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PI3K in cancer: divergent roles of isoforms, modes of activation, and therapeutic targeting

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Preface

Phosphatidylinositol 3-Kinases (PI3Ks) are critical coordinators of intracellular signaling in response to extracellular stimuli. Hyperactivation of PI3K signaling cascades is one of the most common events in human cancers. In this Review, we discuss recent advances in our knowledge of the roles of distinct PI3K isoforms in normal and oncogenic signaling, the different ways in which PI3K can be upregulated, and the current state and future potential of targeting this pathway in the clinic.

Introduction

Phosphatidylinositol 3-Kinases (PI3Ks) are a family of lipid kinases that integrate signals from growth factors, cytokines, and other environmental cues, translating them into intracellular signals that regulate multiple signaling pathways. These pathways control many physiological functions and cellular processes, including cell proliferation, growth, survival, motility, and metabolism[1-3]. Activating alterations in PI3K are frequent in a variety of cancers (Table 1; for a fully referenced version see Supplemental Table 1), making this class of enzymes a prime drug target[2, 4]. Tremendous efforts have been devoted to the development of effective PI3K inhibitors for cancer therapy. Initial PI3K-directed drugs in clinical trials, consisting largely of non-isoform-selective pan-PI3K inhibitors, have not yielded exciting results. However, recent preclinical studies have demonstrated that different PI3K isoforms play divergent roles in cellular signaling and cancer, suggesting that inhibitors targeting individual isoforms may be able to achieve greater therapeutic efficacy. Isoform-selective inhibitors are now emerging in the clinic, and have had promising success. In this Review, we provide an update on what has been learned in recent years about PI3K

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isoform-specific functions, differences in the modes of PI3K isoform activation, and the progress of isoform-selective inhibitors in preclinical and early clinical studies.

Multiple PI3K classes and isoforms

PI3Ks phosphorylate the 3′;-hydroxyl group of phosphatidylinositides (PtdIns). They are divided into three classes based on their structures and substrate specificities (Figure 1). In mammals, class I PI3Ks are further divided into subclasses IA and IB based on their modes of regulation. Class IA PI3Ks are heterodimers of a p110 catalytic subunit and a p85 regulatory subunit. The genes *PIK3CA, PIK3CB,* and *PIK3CD* respectively encode three highly homologous class IA catalytic isoforms, p110α, p110β, and p110δ. These isoforms associate with any of five regulatory isoforms, p85α (and its splicing variants p55α and p50α, encoded by *PIK3R1*), p85β (*PIK3R2*), and p55γ (*PIK3R3*), collectively called p85 type regulatory subunits (reviewed in [1, 5]). Class IB PI3Ks are heterodimers of a p110γ catalytic subunit (encoded by *PIK3CG*) coupled with regulatory isoforms p101 (*PIK3R5*) or p87 (p84 or $p87^{PIKAP}$, encoded by *PIK3R6*). While p110α and p110β are ubiquitously expressed, p110δ and p110γ expression is largely restricted to leukocytes[6].

In the absence of activating signals, p85 interacts with p110, inhibiting p110 kinase activity. Upon receptor tyrosine kinase (RTK) or G-protein coupled receptor (GPCR) activation, class I PI3Ks are recruited to the plasma membrane, where p85 inhibition of p110 is relieved and p110 phosphorylates PtdIns 4,5-bisphosphate (PtdIns(4,5)P_2) to generate PtdIns(3,4,5)P_3 (Figure 2A). This lipid product acts as a second messenger, activating AKT-dependent and –independent downstream signaling pathways (reviewed in [1-3]). The phosphatase and tensin homolog (PTEN) lipid phosphatase removes the 3′ phosphate from PtdIns(3,4,5)P_3 to inactivate PI3K signaling.

Relatively little is known about class II PI3Ks. There are three class II isoforms, PI3K-C2α, PI3K-C2β, and PI3K-C2γ, respectively encoded by *PIK3C2A, PIK3C2B,* and *PIK3C2G.* These monomeric lipid kinases do not possess a regulatory subunit. PI3K-C2α and PI3K-C2β are broadly expressed, while PI3K-C2γ expression is limited to the liver, prostate, and breast[7]. Although early experiments indicated that PI3K-C2α and PI3K-C2β could phosphorylate both PtdIns and PtdIns(4)P, *in vivo* PtdIns may be the preferred substrate, generating PtdIns(3)P[8-10]. The physiological roles of class II PI3Ks are not fully understood, but recent studies suggest that PI3K-C2α is important in angiogenesis[10] and primary cilium function[11]. In addition, PI3K-C2α and PI3K-C2β have been reported to regulate cellular functions including growth and survival (reviewed in [3, 7]) (Figure 2B).

The single class III PI3K, VPS34, is encoded by *PIK3C3.* VPS34 forms a constitutive heterodimer with the myristoylated**[G]**, membrane-associated VPS15 (encoded by *PIK3R4*), and phosphorylates PtdIns to produce PtdIns(3)P[12, 13]. In mammals, VPS34 is ubiquitously expressed[13]. The VPS34-VPS15 dimer is found in distinct multiprotein complexes, which have critical roles in intracellular trafficking and autophagy (reviewed in [3, 14]) (Figure 2C). The myotubularin (MTM) family phosphatases MTM1 and MTMR2 remove the 3′ phosphate from PtdIns(3)P, regulating the lipid products of class II and III PI3Ks[15-18].

Alterations of PI3K isoforms in cancer

Overactivation of the PI3K pathway is one of the most frequent events in human cancers. The most common mechanism leading to aberrant PI3K signaling is somatic loss of PTEN via genetic or epigenetic alterations (reviewed in [19, 20]). The PI3K pathway can also be upregulated by activation of RTKs, or alterations in isoforms of PI3K itself (Table 1).

Class I PI3K catalytic isoform alterations

The transforming potential of class I PI3K catalytic isoforms was first demonstrated by studies in the late 1990s and early 2000s, which showed that fusion of p110α to viral sequences[21] or the SRC myristoylation sequence[22-24] was activating and highly oncogenic. The 2004 discovery of frequent *PIK3CA* mutations in human cancers[25] brought PI3K to the forefront as a major cancer driver and potential drug target. *PIK3CA* mutation has since been firmly established as causative in many cancer types (Table 1). Missense mutations occur in all domains of p110α, but the majority cluster in two hotspots, the most common being E542K and E545K in the helical domain and H1047R in the kinase domain. Cell-based analyses confirmed that these hotspot mutations confer transformation via constitutive activation of p110α[23, 26, 27]. Subsequently, several studies using genetically engineered mouse models (GEMMs) demonstrated roles for mutant *PIK3CA* in tumor initiation, progression, and maintenance[28-32] (Supplemental Table 2). Helical domain mutations reduce inhibition of p110α by p85[33-36] or facilitate direct interaction of p110α with insulin receptor substrate 1 (IRS1)[37], while kinase domain mutations increase interaction of p110α with lipid membranes[33, 36, 38]. Other *PIK3CA* mutations mimic distinct structural conformation changes that occur during activation of PI3K[36]. Interestingly, some of these mutations in *PIK3CA* have also been reported in congenital mosaic overgrowth syndromes**[G]**[39-42].

In contrast, mutations in other class I catalytic isoforms are rare. While activating *PIK3CD* mutations have been described in immune deficiencies[43, 44], they have not been linked to cancer. One *PIK3CB* mutation was detected in a single case of breast cancer[45]; this helical domain substitution enhances basal PI3K activation, potentially by increasing p110β association with membranes[46]. Recent structural studies have indicated that p110β may be less inhibited by p85[47-49] and thus has higher basal transforming potential. Interestingly, p110δ expression has been detected in some human solid cancer cell lines[50], and overexpression of wildtype p110β, p110δ, or p110γ, but not p110α, transforms cells *in vitro*[51]. This is consistent with the fact that *PIK3CB*, *PIK3CD*, and *PIK3CG* are generally amplified or overexpressed, but not mutated, in cancers (Table 1).

Class I PI3K regulatory isoform alterations

Recent studies have converged to implicate the p85 regulatory isoforms in tumorigenesis. Since the initial discovery of *PIK3R1* mutations in human cancer cell lines and primary tumors[52], somatic mutations in *PIK3R1* have been identified in a number of different cancers[53-57] (Table 1). The majority are substitutions or in-frame insertions or deletions in the inter-SH2 (iSH2) domain**[G]** of p85α[53-56], the region of the protein that makes contact with p110[33], indicating this domain as a mutation hotspot[53]. A number of these iSH2

domain mutants retain the ability to bind and stabilize p110 isoforms, but promote enhanced PI3K activity and transformation due to reduced ability to inhibit p110[53-55, 58, 59].

In addition, reduced expression of *PIK3R1* has been reported in some cancers[57, 60] (Table 1). *PIK3R1* mRNA levels inversely correlated with malignancy grade and incidence of metastasis in both breast and liver cancers[57, 60]. In mice, *Pik3r1* ablation increased epithelial neoplasia driven by *Pten* loss[61] and led to spontaneous development of aggressive liver tumors[60]. This work indicates that p85α can negatively regulate PI3K signaling in cancer, and suggests that p85α has tumor suppressive functions in certain tissues[62].

Alterations in genes encoding other regulatory isoforms have also been detected, albeit at a lower frequency. Increased *PIK3R2* expression has been reported in breast and colon cancers[63] (Table 1). Consistent with this, overexpression of wildtype p85β increased PI3K pathway activation in cells and tumor formation in mice[63]. Somatic *PIK3R2* mutations have been found in endometrial and colorectal cancers[53, 55], and causative germline *PIK3R2* mutations have been reported in megalencephaly syndromes**[G]**[41]. All *PIK3R2* mutations described to date are substitutions with no apparent hotspot region, and similar to some p85α mutants, mutations in p85β increase PI3K activation without affecting p110 binding[53]. Together these studies indicate that PI3K regulatory isoforms may contribute to tumorigenesis by multiple mechanisms.

