The PI3K/AKT Pathway as a Target for Cancer Treatment

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Annu. Rev. Med. 2016. 67:11–28

First published online as a Review in Advance on October 14, 2015

The *Annual Review of Medicine* is online at med.annualreviews.org

This article's doi: 10.1146/annurev-med-062913-051343

Keywords

phosphoinositide 3-kinase (PI3K)/AKT, mammalian target of rapamycin (mTOR), pathway inhibitors, breast cancer, lymphoproliferative disorders, mutation

Abstract

Anticancer targeted therapies are designed to exploit a particular vulnerability in the tumor, which in most cases results from its dependence on an oncogene and/or loss of a tumor suppressor. Genes in the phosphoinositide 3-kinase (PI3K)/AKT pathway are the most frequently altered in human cancers. Aberrant activation of this pathway, as a result of these somatic alterations, is associated with cellular transformation, tumorigenesis, cancer progression, and drug resistance. Several drugs targeting PI3K/ATK are currently in clinical trials, alone or in combination, in both solid tumors and hematologic malignancies. These drugs are the focus of this review.

INTRODUCTION

Advances in tumor genetics and drug development have led to the generation of a wealth of anticancer targeted therapies. These drugs are intended to target a particular vulnerability in the tumor, generated in most cases by its dependence on an oncogene and/or loss of a tumor suppressor. Several recent examples indicate that these drugs are mainly, if not exclusively, active against tumors of a particular genotype that can be identified by a diagnostic test, usually by detecting a somatic alteration in tumor DNA. Drugs that target the phosphoinositide 3-kinase (PI3K)/AKT pathway, which is frequently mutated in both solid tumors and hematologic malignancies, are the focus of this review.

Multiple PI3K families exist in higher eukaryotes. To date, mainly class I_A PI3Ks have been implicated in cancer (1). Class I_A PI3Ks are heterodimers consisting of a p85 regulatory subunit and a p110 catalytic subunit. Growth factor receptor tyrosine kinases (RTKs), such as EGFR, HER2, insulin receptor (InsR), IGF-IR, MET, FGFR, etc. also signal via class I_A PI3K. These transmembrane receptors do not bind or activate PI3K directly; they phosphorylate adaptor proteins such as GAB1/2, IRS-1/2, FRS2, and HER3 (ERBB3), which, in turn, via YXXM motifs, bind the amino-terminal domain of p85. This binding relieves the inhibition of p110 by p85 and recruits the p85–p110 heterodimer to its substrate, the lipid phosphatidylinositol-4,5-bisphosphate (PIP2), at the plasma membrane (2). PI3K (p110) then phosphorylates PIP2 to produce the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). A Ras-binding domain in p110α also mediates activation by RAS. PTEN (phosphatase and tensin homologue) and INPP4B dephosphorylate PIP3 in positions 3 and 5 of the inositol ring, respectively, thereby negatively regulating PI3K signaling output (3). Several pleckstrin homology (PH) domain–containing proteins, including AKT, SGK, and PDK1, bind to PIP3 at the plasma membrane. The phosphorylation of AKT at Thr^{308} by PDK1 and at Ser^{473} by a complex involving mTOR/Rictor (TORC2) results in full activation of this enzyme. AKT phosphorylates a host of cellular proteins, including GSK3α, GSK3β, FoxO transcription factors, MDM2, BAD, and $p27^{KIP1}$ to facilitate survival and cell cycle entry (4). In addition, AKT phosphorylates and inactivates Tuberin, a GTPase-activating protein (GAP) for the Ras homologue Rheb. Inactivation of Tuberin allows GTP-bound Rheb to accumulate and activate the mTOR/Raptor (TORC1) complex, which ultimately regulates protein synthesis, RNA translation, cell growth, and autophagy (5) (**Figure 1**).

GENETIC ALTERATIONS IN PI3K/AKT: ONCOGENE DEPENDENCE

The PI3K/AKT pathway is frequently mutated in human cancers. These alterations (reviewed in 6) include loss of the lipid phosphatases PTEN and INPP4B, as well as mutation and/or amplification of the genes encoding the PI3K catalytic subunits p110α (*PIK3CA*) and p110β (*PIK3CB*); the PI3K regulatory subunits p85α, p55α, p50α (all encoded by *PIK3R1*), and p85β (*PIK3R2*); RTKs such as HER2 (*ERBB2*); the PI3K activator K-RAS; and the PI3K effectors AKT1, AKT2, AKT3, and PDK1. The genes *PIK3CA*, *PIK3CB*, *PIK3CD*, and *PIK3CG* encode the homologous p110α, p110β, p110δ, and p110γ isozymes, respectively. Expression of p110δ and p110γ is largely restricted to immune cells and leukocytes, whereas p110α and p110β are ubiquitously expressed. Studies with genetically engineered mice (GEMs) have suggested these isoforms mediate distinct roles in PI3K signaling in both physiology and oncogenicity. For example, germline ablation of *Pik3ca* is embryonically lethal, whereas mice carrying a kinase-dead *Pik3cb* knock-in allele develop to maturity but with reduced size and male fertility and defective glucose homeostasis (7). Loss of p110δ results in functional defects in lymphocytes, neutrophils, and mast cells (8), whereas loss of p110γ impairs thymocyte development, T cell activation, and neutrophil migration (9).

