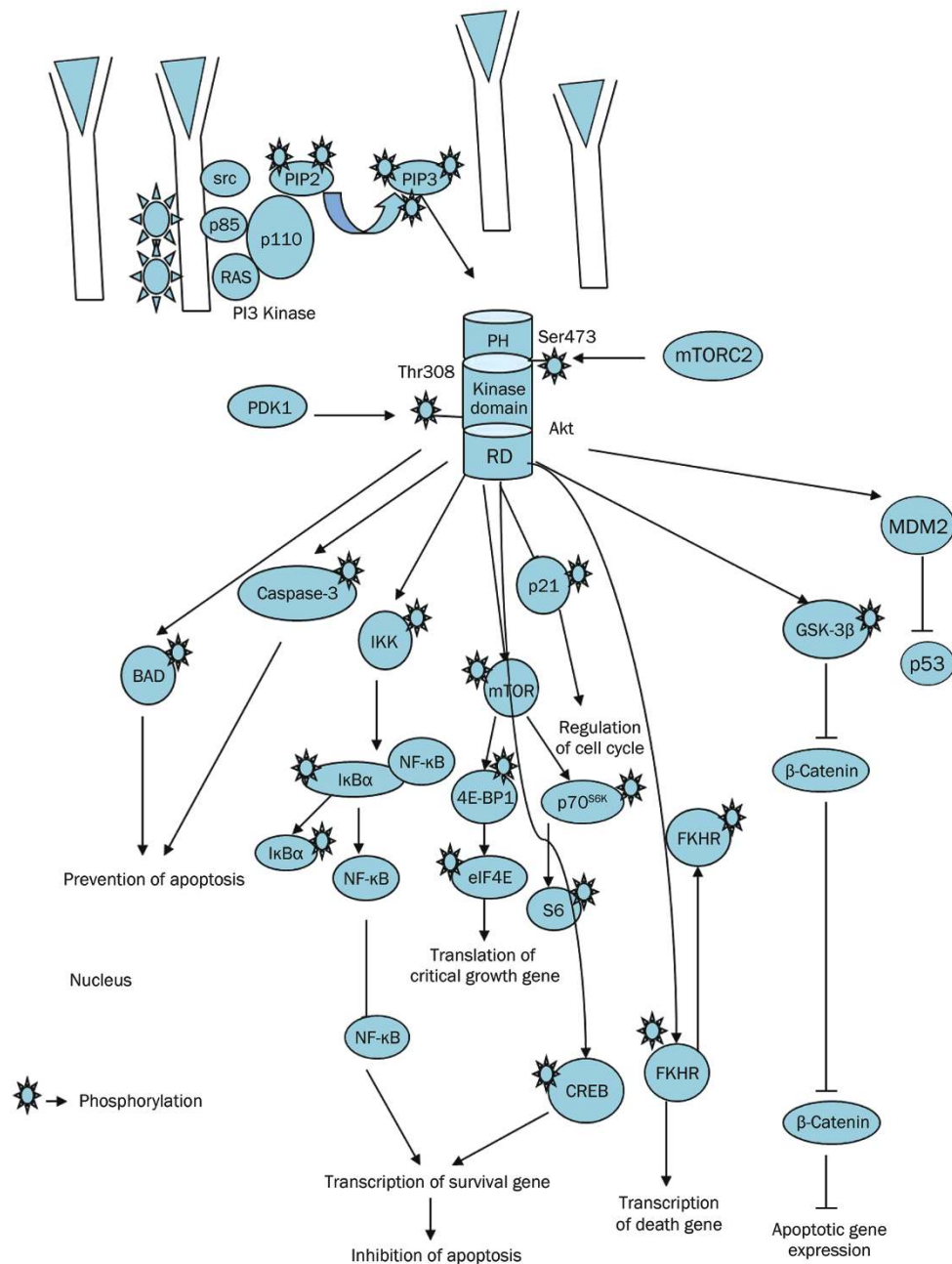


Figure 1. Schematic representation of PI3K-AKT pathway and associated cellular activities. PDK2 is shown as mTORC2. Continuous lines represent direct AKT phosphorylation, leading to activation (arrow end) or inhibition (blunt end). PI3K kinase consists of p110 catalytic and p85 regulatory subunits. PI3K is activated by receptor tyrosine kinases. Once, activated PI3K converts phosphatidylinositol 3,4-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 helps to recruit PH domain binding protein like Akt to cell membrane and thus activates Akt. The recruitment of Akt to cell membrane leads to the conformational changes in Akt and helps PDK1 for phosphorylation at Thr308 residue. The complete activation of Akt requires phosphorylation of AKT at the hydrophobic C-terminal domain Ser473 residue. This phosphorylated AKT regulates apoptosis and various cellular activities by phosphorylating various downstream substrates.

