Targeting the PI3K/AKT/mTOR Pathway: Biomarkers of Success and Tribulation

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OVERVIEW

PI3K/AKT/mTOR pathway is an established oncogenic driver in humans. Targeted biologic agents against components of this pathway have shown promising activity leading to the approval of the allosteric inhibitors of mTOR, everolimus, and temsirolimus for the treatment of advanced cancers of the kidney, breast, and pancreas. Despite the established and promising activity of this therapeutic strategy, the duration and quality of benefit remains suboptimal in unselected patients. Improved understanding of the biologic consequence of altered PI3K/AKT/mTOR signaling is informing the development of protein (phosphorylated forms of S6, AKT, eIF4e) and genetic (*PIK3CA* mutation, *PTEN* loss of function, *TSC1* and *TSC2* mutation, *PIK3CA-GS* genetic profile) biomarkers to identify patients most likely to benefit from this therapeutic strategy. This review provides an overview of the biologic rational and promising results of protein and genetic biomarkers for selecting patients appropriate for therapy with inhibitors of this pathway.

PHOSPHOINOSITIDE 3-KINASE, AKT, AND MAMMALIAN TARGET OF RAPAMYCIN SIGNALING PATHWAY

The PI3K/AKT/mTOR signaling pathway has been well characterized and recognized to play essential roles in normal cellular functions including nutrition and energy balance, protein synthesis, and growth control in mammalian cells. Eight classes of PI3K kinases have been described in mammalian cells, but only the class I product that can function as second-messenger in intracellular signaling has been implicated in oncogenesis. The class I PI3K protein consists of two main subunits of different sizes, p85 and p110, which, respectively, mediate regulatory and catalytic activity of the enzymes. There are three different isoforms of the p110 catalytic subunit: p110α, β, and δ, encoded by their specific genes, *PIK3CA*, *PIK3CB,* and *PIK3CD*. The p85 regulatory subunit is encoded by three genes: *PIK3R1*, *PIK3R2,* and *PIK3R3*. The PI3K family of lipid and protein kinases is activated by receptor-associated tyrosine kinases and phosphorylate the 3′-hydroxyl group of phosphoinositides to generate phoshatidylinositol-3,4,5-trisphosphate (PIP3).[1–3] PIP3 is an important second messenger that signals through AKT to downstream activators of cellular

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growth and survival, whereas PTEN acts as a negative regulator of PIP3 activity by catalyzing its dephosphorylation. Figure 1 provides a schematic illustration of the interaction and regulation of the PI3K/AKT/mTOR pathway protein members.

Mammalian target of rapamycin (mTOR) is a serinethreonine kinase that is amaster regulator of protein synthesis, along with its important roles in other biologic processes such as cell growth and survival. mTOR activity in the cell is carried out by two distinct complexes. mTORC1 complex is made up of mTOR, raptor, mLST8, and PRAS40. It is very sensitive to rapamycin and activates S6K and inactivates 4E-BP1, leading to protein translation and cell growth. mTORC2 complex is composed of mTOR, rictor, Sin1, and mLST8. It is less sensitive to rapamycin, and its role in normal cell function and oncogenesis has not been well elucidated. However, it is known to activate AKT, thereby promoting cell proliferation and survival. The canonical pathway of mTOR activation depends on mitogen-driven signaling through PI3K/AKT, although alternative non-AKT dependent activation through the Ras/MEK/ERK pathway is now recognized.[4]

ESTABLISHED AND EMERGING THERAPEUTIC ANTICANCER AGENTS TARGETING THE PI3K/AKT/MTOR PATHWAY

Randomized phase III studies have confirmed the efficacy of agents targeting the PI3K/AKT/mTOR axis, leading to the approval of mTOR inhibitors, everolimus, and temsirolimus, for an increasing array of solid malignancies including renal cell carcinoma, hormone refractory breast cancer, pancreatic neuroendocrine cancer, and subependymal giant cell astrocytoma (Table 1). Temsirolimus binds to an intracellular protein, FKBP-12, to form an inhibitory complex that abrogates mTOR signaling. In a phase III study in poor-risk advanced kidney cancer, 626 patients were randomized to receive interferon-alpha alone, temsirolimus alone, or the combination of both agents.[5] Temsirolimus showed superior overall survival (OS) over interferon alone (HR: 0.73; 95% CI: 0.58–0.92; p = 0.008), whereas the 2-drug combination failed to show any additional benefit to patients treated with IFN-α (HR: 0.96; 95% CI: 0.76–1.20; p = 0.70). The median OS for interferon alone, temsirolimus alone, and the 2-drug combination were 7.3, 10.9, and 8.4 months, respectively. The outcome of this study was the basis for the FDA approval of temsirolimus for advanced poor-risk kidney cancer.