Class II PI3K isoform alterations

Although class II PI3Ks are not well understood, *PIK3C2A* or *PIK3C2B* expression has been implicated in physiological functions important to tumorigenesis[9, 64-67]. *PIK3C2B* amplification has been reported in glioblastoma[68-70], and somatic *PIK3C2B* mutations were detected in non-small cell lung cancer[71], but the functional consequence of these mutations is unknown. Perhaps the most convincing evidence towards a role for class II PI3Ks in tumorigenesis comes from a recent study demonstrating that mice with *Pik3c2a* ablation had compromised angiogenesis and vascular barrier integrity, and significant reduction in the size and microvessel density of implanted tumors[10]. Since mice with embryonic *Pik3c2a* or *Pik3c2b* knockout (KO) are viable[72, 73], a class II-selective PI3K inhibitor might target tumor angiogenesis with tolerable side effects, although toxicity due to the critical role of PI3K-C2α in maintaining normal renal homeostasis[73] would need to be considered.

Type II inositol 3,4-bisphosphate 4-phosphatase (INPP4B), the phosphatase responsible for dephosphorylation of PtdIns(3,4)P_2 to PtdIns(3)P[74, 75], has also been implicated in cancer. In human mammary cell lines, *INPP4B* knockdown increased AKT activation and transformation[75, 76]. *INPP4B* loss-of-heterozygosity has been detected in cancers[75, 77], and reduced INPP4B expression has been correlated with high tumor grade, earlier recurrence, and decreased survival[75, 76, 78]. Identification of INPP4B as a tumor suppressor suggests that deregulation of the class II PI3K lipid products may contribute to tumorigenesis.

Class III PI3K isoform alterations

There is currently little evidence indicating an oncogenic role for VPS34. One recent study suggested that VPS34 is tyrosine-phosphorylated and activated downstream of SRC, and its

lipid kinase activity is required for SRC-mediated transformation[79]. However, overexpression of wildtype or myristoylated VPS34 was not sufficient to induce cellular transformation[80]. Another study indicated that VPS34 activity might be decreased in the context of activated epidermal growth factor receptor (EGFR)[81]. Further investigation is needed to determine whether VPS34 plays a role in transformation.

Divergent roles of class I PI3K catalytic isoforms

Class I PI3K catalytic isoforms share a conserved domain structure. They utilize the same lipid substrates and generate the same lipid products. Despite their similarities, accumulating evidence indicates these isoforms have distinct roles in mediating PI3K signaling in physiological and oncogenic contexts.

GEMMs have been used to elucidate the roles of individual class I PI3K isoforms. Mice with germline KO of *Pik3ca* or knock-in (KI) of a kinase-dead *Pik3ca* allele die at day E10.5[82, 83]. Interestingly, *Pik3cb* KO mice die much earlier at day E3.5[82], while kinase-dead *Pik3cb* KI mice develop to maturity with minor defects in size and glucose metabolism, and major defects in male fertility[84, 85]. These differences suggest an important kinase-independent scaffolding role for p110β[84]. Germline inactivation of *Pik3cd* or *Pik3cg* by KO or KI of kinase-dead alleles yields viable mice that grow to adulthood; however, loss of p110δ results in functional defects in lymphocytes, neutrophils, and mast cells[86-89], while loss of p110γ impairs thymocyte development, T cell activation, and neutrophil migration[90-92]. These studies indicate non-redundant roles in mouse embryonic development for p110α and p110β, the two ubiquitously expressed class I PI3K isoforms, and distinct roles in the immune system and inflammatory response for p110δ and p110γ, the two leukocyte-restricted isoforms.

Technological developments have facilitated further insight into the individual roles of PI3K enzymes. The generation of conditional KO animals using the Cre/loxP recombination system has allowed the functions of each isoform to be studied in different tissues, stages of development, and pathological settings (Supplemental Table 2). Additional progress has come from studies using RNA interference (RNAi) and a new generation of isoform-selective PI3K inhibitors. These have advanced our understanding of the roles of class I catalytic isoforms in mediating signaling downstream of RTKs, GPCRs, and small GTPases (Figure 3), and in the context of PTEN deficiency (Figure 4A).

In mediating RTK signaling

Binding of growth factor ligands induces RTK dimerization, activation, and auto-phosphorylation of tyrosine-containing YXXM motifs on the receptors or their associated adaptor proteins. Class IA p110-p85 heterodimers are then recruited to activated RTKs through direct interaction of p85 SH2 domains**[G]** with these phosphorylated YXXM motifs[93-95] (Figure 2A). Accordingly p110α, p110β, and p110δ can complex with activated RTKs (Figure 3A), and might be expected to mediate growth factor signaling.

Studies using isoform-selective pharmacological inhibitors and genetic inactivation or ablation indicated that loss of p110α activity was sufficient to largely block PI3K signaling

in response to a number of growth factors[96-101]. Notably, genetic ablation or inactivation of p110β had only a modest effect on PI3K signaling following acute RTK activation[84, 102, 103]. It was suggested that the relative abundance of catalytic isoforms in a particular tissue might dictate which isoforms are dominant in mediating RTK signaling[104]. This may explain the role of p110δ, which is mainly expressed in leukocytes and is the primary isoform regulating PI3K signaling downstream of certain RTKs in mast cells and macrophages[87, 105, 106]. However, differential expression does not completely explain isoform dependence, as in many tissues p110β levels are comparable to or even higher than levels of p110α[107].

The involvement of p110β in RTK signaling remained puzzling, until a recent study from our group suggested a new model. In mice, while p110α ablation blocked normal mammary development and mammary tumorigenesis driven by polyoma middle T (PyMT) or HER2 (also known as ERBB2), p110β ablation increased mammary gland outgrowth and accelerated tumor formation driven by these oncogenic RTKs[96]. To explain this negative role of p110β, a competition model was proposed: if p110α has higher RTK-associated lipid kinase activity than p110β, the less-active p110β could compete with p110α for phosphorylated YXXM sites on receptors to modulate PI3K signal strength downstream of RTKs[96] (Figure 3B). Although direct comparison of RTK-associated p110α and p110β lipid kinase activity has not been shown, the maximal specific activity and enzymatic rate of p110α are higher than that of p110β[108, 109]. Biochemical data were consistent with this proposed model, demonstrating that in p110β KO cells, activated RTKs had more bound p110α and higher associated lipid kinase activity[96]. Furthermore, pharmacologically inactivated p110β could still compete with p110α for binding sites on activated receptors, modestly reducing signaling and tumor growth driven by PyMT or HER2[96]. This model also explains moderately decreased AKT activation, mild hyperglycemia, and delayed HER2-driven tumor formation observed in mice with KI of kinase-dead p110β[84], a scenario mimicking p110β-selective kinase inhibition. These studies not only reveal a novel p110β-based regulatory mechanism in RTK-mediated PI3K signaling, but also identify p110α as an important target in cancers driven by oncogenic RTKs.

Initial studies suggested that class IA isoforms mediated signaling downstream of RTKs, while the class IB isoform signaled downstream of GPCRs. Although p110γ activation by GPCRs is well established, a recent report suggested that this class IB isoform might also function downstream of RTKs through regulatory isoform p87 in mouse myeloid cells[110] (Figure 3A). Given that p87 and p101 may have distinct tissue distribution[111-113] and non-redundant functions[110, 111, 113, 114], this suggests that the two class IB regulatory isoforms may mediate p110γ activation in response to specific upstream signals.

In mediating GPCR signaling

GPCRs are a family of seven-transmembrane domain receptors that associate with heterotrimeric G proteins composed of the G_{α} and $G_{\beta\gamma}$ subunits. Ligand binding to GPCRs results in allosteric activation and disassociation of bound G proteins into their separate subunits, which can then act on intracellular targets.

The single class IB PI3K isoform, p110γ, is activated by G proteins[115-117] (Figure 2A). Although association of p110γ with either its p101 or p87 regulatory isoforms increased its activation in response to $G_{\beta\gamma}$[115, 118, 119], recent evidence indicated that p101 is the main regulatory isoform involved in GPCR-mediated p110γ signaling[110, 114] (Figure 3A). Both p110γ and p101 interact directly with $G_{\beta\gamma}$ heterodimers, and these contacts are critical for signaling and transformation mediated by p110γ[115, 120]. Recent studies have shown that in myeloid cells, p110γ can be activated by GPCR and RTK signals in a RAS- or RAP1A-dependent manner to mediate integrin α4β1 activity, leading to tumor inflammation and progression[110, 121]. Thus p110γ-mediated signaling may contribute to tumorigenesis by controlling both tumor cell characteristics and the tumor microenvironment.

Interestingly, *in vitro* experiments[117, 122-124] and subsequent GEMM studies[84, 102, 103] demonstrated a role for p110β in G protein-mediated PI3K signaling (Figure 2A). Recently a region in the C2-helical domain linker of p110β was shown to bind $G_{\beta\gamma}$ subunits (Figure 3A); this region is not present in other class IA isoforms[125], and is similar to the region of p110γ that binds $G_{\beta\gamma}$[120]. Abrogation of p110β-$G_{\beta\gamma}$ interaction blocked p110β-mediated signaling and transformation downstream of GPCRs, and inhibited the proliferation and invasiveness of cancer cells[125]. Although p110δ does not directly interact with G proteins, a non-redundant role for this isoform in GPCR-mediated leukocyte migration has been demonstrated in certain contexts[126-128]; however, the mechanism of p110δ activation downstream of GPCRs is unknown. It has also been reported that some G_{α} proteins directly bind and inhibit p110α[129-131]. Clearly, class I PI3K isoforms cooperate with GPCRs in a number of different ways to regulate signaling and transformation.

