Targeting the PI3K-AKT-mTOR signalling network in cancer

Khurum H. Khan¹, Timothy A. Yap², Li Yan³,⁴,⁵ and David Cunningham¹

Abstract

The phosphoinositide 3-kinase-AKT-mammalian target of rapamycin (PI3K-AKT-mTOR) pathway is a frequently hyperactivated pathway in cancer and is important for tumor cell growth and survival. The development of targeted therapies against mTOR, a vital substrate along this pathway, led to the approval of allosteric inhibitors, including everolimus and temsirolimus, for the treatment of breast, renal, and pancreatic cancers. However, the suboptimal duration of response in unselected patients remains an unresolved issue. Numerous novel therapies against critical nodes of this pathway are therefore being actively investigated in the clinic in multiple tumor types. In this review, we focus on the progress of these agents in clinical development along with their biological rationale, the need of predictive biomarkers and various combination strategies, which will be useful in counteracting the mechanisms of resistance to this class of drugs.

Key words PI3K-AKT-mTOR, PTEN, RTK, signaling pathways, molecular therapeutics

Despite significant advancements in the management of solid malignancies over the last six decades, the prognosis of advanced solid malignancies is generally limited to months. In this era of personalized medicine, the focus of treatment has been to exploit the principles of oncogenic addiction and synthetic lethality in order to identify optimal targets for various cancers. Over the last two decades, the phosphoinositide 3-kinase (PI3K) pathway has been studied extensively in view of the growing evidence supporting the critical role of this pathway in cancer progression, as it influences metabolism, tumor growth, survival, and the development of metastases.¹⁻⁴ Consequently, several drugs have been developed to target this pathway to treat human cancers. These agents have shown some preclinical and clinical activity; however, they have largely failed to live up to the expectations of clinicians and scientists. Further significant efforts are therefore required to target this pathway effectively. In this review, we examine the current literature demonstrating the role of the PI3K pathway in the development and progression of cancer. We review various existing inhibitors of this pathway; their preclinical activity and clinical efficacy, along with possible reasons for their failure; and potential treatment strategies for overcoming resistance to inhibitors of this pathway.

The PI3K-AKT-mTOR Pathway in Cancer

The PI3K-AKT-mTOR signaling in cancer cell growth and survival

The family of lipid kinases termed PI3K are regarded as key regulators in many essential cellular processes, including cell survival, growth, and differentiation.¹⁻⁴ The PI3K pathway has several important nodes that play a crucial role in this pathway, resulting in a diversity of functional outcomes. The AKT-mediated activation of downstream targets, including mammalian target of rapamycin (mTOR), stimulates cell proliferation and the regulation of translation in response to growth factors by the phosphorylation of the protein synthesis machinery (Figure 1) [⁷]. This translational promotion by mTOR
includes the phosphorylation of ribosomal protein S6 kinases (S6K) and 4E-binding protein 1 (4E-BP1), the latter of which results in the release of eukaryotic translation initiation factor 4E (eIF4E), which has known anti-apoptotic activities in vitro. These effects are counteracted by the tuberous sclerosis complex-1 (TSC1)-TSC2 complex, which has inhibitory effects on 4E-BP1 and eIF4E. AKT also phosphorylates and inhibits TSC2, which demonstrates the complexity of this pathway. Intriguingly, rapamycin and its analogues can inhibit mTOR, but this can lead to activation of upstream proteins such as AKT, due to the loss of a feedback loop mechanism.

In addition, the PI3K-AKT pathway interacts with the complex molecular mechanism that controls cellular energy control and glucose metabolism. AKT phosphorylates and inhibits glycogen synthase kinase-3 (GSK3), phospho-diesterase-3B, protein phosphatase 2A, and Raf-1. The PI3K signaling also controls growth, proliferation, senescence, and angiogenesis. These processes are regulated by vascular endothelial growth factor (VEGF) transcriptional activation and hypoxia-inducible factor-1alpha (HIF-1α) expression.

This brief summary of the PI3K-AKT pathway involvement in various important cell growth mechanisms highlights the complexity and importance of several nodes within this pathway.

Resistance to anti-cancer therapy

It is well established that inhibition of any of the nodes of the PI3K-AKT pathway can restore vulnerability to chemotherapy, radiotherapy, and hormonal treatment. Several studies have demonstrated a link between drug resistance and altered AKT signaling. Knuefermann et al. showed that the cell lines expressing both HER2 and HER3 in breast adenocarcinoma had a higher

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**Figure 1** Schematic diagram of signaling through the phosphoinositide 3-kinase-AKT (PI3K-AKT) pathway with current and future treatment strategies. PI3K signaling impacts on cell growth, survival, differentiation, and proliferation. The pointed arrows represent activation of substrates, while blunt arrows represent inhibition. Activation of membrane kinases, such as insulin-like growth factor-1 (IGF1), by external growth factors can initiate activation of intracellular signaling pathways. AKT is phosphorylated downstream of PI3K with various effects, including the activation of mammalian target of rapamycin (mTOR). mTOR phosphorylates p70S6 and 4E-binding protein 1 (4EBP-1), which then leads to an increased translation of mRNA that encodes several cell cycle regulators. These effects are controlled by the tuberous sclerosis 1 (TSC1)-TSC2 complex. The Ras-Raf-MEK-MAPK pathway has its own distinct downstream effects, but also converges with the PI3K-AKT pathway. Future combination regimens involving targeted agents against this signaling network include the concomitant or sequential blockade of these pathways. PTEN, phosphatase and tensin homolog; HIF-1α, hypoxia-inducible factor-1α; MAPK, mitogen-activated protein kinase.
phosphorylation level of AKT and were associated with increased resistance to multiple chemotherapeutic agents. Selective inhibition of PI3K or AKT activity leads to induction of apoptosis; therefore, the proposed mechanism for this synergy was the potentiation of apoptosis.