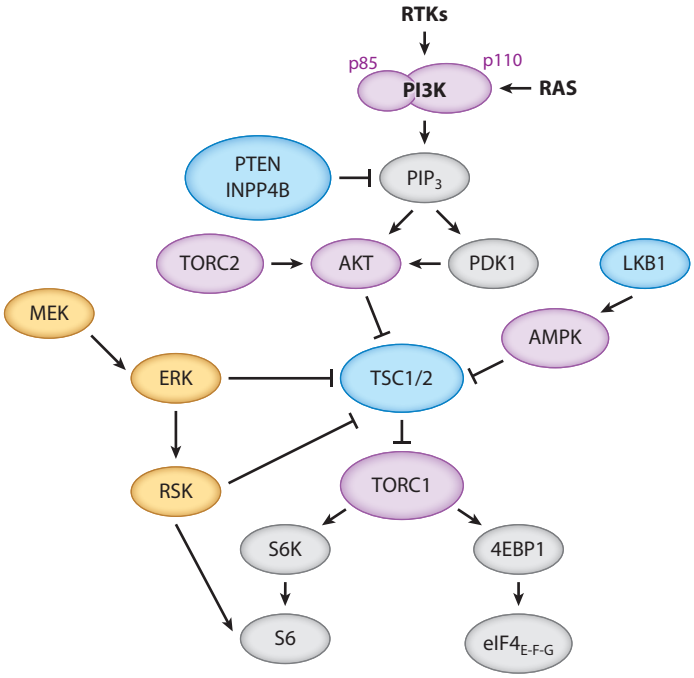

Figure 1

Schema of the PI3K/AKT pathway. Mechanisms of aberrant activation include amplification/mutation of receptor tyrosine kinases and oncogenes (ERRB2, KRAS), mutations in PIK3CA (p110), p85, AKT, TSC1/2, mTOR, and/or loss of tumor suppressors such as PTEN, INPP4B, and LKB1. The two complexes nucleated by the mTOR kinase, TORC1 and TORC2, are downstream and upstream AKT, respectively. Oncogenes are noted in pink and tumor suppressors in blue. The RAS/RAF/MEK/ERK pathway, either directly or via RSK, confers an alternative pathway for activation of TORC1.

PIK3CA mutations are the most common genetic alterations of this pathway, where ≥80% occur within the helical (E542K and E545K) and kinase (H1047R) domains of p110α. Helical domain mutations increase catalytic activity by reducing the repression of p110α by p85 (10) or facilitating the interaction of p110α with IRS-1 (11), whereas kinase domain mutations increase the retention of p110α at the plasma membrane (12). These gain-of-function mechanisms induce cellular transformation, growth factor- and anchorage-independent growth, and resistance to anoikis. Several studies using GEMs subsequently showed that tissue-specific expression of mutant *PIK3CA* can induce tumor formation and accelerate cancer progression (13–15). *PIK3CD* mutations have been described in immune deficiencies but not in human cancers. *PIK3CB* and *PIK3CD*, unlike *PIK3CA*, are amplified or overexpressed but not mutated in human neoplasias (6).

Somatic mutations in *PIK3R1* have also been identified in a variety of tumors. In general, they promote PI3K activity and cellular transformation as a result of their reduced ability to inhibit p110 (16, 17). Suggestive of a tumor suppressor role, reduced *PIK3R1* mRNA levels negatively correlate with tumor grade and incidence of metastases in breast cancers and hepatocellular carcinomas (18). In addition, genetic ablation of *Pik3r1* in mice accelerates tumors induced by loss of *PTEN* and results in spontaneous liver cancers (19). Increased *PIK3R2* expression has been reported in breast and colon cancers, and its overexpression in cells increases PI3K activity and results in tumor formation in mice (20). Similar to *PIK3R1*, somatic mutations in *PIK3R2* have been found in endometrial and colorectal cancers, where they increase PI3K activation (17).

ER+: estrogen receptor positive

Recruitment of p110–p85 heterodimers to phosphorylated YXXM motifs in activated RTKs is critical for growth factor signaling and cellular transformation. Studies with p110 isoform-specific inhibitors and ablation of p110α in GEMs have shown that the p110α isozyme is essential for PI3K signaling and growth of tumors driven by *PIK3CA* mutations, RTKs, and mutant RAS. Interestingly, whereas tissue-specific ablation of p110α retards mammary gland development and mammary cancers induced by polyoma virus middle T and ERBB2 (NEU), p110β ablation accelerates mammary ductal growth and tumor formation driven by these oncogenes (21). In mice with a knock-in allele of *Pik3ca* with a Ras-binding domain mutation or loss of endogenous *Pik3ca*, development of myeloid leukemias and the formation and progression of lung cancer induced by mutant RAS are all delayed (22, 23).

Conversely, ablation of p110β inhibits transformation induced by G protein–coupled receptors as well as the proliferation and invasiveness of urothelial cancer cells in GEMs (24). Interestingly, GEMs with conditional knockout of *PTEN* in the prostate develop prostatic intraepithelial neoplasias. Ablation of p110β but not p110α abrogates prostatic intraepithelial neoplasias induced by loss of *PTEN* (25). This reliance of PTEN-deficient tumors on p110β is not universal and may depend on the tissue type and on its pathology. For example, mice with *PTEN* ablation in the basal epidermis require both p110α and p110β for the development of hamartomas (26). Furthermore, *PTEN*-mutant endometrial cancer cell lines harboring other *PIK3CA* activating mutations are resistant to PI3Kβ inhibitors (27). In other cases, cancer cells may activate p110β to counteract the blockade of p110α. For example, inhibition of *PIK3CA* with the p110α-specific small-molecule antagonist BYL719 in *PIK3CA*-mutant and HER2-overexpressing cancer cells is dampened by rapid reaccumulation of PIP3 mediated by p110β. Addition of a p110β inhibitor to BYL719 prevents the rebound of PIP3 and induces greater antitumor activity (28). In a second recent example, a patient with ER+/*PIK3CA*-mutant breast cancer rapidly progressed and died after an initial excellent response to BYL719. Analysis of all drug-resistant metastatic lesions collected at autopsy revealed several genetic alterations uniformly resulting in loss of PTEN expression. PTEN-deficient xenografts derived from the metastatic lesions were resistant to BYL719, but the addition of a p110β inhibitor reversed resistance to the p110α inhibitor (29).