Downstream of RAS and other small GTPases

RAS superfamily proteins**[G]** are direct activators of the PI3K pathway. All class I PI3K catalytic isoforms possess an N-terminal RAS-binding domain (RBD) (Figure 1) allowing them to interact with RAS GTPases**[G]** or other RAS superfamily members (Figure 3A).

Activated or oncogenic mutant RAS proteins directly bind and increase the enzymatic activity of both p110α[132, 133] and p110γ[134-136]. Cellular and structural studies suggest that p110γ association with RAS might both increase its membrane translocation[114, 135] and allosterically increase p110γ kinase activity[135]. Interestingly, RAS is required for activation of p110γ bound to regulatory isoform p87, but not p101[114]. *In vitro*, the transforming capability of both helical domain p110α mutants[34, 137] and of overexpressed wildtype p110γ[51, 138] are dependent on their association with RAS. GEMM studies using KI of *Pik3ca* with an RBD mutation or KO of endogenous *Pik3ca* revealed that the p110α-RAS interaction is critical for both the initiation and maintenance of lung tumors[139, 140] and the development of myeloid leukemia[141] driven by oncogenic KRAS. In mice, p110γ-RAS binding is required for inflammation-induced PtdIns(3,4,5)P_3 accumulation[142] and inflammation-associated tumor progression[110, 121]. These studies highlight the importance of p110α or p110γ interaction with RAS in both normal PI3K signaling and transformation.

Although p110δ was shown to bind RAS *in vitro*[143, 144], some studies indicated that p110δ kinase activity was not stimulated by HRAS, NRAS, or KRAS, but instead by RRAS and TC21 (also known as RRAS2)[145, 146]. Furthermore, B and T cells derived from *Tc21* KO

mice displayed diminished PI3K activity and recruitment of p110δ to T cell receptors (TCRs) and B cell receptors (BCRs), suggesting that TC21 might function upstream of p110δ[147]. Thus PI3K signaling through p110δ may be regulated by additional RAS subfamily members.

It was initially anticipated that all p110 isoforms bearing a RBD might interact with RAS GTPases. Surprisingly, *in vitro* studies determined that p110β kinase activity was not stimulated by any RAS subfamily members[146]. A recent extensive biochemical study demonstrated that p110β is instead regulated by RAC1 and CDC42 of the RHO GTPase**[G]** subfamily[143] (Figure 3A). Direct interaction between the p110β RBD and RAC1 is important for GPCR-mediated activation of p110β[143], indicating cooperative $G_{\beta\gamma}$ and RHO GTPase signaling through p110β. Previous studies reported that an intact RBD was required for signaling and oncogenic transformation by wildtype p110β in cultured cells[51, 138], suggesting a potential role for RHO GTPase interaction with p110β in transformation. Notably, RAC1 and CDC42 can also be activated downstream of PI3K by PtdIns(3,4,5)P_3-dependent guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs)[148-150]. The finding of distinct p110β regulation by RAC1 and CDC42 expands PI3K signaling input by GTPases beyond the RAS subfamily, and also supports the notion that PI3K can act both upstream and downstream of GTPases, potentially allowing for positive feedback loops in cancer settings.

In PTEN deficiency

The PTEN lipid phosphatase counteracts class I PI3K activity, making it an important tumor suppressor. Somatic loss of PTEN in human cancers is common. Germline *PTEN* mutations are also found in several genetic disorders characterized by multiple hamartomas with overgrowth phenotypes, collectively termed PTEN hamartoma tumor syndromes (PHTS)[151].

Pten KO mouse models provided a tool to explore the molecular mechanisms underlying diseases caused by PTEN loss. While embryonic *Pten* KO is lethal[152, 153], heterozygous or conditional *Pten* KO animals recapitulated human disease phenotypes, including development of prostate cancer[154-156]. Surprisingly, ablation of p110β, but not p110α, blocked prostatic intraepithelial neoplasia (PIN) induced by PTEN loss[102]. Subsequent studies demonstrated a correlation between PTEN deficiency and sensitivity to p110β knockdown or inhibition in human cancer cell lines both *in vitro* and in mouse xenografts[157-159]. However, the mechanism governing the specific importance of p110β in the context of PTEN loss remains elusive. Perhaps the unique role for p110β as a convergence point for GPCR and RAC1 or CDC42 signals (Figure 4A) contributes to transformation induced by PTEN deficiency. Structural studies have also suggested that compared to p110α, p110β is less inhibited by p85, and may supply a basal level of PtdIns(3,4,5)P_3 [47-49]. This may explain why wildtype p110β can be oncogenic when it is overexpressed[51, 138] or when PTEN is lost.

Although p110β is the primary PI3K isoform involved in many cases of tumorigenesis driven by PTEN loss, studies have shown that depending on the tissue type and pathology both p110α and p110β may be involved[160-162]. Mice with *Pten* ablation in the basal epidermal compartment require both p110α and p110β for the development of

hyperproliferative epidermal lesions closely resembling PHTS[163, 164]. In this model, spatially distinct roles for these isoforms in epidermal compartments were identified: p110α is responsible for RTK signaling in and survival of suprabasal cells, whereas p110β is important for GPCR signaling in and proliferation of basal cells[164]. In mice with thymocyte-specific *Pten* KO, not surprisingly both p110δ and p110γ were required for the development of T cell acute lymphoblastic leukemia (T-ALL)[165]. This suggests that in certain contexts, transformation driven by PTEN loss may be governed by the PI3K isoforms that are dominant in that tissue or compartment.

Since PTEN loss removes one mechanism of PI3K pathway negative regulation, the specific roles of p110 isoforms in this pathogenic context can be influenced by other activating inputs. These can be cues from the tissue microenvironment, or other coexisting genetic events. A recent GEMM study demonstrated that concomitant activation of oncogenic KRAS in ovarian endometrioid adenocarcinoma driven by *Pten* ablation shifted the PI3K isoform reliance from p110β to p110α[162] (Figure 4A). Consistent with this, a subset of *PTEN*-mutant human endometrioid endometrial cancer cell lines harboring other PI3K-activating mutations were found to be resistant to p110β inhibition[166]. It is also possible that other genetic events downstream of PI3K or in PI3K-independent pathways may render PTEN-null tumors less reliant on PI3K. Thus determination of isoform dependency in PTEN-deficient tumors remains a challenge.

Therapeutic targeting of PI3K isoforms in cancer

The central role of PI3K in cancer makes it an attractive therapeutic target. Enormous efforts have focused on the development of drugs targeting PI3K, many of which are undergoing clinical evaluation (Tables 2-4). Unlike drugs targeting other oncogenic kinases, such as EGFR, BRAF, and ALK, PI3K inhibitors have shown limited efficacy as mono-therapies in early trials on patients with tumors harboring PI3K pathway activation[167]. The effectiveness of these early PI3K inhibitors may have been limited by their lack of specificity, and by compensatory signaling feedback loops and co-existing genetic and epigenetic alterations. The development of novel isoform-selective PI3K inhibitors (Figure 4A) and their rational combination with other therapeutics (Figure 4B and Supplemental Table 3) may substantially improve therapeutic outcomes.

Emerging isoform-selective PI3K inhibitors

Most PI3K inhibitors in early clinical trials are ATP-competitive agents that target all class I isoforms with similar potencies. These include pan-PI3K inhibitors (Table 2) such as GDC0941[168] and dual pan-PI3K and mTOR inhibitors (Table 3) such as BEZ235[169]. Though these drugs display potent preclinical anti-tumor activity, their success in clinical trials as single agents has been modest[167]. The therapeutic window and efficacy of pan-PI3K inhibitors are limited in some cases by adverse effects arising from a broader spectrum of off-target effects[170]. Furthermore, while both pan-PI3K and isoform-selective inhibitors have on-target effects from suppression of essential PI3K functions, for example glucose homeostasis, pan-PI3K inhibitors likely have additional on-target effects from inhibiting isoforms that are not contributing to tumorigenesis. Isoform-selective inhibitors may achieve greater efficacy with fewer toxic effects, and are emerging in the clinic (Table 4).

The most effective single agent PI3K-based therapy to date is idelalisib (CAL101 or GS1101), a p110δ-selective inhibitor. Idelalisib has achieved notable success in early trials for patients with chronic lymphocytic leukemia or indolent lymphoma, and is currently in phase III clinical trials[171, 172]. Interestingly, this dramatic response is not due to genetic activation of the PI3K pathway, as neither PI3K mutation nor PTEN loss is common in these malignancies. Given the important role of p110δ in signaling downstream of BCRs[86, 88, 89] and the fact that leukemic B cells have been shown to be dependent on BCR signaling, it is likely that idelalisib functions by blocking essential BCR signals. Two recent articles provide great insight into the success of idelalisib trials (see [173 and 174]).

