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TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Targeting the PI3K/Akt signaling pathway in gastric carcinoma: A reality for personalized medicine?

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Abstract

Frequent activation of phosphatidylinositol-3 kinases (PI3K)/Akt/mTOR signaling pathway in gastric cancer (GC) is gaining immense popularity with identification of mutations and/or amplifications of *PIK3CA* gene or loss of function of PTEN, a tumor suppressor protein, to name a few; both playing a crucial role in regulating this pathway. These aberrations result in dysregulation of this pathway eventually leading to gastric oncogenesis, hence, there is a need for targeted therapy for more effective anticancer treatment. Several inhibitors are currently in either preclinical or clinical stages for treatment of solid tumors like GC. With so many inhibitors under development, further studies

on predictive biomarkers are needed to measure the specificity of any therapeutic intervention. Herein, we review the common dysregulation of PI3K/Akt/mTOR pathway in GC and the various types of single or dual pathway inhibitors under development that might have a superior role in GC treatment. We also summarize the recent developments in identification of predictive biomarkers and propose use of predictive biomarkers to facilitate more personalized cancer therapy with effective PI3K/Akt/mTOR pathway inhibition.

Key words: Epidemiology; Gastric cancer; PI3K/Akt/mTOR pathway; Predictive biomarkers; Targeted therapy

Core tip: Gastric cancer (GC) is the fifth most common cancer in the world with highest incidence rate in Eastern Asia and Latin America. With increase in GC patient relapse and drug resistance, targeted therapy is gaining immense popularity for GC treatment. One of the pathways which has been reported to be dysregulated is phosphatidylinositol-3 kinases (PI3K)/Akt signaling pathway. This review focuses on how this pathway is crucial in GC oncogenesis. We also summarize the single or dual PI3K/Akt pathway inhibitors under investigation for GC treatment. Thereby, we discuss the plausible novel biomarkers under investigation for a more tailored approach for GC treatment.

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INTRODUCTION

The phosphatidylinositol-3 kinases/Akt (PI3K/Akt) signaling pathway is activated by several cellular stimuli regulating various physiological functions such as cell growth, cell survival, cell cycle progression, protein translation, and metabolism. Dysregulation of this pathway is frequently observed in several cancers including gastric cancer (GC). Hence, a deeper understanding of this signaling pathway would help target this pathway effectively using different therapeutic approaches. In this review we will focus on how this pathway is regulated in GC and the current status of using PI3K/Akt/mammalian target of rapamycin (mTOR) targeted therapy for GC treatment.

PI3K/AKT/MTOR PATHWAY

Several members of PI3K/Akt/mTOR pathway play a crucial role in regulating this pathway and hence, maintaining cellular homeostasis under normal physiological conditions. Some of these essential components are described below.

PI3K

PI3K are a family of lipid kinases known to phosphorylate the inositol ring of the 3-OH group in inositol phospholipids. They are further classified into three classes: Class Ⅰ, Ⅱ, and Ⅲ based on primary structure and regulation[1]. However, till date only Class Ⅰ, assisting in tight regulation of this pathway, has been shown to be associated with cancer. Class Ⅰ PI3K is a heterodimeric enzyme, with a catalytic and a regulatory subunit. The catalytic subunits for class Ⅰ PI3Ks are p110α, p110β, p110γ, and p110δ. It is further subdivided into class1A, encompassing p110α, p110β, and p110δ with a p85α, p85β, and p55γ regulatory subunit, and class1B consisting of only p110γ with p101, p84, and p87PIKAP regulatory subunits. A typical regulatory subunit has several protein-protein interacting domains, one of them, the inter-SH2 domain (iSH2) interacts with the p110 catalytic subunit, stabilizing p110 and its activities[2]. Reports have suggested the p110α subunit encoded by the PIK3CA gene as being the only catalytic subunit associated with several cancers[3]. p110α typically links with p85α, which is the most highly expressed regulatory subunit. The substrate for class1 PI3K, phosphatidylinositol-4,5-biphosphate (PI-4,5-P_2), generates the second messenger phosphatidylinositol-3,4,5-triphosphate (PI-3,4,5-P_3).

Akt

Serine/threonine protein kinase Akt belongs to the AGC [named after the protein kinase A, G, and C families (PKA, PKC, PKG)] family of kinases. Three highly homologous isoforms (Akt1, Akt2, and Akt2) of Akt have been identified so far. Structurally, Akt is mainly comprised of three domains: an N-terminal pleckstrin homology (PH) domain, a central kinase catalytic domain (CAT), and a C-terminal extension (EXT) containing a regulatory hydrophobic motif (HM). Phosphorylation of residues in both the catalytic and C-terminal extension domain is essential for complete activation of Akt downstream of PI3K signaling. PDK1 selectively phosphorylates Thr (308) on the CAT domain of Akt, while the kinases responsible for phosphorylation of Ser (473) on the EXT domain of Akt are still unknown[4]. Thus, phosphorylation of both Ser (473) and Thr (308) residues on Akt is required for its complete activation[5,6]. Fully activated Akt further regulates several processes downstream, along with positive regulation of mTOR and thereby mediating

mTOR activation.

mTOR

The mTOR protein, a 289-kDa serine/threonine kinase, is a master regulator of cell growth. It can be distinguished into two distinct multi-protein complexes; mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The mTORC1 complex is composed of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein8 (MLST8), PRAS40, and DEPTOR. It functions downstream of Akt, integrating the Akt and mTOR pathway with 4E-BP1 and S6K, which are immediate downstream targets of the mTORC1complex. The mTORC2 complex, on the other hand, is composed of rapamycin-insensitive companion of mTOR, MLST8 and mammalian stress-activated protein kinase interacting protein 1. This complex functions upstream of Akt/PKB and plays a role in complete activation of Akt by phosphorylating Akt at the Ser473 residue. Hence, downstream substrates of the mTORC2 complex include Akt[7] as well as PKC[8,9]. Hence, both complexes are important for effective regulation of the Akt/mTOR dual pathway, with the mTORC1 complex responsive towards growth factors, nutrients, energy, or oxidative stress indirectly while the mTORC2 complex plays an important role towards Akt activation to assist in complete activation of the PI3K/Akt/mTOR pathway.

ACTIVATION MECHANISM

Several receptor tyrosine kinases regulate the activation of the PI3K/Akt/mTOR pathway upon growth factor stimulation. Growth factors such as Insulin growth factor (IGF), epidermal growth factor (EGF), and Hepatocyte growth factor activate receptor tyrosine kinases (RTKs) *via* autophosphorylation on their tyrosine residues. Lipid kinases, such as PI3K, then associate with these phosphorylated tyrosine residues to activate the catalytic subunit of PI3Ks. For PI3Ks of class1A, the p110α catalytic subunit is activated upon p85α associating with the RTKs Activated PI3Ks further phosphorylate substrates like phosphatidylinositol 4,5-biphosphate to phosphatidylinositol 3,4,5-triphosphate (PIP3) within a few seconds. Secondary messengers such as PIP3 further recruit Akt to the membrane by interacting with the PH-domain of Akt. Upon membrane translocation, AKT gets activated by phosphorylation of its Ser473 and Thr308 residues by the PDK1 and mTORC2 complex respectively. Fully activated Akt then regulates several cellular processes by interacting with different substrates downstream of Akt. In the meanwhile, PTEN, a PIP3 phosphatase, acts a regulator of this pathway by maintaining homeostasis for this pathway activation. Activated Akt stimulates the mTORC1 complex by phosphorylating tuberous sclerosis complex2 (TSC2) and PRAS40, which are both negative regulators of mTOR. The mTORC1 complex controls protein translation and cell growth by phosphorylating ribosomal S6 kinase and the inhibitory partner of the translation initiation factor 4E (4E-BP1), which are regulators of protein synthesis[10]. Thus, under normal physiological conditions, Akt regulates cellular dynamics such as cell growth, cytoskeletal reorganization, cell cycle progression, cell survival, cell proliferation, protein translation, and cellular metabolism by interacting with various substrates, which will now be discussed in more detail.

CELLULAR ROLE OF THE AKT/mTOR PATHWAY

Cell survival and cell cycle progression

Akt acts as a central regulator of cell survival by interacting with anti-apoptotic signals both transcriptionally and post translationally. Akt phosphorylates Bad, a Bcl-2 family of anti-apoptotic proteins at Ser-136 and Caspase-9, a protease at Ser-196, thereby partially blocking cell death and supporting cell survival signals. Akt also regulates anti-apoptotic functions transcriptionally by translocating into the nucleus and regulating the transcription of the forkhead box O (FoxO) family of transcription factors. The FoxO family of transcription factors regulate cell death signals *via* expression of various members of both intrinsic and extrinsic modes of apoptosis as well as cyclin-dependent kinase inhibitors. Upon nuclear translocation, Akt represses the transcription of FoxO1, FoxO3, and FoxO4, thereby enhancing cell survival signals[11].

Akt also plays an important role in regulating cell cycle progression in normal cells. It either directly phosphorylates or indirectly regulates the protein expression levels of several molecules of cell cycle progression at the G1/S and G2/M phase of the cell cycle. These substrates are mentioned in Table 1.

