Challenges for the Clinical Development of PI3K Inhibitors: Strategies to Improve Their Impact in Solid Tumors

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ABSTRACT The PI3K pathway is mutated and aberrantly activated in many cancers and plays a central role in tumor cell proliferation and survival, making it a rational therapeutic target. Until recently, however, results from clinical trials with PI3K inhibitors in solid tumors have been largely disappointing. Here, we describe several factors that have limited the success of these agents, including the weak driver oncogenic activity of mutant PI3K, suboptimal patient selection in trials, drug-related toxicities, feedback upregulation of compensatory mechanisms when PI3K is blocked, increased insulin production upon PI3Kα inhibition, lack of mutant-specific inhibitors, and a relative scarcity of studies using combinations with PI3K antagonists. We also suggest strategies to improve the impact of these agents in solid tumors. Despite these challenges, we are optimistic that isoform-specific PI3K inhibitors, particularly in combination with other agents, may be valuable in treating appropriately selected patients with PI3K-dependent tumors.

Significance: Despite the modest clinical activity of PI3K inhibitors in solid tumors, there is an increasing understanding of the factors that may have limited their success. Strategies to ameliorate drug-related toxicities, use of rational combinations with PI3K antagonists, development of mutant-selective PI3K inhibitors, and better patient selection should improve the success of these targeted agents against solid tumors.

INTRODUCTION

The PI3K/AKT/TOR signaling network is commonly altered in several human cancers. Gain-of-function mutations in *PIK3CA*, the gene encoding the p110α catalytic subunit of PI3K, are among the most common somatic alterations in solid tumors. Other alterations in the pathway include mutations in *PIK3R1*, encoding the PI3K regulatory subunit p85α, the PI3K effectors *AKT1/2/3*, and loss of the lipid phosphatases PTEN and INPP4B (reviewed in ref. 1). Further, PI3K is aberrantly activated by activated oncogenes and/or amplified/mutated tyrosine kinases such as mutant *RAS*, *ERBB2* (HER2), *MET*, *BCR–ABL*, and *KIT*, among others. The association of these alterations with a transformed phenotype both *in vitro* and *in vivo* has led to the development of a plethora of PI3K antagonists. These include pan-PI3K inhibitors, inhibitors of all PI3Ks and mTOR, and other ATP mimetics with variable selectivity to the p110α (PI3Kα) isozyme (reviewed in ref. 2). Despite the initial enthusiasm for and significant investment in the development of PI3K inhibitors for solid tumors, they have not yielded the outstanding clinical activity observed with other approved targeted therapies.

In this review, we present a critical analysis for this modest outcome and speculate on possible directions to improve the therapeutic targeting of this oncogenic pathway, with a focus on PI3Kα (Table 1). Of note, the PI3Kδ inhibitors idelalisib and copanlisib are effective and currently approved for the treatment of non-Hodgkin lymphoma, and are not the focus of this review. For a comprehensive review of recent progress in targeting all PI3K isoforms, AKT, and mTOR, we refer the reader to Janku and colleagues (3). We also focus this review on inhibition of PI3K within tumor cells. However, we note that there is increasing evidence that interfering with stromal PI3K activity may contribute to the antitumor effects of PI3K inhibitors, particularly through inhibition of angiogenesis (PI3Kα inhibitors) and through modulating the immune system (PI3Kγ/δ inhibitors; reviewed in ref. 4).

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Table 1. Clinical obstacles to PI3K inhibitor efficacy and proposed solutions

Clinical obstacles	Proposed solutions
Suboptimal patient selection	• Selection of patients with tumors harboring activating *PIK3CA* mutations • Identification of PI3K-dependent cancers (i.e., endocrine-resistant ER$^+$ breast cancers with *PIK3CA* mutations) • Exclusion of tumors harboring biomarkers of resistance in *PIK3CA*-mutant tumors (i.e., *KRAS*, *TP53*, or *FGFR1*) • Identification of other genotypes that may benefit from PI3K inhibitors (i.e., *PIK3R1* mutations or *PIK3CA* amplification)
Drug-related toxicity limits target inhibition	• Focus on isoform-specific inhibitors • Development of *PIK3CA* mutant–selective inhibitors
Feedback upregulation of compensatory mechanisms	• Use of combinations that limit adaptive response (i.e., with antiestrogens, RTK and PI3Kβ inhibitors, CDK4/6 inhibitors) • Optimizing dosing schedules of combinations to ameliorate toxicities
Increase in insulin production upon systemic inhibition of PI3Kα	• Combinations with SGLT2 inhibitors or ketogenic diet • Development of *PIK3CA* mutant–selective inhibitors