**Deregulation of the PI3K-AKT-mTOR pathway in cancer**

The PI3K-AKT pathway can be inappropriately activated in various cancers. The two major observed mechanisms of PI3K-AKT activation in human cancers are somatic alterations in specific nodes of the pathway and activation by receptor tyrosine kinases (RTKs). Our understanding of these mechanisms of PI3K pathway activation is crucial for developing effective therapeutic approaches and gaining a clinical benefit from PI3K inhibition.

**Somatic alterations of PI3K pathway components in cancer**

Genetic mutations/loss of function

Several genetic alterations are known to activate the PI3K-AKT signaling, but the second most common genetic abnormality found in human cancer is inactivation of the PTEN tumor suppressor gene. PI3K signaling is inhibited by PTEN through the dephosphorylation of phosphatidylinositol-3,4,5-triphosphate (PIP3), which is the lipid-signaling product of the class I PI3Ks. The vast majority of these PTEN mutations are protein truncations, whereas missense mutations are also common. Transcriptional repression and epigenetic silencing of PTEN are other observed mechanisms of PTEN inactivation. Preclinical studies have shown that the heterozygous loss of PTEN in mice resulted in neoplasia of multiple epithelia, including the prostate, intestine and mammary gland. Homozygous deletion of PTEN in the prostate epithelium can lead to aggressive prostate carcinoma. It has been shown that cancers with high Gleason scores in primary tumors tend to be associated with PTEN loss in metastases. More recently, Mueller et al. examined the signaling pathways that underlie the pathogenesis of pediatric gliomas and assessed the activation of the PI3K-AKT-mTOR pathway by using immunohistochemistry. They evaluated the downstream signaling molecules phosphorylated p-S6, p-PRAS40, and PTEN, as well as PTEN promoter methylation and the MIB labeling index. They found that the majority (80%) of high-grade gliomas showed activation of the PI3K-AKT-mTOR pathway and that 50% had PTEN promoter methylation. Tumor grade correlated negatively with PTEN expression and positively with p-S6 and p-4EBP1 levels. Trends toward an inverse correlation of PTEN promoter methylation with PTEN protein expression and a direct correlation of p-S6 and p-4EBP1 levels with poor clinical outcomes, as measured by progression-free survival, were also noted. It was concluded that the majority of pediatric gliomas show activation of the PI3K-AKT-mTOR pathway, with PTEN promoter methylation being a common feature of these tumors. Germline mutations in the PTEN gene can result in Cowden disease and Bannayan-Riley-Ruvalcaba syndrome (associated with macrocephaly, multiple lipomas, and hemangiomas), two conditions that are associated with high risk of malignancies. Unlike other tumor suppressor genes, such as p53, biallelic inactivation is not required for the suppression of PTEN activity; rather, haplo-insufficiency may suffice in promoting tumorigenesis. This suggests that reduced PTEN protein expression without actual mutations may be another mechanism of PTEN hindrance leading to cancer growth.

Genetic amplification of PIK3CA and AKT1/2

Recent studies have shown that somatic mutations in PIK3CA are common in a variety of human tumors, including breast, colon, and endometrial cancers and glioblastoma. The two common mutation regions are clustered in exons 9 and 20, which encode the helical and catalytic domains of p110α, respectively. A small cluster of mutations is also found in the N-terminal p85α-interacting domain, which can increase the lipid kinase activity of p110α. However, these mutations do not alter the interaction between p110α and p85α subunits. The PIK3CA mutations increase in vitro PI3K activity, and the expression of p110α mutants in cells confers AKT activation in the absence of growth factor stimulation, which in turn leads to oncogenesis. So far, no other p110 isoform mutations have been identified, indicating that p110α harbors the main oncogenic potential. Preclinical studies have shown that transgenic mice with induction of kinase domain mutant p110α H1047R developed lung adenocarcinoma. Likewise, similar mouse-knockout and transgenic models confirm the tumorigenic potential of hyperactivation of the PI3K pathway.

AKT overexpression

There is now growing evidence that different AKT isoforms have non-overlapping functions in cancer. A single amino acid substitution, E17K, in the lipid-binding PH domain of AKT-1 has been identified in various human cancers including breast, colorectal, endometrial, and ovarian cancers. AKT-2 overexpression has been observed in colorectal cancers and metastases. It is proposed that AKT-2 promotes cellular survival and growth. Interestingly, it was noted that the loss of AKT-1 promoted cellular invasion and metastases, possibly by shifting the balance of signaling through AKT-2. The E17K mutation has been found in some melanomas.
Mutations in various AKT isoforms suggest a potential role for AKT inhibitors in therapy, which is discussed below. Notably, in addition to somatic mutations of PTEN, PIK3CA, PIK3R1, and AKT, some cancers have amplifications of AKT-1, AKT-2, and PIK3CA; however, it is not entirely clear if these amplifications have a significant impact on clinical outcome.