In addition to the alterations discussed above, somatic alterations in other genes in the PI3K/AKT/mTOR pathway, such as *PTEN*, *INPP4B*, *AKT1-3*, *mTOR*, and *TSC1/2*, are present in variable frequencies across several tumor types (30, 31). Based on their functional role, all of these should result in aberrant activation of the PI3K pathway and, thus, generate PI3K dependence in tumors which, in turn, may translate into increased sensitivity to PI3K inhibitors. Suggesting an association of these alterations and tumor reliance on this pathway, exceptional responses to TORC1 inhibitors have been reported recently in patients with mutations in TSC1/2 and mTOR (32–34). A full understanding of the suite of tumor genomic alterations and the degree of PI3K pathway activity they generate is incomplete at this time. Nonetheless, the development of therapeutic PI3K inhibitors has been at least partially focused on tumors with alterations in genes in PI3K/AKT/mTOR with the hope of targeting a tumor population more likely to respond to these drugs. Reference 6 summarizes the frequency of these alterations by tumor type.

FEEDBACK MECHANISMS INFORM COMBINATION THERAPIES

Overall, clinical results with single-agent PI3K inhibitors have been modest to date. These may be explained by compensatory mechanisms in cancer cells and host tissues that limit the net effect of these drugs when used as single agents. For example, multiple studies (35–40) have shown that upon therapeutic inhibition of the PI3K/AKT/mTOR pathway, cancer cells upregulate transcription of several RTKs (ERBB3, InsR, IGF-IR, FGFRs, EGFR, ERBB2, etc.) by a

FoxO-dependent mechanism and partially maintain receptor phosphorylation and activity. Of note, these RTKs engage p85–p110 heterodimers as a means to partially maintain the production of PIP3. This increased expression and compensatory phosphorylation of RTKs following PI3K inhibition represent a mechanism of adaptation by cancer cells, which may be required for the subsequent development of drug resistance. Genes encoding most glycolytic enzymes are under dominant transcriptional control by PI3K/AKT (41). Further, activation of AKT stimulates glucose import and metabolism. Thus, drug-induced inhibition of PI3K/AKT reduces glucose uptake, resulting in increased insulin levels (42) that in turn can activate Ins/IGF-IR and provide a survival mechanism to tumor cells (43). Evidence these are mechanisms of compensation is provided, first, by several examples where the simultaneous inhibition of RTKs sensitizes PI3K-dependent cancer cells to PI3K pathway inhibitors (35, 36, 44–46); and second, by anecdotal data suggesting that the addition of hypoinsulinemic agents may improve the efficacy of PI3K inhibitors. These data have also provided a strong basis for the evaluation of RTK neutralizing antibodies and tyrosine kinase inhibitors in combination with anti-PI3K therapies.

Preclinical studies and retrospective analysis of some clinical trials have suggested ER+/*PIK3CA*-mutant tumors exhibit a lower response to antiestrogens than do ER+/*PIK3CA* wild-type tumors (47, 48). In addition, some patients with ER+ breast cancer who progress on antiestrogen therapy respond clinically to PI3K inhibitors (49). ER+ human breast cancer cell lines that adapt to estrogen deprivation exhibit amplification of PI3K/AKT/mTOR signaling, and PI3K pathway inhibitors prevent acquired hormone independence (50). Low levels of estradiol can rescue ER+/*PIK3CA*-mutant cells from the lethal effect of PI3K inhibitors (51). Further, inhibition of PI3K/AKT results in upregulation of ERα mRNA and protein and ER transcriptional activity (43, 52, 53), also suggesting coregulation of ER and PI3K pathways. Finally, combined inhibition of ER and PI3K is synergistic against ER+/*PIK3CA* mutant xenografts (53, 54). Taken together, these data strongly suggest that combined inhibition of ER and PI3K is the correct therapeutic approach to target these cancers.

Vora et al. (55) conducted a combinatorial drug screen on several *PIK3CA*-mutant cancer cells that had been selected for acquired resistance to PI3K inhibitors. In these cells, combined inhibition of CDK4/6 and PI3K reduced cell viability and growth of drug-resistant *PIK3CA*-mutant xenografts. Notably, sensitive cancer cells and patients' tumors exhibited suppression of Rb phosphorylation upon treatment with the PI3Kα inhibitor BYL719, whereas drug-resistant cancer cells and patients' tumors with lower sensitivity failed to do so.

Another study using GEMs deficient in *BRCA1* and *p53*, a genotype observed in triple-negative, basal-like breast cancer, showed that treatment with the pan-PI3K inhibitor buparlisib increases indicators of DNA damage, poly-ADP-ribosylation (PAR), and γ-H2AX, but decreases Rad51 focus formation. These results suggested that class I_A PI3K catalytic activity is required for recruitment of Rad51 into sites of DNA damage in *BRCA1*-deficient cancer cells. The PARP inhibitor olaparib attenuated *BRCA1/p53*-mutant tumor growth modestly; however, the combination of buparlisib and olaparib was markedly synergistic (56), suggesting that combined PI3K and PARP inhibition might be an effective treatment of *BRCA1*-related tumors with homologous recombination deficiency. At this time, the combination of olaparib and buparlisib is being investigated in phase II trials in breast and ovarian cancer.