PIK3CA MUTATIONS ARE WEAK ONCOGENES

Genetically engineered mouse models (GEMM) are a powerful approach used to "graduate" a dominant oncogene as an inducer or driver of a cancer. Indeed, several studies using GEMMs have demonstrated a causal role of mutant *Pik3ca* in tumor initiation, progression, and maintenance *in vivo*. However, many of these studies have relied on tissue-specific overexpression of mutant p110α (5, 6), which in human tumors is not found to be amplified and/or overexpressed. Thus, these models may not represent an otherwise low signaling output and transforming potential of these oncogenes. Indeed, knock-in of *Pik3ca*^{H1047R} into the endogenous *Pik3ca* locus of mouse ovary cells results in ovarian epithelial hyperplasia, but no invasive cancers. However, concomitant deletion of *Pten* in mouse ovary leads to the development of serous adenocarcinomas (7). This oncogenic cooperativity is reminiscent of frequent coexistence of *PIK3CA* mutations with KRAS and/or PTEN alterations in human ovarian (8) and endometrial (9) cancers, suggesting that mutations in either *PIK3CA* or *PTEN* alone are not sufficient to hyperactivate PIP3-induced signaling. Mutations in *Trp53* have also been shown to be required to cooperate with mutant *Pik3ca* to induce ovarian cancer and breast cancer in GEMMs (10, 11). Knock-in of *Pik3ca*^{H1047R} in the mouse mammary gland results in hyperplasia and eventual mammary tumorigenesis, but with very long latencies (>12 months) and incomplete penetrance in some models (12, 13), suggesting that time is needed for additional mutational events to trigger tumorigenesis. Cancer development is accelerated by estrogen supplementation, leading to estrogen receptor–positive (ER$^+$) mammary tumors, whereas tumors in *Pik3ca*^{H1047R} mice that did not receive exogenous estrogen were predominantly ER-negative (14). Translationally, these data suggest that mutant *PIK3CA* may not be able to induce invasive cancer progression on its own. Thus, although *PIK3CA* mutations may play a partial role in the progression of carcinomas, its pharmacologic inhibition should be coupled with other therapies in order to exert an important antitumor effect.

PIK3CA mutations that occur early in embryonic development lead to tissue overgrowth in a mosaic-like pattern or *PIK3CA*-related overgrowth spectrum (PROS), venous malformations, epidermal nevi, and brain malformations associated with epilepsy (15). One of these syndromes is CLOVES, a complex disorder characterized by tissue overgrowth and malformations affecting the epidermis, skeleton, internal organs, and central nervous system. PROS disorders do not appear linked to an increased risk of cancer and are not associated with progression to invasive tumors. Further, treatment with PI3Kα inhibitors, at doses much lower than those used in adult cancers, results in marked objective and functional benefits in patients with multiple affected organs (16).

PI3K consists of a catalytic subunit, p110α, and one of several regulatory subunits, a major one being p85α. In the basal state, p85 stabilizes p110α and inhibits its enzymatic activity. Upon stimulation by growth factors, the SH2 domains of p85 bind phosphotyrosine residues on receptor tyrosine kinases (RTK) or signaling adaptors, such as IRS1 and HER3, thus relieving p110α from inhibitory contacts and facilitating its lipid kinase activity at the plasma membrane, where it can access its substrate and receive other inputs from RAS. Oncogenic mutations in *PIK3CA* have been shown to enhance the natural activation of p110α (17). For example, the helical domain mutation E545K can associate with IRS1 independent of p85, thus increasing response to insulin and IGFs (18, 19). Less common deletions in the C2 domain also relieve inhibitory contacts with p85 and enhance p110α activity (20). The most common *PIK3CA* mutation, H1047R in the kinase domain, has higher affinity for cellular membranes, thus bypassing the requirement for association with RAS and resulting in greater access to the PI3K substrate PIP2 (21).

These data suggest that in cancers with *PIK3CA* mutations, these alterations are permissive for growth factor signaling but not potent signaling units or driver oncogenes *per se*.

Further circumstantial evidence in support of this notion is the subclonal nature of mutations in the PI3K pathway in metastatic versus primary lesions from the same patient (22, 23). A large study of the evolution of cancer heterogeneity showed that subclonal mutations in the PI3K/AKT pathway were more frequent and less ubiquitously expressed across tumor subclones than subclonal mutations in the more potent RAS/RAF/MEK/ERK pathway (24). This could have a negative impact in patient selection for a clinical trial targeting *PIK3CA* mutations, as these can be missed if a "PI3K normal" metastasis is profiled. On the other hand, the discordant "dependence" on PI3K signaling of these lesions as a result of this heterogeneity may result in muted clinical responses to a PI3K inhibitor. A possible exception to this generalization is breast cancer, where recent genomic analyses suggest that *PIK3CA* mutations are primarily clonal (24, 25).