PI3K inhibitors

The PI3K inhibitor LY294002 and wortmannin are first generation pan-PI3K inhibitors that act as ATP competitive manner. Wortmannin inhibits PI3K activity by binding covalently to a conserved lysine residue in the ATP binding site of the enzyme^[55]. LY294002 is a flavonoid derivative and a revers-

ible, ATP-competitive inhibitor that has an IC_{50} for recombinant PI3K in the low micromolar range^[56]. Wortmannin and LY294002 both have anti-proliferative and apoptotic effects *in vitro* and *in vivo*^[56-58]. Wortmannin and LY294002 are effective inhibitors of PI3K, but the use of these two compounds is limited to the preclinical level due to their instability in aque-

ous solutions, toxic side effects, poor pharmaceutical properties, and lack of selectivity for the oncogenic class I PI3K isoforms^[59] (Table 1).

To overcome these pharmacological problems, including poor solubility and lack of selectivity, LY294002 was recently conjugated with an RGD-containing peptide. This conjugation of LY294002 to RGDs results in increased solubility and binding to specific integrins (primarily $\alpha v\beta 3$ and $\alpha 5\beta 1$) in the tumor compartment, which helps to target stromal endothelial and tumor cells, depending upon effect of LY294002 on the microenvironment. The anticancer effects of SF1126 (Figure 2) have been observed in a wide variety of *in vitro* and *in vivo* systems, including renal cell carcinoma, glioblastoma, neuroblastoma, and rhabdomyosarcoma, as well as in breast and prostate cancer xenografts^[60]. Recent studies have found that SF1126, in combination with other chemotherapeutic agents,

has a more profound anticancer effect. SF1126 in combination with trastuzumab synergistically inhibits the growth of HER2-overexpressing breast cancers, including those that are trastuzumab resistant^[61]. SF1126, either alone or in combination with anthracyclines, has profound anticancer effects in neuroblastoma and tumors with a highly activated PI3K-Akt-MDM2 pathway (Table 2). SF1126 inhibits tumor growth by blocking the production of PIP2 and PIP3. Ultimately, SF1126 prevents the conversion of inactive Rac1-GDP to active Rac1-GTP, which deregulates actin cytoskeleton polymerization^[62].

Achievements in clinical trials

SF1126 has recently entered phase I clinical studies. The current phase I clinical trial of SF1126 in adults is at maximum dose 1, 110 mg/m² per dose (40 mg/kg per dose) and shows minimal toxicity. SF1126 is in development in multiple phase

Table 1. Chemical nature, mode of action and clinical stage of different PI3K inhibitors.

PI3K inhibitor	Chemical nature	Target	Clinical trial	Reference
LY294002	Flavonoid derivative	Pan PI3K	-	[57]
Wortmanin	Furansteroid	PI3K, Polo like kinase	-	[171]
SF1126	Morpholino derivative	Pan PI3K, mTOR, DNA-PK	I	[61]
PX-866	Viridian derivative	PI3K	II	Clinicaltrials.gov
GDC-0941	Morpholino derivative	Pan PI3K, mTOR	II	[67]
GDC-0980	Morpholino derivative	PI3K (p110 α), mTOR	II	[74]
PKI-402	Morpholino derivative with fused pyrimidines	Pan PI3K, mutant PI3K, mTOR	I	[76]
PKI-587	Morpholino derivative with fused pyrimidines	Pan PI3K, mTOR	I	[78]
NVP-BKM120	Bismorpholino derivative	Pan PI3K, VPS34, mTOR, DNA-PK, and PI4K	I	[80]
BGT-226	Structure undisclosed	PI3K, mTOR	I/II	[96]
NVP-BEZ235	Imidazoquinazoline derivative	Pan PI3K, mTOR	I/II	[84]
XL147	Structure undisclosed	Class-I PI3K	I	[98]
XL765	Structure undisclosed	Class-I PI3K, DNAPK, mTOR	II	[98]

Table 2. Combinatorial effects of PI3K inhibitors on several cancers.

PI3K inhibitors	Combination agents	Tumor types	Reference
SF1126	Trastuzumab	HER-2 overexpressing breast cancer	[60]
	Doxorubicin	Neuroblastoma	[61]
GDC-0941	Trastuzumab and pertuzumab	Her-2 specific breast cancer	[69]
	Doxorubicin	Breast and ovarian cancer	[70]
NVP-BEZ235	AZD6244	Melanoma	[82]
	Gemcitabine	Pancreatic ductal adenocarcinoma	[172]
	Sorafenib	Renal cell carcinoma	[173]
	RAD001	Non-small cell lung cancer	[174]
		Neuroendocrine tumors	[175]
	GSK2126458	Breast cancer	[176]
	Taxotere	Prostate cancer	[177]
	PD0325901	Glioma	[178]
	Vincristine	Sarcoma	[92]
XL147	Erlotinib	Solid tumors	Bioportfolio.com
	Trastuzumab or paclitaxel	Metastatic breast cancer	Exelixis.com
XL765	Temozolomide	Glioma	Exelixis.com
	Erlotinib	Solid tumor	