Pathway activation by receptor tyrosine kinases (RTKs) and Ras

It is well established that RTK-mediated activation of PI3K is of crucial importance for its oncogenic activity and that it is clearly linked to the RTK signaling. The p85 regulatory subunit is vital in mediating PI3K activation by RTKs. When certain therapies are effective in targeting RTKs, they invariably lead to suppression of PI3K signaling. Examples include PI3K activation by epithelial growth factor receptor (EGFR) in lung cancers harboring somatic activating mutations in EGFR[36] and human epidermal growth factor receptor 2 (HER2) mutations in breast cancers with HER2 amplification[37]. Thus when these cancers are successfully treated, the PI3K signaling is switched off as a result of targeting RTKs. Unfortunately, in some cancers, multiple RTKs activate PI3K signaling, and these cancers tend to be resistant to single RTK-targeted therapies[38]. PI3K is also an effector of Ras-mediated oncogenic signaling, which is a small GTPase that is frequently mutated in human cancers. Studies suggest that a direct link exists between Ras and PI3K. Preclinical studies showed that mutant p110α-inhibited K-Ras–induced lung adenocarcinoma in genetically engineered mouse models[39]. This approach has been rationalized in early phase human clinical trials where a combination of MEK and AKT inhibitors has been examined in patients with K-Ras mutated lung adenocarcinoma. However, it remains unclear whether mutated Ras is sufficient to directly activate PI3K and thereby bypass its engagement with phosphotyrosines.

Inhibitors in Clinical Stage Development

There are five major classes of inhibitors designed to target various nodes of the PI3K-AKT-mTOR pathway. Agents in active clinical development are summarized in Table 1.