SUBOPTIMAL PATIENT SELECTION IN CLINICAL TRIALS

Trials with PI3K inhibitors have suggested preferential clinical activity in patients with *PIK3CA*-mutant cancers. The phase I study of alpelisib included 134 patients with all cancer types; 64 of 76 patients in this trial whose tumors were tested contained hotspot *PIK3CA* mutations in their cancers. The clinical benefit rate was 44% in tumors with *PIK3CA* mutations versus 20% among those patients with *PIK3CA* wild-type (WT) cancers (26). In the phase I trial of taselisib, the overall response rate was 36% among patients with *PIK3CA*-mutant tumors, all with the H1047R variant, versus 0% in the group with *PIK3CA* WT tumors (27). BELLE-2 was the first phase III randomized clinical trial comparing fulvestrant and placebo versus fulvestrant and the pan-PI3K inhibitor buparlisib in patients with ER^+ metastatic breast cancer who had progressed on an aromatase inhibitor (28). In the overall group, treatment with buparlisib and fulvestrant resulted in a modest prolongation of progression-free survival (PFS) by 1.9 months compared with placebo and fulvestrant (6.9 vs. 5 months; HR, 0.79; 95% CI, 0.67–0.89; $P < 0.01$). In the PI3K-activated group (defined as any mutation detected by Sanger sequencing in *PIK3CA* exons 1, 7, 9, or 20, or PTEN expression by IHC in <10% of cells), the PFS in the investigational arm was 6.8 months versus 4 months in the control arm (HR, 0.76; 95% CI, 0.6–0.97; $P = 0.014$). In this trial, 446 patients had paired *PIK3CA* mutation status in tumor and plasma circulating tumor DNA (ctDNA), with 77% concordance between both. Notably, among 307 patients with *PIK3CA* WT tumor tissue, 64 (21%) had *PIK3CA* mutations detected in ctDNA, suggesting that the cancer evolved between the original diagnosis and the development of metastatic disease and treatment in this trial. In an exploratory analysis, a significant difference in PFS in the buparlisib versus the placebo arm was observed in patients with ctDNA *PIK3CA* mutations (7 vs. 3.2 months; HR, 0.56; 95% CI, 0.39–0.8; $P = 0.0005$) but not those with WT ctDNA.

The recent phase III SOLAR-1 trial of alpelisib plus fulvestrant versus placebo plus fulvestrant in patients with metastatic ER^+ breast cancer showed that *PIK3CA*-mutant cancers were more likely to respond to alpelisib. In the *PIK3CA*-mutant cohort ($n = 341$), the median PFS was 11 months in the alpelisib arm versus 5.7 months in the fulvestrant arm (HR, 0.65; 95% CI, 0.5–0.85; $P = 0.00065$). In contrast, in the *PIK3CA* WT cohort ($n = 231$), alpelisib only modestly extended PFS (7.4 vs. 5.6 months; HR, 0.85; 95% CI, 0.58–1.25; ref. 29). A statistically significant benefit in PFS was also observed for alpelisib in patients with *PIK3CA*-mutant ctDNA (10.9 vs. 3.6 months; HR, 0.55; 95% CI, 0.39–0.79; $P = 0.0005$; ref. 30). These data strongly suggest that the development of PI3Kα inhibitors should be focused on *PIK3CA*-mutant tumors.

However, not all patients with *PIK3CA* mutations have similar benefit from PI3K inhibitors. In a phase Ib trial by Mayer and colleagues, patients with *PI3KCA* mutations and concurrent alterations in *KRAS, TP53*, or *FGFR1* did not benefit from alpelisib (31). Larger studies are needed to confirm these associations and to identify other alterations that promote intrinsic resistance. Further, the most frequent mutation in *PIK3CA*, H1047R, appeared to be associated with higher clinical benefit from alpelisib compared with mutations in the helical domain (31). However, this association was not confirmed in the larger SOLAR-1 trial (29). In most studies, there is a small fraction of patients without detectable *PIK3CA* hotspot mutations that respond clinically to PI3K inhibitors. The molecular basis for a potential dependence on PI3K signaling by these tumors has not always been investigated. For example, *PIK3CA* C-terminal truncations and deletion mutants that disrupt the coupling to the p85 regulatory subunit result in hyperactivation of p110α and transformation and have been associated with an excellent clinical response to alpelisib (20). Interestingly, mutations in the corresponding domains of p85 also activate p110α and are oncogenic (32). *PIK3CA* C2 domain mutations and others outside the "hotspots" as well as *PIK3R1* (p85) mutations are generally not included in the DNA-sequencing panels used for patient stratification in trials with PI3K inhibitors. All this would suggest that *PIK3CA* hotspot mutations do not necessarily capture all PI3K-dependent tumor genotypes that can potentially respond to PI3K inhibitors.

Therefore, we propose that only patients with cancers with activating *PIK3CA* mutations and other lesions conferring PI3K pathway dependence (potentially *PIK3R1* mutations, ref. 32; or *PIK3CA* amplifications, ref. 33) should be included in trials with PI3Kα inhibitors. Further selection could be refined by analyzing *PIK3CA* mutation clonality, determining the full repertoire of activating *PIK3CA* mutations (both canonical "hotspot" and less frequent recurrent mutations), and identifying biomarkers of intrinsic resistance to PI3Kα inhibitors (Table 1).

DRUG-RELATED TOXICITY LIMITS SUSTAINED TARGET INHIBITION

A major hurdle for the development of PI3K pathway inhibitors has been the inability to achieve optimal drug-target blockade in tumors while avoiding undue toxicities in patients. Pan-PI3K inhibitors share common, dose-dependent toxicities such as rash, fatigue, hyperglycemia, and diarrhea. In general, toxicity from small-molecule PI3K inhibitors depends on their PI3K isozyme specificity. For example, PI3Kα inhibitors are associated with hyperglycemia and rash, whereas PI3Kδ inhibitors are associated with gastrointestinal side effects, myelosuppression, and transaminitis. This

toxicity profile is even broader with pan-PI3K/mTOR inhibitors. At this time, the development of these nonspecific drugs, which will not be discussed herein, appears to be stalled.