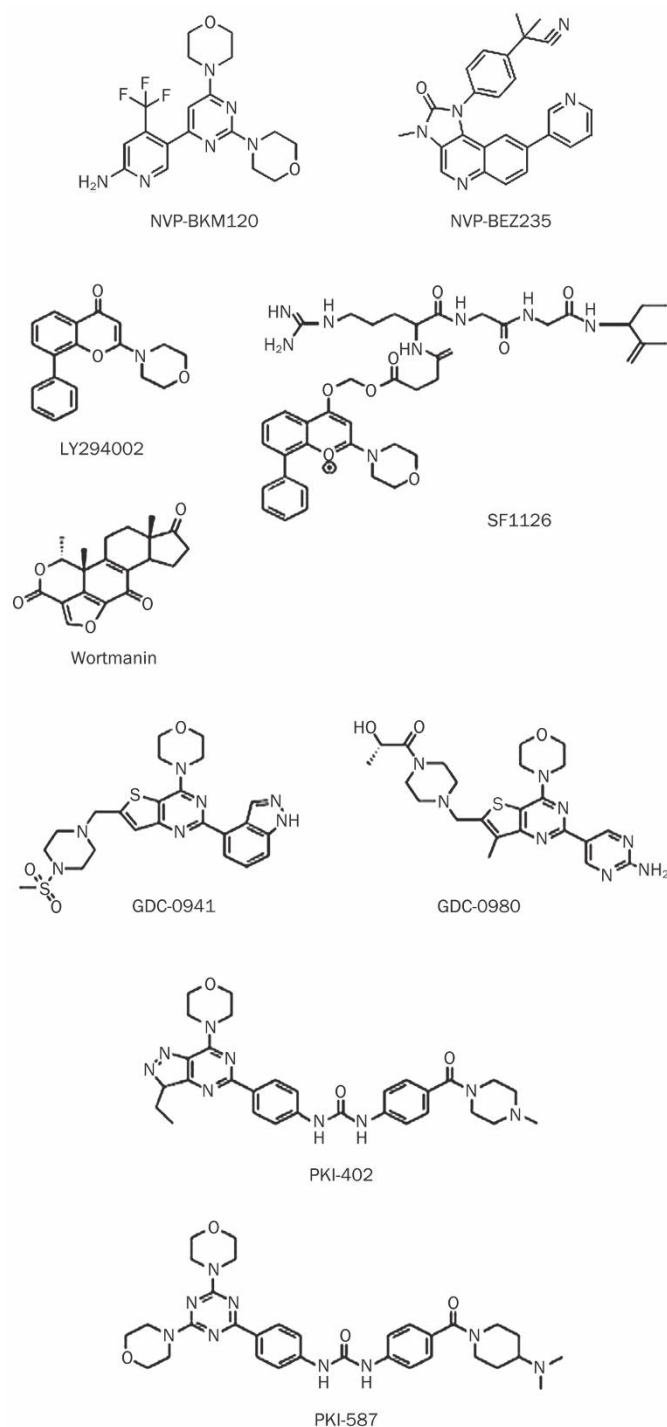


Figure 2. Chemical structure of the PI3K inhibitors.

I clinical trials as a single agent, and interim results have been presented recently from patients with solid tumors^[62] and multiple myelomas^[63]. In an ongoing phase I dose escalation study in patients with solid tumors, SF1126 is administered weekly on days 1, 4 weekly by 90-min IV infusions in cycles of 4-week cycles. To date, 18 of 39 (46%) of dosed patients showed stable disease as the best response, with a median duration of 13

weeks (range 8 to 64+ weeks) and a mean duration of approximately 19 weeks. SF1126 is well tolerated, with the most common grade 1 adverse events being nausea, vomiting, diarrhea, fever, fatigue, chills, and pruritus. SF1126 is dual PI3K/mTOR inhibitor. However, SF1126 exerts its effects mainly as a PI3K inhibitor^[62].

Previous findings suggest that the morpholino group on the related quinazoline template of LY294002 was critical for the p110 α inhibitory activity of LY294002. It was found that this morpholine oxygen helps in the hydrogen bonding interaction in the ATP pocket of both class of PI3K and mTOR^[59]. Depending upon this phenomenon various analogs of LY294002 were discovered which interacts with PI3K and mTOR in a very similar manner^[63].

PX-866 is a semi-synthetic viridian derivative that potently and irreversibly inhibits PI3K α , γ , and δ (IC_{50} = 5.5, 9.0, and 2.7 nmol/L, respectively). PX-866 shows both increased selectivity towards PI3K β (IC_{50} > 300 nmol/L) and improved stability and reduced hepatotoxicity, compared to wortmannin. The compound has also demonstrated antitumor activity in several human solid tumor models (eg, OvCar-3, HT-29, and A-549) when used alone (2 mg/kg, *po*) or in combination with chemotherapeutic or targeted anticancer agents (eg, paclitaxel or gefitinib)^[64]. PX-866 has also exhibited inhibitory effects on human glioblastoma by inducing autophagy and exploiting the aberrant PTEN/PI3K expression^[65]. One of the major side effects of PX-866 is hyperglycemia. The data demonstrate that administration of PX-866 to SCID mice causes hyperglycemia with a decreased glucose tolerance. Recent findings have shown ways to circumvent this problem. Pioglitazone, which is a peroxisome proliferator-activated receptor gamma agonist, prevents the hyperglycemia caused by PX-866 without affecting its antitumor activity^[66]. PX-866 had entered phase I clinical trials in 2008 and is currently in phase II clinical trials for prostate cancer treatment.