THERAPEUTIC INHIBITORS OF PI3K

Several drugs targeting PI3K have been developed and are currently in clinical trials in different phases of clinical development (**Table 1**), alone or in combination, in both solid tumors and

Table 1 Select PI3K inhibitors in clinical development

Drug target	Drug	Source	Phase of development	Tumor types
Pan-PI3K	Buparlisib (BKM120)	Novartis	Phase 3	Advanced solid cancers, NSCLC, endometrial, prostate, breast, colorectal, pancreatic, renal cell, GIST, melanoma, glioblastoma, leukemia, HNSCC, TCC
	Pictilisib (GDC0941)	Roche/Genentech	Phase 2	Advanced solid cancers, breast, non-Hodgkin's lymphoma, NSCLC
	XL-147 (SAR245408)	Sanofi/Exelixis	Phase 2	Solid tumors, endometrial, ovarian, breast, NSCLC, lymphoma, glioblastoma
	PX-866	Oncothyreon	Phase 2	Solid cancers, prostate, NSCLC, HNSCC colorectal, glioblastoma, melanoma
	BAY80-6946	Bayer	Phase 2	Solid cancers, breast, NSCLC, non-Hodgkin's lymphoma
	ZSTK474	Zenyaku Kogyo	Phase 1/2	Advanced solid cancers
	CH5132799	Chugai Pharma	Phase 1	Solid cancers
p110α	Taselisib (GDC0032)	Genentech	Phase 3	Solid cancers, breast, NSCLC
	Alpelisib (BYL719)	Novartis	Phase 2	Solid cancers, breast, renal, pNET, pancreatic, HNSCC, esophageal, gastric, colorectal, AML, MDS, multiple myeloma, GIST
	MLN1117 (INK1117)	Millennium	Phase 1	Advanced solid tumors
p110β	GSK2636771	GlaxoSmithKline	Phase 1/2a	Advanced solid cancers (PTEN deficient)
	AZD8186	AstraZeneca	Phase 1	NSCLC, TNBC
p110δ	Idelalisib (CAL-101)	Gilead/Calistoga	Phase 3	CLL, lymphomas, AML, multiple myeloma
	Duvelisib (IPI-145)	Infinity	Phase 3	Advanced hematologic malignancies, CLL, lymphoma
PI3K/mTOR	BEZ235	Novartis	Phase 2	Advanced solid cancers, breast, RCC, TCC, prostate, leukemias, pNET
	GDC0980	Genentech	Phase 2	Solid cancers, non-Hodgkin's lymphoma, breast, prostate, RCC
	PKI-587	Pfizer	Phase 2	Solid cancers, endometrial, colorectal
	XL-765 (SAR245409)	Sanofi/Exelixis	Phase 2	Solid tumors, ovarian, lymphoma, CLL, breast, melanoma, NSCLC, colorectal, glioblastoma, astrocytoma
	BGT226	Novartis	Phase 1/2	Advanced solid cancers, breast
	DS-7423	Daiichi Sankyo	Phase 1	Solid tumor, colorectal, endometrial
	PWT33597	Pathway	Phase 1	Advanced solid cancers, lymphoma
	SF1126	Semafore	Phase 1	Advanced solid cancers

Abbreviations: AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; GIST, gastrointestinal stromal tumor; HNSCC, head and neck squamous cell carcinoma; MDS, myelodysplastic syndrome; NSCLC, non–small cell lung cancer; pNET, pancreatic neuroendocrine tumor; RCC, renal cell carcinoma; TCC, transitional cell carcinoma of the urothelium; TNBC, triple-negative breast cancer.

hematologic malignancies. The development of these drugs has focused on tumors with frequent somatic alterations in the PI3K pathway, such as breast cancer.

Pan-PI3K Inhibitors

Pan-PI3K inhibitors [such as pictilisib (GDC-0941), XL147, buparlisib (BKM120), and the irreversible PX-866] target all p110 isoforms. Because of its narrower activity and broader specificity, this class of drugs may be better suited to treat tumors regardless of a *PIK3CA* mutation (such as triple-negative breast, prostate, and endometrial cancers), although Janku et al. (57) showed a favorable response to PI3K/AKT/mTOR inhibitors in patients with *PIK3CA*-mutant tumors who had failed conventional therapy. Phase I trials with buparlisib (BKM120) and XL-147 showed that treatment partially inhibited PI3K as measured by levels of p-S6 and P-AKT in patients' skin or tumors, and by 2-deoxy-2-[^{18}F]fluoro-D-glucose uptake measured by positron emission tomography (FDG-PET). Few clinical responses have been observed in patients with and without detectable PI3K pathway mutations, although screening for genetic lesions in this pathway was limited (42).

Overall, the adverse-event profile of pan-PI3K inhibitors has been acceptable, with no unexpected toxic effects. Toxic effects have been primarily mild to moderate and manageable with supportive medication. Dose-limiting toxic effects reported with multiple agents include hyperglycemia, maculopapular rash, gastrointestinal intolerance (anorexia, nausea, vomiting, dyspepsia, diarrhea), and stomatitis. Although some of these are "off-target" effects, others may be related to target engagement and directly related to mechanisms of action, such as hyperglycemia, which is more commonly seen upon more sustained inhibition of p110α (42). In the case of sustained inhibition of p110δ, lymphopenia is common, since the δ isozyme is highly expressed in lymphocytes.