In BELLE-2, the combination of the pan-PI3K inhibitor buparlisib with fulvestrant was found to be superior to fulvestrant and placebo in patients with *PIK3CA*-mutated tumors, even though the median time on buparlisib was <2 months (28). Grade 3–4 toxicities were significant and included hyperglycemia (15%), increased alanine aminotransferase (ALT; 26%), rash (8%), depression (5%), and anxiety (5%), leading to the discontinuation of further drug development. Pictilisib, another pan-PI3K inhibitor, was evaluated in the randomized phase II FERGIE trial that compared pictilisib and fulvestrant versus placebo and fulvestrant. In this study, the investigational arm did not significantly improve progression-free survival, possibly due to its suboptimal dosing limited by toxicity. Toxic side effects included grade 3–4 fatigue (8%) and diarrhea (8%), but rare hyperglycemia and rash (34), further suggesting limited drug-target engagement. Copanlisib, a small-molecule inhibitor with predominant activity against PI3Kα and PI3Kδ, was associated with grade 3–4 hyperglycemia (41%), hypertension (24%), lung infection (16%, including one death on study), and diarrhea (5%; ref. 35). Alpelisib, a PI3Kα-selective inhibitor, at a dose of 300 mg daily, had a better safety profile with grade 3–4 diarrhea and hyperglycemia seen in 10% of patients and grade 3–4 elevation of transaminases seen in 5% of patients (26). The rate of grade 3–4 hyperglycemia was even higher in combination with fulvestrant in the SOLAR-1 trial (36.7%; ref. 29). Taselisib, a PI3Kβ-sparing small-molecule inhibitor, was associated with grade 3–4 hyperglycemia (14.7%), rash (11.8%), diarrhea (5.9%), fatigue (5.9%), and pruritus (5.9%; ref. 27). We speculate that the differences in safety between alpelisib and taselisib are due to inhibition of PI3Kγ/δ by taselisib. Finally, idelalisib, a PI3Kδ selective inhibitor, was associated with grade 3–4 diarrhea (13%), increased ALT (13%), and pneumonia (7%; ref. 36). The increased success of p110δ inhibitors in hematologic malignancies relative to PI3K inhibitors in solid tumors may be due, in part, to the different toxicity profiles.

Due to the safety profile associated with continuous dosing of PI3Kα inhibitors, preclinical studies have experimented with intermittent dosing schedules. Intermittent high doses of the PI3Kα/β inhibitor AZD8835 effectively blocked pAKT, induced apoptosis, and induced regressions in breast cancer xenografts, and facilitated combinations with fulvestrant and palbociclib *in vivo* (37). Likewise, treatment of tumor-bearing mice with high doses of pictilisib every 3 days enabled it to be combined with a MEK inhibitor and blocked growth of xenografts with *KRAS* or *BRAF* and *PTEN* or *PIK3CA* alterations (38). Further exploration of intermittent dosing of PI3K inhibitors in the clinic is warranted. The toxicities from PI3K inhibitors have limited the ability of clinical trials with these small molecules to adequately assess the dose required to optimally inhibit PI3K in cancers *in situ*. On-treatment glucose uptake in primary tumors, as measured by [^{18}F]-FDG-PET, has been used as a surrogate of therapeutic inhibition of PI3K in several early-phase studies (26, 27, 39). Of note, however, the best timing for this pharmacodynamic assessment is not clear, and overall the observed drug-induced inhibition of FDG uptake has been only partial. Thus, on-target toxicities from PI3K inhibitors and suboptimal doses and dosing schedules have limited complete and sustained PI3K inhibition and may explain the discrepancies between the results of preclinical studies and those in clinical trials. In addition, the toxicity profile of PI3K inhibitors makes combinations with some other small molecules quite challenging (see below).

