Published in final edited form as:

Surg Oncol Clin N Am. 2013 October ; 22(4): . doi:10.1016/j.soc.2013.06.008.

Targeting the PI3-kinase/Akt/mTOR Signaling Pathway

Burhan Hassan, MD¹, Argun Akcakanat, MD PhD², Ashley M. Holder, MD³, and Funda Meric-Bernstam, MD⁴

Argun Akcakanat: aakcakanat@mdanderson.org; Ashley M. Holder: AMHolder@mdanderson.org

¹Post Doctorate Research Fellow, Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

²Assistant Professor Research, Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

³Post Doctorate Research Fellow, Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

⁴Professor, Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Synopsis

This article presents an overview of the PI3K/Akt/mTOR signaling pathway. As a central regulator of cell growth, protein translation, survival, and metabolism, activation of this signaling pathway contributes to the pathogenesis of many tumor types. Biochemical and genetic aberrations of this pathway observed in various cancer types will be explored. Lastly, pathway inhibitors both in development and already FDA-approved will be discussed.

Keywords

PI3K/Akt/mTOR signaling pathway; PTEN; cell signaling; molecular targeted therapy

Cells communicate with each other and respond to environmental conditions through signal transduction pathways. In cancer, deregulation of these pathways results in altered responses, such as increased cell survival and proliferation under conditions that would usually promote cell death or cell cycle arrest. The Phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signaling pathway assimilates both intracellular and extracellular signals to control cell metabolism, growth, proliferation and survival (1). Activation of PI3K/Akt/mTOR signaling contributes to the pathogenesis of many tumor types, suggesting that targeted inhibition of individual players in this pathway, including PI3K, phosphoinositide dependent kinase-1 (PDK-1), Akt and mTOR (mammalian Target of Rapamycin) is a

© 2013 Elsevier Inc. All rights reserved

Corresponding Author: Funda Meric-Bernstam, MD, Chair, Department of Investigational Cancer Therapeutics, and Professor, Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, 1400 Holcombe Blvd., FC8.3044, Houston, TX 77030, Tel#: 713/794-1226, Fax#: 713/563-0566.

Co-Authors ¹Burhan Hassan M.D., Department of Surgical Oncology, Unit b4.4938, The University of Texas MD Anderson Cancer Center, 1400 Pressler Street, Houston, TX, 77030 USA, bhassan@mdanderson.org, Tel: 713/563-7528, Fax: 713/792-6898

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest: Funda Meric-Bernstam has research funding from AstraZeneca and Celgene.

potential strategy for cancer therapy (1–3). This article offers an overview of pathway, a review of the biochemical and genetic aberrations of the pathway observed in cancer, and a description of pathway inhibitors that are approved and in clinical development.

PI3K/Akt/mTOR Signaling Pathway

Initiation of signaling through the PI3K/Akt/mTOR pathway occurs through several mechanisms, all of which result in increased activation of the pathway, as commonly seen in many cancer subtypes. Once PI3K signaling is activated, it can act upon a diverse array of substrates including mTOR, a master regulator of protein translation (4). The PI3K/Akt/mTOR pathway is an attractive therapeutic target in cancer not only because it is the second most frequently altered pathway after p53 (5, 6) but also because as it serves as a convergence point for many stimuli. Through its downstream substrates, this pathway controls key cellular processes such as transcription, apoptosis, cell cycle progression, and translation (Figure 1).

PI3 Kinase

PI3K signaling can be activated by multiple stimuli—activated tyrosine kinase growth factor receptors; cell adhesion molecules, such as integrins and G-protein-coupled receptors (GPCR); and oncogenes, such as *Ras*. PI3K is a member of lipid kinase family that is divided into three classes, each differentially activated by stimuli. Class IA isoforms are activated by tyrosine kinase receptors or *Ras*; class IB are activated by heterotrimeric G proteins or *Ras*; class II PI3K isoforms are activated through insulin receptors, growth factor receptors, and integrins; and class III kinases are thought to be constitutively active (7). Despite all isoforms having unique lipid substrate specificity, all initiate downstream signaling by phosphorylating the D3 position of phosphatidylinositol rings at the cell membrane.

The class IA PI3K enzymes are the most relevant to activation of the PI3K/Akt/mTOR pathway, because they catalyze the generation of phosphatidylinositol-3, 4, 5-trisphosphate (PIP3) from phosphatidylinositol-4, 5-bisphosphate (PIP2). PI3K activation occurs through engagement of Src homology 2 (SH2) domains with phosphotyrosine residues on activated growth factor receptors or through direct interaction with activated *Ras* (8, 9). PDK-1 and Akt are the crucial downstream kinases, and their activation depends upon the generation of PIP3 and PIP2. These 3l-phosphoinositides bind to the pleckstrin homology (PH) domains of PDK-1 and Akt to cause translocation of each kinase to the plasma membrane, where both are subsequently activated.

Akt

The phosphatase and tensin homolog deleted from chromosome 10 (PTEN), a tumor suppressor that dephosphorylates membrane phosphatidylinositols, is a key negative regulator of the effects of PI3K (10, 11). Once PDK-1 is activated by PIP3, it propagates the signal to the serine/threonine kinase Akt by phosphorylating its catalytic domain. Akt has three isoforms (Akt1, 2 and 3), which are structurally similar and are expressed in most tissues (12). PDK-1 phosphorylates Akt1 in its activation loop on threonine 308 (T308), an event that alone stimulates partial activation of Akt (13, 14). Full activation of Akt1 also requires phosphorylation at serine 473 (S473) in its regulatory domain. Phosphorylation of homologous residues in Akt2 and Akt3 occurs by the same mechanism. Several kinases are capable of phosphorylating Akt at S473, including PDK-1 (15), integrin-linked kinase (ILK), an ILK-associated kinase (16, 17), Akt itself (18), DNA-dependent protein kinase (DNA-PK) (19, 20), and mTORC2 (21). Since many kinases are capable of S473

phosphorylation, this suggests that cell type-specific mechanisms of regulating Akt activity may exist or that different S473 kinases may be stimulated under different conditions.

Akt can be regulated by phosphorylation at other sites or by binding to other proteins in addition to phosphorylation at T308 and S473 (22). For example, PKC- ζ , an isoform of protein kinase C, inhibits phosphorylation of Akt at T34 in the PH domain (23). Tyrosine (Y) phosphorylation at Y474 can also affect activation of Akt (24). Inositol polyphosphate 4-phosphatase type II (INPP4B), a tumor suppressor in human epithelial cells, is another inhibitor of PI3K/Akt signaling. In addition, S6 kinase 1 (S6K1), a downstream substrate of mTOR plays an important role in negative feedback regulation of Akt by catalyzing an inhibitory phosphorylation on insulin receptor substrate (IRS) proteins, abolishing their association and activation of PI3K, adding further complexity to the regulation of Akt kinase activity (25–27). In addition, Akt activity can also be modulated by Akt-binding proteins such as heat shock protein 90 (28), T cell leukemia/lymphoma protein-1 (29), carboxyterminal modulator protein (30), c-Jun N-terminal kinase (JNK)-interaction protein (31), and Tribbles homolog 3 (32). Whether these mechanisms play an important role in cancer biology is not clearly known. However, the fact that multiple mechanisms of modulating Akt activity exist suggests that cell- and context-specific modes of regulation are involved; likewise, targeting these may lead to developments in PI3K/Akt pathway inhibitors.

Akt has numerous substrates that have been identified and validated through bioinformatics approaches (33). These substrates control key cellular processes such as growth, including transcription, translation, cell cycle progression and survival including apoptosis, autophagy, and metabolism. With a few exceptions, Akt has an inhibitory effect on its multiple targets. However, as most Akt targets are negative regulators, the net result of Akt activation is cellular activation. For example, Akt phosphorylates forkhead box O1 (FoxO1) and other forkhead family members and results in inhibition of transcription of pro-apoptotic genes such as *Fas* ligand, insulin-like growth factor binding protein 1 (*IGFBP1*) and bisindolyl maleimide (*Bim*) (34, 35). Conversely, the inflammatory kinases (IKK), following Akt phosphorylation, increase NF- κ B activity and the transcription of pro-survival genes (36, 37). Akt, by phosphorylating and inactivating pro-apoptotic proteins, such as BCL2-associated agonist of cell death (Bad), directly regulates apoptotic machinery through Bad's regulation and control of cytochrome c release from mitochondria. Akt is also involved in the regulation of apoptosis signal-regulating kinase-1 (ASK-1) and mitogen-activated protein kinase (MAPK) kinase, both of which are involved in stress and cytokine-induced cell death (38–40). Akt regulates cell cycle progression through the cyclin-dependent kinase inhibitors, p21WAF1/CIP1 and p27KIP1 (36–39, 41). Inhibition of glycogen synthase kinase-3 β (GSK-3 β) by Akt also stimulates cell cycle progression by stabilizing cyclin D1 expression (40). Akt also plays an important role in protein translation by phosphorylating tuberous sclerosis complex 2 (TSC2, also called tuberlin) and mTOR. Thus, Akt inhibition can result in numerous effects on cancer cells that could contribute to an antitumor response.

mTOR

mTOR is an atypical serine/threonine protein kinase that belongs to the PI3K-related kinase family. mTOR exists in two multiprotein complexes: mTOR complex 1 and 2 (mTORC1 and mTORC2). mTORC1 is a complex of the mTOR protein, mammalian LST8, proline-rich Akt1 substrate 40 (PRAS40) and raptor (42, 43), while mTORC2 consists of a complex of mTOR, mLST8, mSIN1, prothor and rictor (44–49). Akt activates mTOR through at least two mechanisms: either directly by phosphorylating mTORC1 at S2448 (50) or indirectly through TSC2. TSC2 inactivation through phosphorylation by Akt results in upregulation of mTORC1 activity through a cascade of signaling molecules (51–53). A GTPase-activating domain of TSC2 catalyzes the conversion of the Ras-like protein, Ras homolog enriched in

brain (Rheb)-GTP to Rheb-GDP leading to inactivation of mTOR function (54, 55). Thus, Akt by decreasing TSC2 activity, increases levels of Rheb-GTP, which then leads to activation of mTORC1. mTORC1 plays a pivotal role in protein translation through its substrate: eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1). Hyperphosphorylation of 4E-BP1 by mTORC1, inhibits its binding to eukaryotic initiation factor 4E (eIF4E), thereby activating cap-dependent translation (54). In addition to protein translation, mTORC1 regulates cell proliferation, survival, and angiogenesis by regulating eIF4E-mediated translation of Bcl-2, Bcl-xL, and vascular endothelial growth factor (VEGF) (56–58). mTORC1 also phosphorylates S6K1 which in turn leads to phosphorylation of S6 ribosomal protein, and other targets including insulin receptor substrate 1 (IRS-1), eukaryotic initiation factor 4B (eIF4B), programmed cell death 4 (PDCD4), eukaryotic elongation factor-2 kinase (eEF2K), mTOR, and glycogen synthase kinase-3, which are implicated in cellular transformation (56, 59, 60). In addition, mTORC1 regulates the transition from G1 to S phase through downregulation of cyclin D1 and c-Myc, which are required for progression through this phase of the cell cycle (61).