In addition to the role of p110δ in B cell malignancies, a recent preclinical study showed that this isoform also contributes to PTEN-null T-ALL[165]. However, p110δ-selective inhibition in this study was insufficient to suppress tumorigenesis; combined inhibition of both p110δ and p110γ was required for effective anti-PI3K therapy[165]. The involvement of p110δ and p110γ in leukocyte signaling and hematological malignancies has drawn great attention, and new inhibitors that target both isoforms simultaneously are in clinical trials for B and T cell lymphomas (Table 4). These isoforms may also mediate immune responses that support the growth of solid tumors. In a mouse model, p110γ inhibition blocked myeloid cell recruitment to tumors, thus suppressing malignancy by targeting the tumor microenvironment[110]. Another study indicated that p110δ inhibition impaired tumor growth by disrupting regulatory T cell-mediated immune tolerance[175]. These findings indicate potential new applications for p110δ- or p110γ-selective therapies in cancer.

The frequency of *PIK3CA* mutations in solid tumors has generated great interest in the potential for p110α-selective inhibitors in targeting these cancers. Data presented at the 2013 San Antonio Breast Cancer Symposium (SABCS) indicated promising early clinical activity of p110α-selective inhibitors BYL719 or GDC0032 as single agents in patients with *PIK3CA*-mutant advanced breast tumors[176]. Recent preclinical findings that HER2-or KRAS-driven tumors rely on p110α[96, 139-141, 162] underscore the need for clinical evaluation of p110α-selective drugs in these disease settings. In these studies, growth of HER2- or KRAS-driven solid tumors is inhibited similarly by pan- and p110α-selective inhibitors[96, 140], but only modestly by p110β-selective inhibition[96, 162]. However, further study is needed to determine the contexts in which simultaneous inhibition of p110α and p110β can improve outcomes of KRAS- or HER2-driven disease.

One drawback of p110α-selective inhibitors is their inevitable on-target adverse effects on insulin signaling and glucose metabolism, since p110α is the major isoform mediating these functions[98, 100]. In the clinic, the effect of p110α-selective inhibitors on glucose homeostasis must be carefully managed[177], and is in some cases limiting[167]. To circumvent this, inhibitors are being developed that specifically target p110α harboring hotspot mutations. Such agents might be used at high doses with low toxicity, similar to mutant-selective BRAF inhibitors that have had great clinical success[178, 179]. A major obstacle to this approach is the heterogeneity of oncogenic *PIK3CA* mutations. Some progress has been made with the discovery of GDC0032, which was reported at the 2013 SABCS to have enhanced potency in *PIK3CA* mutant breast cancer models[180]; one preclinical study also reported success using stapled peptides to specifically disrupt the interaction of p110α-

E545K with IRS1[37]. However, devising strategies to selectively interrupt mutant-specific function remains challenging. If developed, this class of inhibitor will likely be most effective in early stage tumors with *PIK3CA* mutations, as advanced *PIK3CA*-mutant tumors may have escaped their dependency on oncogenic p110α[29]. Such drugs would also be ideal for treating congenital overgrowth syndromes caused by *PIK3CA* mutations occurring during early embryonic development[39-42]. In these contexts, p110α mutant-selective inhibitors may yield improved therapeutic index.

Several preclinical studies have documented that certain PTEN-deficient tumors depend on p110β[102, 157, 159], prompting a new clinical trial with the p110β-selective inhibitor GSK2636771 in patients with PTEN-deficient advanced solid tumors (NCT01458067). However, since PTEN is a negative regulator of PI3K, isoform-dependency of PTEN-deficient tumors can be complicated as it can be affected by tissue type, co-existing genetic events, and microenvironmental cues that fuel cancer cells. In model systems where PTEN-deficient tumors are found to be dependent on p110β, addition of oncogenic RTKs, RAS, or mutant *PIK3CA* can shift dependency partially or totally to p110α (Figure 4A). Recent studies also show that prolonged treatment of PTEN-deficient tumor cells with p110β-selective inhibitors can shift isoform dependency from p110β to p110α (N. Rosen, unpublished observations). Therefore in most PTEN-deficient solid tumors, both p110α and p110β should be targeted.

Although development of dual p110α- and p110β-selective inhibitors has proven difficult[98], combination of individual p110α- and p110β-selective inhibitors might offer flexibility in the dosing of each isoform-selective inhibitor to further reduce toxicity and increase the therapeutic window. One approach could involve continuous inhibition of p110β to suppress elevated basal PI3K activity due to PTEN loss, combined with pulsatile inhibition of p110α to avoid toxicity due to glucose elevation. Such a strategy might also avoid the reported shift in isoform dependency of tumors from p110α to p110β after prolonged treatment with the p110α-selective inhibitor BYL719 (J.A. Engelman, unpublished observations). Ultimately, the success of targeting PI3K in cancer will likely require better understanding of which PI3K isoforms to target in a given disease setting, improved inhibitors, and more careful dosing strategies.

Resistance mechanisms and combination therapeutic strategies

PI3K-based therapeutic approaches have encountered a number of roadblocks in the form of intrinsic and acquired resistance mechanisms. A large body of work has identified multiple signaling feedback loops, compensatory parallel signaling pathways, and modes of downstream pathway activation that may result in clinical resistance to PI3K inhibitors. Consequently, combination therapies are being developed and evaluated in both preclinical and clinical settings (Figure 4B and Supplemental Table 3), and will be necessary to maximize clinical efficacy of PI3K inhibitors.

The first indication of feedback loops in the PI3K pathway came from experiments with mTOR inhibitors. In early studies mTOR inhibition led to p70 ribosomal protein S6 kinase (S6K) suppression, IRS1 upregulation, and PI3K-AKT activation[181]. This prompted the development of dual pan-PI3K and mTOR inhibitors that are currently in clinical trials

(Table 3). Interestingly, feedback loops can also arise from dual PI3K and mTOR inhibition. A recent preclinical report suggested that PI3K and mTOR blockade activated the Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) signaling axis via IRS1, generating resistance to PI3K and mTOR inhibition, which could be overcome by targeting JAK2[182]. Similarly, in another preclinical study treatment with BEZ235 increased phosphorylation of multiple signaling molecules, including STAT3, STAT5, JUN, and p90 ribosomal S6 kinase (p90RSK)[183]. Isoform-selective PI3K inhibitors can also generate feedback loops: in a recent study of *PIK3CA* mutant breast tumors, mTOR complex 1 (mTORC1) reactivation by insulin-like growth factor 1 (IGF1) and neuregulin 1 (NRG1) was associated with tumor resistance to the p110α-selective agent BYL719, necessitating concurrent mTORC1 inhibition using RAD001[184]. Inhibiting both PI3K and mTOR, possibly in conjunction with additional signaling pathways, may be required to achieve effective anti-tumor activity.

Another important resistance mechanism to PI3K pathway inhibition is increased expression of RTKs, such as HER3, IGF1R, insulin receptor (IR), and EGFR, via forkhead box O (FOXO)-mediated transcriptional upregulation[185]. Robust HER3 induction in response to PI3K inhibition has been reported in several tumor types[183, 186, 187]. While HER3 itself does not possess tyrosine kinase activity, it dimerizes with EGFR, HER2, or HER4, hyperactivating the PI3K pathway and dampening the efficacy of PI3K drugs. A preclinical study demonstrated that combination of the HER3-neutralizing antibody LJM716 and the p110α-selective inhibitor BYL719 potently blocked PI3K signaling and growth of HER2-positive breast tumor xenografts, even without a direct HER2 antagonist[188]. Similarly, combination of the dual EGFR and HER3 inhibitor MEHD7945A with a PI3K inhibitor (GDC0941) or AKT inhibitor (GDC0068) effectively blocked the growth of triple-negative breast cancer cells *in vitro* and in xenografts in a preclinical study[189]. Blockade of PI3K along with upstream RTKs may therefore circumvent certain PI3K therapy resistance mechanisms (Figure 4B).

Activation of convergent signaling pathways, for example the RAS-RAF-MEK-ERK pathway, can also lead to PI3K pathway inhibition resistance. Mutant RAS can activate both the RAF-ERK and PI3K-AKT-mTOR pathways in cancer cells; blocking the PI3K pathway in such cells leads to upregulation of the ERK pathway[190]. Inhibition of both PI3K and ERK pathways successfully suppressed the growth of cancer cells in mouse models[28, 140, 191], and combinations of MEK inhibitors and pan- or isoform-selective PI3K agents are being evaluated in clinical trials. However, there is preclinical evidence that some of these combinations may be limited due to synergistic toxicity[140]. Preclinical studies indicate that pulsatile inhibition of both PI3K and ERK pathways may provide more effective anti-tumor activity while limiting toxic effects[191], suggesting that optimization of such combinations in the clinic will require careful dosing strategies.

Another mode of resistance to PI3K-directed therapies arises from the activation of transcription downstream or outside of the PI3K pathway. Several reports have indicated MYC amplification or overexpression[29, 192] or activation of the Notch and WNT/β-catenin pathways[193, 194] as mechanisms of resistance to PI3K inhibition. Recently, the bromodomain and extraterminal (BET) inhibitor JQ1 has been shown to downregulate

transcription of *MYC*, among other targets[195]. XAV939 has also been identified as an inhibitor of WNT/β-catenin-mediated transcription[196]. Combination of PI3K inhibition with these agents is being actively pursued in preclinical settings.