FEEDBACK UPREGULATION OF COMPENSATORY MECHANISMS

Pharmacologic inhibition of the PI3K pathway in cancer cells is followed within hours to days by nongenetic mechanisms of adaptation, eventually leading to drug resistance. This adaptation can be explained in good part by RTK-induced activation of PI3K/AKT/TOR, resulting in AKT-mediated phosphorylation of FOXO proteins (Fig. 1). In turn, FOXO proteins transcriptionally repress RTKs and/or adaptors that activate PI3K, such as HER3, EGFR, IGF1R, insulin receptors (InsR), and FGFRs (40, 41). Further, AKT activates TORC1 and S6K, which repress IRS1 expression in order to regulate pathway signaling output (42). In addition, activated TORC1, downstream of AKT, phosphorylates and activates GRB10, which binds and downregulates InsR (43). Hence, inhibition of PI3K/AKT blocks FOXO phosphorylation and transcriptional repression of RTKs and leads to derepression of S6K and GRB10, resulting in activation of multiple RTKs and partial maintenance of PIP3 formation. In some luminal breast cancer cells with *PIK3CA* mutations or with HER2 gene amplification—where PI3K is hyperactivated as a result of signaling by HER2–HER3 dimers—there is reaccumulation of PIP3 mediated by p110β (44). Using ovarian cancer spheroids, Muranen and colleagues elegantly showed that inhibition of PI3K/mTOR results in death of inner matrix-deprived cells, but cells attached to the matrix survived. This matrix-associated resistance occurs as a result of FOXO-mediated transcription and cap-independent translation of survival factors such as ERα, BCL2, and IGF1R (45). Another FOXO-mediated adaptive response to PI3K inhibition is upregulation of Rictor, resulting in increased AKT phosphorylation in renal cancer cells (46). Finally, inhibition of PI3K/mTOR increases IRS1-dependent activation of JAK2/STAT5 and secretion of IL8 in triple-negative breast cancer cells and primary tumors, with cotreatment with a JAK inhibitor abrogating this feedback loop (47).

These compensatory mechanisms following inhibition of PI3K have been extensively investigated in ER$^+$ human breast cancer cells and primary tumors. Treatment with the AKT inhibitor AZD5363 upregulates several RTKs as well as *ESR1* mRNA and ERα-dependent transcription of IGF1 and IGF2 ligands (48). Bosch and colleagues reported increased *ESR1* mRNA and ER-dependent gene-expression signature in tumors from patients treated with the PI3Kα inhibitor alpelisib. These drug-induced transcriptional changes were abrogated by the anti-ER drugs tamoxifen and fulvestrant (49). Finally, Toska and colleagues elegantly showed that treatment with alpelisib of ER$^+$ breast cancer cells and primary tumors in patients triggers activation of the lysine methyltransferase KMT2D, which, in turn, activates ERα transcriptional activity by facilitating assembly of an ERα–FOXA1–PBX1 complex (50). Taken together, these data

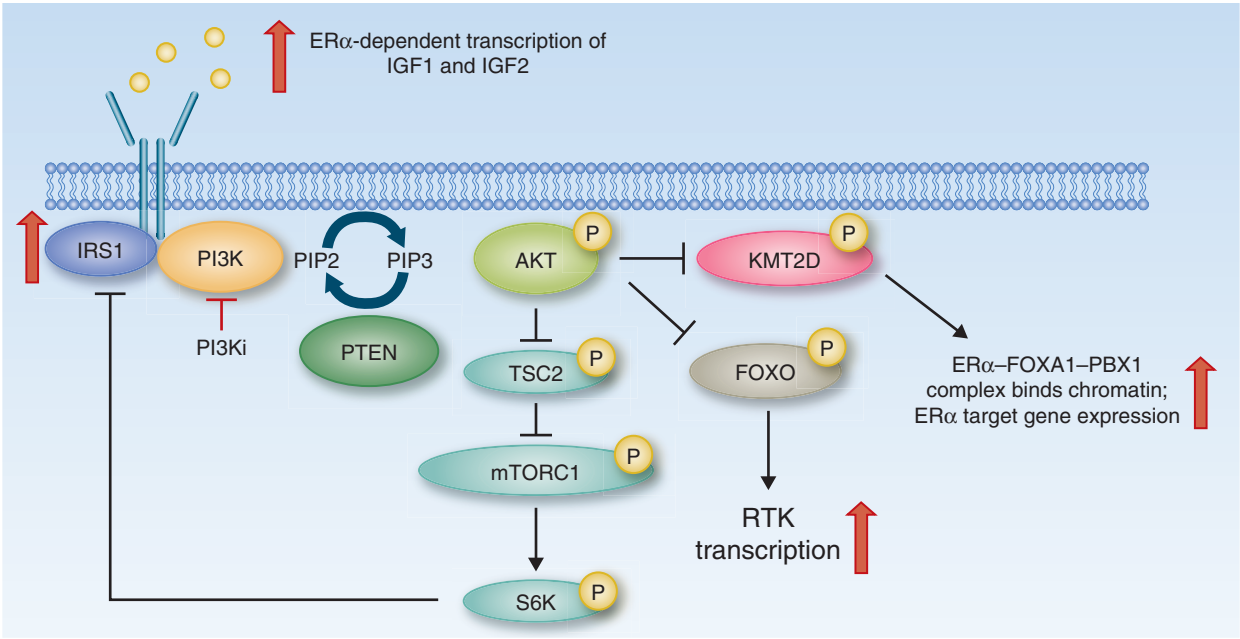

Figure 1. Adaptive upregulation of compensatory pathways limits the efficacy of PI3K inhibitors. Several adaptive feedback mechanisms that limit complete suppression of PIP3 and the cellular response to PI3K inhibitors have been described. These include FOXO-mediated derepression of RTKs and derepression of IRS1, leading to partial maintenance of PIP3. In ER$^+$ breast cancer cells, treatment with PI3K inhibitors induces ERα transcriptional activity via the histone methyltransferase KMT2D.

suggest that in patients with ER$^+$ breast cancer, PI3K inhibitors should be developed in combination with endocrine therapy, thus leading to the current registration trials SANDPIPER and SOLAR-1, discussed below.