Cellular metabolism and protein synthesis

Cellular metabolism of carbohydrates into proteins, nucleotides, and lipids is a fundamental aspect of cell growth and proliferation, with nutrients acting as a fuel for cell growth. mTOR plays a crucial role in regulating this metabolism in response to nutrient availability. Of the two mTOR complexes, the mTORC1 complex plays a key role in regulating cellular metabolism[12]. It receives signals of activation from nutrients and growth factors. For example, during metabolism of carbohydrates, there is a spike in insulin levels, which activates the mTORC1 complex of the Akt/mTOR pathway *via* inhibition of the TSC1/2 complex by phosphorylation of TSC2 at multiple sites to inhibit TSC1[13]. In this process, eventually Ras homolog enriched in brain (Rheb), a small GTPase belonging to the Ras family of guanine-nucleotide

Table 1 Role of Akt in regulating cell cycle

Cell cycle regulation by Akt upon mitogen stimulation			
Cell cycle phase	**Direct regulation**	**Indirect regulation**	**Downstream effect**
G1 → S phase	P-p21 at Thr145 residue[107]	↑ Transcription of c-MYC[108]	Increase in CyclinD expression Decrease in cdk inhibitors: $p21^{cip1}$, $p21^{kip1}$, $p15^{INK4B}$
G2 → M phase	P-Cdc25B at Ser353[109]		Cdc-25B inactivation Cyclin B activation
	P-Wee1Hu at Ser642[110]		Inactivation of Wee1Hu results in G2/M cell cycle progression
	P-Myt1 at Ser75[111]		Activation of Cyclin B-associated Cdk1 kinase activity
S → G2 phase	P-Cdk2 at Thr39[112]		Cytoplasmic shuttling of Cdk2

binding proteins that enhances apoptotic signalling at cellular levels[14], is inhibited upon TSC1 complex inactivation. The mTORC1 complex is also stimulated in the presence of amino acids by promoting the conversion of Ras-related GTP-binding protein (RAG) heterodimers (RAGA or RAGB, and RAGC or RAGD) into their active conformation, which further assists in mTORC1 complex cellular localization from the cytoplasm to the surface of the lysosome where it binds to inactivated RHEB[15-17]. The activated mTORC1 complex also tightly regulates pathways such as the AMP-activated protein kinase (AMPK) pathway by preventing its activation in the presence of a high ATP/AMP ratio. However, in the absence of energy in cells, AMPK gets activated by phosphorylating TSC2 at Ser1387 and Raptor from the mTORC1 complex at Ser-792, resulting in mTORC1 inactivation[18,19]. After mTORC1 activation and subsequent complete activation of the Akt/mTOR pathway, immediate downstream substrates of mTORC1 complex such as S6K (ribosomal S6 kinase), 4E-BP1, and ULK1 (UNC-51 like kinase) are phosphorylated at different residues. Interestingly, activated S6K further phosphorylates Insulin receptor substrate-1 (IRS-1), upstream of mTORC1. Phosphorylation of IRS-1 at serine residues by S6 kinases prevents IRS-1 functions and thereby PI3K activation[20]. This negative feedback loop of the PI3K/Akt/mTOR pathway is an important aspect of maintaining homeostasis in cellular metabolism, protein synthesis, and cell growth.

ONCOGENIC POTENTIAL OF PI3K/AKT/mTOR PATHWAY IN GC

Dysregulations caused by genetic alterations of the PI3K/Akt/mTOR pathway have been recently identified to play a crucial role in gastric oncogenesis. GC is the second most common cause of cancer-related death worldwide. *PIK3CA*, the gene encoding the catalytic subunit p110 of PI3K, is frequently mutated in gastric carcinoma cell lines and tumor tissues. Some reports identify mismatch repair deficiency as one of the factors contributing towards the PIK3CA mutations[21-23]. Another study suggested PIK3CA amplifications contributing towards the PI3K/Akt/mTOR pathway deregulation in GC[23]. This PIK3CA amplification was also associated with poor prognosis of GC patients.

PTEN (Phosphatase and tensin homolog), a tumor suppressor of the PI3K/Akt/mTOR pathway is frequently mutated or abnormally expressed in GC, with eventual functional inactivation of this gene product. Its inactivation is associated with increased progression towards gastric tumorigenesis. This inactivation is attributed to many possible mechanisms. While PTEN gene mutations are a rare phenomenon of PTEN inactivation, loss of heterozygosity of PTEN is more common in GC[24,25]. Abnormal PTEN promoter hypermethylation at the CpG islands also inhibits PTEN expression in GC tissues[26]. Post-transcriptional repression of PTEN by microRNAs is another well studied mechanism of PTEN repression in GC. miR21 and miR-221/222 have been identified as PTEN targets repressing PTEN expression by complexing with its 3'-UTR region[27-29]. PTEN also undergoes post-translational modifications like phosphorylation for its regulation. A recent study indicated that increased phosphorylation of PTEN at the Ser380 residue and reduced expression of PTEN could contribute to PTEN inactivation in gastric tumor tissues[30]. Overall, PTEN inactivation has several functional consequences that fall in the category of hallmarks of cancer, such as angiogenesis and evading apoptosis. PTEN has been shown to elevate the apoptotic cascade *via* Fas/FasL or cytochrome-c mediated activation of capsase-3 under normal physiological functions. Hence, with PTEN inactivation in GC cells, bypassing apoptosis *via* dysregulation of the apoptotic cascade will result in "evading apoptosis", a hallmark of cancer[31,32]. PTEN is also known to be a negative modulator of endogenous VEGF-mediated signaling. Thus, PTEN inactivation is associated with increased VEGF expression, and thereby potentiating angiogenesis in GC cells[33]. PTEN inactivation also results in constitutive activation of Akt, a PKB kinase regulating cell growth, cell death, and cell cycle. Using gastric tumor specimens, there has been a statistically significant correlation demonstrated between increased phosphorylation of Akt with poor prognosis of GC patients[34-36]. Functionally, this constitutive expression of phosphorylated Akt further contributes towards hallmarks of cancer such as

escaping cellular death pathways, cell cycle inhibition, and promoting survival and angiogenesis. One of the immediate downstream substrates of Akt is the FoxO family of transcription factors, which promotes growth inhibitory or/and pro-apoptotic signals by either regulating cell cycle inhibitory proteins such as $p21^{KIP1}$ or $p27^{WAF1/CIP1}$ or pro-apoptotic proteins of the Bcl-2 family of proteins[37,38]. Activated Akt phosphorylates FoxO and thereby inhibits transcriptional functions of this family of proteins, resulting in increased cell survival and proliferation[37]. Constitutive phosphorylation of FoxO is also correlated with increased expression of angiogenesis-related molecules in gastric tumor tissues[37]. Akt also directly phosphorylates anti-apoptotic proteins such as Bad at Ser-136. Thus, GCs with increased Akt expression show elevated levels of P-Bad at Ser136 that are sufficient to promote cell survival[39]. Another important substrate of Akt that acts as an initiator of the mitochondrial apoptotic pathway is caspase-9. Phosphorylation of capsase-9 at Ser-196 results in its inactivation. There is a significant correlation between constitutive phosphorylation of Akt with caspase-9 phosphorylation in gastric tumor tissues, although the mechanism still remains unclear[40]. This apoptosis resistance conferred by p-Akt also occurs by regulating increased expression of survivin, an inhibitor of apoptosis protein with a significant correlation between p-Akt and survivin expression levels in gastric tumor staging[39]. Pro-survival signals by Akt can also be intensified with its interaction with components of other signaling pathways such as the NF-κB pathway. Akt can phosphorylate NF-κB kinases such as IκB kinase (IKKα) at Thr23, resulting in a stimulatory phosphorylation and thereby NF-κB activation. This further augments the expression of pro-survival signals in GC cells[41,42]. Another immediate downstream target of Akt is the mTORC1 complex, which is activated by phosphorylation of TSC2 by Akt and subsequent TSC1/2 complex formation, which acts on RHEB (Ras homolog enriched in brain) to further phosphorylate mTOR at Ser2448 and thereby resulting in mTOR activation. Therefore, low expression or mutations in TSC1 are associated with a dysfunctional TSC1/2 complex and constitutively activated mTOCR1 complex[43]. Recent studies have identified high p-mTOR expression to be associated with poor prognosis and with some clinicopathological characteristics in GC tumor specimens both independently and in combination with low TSC1 expression[44,45]. A preliminary epidemiological study identified functional polymorphisms of mTORC1 contributing towards GC susceptibility in Eastern Chinese population[46]. With studies focussing on mTORC1 complex dysregulation and functional consequences, further studies are important to understand mTORC2 dysregulations, the cause of which still remains unclear. Immediate downstream effectors for the activated mTORC1 complex are p70S6K and 4E-BP1. p70S6K phosphorylation and activation, which mainly occurs in the cytoplasm, results in translation of 40s ribosomal S6 protein, while phosphorylated 4E-BP1 acts as a translational repressor. Recent studies have also shown p70S6K and mTOR regulating each other, with p70S6K also acting as a kinase for mTOR phosphorylation at Ser2448[47]. Aberrant expression of p-p70S6K is linked to GC carcinogenesis and its aggressiveness. Nuclear localization of this protein could have some inhibitory effects towards GC pathogenesis[48].

PRECLINICAL STUDIES AND ONGOING CLINICAL TRIALS WITH PI3K/AKT INHIBITORS

Involvement of several molecules of the PI3K/Akt/mTOR pathways in GC carcinogenesis has eventually led to development of both single, as well as recently, dual inhibitors essential for molecular targeted therapy for GC.

PI3K inhibitors and GC

PI3K inhibitors are classified into three classes based on their selectivity for the ATP-binding cleft on PI3K and pharmacokinetic properties: pan-class Ⅰ, isoform-selective, and dual PI3K/mTOR inhibitors[49,50].

Pan-Class Ⅰ inhibitors

Pan-class Ⅰ inhibitors have inhibitory effects against each isoform of p110 (PIK3CA). Several pan-class Ⅰ inhibitors are under investigation for GC targeted therapy, since PIK3CA gene mutations comprise 25% of gastric tumors, resulting in PI3K dysregulation in GC[51]. The first report of a molecular agent inhibiting PI3K was quecertin, which was, however, a non-specific kinase inhibitor[52]. Eventually, more specific pan-class Ⅰ inhibitors were identified, such as Wortmannin and a quecertin analogue, LY294002. Although both LY294002 and Wortmannin exhibited potent PI3K-inhibitory properties, there were considerable limitations for them to proceed towards clinical trials[52-54]. LY294002 showed non-specific targeting, a short half-life, and toxicity *in vivo*[55,56], while Wortmannin had limitations involving biological stability and short half-life[57].