Interestingly, buparlisib (BKM120) can cross the blood–brain barrier (Novartis, data on file), potentially inhibiting PI3K in the central nervous system (58, 59). In phase I clinical trials, some patients treated with buparlisib developed mood alterations (anxiety, irritability, or depression) likely resulting from inhibition of PI3K in the central nervous system. These side effects were generally mild and responsive to dose reductions or interruptions (suggestive of dose dependence), as well as treatment with selective serotonin reuptake inhibitors and anxiolytics (42, 49).

Isoform-Specific PI3K Inhibitors

The initial rationale for the development of isozyme-specific antagonists was to allow anti-p110α, anti-p110β, and anti-p110δ agents to be delivered at maximal target-inhibitory doses while potentially avoiding the side effects of pan-PI3K inhibitors. Specific inhibitors of p110α [e.g., alpelisib (BYL719) and MLN1117] and p110β-sparing inhibitors [e.g., taselisib (GDC-0032)] were also designed to work better against *PIK3CA*-mutant tumors, whereas p110β inhibitors (e.g., GSK2636771) would work better in *PTEN*-deficient tumors, such as endometrial cancer and lymphoblastic leukemia. Because p110δ is highly expressed in lymphocytes, p110δ inhibitors [e.g., idelalisib (CAL-101)] were initially developed in hematologic malignancies.

Dual PI3K/mTOR Inhibitors

Laboratory studies had clearly shown that treatment with allosteric inhibitors of TORC1 (rapalogs) releases IRS-1 suppression by S6 kinase. The release of this feedback mechanism results in IRS-1-mediated reactivation of AKT, which, in turn, counteracts and limits the antitumor effect of rapalogs (40). This mechanistic science may explain the limited clinical activity of

rapalogs against solid tumors (60). These data also support the development of dual PI3K/mTOR inhibitors in the hopes of overcoming the reactivation of PI3K/IRS-1/AKT upon inhibition of TORC1 with rapalogs and more comprehensively inhibiting PI3K/AKT/mTOR at multiple signaling hubs. Dual PI3K/mTOR inhibitors (such as SF1126, BEZ235, XL765, and GSK1059615) inhibit all p110 isoforms as well as the mTOR kinase, thus inhibiting both TORC1 and TORC2 complexes. They should be an effective approach to block AKT by inhibiting TORC2-mediating activation of AKT as well as AKT-mediated stimulation of TORC1 downstream. In accord with this expectation, they exhibit a broad activity profile and significantly higher toxicity. In general, this class of drugs would be quite suitable to treat tumors with broad genetic abnormalities or loss of function in *PTEN*, *TSC1/2*, and *STK11*. Of note, alterations of *STK11/LKB1* in conjunction with *KRAS* mutations are seen in one-third of non–small cell lung cancers (61).

PFS: progression-free survival

Other PI3K Pathway Inhibitors

Inhibitors of mTOR such as rapamycin (sirolimus) and the rapalogs temsirolimus (CCI-779), everolimus (RAD001), and deforolimus (AP23573) have been extensively and successfully evaluated in hematologic malignancies and transplant rejection treatment. In solid tumors, clinical benefit has also been shown in trials incorporating TORC1 inhibitors. Two phase III randomized trials led to the FDA approval of mTOR inhibitors in the treatment of renal cell carcinoma. First, a trial comparing temsirolimus to interferon in patients with poor-prognosis advanced renal cancer showed a survival benefit in favor of temsirolimus (62). In a second trial, everolimus was associated with improved progression-free survival (PFS) compared with best supportive care in patients with metastatic renal disease previously exposed to sunitinib or sorafenib (63).

The BOLERO-2 phase III trial led to FDA approval of the combination of everolimus with the aromatase inhibitor exemestane in patients with ER+ metastatic breast cancer refractory to aromatase inhibitors. Treatment with the combination resulted in significantly higher PFS than was seen in the exemestane arm (64). A subsequent analysis, however, did not show an improvement in overall survival. In at least one-third of the patients' tumors, DNA was extracted and analyzed by targeted-capture next-generation sequencing (NGS). Benefit from treatment with the combination over the control exemestane arm was maintained in the subgroups defined by genes with a mutation rate >10% (e.g., *PIK3CA*, *FGFR1*, *CCND1*). In patients whose tumors harbored zero or one genetic alteration in PI3K or FGFR pathways or CCND1, the combination had a greater treatment effect than did everolimus (HR = 0.27; 95% CI, 0.18–0.41, adjusted by covariates in 76% of the NGS population), suggesting a predictive value of these somatic alterations (65).

Other novel pathway inhibitors, such as the AKT inhibitors AZD5363, GDC0068, and MK2006, are also in early clinical trials. Investigation of these drugs has focused on PTEN-deficient cancers as well as *AKT1*- and *PIK3CA*-mutant tumors. Dual TORC1/TORC2 inhibitors, including MLN128 and AZD2014, have also been developed in the hope of overcoming the reactivation of PI3K/AKT upon inhibition of TORC1 with rapalogs such as everolimus. Several clinical trials are now testing if dual TORC1/TORC2 inhibitors have better activity against solid tumors known to initially respond to TORC1 inhibitors (such as renal carcinoma) or tumors with high proportion of mutations in *TSC1/2* (such as astrocytomas associated with tuberous sclerosis complex and bladder cancer).

CLINICAL TRIALS IN CANCER

Multiple phase I, II, and III clinical trials are evaluating the role of PI3K pathway inhibitors, alone or in combination, in both solid tumors and hematologic malignancies. This section highlights

some of the preclinical and clinical work in breast cancer and some hematologic malignancies, where several phase II and phase III registration trials are either completed or ongoing (**Table 2**).