The adaptive responses to the inhibition of PI3K in cancer cells have suggested other logical combinations with small molecules or antibodies against RTK signaling pathways, including inhibitors of IGF1R or HER3 (48, 51–53). However, because of the lack of selectivity of the partner drugs and/or mainly toxicity in patients, these combinations have been challenging. For example, a phase Ib trial of the PI3K inhibitor alpelisib with the IGF1R mAb ganitumab (AMG 479; clinicaltrials.gov NCT 01708161) resulted in excessive rash and hyperglycemia and no evidence of clinical activity (https://clinicaltrials.gov/ct2/show/NCT01708161?term=NCT01708161&rank=1). This can probably be explained by elevation of growth hormone (GH) levels upon IGF1R mAb-mediated blockade of IGF1, a ligand necessary for negative regulation of GH in the brain. Elevated GH levels result in insulin resistance partially from increased fatty-acid efflux from the liver leading to enhanced insulin production (54). These elevated insulin and IGF1 levels, in turn, may stimulate tumor growth via activation of uninhibited insulin receptors in cancer cells (55), thus limiting any potential clinical effect of the combination.

Significant gastrointestinal and metabolic toxicities were also observed with the combination of alpelisib, trastuzumab, and the HER3 monoclonal antibody LJM716. These toxicities severely limited drug delivery and dose escalation, thus allowing 72% and 83% of the planned doses of alpelisib and LJM716, respectively (56). As mentioned above, intermittent dosing schedules may optimize the therapeutic index of combination therapies, enabling combinations that are unachievable with continuous dosing.

INCREASE IN INSULIN PRODUCTION UPON INHIBITION OF PI3K

The p110α isozyme and AKT2 mediate insulin-driven glucose uptake in muscle, liver, and fat cells, mainly attributable to the translocation of glucose transporters (GLUT) to the plasma membrane (57). As a result, therapeutic inhibition of PI3K/AKT blocks insulin action, thus preventing glucose uptake in adipose tissue and skeletal muscle, and promoting glycogen breakdown in the liver (Fig. 2). This generates hyperglycemia, which in turn leads to insulin release from the pancreas with potential normalization of glucose levels (reviewed in ref. 58). Therefore, a dose-dependent increase in the plasma levels of fasting C-peptide and insulin, most of the time associated with hyperglycemia, is an obligatory on-target pharmacodynamic surrogate of PI3K inhibition in trials with PI3K inhibitors (26, 59). This obligatory surge in insulin secretion may activate InsR and PI3K, particularly in tumors rich in InsR, and limit the clinical activity of PI3K antagonists. This is supported by the correlation with glucose uptake in primary tumors as measured by [^{18}F]-FDG-PET following treatment with PI3K inhibitors. In the phase Ib trial of letrozole and the pan-PI3K small-molecule inhibitor buparlisib, 50% of patients exhibiting a reduction in FDG tumor uptake derived clinical benefit, whereas increased FDG uptake preceded rapid tumor progression (39). These data suggest, first, that an increase in tumor FDG uptake