To further improve on the pharmacological availabilities, a structural analogue of Wortmannin, PX-866 was developed.

PX-866: PX-866 is a semisynthetic pan-class-1, Wortmannin analogue with inhibitory concentrations in nanomolar ranges and better efficacy and a safer pharmacokinetic profile than Wortmannin. Preclinical *in vivo* studies have shown its anti-cancer effect against several xenograft models of various cancers[57,58]. It is currently in Phase Ⅱ clinical trials for patients with glioblastoma and head and neck cancer[59,60].

PX-866 recently also came under limelight for a multicenter trial for advanced solid tumors including gastric tumors. Data from the trial show that PX-866 can be administered with endurable toxicity for patients with advanced solid tumors[61].

NVP-BKM120 (Buparlisib): NVP-BKM120 is a potent pan-class Ⅰ PI3K inhibitor with its activity in nanomolar ranges for all the isoforms of Class Ⅰ PI3K. Preclinical investigations have revealed its effectiveness in a diverse range of cancer cell lines, with increased sensitivity in tumors harboring PIK3CA mutations[62]. Similar results were also observed in a panel of GC cell lines[63]. Additionally, combination therapy using PI3K and STAT3 inhibitors showed better efficacy and a synergistic effect in GC cell lines harboring KRAS mutations. The STAT3 pathway is also known to be constitutively activated in GC[64]. Although preclinical studies on BKM120 in GC are still ongoing, it has reached Phase Ⅱ clinical trials for other cancers such as brain, breast, colorectal, endometrial, NSCLC, and renal cell carcinoma[49,65-67]. Thus, BKM120 has a potential to progress into clinical trials for GC treatment using targeted therapy.

ZSTK474: ZSTK474, a pan-class 1 PI3K inhibitor inhibits all the four isoforms of PI3K and exhibits anti-tumor activity *in vivo* against human tumor xenograft models[68-70]. *In vitro* studies in GC cell lines suggest combination therapy of ZST474 and IGFR inhibitors for treating IGFR-positive cancers to overcome any intrinsic resistance to inhibition of PI3K/Akt/mTOR signaling, since over-expression of IGFR correlated with increased tyrosine phosphorylation on Insulin Receptor substrate, leading to increased PI3K activation. Hence, combination therapy with both ZST474 and IGFR inhibitors on GC cells with high IGFR expression exerted a superior therapeutic response[71].

BAY80-6946: BAY80-6946 is synthesized by Bayer healthcare and is a highly potent, selective, and reversible pan-class Ⅰ inhibitor working in nanomolar concentrations against all the isoforms of p110. However, it shows preferential activity against p110α and β than p110 γ and δ in tumor cell lines and xenograft models[72]. BAY80-6946 demonstrated acceptable safety profiles in phase Ⅰ clinical trials for advanced solid tumors, and therefore, exhibiting a potential to be progressed to phase Ⅱ clinical trials for patients with advanced solid tumors.

Isoform specific PI3K inhibitors

PI3K isoform specific inhibitors were designed with an aim to provide comparable or superior efficacy than pan-class Ⅰ inhibitors. Some of them under investigation for GC treatment will now be discussed.

BYL719: BYL719 is an α-isoform specific PI3K inhibitor working at nanomolar concentrations with minimal activity against other PI3K isoforms[73]. With the PI3K/Akt/mTOR pathway being frequently dysregulated in GC, BYL719 exhibited its inhibitory effects in synergy with another inhibitor LJM716, a ligand dependent as well as independent HER3 inhibitor, in GC xenograft models[74]. Interestingly, the combination study was done in HER2 positive GC cell lines, suggesting the sensitivity of this drug towards HER amplifications. BYL719 also recently completed Phase1b clinical trial for advanced stage GC in a combinational study with the HSP90 inhibitor AUY922 in patients whose tumors either harbour molecular alterations of PIK3CA or HER2 amplification[75].

INK1117: INK117 is another novel, selective p110α inhibitor. It is particularly more effective and sensitive to tumors bearing PIK3CA mutations. With good oral bioavailability in preclinical xenograft studies, it has entered a phase- Ⅰ study for advanced solid tumors including GC, to evaluate its safety, tolerability, pharmacokinetic and pharmacodynamic properties[76].

Dual PI3K/mTOR inhibitors

PI3K/mTOR dual inhibitors inhibit PI3K and the downstream mTOR kinase activity by binding to the ATP-binding cleft of these enzymes. Relative to the single inhibitors, these drugs have the benefit of inhibiting mTORC1 and mTOCR2, as well as all the isoforms of PI3K. Evidence has suggested that the mTORC1/S6K axis has a "two-edge sword"-like function in activation of the PI3K/mTOR pathway by promoting growth signals downstream of Akt, as well as mediating a potent negative feedback loop that restrains signaling *via* the insulin/IGFR and other RTKs. Dysregulation of this negative feedback loop has been reported to contribute towards resistance in cancers subjected to single inhibitors[77]. Hence, the need of dual PI3K/mTOR inhibitors arises with an aim to discover drugs with low toxicity and good pharmacokinetic profile.

NVP-BEZ235: NVP-BEZ235 is a novel dual ATP-competitive PI3K and mTOR inhibitor for p110α/β/γ/δ and mTOR kinase, with inhibitory doses at nanomolar ranges. It first entered phase trials for breast cancer[78]. The effectiveness of BEZ235 has been investigated in both PIK3CA mutated and wild type cell lines *in vitro* and in xenograft models *in vivo*. The first group reporting an effect of BEZ235 on gastric xenografts showed reduced tumor growth for NCI-N87 but not MKN-45 or MKN-28 xenografts. Interestingly, the reduction in tumor growth correlated with thymidine kinase expression and not PI3K/mTOR pathway inhibition[79]. Another group demonstrated *in vitro* increased sensitivity of AGS, PIK3CA mutated cells than for NCI-N87 and MKN-45, wild type PIK3CA GC cells[63]. Clinically, the response rate for BEZ235 was

highest for patients with PIK3CA mutations than those without this mutation[80]. However, another recent study showed an increased anti-tumor response with BEZ235 alone or in combination with nab-paclitaxel in NCI-N87, AGS, and SNU-16 GC cells, independent of PIK3CA mutation status *in vitro* and in a SNU16 xenograft model *in vivo*[81]. Hence, with increasing preclinical studies focusing on using NVP-BEZ235 for GC targeted therapy, and its gaining popularity in clinical trials for other cancers, NVP-BEZ235 might be a good potential candidate drug to be considered for clinical trials for solid tumors such as GC.

VS-5884: VS-5884 is a dual PI3K and mTOR inhibitor, inhibiting all the isoforms of PI3K and both mTOR complexes (mTORC1 and mTORC2), with nanomolar inhibitory concentrations for a panel of cancer cell lines *in vitro* and increased sensitivity towards cell lines harboring PIK3CA mutations. It also exhibits a favourable pharmacokinetic profile *in vivo*. VS-5884 shows a statistically significant inhibition of tumor growth in HER-over-expressing GC xenograft models (NCI-N87). This drug also exhibits a synergistic response in these xenograft models with geftinib, an EGFR inhibitor (EGFRi), currently in phase Ⅱ trials for GC treatment. Since this drug has proven its efficacy for monotherapy and combination therapy in GC xenograft models[82], these data provide a rationale for testing VS-5884 in early phase clinical trials for GC patients.

PI-103: PI-103 is an ATP-competitive PI3K and mTOR inhibitor with variable sensitivities towards different isoforms of p110 and mTOR at nanomolar concentrations. It was recently assessed for its synergistic effect to enhance the anti-tumor response for GC both *in vitro* and *in vivo* with 5-FU treatment. This study suggested PI-103 usage for enhancing 5-FU chemotherapy for GC; with 5-FU currently being used to treat GC patients but demonstrating limitations due to inter-variability in response rate of these patients. This synergistic effect of PI-103 with 5-FU was also associated with PIK3CA mutations and reduction of downstream effectors of PI3K/Akt/mTOR pathway and thymidylate synthase, an enzyme that generates thymidylate precursors for DNA synthesis *in vitro*[83].

Akt inhibitors and GC

The Akt signaling cascade controls a spectrum of tumorigenesis events such as cell growth, proliferation, survival, angiogenesis, invasion, and metastasis, as well as activation of the mTOR signaling cascade. Several mutations or amplifications in the Akt/mTOR signaling cascade contribute towards constitutive activation of Akt, which includes PTEN mutations and PIK3CA mutations, as well as over-expression/amplification of Akt itself[84-87]. With activated Akt playing a crucial role in tumorigenesis, several Akt inhibitors have been designed that have entered preclinical as well as clinical trials. Akt inhibitors that are currently being investigated for GC treatment include:

AZD5363: AZD5363 binds to and inhibits all the isoforms of Akt (Akt1, Akt2, and Akt3) with potency in the nanomolar range. Reports have suggested increased sensitivity towards AZD5363 in cancer cells harboring PIK3CA mutations, PTEN mutations, or HER-2 amplifications both *in vitro* and *in vivo*[88]. This sensitivity of AZD5363 towards the activating mutations was also tested in GC xenograft models, one with a PIK3CA mutation and another with PTEN loss. AZD5363 exhibited a relatively more significant anti-tumor response towards PIK3CA mutant GC xenografts than those with PTEN loss alone. Interestingly, for GC xenografts with inactivated PTEN treated with a combination of AZ5363 and taxotrene, a synergistic and potent anti-tumor response was observed rather than monotherapy with AZD5363[89]. Hence, selection of patients based on their mutational status would be beneficial for targeted therapy, which can eventually lead to more effective and tailored therapy either with single agents or in combination.