In comparison with mTORC1, little is known about mTORC2 signaling. mTORC2 responds to growth factors such as insulin through a poorly defined mechanism that requires PI3K and ribosomes, since ribosomes are needed for mTORC2 activation and mTORC2 binds them in a PI3K-dependent fashion (62). mTORC2 controls several members of the AGC subfamily of kinases, including Akt, serum- and glucocorticoid-induced protein kinase 1 (SGK1) and protein kinase C- β (PKC- β). Akt is phosphorylated by mTORC2 at its hydrophobic motif (S473), a site required for its maximal activation (21). mTORC2 deletion, associated with defective Akt-Ser473 phosphorylation, impairs the phosphorylation of some Akt targets, including forkhead box O1/3a (FoxO1/3a), whereas other Akt targets such as TSC2 and GSK-3 β remain unaffected (44, 63). mTORC2 also directly activates SGK1, a kinase controlling ion transport and growth (64). In contrast to Akt, mTORC2 deletion results in complete loss of SGK-1 activity. As SGK1 phosphorylates FoxO1/3a residues that are also phosphorylated by Akt, loss of SGK1 activity is probably responsible for the reduction in FoxO1/3a phosphorylation in mTORC2-depleted cells. PKC- β is another AGC kinase regulated by mTORC2. Along with other effectors, such as paxillin and Rho GTPases, mTORC2 plays a role in cell migration by regulating phosphorylation of PKC- β and control of the actin cytoskeleton in cell-type-specific fashion (49, 65, 66).

Pathogenesis of Cancer by Aberrations in the PI3K/Akt/mTOR Pathway

Aberrations in the PI3K/Akt/mTOR pathway can occur through multiple mechanisms, resulting in pathway activation and contributing to the development of many human cancer types. Genomic aberrations affecting the PI3K pathway include germline and somatic mutations, amplifications, rearrangements, methylation, overexpression, and aberrant splicing resulting in decreased expression or function of PTEN, amplification or mutation of *PIK3CA* or amplification of *Akt* (67–70). The pathway is also triggered by activation of growth factor receptors, including human epidermal growth factor receptor 2 (HER2) and insulin-like growth factor receptor (IGFR), through autocrine growth loops, through mutations or overexpression of the growth factor receptors themselves, or by additional intracellular signaling molecules (10, 71, 72) (Table 1).

Loss of PTEN function

PTEN is targeted by a number of regulatory events, many of which are aberrant in cancer, suggesting that PTEN is a critical regulator of pathway function. PTEN functions both as a protein and lipid phosphatase. Its tumor suppressive function is attributed to lipid phosphatases, as loss of lipid phosphatase function of PTEN results in increased PI3k/Akt/mTOR pathway activation. Loss of PTEN function and its activity in cancer occurs through

multiple mechanisms, which include mutations, loss of heterozygosity, methylation, aberrant expression of regulatory microRNA, protein instability, and protein phosphorylation (54). PTEN is the second most frequently mutated tumor suppressor gene (73) and, as shown in multiple studies, it is mutated or deleted in many human cancer types including brain, bladder, breast, prostate and endometrial cancers (74–85). Epigenetic silencing is seen in some tumor types, in which PTEN mutations are rare (86–88); methylation of PTEN has been observed in 69% of non-small cell lung cancer (89). Methylation of the promoter region in PTEN is also seen in endometrial and gastric cancer (90–92).

PIK3CA Amplification or Mutation

PI3K can be activated as a result of overexpression of structurally normal protein or from mutations in the catalytic p110 or regulatory p85 subunits. *PIK3CA* is the gene that encodes the p110a catalytic subunit and is overexpressed in 40% of ovarian (93) and 50% of cervical cancers (94). In several cancer types, somatic mutations of this gene have been detected that result in increased kinase activity. Nonsynonymous mutations that encode the helical and kinase domains of the protein have been seen in 32% of colorectal cancers. In breast cancer, *PIK3CA* mutations have been observed in 21.4% of tumors (10). PIK3A mutations have also been detected in 27% of glioblastomas and 25% of gastric cancers (95). Mutations in the regulatory subunit p85 have also been detected. For example, p65, a truncated version of p85, was isolated from a tumor cell line that has shown to cause constitutive activation of PI3K and cellular transformation (96). Moreover, a constitutively active p85 mutant, as a result of SH2 domain deletion, has been detected in colon and ovarian cancers (97). Notably, mutations, particularly in exons 9 and 20 of *PIK3CA*, encoding the helical and kinase domains respectively, activate Akt signaling in some models (98–100). However, *PIK3CA* mutations are not always associated with PI3K/Akt/mTOR pathway activation *in vitro* and were not associated with PI3K/Akt/mTOR pathway activation in breast cancers in The Cancer Genome Atlas (101). This suggests that the effect of PIK3CA mutations may also be cell context-dependent, and in certain cancer types, such as in breast cancer, other major regulators of the pathway may need to be considered.

Amplification of Akt

Amplification of Akt isoforms has been observed in some cancer subtypes. *Akt1* amplification has been detected in gastric carcinomas (102), and amplification of *Akt2* has been observed in 12% of ovarian cancers, 10–20% of pancreatic carcinomas (103, 104), and a subset of pancreatic cancers (93, 104, 105). In breast cancer, amplification of either *Akt1* or *Akt2* has been reported. *Akt3* amplification has been observed in prostate cancer and hormone independent breast cancer cell lines (106). E17K mutation in the Pleckstrin homology (PH) protein domain of Akt has been identified (5, 107). This mutation allows Akt1 recruitment to the cellular membrane independent of PI3K, conferring transforming activity. Sequencing based on mass spectroscopy showed mutations in *Akt1-E17K* in <2% of the breast tumors evaluated, and in none of the cell lines evaluated (10).

Activation of Growth Factor Receptors

The PI3K/Akt/mTOR pathway is also activated by cell surface growth factor receptors. Increased activation of growth factor receptors in cancer can occur via amplification, activating mutations, or the release of growth factors that stimulate their receptors in an autocrine fashion. The ERBB family of receptor tyrosine kinases is the most important family of growth factor receptors that is frequently activated in cancer cells. ERBB includes ERBB1/epidermal growth factor receptor (EGFR), HER2, HER3 and HER4. These receptors dimerize to activate the PI3K/Akt/mTOR pathway. HER2/HER3 heterodimers are especially considered to be strong activators of the PI3K/Akt pathway (108), while tumor cells with HER2 overexpression exhibit constitutive activation of the pathway (71). HER2

receptor mutations can also lead to constitutive activation of the pathway. EGFRvIII, a truncated version of EGFR, lacks the extracellular ligand-binding domain and is constitutively active, resulting in activation of the PI3K/Akt pathway (109). Mutations in the kinase domains of EGFR and HER2, but not in HER3 or HER4, result in pathway activation and have also been described in lung cancer (110–113). Aberrant autocrine growth loops can also result in increased pathway activation via activation of ERBB family members. For example, overexpression of two ligands of EGFR, transforming growth factor- α (TGF- α) and amphiregulin, is associated with activation of EGFR and the PI3K/Akt pathway (114, 115).

Pathway Activation in Human Dysplastic Lesions

PI3K/Akt/mTOR pathway activation is an early event in the tumorigenesis of multiple cancers as activation of the pathway has been described in multiple preneoplastic lesions. Hamartoma syndromes such as Cowden's syndrome (*PTEN* mutations), Peutz-Jeghers syndrome (*LKB1* mutations), tuberous sclerosis (*TSC1/2* mutations), neurofibromatosis (*NF1* mutations), and probably Birt-Hogg-Dubé (*BHD/Folliculin* mutations) (116–120) have increased activation of PI3K/Akt/mTOR pathway. Compared to a normal nevus, a dysplastic nevus has increased Akt activation (121), while in case of breast tissue, phosphorylation of Akt, mTOR and 4E-BP1 increases progressively from normal breast epithelium to hyperplasia and from abnormal hyperplasia to tumor invasion (122).

PI3K/Akt/mTOR Pathway Targeted Therapy

The PI3K/Akt/mTOR pathway holds multiple putative therapeutic targets. Because homeostasis of the pathway is tightly regulated, it is necessary to identify mechanistic feedback loops and cross-talk with other signaling cascades to anticipate mechanisms of adaptive response and acquired resistance and thereby to develop rational combination therapies. The PI3K/Akt/mTOR pathway can be targeted by a variety of approaches including: (i) targeting kinases that lead to activation of Akt, PI3K and PDK-1, (ii) directly targeting PI3K, (iii) directly inhibiting Akt, (iv) targeting downstream effectors of Akt such as mTOR, or (v) combination approaches (Table 2).

PI3K kinase inhibitors

Wortmannin, an irreversible pan-isoform PI3K inhibitor, and LY294002, a reversible inhibitor of mTOR and PI3K, are first generation PI3K inhibitors. Although these commercially available inhibitors were effective in treating cancer xenografts by inhibiting PI3K, poor solubility and high toxicity limited their clinical application. Both Wortmannin and LY294002 failed to enter clinical trials in humans. Meanwhile, derivatives of both drugs, as well as inhibitors of the p110 catalytic subunit of PI3K and isoform specific inhibitors, are in development and even in clinical trials.