Everolimus is one of the most investigated mTOR inhibitor to date and has received regulatory approval for various tumor types. In a phase III study in patients with breast cancer previously treated with nonsteroidal hormone therapy, 724 patients randomized to receive everolimus plus exemestane or exemestane plus placebo were compared for progression free survival (PFS). At preplanned interim analysis, the everolimus-containing arm was superior, with median PFS of 6.9 months versus 2.8 months (HR: 0.43; 95% CI: 0.35–0.54; p<0.001) by investigator assessment and 10.6 months versus 4.1 months (HR: 0.36; 95% CI: 0.27–0.47; p < 0.001) by central review.[6]

The RADIANT3 study randomized 410 patients with advanced, progressing, low-grade, or intermediate-grade pancreatic neuroendocrine tumors to everolimus or placebo. There was significant clinical benefit of everolimus therapy, evidenced by amedian PFS of 11.0 months versus 4.6 months with placebo (HR: 0.35; 95% CI: 0.27–0.45; p < 0.001).[7] Based on these positive results, everolimus is now approved for the treatment of this disease.

Similarly, 410 patients with metastatic renal cell carcinoma previously treated with antiangiogenic agents were randomized to receive everolimus or placebo in conjunction with best supportive care. The study was terminated after a specified interim analysis demonstrated a significant PFS benefit in favor of everolimus (median PFS of 4.0 months

[95% CI: 3.7–5.5] vs. 1.9 months [1.8–1.9] months; HR: 0.30, 95% CI: 0.22–0.40, p < 0.0001).[8]

Tuberous sclerosis complex (TSC) is amultisystem disease mediated by autosomal dominant genetic mutations in *TSC1* (hamartin) and *TSC2* (tuberin) genes. The disorder is characterized by benign hamartomatous growths in different organs, most commonly skin, brain, kidney, lung, heart, and retina. The *TSC* genes encode a tumor-suppressor complex that controls activation of the mTOR pathway through the Ras homolog enriched in brain (RHEB) protein. Loss of this suppressor activity as a result of mutation in either *TSC1* or *TSC2* allows for constitutive signaling and activation of the mTOR pathway, leading to abnormal cellular growth, proliferation, and protein synthesis.[9] Elucidation of the TSC1 signaling cascade and its role as a critical node that negatively modulates the propagation of signals from upstream PI3K and AKT to the mTOR complex informed the clinical evaluation of mTOR inhibitors in patient groups with symptomatic manifestations of the TSC. The EXIST-1 study randomized 78 pediatric and adult patients with progressive or symptomatic subependymal giant cell astrocytoma to everolimus and 39 to placebo. Objective response (minimum of 50% reduction in tumor volume) was seen in 35% of patients in the everolimus group compared with 0% in the placebo group (difference 35%, 95% CI: 15–52; p<0.0001).[10] In the EXIST-2 study, patients 18 years or older with angiomyolipoma measuring at least 3 cmor larger in diameter (defıned by radiological assessment) in the setting of a defınite TSC diagnosis or sporadic lymphangioleiomyomatosis were assigned to oral everolimus (79 patients) or placebo (39 patients). Similar to the EXIST-1 study, patients treated with everolimus achieved a response rate of 42% (95% CI 31% to 53%) versus 0% for placebo (response rate difference 42% [24% to 58%]; p < 0.0001).[11] Everolimus received FDA approval for the treatment of these TSC-associated diseases based on the positive outcome of these studies.

Many other agents currently in preclinical and clinical evaluation specifıcally target *PI3K* or the *AKT* protein (Table 2). Although encouraging activity against various cancer types has been recorded, none of these agents has demonstrated suffıcient effıcacy for regulatory approval.