GDC-0941 is a morpholino derivative (Figure 2) that is a potent, selective, and orally available Pan-PI3K and dual pan-PI3K/mTOR inhibitor, which has recently entered into phase I clinical trials. GDC-0941 has a prominent inhibitory effect against all four isoforms of PI3K, but it is the most effective against p110 α and p110 δ . GDC-0941 has selectivity against 228 kinases of the PI3K family. However, greater than 50% inhibition occurs in the case of only two enzymes, Flt3 (59% inhibition) and the human kinase TrkA (61% inhibition)^[67]. It has been previously shown that GDC-0941 has inhibitory effects on U87MG, PC3, SKOV-3, IGROV-1, Detroit 562, HCT116, SNUC2B, and LoVo cell lines with very low IC_{50} values. GDC-0941 also inhibits U87MG glioblastoma and IGROV-1 human ovarian cancer xenograft models in athymic mice^[68]. GDC-0941 either alone or in combination with trastuzumab and pertuzumab inhibits HER-2 specific breast cancer effectively *in vitro* and *in vivo*^[69] (Table 2). In combination with DNA damaging agents, GDC-0941 has inhibitory effects on breast and ovarian cancer cells^[70] (Table 2). The drug also shows remarkable inhibitory effects on B-cell lymphomas in PTEN/LKB1 deficient mice. GDC-0941 suppresses the

phosphorylation of Akt at Ser473 and Thr308, as well as the phosphorylation of PRAS 40 and ribosomal S6 kinase, which resides in the downstream of the PI3K pathway. However, GDC-0941 does not have a significant effect on the phosphorylation of 4E-Bp1. It is still unclear whether PTEN status affects the activity GDC-0941 in B-cell lymphomas^[71]. Recently, it has been shown that the metastatic abilities of thyroid cancer cells are suppressed by GDC-0941. The mechanism of this inhibition is solely dependent upon the hypoxia-inducible factor-1 α (HIF-1 α). It has also been suggested that the anti-proliferative effects of GDC-0941 depend on PTEN status in thyroid cancer^[72], but the mechanism is still unclear. Thus, the role of PTEN status in the effectiveness of GDC-0941 is distinct for different cancer types. Further investigations are required to reach definitive conclusions regarding the effect of PTEN status on GDC-0941 efficacy.

Another morpholino derivative that acts as dual PI3K/mTOR inhibitor and is going through phase II clinical trials is GDC-0980 (Figure 2). GDC-0980 is synthesized by substituting the indazole in GDC-0941 for a 2-aminopyrimidine^[73]. This substitution also enhances the efficacy of mTOR inhibition. GDC-0980 has a very low IC₅₀ (4.8 nmol/L for p110 α /p85). The drug shows great potency against a wide array of cell lines, including breast, prostate, and lung cancers; however, it demonstrates less activity in melanoma and pancreatic cancers, which is consistent with KRAS and BRAF acting as resistance markers in these tumors^[74]. GDC-0980 shows inhibitory effects against human xenograft models, including PC3 and MCF-7 neo/HER2 xenografts and xenografts with activated PI3K, loss of LKB1 or PTEN. The drug also elicited an exposure-related decrease in PD biomarkers^[74].

Current progress in clinical trials

In phase I trials, to date it has been found that twelve patients have been enrolled in four successive dose-escalation cohorts with daily dose levels of 2, 4, 8, and 16 mg of GDC-0980. The most frequently reported adverse events were nausea (25%), fatigue (50%), diarrhea (42%), and flatulence (25%). No drug-related grade 3 or higher events or DLT have been reported to date. Preliminary PK data revealed dose-proportional increases in C_{max} and AUC. Preliminary PD data show >50% inhibition of pAkt levels assayed in PRP (relative to baseline) following a single dose of 8 mg or higher GDC-0980. Potential signs of antitumor activity have been observed in one mesothelioma patient (previously treated with radiation and cisplatin/pemetrexed). This patient had a decrease in target lesions of ~32%, as measured by RECIST after one cycle of 8 mg GDC-0980 QD. A soft-tissue-sarcoma patient also had a decrease in tumor mean (SUV_{max} of ~31%), as assessed by FDG-PET after 2 weeks of 16 mg GDC-0980 QD^[75]. GDC-0941 and GDC-0980 will enter into phase 2 clinical trials with advanced and metastatic breast cancer patients (www.clinicaltrials.gov).