MK-2206: MK-2206 is a highly selective, allosteric Akt inhibitor, with higher potency for Akt1 and Akt2 isoforms than Akt3. Its efficacy was investigated both *in vitro* and *in vivo* as a single agent as well as in combination with several chemotherapeutic drugs or molecular targeted drugs (EGFRi) to overcome any potential resistance. This drug enhances the anti-tumor response in combination therapy, making it a suitable and promising agent for the second line of therapy in cancer patients receiving chemotherapy or targeted therapy[90]. This drug is currently used in phase Ⅱ trials as a second-line therapy for gastric and gastroesophageal cancer[91].

Perifosine: An oral anti-cancer agent and an Akt inhibitor, Perifosine has entered clinical trials for major human cancers. eIF4E is a downstream effector of the Akt/mTOR pathway, and increased levels of phosphorylated eIF4E and total-eIF4E correlate with increased GC in tumor tissues. A recent study showed that Perifosine treatment of GC cells with increased eIF4E expression (p-eIF4E) down-regulated eIF4E expression, and thereby exerting an inhibitory effect on the Akt/mTOR pathway. Also, the combination of eIF4E inhibitor with Perifosine in these GC cells further sensitized the cells towards more effective treatment[92]. Another study of combination therapy in GC revealed the effectiveness of Perifosine in combination with a miR-27a inhibitor, an oncogene that contributes to drug resistance in GC cells[93]. With more studies identifying the molecular mechanisms of Perifosine inhibition in GC, a recent study shows Perifosine inhibiting tumor growth in GC cells *via* inhibition of the Akt/GSK3β/C-MYC signaling pathway,

with significant down-regulation of AEG-1 (Astrocyte elevated gene), a gene reported to play an important role in cellular processes such as proliferation, apoptosis, and invasion[94]. Hence, Perifosine is a good potential therapeutic Akt inhibitor when used in combination therapies to overcome drug resistance, with scope for further progression into clinical trials.

TCN-PM: Triciribine Phosphate Monohydrate (TCN-PM) is a potent Akt inhibitor inhibiting all the three isoforms of Akt. In Phase Ⅰ studies of patients with solid tumors, where TCN-PM was administered to patients with increased p-Akt levels (as assessed by immuno-histochemical staining), a moderate reduction in p-Akt was observed after single TCN-PM therapy, which may have been possibly due to a small sample size. Further studies to confirm its availability as a single agent as well as its efficacy in combination treatments would help to promote its importance for phase Ⅱ clinical trials for GC[95].

mTOR inhibitors

mTOR is often dysregulated in GC, with several preclinical studies suggesting mTOR as a potential therapeutic target. mTOR forms two types of complexes to perform its cellular function based on its interacting partner and substrate specificity, these being the mTORC1 and mTOR2 complex. The mTORC1 complex is rapamycin sensitive[96]; rapamycin being the first mTOR inhibitor developed, while the mTORC2 complex is rapamycin insensitive. mTOR inhibitors are classified into two types based on their specificity for mTOR complexes: Rapalogs and mTORC1/2 inhibitors.

Rapalogs: Rapamycin and its analogs (referred as rapalogs), first form a complex with the intracellular receptor FK506 binding protein 12 (FKBP12) and then bind to a domain separate from the catalytic site of mTOR, preventing mTOR function. Rapalogs are effective against the mTOCR1 complex[97]. Some of the rapalogs under preclinical and clinical studies for GC treatment are as follows:

Temsirolimus: Temsirolimus binds to FKBP12, and the resultant protein-drug complex prevents mTORC1 activity. A Phase Ⅰ clinical study determined a favorable toxicity profile, maximum tolerated dose, pharmacokinetics, and anti-tumor efficacy in patients with advanced cancer including GC, making it a favorable drug to proceed towards phase Ⅱ trials[98].
Everolimus - Everolimus is another oral mTORC1 complex inhibitor that has demonstrated good safety and clinical tolerability profile in Phase Ⅰ trials for several cancers including GC. Phase Ⅱ trials for patients with advanced GC treated with Everolimus exhibited a good median progression free survival (PFS). Phase Ⅲ trials for previously treated GC did not show a significant overall survival benefit *vs* best supportive care patients. However, the PFS for six-months and the safety profile was significant, which highlights the need for predictive biomarkers for Everolimus treatment response in order to obtain better efficacy for this drug[99].

Ridaforolimus: Ridaforolimus, a rapamycin analog, is under clinical investigation with its well defined anti-tumor response in preclinical studies. It showed a synergistic anti-tumor response in patients with solid tumors including GC, in a Phase Ib trial, where ridaforolimus was given in combination with capecitabine, a prodrug that converts into FU[100].

mTORC1/2 inhibitors

Although mTORC1 inhibition by rapamycin analogs results in substantial tumor growth inhibition, drug resistance has been reported due to a negative feedback loop in the PI3K/Akt/mTOR pathway either *via* RTKs upregulation with increased Akt activation or crosstalk of PI3K with Ras signaling, leading to MAPK pathway activation. Hence, mTORC1/2 complex inhibitors have gained interest owing to their ability to act as ATP-competitive inhibitors of mTOR kinase activity.

PP242: PP242 significantly inhibits mTOR kinase activity, inhibiting both mTORC1 and mTORC2 complex activities. It shows superior anti-proliferative and anti-angiogenesis effects in GC cell lines *in vitro* relative to rapamycin, indicating its promising potential as a therapeutic drug in future for GC[101].

Other mTORC1/2 inhibitors that have been under investigation for solid tumor treatment include AZD2014, AZD8055, and OSI-027; however, there are currently no reports on their usage for GC treatment.

POTENTIAL BIOMARKERS FOR TARGETED THERAPY

The PI3k/Akt/mTOR pathway is activated in approximately 30%-60% of GC tumors. Hence, targeted therapy using either single or dual Akt/mTOR inhibitors is under investigation in several clinical trials as summarized in Table 2, with Everolimus, an mTOR inhibitor, being the only drug to date that has progressed towards phase Ⅲ trials for advanced GC patients[99]. Unfortunately, overall survival (OS) for GC patients treated with Everolimus was not significant; hence, identification of specific biomarkers for patient selection for Everolimus treatment would aid in more personalized therapy, with a potential for better efficacy and anti-tumor response. Although Everolimus failed to significantly improve the overall survival of patients with refractory advanced gastric cancer (AGC), an interesting case study showed satisfactory Everolimus monotherapy in a patient with refractory AGC harboring PIK3CA mutations and pS6 aberrations[102]; indicating the

Table 2 Summary of phosphatidylinositol-3 kinases/Akt/mTOR inhibitors under investigation for gastric cancer treatment

Classification of PI3K/Akt/mTOR inhibitors	Inhibitors under investigation for GC	Clinical status for GC
PI3K inhibitors: 3 classes of PI3K inhibitors		
Pan-class Ⅰ inhibitors	PX-866	Phase-Ⅱ study for solid tumors
	NVP-BKM120	Phase-Ⅰ study for advanced solid tumors
	ZSTK474	Preclinical studies
	BAY80-6946	Phase-Ⅱ study for advanced solid tumors
Isoform specific PI3K inhibitors	BYL719	Phase-Ⅰ study
	INK117	Phase-Ⅰ study
Dual Akt/mTOR inhibitors	NVP-BEZ235	Preclinical studies
	VS-5884	Phase-Ⅱ study
	PI-103	Phase-Ⅰ study
Akt inhibitors	AZD5363	Preclinical studies
	MK-2206	Phase-Ⅱ study
	Perifosine	Preclinical studies
	TCN-PM	Phase-Ⅰ study for solid tumors
mTOR inhibitors: 2 types		
Rapalogs	Everolimus	Phase-Ⅲ study
	Ridaforolims	Preclinical studies
	Sirolimus	Phase-Ⅰ study
	Temsirolimus	Phase-Ⅱ study
mTORC1/2 inhibitors	PP242	Preclinical studies

GC: Gastric cancer; PI3K: Phosphatidylinositol-3 kinases.

importance of predictive biomarkers for various subpopulations of AGC for effective treatment. With studies ongoing for biomarker discovery for better prognosis with Everolimus treatment for AGC patients, some biomarkers that have been explored to predict Everolimus sensitivity in other cancers are PIK3CA/PTEN genomic aberrations[103,104].

With the lack of predictive biomarkers, the need arises to discover new molecules as surrogate markers to further segregate the AGC patient population for more efficient PI3K/Akt/mTOR pathway inhibition. Recent reports have also shown constitutive activation of the Akt/mTOR pathway being regulated by receptor interacting protein-1 (RIP1), a key enzyme in the activation of survival pathways such as Akt/mTOR as well as NF-κB[105]. RIP-1 down-regulates PTEN expression as well as suppresses the mTOR/PI3K feedback inhibitory loop, leading to potent activation of this pathway. At the same time, RIP1 also mediates activation of NF-κB pathway, where TAK-1 (TGF-β), a key regulator of the signaling cascade, is recruited to the TNF-α receptor complex, which is a pivotal step for IKK (IκB kinase) activation. The NF-κB pathway is aberrantly activated in several cancers including breast and GC, and recently, an oncogene DP103 (a DEAD-box RNA helicase), was identified by our group to be upregulated in breast, prostate, gastric and colon cancers, and was shown to define the metastatic potential *via* activation of the NF-κB pathway in two independent breast cancer cohorts[106]. DP103 regulates the NF-κB pathway *via* direct interaction with TAK1 and thus, the DP103-TAK1 protein complex regulates activation of NF-κB in breast cancer[106]. With TAK1 activation also being RIP-1 dependent, cross talk between the Akt/mTOR and NF-κB pathways suggests exploring the role of DP103 as a future biomarker for GC upon aberrant PI3K/Akt/mTOR pathway activation.