| PI3K inhibitors |
|------------------|
| Pan-PI3K inhibitors |
| Pan-PI3K inhibitors target all Class IA PI3Ks and are represented by several small molecule drugs, XL147, PX-866, BKM120, GDC-0941, BAY806946, GSK2126458, and CH5132799, and one RNA interfering (RNAi) agent, ATU027. In general, the single agent |
| activities of these pan-PI3K inhibitors have been limited to the prolonging of RECIST stable disease rather than tumor regression, in line with being cytostatic or cytoreductive as monotherapy in preclinical models. Mechanism-based side effects may limit dose escalation further, resulting in suboptimal target engagement and impaired efficacy. |
| XL147, a selective inhibitor of Class I PI3K isoforms, has a maximally tolerated dose (MTD) of 600 mg in both the 21-day-on/7-day-off schedule and the continuous dosing schedule (CDD)[39]. Dose limiting toxicity (DLT) was grade 3 skin rash. Only 1 partial response in NSCLC (non-PI3K/PTEN mutated) was observed in 68 patients treated in the phase I monotherapy trial. |
| PX-866 is a derivative of wortmannin and acts as an irreversible pan-PI3K Class 1A and B inhibitor. The MTD for intermittent schedule (once daily on days 1–5 and 8–12 of a 28-day cycle) was 12 mg, and that for continuous schedule was 8 mg, with DLTs including diarrhea and ALT/AST elevation[39-42]. No objective response was reported in 84 patients treated; however, stable disease was achieved in 22% of the patients[39-42]. |
| BKM120 is a highly specific pan-Class I PI3K inhibitor without overlapping inhibitory activity against mTOR or Vps34. The MTD for daily dosing schedule was 100 mg with DLTs including hyperglycemia, skin rash, and mood alteration[43]. In the Phase I trial of 35 evaluable patients, there was 1 confirmed and 1 unconfirmed partial response, and 7 patients achieved stable disease for >8 months[44]. Both women with partial responses had breast cancer—one had triple negative breast cancer [estrogen receptor (ER), progesterone receptor (PR)-, and HER2-negative] with wild-type PIK3CA and mutant KRAS, without PTEN loss; the other had an ER/PR-positive, HER2-negative tumor with a confirmed PIK3CA mutation (E545K)[41-42]. |
| GDC-0941 is another pan-Class I PI3K inhibitor that is derived from PI103, an early generation PI3K inhibitor. In phase I trials, two dosing schedules, 3-week-on/1-week-off once daily (QD) or twice a day (BID), were investigated[45]. No MTD has been determined with DLTs consisting of headache, pleural effusion, and decreased diffusion capacity of the lung for carbon monoxide (DLCO). A partial response was observed in 1 patient with breast cancer, with additional anti-cancer signals seen in ovarian cancer. |
| BAY80-6946 (BAY) is a potent and highly selective reversible pan-Class I PI3K inhibitor. In a phase I trial, it was reported to be tolerated as an infusion at a dose of 0.8 mg/kg on days 1, 8, and 15 in a 28-day cycle[46]. Grade 2/3 hyperglycemia in the first 24 h after receiving a dose was commonly observed at the MTD. In the MTD expansion, 23 patients with solid tumors and 5 patients with follicular lymphoma (FL) were treated with BAY at |
| Generic name / code number (trade name) | Company | Stage of development | Target disease | Target and mechanism |
|----------------------------------------|---------|----------------------|----------------|---------------------|
| **PI3K inhibitors**                    |         |                      |                |                     |
| Pan-PI3K inhibitors                     |         |                      |                |                     |
| XL-147/EXEL6147                         | Exelixis / Sanofi-Aventis | PII | Endometrial cancer | PI | Class I PI3K / EGFR inhibitor |
|                                        |         |                      | Breast cancer | PI                  |                     |
|                                        |         |                      | NSCLC | PII               |                     |
|                                        |         |                      | Ovarian cancer | PI                  |                     |
|                                        |         |                      | Solid tumors | PI                  |                     |
|                                        |         |                      | Lymphoma | PII               |                     |
|                                        |         |                      | Glioblastoma | PII               |                     |
| PX866                                  | Oncothyreon | PII | Solid tumors | PI | PI3K inhibitor |
|                                        |         |                      | Lung cancer | Preclinical        |                     |
|                                        |         |                      | Ovarian cancer | Preclinical      |                     |
|                                        |         |                      | Glioblastoma | Preclinical      |                     |
|                                        |         |                      | Metastatic tumors | PII   |                     |
| BKM120                                 | Novartis | PII | Solid tumors | PI | PI3K inhibitor |
|                                        |         |                      | Breast cancer | PI                  |                     |
|                                        |         |                      | Colorectal cancer | PI            |                     |
| RG7321/GDC0941                         | Roche | PII | Breast cancer | PI | PI3K inhibitor |
|                                        |         |                      | NSCLC | PI                  |                     |
|                                        |         |                      | Ovarian cancer | PI                  |                     |
|                                        |         |                      | Non-Hodgkin’s lymphoma | PI |                     |
| BAY806946                              | Bayer Schering Pharma | PI | Solid tumors/follicular lymphomas | PI | PI3K inhibitor |
| GSK2126458                             | GlaxoSmithkline | PI | Solid tumors | PI | PI3K inhibitor |
|                                        |         |                      | Lymphoma | PII               |                     |
| CHS132799                              | Roche | PI | Solid tumors | PI | PI3K inhibitor |
| ATU027                                 | Silence Therapeutics | PI | Solid tumors | PI | PI3K inhibitor |
|                                        |         |                      | Pancreatic cancer | Preclinical | PKN3 inhibitor (RNA interference agent) |
|                                        |         |                      | Gastric cancer | Preclinical |                     |
|                                        |         |                      | Prostate cancer | Preclinical |                     |
| **PI3K isoform-specific inhibitors**   |         |                      |                |                     |
| CAL101                                 | Calistoga Pharmaceuticals | PII | CLL | PII | PI3K delta inhibitor |
|                                        |         |                      | Non-Hodgkin’s lymphoma | PI |                     |
|                                        |         |                      | Allergic rhinitis | Discontinued |                     |
|                                        |         |                      | Inflammation | Discontinued |                     |
|                                        |         |                      | Asthma | Discontinued |                     |
| BYL719                                 | Novartis | PI | Neoplasm | PI | PI3K alpha inhibitor |
| AZD6482                                | AstraZeneca | Preclinical | Hematologic malignancies | Preclinical | PI3K beta inhibitor |
| GSK2636771                             | GlaxoSmithkline | PII/II | Solid tumors | PI | PI3K beta inhibitor |
| **AKT inhibitors**                     |         |                      |                |                     |
| Allosteric AKT inhibitors               |         |                      |                |                     |
| MK-2206                                | Merck & Co., Inc. | PII | Endometrial cancer | PI | AKT inhibitor |
|                                        |         |                      | Leukemia | PI |                     |
|                                        |         |                      | Ovarian cancer | PI                  |                     |
|                                        |         |                      | Breast cancer | PI                  |                     |
|                                        |         |                      | Pancreatic cancer | PI                |                     |
|                                        |         |                      | Melanoma | PI                  |                     |
|                                        |         |                      | Multiple myeloma | PI                |                     |
|                                        |         |                      | AML | PI                  |                     |
|                                        |         |                      | Hematologic tumors | PI |                     |
|                                        |         |                      | CLL | PI                  |                     |
|                                        |         |                      | Myelodysplastic syndrome | PI |                     |

(To be continued)
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| Generic name /code number (trade name) | Company                        | Stage of development | Target disease | stage | Target and mechanism                       |
|---------------------------------------|--------------------------------|----------------------|----------------|-------|-------------------------------------------|
| GSK690693                             | GlaxoSmithkline                 | PI                   | Melanoma       | PI    | AKT inhibitor                             |
| RX0201 (Archexin)                     | Rexahn Pharmaceuticals           | PII                  | Solid tumors   | PI    | AKT Antisense                             |
| ATP-competitive AKT inhibitors         |                                |                      |                |       |                                           |
| Triciribine phosphate                  | VioQuest Pharmaceuticals / Cahaba| PII                  | Leukemia       | PII   | AKT inhibitor                             |
|                                       | Phoenix Biosciences             |                      | Solid tumors   | PII   |                                           |
|                                       | Stengent Arno                   | Preclinical          | Hematological  | PII   | AKT inhibitor                             |
|                                       | GlaxoSmithkline                 | PI                   | Solid tumors   | PII   | AKT inhibitor                             |
|                                       | Pancreatic cancer               | PII                  | Multiple myeloma| PII   | AKT inhibitor                             |
|                                       | AML                             | PII                  | Myelodysplastic| syndrome | AKT inhibitor                             |
|                                       | Melanoma                        | PII                  | KSCL           | Preclinical | AKT inhibitor, FGF-2, NF-κB, and p70S6K inhibitors |
| PBI-05204 (oleandrin)                 | Phoenix Biosciences             | PI                   | Neoplasm       | PI    | AKT, PDK-1, PI3                                |
|                                       | Stengent Arno                   | Preclinical          | Hematological  | tumors | AKT inhibitor                             |
|                                       | GlaxoSmithkline                 | PI                   | Lymphoma       | PI    | AKT inhibitor                             |
|                                       | Pancreatic cancer               | PII                  | Endometrial    | cancer | AKT inhibitor                             |
|                                       | AML                             | PII                  | Pancreatic     | cancer | AKT inhibitor                             |
|                                       | Ovarian cancer                  | PII                  | Ovarian cancer | PII   | AKT inhibitor                             |
| RG7440                                | Roche                           | PI                   | Solid tumors   | PII   | AKT inhibitor                             |
| GD0058                                | Array Biopharma                 | PI                   | Solid tumors   | PII   | AKT inhibitor                             |
| SR13668                               | Pan-AKT inhibitor               | Preclinical          | Preclinical    |       | AKT inhibitor                             |
| AZD5363                               | Astra Zeneca                    | PI                   | Solid tumors   | PII   | Pan-AKT inhibitor                         |

