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REVIEW

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Targeting PI3K family with small-molecule inhibitors in cancer therapy: current clinical status and future directions

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Abstract

The Phosphatidylinositol-3-kinase (PI3K) family is well-known to comprise three classes of intracellular enzymes. Class I PI3Ks primarily function in signaling by responding to cell surface receptor stimulation, while class II and III are more involved in membrane transport. Under normal physiological conditions, the PI3K signaling network orchestrates cell growth, division, migration and survival. Aberrant activation of the PI3K signaling pathway disrupts cellular activity and metabolism, often marking the onset of cancer. Currently, the Food and Drug Administration (FDA) has approved the clinical use of five class I PI3K inhibitors. These small-molecule inhibitors, which exhibit varying selectivity for different class I PI3K family members, are primarily used in the treatment of breast cancer and hematologic malignancies. Therefore, the development of novel class I PI3K inhibitors has been a prominent research focus in the field of oncology, aiming to enhance potential therapeutic selectivity and effectiveness. In this review, we summarize the specific structures of PI3Ks and their functional roles in cancer progression. Additionally, we critically evaluate small molecule inhibitors that target class I PI3K, with a particular focus on their clinical applications in cancer treatment. Moreover, we aim to analyze therapeutic approaches for different types of cancers marked by aberrant PI3K activation and to identify potential molecular targets amenable to intervention with small-molecule inhibitors. Ultimately, we propose future directions for the development of therapeutic strategies that optimize cancer treatment outcomes by modulating the PI3K family.

Keywords PI3K family, Class I PI3K, Clinical applications, Therapeutic approach, Molecular target, Small-molecule inhibitor, Cancer therapy

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Introduction

Since its discovery in the 1980s, the structures and functions of three classes PI3Ks have gradually been uncovered, following the expanding research of upstream and downstream corresponding factors of the PI3K-centered pathway and its activation mechanisms until the end of the twentieth century. PI3K has garnered increasing attention in scientific research due to its pivotal role in cellular regulation and its significant involvement in cancer progression. Current studies in PI3K signaling predominantly investigate upstream activating stimuli and their subsequent effects on downstream substrates, illuminating the intricate pathways through which PI3K exerts its influence on cellular behavior, particularly in the context of cancer growth and proliferation. Meanwhile, to address the over-activated PI3K pathway in cancer, the inhibitors targeting the PI3K family have always been a hot topic.

The PI3K family encompasses three primary classes: class I, II, and III [1]. Within class I, further subdivisions include class IA (p110α, p110β, and p110δ) and class IB (p110γ), with particular relevance to cancer [2]. Class II PI3Ks are divided into PI3KC2α, PI3KC2β, and PI3KC2γ. Class III PI3Ks indeed form two complexes, with VPS34 serving as the central component [3]. The PI3K/AKT/mTOR signaling pathway stands as one of the most crucial intracellular pathways, governing vital cellular processes like survival, proliferation, differentiation, and metabolism. Its dysregulation is often linked with cancer development [1]. Other common oncoproteins frequently intervene, either directly or indirectly, to upregulate or disrupt this pathway. This interference often marks a pivotal event in cancer progression because the dysregulation of the pathway leads to an imbalance in cellular activities [4]. The aberrant expression of PI3K is prevalent across various types of cancer. Frequent epigenetic alterations in genomes, such as *PIK3CA, PIK3R1, PTEN*, and *AKT1*, offering perspectives and therapeutic targets for the management of cancer by targeting the dysregulated PI3K pathway [5]. Besides, with the progressive discovery of class II and III PI3K's regulations in cancer, their targeted small-molecule have also become a hot-spot in studies to date.

First generation of PI3K inhibitors was discovered in 1990s with disadvantages of non-selectivity, poor pharmacokinetic and intolerable side-effect, thus setting off a process of everlasting development and optimization. Class I PI3K inhibitors are categorized based on target affinity as pan-PI3K inhibitors, isoform-specific PI3K inhibitors, and dual PI3K/mTOR inhibitors. Over the last two decades, precision-targeted PI3K inhibitors have been developed, expanding their clinical applications [6]. The PI3K alpha-specific inhibitor 26 (BYL719) received initial FDA approval for use in combination with fulvestrant for the treatment of hormone receptor (HR)-positive, Human epidermal growth factor receptor 2 (HER2)-negative breast cancer in patients harboring a *PIK3CA* mutation. This approval was based on its demonstrated ability to extend progression-free survival (PFS) [7, 8]. Furthermore, novel approaches in drug design such as PROTACs and allosteric inhibitors have propelled the development of PI3K inhibitors to new heights (Fig. 1).