PR+: progesterone receptor positive

ER+ Breast Cancer

Activation of PI3K/AKT has been shown to confer resistance to antiestrogens in various models of breast cancer, including PTEN- and INPP4B-deficient cells and cells overexpressing HER2, IGF-IR, and mutant AKT1 (48). Inhibitors of the PI3K pathway have also been shown to trump the adaptation of ER+ breast cancer cells to estrogen deprivation (51). In some retrospective series, activating mutations in *PIK3CA* correlate with good patient prognosis (66). However, PI3K has been shown to interact with ER directly and indirectly, resulting in ER phosphorylation and an increase in estrogen- and tamoxifen-induced as well as ligand-independent ER transcription. Further, preclinical evidence with ER+/*PI3K*-mutant breast cancer cells and xenografts suggests that simultaneous inhibition of PI3K and ER exerts synergistic antitumor activity (51, 53, 54), providing a rationale for combining antiestrogens with PI3K pathway inhibitors in hormone-receptor-positive breast cancer.

The first reported clinical trial of a PI3K inhibitor combined with endocrine therapy was a phase Ib study of the oral reversible pan-PI3K inhibitor buparlisib with letrozole in postmenopausal patients with ER+ metastatic breast cancer (49). Two patients had an objective response to treatment, and ~30% of patients remained progression free for ≥6 months. Of note, *PIK3CA* mutation status did not predict clinical activity. Buparlisib is now in two ongoing phase III trials, in combination with fulvestrant or placebo, in postmenopausal ER+/HER2– metastatic breast cancer refractory to aromatase inhibitors (BELLE-2; ClinicalTrials.gov NCT01610284) and refractory to aromatase inhibitors and everolimus (BELLE-3; ClinicalTrials.gov NCT01633060). Recently, the results of FERGI (67), the first phase II trial of fulvestrant with or without the pan-PI3K inhibitor pictilisib (GDC-0941) for ER+ metastatic breast cancer refractory to aromatase inhibitors, were reported. The addition of pictilisib to fulvestrant failed to improve PFS in either *PIK3CA*-mutant or wild-type breast cancers. A more significant benefit was seen in PR+ patients, regardless of *PIK3CA* mutation, suggesting that in tumors with a luminal A intrinsic phenotype, PI3K inhibition might be enough to cooperate with endocrine therapy in reversing resistance. The incidence of hyperglycemia—a surrogate biomarker of PI3K inhibition—was low, suggesting pictilisib did not strongly inhibit PI3K in tumors and, therefore, FERGI may not have been a definitive test of the clinical value of PI3K inhibitors in ER+ breast cancer.

No known biomarkers can reliably predict benefit (or lack thereof) from PI3K/mTOR inhibitors in ER+ breast cancer. This has been a limitation for clinical trial development. An ongoing phase II neoadjuvant trial of letrozole with or without the pan-PI3K inhibitor buparlisib (BKM120) or the PI3Kα inhibitor alpelisib (BYL719) for operable ≥T1c breast cancers (ClinicalTrials.gov NCT01923168) not only could help elucidate differences in outcomes based on genomic profiling, such as treatment effect of the two different PI3K inhibitors in ER+/*PIK3CA*-mutant versus ER+/*PIK3CA* wild-type tumors, but also could help define mechanisms of resistance to novel therapies in cancers that remain in the breast after neoadjuvant treatment.

Numerous other trials evaluating other pan-PI3K and isoform-specific PI3K inhibitors in ER+ breast cancer are ongoing (**Table 2**), including attempts to circumvent the activation of a compensatory pathway in cancers that become resistant to both endocrine- and PI3K-targeted therapies. For instance, combinations with PARP inhibitors and triple combinations of endocrine therapy, PI3K pathway inhibitors, and CDK4/6 inhibitors are under investigation.

Table 2 Select phase II and III clinical trials with PI3K inhibitors by tumor and target type

Drug target	Drug	Tumor type	Phase of development	Title	ClinicalTrials.gov NCT Number
Pan-PI3K	Buparlisib (BKM120)	Head and neck cancer	Phase 1/Phase 2	PI3K Inhibitor BKM120 and Cetuximab in Treating Patients With Recurrent or Metastatic Head and Neck Cancer	NCT01816984
			Phase 2	Activity and Safety Study of BKM120 in Monotherapy in Patient With Metastatic Head and Neck Cancer Recurrent or Progressive	NCT01737450
		Glioblastoma multiforme	Phase 1/Phase 2	Safety and Efficacy of INC280 and Buparlisib (BKM120) in Patients With Recurrent Glioblastoma	NCT01870726
			Phase 1/Phase 2	Combination of BKM120 and Bevacizumab in Refractory Solid Tumors and Relapsed/Refractory Glioblastoma Multiforme	NCT01349660
		Breast cancer	Phase 2	NeoPHOEBE: Neoadjuvant Trastuzumab + BKM120 in Combination With Weekly Paclitaxel in HER2-positive Primary Breast Cancer	NCT01816594
			Phase 2	Trial of BKM120/Tamoxifen-combination in Patients With HR-pos, HER2-neg Breast Cancer	NCT02404844
			Phase 3	A Phase III Study of BKM120 With Fulvestrant in Patients With HR+, HER2-, AI Treated, Locally Advanced or Metastatic Breast Cancer Who Progressed on or After mTORi	NCT01633060
		Non-Hodgkin's lymphoma	Phase 2	Safety and Efficacy of BKM120 in Relapsed and Refractory NHL	NCT01693614
			Phase 2	Buparlisib (BKM120) in Patients With Recurrent/Refractory Primary Central Nervous System Lymphoma (PCNSL) and Recurrent/Refractory Secondary Central Nervous System Lymphoma (SCNSL)	NCT02301364
		Non–small cell lung cancer	Phase 2	Trial of Erlotinib and BKM120 in Patients With Advanced Non Small Cell Lung Cancer Previously Sensitive to Erlotinib	NCT01487265
	BAY80-6946	Non-Hodgkin's lymphoma	Phase 2	Open-label, Uncontrolled Phase II Trial of Intravenous PI3K Inhibitor BAY80-6946 in Patients With Relapsed, Indolent or Aggressive Non-Hodgkin's Lymphomas	NCT01660451