mTOR inhibitors

| Rapalog mTOR inhibitors               | Novartis                        | Launched             | Renal cell carcinoma | Launched | mTOR inhibitor                             |
|                                       |                                 |                      | Pancreatic cancer    | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | Breast cancer        | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | Gastric cancer       | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | Non-Hodgkin’s lymphoma| PII   | mTOR inhibitor                             |
|                                       |                                 |                      | Liver cancer         | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | AML                  | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | GIST                 | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | Prostate cancer      | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | NSCLC                | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | Colon cancer         | PII      | mTOR inhibitor                             |
|                                       |                                 |                      | Metastatic bone disease| PII   | mTOR inhibitor                             |

(To be continued)
### Table 1. PI3K, AKT, and mTOR Inhibitors in Clinical Development (continued)

| Generic name/code number (trade name) | Company | Stage of development | Target disease | Target and mechanism |
|--------------------------------------|---------|----------------------|----------------|----------------------|
| Temsirolimus (TORISEL®)              | Pfizer  | Launched             | Melanoma | PII                  |
|                                      |         |                      | Sarcoma   | PII                  |
|                                      |         |                      | Myelodysplastic syndrome | PII |
|                                      |         |                      | Thyroid cancer | PII |
|                                      |         |                      | Head & neck cancer | PII |
|                                      |         |                      | Glioblastoma | PII |
|                                      |         |                      | SCLC | PII |
|                                      |         |                      | Macular degeneration | PII |
|                                      |         |                      | ALL | PII |
|                                      |         |                      | Ovarian cancer | PII |
|                                      |         |                      | Renal cell carcinoma | Launched |
|                                      |         |                      | Non-Hodgkin’s lymphoma | Registered |
|                                      |         |                      | Lymphoma | PII |
|                                      |         |                      | Prostate cancer | PII |
|                                      |         |                      | Multiple myeloma | PII |
|                                      |         |                      | Melanoma | PII |
|                                      |         |                      | CLL | PII |
|                                      |         |                      | Ovarian cancer | PII |
|                                      |         |                      | NSCLC | PII |
|                                      |         |                      | Head & neck cancer | PII |
|                                      |         |                      | Breast cancer | Discontinued |
| ridaforolimus                        | ARIAD Pharmaceuticals/ Merck | PIII | Sarcoma | PII |
|                                      |         |                      | Prostate cancer | PII |
|                                      |         |                      | Solid tumors | PII |
|                                      |         |                      | Hematological tumors | PII |
|                                      |         |                      | Endometrial cancer | PII |
|                                      |         |                      | Breast cancer | PI |
| sirolimus ABI009 TORC1/2 inhibitors  | Celgene | PI | Solid tumors | PI |
| OSI027                                | Astellas Pharma | PI | Solid tumors | PI |
|                                      |         |                      | Lymphoma | PII |
| AZD8055                               | AstraZeneca | PII | Liver cancer | PII |
| AZD2014                               | AstraZeneca | PI | Solid tumors | PII |
| INK128                                | Intellikine | PI | Solid tumors | PII |
| CC223                                 | Celgene | PII | Multiple myeloma | PII |
| Dual PI3K-mTOR inhibitors             | Novartis | PII | Solid tumors | PII |
| BEZ235                                |         |                      | Breast cancer | PI |
|                                      |         |                      | Glioma | PI |
|                                      |         |                      | Prostate cancer | PII |
|                                      |         |                      | Colorectal cancer | PII |
|                                      |         |                      | Renal cell carcinoma | PII |

*(To be continued)*
Targeting the PI3K-AKT-mTOR pathway in cancer

### Table 1. PI3K, AKT, and mTOR Inhibitors in clinical development (continued)

| Generic name / code number (trade name) | Company | Stage of development | Target disease | stage | Target and mechanism |
|----------------------------------------|---------|----------------------|----------------|-------|----------------------|
| SAR245409/XL765 Exelixis / Sanofi-Aventis | PII | NSCLC | PI | PII | mTOR-ERK inhibitor |
| SF1126 Semafore Pharmaceuticals | PI | Solid tumors | PI | Prostate cancer | mTOR-PiN inhibitor |
| RG7422 Roche / Piramal | PI | Non-Hodgkin’s lymphoma | PI | P3K-mTOR inhibitor |
| GDC0980 Life Sciences | PII | Solid tumors | PI | P3K-mTOR inhibitor |
| PF05212384 Pfizer | PI | Solid tumors | PI | P3K-mTOR inhibitor |
| PF4691502 Pfizer | Solid tumors | P3K-mTOR inhibitor |
| PP-242 Pfizer | Solid tumors | P3K-mTOR inhibitor |