Other combination therapies have been suggested by assessing pathways that may synergize with PI3K (Figure 4B). As presented at the 2012 and 2013 SABCS, anti-estrogen therapies are being tested in combination with PI3K inhibitors in clinical trials for breast cancer patients[176, 197, 198]. In a brain tumor study, coordinate activation of sonic hedgehog (SHH) and PI3K signaling was found in PTEN-deficient glioblastoma; combination of BKM120, a pan-PI3K inhibitor, and LED225, a smoothened (SMO) inhibitor that blocks SHH signaling, resulted in synergistic anti-tumor effects[199]. Poly-(ADP-ribose) polymerase (PARP) and PI3K inhibitors have been found to cooperate in prostate and triple-negative breast cancers[200-202]. It appears that PI3K inhibition downregulates BRCA1 and BRCA2, impairing homologous recombination and sensitizing BRCA-wildtype cancer cells to PARP inhibition. Another attractive approach is combination of PI3K-targeted agents with drugs that suppress anti-apoptotic factors. B cell lymphoma 2 (BCL2), myeloid cell leukemia sequence 1 (MCL1), and other pro-survival proteins are frequently upregulated in cancer, and may explain why PI3K inhibition is often cytostatic in tumor cells. BCL2 or MCL1 suppression may induce cytotoxicity in response to PI3K inhibition[203]. Finally, an emerging approach is to combine PI3K inhibitors with agents that disrupt cell cycle machinery[204]. The p16-Cyclin D-cyclin-dependent kinase 4 (CDK4)-CDK6 pathway is frequently dysregulated in cancer. A number of CDK4 and CDK6 inhibitors, including LEE011 and palbociclib (PD0332991), are entering clinical trials for combination with pan- or p110α-selective inhibitors. Such rational combination therapies will be required to increase the success of PI3K inhibitors.

Conclusions and perspective

Targeting the PI3K pathway remains both an opportunity and a challenge for cancer therapy. Recent advances have provided the framework and rationale for inhibiting select class I PI3K catalytic isoforms. We have learned a great deal about the divergent roles of these isoforms in different signaling contexts, and are beginning to understand the importance of each isoform in various tissues, compartments, and cancer types. These findings have informed preclinical and clinical studies with isoform-selective PI3K agents, which offer improved specificity and reduced toxicity over first-generation pan-PI3K drugs. Isoform-selective PI3K inhibitors have seen promising success in early- and late-stage clinical trials for solid and hematological malignancies, highlighting the potential for isoform-selective PI3K therapeutics.

Although we have made substantial progress, further efforts are needed. We have only recently begun to appreciate the importance of class I regulatory isoforms in tumorigenesis. The different ways in which p85 subunits contribute to cancer, and the effective means to pharmacologically inhibit these mechanisms, are still not fully understood. Similarly, while a recent study indicates that class II isoform PI3K-C2α is important for pathophysiological angiogenesis, the roles of class II and III PI3Ks in cancer remain unclear.

For the class I catalytic isoforms, we must continue to precisely define the disease settings in which different PI3K isoforms will need to be targeted. To better inform isoform-selective therapeutic strategies, a set of biomarkers to predict the active p110 isoforms in a given tumor would be ideal, but development of this will require systematic studies. Continued work to understand the underlying cellular programs that protect tumors with aberrant PI3K activation from PI3K-targeted therapy will also be important. This will allow for better rational design of combination therapies, which will be necessary to overcome compensatory pathway activation and acquired resistance mechanisms and maximize the anti-tumor activity of PI3K inhibitors. Dosing strategies will also need to be carefully considered, as recent studies suggest that in some cases pulsatile inhibition may reduce toxicity without sacrificing efficacy. Progress in these areas should increase the effectiveness of PI3K-directed therapies in the clinic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Myristoylated	irreversible co-translational modification of proteins in which a myristoyl group is covalently attached to an N-terminal amino acid of a nascent polypeptide, promoting membrane localization of the modified protein
Congenital mosaic overgrowth syndromes	a clinically heterogeneous group of genetic disorders characterized by abnormal progressive localized growth. They are caused by diverse somatic mutations and associated with increased cancer risk
Inter-SH2 (iSH2) domain	the domain of p85 regulatory PI3K isoforms that is located between the C- and N-terminal SH2 domains and directly interacts with class IA p110 catalytic isoforms
Megalencephaly syndromes	a collection of sporadic overgrowth disorders characterized by enlarged brain size and other distinct features
SH2 domain	SRC homology 2 domain; a structurally conserved protein–protein interaction domain that facilitates interaction with phosphorylated tyrosine residues on other proteins
RAS superfamily proteins	small monomeric membrane-associated GTPases, which are divided into the RAS, RHO, RAB, ARF, and RAN subfamilies based on structure and function
RAS GTPases	subfamily of RAS superfamily GTPases that plays critical roles in signal transduction. In mammals, the three major RAS subfamily members are HRAS, KRAS, and NRAS
RHO GTPases	subfamily of RAS superfamily proteins that shares similar roles in signal transduction to RAS GTPases and is best characterized for the regulation of cell shape, movement, and polarity

Biographies

Lauren M. Thorpe received her BS in Biological Sciences in 2008 from Carnegie Mellon University, and is now completing her PhD at Harvard University in the lab of Dr. Jean J. Zhao. There, her work has focused on understanding the role of p85 regulatory isoforms in modulating physiological and pathophysiological PI3K signals.

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Key points

- Oncogenic mutation of PI3K catalytic isoform p110α is frequent in human cancers, while catalytic isoforms p110β, p110δ, and p110γ are rarely mutated but can be overexpressed. Mutation or loss of expression of regulatory isoform p85α is also associated with cancer.
- Although class IA PI3K catalytic isoforms share structural and substrate similarities, they have distinct roles in mediating PI3K signaling in different physiological and oncogenic contexts.
- Cancer cells with upregulation or mutation of receptor tyrosine kinases (RTKs), oncogenic RAS mutations, or activating p110α mutations are highly dependent on p110α, even in the presence of mutation or loss of PTEN.
- In many cases, tumorigenesis driven by PTEN loss depends on p110β. However, PI3K isoform dependence in PTEN-deficient transformation may be governed by other PI3K isoforms that are dominant in a tissue or compartment, or shifted by coexisting oncogenic mutations.
- Isoforms p110α, p110δ, and p110γ bind to and are activated by RAS subfamily GTPases, while p110β binds and is activated by RHO subfamily GTPases RAC1 and CDC42.
- Non-isoform-selective pan-PI3K inhibitors have not yielded exciting clinical results, but second-generation PI3K drugs targeting individual PI3K isoforms may be able to achieve greater therapeutic efficacy by offering improved specificity and reduced toxicity.
- The p110δ-selective inhibitor idelalisib has been remarkably effective in clinical trials for patients with B cell malignancies, while p110α-selective inhibitors have shown promise in early phase trials for patients with solid tumors bearing *PIK3CA* mutations or *HER2* amplification.
- Intrinsic and acquired resistance mechanisms are an ongoing challenge for PI3K-directed therapeutic approaches. To overcome this, combination therapies or alternative dosing strategies are being developed and evaluated in both preclinical and clinical settings.

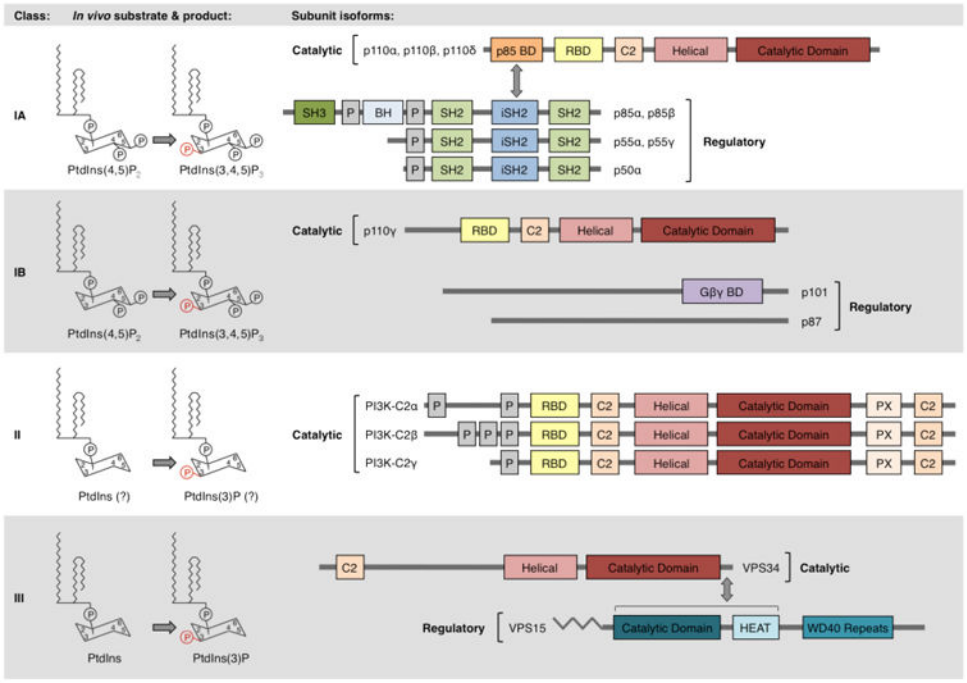
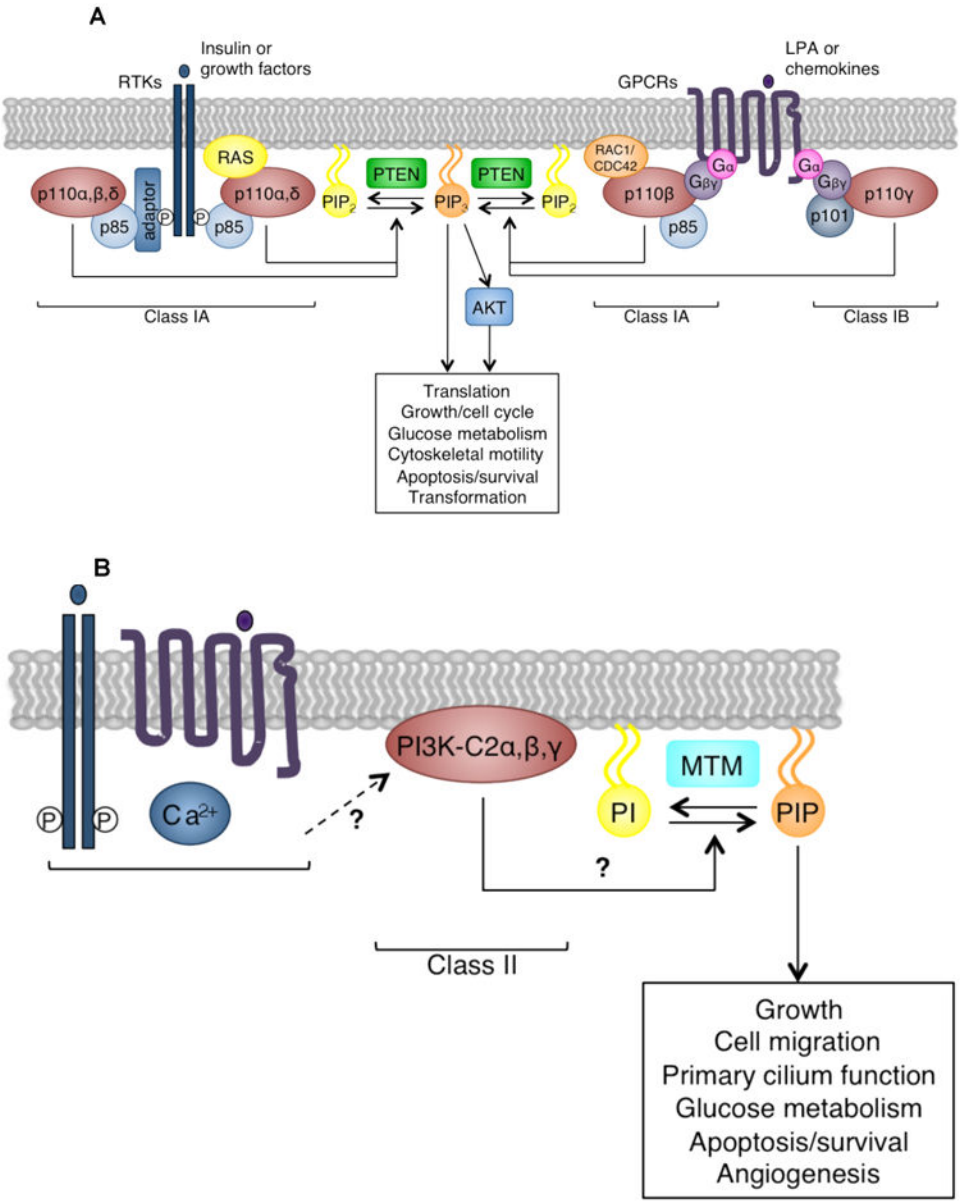