The majority of the PI3K inhibitors under development target all three isoforms of the p110 catalytic subunit of class IA PI3K. Most of them have been effective in cancer cell lines *in vitro* settings, particularly in those harboring *PIK3CA* mutations. Severe adverse effects, which were the limiting factor in cases of the Wortmannin and LY294002 drug, were not observed in newer drugs. In BKM120, mood disorders have been reported as the doselimiting toxicity, when used in combination with endocrine therapy (123). Prolonged stable disease has been observed even in heavily pretreated patients. The inhibitors currently in development include: NVP-BKM120, BAY80-6946, PX866, XL147, and GDC-0941. Some of these inhibitors are currently tested in association with chemotherapy (PX866 and docetaxel; XL147 or GDC-0941 and carboplatin/paclitaxel), with RTK inhibitors (XL147 and erlotinib; NVP-BKM120 and trastuzumab), and with other new agents (GDC-0941 and GDC-0973, an oral MAPK inhibitor) (124).

PI3K isoform specific inhibitors

To limit unintended off-target effects, isoform specific inhibitors of PI3K have also been developed. CAL-101, BYL719 and MLN1117 (formerly INK1117) are a few examples of such inhibitors. They have been shown to be more effective in cancer cells with PIK3CA activation than in PTEN-deficient tumors. CAL-101 is an isoform-specific inhibitor with high selectivity for the PI3K α isoform and low nanomolar IC₅₀. It is available in oral formulation and has displayed an acceptable safety profile and promising clinical activity in patients with advanced chronic lymphocytic leukemia (125), mantle cell lymphoma, and indolent non-Hodgkin lymphoma (NHL). In B-cell malignancies, CAL-101, in association with anti-CD20 monoclonal antibodies and/or bendamustine, has shown promising results and acceptable toxicity in phase 1 clinical trials (126). MLN1117 is a potent and orally efficacious PI3K γ -selective kinase inhibitor currently in clinical development. BYL719, another PI3K β -selective kinase inhibitor, has shown promising preliminary clinical activity as a single agent in patients with PIK3CA mutant ER+ metastatic breast cancer (127).

PDK-1 inhibitors

To be completely active, Akt requires phosphorylation at two sites—one in its catalytic domain (T308) and one in its regulatory domain (S473). PDK-1, once activated by the products of PI3K, PIP2, and PIP3, phosphorylates Akt at its T308 residue (34, 128). Since PDK-1 has a central role in the activation of the pathway, it is possible that PDK-1 inhibitors could be an effective therapeutic option in cancer types that rely on this pathway. PDK-1 has been shown to be inhibited by two groups of drugs, staurosporine and its analogs and celecoxib and its analogs.

Staurosporine, a broad-spectrum kinase inhibitor, was isolated from *Streptomyces staurosporeus* in an attempt to identify inhibitors of PKC. Derivatives of staurosporine were developed, as its activity was limited to *in vitro* settings. 7-Hydroxystaurosporine (UCN-01) is a PKC inhibitor that inhibits a variety of kinases, including cdc2/CDK1 (129), PDK-1 (130), and PKC (131). UCN-01 has shown antiproliferative effects in head and neck cancer cells (132). Clinical trials have shown promising results for UCN-01, with preliminary evidence of antitumor activity in melanoma and large cell lymphoma (133). Despite limited promising results in clinical trials, the nonspecific nature of UCN-01 and its insufficient efficacy as a single agent (134) make it less attractive as a targeted, single-agent therapy.

Celecoxib derivatives—OSU-03012 and OSU-0301—are another group of drugs that are PDK-1 inhibitors, with IC₅₀'s in the millimolar range in sixty different human cancer cell lines. Their biologic efficacy stems from their ability to delay G2/M cell cycle progression and induce apoptosis independently of PDK-1 inhibition (135). While celecoxib derivatives may be very effective in growth inhibition and apoptosis, their COX-independent mechanism of action likely involves the inhibition of other pathways in addition to PDK-1 inhibition.

Akt inhibitors

Inhibition of Akt by biochemical and genetic means induces apoptosis and increases a cancer's responsiveness to chemotherapy or radiation in both *in vitro* and *in vivo* settings. Thus, the rationale to target Akt in cancer is strong. Lipid-based Akt inhibitors, small molecules that target the ATP-binding domain of Akt or the PH domain of Akt, or peptides that inhibit Akt function are a few of the approaches employed in the development of Akt inhibitors.

Lipid-based Akt inhibitors

The alkylphospholipid (ALK) perifosine bears structural similarity to ceramide – a sphingomyelin derivative that has been shown to inhibit the Akt pathway (136). Perifosine inhibits Akt translocation and activity (137). Perifosine has shown a broad spectrum of activity in melanoma, lung, prostate, colon, and breast cancer cells *in vitro* (138). In a phase I clinical trial, gastrointestinal toxicity proved to be dose-limiting (138). In another clinical trial, a loading dose/maintenance dose schedule was employed that limited the side effects observed earlier. Perifosine is currently in Phase II/III clinical trials. Other lipid-based Akt inhibitors, such as Phosphatidylinositol Ether Lipid Analogs (PIAs) and d-3-deoxy-phosphatidyl-myoinositol-1-[(R)-2-methoxy-3-octadecyloxypropyl hydrogen phosphate] (PX-316), are in the early phases of development.

MK-2206 is a novel, selective allosteric inhibitor of Akt. MK-2206 inhibits Akt signaling and cell cycle progression and increases apoptosis in a dose-dependent manner. MK-2206 sensitivity was significantly greater in cell lines with PTEN or PIK3CA mutation; however, not all cell lines with PI3K pathway aberrations were sensitive. MK-2206 had a growth-inhibitory effect *in vivo* and enhanced the antitumor activity of paclitaxel (98). Phase II clinical trials of MK-2206 have begun for the treatment of a variety of tumor types, including endometrial cancer, breast cancer, and colon cancer.

AZD5363 is a potent inhibitor of all isoforms of Akt. Cell lines with *PIK3CA* mutation, *PTEN* mutation, or *HER2* amplification, without coincident *RAS* mutation, showed the highest frequency of response to AZD5363 *in vitro* (139). This drug is currently in phase I clinical trials for advanced metastatic breast cancer, prostate cancer, and solid tumors.

Isoform-specific Akt inhibitors

High-throughput kinase activity screen has helped to identify isoform-specific Akt inhibitors. Akti1 inhibits Akt1; Akti2 inhibits Akt2; and Akti1,2 inhibits both Akt1 and Akt2 *in vitro* with IC₅₀'s in the micromolar range (140, 141). These inhibitors act through a PH domain-dependent mechanism that prevents phosphorylation of the enzyme. Treatment with either Akti1 or Akti2 sensitizes cancer cells to chemotherapy-induced cell death, but inhibition of both isoforms by Akti1,2 was necessary for maximum sensitization (142).

Allosteric mTOR inhibitors

mTOR inhibitors are the best-characterized PI3K/Akt/mTOR pathway inhibitors, as they target the most distal actor. In 1975, rapamycin, the prototypic mTOR inhibitor, was discovered as a potent antifungicide (143, 144). Originally, it was FDA-approved to prevent allograft rejection, but later on its potential as an antiproliferative drug in cancer cell lines was discovered (145–147).

Temsirolimus, everolimus, and ridaforolimus are rapamycin analogs that have been explicitly designed as cancer drugs. These inhibitors bind to the FK506-binding protein, FKBP-12, which then binds to and inhibits mTOR resulting in activation of mTOR signaling. Rapamycin, temsirolimus, ridaforolimus, and everolimus have potent activity as single agents and in combination with cytotoxic chemotherapy *in vitro*. Rapalogs promote cell cycle arrest in some cell lines, while it promotes apoptosis in others by sensitizing tumor cells to DNA damage-induced apoptosis through inhibition of p21 translation. They also sensitize cancer cells to chemotherapeutic agents, including cisplatin, paclitaxel and camptothecin (147–150). Rapamycin analogs have been FDA-approved for the treatment of pancreatic neuroendocrine tumors, renal cell carcinoma, breast cancer (in combination with exemestane) and subependymal giant cell astrocytoma associated with tuberous sclerosis;

they have also shown promise in clinical trials in other tumor types, such as mesothelioma and endometrial cancer (54, 151).

Several trials have examined the efficacy of rapamycin analogs in combination with other anticancer agents in addition to being investigated as single agents. For example, a recent phase I/II trial examined the effect of everolimus administration in combination with imatinib mesylate in patients with gastrointestinal stromal tumors. Patients whose tumors were refractory to imatinib alone were responsive to combination therapy (152). This result provides evidence that, in cancer types that exhibit chemotherapeutic resistance, the use of signal transduction inhibitors in combination may be an effective treatment strategy. There are other clinical trials going on that are looking at the therapeutic benefit of combining temsirolimus with other signal transduction inhibitors in solid tumors. Since the PI3K/Akt/mTOR pathway is one of the most frequently activated pathways in cancer cells, targeting it seems natural. However, the possibility of feedback activation of Akt due to mTOR inhibition suggests that combination therapy or a newer class of drugs, such as mTOR kinase inhibitors, might be more effective than rapalogs alone.

mTOR kinase inhibitors

mTOR kinase inhibitors are ATP-competitive inhibitors of mTOR, including Torin1, PP242, PP30, Ku-0063794, AZD8055, AZD2014, and MLN0128. These drugs share remarkable selectivity toward mTOR with IC₅₀ values in the low nanomolar range (153–156). mTOR kinase inhibitors are potent inhibitors of both mTORC1 and mTORC2. They effectively inhibit both protein translation and S473 Akt phosphorylation via direct inhibition of mTORC2. PP242 has been observed to cause cell death in models of acute leukemia harboring the Philadelphia chromosome (Ph) translocation and to delay leukemia onset *in vivo* (157). mTOR kinase inhibitors induce cell death in several hematologic malignancies, such as AML (158, 159) and T-ALL (160), and in a broad range of *in vitro* cancer settings (154, 161). Some of these second-generation mTOR inhibitors are currently in phase I/II trials (OSI-027, MLN0128 and AZD2014). The mTOR kinase inhibitors strongly inhibit phosphorylation of Akt at S473 residue but with however a biphasic regulation of Akt, characterized by a rapid but only transient dephosphorylation of Akt T308 (162). Moreover, the loss of mTORC1-mediated feedback on PI3K might activate PIP3-dependant PI3K effectors other than Akt. These potential mechanisms of resistance have encouraged the development of dual PI3K/mTOR kinase inhibitors.