REFERENCES

1 **Leevers SJ**, Vanhaesebroeck B, Waterfield MD. Signalling through phosphoinositide 3-kinases: the lipids take centre stage. *Curr Opin Cell Biol* 1999; **11**: 219-225 [PMID: 10209156]

2 **Yu J**, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA, Backer JM. Regulation of the p85/p110 phosphatidylinositol 3'-kinase: stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. *Mol Cell Biol* 1998; **18**: 1379-1387 [PMID: 9488453]

3 **Yuan TL**, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 2008; **27**: 5497-5510 [PMID: 18794884 DOI: 10.1038/onc.2008.245]

4 **Manning BD**, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* 2007; **129**: 1261-1274 [PMID: 17604717 DOI: 10.1016/j.cell.2007.06.009]

5 **Gonzalez E**, McGraw TE. The Akt kinases: isoform specificity in metabolism and cancer. *Cell Cycle* 2009; **8**: 2502-2508 [PMID: 19597332]

6 **Kumar CC**, Madison V. AKT crystal structure and AKT-specific inhibitors. *Oncogene* 2005; **24**: 7493-7501 [PMID: 16288296 DOI: 10.1038/sj.onc.1209087]

7 **Sarbassov DD**, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005; **307**: 1098-1101 [PMID: 15718470 DOI: 10.1126/science.1106148]

8 **Facchinetti V**, Ouyang W, Wei H, Soto N, Lazorchak A, Gould C, Lowry C, Newton AC, Mao Y, Miao RQ, Sessa WC, Qin J, Zhang P, Su B, Jacinto E. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. *EMBO J* 2008; **27**: 1932-1943 [PMID: 18566586 DOI: 10.1038/emboj.2008.120]

9 **Ikenoue T**, Inoki K, Yang Q, Zhou X, Guan KL. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *EMBO J* 2008; **27**: 1919-1931 [PMID: 18566587 DOI: 10.1038/emboj.2008.119]

10 **Hemmings BA**, Restuccia DF. The PI3K-PKB/Akt pathway. *Cold Spring Harb Perspect Biol* 2015; **7**: pii a026609 [PMID: 25833846 DOI: 10.1101/cshperspect.a026609]

11 **Liu P**, Begley M, Michowski W, Inuzuka H, Ginzberg M, Gao D, Tsou P, Gan W, Papa A, Kim BM, Wan L, Singh A, Zhai B,

Yuan M, Wang Z, Gygi SP, Lee TH, Lu KP, Toker A, Pandolfi PP, Asara JM, Kirschner MW, Sicinski P, Cantley L, Wei W. Cell-cycle-regulated activation of Akt kinase by phosphorylation at its carboxyl terminus. *Nature* 2014; **508**: 541-545 [PMID: 24670654 DOI: 10.1038/nature13079]

12 **Wullschleger S**, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006; **124**: 471-484 [PMID: 16469695 DOI: 10.1016/j.cell.2006.01.016]

13 **Inoki K**, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 2002; **4**: 648-657 [PMID: 12172553 DOI: 10.1038/ncb839]

14 **Karassek S**, Berghaus C, Schwarten M, Goemans CG, Ohse N, Kock G, Jockers K, Neumann S, Gottfried S, Herrmann C, Heumann R, Stoll R. Ras homolog enriched in brain (Rheb) enhances apoptotic signaling. *J Biol Chem* 2010; **285**: 33979-33991 [PMID: 20685651 DOI: 10.1074/jbc.M109.095968]

15 **Jewell JL**, Russell RC, Guan KL. Amino acid signalling upstream of mTOR. *Nat Rev Mol Cell Biol* 2013; **14**: 133-139 [PMID: 23361334 DOI: 10.1038/nrm3522]

16 **Long X**, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J. Rheb binds and regulates the mTOR kinase. *Curr Biol* 2005; **15**: 702-713 [PMID: 15854902 DOI: 10.1016/j.cub.2005.02.053]

17 **Ma XM**, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* 2009; **10**: 307-318 [PMID: 19339977 DOI: 10.1038/nrm2672]

18 **Gwinn DM**, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* 2008; **30**: 214-226 [PMID: 18439900 DOI: 10.1016/j.molcel.2008.03.003]

19 **Hahn-Windgassen A**, Nogueira V, Chen CC, Skeen JE, Sonenberg N, Hay N. Akt activates the mammalian target of rapamycin by regulating cellular ATP level and AMPK activity. *J Biol Chem* 2005; **280**: 32081-32089 [PMID: 16027121 DOI: 10.1074/jbc.M502876200]

20 **Gual P**, Le Marchand-Brustel Y, Tanti JF. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* 2005; **87**: 99-109 [PMID: 15733744 DOI: 10.1016/j.biochi.2004.10.019]

21 **García-Rostán G**, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R, Hermsem MJ, Herrero A, Fusco A, Cameselle-Teijeiro J, Santoro M. Mutation of the PIK3CA gene in anaplastic thyroid cancer. *Cancer Res* 2005; **65**: 10199-10207 [PMID: 16288007 DOI: 10.1158/0008-5472.CAN-04-4259]

22 **Li VS**, Wong CW, Chan TL, Chan AS, Zhao W, Chu KM, So S, Chen X, Yuen ST, Leung SY. Mutations of PIK3CA in gastric adenocarcinoma. *BMC Cancer* 2005; **5**: 29 [PMID: 15784156 DOI: 10.1186/1471-2407-5-29]

23 **Samuels Y**, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004; **304**: 554 [PMID: 15016963 DOI: 10.1126/science.1096502]

24 **Byun DS**, Cho K, Ryu BK, Lee MG, Park JI, Chae KS, Kim HJ, Chi SG. Frequent monoallelic deletion of PTEN and its reciprocal associatioin with PIK3CA amplification in gastric carcinoma. *Int J Cancer* 2003; **104**: 318-327 [PMID: 12569555 DOI: 10.1002/ijc.10962]

25 **Sato K**, Tamura G, Tsuchiya T, Endoh Y, Sakata K, Motoyama T, Usuba O, Kimura W, Terashima M, Nishizuka S, Zou T, Meltzer SJ. Analysis of genetic and epigenetic alterations of the PTEN gene in gastric cancer. *Virchows Arch* 2002; **440**: 160-165 [PMID: 11964046]

26 **Kang YH**, Lee HS, Kim WH. Promoter methylation and silencing of PTEN in gastric carcinoma. *Lab Invest* 2002; **82**: 285-291 [PMID: 11896207]

27 **Chun-Zhi Z**, Lei H, An-Ling Z, Yan-Chao F, Xiao Y, Guang-Xiu W, Zhi-Fan J, Pei-Yu P, Qing-Yu Z, Chun-Sheng K. MicroRNA-221 and microRNA-222 regulate gastric carcinoma cell proliferation and radioresistance by targeting PTEN. *BMC Cancer* 2010; **10**: 367 [PMID: 20618998 DOI: 10.1186/1471-2407-10-367]

28 **Guo J**, Miao Y, Xiao B, Huan R, Jiang Z, Meng D, Wang Y. Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues. *J Gastroenterol Hepatol* 2009; **24**: 652-657 [PMID: 19175831 DOI: 10.1111/j.1440-1746.2008.05666.x]

29 **Wang ZX**, Lu BB, Wang H, Cheng ZX, Yin YM. MicroRNA-21 modulates chemosensitivity of breast cancer cells to doxorubicin by targeting PTEN. *Arch Med Res* 2011; **42**: 281-290 [PMID: 21820606 DOI: 10.1016/j.arcmed.2011.06.008]

30 **Yang Z**, Yuan XG, Chen J, Luo SW, Luo ZJ, Lu NH. Reduced expression of PTEN and increased PTEN phosphorylation at residue Ser380 in gastric cancer tissues: a novel mechanism of PTEN inactivation. *Clin Res Hepatol Gastroenterol* 2013; **37**: 72-79 [PMID: 22521126 DOI: 10.1016/j.clinre.2012.03.002]

31 **Carson JP**, Kulik G, Weber MJ. Antiapoptotic signaling in LNCaP prostate cancer cells: a survival signaling pathway independent of phosphatidylinositol 3'-kinase and Akt/protein kinase B. *Cancer Res* 1999; **59**: 1449-1453 [PMID: 10197612]

32 **Wick W**, Furnari FB, Naumann U, Cavenee WK, Weller M. PTEN gene transfer in human malignant glioma: sensitization to irradiation and CD95L-induced apoptosis. *Oncogene* 1999; **18**: 3936-3943 [PMID: 10435616 DOI: 10.1038/sj.onc.1202774]

33 **Huang J**, Kontos CD. PTEN modulates vascular endothelial growth factor-mediated signaling and angiogenic effects. *J Biol Chem* 2002; **277**: 10760-10766 [PMID: 11784722 DOI: 10.1074/jbc.M110219200]

34 **Cinti C**, Vindigni C, Zamparelli A, La Sala D, Epistolato MC, Marrelli D, Cevenini G, Tosi P. Activated Akt as an indicator of prognosis in gastric cancer. *Virchows Arch* 2008; **453**: 449-455 [PMID: 18841391 DOI: 10.1007/s00428-008-0676-8]

35 **Kobayashi I**, Semba S, Matsuda Y, Kuroda Y, Yokozaki H. Significance of Akt phosphorylation on tumor growth and vascular endothelial growth factor expression in human gastric carcinoma. *Pathobiology* 2006; **73**: 8-17 [PMID: 16785763 DOI: 10.1159/000093087]