Figure 1. The PI3K family comprises multiple classes and isoforms

PI3Ks are classified based on their substrate specificities and structures. *In vivo*, class IA and IB PI3Ks phosphorylate PtdIns(4,5)P_2, while class III PI3Ks phosphorylate PtdIns. Some evidence suggests that class II PI3Ks may also preferentially phosphorylate PtdIns *in vivo*[8-10]. Class IA PI3Ks are heterodimers of a p110 catalytic subunit and a p85 regulatory subunit. Class IA catalytic isoforms (p110α, p110β, and p110δ) possess a p85-binding domain (p85-BD), RAS-binding domain (RBD), helical domain, and catalytic domain. Class IA p85 regulatory isoforms (p85α, p85β, p55α, p55γ, and p50α) possess an inter-SH2 (iSH2) domain that binds class IA catalytic subunits, flanked by SH2 domains that bind phosphorylated YXXM motifs. The longer isoforms, p85α and p85β, additionally possess N-terminal SH3 and breakpoint cluster homology (BH) domains. Class IB PI3Ks are heterodimers of a p110γ catalytic subunit and a p101 or p87 regulatory subunit. p110γ possesses an RBD, helical domain, and catalytic domain. The domain structures of p101 and p87 are not fully known, but a C-terminal region of p101 has been identified as binding $G_{\beta\gamma}$ subunits[120]. The monomeric class II isoforms (PI3K-C2α, PI3K-C2β, and PI3K-C2γ) possess an RBD, helical domain, and catalytic domain. VPS34, the only class III PI3K, possesses helical and catalytic domains. VPS34 forms a constitutive heterodimer with the myristoylated, membrane-associated VPS15 protein. Other indicated domains include proline-rich (P) domains and membrane-interacting C2 domains. Modified with permission from Reference 2.



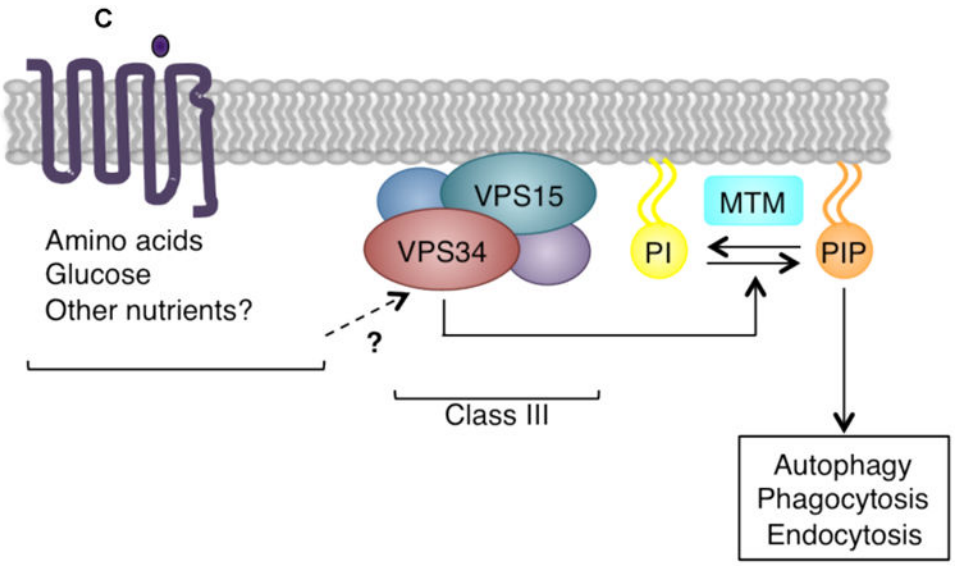


Figure 2. Signaling by class I, II, and III PI3K isoforms

(A) Upon receptor tyrosine kinase (RTK) or G-protein coupled receptor (GPCR) activation, class I PI3Ks are recruited to the plasma membrane by interaction with phosphorylated YXXM motifs on RTKs or their adaptors, or with GPCR-associated $G_{\beta\gamma}$ subunits. There they phosphorylate PtdIns(4,5)P_2 (PIP2) to generate PtdIns(3,4,5)P_3 (PIP_3), a second messenger which activates a number of AKT-dependent and –independent downstream signaling pathways regulating diverse cellular functions including growth, metabolism, motility, survival, and transformation. The phosphatase and tensin homolog (PTEN) lipid phosphatase removes the 3′ phosphate from PtdIns(3,4,5)P_3 to inactivate class I PI3K signaling. Modified with permission from Reference 2.

(B) Class II PI3Ks are not well understood, but may be activated by a number of different stimuli, including hormones, growth factors, chemokines, cytokines, phospholipids, and calcium (Ca^{2+}). Although *in vitro* class II PI3Ks can phosphorylate both PtdIns and PtdIns(4)P, *in vivo* this class may preferentially phosphorylate PtdIns (PI) to generate PtdIns(3)P (PIP)[8-10]. Class II PI3Ks regulate cellular functions including glucose transport, endocytosis, cell migration, and survival. Myotubularin (MTM) family phosphatases remove the 3′ phosphate from PtdIns(3)P to inactivate class II PI3K signaling.

(C) The class III VPS34-VPS15 heterodimer is found in distinct multiprotein complexes, which perform specific cellular functions. VPS34 may be activated by stimuli including amino acids, glucose, and other nutrients, and phosphorylates PtdIns (PI) to generate PtdIns(3)P (PIP). It plays critical roles in autophagy, endosomal trafficking, and phagocytosis. MTM family phosphatases remove the 3′ phosphate from PtdIns(3)P to inactivate class III PI3K signaling.