PI3K/mTOR dual inhibitors

The PI3K/mTOR dual inhibitors hypothetically have the potential to be more effective than rapamycin yet may have more toxicity. The dual pathway inhibitors are more efficient in blocking Akt activity than pure ATP-competitive mTOR inhibitors. Akt is more active when it is phosphorylated at both T308 and S473 residues. PP242 (pure mTOR inhibitor) has no effect on Akt T308 phosphorylation, Akt1 activity, or phosphorylation of the Akt substrate GSK-3 β in human platelets (163). PI-103 and NVP-BEZ235 are two dual PI3K/mTOR inhibitors that have been tested in a wide range of tumors in preclinical studies (164–169). They efficiently inhibit Akt phosphorylation at both T308 and S473 residues. NVP-BEZ235 and other dual inhibitors (GDC-0980, XL675) have demonstrated biological activity in clinical trials. Side effects included nausea, vomiting, diarrhea, hyperglycemia, and loss of appetite. Furthermore, the concomitant use of rapalogs and dual PI3K/mTOR inhibitors at low concentrations has demonstrated decreased toxicity along with synergistic treatment effects *in vitro* and in xenograft models (170–172).

SF116, a multimodal inhibitor (PI3K, mTOR, DNA-PK, PIM1, and HIF-1 α) has been developed as a RGDS-conjugated LY294002 prodrug with site selectivity. SF116 binds to

specific integrins (173). SF1126 has shown significant disease stabilization in phase I study (174) and is now being tested in B-cell malignancies.

Summary

The PI3K/Akt/mTOR pathway plays a central role in cell growth, protein translation, survival, and metabolism. Activation of PI3K/Akt/mTOR signaling contributes to the pathogenesis of many tumor types. Because it is the second most frequently activated signaling pathway, there is intense interest in targeting this pathway for cancer therapy. Numerous preclinical studies and clinical trials are ongoing with inhibitors targeting PI3K, PDK-1, Akt, and mTOR. Though the results of these trials are eagerly anticipated, it is likely that the optimal activity of PI3K/Akt/mTOR pathway inhibitors will only be manifested through the development, evaluation, and implementation of rational combination therapies based on biomarkers of response.

Acknowledgments

Funding sources: This work was supported in part by the National Cancer Institute T32 CA009599-23 (AH, FMB), Susan G. Komen for the Cure SAC10006 (FMB), Stand Up to Cancer Dream Team Translational Research Grant, a Program of the Entertainment Industry Foundation (SU2C-AACR-DT0209) (FMB, AA), National Cancer Institute 5R21 CA159270, the Kleberg Center for Molecular Markers at The University of Texas MD Anderson Cancer Center, the National Center for Research Resources Grants 3UL1RR024148 and UL1TR000371 (FMB and AA).

References

1. Meric-Bernstam F, Akcakanat A, Chen HQ, et al. PIK3CA/PTEN Mutations and Akt Activation As Markers of Sensitivity to Allosteric mTOR Inhibitors. *Clin Cancer Res*. 2012; 18:1777–1789. [PubMed: 22422409]
2. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase-AKT pathway in human cancer. *Nat Rev Cancer*. 2002; 2:489–501. [PubMed: 12094235]
3. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell*. 2007; 12:9–22. [PubMed: 17613433]
4. Laplante M, Sabatini DM. mTOR Signaling in Growth Control and Disease. *Cell*. 2012; 149:274–293. [PubMed: 22500797]
5. Brugge J, Hung MC, Mills GB. A new mutational activation in the PI3K pathway. *Cancer Cell*. 2007; 12:104–107. [PubMed: 17692802]
6. Agarwal R, Carey M, Hennessy B, et al. PI3K pathway-directed therapeutic strategies in cancer. *Curr Opin Investig Drugs*. 2010; 11:615–628.
7. Vanhaesebroeck B, Leevers SJ, Panayotou G, et al. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem Sci*. 1997; 22:267–272. [PubMed: 9255069]
8. Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J*. 2000; 346:561–576. [PubMed: 10698680]
9. Vanhaesebroeck B, Waterfield MD. Signaling by distinct classes of phosphoinositide 3-kinases. *Exp Cell Res*. 1999; 253:239–254. [PubMed: 10579926]
10. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al. Integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res*. 2008; 68:6084–6091. [PubMed: 18676830]
11. Stocker H, Andjelkovic M, Oldham S, et al. Living with lethal PIP3 levels: Viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. *Science*. 2002; 295:2088–2091. [PubMed: 11872800]
12. Zinda MJ, Johnson MA, Paul JD, et al. AKT-1,-2, and-3 are expressed in both normal and tumor tissues of the lung, breast, prostate, and colon. *Clin Cancer Res*. 2001; 7:2475–2479. [PubMed: 11489829]

13. Alessi DR, Saito Y, Campbell DG, et al. Identification of the Sites in Map Kinase Kinase-1 Phosphorylated by P74(Raf-1). *Embo J*. 1994; 13:1610–1619. [PubMed: 8157000]
14. Andjelkovic M, Alessi DR, Meier R, et al. Role of translocation in the activation and function of protein kinase B. *J Biol Chem*. 1997; 272:31515–31524. [PubMed: 9395488]
15. Balendran A, Currie R, Armstrong CG, et al. Evidence that 3-phosphoinositide-dependent protein kinase-1 mediates phosphorylation of p70 S6 kinase in vivo at Thr-412 as well as Thr-252. *J Biol Chem*. 1999; 274:37400–37406. [PubMed: 10601311]
16. Lynch DK, Ellis CA, Edwards PAW, et al. Integrin-linked kinase regulates phosphorylation of serine 473 of protein kinase B by an indirect mechanism. *Oncogene*. 1999; 18:8024–8032. [PubMed: 10637513]
17. Delcommenne M, Tan C, Gray V, et al. Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. *Proc Natl Acad Sci U S A*. 1998; 95:11211–11216. [PubMed: 9736715]
18. Tokar A, Newton AC. Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. *J Biol Chem*. 2000; 275:8271–8274. [PubMed: 10722653]
19. Hill MM, Feng JH, Hemmings BA. Identification of a plasma membrane raft-associated PKB Ser473 kinase activity that is distinct from ILK and PDK1. *Curr Biol*. 2002; 12:1251–1255. [PubMed: 12176337]
20. Feng JH, Park J, Cron P, et al. Identification of a PKB/Akt hydrophobic motif Ser-473 kinase as DNA-dependent protein kinase. *J Biol Chem*. 2004; 279:41189–41196. [PubMed: 15262962]
21. Sarbassov DD, Guertin DA, Ali SM, et al. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*. 2005; 307:1098–1101. [PubMed: 15718470]
22. Brazil DP, Park J, Hemmings BA. PKB binding proteins: Getting in on the akt. *Cell*. 2002; 111:293–303. [PubMed: 12419241]
23. Powell DJ, Hajdich E, Kular G, et al. Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Mol Cell Biol*. 2003; 23:7794–7808. [PubMed: 14560023]
24. Conus NM, Hannan KM, Cristiano BE, et al. Direct identification of tyrosine 474 as a regulatory phosphorylation site for the Akt protein kinase. *J Biol Chem*. 2002; 277:38021–38028. [PubMed: 12149249]
25. Harrington LS, Findlay GM, Gray A, et al. The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J Cell Biol*. 2004; 166:213–223. [PubMed: 15249583]
26. Shah OJ, Wang ZY, Hunter T. Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. *Curr Biol*. 2004; 14:1650–1656. [PubMed: 15380067]
27. Tremblay F, Brule S, Um SH, et al. Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. *Proc Natl Acad Sci USA*. 2007; 104:14056–14061. [PubMed: 17709744]
28. Basso AD, Solit DB, Chiosis G, et al. Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. *J Biol Chem*. 2002; 277:39858–39866. [PubMed: 12176997]
29. Pekarsky Y, Koval A, Hallas C, et al. Tcl1 enhances Akt kinase activity and mediates its nuclear translocation. *Proc Natl Acad Sci USA*. 2000; 97:3028–3033. [PubMed: 10716693]
30. Maira SM, Galetic I, Brazil DP, et al. Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. *Science*. 2001; 294:374–380. [PubMed: 11598301]
31. Kim AH, Sasaki T, Chao MV. JNK-interacting protein 1 promotes Akt1 activation. *J Biol Chem*. 2003; 278:29830–29836. [PubMed: 12783873]
32. Du KY, Herzig S, Kulkarni RN, et al. TRB3: A tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science*. 2003; 300:1574–1577. [PubMed: 12791994]
33. Obenauer JC, Cantley LC, Yaffe MB. Scansite 2.0: proteome-wide prediction of cell signaling interactions using short sequence motifs. *Nucleic Acids Res*. 2003; 31:3635–3641. [PubMed: 12824383]

34. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. *Gene Dev.* 1999; 13:2905–2927. [PubMed: 10579998]
35. Nicholson KM, Anderson NG. The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal.* 2002; 14:381–395. [PubMed: 11882383]
36. Ozes ON, Mayo LD, Gustin JA, et al. NF-kappa B activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature.* 1999; 401:82–85. [PubMed: 10485710]
37. Romashkova JA, Makarov SS. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature.* 1999; 401:86–90. [PubMed: 10485711]
38. Shin I, Yakes FM, Rojo F, et al. PKB/Akt mediates cell-cycle progression by phosphorylation of p27(Kip1) at threonine 157 and modulation of its cellular localization. *Nat Med.* 2002; 8:1145–1152. [PubMed: 12244301]
39. Zhou BP. Cytoplasmic localization of p21(Cip1/WAF1) by Akt-induced phosphorylation in HER-2/neuoverexpressing cells. (vol 3, pg 245, 2001). *Nat Cell Biol.* 2001; 3:438–438.
40. Diehl JA, Cheng MG, Roussel MF, et al. Glycogen synthase kinase 3 beta regulates cyclin D1 proteolysis and subcellular localization. *Gene Dev.* 1998; 12:3499–3511. [PubMed: 9832503]
41. Liang J, Zubovitz J, Petrucelli T, et al. PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. *Nat Med.* 2002; 8:1153–1160. [PubMed: 12244302]
42. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell.* 2006; 124:471–484. [PubMed: 16469695]
43. Wang L, Harris TE, Lawrence JC. Regulation of proline-rich Akt substrate of 40 kDa (PRAS40) function by mammalian target of rapamycin complex 1 (mTORC1)-mediated phosphorylation. *J Biol Chem.* 2008; 283:15619–15627. [PubMed: 18372248]
44. Jacinto E, Facchinetti V, Liu D, et al. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell.* 2006; 127:125–137. [PubMed: 16962653]
45. Yang Q, Inoki K, Ikenoue T, et al. Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. *Gene Dev.* 2006; 20:2820–2832. [PubMed: 17043309]
46. Pearce LR, Huang X, Boudreau J, et al. Identification of Protor as a novel Rictor-binding component of mTOR complex-2. *Biochem J.* 2007; 405:513–522. [PubMed: 17461779]
47. Frias MA, Thoreen CC, Jaffe JD, et al. mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr Biol.* 2006; 16:1865–1870. [PubMed: 16919458]
48. Martin J, Masri J, Bernath A, et al. Hsp70 associates with Rictor and is required for mTORC2 formation and activity. *Biochem Biophys Res Commun.* 2008; 372:578–583. [PubMed: 18505677]
49. Sarbassov DD, Ali SM, Kim DH, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol.* 2004; 14:1296–1302. [PubMed: 15268862]
50. Nave BT, Ouwens DM, Withers DJ, et al. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem J.* 1999; 344:427–431. [PubMed: 10567225]
51. Inoki K, Li Y, Zhu TQ, et al. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol.* 2002; 4:648–657. [PubMed: 12172553]
52. Manning BD, Tee AR, Logsdon MN, et al. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberlin as a target of the phosphoinositide 3-Kinase/Akt pathway. *Mol Cell.* 2002; 10:151–162. [PubMed: 12150915]
53. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. *Nat Cell Biol.* 2002; 4:658–665. [PubMed: 12172554]
54. Meric-Bernstam F, Gonzalez-Angulo AM. Targeting the mTOR Signaling Network for Cancer Therapy. *J Clin Oncol.* 2009; 27:2278–2287. [PubMed: 19332717]
55. Garami A, Zwartkruis FJT, Nobukuni T, et al. Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. *Mol Cell.* 2003; 11:1457–1466. [PubMed: 12820960]

56. De Benedetti A, Graff JR. eIF-4E expression and its role in malignancies and metastases. *Oncogene*. 2004; 23:3189–3199. [PubMed: 15094768]
57. Soni A, Akcakanat A, Singh G, et al. eIF4E knockdown decreases breast cancer cell growth without activating Akt signaling. *Mol Cancer Ther*. 2008; 7:1782–1788. [PubMed: 18644990]
58. Culjkovic B, Tan K, Orolicki S, et al. The eIF4E RNA regulon promotes the Akt signaling pathway. *J Cell Biol*. 2008; 181:51–63. [PubMed: 18391071]
59. Jastrzebski K, Hannan KM, Tchoubrieva EB, et al. Coordinate regulation of ribosome biogenesis and function by the ribosomal protein S6 kinase, a key mediator of mTOR function. *Growth Factors*. 2007; 25:209–226. [PubMed: 18092230]
60. Barlund M, Forozan F, Kononen J, et al. Detecting activation of ribosomal protein S6 kinase by complementary DNA and tissue microarray analysis. *J Natl Cancer Inst*. 2000; 92:1252–1259. [PubMed: 10922410]
61. Sawyers CL. Will mTOR inhibitors make it as cancer drugs? *Cancer Cell*. 2003; 4:343–348. [PubMed: 14667501]
62. Zinzalla V, Stracka D, Oppliger W, et al. Activation of mTORC2 by Association with the Ribosome. *Cell*. 2011; 144:757–768. [PubMed: 21376236]
63. Guertin DA, Stevens DM, Thoreen CC, et al. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKC alpha but not S6K1. *Dev Cell*. 2006; 11:859–871. [PubMed: 17141160]
64. Garcia-Martinez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J*. 2008; 416:375–385. [PubMed: 18925875]
65. Jacinto E, Loewith R, Schmidt A, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*. 2004; 6:1122–1128. [PubMed: 15467718]
66. Hernandez-Negrete I, Carretero-Ortega J, Rosenfeldt H, et al. P-Rex1 links mammalian target of rapamycin signaling to Rac activation and cell migration. *J Biol Chem*. 2007; 282:23708–23715. [PubMed: 17565979]
67. Hennessy BT, Smith DL, Ram PT, et al. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov*. 2005; 4:988–1004. [PubMed: 16341064]
68. Karni R, de Stanchina E, Lowe SW, et al. The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nat Struct Mol Biol*. 2007; 14:185–193. [PubMed: 17310252]
69. Kumar R, Hung NC. Signaling intricacies take center stage in cancer cells. *Cancer Res*. 2005; 65:2511–2515. [PubMed: 15805240]
70. Manning BD, Cantley LC. AKT/PKB signaling: Navigating downstream. *Cell*. 2007; 129:1261–1274. [PubMed: 17604717]
71. Zhou BP, Hu MCT, Miller SA, et al. HER-2/neu blocks tumor necrosis factor-induced apoptosis via the Akt/NF-kappa B pathway. *J Biol Chem*. 2000; 275:8027–8031. [PubMed: 10713122]
72. Cui XJ, Zhang P, Deng WL, et al. Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/akt/mammalian target of rapamycin pathway: Progesterone receptor as a potential indicator of growth factor activity in breast cancer. *Mol Endocrinol*. 2003; 17:575–588. [PubMed: 12554765]
73. Stokoe D. *Pten*. *Curr Biol*. 2001; 11:R502. [PubMed: 11470419]
74. Wang SI, Puc J, Li J, et al. Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res*. 1997; 57:4183–4186. [PubMed: 9331071]
75. Chiariello E, Roz L, Albarosa R, et al. PTEN/MMAC1 mutations in primary glioblastomas and short-term cultures of malignant gliomas. *Oncogene*. 1998; 16:541–545. [PubMed: 9484844]
76. Feilottter HE, Coulon V, McVeigh JL, et al. Analysis of the 10q23 chromosomal region and the PTEN gene in human sporadic breast carcinoma. *Br J Cancer*. 1999; 79:718–723. [PubMed: 10070859]
77. Freihoff D, Kempe A, Beste B, et al. Exclusion of a major role for the PTEN tumour-suppressor gene in breast carcinomas. *Br J Cancer*. 1999; 79:754–758. [PubMed: 10070865]

78. Smith JS, Tachibana I, Passe SM, et al. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. *J Natl Cancer Inst.* 2001; 93:1246–1256. [PubMed: 11504770]
79. McMenamin ME, Soung P, Perera S, et al. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer Res.* 1999; 59:4291–4296. [PubMed: 10485474]
80. Rasheed BKA, Stenzel TT, McLendon RE, et al. PTEN gene mutations are seen in high-grade but not in lowgrade gliomas. *Cancer Res.* 1997; 57:4187–4190. [PubMed: 9331072]
81. Ali IU, Schriml LM, Dean M. Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. *J Natl Cancer Inst.* 1999; 91:1922–1932. [PubMed: 10564676]
82. Aveyard JS, Skilleter A, Habuchi T, et al. Somatic mutation of PTEN in bladder carcinoma. *Br J Cancer.* 1999; 80:904–908. [PubMed: 10360673]
83. Dahia PLM. PTEN, a unique tumor suppressor gene. *Endocr Relat Cancer.* 2000; 7:115–129. [PubMed: 10903528]
84. Dreher T, Zentgraf H, Abel U, et al. Reduction of PTEN and p27(kip1) expression correlates with tumor grade in prostate cancer. Analysis in radical prostatectomy specimens and needle biopsies. *Virchows Arch.* 2004; 444:509–517. [PubMed: 15118854]
85. Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science.* 1997; 275:1943–1947. [PubMed: 9072974]
86. Yokomizo A, Tindall DJ, Drabkin H, et al. PTEN/MMAC1 mutations identified in small cell, but not in nonsmall cell lung cancers. *Oncogene.* 1998; 17:475–479. [PubMed: 9696041]
87. Forgacs E, Biesterveld EJ, Sekido Y, et al. Mutation analysis of the PTEN/MMAC1 gene in lung cancer. *Oncogene.* 1998; 17:1557–1565. [PubMed: 9794233]
88. Kohno T, Takahashi M, Manda R, et al. Inactivation of the PTEN/MMAC1/TEPI gene in human lung cancers. *Genes Chromosomes Cancer.* 1998; 22:152–156. [PubMed: 9598803]
89. Soria JC, Lee HY, Lee JI, et al. Lack of PTEN expression in non-small cell lung cancer could be related to promoter methylation. *Clin Cancer Res.* 2002; 8:1178–1184. [PubMed: 12006535]
90. Salvesen HB, MacDonald N, Ryan A, et al. PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. *Int J Cancer.* 2001; 91:22–26. [PubMed: 11149415]
91. Kang YH, Lee HS, Kim WH. Promoter methylation and silencing of PTEN in gastric carcinoma. *Lab Invest.* 2002; 82:285–291. [PubMed: 11896207]
92. Tamura G. Promoter methylation status of tumor suppressor and tumor-related genes in neoplastic and non-neoplastic gastric epithelia. *Histol Histopathol.* 2004; 19:221–228. [PubMed: 14702190]
93. Shayesteh L, Lu YL, Kuo WL, et al. PIK3CA is implicated as an oncogene in ovarian cancer. *Nat Genet.* 1999; 21:99–102. [PubMed: 9916799]
94. Ma YY, Wei SJ, Lin YC, et al. PIK3CA as an oncogene in cervical cancer. *Oncogene.* 2000; 19:2739–2744. [PubMed: 10851074]
95. Samuels Y, Wang ZH, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science.* 2004; 304:554–554. [PubMed: 15016963]
96. Jimenez C, Jones DR, Rodriguez-Viciana P, et al. Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. *Embo J.* 1998; 17:743–753. [PubMed: 9450999]
97. Philp AJ, Campbell IG, Leet C, et al. The phosphatidylinositol 3'-kinase p85 alpha gene is an oncogene in human ovarian and colon tumors. *Cancer Res.* 2001; 61:7426–7429. [PubMed: 11606375]
98. Sangai T, Akcakanat A, Chen HQ, et al. Biomarkers of Response to Akt Inhibitor MK-2206 in Breast Cancer. *Clin Cancer Res.* 2012; 18:5816–5828. [PubMed: 22932669]
99. Bachman KE, Argani P, Samuels Y, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther.* 2004; 3:772–775. [PubMed: 15254419]
100. Kang SY, Bader AG, Vogt PK. Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci USA.* 2005; 102:802–807. [PubMed: 15647370]