PI3K, phosphatidylinositol 3-kinase; AKT, serine/threonine kinase; mTOR, mammalian target of rapamycin; PI, phase I; PII, phase II; PII, phase III; NSCLC, non–small cell lung cancer; EGFR, epidermal growth factor receptor; CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; EGF-2, epidermal growth factor-2; NF-κB, nuclear factor-kappa B; PDK1, phosphoinositide-dependent kinase-1; GIST, gastrointestinal stromal tumor; SCLC, small cell lung cancer; ALL, acute lymphocytic leukemia; ERK, extracellular signal-regulated kinases; PKN3, protein kinase N3.

MTD [46]. All had PI3K mutations. All these patients had grade 2/3 hyperglycemia, as noted in the dose escalation phase. Two patients developed interstitial pneumonitis. Hypertension for less than 24 h was commonly seen in patients with pre-existing hypertension. Three of 4 patients with FL achieved partial response after 2 cycles. Two patients with breast cancer also had partial responses. However, the response to the treatment did not correspond with the PI3K mutation status of these patients [46].

ATU027 is a novel RNAi therapeutic directed against protein kinase N3 (PKN3), a protein kinase C–related kinase downstream in the PI3K signaling pathway. Preclinically, systemic administration of ATU027 siRNA results in specific silencing of PKN3 expression, inhibition of tumor growth and lymph node metastasis, and a reduction in lymph vessel density [46]. A phase I trial in cancer patients is ongoing. If successful, ATU027 and other siRNA-based therapeutics will become a novel approach for targeting individual components of the PI3K pathway.

PI3K isoform-specific inhibitors

These PI3K isoform–specific inhibitors selectively inhibit PI3K p110α, β, δ, or γ catalytic subunits and may therefore have an advantage of more complete target inhibition with fewer side effects.

CAL101 is a specific inhibitor of PI3Kδ, the isoform that is predominantly expressed in leukocytes. Early trials generated impressive response rates of 57%, 67%, and 30% in patients with relapse or recurrent indolent non-Hodgkin’s lymphoma (NHL), mantle cell lymphoma (MCL), and chronic lymphocytic leukemia (CLL), respectively [46]. The compound is generally well tolerated with reversible spikes in serum transaminases as DLT. Phase II/III trials in B-cell malignancies are currently ongoing.

AKT inhibitors

There are two main classes of AKT inhibitors in clinical development: ATP-competitive and allosteric AKT inhibitors.
ATP-competitive AKT inhibitors

ATP-competitive AKT inhibitors are pan-AKT kinase inhibitors. Because of the shared homology of the ATP-binding pocket among various kinases, this class of AKT inhibitors often has overlapping activity against other AGC kinases such as p70S6 kinase, protein kinase C (PKC), and Rho kinase. It remains to be determined whether such an inhibitory profile will result in undesirable side effects in clinical settings.

AZD5363 is a potent pan-AKT kinase inhibitor that has pharmacologic properties consistent with AKT inhibition in vivo. It inhibited the growth of a range of human tumor xenografts and caused significant regression in combination with docetaxel, particularly in breast cancer xenografts. AZD5363 is currently being investigated in phase 1 clinical trials.

Allosteric AKT inhibitors

Allosteric AKT inhibitors bind to the PH domain of the AKT enzyme-forming drug-enzyme complexes. Due to conformational changes, translocation of AKT to the plasma membrane, a step essential for AKT activation, is disrupted. Such a conformation-based approach circumvents the issue of kinase selectivity often seen with ATP-competitive AKT inhibitors.

MK-2206 is an allosteric AKT inhibitor that selectively inhibits AKT-1, -2, and -3 isoforms with nano-molar (nM) potency. Two dosing schedules were investigated in phase I monotherapy trials. The MTD for once every other day (QOD) schedule is 60 mg, and the MTD for once every week (QW) is preliminarily determined to be 200 mg. The main DLT was skin rash. No objective responses were observed. Evidence of target inhibition measured by changes in p-AKT before and after treatment was confirmed in tumor biopsies as well as surrogate tissues.

Other AKT inhibitors

Triciribine phosphate (TCN-PM) is a potent pan-AKT inhibitor. TCN-PM was administered to subjects whose tumors displayed evidence of increased AKT phosphorylation (p-AKT), as measured by immunohistochemical analysis (IHC), over 30 min on days 1, 8, and 15 of a 28-day cycle. Modest decreases in tumor p-AKT were detected following treatment with TCN-PM. No MTD was determined, and no objective response was observed.

PBI-05204 contains oleandrin, a cardiac glycoside, which inhibits the α-3 subunit Na-K ATPase pump. Oleandrin inhibits the export of fibroblast growth factor-2 (FGF-2), activation of necrosis factor-αB (NF-αB) and phosphorylation of AKT. PBI-05204 also inhibits p70S6K, decreasing mTOR activity. PBI-05240 was given orally for 21 days of a 28-day cycle. No MTD has been determined, with no DLT reported. Only minor responses or stable disease were observed. Western blotting of peripheral blood mononuclear cells (PBMCs) showed that PBI-05204 markedly reduced the phosphorylation of AKT, p70S6K, and S6 in a time-dependent manner, suggesting target engagement.