p110α	Taselisib (GDC0032)	Breast cancer	Phase 3	SANDPIPER Study: A Study of Taselisib + Fulvestrant Versus Placebo + Fulvestrant in Patients With Advanced or Metastatic Breast Cancer Who Have Disease Recurrence or Progression During or After Aromatase Inhibitor Therapy	NCT02340221
	Alpelisib (BYL719)	Breast cancer	Phase 2	Study of Letrozole With or Without BYL719 or Buparlisib, for the Neoadjuvant Treatment of Postmenopausal Women	NCT01923168
			Phase 2	A Phase II Study With BYL719 in Premenopausal Patients With Locally Advanced or Metastatic Breast Cancer	NCT02058381
			Phase 2	Study of LEE011 With Fulvestrant and BYL719 or BKM120 in Advanced Breast Cancer	NCT02088684
			Phase 3	Study Assessing the Efficacy and Safety of Alpelisib Plus Fulvestrant in Men and Postmenopausal Women With Advanced Breast Cancer Which Progressed on or After Aromatase Inhibitor Treatment	NCT02437318
			Phase 1/Phase 2	BYL719 and Nab-Paclitaxel in Locally Recurrent or Metastatic HER-2 Negative Breast Cancer	NCT02379247
			Phase 1/Phase 2	Study of LEE011, BYL719 and Letrozole in Advanced ER+ Breast Cancer	NCT01872260
		Head and neck squamous cell carcinoma	Phase 2	An Open Label, Single Arm, Multicenter Phase II Study of BYL719 in Patients With Recurrent or Metastatic Squamous Cell Carcinoma of Head and Neck Who Failed to Respond to Platinum-based Therapy	NCT02145312
			Phase 1/Phase 2	A Phase Ib/II Study of BYL719 and Cetuximab in Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma	NCT01602315
		Multiple myeloma	Phase 1/Phase 2	Study of the Safety and Effectiveness of LGH447 and BYL719 in Patients With Relapsed and Refractory Multiple Myeloma	NCT02144038
		Advanced solid tumors	Phase 1/Phase 2	A Phase Ib Study of MEK162 Plus BYL719 in Adult Patients With Selected Advanced Solid Tumors	NCT01449058

(*Continued*)

Table 2 (*Continued*)

Drug target	Drug	Tumor type	Phase of development	Title	ClinicalTrials.gov NCT Number
p110δ	Idelalisib (CAL-101)	Waldenström macroglobulinemia	Phase 2	Study of Phosphatidylinositol-3-kinase (PI3K) Inhibitor Idelalisib (GS-1101) in Waldenström Macroglobulinemia	NCT02439138
		Chronic lymphocytic leukemia/small lymphocytic lymphoma	Phase 2	A Study of Idelalisib (GS1101, CAL101) + Ofatumumab in Previously Untreated CLL/SLL	NCT02135133
	Duvelisib (IPI-145)	Non-Hodgkin's lymphoma	Phase 2	A Phase 2 Study of Duvelisib in Subjects With Refractory Indolent Non-Hodgkin Lymphoma (DYNAMO)	NCT01882803
			Phase 1/Phase 2	A Study of Duvelisib in Combination With Rituximab or Obinutuzumab in Subjects With Previously Untreated CD20+ Follicular Lymphoma (CONTEMPO)	NCT02391545
		Chronic lymphocytic leukemia/small lymphocytic lymphoma	Phase 1/Phase 2	A Phase 1b/2 Study of IPI-145 Plus FCR in Previously Untreated, Younger Patients With CLL	NCT02158091
			Phase 3	A Phase 3 Study of Duvelisib Versus Ofatumumab in Patients With Relapsed or Refractory CLL/SLL (DUO)	NCT02004522
			Phase 3	A Phase III Extension Study of Duvelisib and Ofatumumab in Patients With CLL/SLL Previously Enrolled in Study IPI-145-07	NCT02049515

Abbreviations: AI, aromatase inhibitors; CLL, chronic lymphocytic leukemia; SLL, small lymphocytic leukemia.

HER2+ Breast Cancer

Aberrant activation of PI3K/AKT has been shown to confer resistance to HER2-targeted therapies in various experimental models (68–70). A recent study showed that transgenic mammary tumors expressing both HER2 and PIK3CA^{H1047R} were highly resistant to the combinations of trastuzumab plus pertuzumab and trastuzumab plus lapatinib. Interestingly, the addition of the pan-PI3K inhibitor buparlisib to each combination restored drug-induced inhibition of tumor growth (71).