101. Koboldt DC, Fulton RS, McLellan MD, et al. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012; 490:61–70. [PubMed: 23000897]
102. Staal SP. Molecular-Cloning of the Akt Oncogene and Its Human Homologs Akt1 and Akt2 - Amplification of Akt1 in a Primary Human Gastric Adenocarcinoma. *Proc Natl Acad Sci USA*. 1987; 84:5034–5037. [PubMed: 3037531]
103. Cheng JQ, Ruggeri B, Klein WM, et al. Amplification of AKT2 in human pancreatic cancer cells and inhibition of ATK2 expression and tumorigenicity by antisense RNA. *Proc Natl Acad Sci USA*. 1996; 93:3636–3641. [PubMed: 8622988]
104. Ruggeri BA, Huang LY, Wood M, et al. Amplification and overexpression of the AKT2 oncogene in a subset of human pancreatic ductal adenocarcinomas. *Mol Carcinog*. 1998; 21:81–86. [PubMed: 9496907]
105. Bellacosa A, Defeo D, Godwin AK, et al. Molecular Alterations of the Akt2 Oncogene in Ovarian and Breast Carcinomas. *Int J Cancer*. 1995; 64:280–285. [PubMed: 7657393]
106. Nakatani K, Thompson DA, Barthel A, et al. Up-regulation of Akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines. *J Biol Chem*. 1999; 274:21528–21532. [PubMed: 10419456]
107. Carpten JD, Faber AL, Horn C, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature*. 2007; 448:439–444. [PubMed: 17611497]
108. Prigent SA, Gullick WJ. Identification of C-ErbB-3 Binding-Sites for Phosphatidylinositol 3'-Kinase and Shc Using an Egf Receptor C-ErbB-3 Chimera. *Embo J*. 1994; 13:2831–2841. [PubMed: 8026468]
109. Moscatello DK, Holgado-Madruga M, Emlet DR, et al. Constitutive activation of phosphatidylinositol 3-kinase by a naturally occurring mutant epidermal growth factor receptor. *J Biol Chem*. 1998; 273:200–206. [PubMed: 9417065]
110. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res*. 2005; 65:1642–1646. [PubMed: 15753357]
111. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA*. 2004; 101:13306–13311. [PubMed: 15329413]
112. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science*. 2004; 304:1497–1500. [PubMed: 15118125]
113. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004; 350:2129–2139. [PubMed: 15118073]
114. Rusch V, Baselga J, Cordoncardo C, et al. Differential Expression of the Epidermal Growth-Factor Receptor and Its Ligands in Primary Nonsmall Cell Lung Cancers and Adjacent Benign Lung. *Cancer Res*. 1993; 53:2379–2385. [PubMed: 7683573]
115. Tateishi M, Ishida T, Mitsudomi T, et al. Immunohistochemical Evidence of Autocrine Growth-Factors in Adenocarcinoma of the Human Lung. *Cancer Res*. 1990; 50:7077–7080. [PubMed: 2208175]
116. Liaw D, Marsh DJ, Li J, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet*. 1997; 16:64–67. [PubMed: 9140396]
117. Johannessen CM, Reczek EE, James MF, et al. The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc Natl Acad Sci USA*. 2005; 102:8573–8578. [PubMed: 15937108]
118. Kamm MA. The small intestine and colon: scintigraphic quantitation of motility in health and disease. *European journal of nuclear medicine*. 1992; 19:902–912. [PubMed: 1451707]
119. Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer*. 2009; 9:563–575. [PubMed: 19629071]
120. Hartman TR, Nicolas E, Klein-Szanto A, et al. The role of the Birt-Hogg-Dube protein in mTOR activation and renal tumorigenesis. *Oncogene*. 2009; 28:1594–1604. [PubMed: 19234517]
121. Dai DL, Martinka M, Li G. Prognostic significance of activated Akt expression in melanoma: A clinicopathologic study of 292 cases. *J Clin Oncol*. 2005; 23:1473–1482. [PubMed: 15735123]

122. Zhou XY, Tan M, Hawthorne VS, et al. Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers. *Clin Cancer Res*. 2004; 10:6779–6788. [PubMed: 15501954]
123. Mayer IA, Abramson VG, Balko JM, et al. SU2C phase Ib study of pan-PI3K inhibitor BKM120 with letrozole in ER+/HER2- metastatic breast cancer (MBC). *ASCO Meeting Abstracts*. 2012; 30:510.
124. Bowles DW, Jimeno A. New phosphatidylinositol 3-kinase inhibitors for cancer. *Expert Opin Inv Drug*. 2011; 20:507–518.
125. Sharman J, de Vos S, Leonard JP, et al. A Phase 1 Study of the Selective Phosphatidylinositol 3-Kinase-Delta (PI3K delta) Inhibitor, CAL-101 (GS-1101), in Combination with Rituximab and/or Bendamustine in Patients with Relapsed or Refractory Chronic Lymphocytic Leukemia (CLL). *Blood*. 2011; 118:779–780.
126. Leonard J, Schreeder M, Coutre S, et al. A Phase 1 Study of Cal-101, an Isoform-Selective Inhibitor of Phosphatidylinositol 3-Kinase P110 δ , in Combination with Anti-Cd20 Monoclonal Antibody Therapy and/or Bendamustine in Patients with Previously Treated B-Cell Malignancies. *Ann Oncol*. 2011; 22:137–137.
127. Juric D, Argiles G, Burris H, et al. Phase I study of BYL719, an α -specific PI3K inhibitor, in patients with PIK3CA mutant advanced solid tumors: preliminary efficacy and safety in patients with PIK3CA mutant ER-positive (ER+) metastatic breast cancer (MBC). *Cancer Res*. 2012; 72 P6-10-07.
128. Kandel ES, Hay N. The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. *Exp Cell Res*. 1999; 253:210–229. [PubMed: 10579924]
129. Graves PR, Yu LJ, Schwarz JK, et al. The Chk1 protein kinase and the Cdc25C regulatory pathways are targets of the anticancer agent UCN-01. *J Biol Chem*. 2000; 275:5600–5605. [PubMed: 10681541]
130. Sato S, Fujita N, Tsuruo T. Interference with PDK1-Akt survival signaling pathway by UCN-01 (7-hydroxystaurosporine). *Oncogene*. 2002; 21:1727–1738. [PubMed: 11896604]
131. Mizuno K, Noda K, Ueda Y, et al. Ucn-01, an Antitumor Drug, Is a Selective Inhibitor of the Conventional Pkc Subfamily. *FEBS Lett*. 1995; 359:259–261. [PubMed: 7867810]
132. Patel V, Lahusen T, Leethanakul C, et al. Antitumor activity of UCN-01 in carcinomas of the head and neck is associated with altered expression of cyclin D3 and p27(KIP1). *Clin Cancer Res*. 2002; 8:3549–3560. [PubMed: 12429646]
133. Sausville EA, Arbuck SG, Messmann R, et al. Phase I trial of 72-hour continuous infusion UCN-01 in patients with refractory neoplasms. *J Clin Oncol*. 2001; 19:2319–2333. [PubMed: 11304786]
134. Li TH, Christensen SD, Frankel PH, et al. A phase II study of cell cycle inhibitor UCN-01 in patients with metastatic melanoma: a California Cancer Consortium trial. *Invest New Drug*. 2012; 30:741–748.
135. Ding HM, Han CH, Zhu JX, et al. Celecoxib derivatives induce apoptosis via the disruption of mitochondrial membrane potential and activation of caspase 9. *Int J Cancer*. 2005; 113:803–810. [PubMed: 15499625]
136. Summers SA, Garza LA, Zhou HL, et al. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol Cell Biol*. 1998; 18:5457–5464. [PubMed: 9710629]
137. Kondapaka SB, Singh SS, Dasmahapatra GP, et al. Perifosine, a novel alkylphospholipid, inhibits protein kinase B activation. *Mol Cancer Ther*. 2003; 2:1093–1103. [PubMed: 14617782]
138. Crul M, Rosing H, de Klerk GJ, et al. Phase I and pharmacological study of daily oral administration of perifosine (D-21266) in patients with advanced solid tumours. *Eur J Cancer*. 2002; 38:1615–1621. [PubMed: 12142051]
139. Davies BR, Greenwood H, Dudley P, et al. Preclinical pharmacology of AZD5363, an inhibitor of AKT: pharmacodynamics, antitumor activity, and correlation of monotherapy activity with genetic background. *Mol Cancer Ther*. 2012; 11:873–887. [PubMed: 22294718]
140. Lindsley CW, Zhao ZJ, Leister WH, et al. Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors. *Bioorg Med Chem Lett*. 2005; 15:761–764. [PubMed: 15664853]