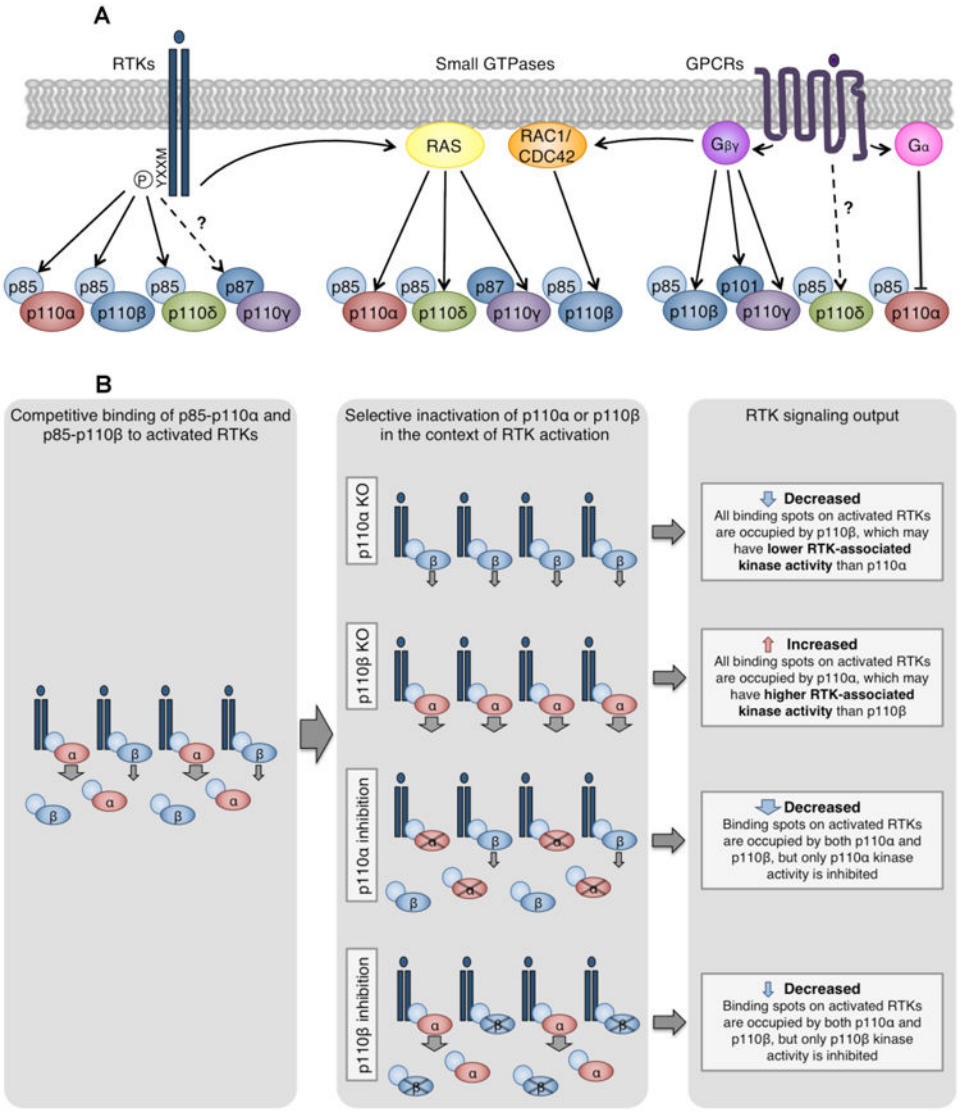


Figure 3. Divergent roles of class I PI3K catalytic isoforms in different signaling contexts

(A) Class I PI3Ks mediate signaling downstream of RTKs, GPCRs, and small GTPases. *Left:* p85 regulatory subunits bind phosphorylated YXXM motifs on activated RTKs. Because p110α, p110β, and p110δ bind p85, these isoforms mediate signaling downstream of RTKs. Recent evidence also suggests that p87-p110γ may be activated by certain RTKs[110]. *Middle:* Small GTPases synergize with RTK and GPCR signals to directly activate PI3Ks by interacting with their RAS-binding domains (RBDs). Isoforms p110α, p110δ, and p110γ bind RAS family GTPases, while p110β binds the RHO family GTPases RAC1 and CDC42[143]. *Right:* G_{α} and $G_{\beta\gamma}$ proteins dissociate from activated GPCRs. Catalytic isoforms p110β and p110γ, and regulatory isoform p101, directly bind and are activated by $G_{\beta\gamma}$. p110δ may be activated downstream of GPCRs, but the mechanism is unknown[126-128]. G_{α} proteins have been reported to directly bind and inhibit p110α[129-131]. Modified with permission from Reference 3.

(B) Competition model for p110α and p110β regulation of RTK signaling[96]. Both p85-p110α and p85-p110β compete for phosphorylated YXXM sites on activated RTKs. However, the maximal specific activity and enzymatic rate of p110α are higher than that of p110β[108, 109], and RTK-associated p110α may have higher lipid kinase activity than p110β[96]. By this model, loss or inactivation of p110α or p110β differentially modulates RTK signaling. Knockout of p110α allows all sites to be occupied by the less active p110β, decreasing RTK output. Conversely, knockout of p110β allows all sites to be bound by the more active p110α, increasing RTK output. Genetically or pharmacologically inactivated p110α or p110β can still bind RTKs but cannot signal, reducing RTK output.

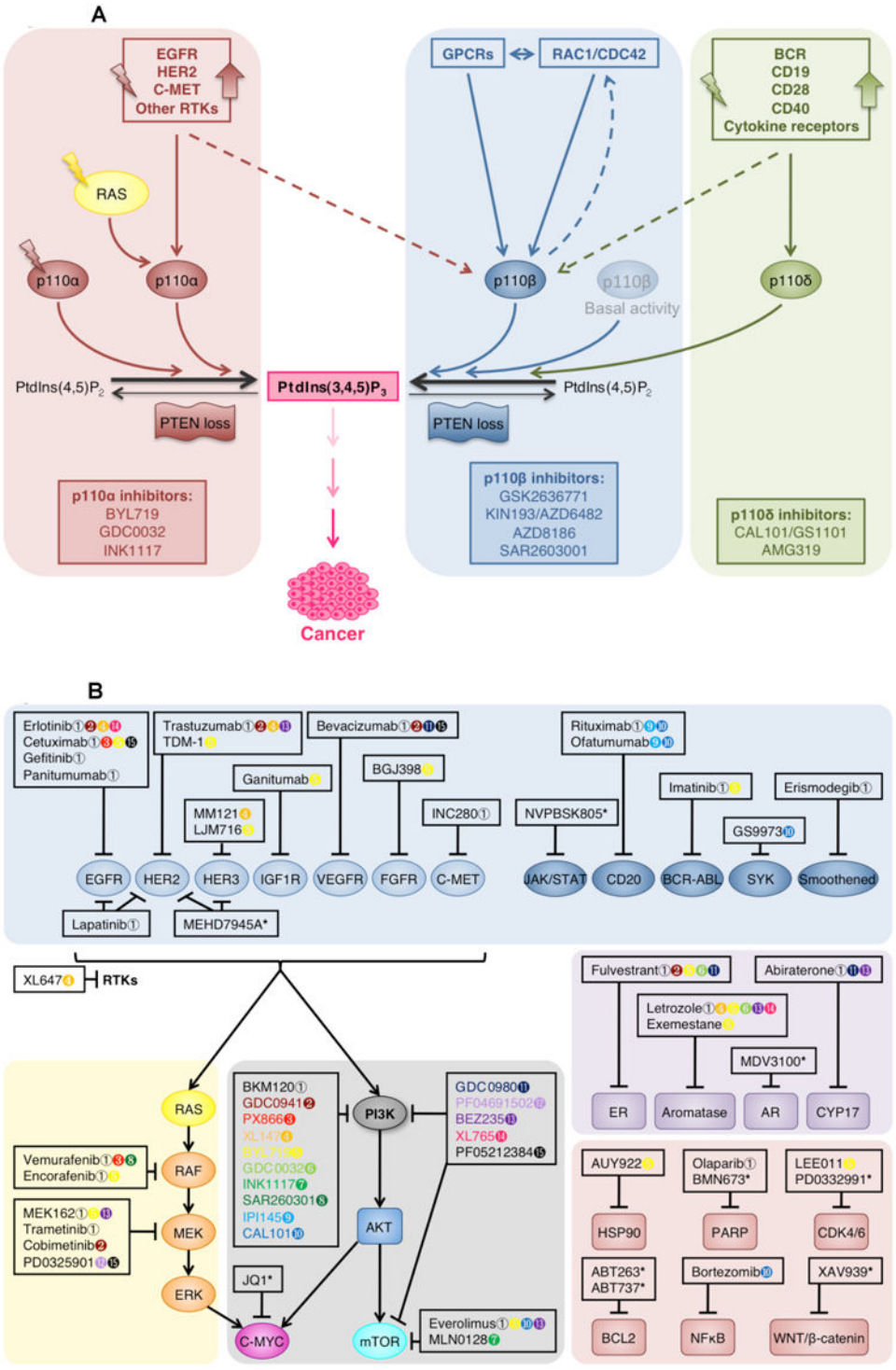


Figure 4. An overview of PI3K inhibitors and their combination with other therapeutics

(A) Molecular contexts dictating applications for isoform-selective PI3K inhibitors. *Light orange boxes:* Upregulation or mutation of receptor tyrosine kinases (RTKs), oncogenic RAS mutations, or activating p110α mutations all increase PtdIns(3,4,5)P_3 production through p110α, which can be amplified by mutation or loss of PTEN. In these contexts use

of p110α-selective inhibitors is effective. *Blue boxes:* In the absence of other oncogenic alterations, PTEN loss or mutation increases PtdIns(3,4,5)P_3 production through p110β, perhaps due to RAC1- or CDC42-mediated p110β activation, or the basal activity of this isoform. In this context use of p110β-selective inhibitors is effective. *Dark orange boxes:* Upregulation or mutation of B cell receptors (BCRs), cytokine receptors, or other immune cell surface markers increases PtdIns(3,4,5)P_3 production through p110δ. In this context use of p110δ-selective inhibitors is effective.

(B) Rational combination of PI3K inhibitors and other targeted therapeutics. Pan-PI3K and dual pan-PI3K and mTOR inhibitors are currently being tested in clinical trials (*white box*). These agents are being combined with mTOR-selective inhibitors (*shown in dark orange*), RAS-RAF-MEK-ERK pathway inhibitors (*shown in light orange*), RTK (*shown in grey*) or other membrane-associated protein inhibitors (*shown in turquoise*), hormone signaling inhibitors (*shown in dark blue*), and other agents inhibiting the cell cycle, apoptosis machinery, or other signaling pathways (*shown in purple*). Colored symbols indicate targeted therapeutics currently in clinical trials for combination with the designated PI3K inhibitor. For further detail, see Supplementary Table 3.