BIOMARKERS OF EFFICACY AND RESISTANCE

Genetic Biomarkers

Germline loss or acquired somatic mutations in the mammalian *phosphatase and tensin homolog (PTEN*) gene locus on chromosome 10q are among the most common aberrations observed in solid malignancies.[3,12] In addition, exon 9 (E542K and E545K) and exon 20 (H1047R) mutations of the *phosphoinositide-3-kinase, catalytic, polypeptide (PIK3CA*) gene also promote oncogenesis.[13] Because tumors that harbor these genetic events have uncontrolled constitutively active PI3K enzyme, they are expected to be sensitive to agents targeting this pathway. Robust evidence supports the predictive capability of these genetic aberrations in preclinical models,[14,15] but validation in the clinical setting remains limited and, at times, contradictory.[16] The interplay between *PTEN* loss and activating *PIK3CA* is an interesting example of how a genetic alteration that predicts for sensitivity in one cancer type (endometrial cancer) may fail to predict for effıcacy in another (in breast cancer).[16] Activating *PIK3CA* mutations or *HER2* amplifıcation conferred remarkable sensitivity to inhibitors of the PI3K/Akt/mTOR such as BKM120, GDC-0941, everolimus and PP24237, whereas PTEN loss was not predictive in a panel of breast cancer cell lines. Combined presence of *HER2* gene amplifıcation along with *PIK3CA* mutation was found to be highly predictive of sensitivity to GDC-0941.[17] The reason for this observation may reside in differences in biologic consequences of each of these specifıc mutations. Although activating *PIK3CA* mutations and *PTEN* loss both result in PI3K/AKT/mTOR pathway

activation, the downstream effects and the mediators recruited by these genetic alterations are dissimilar. For instance, *PIK3CA*-mutant tumors require p110-α activation to sustain cellular proliferation, which may occur through AKT or via PDK1 and its substrate SGK3.[18] *PTEN*-deficient cancer cells, on the other hand, depend on the kinase activity of p110-β, which is not sufficient to induce sustained cellular proliferation.[19] This difference is biologically relevant and demonstrates the need for careful patient and therapeutic agent selection for optimal clinical benefit. This observation has informed the development of isoform-specific PI3K inhibitors and studies designed to evaluate them in PTEN-deficient tumors.

Gene expression and protein data from approximately 1,800 patients with breast cancer were used to develop a *PIK3CA* mutation-associated gene signature (PIK3CA-GS). This signature predicted the *PIK3CA* mutation status in two independent datasets and also identified rapamycin-resistant cell lines in preclinical studies.[20] The ability of this gene signature to estimate PI3K pathway activation was assessed in tumor samples from patients with breast cancer enrolled in two prospective neoadjuvant clinical trials of everolimus. Relative change from baseline to day 15 in Ki67 (a proliferative and prognostic marker in breast cancer) and pS6 was correlated with the baseline PIK3CA-GS profile. Patients with the largest relative decreases in Ki67 following combined letrozole/everolimus therapy were identified ($R = -0.43$, $p = 0.008$) by the PIK3CA-GS profile. In contrast, there was no significant correlation between PIK3CA-GS profile and Ki67 in the letrozole/placebo group ($R = 0.07$, $p=0.58$). Similarly, PIK3CA-GS profile was inversely correlated ($R=-0.46$, $p = 0.028$) with relative change in pS6 in patients treated with single agent everolimus using an independent dataset obtained from patients enrolled in a neoadjuvant study of everolimus in surgically resectable breast cancer. Although there was no significant correlation of the PIK3CA-GS profile with any survival end point, the results indicate that the profile outperforms PIK3CA genotyping as a marker of pathway activation and may be useful for identifying patients with breast and other types of cancer who are likely to respond to this therapeutic strategy.[21]

De novo and acquired genetically mediated treatment resistance—The existence of de novo resistance to inhibitors of the PI3K/AKT/mTOR pathway is indicated by the large number of patients treated on studies who derive no meaningful clinical benefit. Understanding the biologic basis of de novo resistance to therapy may therefore help in identifying patients unlikely to benefit from this class of anticancer agents. Preclinical work showed that an activated MAPK pathway, induced by KRAS mutation, selects for cell lines unlikely to respond to this class of agents, whereas isolated oncogenic *PIK3CA* alterations sensitized cells to everolimus, both in vitro and in vivo. Concomitant or exogenous introduction of *KRAS* mutations, however, rendered the cells insensitive, although genetic ablation of mutant *KRAS* from cells with coexisting *PIK3CA* and *KRAS* reinstated drug sensitivity. This lack of benefit of mTOR inhibitor therapy in the presence of *KRAS* mutation was reproduced in a cohort of patients treated with everolimus.[22] Interestingly, de novo resistance mediated by *KRAS* is mitigated by coexisting LKB1-deficiency as well as p53 loss.[23] Given the co-occurrence of *LKB1* and *KRAS* alterations in lung cancer, this finding will be especially useful in therapeutic studies of PI3K/AKT/mTOR inhibitors in this population.