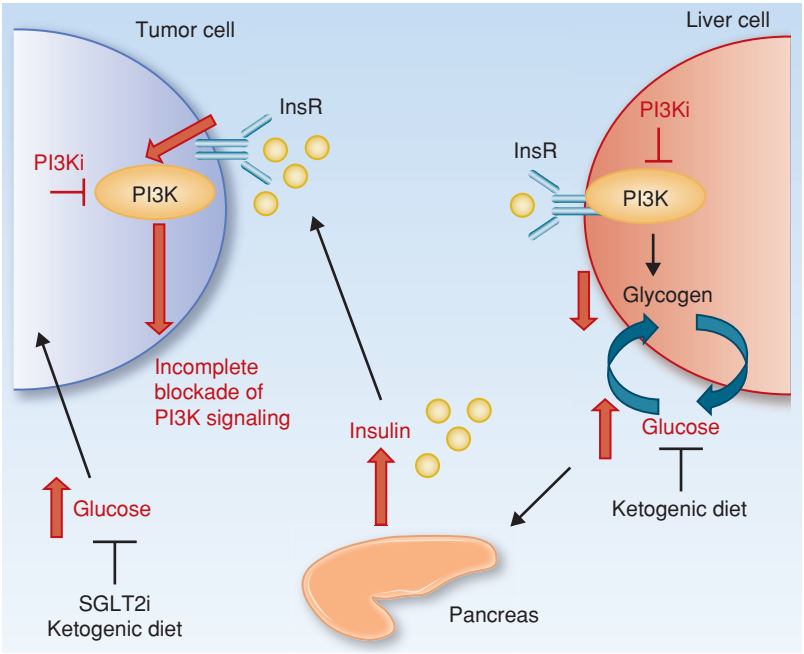

Figure 2. Insulin feedback limits the antitumor effects of PI3K inhibitors. PI3Kα mediates insulin-driven glucose uptake in muscle, liver, and fat cells. Upon inhibition of PI3Kα, glucose uptake in muscle and fat cells is blocked, and breakdown of liver glycogen is stimulated, thus resulting in hyperglycemia. Elevated glucose levels, in turn, drive insulin release from the pancreas. Elevated insulin levels activate the insulin receptor (InsR) in tumor cells, leading to enhanced stimulation of PI3K, and limiting the effects of PI3K inhibitors. Tumors with *PIK3CA* mutations would be particularly sensitive to insulin. In preclinical models, SGLT2 inhibitors or a ketogenic diet restore homeostasis and synergize with PI3K inhibitors to reduce tumor growth. Red arrows indicate response to PI3K inhibitors.

shortly after treatment initiation, potentially explained by an insulin surge following PI3K inhibition in an InsR-rich tumor, can be used as a signal for early treatment discontinuation. Second, the data suggest that the level of on-treatment FDG uptake would reflect the net effect of PI3K inhibition in the cancer counteracted by the insulin-mediated activation of tumor InsR. Thus, the magnitude of inhibition of FDG uptake in tumors on therapy can be used as a metric to score interventions aimed at abrogating the insulin rebound in patients on PI3K inhibitors.

In a recent paper using preclinical animal models, Hopkins and colleagues (60) reported dietary and pharmacologic strategies aimed at preventing insulin feedback that, in turn, enhance the efficacy and reduce the toxicity of PI3K inhibitors. These included the antidiabetic drug metformin, which increases insulin sensitivity and reduces insulin levels; sodium glucose cotransporter 2 (SGLT2) inhibitors, which reduce glucose reabsorption in kidney tubules; and a ketogenic diet, which depletes glycogen stores and thus limits the acute efflux of glucose from the liver upon inhibition of PI3K (60). SGLT2 inhibitors and a ketogenic diet prevented insulin feedback and enhanced the antitumor effect of PI3K pathway inhibitors in both *PIK3CA*-mutant and *PIK3CA* WT tumors. These combinations remain to be tested in the clinic and are of particular importance considering that, pending their approval, long-term use of PI3K inhibitors would be expected to induce insulin resistance and potentially type II diabetes, unless insulin feedback is controlled.

LACK OF MUTANT *PIK3CA*-SPECIFIC INHIBITORS

An outstanding demonstration of the driver oncogenic role of *PIK3CA* mutations was provided by Juric and colleagues. Upon development of acquired clinical resistance to the PI3Kα inhibitor alpelisib in a patient with *PIK3CA*-mutant breast cancer, these authors identified six distinct subclonal mutations in *PTEN* in multiple metastatic lesions, all resulting in loss of PTEN function and on a common background of a clonal monoallelic deletion of *PTEN* (61). In the absence of PTEN, cells become dependent on p110β to maintain PI3K pathway activity when p110α is blocked. In this report, a xenograft derived from a *PTEN*-null lung metastasis from the patient progressing on alpelisib was sensitive to the combination of alpelisib with the p110β inhibitor AZD6482 (61). This result is remarkable considering the convergent evolution of drug-resistant mutations occurred after treatment with a drug that may not have blocked mutant *PIK3CA* completely and/or in sustained fashion. This report also suggests that for tumors highly dependent on mutant *PIK3CA* and PI3K signaling, mutant-specific inhibitors should be able to exert an even stronger selective pressure. Theoretically, drugs that specifically target mutant p110α (H1047R, E542K, etc.) should spare endogenous p110α and downstream effectors that maintain normal homeostasis, thus limiting toxicities and permitting higher doses and more complete inhibition of the drug target.

Taselisib is a small-molecule inhibitor of p110α that induces ubiquitin-mediated, proteasome-dependent degradation of

PIK3CA^{H1047R} in cancer cells in culture and patient-derived xenografts (PDX) without significant change in WT p110α (62). It spares p110β but also inhibits p110γ and p110δ. This relative selectivity for mutant *PIK3CA* was recently tested in the SANDPIPER randomized trial in patients with ER$^+$/*PIK3CA*-mutant breast cancer (63). Patients treated with the ER antagonist fulvestrant plus taselisib exhibited a modestly improved progression-free survival compared with those treated with fulvestrant plus placebo. Main toxicities included diarrhea, hyperglycemia, rash, stomatitis, and colitis, thus limiting the median time on treatment to less than 5 months. We speculate that the prominent gastrointestinal side effects could have been secondary to inhibition of p110δ as it has been seen in trials with the p110δ inhibitors idelalisib and copanlisib (35, 36) and may have compromised selective inhibition of mutant *PIK3CA* in primary tumors *in vivo*. Another clinical candidate is the ATP mimetic GDC-0077, with more than 300-fold selectivity against p110α (IC_{50} 0.038 nmol/L) over the β, γ, and δ class I PI3K isoforms (64). GDC-0077, which also selectively degrades mutant PI3K, has shown remarkable preclinical activity against *PIK3CA*-mutant breast cancer cells and PDXs (64). GDC-0077 is now in early clinical development as a single agent and in combination with endocrine and other targeted therapies in patients with advanced breast cancer who harbor *PIK3CA* mutations.