36 **Nam SY**, Lee HS, Jung GA, Choi J, Cho SJ, Kim MK, Kim WH, Lee BL. Akt/PKB activation in gastric carcinomas correlates with clinicopathologic variables and prognosis. *APMIS* 2003; **111**: 1105-1113 [PMID: 14678019]

37 **Benayoun BA**, Caburet S, Veitia RA. Forkhead transcription factors: key players in health and disease. *Trends Genet* 2011; **27**: 224-232 [PMID: 21507500 DOI: 10.1016/j.tig.2011.03.003]

38 **Fu Z**, Tindall DJ. FOXOs, cancer and regulation of apoptosis. *Oncogene* 2008; **27**: 2312-2319 [PMID: 18391973 DOI: 10.1038/onc.2008.24]

39 **Datta SR**, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 1997; **91**: 231-241 [PMID: 9346240]

40 **Sangawa A**, Shintani M, Yamao N, Kamoshida S. Phosphorylation status of Akt and caspase-9 in gastric and colorectal carcinomas. *Int J Clin Exp Pathol* 2014; **7**: 3312-3317 [PMID: 25031754]

41 **Bai D**, Ueno L, Vogt PK. Akt-mediated regulation of NFkappaB and the essentialness of NFkappaB for the oncogenicity of PI3K and Akt. *Int J Cancer* 2009; **125**: 2863-2870 [PMID: 19609947 DOI: 10.1002/ijc.24748]

42 **Chao X**, Zao J, Xiao-Yi G, Li-Jun M, Tao S. Blocking of PI3K/AKT induces apoptosis by its effect on NF-κB activity in gastric carcinoma cell line SGC7901. *Biomed Pharmacother* 2010; **64**: 600-604 [PMID: 20947290 DOI: 10.1016/j.biopha.2010.08.008]

43 **Zoncu R**, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol* 2011; **12**: 21-35 [PMID: 21157483 DOI: 10.1038/nrm3025]

44 **Byeon SJ**, Han N, Choi J, Kim MA, Kim WH. Prognostic implication of TSC1 and mTOR expression in gastric carcinoma. *J Surg Oncol* 2014; **109**: 812-817 [PMID: 24615476 DOI: 10.1002/jso.23585]

45 **Yu G**, Wang J, Chen Y, Wang X, Pan J, Li G, Jia Z, Li Q, Yao JC, Xie K. Overexpression of phosphorylated mammalian target of rapamycin predicts lymph node metastasis and prognosis of chinese

patients with gastric cancer. *Clin Cancer Res* 2009; **15**: 1821-1829 [PMID: 19223493 DOI: 10.1158/1078-0432.CCR-08-2138]

46 **He J**, Wang MY, Qiu LX, Zhu ML, Shi TY, Zhou XY, Sun MH, Yang YJ, Wang JC, Jin L, Wang YN, Li J, Yu HP, Wei QY. Genetic variations of mTORC1 genes and risk of gastric cancer in an Eastern Chinese population. *Mol Carcinog* 2013; **52** Suppl 1: E70-E79 [PMID: 23423739 DOI: 10.1002/mc.22013]

47 **Chiang GG**, Abraham RT. Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *J Biol Chem* 2005; **280**: 25485-25490 [PMID: 15899889 DOI: 10.1074/jbc.M501707200]

48 **Xiao L**, Wang YC, Li WS, Du Y. The role of mTOR and phospho-p70S6K in pathogenesis and progression of gastric carcinomas: an immunohistochemical study on tissue microarray. *J Exp Clin Cancer Res* 2009; **28**: 152 [PMID: 20003385 DOI: 10.1186/1756-9966-28-152]

49 **Martini M**, Ciraolo E, Gulluni F, Hirsch E. Targeting PI3K in Cancer: Any Good News? *Front Oncol* 2013; **3**: 108 [PMID: 23658859 DOI: 10.3389/fonc.2013.00108]

50 **Vadas O**, Burke JE, Zhang X, Berndt A, Williams RL. Structural basis for activation and inhibition of class I phosphoinositide 3-kinases. *Sci Signal* 2011; **4**: re2 [PMID: 22009150 DOI: 10.1126/scisignal.2002165]

51 **Samuels Y**, Ericson K. Oncogenic PI3K and its role in cancer. *Curr Opin Oncol* 2006; **18**: 77-82 [PMID: 16357568]

52 **Matter WF**, Brown RF, Vlahos CJ. The inhibition of phosphatidylinositol 3-kinase by quercetin and analogs. *Biochem Biophys Res Commun* 1992; **186**: 624-631 [PMID: 1323287]

53 **Nakanishi S**, Kakita S, Takahashi I, Kawahara K, Tsukuda E, Sano T, Yamada K, Yoshida M, Kase H, Matsuda Y. Wortmannin, a microbial product inhibitor of myosin light chain kinase. *J Biol Chem* 1992; **267**: 2157-2163 [PMID: 1733924]

54 **Vlahos CJ**, Matter WF, Hui KY, Brown RF. A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* 1994; **269**: 5241-5248 [PMID: 8106507]

55 **Bain J**, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, Klevernic I, Arthur JS, Alessi DR, Cohen P. The selectivity of protein kinase inhibitors: a further update. *Biochem J* 2007; **408**: 297-315 [PMID: 17850214 DOI: 10.1042/BJ20070797]

56 **Gharbi SI**, Zvelebil MJ, Shuttleworth SJ, Hancox T, Saghir N, Timms JF, Waterfield MD. Exploring the specificity of the PI3K family inhibitor LY294002. *Biochem J* 2007; **404**: 15-21 [PMID: 17302559 DOI: 10.1042/BJ20061489]

57 **Ihle NT**, Paine-Murrieta G, Berggren MI, Baker A, Tate WR, Wipf P, Abraham RT, Kirkpatrick DL, Powis G. The phosphatidylinositol-3-kinase inhibitor PX-866 overcomes resistance to the epidermal growth factor receptor inhibitor gefitinib in A-549 human non-small cell lung cancer xenografts. *Mol Cancer Ther* 2005; **4**: 1349-1357 [PMID: 16170026 DOI: 10.1158/1535-7163.MCT-05-0149]

58 **Ihle NT**, Williams R, Chow S, Chew W, Berggren MI, Paine-Murrieta G, Minion DJ, Halter RJ, Wipf P, Abraham R, Kirkpatrick L, Powis G. Molecular pharmacology and antitumor activity of PX-866, a novel inhibitor of phosphoinositide-3-kinase signaling. *Mol Cancer Ther* 2004; **3**: 763-772 [PMID: 15252137]

59 **Jimeno A**, Shirai K, Choi M, Laskin J, Kochenderfer M, Spira A, Cline-Burkhardt V, Winquist E, Hausman D, Walker L, Cohen RB. A randomized, phase II trial of cetuximab with or without PX-866, an irreversible oral phosphatidylinositol 3-kinase inhibitor, in patients with relapsed or metastatic head and neck squamous cell cancer. *Ann Oncol* 2015; **26**: 556-561 [PMID: 25524478 DOI: 10.1093/annonc/mdu574]

60 **Pitz MW**, Eisenhauer EA, MacNeil MV, Thiessen B, Easaw JC, Macdonald DR, Eisenstat DD, Kakumanu AS, Salim M, Chalchal H, Squire J, Tsao MS, Kamel-Reid S, Banerji S, Tu D, Powers J, Hausman DF, Mason WP. Phase II study of PX-866 in recurrent glioblastoma. *Neuro Oncol* 2015; **17**: 1270-1274 [PMID: 25605819 DOI: 10.1093/neuonc/nou365]

61 **Hong DS**, Bowles DW, Falchook GS, Messersmith WA, George GC, O'Bryant CL, Vo AC, Klucher K, Herbst RS, Eckhardt SG, Peterson S, Hausman DF, Kurzrock R, Jimeno A. A multicenter phase I trial of PX-866, an oral irreversible phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors. *Clin Cancer Res* 2012; **18**: 4173-4182 [PMID: 22693357 DOI: 10.1158/1078-0432.CCR-12-0714]

62 **Maira SM**, Pecchi S, Huang A, Burger M, Knapp M, Sterker D, Schnell C, Guthy D, Nagel T, Wiesmann M, Brachmann S, Fritsch C, Dorsch M, Chène P, Shoemaker K, De Pover A, Menezes D, Martiny-Baron G, Fabbro D, Wilson CJ, Schlegel R, Hofmann F, García-Echeverría C, Sellers WR, Voliva CF. Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-kinase inhibitor. *Mol Cancer Ther* 2012; **11**: 317-328 [PMID: 22188813 DOI: 10.1158/1535-7163.MCT-11-0474]

63 **Mueller A**, Bachmann E, Linnig M, Khillimberger K, Schimanski CC, Galle PR, Moehler M. Selective PI3K inhibition by BKM120 and BEZ235 alone or in combination with chemotherapy in wild-type and mutated human gastrointestinal cancer cell lines. *Cancer Chemother Pharmacol* 2012; **69**: 1601-1615 [PMID: 22543857 DOI: 10.1007/s00280-012-1869-z]

64 **Park E**, Park J, Han SW, Im SA, Kim TY, Oh DY, Bang YJ. NVP-BKM120, a novel PI3K inhibitor, shows synergism with a STAT3 inhibitor in human gastric cancer cells harboring KRAS mutations. *Int J Oncol* 2012; **40**: 1259-1266 [PMID: 22159814 DOI: 10.3892/ijo.2011.1290]

65 **Bendell JC**, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanse D, De Buck SS, Ru QC, Peters M, Goldbrunner M, Baselga J. Phase I, dose-escalation study of BKM120, an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. *J Clin Oncol* 2012; **30**: 282-290 [PMID: 22162589 DOI: 10.1200/JCO.2011.36.1360]