It has previously been reported that several morpholine group bearing fused pyrimidines, such as imidazolopyrimidines, pyrrolopyrimidines and triazolopyrimidines, act as PI3K/mTOR dual inhibitors. PKI-402 and PKI-587 are new

additions to this group (Figure 2). PKI-402 is another dual PI3K/mTOR inhibitor that behaves in an ATP competitive manner. PKI-402 exerts inhibitory effects against all of the isoforms of PI3K, including the E545K and H1047R PI3K- α mutants and mTOR. Analyses of the inhibitory effects of PKI-402 against a panel of 236 human kinases showed a highly selective profile. However, only C-Raf and B-Raf (wild-type and V600E mutant) were inhibited by PKI-402 with a low IC₅₀ value. PKI-402 has exhibited growth inhibition *in vitro* and *in vivo* against breast, brain, pancreas, and non-small cell lung cancer (NSCLC) cells^[76]. The mechanism behind the inhibitory effects of PKI-402 was investigated in MDA-MB-361 cells. It was found out that PKI-402 inhibits MDA-MB-361 cell line growth *in vitro* and *in vivo* by inhibiting the phosphorylation of Akt at Ser473 and Thr308, as well as by inhibiting the downstream target molecules of Akt phosphorylation. PKI-402 induces apoptosis in MDA-MB-361 cells by a caspase-3 dependent pathway^[77].

PKI-587 has a similar *in vitro* and *in vivo* profile to PKI-402. PKI-587 has broad *in vivo* efficacy in the MDA-MB-361, BT474, H1975, U87MG, and HCT116 tumor models^[78]. PKI-587 has shown great potency against EGFR- and HER2-inhibitor-resistant lung tumors. PKI-587 is less effective in HCT116 colon cancers bearing K-Ras and *PIK3CA* mutations. When combined with the MEK inhibitor PD0325901, PKI-587 shows enhanced efficacy in HCT116 cells *in vitro* and *in vivo*^[79].

NVP-BKM120, a bismorpholino derivative, inhibits a range of PI3K deregulated cell lines from a variety of tumor types, including glioblastoma and ovarian, breast, and prostate cancer. NVP-BKM120 inhibits all four isoforms of PI3K with the following low IC₅₀ values: 0.052 μ mol/L (p110 α), 0.166 μ mol/L (p110 β), 0.116 μ mol/L (p110 δ) and 0.262 nmol/L (p110 γ). NVP-BKM120 has lower potency against class III and class IV PI3K. The biochemical activity of NVP-BKM120 was observed for the inhibition of VPS34, mTOR, DNAPK, and PI4K. The drug exerts antitumor activity against two models of PI3K/Akt pathway driven cancers: the A2780 ovarian carcinoma model and the U87MG glioma model, which carry a PTEN deletion^[80]. It has also been observed that the inhibition of glioma cells by NVP-BKM120 depends upon the mutational status of p53. Wild-type p53 cells are more sensitive towards apoptosis than cells with mutated or deleted p53, which undergo mitotic catastrophe cell death^[81]. NVP-BKM120 also enhances the efficacy of other antitumor agents in *in vitro* combination studies (Figure 2)^[82].

Another dual PI3K/mTOR inhibitor NVP-BEZ235, which is currently in phase I/II clinical trials, is the most effective small molecule inhibitor of PI3K to date. NVP-BEZ235 has an imidazoquinazoline moiety (Figure 2) that helps to mimic the H-bond interactions between the adenine moiety of ATP and the hinge region using several binding modes, which inhibits the binding of ATP^[83]. NVP-BEZ235 is a pan-PI3K inhibitor that acts against all of the isoforms of PI3K and PI3K mutants. Previous studies have demonstrated the efficacy of NVP-BEZ235 as an antitumor agent *in vitro* and *in vivo* in glioblastoma, multiple myeloma, melanoma, lymphoma,

sarcoma, breast, lung, and ovarian cancer models^[84-93]. In the case of colon cancer, NVP-BEZ23 decreases cellular proliferation and causes sustained inhibition of mTORC1 and mTORC2 with a transient PI3K blockade with no subsequent effect on apoptosis either *in vitro* or *in vivo* in a GEM model. The effect of NVP-BEZ235 does not depend on the mutational status of PIK3CA in *in vitro* CRC cell lines. The GEM model also suggests that treatment with NVP-BEZ23 causes tumor regression and a decrease in tumor angiogenesis^[94]. NVP-BEZ235 shows remarkable efficacy in a wide array of cancer types in combination with several chemotherapeutic agents (Table 2).

BGT226, a dual PI3K/mTOR inhibitor, is currently in phase I/II clinical trials for solid tumor, breast cancer, and Cowden syndrome patients (www.clinicaltrials.gov). It has shown efficacy in inhibiting estrogen-deprived ER positive breast cancer cell lines, multiple myelomas and head and neck cancers^[95-97]. The modes of inhibition by BGT226 vary in different types of cancer cell lines. BGT226 inhibits breast cancer by an apoptosis dependent manner which is solely dependent upon the PIK3CA mutational status^[95]. But, on the contrary, BGT226 inhibits head and neck cancer in an apoptosis-independent manner. Instead of apoptosis, BGT226 induces autophagy, which is regulated by upregulation of the microtubule-associated protein light chain 3B-II and p62 degradation^[97].