However, the utilization of PI3K inhibitors is beset by several challenges. The complex PI3K signaling network is marked by numerous feedback loops and extensive crosstalk with various compensatory pathways, exerting influence on and regulating each other [9]. The use of PI3K inhibitors can inadvertently lead cancer cells to favor mutations in PI3K regulatory genes or in upstream and downstream signaling pathways, as parallel gene evolution under PI3Kα selective therapeutic leads to progressive loss of *PTEN* expression [10]. This often results in the overexpression of PI3K, allowing cells to become resistant to the standard doses of PI3K inhibitors, or even to negate the requirement for PI3K activity altogether. These compensatory regulatory mechanisms pose significant obstacles to overcoming drug resistance in clinical applications. Additionally, alterations in regulatory genes can lead to intrinsic or acquired resistance to PI3K inhibitors, presenting challenges like off-target adverse effects and patient resistance in clinical use [11]. Thus, PI3K inhibitors with greater efficacy, fewer adverse reactions, and more precise treatment capabilities are always the future direction of small molecule inhibitor development.

Subsequently, we will delve into the specific structures of PI3K and their functional roles in cancer signaling and examine small molecule PI3K inhibitors and their applications in cancer treatment. We aim to explore the challenges associated with PI3K inhibitors and propose potential future directions for therapeutic strategies in cancer treatment.

Molecular structures and biological functions of PI3K family

PI3Ks family classes are conserved across mammals, contrasting with yeast, which only have one type of PI3K isoform which is similar to human class III PI3Ks [5, 12]. Members of the PI3K family share structural and functional similarities, each characterized by a conserved core that includes a C2 domain, a helical domain, and a kinase domain. Notable differences among these domains include substrate specificity, structural variations, and regulatory mechanisms [13].

The localization and recruitment of these PI3K classes vary significantly. Class I PI3Ks are primarily associated