Retrospective reports show that patients with *HER2*-amplified/*PIK3CA*-mutant breast cancer exhibit a lower clinical response and PFS in the metastatic setting, and worse disease-free and overall survival in the adjuvant setting (70, 72–77). However, there is still controversy over the predictive value of *PIK3CA* mutations for benefit from HER2-targeted therapies. A prospective analysis of 737 patients with HER2+ breast cancer in two large European neoadjuvant studies (GeparQuinto and GeparSixto trials) that utilized trastuzumab, lapatinib, or the combination of these drugs with chemotherapy, revealed that about 20% of tumors had a *PIK3CA* mutation. The rate of pathologic complete response was significantly lower in patients with *PIK3CA*-mutant cancer than in those with wild-type tumors, particularly for patients with HER2+/ER+ breast cancer and those who received both lapatinib and trastuzumab (78). Consistent with GeparQuinto and GeparSixto, analyses of the Neo-ALTTO (77, 79), Neosphere (80), and TBCRC006 (81) phase II trials also observed a significantly lower pathologic complete response rate for *PIK3CA*-mutant tumors than for wild type. However, a large phase III adjuvant trial (NSABP B-31 trial) that randomly assigned women with HER2+ stage II–III breast cancer to adjuvant chemotherapy with or without 12 months of trastuzumab found no difference in outcome between the *PIK3CA*-mutant (25% of patients) and wild-type subgroups (disease-free survival HR 0.44 versus 0.51) (82). Following all these retrospective studies, NeoPHOEBE (ClinicalTrials.gov NCT01816594) is a prospective, phase II randomized trial of neoadjuvant paclitaxel and trastuzumab with or without the pan-PI3K inhibitor buparlisib (BKM120) for treatment of stage II and III HER2+ breast cancers, which will try to clarify these discrepancies.

In summary, *PIK3CA* mutation status may not be a reliable predictive biomarker for selection of HER2-targeted therapies, particularly in early-stage disease. We recognize, however, there may be differences in the interactions between *PIK3CA* mutation status and HER2 overexpression in the primary tumor versus metastases.

B Cell Lymphoproliferative Disorders

Several preclinical studies provided a strong rationale for the use of p110δ inhibitors as a targeted therapy for B cell lymphoproliferative disorders. Idelalisib (CAL-101, GS-1101) is an oral, first-in-class, highly selective inhibitor of p110δ with proapoptotic activity in B cell acute lymphoblastic leukemia and chronic lymphocytic leukemia (CLL) cell lines (83, 84), as well as in Hodgkin's lymphoma cell lines (85). On the basis of single-agent activity observed in (*a*) early-phase trials in patients with relapsed/refractory CLL (86, 87) and relapsed/refractory mantle-cell lymphoma and (*b*) combination trials with rituximab and/or bendamustine in patients with relapsed/refractory CLL (88, 89), a phase III randomized double-blind placebo-controlled study is currently assessing the efficacy and safety of bendamustine and rituximab with or without idelalisib for patients with previously treated CLL (90). Based on a study that evaluated idelalisib plus ofatumumab as salvage therapy in relapsed/refractory CLL (89), another phase III randomized controlled study is exploring ofatumumab with or without idelalisib in patients with refractory/relapsed CLL who had progressed after treatment with a purine analog and/or bendamustine. Finally, based on response rates of idelalisib plus rituximab in patients with relapsed or refractory CLL, this combination

is now being tested in treatment-naive, elderly patients with CLL/small lymphocytic lymphoma. Idelalisib is FDA approved for the treatment of relapsed CLL in combination with rituximab. Additionally, accelerated approval has been granted for treatment of relapsed follicular B cell non-Hodgkin's lymphoma (NHL) and relapsed small lymphocytic lymphoma.

The combination of idelalisib with rituximab, bendamustine, or both was noted to be very active in phase I studies in patients with NHL. Currently a phase III randomized trial comparing idelalisib plus bendamustine and rituximab versus placebo plus bendamustine and rituximab in heavily pretreated patients with NHL has been initiated, with PFS as its main endpoint.

CONCLUSION

The PI3K/AKT pathway is the most frequently mutated network in human cancer. Aberrant activation of this pathway is clearly associated with tumorigenesis, cancer progression, and drug resistance. The diversity of alterations in this pathway (p110, p85, AKT, mTOR, PTEN, etc.) provides multiple molecular targets for therapy and raises the challenge of identifying the key hub or hubs where a targeted therapeutic intervention would be most effective.

Following the approval of TORC1 inhibitors, which disable one of the pathways downstream of PI3K, pan-PI3K and isozyme-specific PI3K inhibitors are poised to be approved for use in human cancer at the time of this writing. The PI3Kδ inhibitor idelalisib has been approved by the FDA for treatment of B cell lymphomas. Biomarkers that identify PI3K-dependent cancers, which as a result of that dependence would be more likely to respond to these drugs, are not yet known. Fortunately, large randomized registration trials with PI3K inhibitors include an adequate number of tumors with activating "hot spot" *PIK3CA* mutations in order to determine if the benefit of these drugs, if present, will be limited to or predominant among cancers of that genotype. On-target toxicities induced by these drugs are manageable but not insignificant. These toxicities should be avoidable by *PIK3CA*-mutant-specific inhibitors, which would spare wild-type *PIK3CA*. Development of these inhibitors is highly anticipated and would represent a major advance in this field.

Despite early signals of clinical activity, several challenges for the therapeutic targeting of PI3K/AKT remain. These include exploring mechanisms of compensation in tumor and host tissues upon treatment with these drugs that limit their optimal biological dosing and antitumor effect but also identify other targeted drugs that should be used in combination with PI3K pathway inhibitors. It is clear that the benefit of these drugs will require development of rational combinations before they become a significant component of the anticancer portfolio across solid and hematologic cancers.

DISCLOSURE STATEMENT

Erratum >

Dr. Arteaga was an Advisory Board member for Novartis (2014) and consultant for Genentech (2015), AstraZeneca (2015), and Millennium Pharmaceuticals (2015).

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