66 **Brachmann SM**, Kleylein-Sohn J, Gaulis S, Kauffmann A, Blommers MJ, Kazic-Legueux M, Laborde L, Hattenberger M, Stauffer F, Vaxelaire J, Romanet V, Henry C, Murakami M, Guthy DA, Sterker D, Bergling S, Wilson C, Brümmendorf T, Fritsch C, Garcia-Echeverria C, Sellers WR, Hofmann F, Maira SM. Characterization of the mechanism of action of the pan class I PI3K inhibitor NVP-BKM120 across a broad range of concentrations. *Mol Cancer Ther* 2012; **11**: 1747-1757 [PMID: 22653967 DOI: 10.1158/1535-7163.MCT-11-1021]

67 **Koul D**, Fu J, Shen R, LaFortune TA, Wang S, Tiao N, Kim YW, Liu JL, Ramnarian D, Yuan Y, Garcia-Echevrria C, Maira SM, Yung WK. Antitumor activity of NVP-BKM120--a selective pan class I PI3 kinase inhibitor showed differential forms of cell death based on p53 status of glioma cells. *Clin Cancer Res* 2012; **18**: 184-195 [PMID: 22065080 DOI: 10.1158/1078-0432.CCR-11-1558]

68 **Kong D**, Yamori T. ZSTK474 is an ATP-competitive inhibitor of class I phosphatidylinositol 3 kinase isoforms. *Cancer Sci* 2007; **98**: 1638-1642 [PMID: 17711503 DOI: 10.1111/j.1349-7006.2007.00580.x]

69 **Kong DX**, Yamori T. ZSTK474, a novel phosphatidylinositol 3-kinase inhibitor identified using the JFCR39 drug discovery system. *Acta Pharmacol Sin* 2010; **31**: 1189-1197 [PMID: 20729870 DOI: 10.1038/aps.2010.150]

70 **Yaguchi S**, Fukui Y, Koshimizu I, Yoshimi H, Matsuno T, Gouda H, Hirono S, Yamazaki K, Yamori T. Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. *J Natl Cancer Inst* 2006; **98**: 545-556 [PMID: 16622124 DOI: 10.1093/jnci/djj133]

71 **Isoyama S**, Kajiwara G, Tamaki N, Okamura M, Yoshimi H, Nakamura N, Kawamura K, Nishimura Y, Namatame N, Yamori T, Dan S. Basal expression of insulin-like growth factor 1 receptor determines intrinsic resistance of cancer cells to a phosphatidylinositol 3-kinase inhibitor ZSTK474. *Cancer Sci* 2015; **106**: 171-178 [PMID: 25483727 DOI: 10.1111/cas.12582]

72 **Liu N**, Rowley BR, Bull CO, Schneider C, Haegebarth A, Schatz CA, Fracasso PR, Wilkie DP, Hentemann M, Wilhelm SM, Scott WJ, Mumberg D, Ziegelbauer K. BAY 80-6946 is a highly selective intravenous PI3K inhibitor with potent p110α and p110δ activities in tumor cell lines and xenograft models.

Mol Cancer Ther 2013; **12**: 2319-2330 [PMID: 24170767 DOI: 10.1158/1535-7163.MCT-12-0993-T]

73 **Furet P**, Guagnano V, Fairhurst RA, Imbach-Weese P, Bruce I, Knapp M, Fritsch C, Blasco F, Blanz J, Aichholz R, Hamon J, Fabbro D, Caravatti G. Discovery of NVP-BYL719 a potent and selective phosphatidylinositol-3 kinase alpha inhibitor selected for clinical evaluation. *Bioorg Med Chem Lett* 2013; **23**: 3741-3748 [PMID: 23726034 DOI: 10.1016/j.bmcl.2013.05.007]

74 **Garrett JT**, Sutton CR, Kurupi R, Bialucha CU, Ettenberg SA, Collins SD, Sheng Q, Wallweber J, Defazio-Eli L, Arteaga CL. Combination of antibody that inhibits ligand-independent HER3 dimerization and a p110α inhibitor potently blocks PI3K signaling and growth of HER2+ breast cancers. *Cancer Res* 2013; **73**: 6013-6023 [PMID: 23918797 DOI: 10.1158/0008-5472.CAN-13-1191]

75 **Wainberg ZA**, Anghel A, Rogers AM, Desai AJ, Kalous O, Conklin D, Ayala R, O'Brien NA, Quadt C, Akimov M, Slamon DJ, Finn RS. Inhibition of HSP90 with AUY922 induces synergy in HER2-amplified trastuzumab-resistant breast and gastric cancer. *Mol Cancer Ther* 2013; **12**: 509-519 [PMID: 23395886 DOI: 10.1158/1535-7163.MCT-12-0507]

76 **Thorpe LM**, Yuzugullu H, Zhao JJ. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nat Rev Cancer* 2015; **15**: 7-24 [PMID: 25533673 DOI: 10.1038/nrc3860]

77 **Burris HA**. Overcoming acquired resistance to anticancer therapy: focus on the PI3K/AKT/mTOR pathway. *Cancer Chemother Pharmacol* 2013; **71**: 829-842 [PMID: 23377372 DOI: 10.1007/s00280-012-2043-3]

78 **Kuger S**, Cörek E, Polat B, Kämmerer U, Flentje M, Djuzenova CS. Novel PI3K and mTOR Inhibitor NVP-BEZ235 Radiosensitizes Breast Cancer Cell Lines under Normoxic and Hypoxic Conditions. *Breast Cancer* (Auckl) 2014; **8**: 39-49 [PMID: 24678241 DOI: 10.4137/BCBCR.S13693]

79 **Fuereder T**, Wanek T, Pflegerl P, Jaeger-Lansky A, Hoeflmayer D, Strommer S, Kuntner C, Wrba F, Werzowa J, Hejna M, Müller M, Langer O, Wacheck V. Gastric cancer growth control by BEZ235 in vivo does not correlate with PI3K/mTOR target inhibition but with [18F]FLT uptake. *Clin Cancer Res* 2011; **17**: 5322-5332 [PMID: 21712451 DOI: 10.1158/1078-0432.CCR-10-1659]

80 **Janku F**, Tsimberidou AM, Garrido-Laguna I, Wang X, Luthra R, Hong DS, Naing A, Falchook GS, Moroney JW, Piha-Paul SA, Wheler JJ, Moulder SL, Fu S, Kurzrock R. PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitors. *Mol Cancer Ther* 2011; **10**: 558-565 [PMID: 21216929 DOI: 10.1158/1535-7163.MCT-10-0994]

81 **Zhang CH**, Awasthi N, Schwarz MA, Schwarz RE. The dual PI3K/mTOR inhibitor NVP-BEZ235 enhances nab-paclitaxel antitumor response in experimental gastric cancer. *Int J Oncol* 2013; **43**: 1627-1635 [PMID: 24042258 DOI: 10.3892/ijo.2013.2099]

82 **Hart S**, Novotny-Diermayr V, Goh KC, Williams M, Tan YC, Ong LC, Cheong A, Ng BK, Amalini C, Madan B, Nagaraj H, Jayaraman R, Pasha KM, Ethirajulu K, Chng WJ, Mustafa N, Goh BC, Benes C, McDermott U, Garnett M, Dymock B, Wood JM. VS-5584, a novel and highly selective PI3K/mTOR kinase inhibitor for the treatment of cancer. *Mol Cancer Ther* 2013; **12**: 151-161 [PMID: 23270925 DOI: 10.1158/1535-7163.MCT-12-0466]

83 **Bhattacharya B**, Akram M, Balasubramanian I, Tam KK, Koh KX, Yee MQ, Soong R. Pharmacologic synergy between dual phosphoinositide-3-kinase and mammalian target of rapamycin inhibition and 5-fluorouracil in PIK3CA mutant gastric cancer cells. *Cancer Biol Ther* 2012; **13**: 34-42 [PMID: 22336586 DOI: 10.4161/cbt.13.1.18437]

84 **Cheng JQ**, Ruggeri B, Klein WM, Sonoda G, Altomare DA, Watson DK, Testa JR. Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. *Proc Natl Acad Sci USA* 1996; **93**: 3636-3641 [PMID: 8622988]

85 **Hsieh AC**, Truitt ML, Ruggero D. Oncogenic AKTivation of translation as a therapeutic target. *Br J Cancer* 2011; **105**: 329-336 [PMID: 21772331 DOI: 10.1038/bjc.2011.241]

86 **Song G**, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. *J Cell Mol Med* 2005; **9**: 59-71 [PMID: 15784165]

87 **Whang YE**, Yuan XJ, Liu Y, Majumder S, Lewis TD. Regulation of sensitivity to TRAIL by the PTEN tumor suppressor. *Vitam Horm* 2004; **67**: 409-426 [PMID: 15110188 DOI: 10.1016/S0083-6729(04)67021-X]

88 **Davies BR**, Greenwood H, Dudley P, Crafter C, Yu DH, Zhang J, Li J, Gao B, Ji Q, Maynard J, Ricketts SA, Cross D, Cosulich S, Chresta CC, Page K, Yates J, Lane C, Watson R, Luke R, Ogilvie D, Pass M. 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 [PMID: 22294718 DOI: 10.1158/1535-7163.MCT-11-0824-T]

89 **Li J**, Davies BR, Han S, Zhou M, Bai Y, Zhang J, Xu Y, Tang L, Wang H, Liu YJ, Yin X, Ji Q, Yu DH. The AKT inhibitor AZD5363 is selectively active in PI3KCA mutant gastric cancer, and sensitizes a patient-derived gastric cancer xenograft model with PTEN loss to Taxotere. *J Transl Med* 2013; **11**: 241 [PMID: 24088382 DOI: 10.1186/1479-5876-11-241]