RX-0201 (Archexin) is a 20-mer oligonucleotide with sequence complementary to Akt-1 mRNA. RX-0201 was administered to patients by up to 2 cycles of continuous infusion; each cycle of infusion lasted for 14 days followed by a 7-day rest. Grade 3 fatigue was DLT. Only stable disease was seen in 2 of 17 patients.

Perifosine is a plasma membrane disrupting agent that inhibits AKT among other membrane-associated kinases by preventing their translocation to the plasma membrane. Perifosine has been evaluated in multiple trials in clinical trials both alone and in combination with various other agents. The most common adverse reactions are fatigue and gastrointestinal toxicity. Like other PI3K-targeting agents, monotherapy activity with perifosine has generally been disappointing, although activity has been observed in patients with sarcoma and Waldenström’s macroglobulinemia.

mTOR inhibitors

Rapalogs

Rapamycin (sirolimus) and its analogs, temsirolimus, everolimus, and deforolimus, inhibit the mTORC1 kinase by binding to an abundant intracellular protein, FKBP-12, forming a complex that inhibits the mTOR signaling. Monotherapy activity was observed in early trials of these rapalogs, serving as a proof-of-concept for targeting the PI3K-AKT-mTOR pathway. Follow-up studies confirmed clinical efficacy and led to the approval of rapalogs in the treatment of renal cell carcinoma. Of note, everolimus, as compared with placebo, significantly prolonged progression-free survival (PFS) among patients with progressive advanced pancreatic neuroendocrine tumors. Everolimus has shown significant improvement in PFS in renal cell carcinoma when compared to the best supportive care in previously treated patients. Other indications with significant clinical activities include mantle cell lymphoma and sarcoma. Combination trials of rapalogs plus hormonal therapy in ER-positive breast cancer have shown the potential of these agents in this patient population.

mTORC1/2 inhibitors

mTOR catalytic site inhibitors directly target the kinase domain of mTOR and therefore impede the activity of both mTORC1 and mTORC2 kinases. A theoretical advantage of dual mTORC1/2 inhibition is to...
prevent compensatory feedback activation of AKT upon mTORC1 inhibition by rapalogs\textsuperscript{[48]}. OSI027 is an oral inhibitor of mTORC1 and mTORC2. Three dosing schedules were tested: days 1–3, once weekly, and continuous once daily\textsuperscript{[49]}. DLTs included decreased left ventricular ejection fraction and fatigue. No objective response was achieved in phase I trials. Decreases in the phosphorylation of 4E-BP1 in PBMCs at threonine 37/46, a rapamycin-insensitive, mTOR-dependent phosphorylation site, confirmed TORC2 inhibition in patients\textsuperscript{[50]}. Other mTORC1/2 inhibitors are still in early phase I stage, and it is premature to predict whether this class of inhibitors will have higher clinical efficacy than rapalogs with acceptable tolerance.

**Dual PI3K-mTOR inhibitors**

The dual PI3K-mTOR inhibitors target the p110 subunit of PI3K as well as mTOR. The potential advantage is more complete inhibition of the PI3K-AKT-mTOR pathway, which may increase clinical efficacy. BEZ235 is an orally available, potent and highly selective reversible PI3K-mTOR dual inhibitor. No DLTs have been observed in the first 59 treated patients of a phase I trial\textsuperscript{[51]}. Of the 51 evaluable patients, 2 achieved partial responses, including 1 ER-positive, HER2-negative breast cancer patient with unknown PI3K pathway status, and 1 patient with Cowden’s syndrome (germline PTEN mutation) who had developed lung cancer. Furthermore, 16 patients achieved minor responses, with 14 achieving stable disease for 4 months or longer. It is of interest to point out that tumors from 6 of these 14 patients showed dysregulation of the PI3K pathway\textsuperscript{[52]}. XL765 is also known as SAR245409, an oral dual inhibitor of PI3K/mTOR. Stable disease in 12 patients for 16 weeks or longer and in 7 patients for 24 weeks or longer was observed among 83 patients in a phase I trial\textsuperscript{[53]}. The most frequently observed toxicities involved elevated liver enzymes, nausea, vomiting, diarrhea, and skin rash. The MTD has been defined as 50 mg twice daily or 90 mg daily. GDC-0980 is a dual PI3K/mTOR inhibitor that targets all PI3K isoforms and mTOR at low nanomolar concentrations. It was investigated in a phase I trial with daily dosing on a 3-week-on/1-week-off schedule\textsuperscript{[54]}. Early data suggested pharmacodynamic target engagement and clinical activity. Toxicity included rash, hyperglycemia, mucositis, and pneumonitis, which resolved with drug cessation. GDC-0980 showed antitumor activity in 3 of 33 patients with mesothelioma. Fluorodeoxyglucose positron emitting tomography (PET-FDG) responses were also observed in gastrointestinal stromal tumor (GIST) and adrenal tumors\textsuperscript{[55]}. SF1126 is composed of the pan-PI3K inhibitor LY294002 conjugated to an RGDS-targeting peptide. It is designed to increase solubility and binding to integrins expressed on tumor vasculature. LY294002 inhibits other kinases, including mTOR, DNA-PK, PIM1, PLK1, and CK2, and induces oxidative stress in cancer cells independent of its PI3K inhibition. Stable disease (≥8 weeks) was seen in 19 of 33 (58%) evaluable patients, with durations of 20 weeks for patients with GIST, endometrial cancer, and prostate cancer\textsuperscript{[56]}. MTD was not reached but the maximum administered dose (MAD) was 1,100 mg/m\textsuperscript{2}. Pharmacodynamic studies showed reduced p-AKT and increased apoptosis, indicating inhibition of the PI3K pathway signaling. Other dual PI3K-mTOR inhibitors in clinical development include the orally administered PF-04691502 and an intravenous agent, PF-05212384.