XL-147 (structure not disclosed) is a low-molecular-weight, potent, orally bioavailable PI3K inhibitor that recently went into phase II clinical trials for advanced or recurrent endometrial cancer (www.clinicaltrials.gov). XL-147 generally inhibits the class I PI3K family of lipid kinases with a nanomolar range of IC₅₀ values^[98]. XL-147 is active against class I PI3Ks (IC₅₀=39, 383, 23, and 36 nmol/L for p110 α , β , γ , and δ , respectively) without inhibiting the kinase activity of Vps34 (IC₅₀=6975 nmol/L), DNA-PK, or mTOR (IC₅₀>15 000 nmol/L)^[98, 99]. XL-147 acts in an ATP competitive and reversible manner^[98]. Preclinical studies suggest that XL-147 has a remarkable inhibitory effect on MCF-7 breast cancer cells and A549 lung adenocarcinoma cells^[98]. The drug also induces striking tumor regression in breast, lung, ovarian, prostate, and glioma tumors^[100]. In combination with several chemotherapeutic agents, XL147 has also shown tumor growth inhibition (Table 2).

XL-765 is a dual PI3K/mTOR inhibitor with IC₅₀s in the low nanomolar range (IC₅₀=39, 113, 9, and 43 nmol/L for p110 α , β , γ , and δ , respectively). Unlike XL-147, XL-765 also inhibits DNA-PK (IC₅₀=150 nmol/L) and mTOR (IC₅₀=157 nmol/L)^[101, 102]. Recent studies suggest that XL-765, in combination with autophagy-inducing agents, shows increased antitumor activity in pancreatic adenocarcinomas^[103]. The combination of XL-765 and other chemotherapeutic agents is now undergoing a screening process and entering into clinical trials (Table 2). The clinical trial data suggest that there is inter-patient variability, with terminal half-life values that range from 2.5 to 8 d for XL-147 and 2 to 15 h for XL-765. Preliminary signs of exposure-dependent pharmacodynamic modulations (changes in plasma insulin levels) are observed at 30 mg/60 mg for XL-147 and 15 mg/30 mg bid for XL-765^[100].

Akt inhibitors

The role of Akt in tumor initiation and progression has been discussed above. Overexpression and activation of Akt are often associated with resistance of tumors to chemotherapy or radiotherapy^[104-106]. Current studies with a dominant negative Akt suggest that introducing a dominant negative Akt reduces Akt activity. These data suggest that Akt activity causes resistance against drug and radiation therapy^[107, 108]. Introduction of pan-Akt or Akt isoform-specific inhibitors exhibit great efficacy in inhibiting tumor growth.

Alkyl-phospholipids (APLs) are a heterogeneous group of unnatural lipids that have been shown to have anticancer activity. APLs are made up of two aliphatic side chains that are linked to glycerol phosphocholine by either an ether or thio-ether bond. Unlike the other anti-cancer agents described, APLs inhibit cancer by targeting cell membranes rather than DNA^[109, 110]. The rapid turnover and nonessential glycerol moiety of APLs restrict their use as anticancer agents. To overcome this problem, next generation molecules called alkylphosphocholines (APCs) have been synthesized.

APCs are derived from APLs by the removal of the glycerol group, which leads to increased stability of the compounds. This allows the APCs to integrate into the lipid bilayer of the cell membrane by accumulating in the lipid rafts where they disrupt the natural balance and metabolism of phospholipids, which in turn leads to alterations in membrane-signaling pathways that are associated with apoptosis^[111]. Examples of anticancer APCs include the prototypic compound edelfosine and the novel drug octadecyl-(1,1-dimethyl-piperidino-4-yl)-phosphate (D-21266, Perifosine), which affect apoptotic signaling. Studies on malignantly transformed hematopoietic cell lines showed that APCs reduce the levels of phosphorylated Akt (Figure 3)^[112].

Perifosine is considered to be one of the first generation of Akt inhibitors. It is a alkylphospholipid derived from the compound miltefosine, which is also an APL that has antitumor activity to a lesser extent^[113] (Figure 3). Mechanistically, Perifosine exhibits antitumor activity by preventing Akt translocation to the cell membrane^[114]. The drug has demonstrated antitumor effects in neuroblastoma, ovarian cancer, Waldenstrom's macroglobulinemia, medulloblastoma, kidney cancer, endometrial cancer, prostate cancer, myelogenous leukemia, multiple myeloma, squamous cell carcinoma, breast cancer, sarcoma, lung cancer, hepatocellular carcinoma, and other solid tumors^[115-127]. With its success in preclinical models, Perifosine reached phase I and II trials for the treatment of only solid tumors, Waldenstrom's macroglobulinemia, and sarcoma^[127-129]. In cases of relapsed or refracted Waldenstrom's macroglobulinemia, Perifosine resulted in at least a minimal response in 35% and stable disease in 54% of patients but had major side effects, including cytopenias and gastrointestinal toxicities. Despite its promising performance in the preclinical models, Perifosine monotherapy does not show efficacy in clinical trials. Disappointingly, it has been showing poor results in advanced or metastatic breast cancer, head and neck cancer, pancreatic adenocarcinoma, and metastatic mela-