Acquired resistance may also be mediated by genetic alteration that develops under selective pressure by the therapeutic agent. Although no such genetic alteration has yet been reported from human trials, basic research in *S. cerevisae* identified a hotspot in Ile800 of the *PIK3CA* gene, which confers a 5 to 10-fold decrease in potency for a large panel of mTOR and PI3K inhibitors. Interestingly, these resistant mutations do not reside in the classic gatekeeper residues, unlike the observation with tyrosine kinase inhibitors.[24]

Protein Biomarkers

Preclinical work showed that sensitivity to the PI3K inhibitor, GDC-0941, was associated with high baseline expression of the mTOR pathway protein, p4E-BP1, and pAkt by gene expression assay in the NCI-60 human tumor cell line screening study.[25] A predictive model for mTOR inhibitor therapy efficacy was investigated by using data obtained from extensive pharmacokinetic and pharmacodynamic measurements in human and animal studies treated with various mTOR inhibitors. [26] Univariate analysis showed significant correlation of efficacy with pS6 ($R=0.53$, $p<0.01$), total S6 ($R=-0.51$, $p < 0.02$), pAKT ($R = 0.66$, $p < 0.001$), pS6/total S6 ($R = 0.76$, $p < 0.0001$). Using logistic regression and ROC algorithm, the combination of high pAKT and high p235-S6/total S6 as a predictor of sensitivity and low pAKT and low p235-S6/total S6 as a predictor of insensitivity demonstrated 73% and 100% sensitivity and specificity, respectively and an AUC of 0.88.[26] Although this combination of biomarkers has not been validated in a prospective human patient study, findings from early phase clinical studies support the utility of pAKT in tumor samples as a predictor of sensitivity to mTOR inhibitor therapy.[27,28]

Complementary pathway protein modulation—mTOR signaling inhibition by nonspecific allosteric inhibitors such as rapamycin suppressed p70S6 kinase activity but paradoxically also increased the activation of AKT and eIF4E, which attenuated the therapeutic efficacy of these agents. This observation provided the initial insight into the role that compensatory feedback loop through AKT may play in the ability of treated cells to overcome mTOR inhibitor therapy. [29] It also supported the impetus to develop TORC1 and TORC2-specific inhibitors as well as dual mTOR/PI3K inhibitors that can inhibit downstream mTOR signaling with minimal compensatory AKT activation. Several such compounds have entered the clinical testing phase but whether they achieve superior efficacy over nonselective mTOR inhibitors is still unclear. Other feedback or alternative signaling loops that mediate resistance involve an mTOR-dependent serine phosphorylation of insulin receptor substrate-1 (IRS-1) that enhances insulin-like growth factor-1 (IGF-1) signaling leading to downstream activation of PI3K/AKT. Efforts to exploit these findings in the clinic include a combination strategy of an mTOR and IGFR inhibitor.[30]

The efficacy of mTOR inhibitors has also been ascribed to the involvement of the mTOR pathway in tumor-related angiogenesis and may be significant in renal cell cancer in which loss of the tumor suppressor *VHL* gene promotes a high level of HIF-α mRNA expression. Similar to observations with cytotoxic chemotherapy in which HIF-1α overexpression induces tumor resistance to chemotherapy, a high level of HIF-1α activity may predict for benefit from mTOR inhibitor therapy but may therefore provide an escape mechanism leading to treatment resistance. Consistent with this theory, the level of expression of the proangiogenic factor, VEGF-D, has been shown to correlate with disease burden and decreases following treatment with sirolimus in patients with TSC.[31]

A newly identified biomarker of resistance is the p21-activated kinase 1 (PAK1) protein, which is involved in pro-survival cell signaling and was previously reported as a marker of poor prognosis in patients with solid malignancies. PAK1 protein expression was shown to mediate resistance to PI3K and PI3K/mTOR inhibitors in lymphoma cell lines.[32] Clinical validation of this biomarker is still awaited.