141. Barnett SF, Defeo-Jones D, Fu S, et al. Identification and characterization of pleckstrin-homology-domain-independent and isoenzyme-specific Akt inhibitors. *Biochem J.* 2005; 385:399–408. [PubMed: 15456405]
142. DeFeo-Jones D, Barnett SF, Fu S, et al. Tumor cell sensitization to apoptotic stimuli by selective inhibition of specific Akt/PKB family members. *Mol Cancer Ther.* 2005; 4:271–279. [PubMed: 15713898]
143. Sehgal SN, Baker H, Vezina C. Rapamycin (Ay-22,989), a New Antifungal Antibiotic .2. Fermentation, Isolation and Characterization. *J Antibiot (Tokyo).* 1975; 28:727–732. [PubMed: 1102509]
144. Vezina C, Kudelski A, Sehgal SN. Rapamycin (Ay-22,989), a New Antifungal Antibiotic .1. Taxonomy of Producing Streptomycete and Isolation of Active Principle. *J Antibiot (Tokyo).* 1975; 28:721–726. [PubMed: 1102508]
145. Douros J, Suffness M. New Anti-Tumor Substances of Natural Origin. *Cancer Treat Rev.* 1981; 8:63–87. [PubMed: 7248995]
146. Houchens DP, Ovejera AA, Riblet SM, et al. Human-Brain Tumor Xenografts in Nude-Mice as a Chemotherapy Model. *Eur J Cancer Clin On.* 1983; 19:799–805.
147. Geoerger B, Kerr K, Tang CB, et al. Antitumor activity of the rapamycin analog CCI-779 in human primitive neuroectodermal tumor/medulloblastoma models as single agent and in combination chemotherapy. *Cancer Res.* 2001; 61:1527–1532. [PubMed: 11245461]
148. Shi YF, Frankel A, Radvanyi LG, et al. Rapamycin Enhances Apoptosis and Increases Sensitivity to Cisplatin in-Vitro. *Cancer Res.* 1995; 55:1982–1988. [PubMed: 7728769]
149. Wan XL, Helman LJ. Effect of insulin-like growth factor II on protecting myoblast cells against cisplatin-induced apoptosis through p70 s6 kinase pathway. *Neoplasia.* 2002; 4:400–408. [PubMed: 12192598]
150. Mondesire WH, Jian W, Zhang H, et al. Targeting mammalian target of rapamycin synergistically enhances chemotherapy-induced cytotoxicity in breast cancer cells. *Clin Cancer Res.* 2004; 10:7031–7042. [PubMed: 15501983]
151. Chan S, Scheulen ME, Johnston S, et al. Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. *J Clin Oncol.* 2005; 23:5314–5322. [PubMed: 15955899]
152. Van Oosterom AT, Dumez H, Desai J, et al. Combination signal transduction inhibition: A phase I/II trial of the oral mTOR-inhibitor everolimus (E, RAD001) and imatinib mesylate (IM) in patients (pts) with gastrointestinal stromal tumor (GIST) refractory to IM. *J Clin Oncol.* 2004; 22:195s–195s.
153. Garcia-Martinez JM, Moran J, Clarke RG, et al. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *Biochem J.* 2009; 421:29–42. [PubMed: 19402821]
154. Chresta CM, Davies BR, Hickson I, et al. AZD8055 Is a Potent, Selective, and Orally Bioavailable ATP-Competitive Mammalian Target of Rapamycin Kinase Inhibitor with In vitro and In vivo Antitumor Activity. *Cancer Res.* 2010; 70:288–298. [PubMed: 20028854]
155. Feldman ME, Apsel B, Uotila A, et al. Active-Site Inhibitors of mTOR Target Rapamycin-Resistant Outputs of mTORC1 and mTORC2. *PLoS Biol.* 2009; 7:371–383.
156. Hsieh AC, Liu Y, Edlind MP, et al. The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature.* 2012; 485 55-U196.
157. Janes MR, Limon JJ, So LM, et al. Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. *Nat Med.* 2010; 16 205-U115.
158. Altman JK, Sassano A, Kaur S, et al. Dual mTORC2/mTORC1 Targeting Results in Potent Suppressive Effects on Acute Myeloid Leukemia (AML) Progenitors. *Clin Cancer Res.* 2011; 17:4378–4388. [PubMed: 21415215]
159. Willems L, Chapuis N, Puissant A, et al. The dual mTORC1 and mTORC2 inhibitor AZD8055 has anti-tumor activity in acute myeloid leukemia. *Leukemia.* 2012; 26:1195–1202. [PubMed: 22143671]
160. Evangelisti C, Ricci F, Tazzari P, et al. Targeted inhibition of mTORC1 and mTORC2 by active-site mTOR inhibitors has cytotoxic effects in T-cell acute lymphoblastic leukemia. *Leukemia.* 2011; 25:781–791. [PubMed: 21331075]

161. Bhagwat SV, Gokhale PC, Crew AP, et al. Preclinical characterization of OSI-027, a potent and selective inhibitor of mTORC1 and mTORC2: distinct from rapamycin. *Mol Cancer Ther.* 2011; 10:1394–1406. [PubMed: 21673091]
162. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, et al. mTOR Kinase Inhibition Causes Feedback-Dependent Biphasic Regulation of AKT Signaling. *Cancer Discov.* 2011; 1:248–259. [PubMed: 22140653]
163. Moore SF, Hunter RW, Hers I. mTORC2 protein complex-mediated Akt (Protein Kinase B) Serine 473 Phosphorylation is not required for Akt1 activity in human platelets [corrected]. *J Biol Chem.* 2011; 286:24553–24560. [PubMed: 21592956]
164. Raynaud FI, Eccles S, Clarke PA, et al. Pharmacologic characterization of a potent inhibitor of class I phosphatidylinositol 3-kinases. *Cancer Res.* 2007; 67:5840–5850. [PubMed: 17575152]
165. Park S, Chapuis N, Bardet V, et al. PI-103, a dual inhibitor of Class IA phosphatidylinositol 3-kinase and mTOR, has antileukemic activity in AML. *Leukemia.* 2008; 22:1698–1706. [PubMed: 18548104]
166. Chiarini F, Fala F, Tazzari PL, et al. Dual Inhibition of Class IA Phosphatidylinositol 3-Kinase and Mammalian Target of Rapamycin as a New Therapeutic Option for T-Cell Acute Lymphoblastic Leukemia. *Cancer Res.* 2009; 69:3520–3528. [PubMed: 19351820]
167. Maira SM, Stauffer F, Brueggen J, et al. Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol Cancer Ther.* 2008; 7:1851–1863. [PubMed: 18606717]
168. Serra V, Markman B, Scaltriti M, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res.* 2008; 68:8022–8030. [PubMed: 18829560]
169. Chapuis N, Tamburini J, Green AS, et al. Dual Inhibition of PI3K and mTORC1/2 Signaling by NVP-BEZ235 as a New Therapeutic Strategy for Acute Myeloid Leukemia. *Clin Cancer Res.* 2010; 16:5424–5435. [PubMed: 20884625]
170. Xu CX, Li YK, Yue P, et al. The Combination of RAD001 and NVP-BEZ235 Exerts Synergistic Anticancer Activity against Non-Small Cell Lung Cancer In Vitro and In Vivo. *Plos One.* 2011; 6
171. Mazzeletti M, Bortolin F, Brunelli L, et al. Combination of PI3K/mTOR Inhibitors: Antitumor Activity and Molecular Correlates. *Cancer Res.* 2011; 71:4573–4584. [PubMed: 21602434]
172. Werzowa J, Koehrer S, Strommer S, et al. Vertical Inhibition of the mTORC1/mTORC2/PI3K Pathway Shows Synergistic Effects against Melanoma In Vitro and In Vivo. *J Invest Dermatol.* 2011; 131:495–503. [PubMed: 21048785]
173. Garlich JR, De P, Dey N, et al. A vascular targeted pan phosphoinositide 3-kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. *Cancer Res.* 2008; 68:206–215. [PubMed: 18172313]
174. Garlich JR, Becker MD, Shelton CF, et al. Phase I Study of Novel Prodrug Dual PI3K/mTOR Inhibitor SF1126 In B-Cell Malignancies. *Blood.* 2010; 116:744–744.
175. Tashiro H, Blazes MS, Wu R, et al. Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. *Cancer Res.* 1997; 57:3935–3940. [PubMed: 9307275]
176. Risinger JI, Hayes K, Maxwell GL, et al. PTEN mutation in endometrial cancers is associated with favorable clinical and pathologic characteristics. *Clin Cancer Res.* 1998; 4:3005–3010. [PubMed: 9865913]
177. Bilbao C, Rodriguez G, Ramirez R, et al. The relationship between microsatellite instability and PTEN gene mutations in endometrial cancer. *Int J Cancer.* 2006; 119:563–570. [PubMed: 16506206]
178. Byun DS, Cho K, Ryu BK, et al. Frequent monoallelic deletion of PTEN and its reciprocal association with PIK3CA amplification in gastric carcinoma. *Int J Cancer.* 2003; 104:318–327. [PubMed: 12569555]
179. Gray IC, Stewart LMD, Phillips SMA, et al. Mutation and expression analysis of the putative prostate tumoursuppressor gene PTEN. *Br J Cancer.* 1998; 78:1296–1300. [PubMed: 9823969]