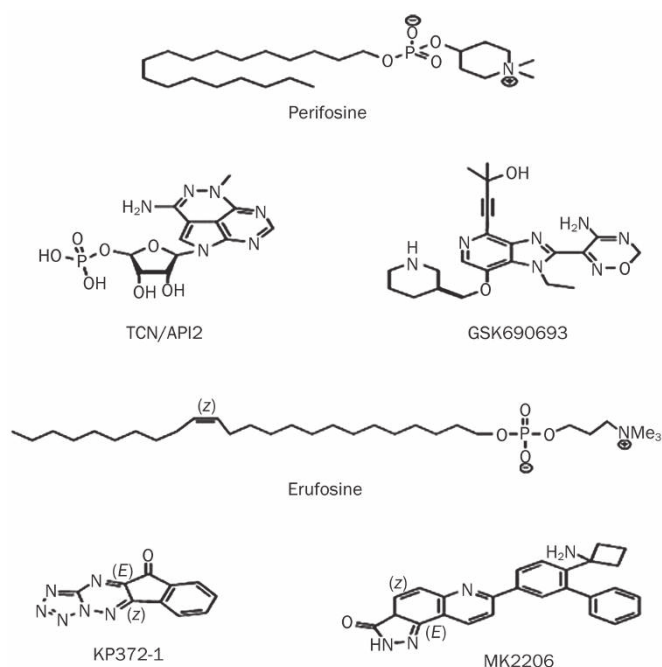


Figure 3. Chemical structure of Akt inhibitors.

noma patients. Recently, preclinical data have informed the attempts to use Perifosine for combination therapy (Table 3). Perifosine has had clinical success when used in combination regimens in metastatic colorectal cancer, relapsed or refractory multiple myeloma and glioma^[130-132]. Perifosine in combination with paclitaxel leads to Akt/mTORC1 inhibition, along with a marked increase in ceramide and ROS generation and an increase in AMPK and JNK that results in apoptosis^[133]. Perifosine induces caspase-dependent apoptosis and downregulates P-glycoprotein expression in multidrug-resistant human T-acute leukemia cells by a JNK-dependent mechanism. In multiple myeloma, the drug phosphorylates Rb^[134] and downregulates β -catenin and survivin^[135]. Perifosine has been given Fast Track status by the FDA in these tumors and is currently in phase III clinical trials.

Erufosine, another APC compound, was introduced in late 1990. However, its lack of efficiency on the leukemia cell line has restricted its use^[136]. Later, Erufosine was improved upon

with a longer (22-carbon) chain containing *cis*-13-14 double bonds (Figure 3). Erufosine shows less hemolytic toxicity upon intravenous application and its cholinomimetic properties are less pronounced compared to perifosine^[137]. It has been previously reported that Erufosine induced apoptosis in human astrocytoma and glioblastoma cell lines *in vitro* and affected rat C6 glioma tumor growth *in vivo*^[138, 139]. Erufosine induces apoptosis in glioma tumor by ROS generation and inhibits the mitochondrial respiratory chain, especially F₁F₀-ATP-synthase^[140]. Erufosine, either alone or in combination with other chemotherapeutic agents and ionic radiation, induces apoptosis in different kinds of cancer^[141] (Table 3). Erufosine combined with etoposide and cytarabine acts additively in acute myeloid leukemia^[142]. Erufosine causes cell death in acute myeloid leukemia by JNK 1/2 activation and ERK 1/2 dephosphorylation, leading to apoptosis^[143]. Cytotoxic effects of Erufosine on chronic lymphocytic leukemia (CLL) and multiple myeloma have also been observed. However, depending upon Akt phosphorylation status, the mode of apoptosis induction is different^[144, 145].

KP372-1, a triazinones derivative (Figure 3), inhibits proliferation and induces apoptosis in thyroid cancer, glioblastoma, squamous cell cancer, and acute myelogenous leukemia cell lines^[146-149]. It is also a PDK1 and FLT3 inhibitor that causes the inhibition of PKB activation through Ser473 phosphorylation, as well as downstream target phosphorylation, which reflects the non-selective mode of action. Recently KP372-1 was incorporated into nanoparticles and used in targeted drug-delivery systems^[150].

Triciribine (TCN/API-2) is a small-molecule Akt inhibitor that was invented in the early 1980 and 1990s. Despite showing efficacy in inhibiting the growth of several cancer cell lines, Triciribine was not widely used due to its poor performance in clinical trials^[151]. This poor performance was due to the drug's minimal efficacy and poor toxicity profile, including hyperglycemia and hypertriglyceridemia at high doses. Recently, Triciribine was rediscovered from a chemical library of 1992 compounds in the NCI Diversity Set as an agent that was capable of inhibiting the growth of Akt2-transformed cell lines but not the parental NIH-3T3 cell line. The drug induces cell death by suppressing the phosphorylation of Akt on both Ser473 and Thr308 residue^[152]. Triciribine suppresses epidermal growth factor-induced kinase activity and phosphoryla-

Table 3. Combinatorial effects of Akt inhibitors on cancer and progression into the clinical trial.