Drug-Induced Toxicities as Surrogate Biomarker

The currently approved agents targeting the PI3K/AKT/mTOR pathway and those in early stage development have demonstrated a tolerable toxicity profile. The most common adverse events reported include fatigue, stomatitis, hyperglycemia, hyperlipidemia, and skin rash. Rare but potentially life-threatening noninfectious pneumonitis with mTOR inhibitors

and mood disorder with PI3K inhibitors have also been encountered. The metabolic consequence of hyperglycemia and hyperlipidemia is, however, an intriguing class effect of uncertain pathophysiology. By attenuating the expected increase in insulin level as a physiologic response to hyperglycemia, rapalogs induce a state of insulin resistance leading to increased glucose level. Similarly, inhibitors of this pathway cause an impaired hepatic fatty acid metabolism leading to increased β-oxidation of fatty acid along with reduced influx of free fatty acid into anabolic storage pathways, resulting in increased serum levels of triglyceride and cholesterol.[33,34] A retrospective analysis of cholesterol, triglycerides, and glucose measurements from patients in a prospective randomized study of IFN-α compared with temsirolimus showed a significant association between increased cholesterol and longer survival (OS: HR = 0.77 per mmol/L, $p<0.0001$; PFS: HR=0.81 per mmol/L; $p<0.0001$). Interestingly, the survival benefit associated with temsirolimus was completely explained by the increase in cholesterol on multivariate analysis. It is plausible that with better understanding of the pathophysiologic basis, these metabolic toxicities may be used as surrogate biomarkers of target modulation similar to the use of skin rash and development of hypertension in patients treated with EGFR and angiogenic inhibitors, respectively.

CONCLUSION

The PI3K/AKT/mTOR pathway is an established driver of oncogenesis in human patients. Agents targeting different components of this pathway are in clinical development with regulatory approval for marketing granted for everolimus and temsirolimus. Increased understanding of the mechanism of action of these agents continues to inform their clinical development including combination approaches to prevent or overcome treatment resistance and the development of isoform specific PI3K inhibitors for specific patient subsets defined by driver molecular aberration. Clinical toxicity of hyperglycemia and hyperlipidemia are easily managed class effects of these agents that may also serve as pharmacodynamic markers of activity.

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KEY POINTS

- The PI3K/AKT/mTOR pathway is an established oncogenic driver in a variety of tumor types.
- Inhibitors of this pathway are already in clinical testing, and U.S. Food and Drug Administration (FDA) approval for everolimus and temsirolimus have validated this pathway as a therapeutic target.
- Predictive biomarkers of efficacy (PIK3CA mutation, PTEN loss, and high pAKT and pS6 protein expression by IHC) and resistance (KRAS) developed in the preclinical setting and early phase clinical trials hold promise pending prospective validation.
- Adverse events such as metabolic toxicity with impaired glycemic and lipid control and mood alterations are class effects that may be exploited to guide appropriate dosing of these agents in the clinic.

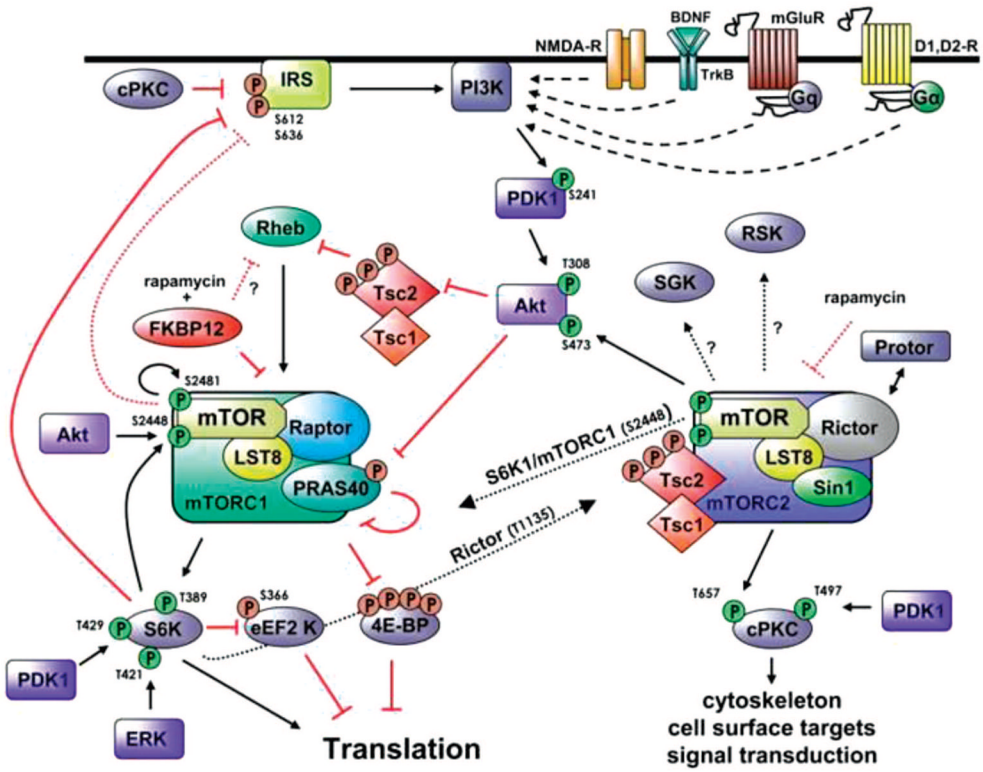

FIG 1.