180. Cairns P, Okami K, Halachmi S, et al. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res.* 1997; 57:4997–5000. [PubMed: 9371490]
181. Pesche S, Latil A, Muzeau F, et al. PTEN/MMAC1/TEP1 involvement in primary prostate cancers. *Oncogene.* 1998; 16:2879–2883. [PubMed: 9671408]
182. Reifemberger J, Wolter M, Bostrom J, et al. Allelic losses on chromosome arm 10q and mutation of the PTEN (MMAC1) tumour suppressor gene in primary and metastatic malignant melanomas. *Virchows Arch.* 2000; 436:487–493. [PubMed: 10881743]
183. Birck A, Ahrenkiel V, Zeuthen J, et al. Mutation and allelic loss of the PTEN/MMAC1 gene in primary and metastatic melanoma biopsies. *J Invest Dermatol.* 2000; 114:277–280. [PubMed: 10651986]
184. Celebi JT, Shendrik I, Silvers DN, et al. Identification of PTEN mutations in metastatic melanoma specimens. *J Med Genet.* 2000; 37:653–657. [PubMed: 10978354]
185. Pollock PM, Walker GJ, Glendening JM, et al. PTEN inactivation is rare in melanoma tumours but occurs frequently in melanoma cell lines. *Melanoma Res.* 2002; 12:565–575. [PubMed: 12459646]
186. Duerr EM, Rollbrocker B, Hayashi Y, et al. PTEN mutations in gliomas and glioneuronal tumors. *Oncogene.* 1998; 16:2259–2264. [PubMed: 9619835]
187. Liu W, James CD, Frederick L, et al. PTEN/MMAC1 mutations and EGFR amplification in glioblastomas. *Cancer Res.* 1997; 57:5254–5257. [PubMed: 9393744]
188. Bedolla R, Prihoda TJ, Kreisberg JI, et al. Determining risk of biochemical recurrence in prostate cancer by immunohistochemical detection of PTEN expression and Akt activation. *Clin Cancer Res.* 2007; 13:3860–3867. [PubMed: 17606718]
189. Depowski PL, Rosenthal SI, Ross JS. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. *Mod Pathol.* 2001; 14:672–676. [PubMed: 11454999]
190. Rhei E, Kang L, Bogomolny F, et al. Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in primary breast carcinomas. *Cancer Res.* 1997; 57:3657–3659. [PubMed: 9288766]
191. Tsao H, Zhang X, Benoit E, et al. Identification of PTEN/MMAC1 alterations in uncultured melanomas and melanoma cell lines. *Oncogene.* 1998; 16:3397–3402. [PubMed: 9692547]
192. Guldberg P, thor Straten P, Birck A, et al. Disruption of the MMAC1/PTEN gene by deletion or mutation is a frequent event in malignant melanoma. *Cancer Res.* 1997; 57:3660–3663. [PubMed: 9288767]
193. Massion PP, Kuo WL, Stokoe D, et al. Genomic copy number analysis of non-small cell lung cancer using array comparative genomic hybridization: implications of the phosphatidylinositol 3-kinase pathway. *Cancer Res.* 2002; 62:3636–3640. [PubMed: 12097266]
194. Bjorkqvist AM, Husgafvel-Pursiainen K, Anttila S, et al. DNA gains in 3q occur frequently in squamous cell carcinoma of the lung, but not in adenocarcinoma. *Genes Chromosomes Cancer.* 1998; 22:79–82. [PubMed: 9591638]
195. Pedrero JM, Carracedo DG, Pinto CM, et al. Frequent genetic and biochemical alterations of the PI 3-K/AKT/PTEN pathway in head and neck squamous cell carcinoma. *Int J Cancer.* 2005; 114:242–248. [PubMed: 15543611]
196. Campbell IG, Russell SE, Choong DY, et al. Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res.* 2004; 64:7678–7681. [PubMed: 15520168]
197. Wu G, Xing M, Mambo E, et al. Somatic mutation and gain of copy number of PIK3CA in human breast cancer. *Breast Cancer Res.* 2005; 7:R609–R616. [PubMed: 16168105]
198. Lee JW, Soung YH, Kim SY, et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene.* 2005; 24:1477–1480. [PubMed: 15608678]
199. Saal LH, Holm K, Maurer M, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res.* 2005; 65:2554–2559. [PubMed: 15805248]
200. Levine DA, Bogomolny F, Yee CJ, et al. Frequent mutation of the PIK3CA gene in ovarian and breast cancers. *Clin Cancer Res.* 2005; 11:2875–2878. [PubMed: 15837735]

201. Samuels Y, Velculescu VE. Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle*. 2004; 3:1221–1224. [PubMed: 15467468]
202. Velho S, Oliveira C, Ferreira A, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. *Eur J Cancer*. 2005; 41:1649–1654. [PubMed: 15994075]
203. Ikenoue T, Kanai F, Hikiba Y, et al. Functional analysis of PIK3CA gene mutations in human colorectal cancer. *Cancer Res*. 2005; 65:4562–4567. [PubMed: 15930273]
204. Knobbe CB, Trampe-Kieslich A, Reifenberger G. Genetic alteration and expression of the phosphoinositol-3-kinase/Akt pathway genes PIK3CA and PIKE in human glioblastomas. *Neuropathol Appl Neurobiol*. 2005; 31:486–490. [PubMed: 16150119]
205. Kang S, Seo SS, Chang HJ, et al. Mutual exclusiveness between PIK3CA and KRAS mutations in endometrial carcinoma. *Int J Gynecol Cancer*. 2008; 18:1339–1343. [PubMed: 18221484]
206. Cheng JQ, Godwin AK, Bellacosa A, et al. AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. *Proc Natl Acad Sci U S A*. 1992; 89:9267–9271. [PubMed: 1409633]
207. Ricarte-Filho JC, Ryder M, Chitale DA, et al. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. *Cancer Res*. 2009; 69:4885–4893. [PubMed: 19487299]
208. Cohen Y, Shalmon B, Korach J, et al. AKT1 pleckstrin homology domain E17K activating mutation in endometrial carcinoma. *Gynecologic oncology*. 2010; 116:88–91. [PubMed: 19853286]
209. Stephens PJ, Tarpey PS, Davies H, et al. The landscape of cancer genes and mutational processes in breast cancer. *Nature*. 2012; 486:400–404. [PubMed: 22722201]
210. Gonzalez-Angulo AM, Blumenschein GR Jr. Defining biomarkers to predict sensitivity to PI3K/Akt/mTOR pathway inhibitors in breast cancer. *Cancer Treat Rev*. 2012; s0305-7372:234–234.
211. Banerji S, Cibulskis K, Rangel-Escareno C, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. *Nature*. 2012; 486:405–409. [PubMed: 22722202]

Key points

- PI3K/Akt/mTOR pathway:
 - Essential role in cell growth, protein translation, survival, and metabolism
 - Activation contributes to pathogenesis of many cancers
 - Second most frequently activated pathway in cancer
- Preclinical studies and clinical trials ongoing with inhibitors targeting PI3K, PDK-1, Akt, and mTOR Rational combination therapies likely key to targeting this pathway effectively

W.B.S. Author Hassan		25/03/13
Journal SOC	Vol : No. 22 : 04	Fig # SOC-22_04-Hassan-fig01.eps
Revised ☒	B x W ☐	2/C ☐ 4/C ☒ PR ☐
High-res files can be downloaded from: ftp://ftp.tnq.co.in/Illustration/SOC/22-04/

Figure 1.

Growth factors, insulin, nutrients and energy status regulate the activation of the PI3K/Akt/mTOR signaling network. Protein synthesis, cell growth and proliferation, and metabolic functions are regulated by downstream effectors of the pathway, such as 4E-BP1 and S6K. Boxes indicate therapies targeting the pathway. Arrows represent activation and bars represent inhibition. Abbreviations: 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; AMPK, adenosine monophosphate-activated protein kinase; ASK1, apoptosis signalregulating kinase 1; ATP, adenosine-5'-triphosphate; BAD, BCL2-associated agonist of cell death; FKBP-12, FK506-binding protein, 12 kD; FoxO, forkhead box O; GDP, guanosine diphosphate; GSK3, glycogen synthase kinase 3; GTP,

guanosine-5'-triphosphate; IRS1, insulin receptor substrate 1; MAP4K3, mitogen-activated protein kinase kinase kinase 3; mLST8, mTOR associated protein, LST8 homolog; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PDK-1, phosphoinositide-dependent kinase 1; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol (4,5) biphosphate; PIP3, phosphatidylinositol (3,4,5) triphosphate; PRAS40, proline-rich Akt1 substrate 40; PTEN, phosphatase and tensin homolog deleted from chromosome 10; Rheb, Ras homolog enriched in brain; RTK, receptor tyrosine kinase; S6K, ribosomal protein S6 kinase; SIN1, stress-activated mitogen-activated protein kinase associated protein 1; TSC1, tuberous sclerosis complex 1; TSC2, tuberous sclerosis complex 2.

Table 1

Pathogenesis of Cancer by Aberrations in the PI3K/Akt/mTOR Pathway

Genetic aberration	Tumor type	Frequency (%)
PTEN loss	Glioblastoma	54–74
	Endometrial	32–83
	Gastric	47
	Prostate	29
	Breast	39
	Melanoma	44–57
PTEN mutation	Glioblastoma	17–44
	Endometrial	36–50
	Gastric	7
	Prostate	12
	Breast	0–4
	Melanoma	7
PIK3CA amplification	Cervix	69
	Gastric	36
	Lung (Squamous)	60
	Head and Neck	37
	Ovary	25
PIK3CA mutation	Breast	21–40
	Colorectal	13–32
	Glioblastoma	5–8
	Endometrial	24–32
	Hepatocellular	6–36
	Ovarian	10
	Gastric	7
Akt amplification	Head and Neck	30
	Gastric	20
	Pancreas	20
	Ovary	12
Akt1 mutation	Thyroid	5
	Endometrial	4
	Breast	4
Akt2 mutation	Breast	0.8–1
Akt3 mutation	Breast	1

Data from Refs 74–80, 85, 94, 95, 99, 102, 104, 105, 175–211.

Table 2

Selected PI3K/Akt/mTOR Pathway Targeted Agents

Molecular Targets	Agent	Company	Phase
PI3K Inhibitors	BAY80-6946	Bayer	I/II
	BKM120	Novartis	II/III
	GDC0941	Genentech	II
	PX866	Oncothyreon	I/II
	XL147	Exelixis	I/II
PIK3Cd	CAL-101	Calistoga	III
PI3Ka	BYL719	Novartis	I/II
	MLN1117	Millennium	I
PDK-1 Inhibitors	OSU-03012 (AR-12)	Arno	I
	UCN-01	Sigma	I/II
Dual PI3K/mTOR inhibitors	BEZ235	Novartis	I/II
	GDC-0980	Genentech	I/II
	PF-04691502	Pfizer	I/II
	PF-05212384	Pfizer	I/II
	XL765	Exelixis	I/II
Multimodal Inhibitor (PI3K, mTOR, DNA-PK, HIF-1)	SF1126	Semafore	I
Akt inhibitors	AZD5363	AstraZeneca	I
	MK-2206	Merck	I/II
	GSK2110183	GlaxoSmithKline	I
	Perifosine	Keryx	III
mTOR Kinase Inhibitors	AZD2014	AstraZeneca	I/II
	AZD8055	AstraZeneca	I
	MLN0128	Millennium	I
	OSI-027	OSI Oncology	I
Rapalogs	Everolimus (RAD001)	Novartis	IV/ FDA approved for breast cancer, RCC, PNET, subependymal giant cell astrocytoma (in TSC)
	Ridaforolimus (MK8669)	ARIAD/MERCK	II
	Sirolimus	Wyeth/Pfizer	III
	Temsirolimus (CCI779)	Wyeth/Pfizer	III/FDA approved for RCC

Abbreviations: PNET, pancreatic neuroendocrine tumor; RCC, renal cell carcinoma; TSC, tuberous sclerosis. Data from <http://clinicaltrials.gov/>.