Table 1
Class I PI3K isoform alterations in cancer

Alteration Type	Cancer Type	Frequency of Alteration	Sample Size Range
Class IA			
***PIK3CA* (p110α)**			
Mutation	Endometrial	10.3-53.0%	29-232
	Breast	7.1-35.5%	65-507
	Ovarian	33.0%	97
	Colorectal	16.9[†]-30.6%	72-195
	Bladder	5.0-20.0%	20-130
	Lung	0.6-20.0%	5-183
	Cervical	13.6%	22
	Glioblastoma	4.3-11.0%	91-291
	Head and neck	8.1-9.4%	32-74
	Esophageal	5.5%	145
	Melanoma	5.0%	121
	Prostate	1.3-3.6%	55-156
	Sarcoma	2.9%	207
	Renal	1.0-2.9%	98-417
	Liver	1.6%	125
	Megalencephaly[‡]	48.0%	50
Copy number gain/amplification	Head and neck	9.1-100%	11-117
	Cervical	9.1-76.4%	22-55
	Lung	9.5-69.6%	3-92
	Lymphoma	16.7-68.2%	22-60
	Ovarian	13.3-39.8%	60-93
	Gastric	36.4%	55
	Thyroid	30.0%	110
	Prostate	28.1%	32
	Breast	8.7-13.4%	92-209
	Glioblastoma	1.9-12.2%	139-206
	Endometrial	10.3%	29
	Thyroid	9.4%	128
	Esophageal	5.7%	87
	Leukemia	5.6%	161
Increased expression	Prostate	40.0%	25
***PIK3CB* (p110β)**			
Mutation	Breast	0.5%	183

Alteration Type	Cancer Type	Frequency of Alteration	Sample Size Range
Copy number gain/amplification	Lung	56.5%	46
	Thyroid	42.3%	97
	Ovarian	5-26.9%	NA-93
	Lymphoma	20.0%	60
	Glioblastoma	5.8%	103
	Breast	4.9-5%	NA-81
Increased expression	Prostate	46.7%	30
	Glioblastoma	3.9%	103
***PIK3CD* (p110δ)**			
Copy number gain	Glioblastoma	40.0%	10
Increased expression	Neuroblastoma	52.6%	19
	Glioblastoma	5.8%	103
***PIK3R1* (p85α, p55α, p50α)**			
Mutation	Endometrial	19.8-32.8%	108-243
	Pancreatic	16.7%	6
	Glioblastoma	7.6-11.3%	91-291
	Colorectal	4.6[†]-8.3%	108-195
	Melanoma	4.4%	68
	Ovarian	3.8%	80
	Esophageal	3.4%	145
	Breast	1.1-2.8%	62-507
	Colon	1.7%	60
Decreased expression	Breast	61.8%	458
	Prostate	17-75%[*]	NA
	Lung	19-46%[*]	NA
	Ovarian	22%[*]	NA
	Breast	18%[*]	NA
	Bladder	18%[*]	NA
Copy number loss	Ovarian	21.5%	93
***PIK3R2* (p85β)**			
Mutation	Endometrial	4.9%	243
	Colorectal	0.9%	108
	Megalencephaly[‡]	22.0%	50
Amplification	Lymphoma	23.3%	60
Increased expression	Colon	55.0%	20

Alteration Type	Cancer Type	Frequency of Alteration	Sample Size Range
	Breast	45.7%	35
***PIK3R3* (p55γ)**			
Copy number gain	Ovarian	15.0%	93
Class IB			
***PIK3CG* (p110γ)**			
Copy number gain	Ovarian	19.3%	93
Increased expression	Breast	77.5%	40
	Prostate	72.4%	29
	Medulloblastoma	52.9%	17
***PIK3R5* (p101)**			
Mutation	Melanoma	38.2%	68
	Gastric	2.7%	37

For further detail and references, see the expanded version of this table online (Supplemental Table 1).

‡Megalencephaly syndromes are a collection of sporadic overgrowth disorders characterized by enlarged brain size and other distinct features.

†Combined number of hypermutated and non-hypermutated colon and colorectal patient samples with mutations in the indicated gene.

*Represents the percent reduction in gene expression.

NA Sample size not available for this study.

Table 2
Pan-PI3K inhibitors and their clinical applications

Agent	Company	Target	Trial stage*	Tumor types*
BKM120	Novartis	Class I PI3Ks	I, II, and III	• NSCLC • Endometrial • Thyroid • CRPC • Breast • Colorectal • Head and neck • GBM • Renal cell • B cell lymphoma • GIST • Melanoma • Ovarian • Prostate • Pancreatic • Leukemia • Esophageal • Cervical • Non-Hodgkin lymphoma • Squamous NSCLC • Adv. solid tumors
GDC0941	Genentech	Class I PI3Ks	I and II	• Breast • NSCLC • Non-Hodgkin lymphoma • Adv. solid tumors
BAY80-6946	Bayer	Class I PI3Ks	I and II	• Non-Hodgkin lymphoma • Adv. solid tumors
ZSTK474	Zenyaku Kogyo Co.	Class I PI3Ks	I and II	• Adv.solid tumors
PX866	Oncothyreon	Class I PI3Ks	I and II	• Colorectal • SCCHN • Melanoma • NSCLC • Prostate • GBM • Adv. solid tumors

Agent	Company	Target	Trial stage*	Tumor types*
XL147	Exelixis/Sanofi-Aventis	Class I PI3Ks	I and II	• Breast • Endometrial • Ovarian • Lymphoma • GBM • NSCLC • Adv. solid tumors
CH5132799	Chugai Pharma Europe	Class I PI3Ks	I	• Adv. solid tumors

*Data taken from an April 2014 search of http://www.clinicaltrials.gov.

NSCLC, non-small cell lung carcinoma; CRPC, castration-resistant prostate cancer; GIST, gastrointestinal stromal tumor; SCCHN, squamous cell carcinoma of the head and neck; GBM, glioblastoma multiforme

Table 3
Dual pan-PI3K and mTOR inhibitors and their clinical applications

Agent	Company	Target	Trial stage*	Tumor types*
GDC0980	Genentech	PI3K and mTOR	I and II	• Prostate • Breast • Endometrial • Renal cell • Non-Hodgkin lymphoma • Adv. solid tumors
PF04691502	Pfizer	PI3K and mTOR	I	• Adv. solid tumors
BGT226	Novartis	PI3K and mTOR	I and II	• Adv. solid tumors
BEZ235	Novartis	PI3K and mTOR	I and II	• Breast • Renal cell • Prostate • TCC • ALL • AML • CML • Pancreatic neuroendocrine • Adv. solid tumors
XL765	Sanofi	PI3K and mTOR	I	• GBM • Adv. solid tumors
GSK2126458	GlaxoSmithKline	PI3K and mTOR	I	• Solid tumors • Lymphoma
DS7423	Daiichi Sankyo	PI3K and mTOR	I	Solid tumors
PWT33597	Pathway Therapeutics	PI3K and mTOR	I	Adv. solid tumors
SF1126	Semafore Pharmaceuticals	PI3K and mTOR	I	Adv. solid tumors
PF05212384	Pfizer	PI3K and mTOR	I and II	Adv. solid tumors

*Data taken from an April 2014 search of http://www.clinicaltrials.gov.

TCC, transitional cell carcinoma; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; GBM, glioblastoma multiforme

Table 4
Isoform-selective PI3K inhibitors and their clinical applications

Agent	Company	Target	Trial stage*	Tumor types*
BYL719	Novartis	p110α	I and II	• SCCHN • ESCC • Colorectal • Breast • GIST • Kidney • Pancreas • Gastric • Adv. solid tumors
GDC0032	Genentech	p110α	I	• Breast • Adv. solid tumors
INK1117	Intellikine/Millenium	p110α	I	• Adv. solid tumors
AZD8186	Astra-Zeneca	p110β	I	• CRPC • sqNSCLC • TNBC • Adv.solid tumors with PTEN deficiency
GSK2636771	GlaxoSmithKline	p110β	I and II	• Adv.solid tumors with PTEN deficiency
SAR260301	Sanofi	p110β	I	• Adv.solid tumors • Lymphoma
IPI145	Infinity	p110δ and p110γ	I, II, and III	• CLL • SLL • ALL • INHL • Hematologic malignancies
AMG319	Amgen	p110δ	I	Lymphoid malignancies
CAL101 (**GS101**)	Gilead Sciences	p110δ	I, II, and III	• INHL • CLL • MCL • SLL • Hodgkin lymphoma • Non-Hodgkin lymphoma • Other lymphomas • AML • MM

Agent	Company	Target	Trial stage*	Tumor types*
				• Hematologic malignancies
GS9820	Gilead Sciences	p110β and p110δ	I	• Lymphoid malignancies

*Data taken from an April 2014 search of http://www.clinicaltrials.gov.

SCCHN, squamous cell carcinoma of the head and neck; ESCC, esophageal squamous cell carcinoma; GIST, gastrointestinal stromal tumor; CRPC, castration-resistant prostate cancer; sqNSCLC, squamous non-small cell lung cancer; TNBC, triple-negative breast cancer; CLL, chronic lymphocytic leukemia; SLL, small lymphocytic leukemia; ALL, acute lymphoblastic leukemia; INHL, indolent non-Hodgkin lymphoma; MCL, mantle cell lymphoma; AML, acute myeloid leukemia; MM, multiple myeloma