90 **Hirai H**, Sootome H, Nakatsuru Y, Miyama K, Taguchi S, Tsujioka K, Ueno Y, Hatch H, Majumder PK, Pan BS, Kotani H. MK-2206, an allosteric Akt inhibitor, enhances antitumor efficacy by standard chemotherapeutic agents or molecular targeted drugs in vitro and in vivo. *Mol Cancer Ther* 2010; **9**: 1956-1967 [PMID: 20571069 DOI: 10.1158/1535-7163.MCT-09-1012]

91 **Molife LR**, Yan L, Vitfell-Rasmussen J, Zernhelt AM, Sullivan DM, Cassier PA, Chen E, Biondo A, Tetteh E, Siu LL, Patnaik A, Papadopoulos KP, de Bono JS, Tolcher AW, Minton S. Phase 1 trial of the oral AKT inhibitor MK-2206 plus carboplatin/paclitaxel, docetaxel, or erlotinib in patients with advanced solid tumors. *J Hematol Oncol* 2014; **7**: 1 [PMID: 24387695 DOI: 10.1186/1756-8722-7-1]

92 **Liang S**, Guo R, Zhang Z, Liu D, Xu H, Xu Z, Wang X, Yang L. Upregulation of the eIF4E signaling pathway contributes to the progression of gastric cancer, and targeting eIF4E by perifosine inhibits cell growth. *Oncol Rep* 2013; **29**: 2422-2430 [PMID: 23588929 DOI: 10.3892/or.2013.2397]

93 **Liu D**, Sun Q, Liang S, Xu L, Luo X, Zhao X, Wang X, Yang L. MicroRNA-27a inhibitors alone or in combination with perifosine suppress the growth of gastric cancer cells. *Mol Med Rep* 2013; **7**: 642-648 [PMID: 23175237 DOI: 10.3892/mmr.2012.1191]

94 **Huang W**, Yang L, Liang S, Liu D, Chen X, Ma Z, Zhai S, Li P, Wang X. AEG-1 is a target of perifosine and is over-expressed in gastric dysplasia and cancers. *Dig Dis Sci* 2013; **58**: 2873-2880 [PMID: 23912246 DOI: 10.1007/s10620-013-2735-5]

95 **Garrett CR**, Coppola D, Wenham RM, Cubitt CL, Neuger AM, Frost TJ, Lush RM, Sullivan DM, Cheng JQ, Sebti SM. Phase I pharmacokinetic and pharmacodynamic study of triciribine phosphate monohydrate, a small-molecule inhibitor of AKT phosphorylation, in adult subjects with solid tumors containing activated AKT. *Invest New Drugs* 2011; **29**: 1381-1389 [PMID: 20644979 DOI: 10.1007/s10637-010-9479-2]

96 **Shimobayashi M**, Hall MN. Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat Rev Mol Cell Biol* 2014; **15**: 155-162 [PMID: 24556838 DOI: 10.1038/nrm3757]

97 **Zhou H**, Luo Y, Huang S. Updates of mTOR inhibitors. *Anticancer Agents Med Chem* 2010; **10**: 571-581 [PMID: 20812900]

98 **Hidalgo M**, Buckner JC, Erlichman C, Pollack MS, Boni JP, Dukart G, Marshall B, Speicher L, Moore L, Rowinsky EK. A phase I and pharmacokinetic study of temsirolimus (CCI-779) administered intravenously daily for 5 days every 2 weeks to patients with advanced cancer. *Clin Cancer Res* 2006; **12**: 5755-5763 [PMID: 17020981 DOI: 10.1158/1078-0432.CCR-06-0118]

99 **Ohtsu A**, Ajani JA, Bai YX, Bang YJ, Chung HC, Pan HM, Sahmoud T, Shen L, Yeh KH, Chin K, Muro K, Kim YH, Ferry D, Tebbutt NC, Al-Batran SE, Smith H, Costantini C, Rizvi S, Lebwohl D, Van Cutsem E. Everolimus for previously treated

advanced gastric cancer: results of the randomized, double-blind, phase III GRANITE-1 study. *J Clin Oncol* 2013; **31**: 3935-3943 [PMID: 24043745 DOI: 10.1200/JCO.2012.48.3552]

100 **Perotti A**, Locatelli A, Sessa C, Hess D, Viganò L, Capri G, Maur M, Cerny T, Cresta S, Rojo F, Albanell J, Marsoni S, Corradino I, Berk L, Rivera VM, Haluska F, Gianni L. Phase IB study of the mTOR inhibitor ridaforolimus with capecitabine. *J Clin Oncol* 2010; **28**: 4554-4561 [PMID: 20855840 DOI: 10.1200/JCO.2009.27.5867]

101 **Xing X**, Zhang L, Wen X, Wang X, Cheng X, Du H, Hu Y, Li L, Dong B, Li Z, Ji J. PP242 suppresses cell proliferation, metastasis, and angiogenesis of gastric cancer through inhibition of the PI3K/AKT/mTOR pathway. *Anticancer Drugs* 2014; **25**: 1129-1140 [PMID: 25035961 DOI: 10.1097/CAD.0000000000000148]

102 **Park JH**, Ryu MH, Park YS, Park SR, Na YS, Rhoo BY, Kang YK. Successful control of heavily pretreated metastatic gastric cancer with the mTOR inhibitor everolimus (RAD001) in a patient with PIK3CA mutation and pS6 overexpression. *BMC Cancer* 2015; **15**: 119 [PMID: 25886409 DOI: 10.1186/s12885-015-1139-7]

103 **Meric-Bernstam F**, Akcakanat A, Chen H, Do KA, Sangai T, Adkins F, Gonzalez-Angulo AM, Rashid A, Crosby K, Dong M, Phan AT, Wolff RA, Gupta S, Mills GB, Yao J. PIK3CA/PTEN mutations and Akt activation as markers of sensitivity to allosteric mTOR inhibitors. *Clin Cancer Res* 2012; **18**: 1777-1789 [PMID: 22422409 DOI: 10.1158/1078-0432.CCR-11-2123]

104 **Janku F**, Hong DS, Fu S, Piha-Paul SA, Naing A, Falchook GS, Tsimberidou AM, Stepanek VM, Moulder SL, Lee JJ, Luthra R, Zinner RG, Broaddus RR, Wheler JJ, Kurzrock R. Assessing PIK3CA and PTEN in early-phase trials with PI3K/AKT/mTOR inhibitors. *Cell Rep* 2014; **6**: 377-387 [PMID: 24440717 DOI: 10.1016/j.celrep.2013.12.035]

105 **Park S**, Zhao D, Hatanpaa KJ, Mickey BE, Saha D, Boothman DA, Story MD, Wong ET, Burma S, Georgescu MM, Rangnekar VM, Chauncey SS, Habib AA. RIP1 activates PI3K-Akt via a dual mechanism involving NF-kappaB-mediated inhibition of the mTOR-S6K-IRS1 negative feedback loop and down-regulation of PTEN. *Cancer Res* 2009; **69**: 4107-4111 [PMID: 19435890 DOI: 10.1158/0008-5472.CAN-09-0474]

106 **Shin EM**, Hay HS, Lee MH, Goh JN, Tan TZ, Sen YP, Lim SW, Yousef EM, Ong HT, Thike AA, Kong X, Wu Z, Mendoz E, Sun W, Salto-Tellez M, Lim CT, Lobie PE, Lim YP, Yap CT, Zeng Q, Sethi G, Lee MB, Tan P, Goh BC, Miller LD, Thiery JP, Zhu T, Gaboury L, Tan PH, Hui KM, Yip GW, Miyamoto S, Kumar AP, Tergaonkar V. DEAD-box helicase DP103 defines metastatic potential of human breast cancers. *J Clin Invest* 2014; **124**: 3807-3824 [PMID: 25083991 DOI: 10.1172/JCI73451]

107 **Zhou BP**, Liao Y, Xia W, Spohn B, Lee MH, Hung MC. Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. *Nat Cell Biol* 2001; **3**: 245-252 [PMID: 11231573 DOI: 10.1038/35060032]

108 **Gartel AL**, Shchors K. Mechanisms of c-myc-mediated transcriptional repression of growth arrest genes. *Exp Cell Res* 2003; **283**: 17-21 [PMID: 12565816]

109 **Baldin V**, Theis-Febvre N, Benne C, Froment C, Cazales M, Burlet-Schiltz O, Ducommun B. PKB/Akt phosphorylates the CDC25B phosphatase and regulates its intracellular localisation. *Biol Cell* 2003; **95**: 547-554 [PMID: 14630392]

110 **Katayama K**, Fujita N, Tsuruo T. Akt/protein kinase B-dependent phosphorylation and inactivation of WEE1Hu promote cell cycle progression at G2/M transition. *Mol Cell Biol* 2005; **25**: 5725-5737 [PMID: 15964826 DOI: 10.1128/MCB.25.13.5725-5737.2005]

111 **Okumura E**, Fukuhara T, Yoshida H, Hanada Si S, Kozutsumi R, Mori M, Tachibana K, Kishimoto T. Akt inhibits Myt1 in the signalling pathway that leads to meiotic G2/M-phase transition. *Nat Cell Biol* 2002; **4**: 111-116 [PMID: 11802161 DOI: 10.1038/ncb741]

112 **Maddika S**, Ande SR, Wiechec E, Hansen LL, Wesselborg S, Los M. Akt-mediated phosphorylation of CDK2 regulates its dual role in cell cycle progression and apoptosis. *J Cell Sci* 2008; **121**: 979-988 [PMID: 18354084 DOI: 10.1242/jcs.009530]

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