OTHER MECHANISMS OF RESISTANCE

In addition to those described above, other mechanisms of compensation and/or resistance to PI3K inhibitors have been reported, primarily derived from laboratory studies and/or clinical correlations. These include CDK4/6 (65), *MYC* amplification (66), *KRAS* mutations (5), *PIK3CB* (p110β) mutations (67), *FGFR1* amplification (31), and overexpression and/or aberrant activation of PIM1 (68), AXL (69), PDK1–SGK1 (70), and SGK3 (71), among others. In this review, however, we have focused on those aspects intrinsic to therapeutic targeting of the PI3K pathway that are unique to it and that make the development of current PI3K inhibitors different and perhaps more challenging than development of other molecularly targeted therapies. Therefore, we do not cover these mechanisms of resistance in any detail herein.

RECENT ADVANCES IN TARGETING THE PI3K/AKT PATHWAY IN SOLID TUMORS

The SOLAR-1 phase III trial was the first to demonstrate a clinically significant effect of PI3Kα inhibition in tumors with *PIK3CA* mutations (29). We speculate that the apparent success of this trial is likely due to the following aspects: (i) a potent, isoform-specific PI3K inhibitor was used; (ii) *PIK3CA*-mutant cancers were included; (iii) endocrine-resistant ER$^+$ breast cancers tend to have clonal *PIK3CA* mutations; and (iv) the PI3K inhibitor was given in combination with an antiestrogen, likely dampening feedback compensation. The increased success of alpelisib versus taselisib in a similar setting in the SANDPIPER trial (PFS prolongation of 5.3 months vs. 2 months, respectively; ref. 63) may be due to more potent inhibition of PI3Kα by alpelisib, as evidenced by the higher rates of hyperglycemia seen in the SOLAR-1 trial compared with the SANDPIPER trial. The high rates of dose reductions and discontinuations in the PI3K inhibitor arms reported in both trials underscore the remaining challenges associated with long-term systemic inhibition of PI3Kα.

Many of the principles outlined above can also be applied to the development of AKT inhibitors. The AKT inhibitor capivasertib (AZD5363) exhibited significant clinical activity in patients with *AKT1*-mutant tumors; the majority of the responses were seen in ER$^+$ breast cancers (72). Likewise, the AKT inhibitor ipatasertib (GDC-0068), in combination with the antiandrogen abiraterone, significantly prolonged PFS in prostate cancers with loss of PTEN (73), and also prolonged PFS in combination with paclitaxel in triple-negative breast cancers with alterations in *PIK3CA, AKT*, or *PTEN* (74). As with PI3Kα inhibitors, the most common adverse events with AKT-selective inhibitors were hyperglycemia, diarrhea, and rash, pointing to the shared roles of PI3Kα and AKT in physiology.

CONCLUSIONS

Several factors have limited the development of PI3K inhibitors, as well as the enthusiasm of the cancer community for this class of drugs. These include (i) adaptive molecular mechanisms upon therapeutic inhibition of PI3K, (ii) our inability to specifically inhibit signaling by *PIK3CA* mutations while sparing endogenous p110α, (iii) the limited use of these therapies in rational combinations, several of them informed by a strong mechanistic background, and (iv) dose-limiting toxicities that prevent sustained PI3K pathway suppression. Despite these limitations, PI3K inhibitors have already shown clinical activity that is superior to that of single-agent trastuzumab (https://clinicaltrials.gov/ct2/show/NCT00842998), a HER2-targeted monoclonal antibody that in "combination" with chemotherapy has significantly improved the survival of patients with HER2-overexpressing breast cancer (75, 76). We posit that, moving forward, combination approaches with PI3Kα inhibitors that can be prioritized are (i) those with CDK4/6 inhibitors (64, 77, 78), (ii) those with drugs that limit insulin feedback, such as SGLT2 inhibitors, or a ketogenic diet (60), and (iii) combinations of p110α and p110β inhibitors (44, 79). For now, we believe that trials of PI3Kα-specific inhibitors in combination with antiestrogens in patients with *PIK3CA*-mutant ER$^+$ breast cancer are the best available test of the hypothesis that *PIK3CA* mutations are a pathogenic driver in cancer. The clinical activity of the PI3Kα inhibitor alpelisib in combination with fulvestrant in patients with advanced ER$^+$ breast cancer who have progressed after primary antiestrogen therapy, reported in the SOLAR-1 trial, provides compelling evidence that PI3K is an important therapeutic target in tumors with PI3K pathway dependence.

Disclosure of Potential Conflicts of Interest

A.B. Hanker reports receiving a commercial research grant from Takeda. V. Kaklamani reports receiving a commercial research grant from Eisai, has received honoraria from the speakers bureaus of Eisai, Pfizer, Novartis, Genentech, Puma, and Celgene, and is a consultant/advisory board member for Amgen, Eisai, Puma, Celldex, AstraZeneca, and Athenex. C.L. Arteaga reports receiving commercial

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