AKT inhibitors	Combination agent	Tumor type	Clinical trial	Reference
Perifosine	Capecitabine	Metastatic colorectal cancer	I	[130]
	Bortezomib	Multiple myeloma	I	[131]
	Bortezomib+dexamethasone	Relapsed/refractory multiple myeloma	II	[131]
	CCI-779	Glioma	I	[132]
Erufosine	Etoposide	Acute myeloid leukemia	I	[141]
	Ionizing radiation	Prostate cancer	I	[141]
MK2206	AZD6244	Colorectal cancer	II	[166]
	Gefitinib	Glioma	I	[168]

tion of all three isoforms of Akt and results in EGF-mediated Akt recruitment to the plasma membrane. However, Triciribine does not inhibit Akt kinase activity *in vitro* and prevents the phosphorylation of Akt in intact cells^[153]. After entering the cell, Triciribine is converted into the active metabolite TCN-P by adenosine kinase^[154]. TCN-P binds with the PH domain of Akt and inhibits PIP3 to bind with it.

Causes for inclusion in clinical trials

One of the main problems for cancer therapeutics is immunoevasion, which occurs when the immune system cannot control tumor growth. The main cause of immunoevasion is supposed to be activation of Akt. TCN modulates immunoevasion efficiently in combination with the E7-specific vaccine^[155]. TCN and TCN-P are currently being tested in clinical trials. They have been showing positive results in soft tissue sarcoma, colorectal cancer, tonsillar carcinoma and cervical squamous cell carcinoma patients^[156, 157].

GSK690693 is a novel ATP competitive low-nanomolar pan-Akt kinase inhibitor (Figure 3) that belongs to the aminofurazan family, and it has IC₅₀ values of 2, 13, and 9 nmol/L for Akt1, Akt2, and Akt 3, respectively^[158]. It is highly selective towards Akt and 13 other kinases. Most of these kinases belong to the AGC kinase family, including the Akt kinases^[159]. GSK690693 is found to be sensitive against the sixty-two hematologic tumor cell lines, including ALL, AML, CLL, CML, Burkitt's lymphoma, non-Hodgkin's lymphoma and Hodgkin's lymphoma cell lines, and induces apoptosis and growth inhibition^[160]. Recent studies using preclinical models suggest that GSK690693 had its greatest efficacy in delaying tumor progression in tumors and tumor derived cell lines with a high degree of Akt activation, including those with myristoylated constitutively active Akt or with PTEN loss^[161]. However, *in vivo* testing of preclinical models suggests that GSK690693 exerts a moderate activity in solid tumor and ALL xenograft models^[162].

MK2206 is an orally active allosteric Akt inhibitor (Figure 3). It shows equal potency toward two isoforms of Akt, purified recombinant human Akt1 (IC₅₀, 5 nmol/L) and Akt2 (IC₅₀, 12 nmol/L), but it is approximately five-fold less potent against human Akt3 (IC₅₀, 65 nmol/L)^[163]. MK2206 shows great potency against several cancer cell lines harboring *PIK3CA* mutations^[164, 165]. In addition, MK-2206, in combination with other chemotherapeutic agents, exerts a greater potency against non-small cell lung cancer, glioma, breast, and ovarian carcinoma in both *in vivo* and *in vitro* models (Table 3)^[166-168]. MK-2206 helps in restoring the efficacy of sorafenib to induce apoptosis in sorafenib-resistant hepatocellular carcinomas^[169]. MK-2206 is now in phase I clinical trials and is demonstrating efficacy with good tolerance levels^[170].

Conclusion

There is an impressive and increasing armamentarium of targeted agents that can inhibit the key components of the PI3K-Akt pathway and many of these drugs are already in clinical trials. As discussed above, deregulation of these signaling

casades by mutation or overexpression of several receptors leads to aberrant activation of these intracellular cascades. This phenomenon is a frequent event in human tumors and provides unique opportunities for therapeutic approaches. Epidemiological data provide information regarding the different components and their roles in the PI3K-Akt pathway, which has allowed for the identification and development of clinical candidates that can control the irregular activation of these pathways. Currently, one of the biggest challenges for researchers designing kinase inhibitors is to identify structural features around the ATP-binding site of the targeted enzyme. Modern kinase inhibitors that are going into clinical trials demonstrate that small molecule inhibitors are highly successful in inducing anticancer effects. This is due to the strong interaction between the inhibitors and groups in the domain adjacent to the ATP-binding site. Despite the lack of complete specificity, with the exception of the PI3K/Akt inhibitors that are among the most selective inhibitors reported to date, the compounds in clinical trials have shown significant *in vivo* antitumor activities with no side effects in preclinical models. It is still unclear whether the selectivity of PI3K/Akt inhibitors, the potential therapeutic benefit, less side effects, greater drug tolerance, and concomitant inhibition of other off-targets and downstream targets will be beneficial in the treatment of cancer. It is imperative that clinical reagents with maximum efficacy and minimum lethal dose and side effects be carefully chosen. This will allow therapies to be made swiftly available for all suitable candidates. We can only hope that novel and safe targeted anticancer agents will come from these drug discovery efforts^[171-178].

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