**Common Challenges in the Clinical Development of PI3K-AKT-mTOR Inhibitors**

**Lack of monotherapy efficacy**

Single agent activity has not been widely observed in clinical trials of these PI3K-AKT-mTOR inhibitors. The mTOR inhibitors, rapalogs, are the most advanced agents in clinical development. Single agent activities in renal cell carcinoma led to market approval of everolimus and temsirolimus\textsuperscript{[57]}. Patient benefit in pancreatic neuroendocrine cancer and sarcoma have been noted for everolimus\textsuperscript{[58]} and ridaforolimus\textsuperscript{[59]}, respectively. However, monotherapy activity in other cancer types has been modest. The lack of single agent activity may be due to (1) incomplete inhibition of target (degree and duration), (2) activation of feedback compensatory loops leading to tumor cell resistance, and (3) lack of predictive biomarkers to prospectively select “sensitive” patient subpopulations.

**Mechanism-based toxicities**

**Skin rash**

Skin rash has been observed in clinical trials of agents targeting the PI3K-AKT-mTOR pathway. Skin rash was reported as the DLT for the allosteric AKT inhibitor MK-2206\textsuperscript{[60]}. This cutaneous toxicity is also associated with several PI3K-AKT-mTOR inhibitors, including XL147, XL764, BKM120, BEZ335, everolimus, and temsirolimus, suggesting a mechanism-based class adverse effect. Skin rash presents as erythematous maculo-papular rash, often first appearing on truncal areas, in contrast to EGFR inhibitor-associated acneiform facial rash. These non-pustular, non-blistering rashes recover fully upon drug interruption or dose.
reduction. Clinical symptom management includes the use of anti-histamines, topical steroids, and moisturizing cream. The pathophysiologic mechanism underlying PI3K-AKT-mTOR inhibitor–induced rash is not yet delineated but is suspected to involve local cytokine and chemokine deregulation upon pathway inhibition.

**Hyperglycemia and hyperlipidemia**

Hyperglycemia and hyperlipidemia have been anticipated as side effects for PI3K-AKT-mTOR pathway inhibitors based on the role of the pathway in regulating the insulin signaling and the physiologic hemo-state of glucose metabolism. Although the exact mechanism of metabolic derangements is not entirely clear, one possibility is that physiologic adaptation to pathway inhibition partially compensates the disrupted insulin-glucose regulatory axis. The pathophysiology of mTOR inhibitors causing dyslipidemia may involve the impaired clearance of lipids from the bloodstream via stimulation of insulin-stimulated lipoprotein lipase (LPL). However, PI3K and AKT inhibitors are less likely to lead to grade 3 or 4 hyperlipidemia, unlike mTOR inhibitors. To date, only mild and reversible hyperglycemia accompanied by elevated insulin C-peptide levels has been frequently observed in clinical trials, confirming pharmacodynamic effects. The detailed management of metabolic disorders induced by PI3K-AKT-mTOR inhibitors is beyond the scope of this review, but the aim of management should be to avoid acute and sub-acute complications of hyperglycemia, while ensuring that hypoglycemia is avoided.

**Lack of predictive biomarkers**

There is strong preclinical evidence that germline loss or acquired somatic mutations of PTEN or mutations within exon 9 (E542K and E545K) or exon 20 (H1047R) of PIK3CA lead to uncontrolled activity of the PI3K enzyme and thus promote oncogenesis. There is also strong preclinical evidence suggesting the predictability of the response to targeted therapies in patients harboring these mutations, but this is yet to be evaluated in a clinical setting. Other preclinical data suggest that efficacy correlates with protein biomarkers such as pS6, pEIF4, rictor, raptor, pAKT and total AKT, but again, this is yet to be validated in prospective human studies. The lack of predictive biomarkers in the clinical setting remains a challenge at this stage, hampering future development of these targeting agents.

**Future Perspectives**

The PI3K-AKT-mTOR pathway is a well-established driver of cancer in humans, and therefore blocking different nodes of the pathway is a relevant treatment strategy for human malignancies. There are several targeting agents in clinical development, but most of them are currently in phase I trial, suggesting that in the near future more data will be available to examine the antitumor properties of PI3K/AKT inhibitors. The activity of these agents in early phase clinical trials has been limited, and it is likely that cancer cells acquire resistance via different feedback loop and crosstalk mechanisms. The future development of these promising inhibitors should therefore focus on combination regimens, including the concomitant or sequential blockade of signaling pathways, e.g., horizontal blockade of the PI3K-AKT-mTOR and RAS-RAF-MEK pathways or vertical blockade of the PI3K/AKT/mTOR and IGF pathways. Indeed, this strategy is now being evaluated in several clinical trials. Finally, there is an urgent need to develop robust predictive biomarkers to increase the efficacy of these drugs by prospectively identifying patients who are most likely to benefit.

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