PI3K/AKT/mTOR signaling pathway showing interacting activation and regulatory feedback loops. Adapted from Hoeffer and Klann.[35]

TABLE 1

Pivotal Clinical Trials Leading to Regulatory Approval of PI3K/AKT/mTOR Inhibitors

Study	Regimen/Design	N	PFS or RR	OS	Indication
Hudes et al.[5]	IFN-alpha versus temsirolimus versus temsirolimus + IFN-alpha 1:1:1 randomization	626	1.9 versus 3.8 versus 3.7 mo	0.73; (95% CI: 0.58 to 0.92); p = 0.008	Advanced RCC with poor prognosis
Baselga et al.[6]	Everolimus + exemestane versus exemestane 2:1 randomization	724	HR: 0.36; (95% CI: 0.27–0.47); p < 0.001 10.6 versus 4.1 mo	Immature data	Hormone-receptor (+) advanced breast cancer patients following failure of a nonsteroidal aromatase inhibitor
Motzer et al.[8]	Everolimus versus placebo 2:1 randomization	410	0.30 (95% CI:0.22–0.40, p < 0.0001); 4.0 versus 1.9	NR	Metastatic RCC previously treated with sunitinib, sorafenib, or both
Bissler et al.[11]	Everolimus versus placebo; 2:1 randomization	118	Response rate difference 42% [24–58%]; p < 0.0001	NR	Angiomyolipoma (sporadic and TSC-associated)
Franz et al.[10]	Everolimus versus placebo; 2:1 randomization	117	RR difference 35% (95% CI: 15–52; p < 0.0001)	NR	Subependymal giant cell astrocytomas associated with TSC
Yao et al.[7]	Everolimus versus placebo 1:1 randomization	410	0.35 (95% CI:0.27–0.45; p < 0.001) 11.0 versus 4.6 mo	1.05 (95% CI: 0.71–1.55; p = 0.59	Low/intermediate-grade advanced pancreatic neuroendocrine tumor

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TABLE 2

Inhibitors of PI3K/AKT/mTOR Pathway in Development

Class	Compound	Target Selectivity	Stage in Development
PI3K			
	AZD6482	PI3Kβ	Preclinical
	AR245408 (XL147)	Pan PI3K	Phase I
	PX-866	Pan PI3K	Phase II
	BKM120	Pan PI3K	Phase II
	GDC-0980	PI3K/mTOR	Phase II
	GDC-0941	PI3K	Phase II
	BYL719	PIK3α	Phase II
	PF-04691502	PI3K/mTOR	Phase II
	GSK2636771	PIK3β	Phase II
	BAY 80–6946	PIK3	Phase I
	ONC-01910	PI3K and Plk1	Phase III
AKT			
	MK-2206	AKT	Phase II
	VIII	AKT 1 &2	Preclinical
	AZD5363	Pan AKT	Phase I
	Triciribine (API-2)	Akt 1, 2, 3	Phase I
	SR13668	Akt	Preclinical
	AR-67 (DB-67)	Akt	Phase I, II
	AR-42	Akt	Preclinical
	GSK690693	Akt1, 2, 3	Phase I
	KP372–1	Akt, PDK-1, Flt3	Preclinical
	VQD-002 (API-2)	Akt	Phase I, II
	A-443654	Akt	Preclinical
mTOR			
	Rapamycin (Sirolimus)	mTORC1	FDA approved (non Oncology) Phase II
	Everolimus	Allosteric TORC1/2 inhibitor	FDA Approved
	Temsirolimus	FKBP-12	FDA approved
	Ridaforolimus	Allosteric TORC1/2 inhibitor	Phase III
	NVP-BEZ235	PIK3/mTOR	Phase II
	BGT226	PIK3/mTOR	Phase II
	WYE-354	mTOR ATP competitive	Preclinical
	AZD-8055	mTORC1/mTORC2	Phase I, II
	OSI-027	mTORC1/mTORC2	Phase I
	INK-128	mTORC1/mTORC2	Phase I
	PP-242	mTORC1/mTORC2	Phase I
	ONC-01910	Non-ATP Plk1 and PI3K	Phase III