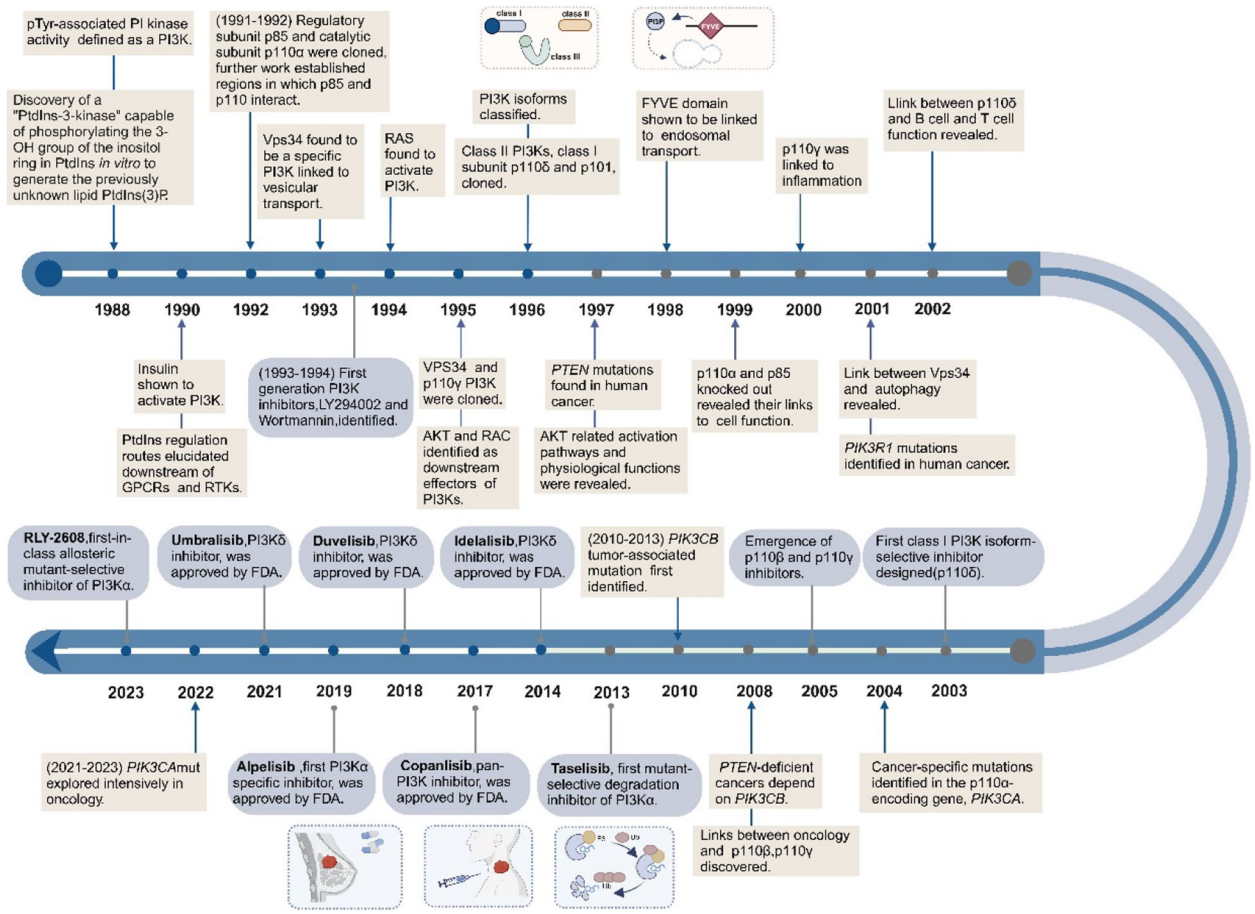


Fig. 1 A Brief History of PI3K family. The journey of PI3K signaling pathway research and key events in its targeted small molecule inhibitors development are shown

with the plasma membrane or act as components of very early endocytic intermediates. Class II PI3Ks are initially recruited to the plasma membrane and peripheral membrane vesicles. In contrast, class III PI3Ks localize to more diverse cellular structures, including endosomes, cytokinetic bridges, and autophagy precursors. This differential localization underscores the unique roles each class plays in cellular signaling and trafficking [14–16].

The PI3K family comprises a diverse group of lipid kinases situated within the plasma membrane, which function as enzymes capable of phosphorylating the 3'-OH groups of phosphatidylinositides (PtdIns). Specifically, this phosphatidylinositide subset includes type PtdIns, PtdIns-4-phosphate (PtdIns4P), and PtdIns-4,5-bisphosphate (PtdIns(4, 5)P2), leading to the synthesis of PtdIns(4, 5)P3. The latter plays a pivotal role in activating a variety of intracellular signaling pathways.

Class I PI3K

Class I PI3Ks are heterodimeric enzymes composed of regulatory and catalytic subunits. The catalytic subunits of Class IA PI3Ks, p110, consist of four isoforms: p110α (encoded by *PIK3CA*), p110β (encoded by *PIK3CB*), p110δ (encoded by *PIK3CD*), and p110γ (encoded by *PIK3CG*) [2]. Above-mentioned isoforms usually associate with various regulatory subunits, such as class IA (p110α, p110β, and p110δ) in combination with p85 regulatory subunits, which can be categorized into five classes: p85α (and its splice variants p55α and p50α, encoded by *PIK3R1*), p85β (encoded by *PIK3R2*), and p55γ (encoded by *PIK2R3*) [3]. Conversely, class IB (p110γ) combines with p101 (encoded by *PIK3R5*) or p84/p87 (encoded by *PIK3R6*) [3]. Among these isoforms, p110δ and p110γ are notably abundant in leukocytes, while the others are ubiquitously expressed [17].

The catalytic subunits of Class IA PI3Ks, p110, share identical structural domains (Fig. 2A): the ABD (facilitating primary interaction with the regulatory subunit), the RBD, a C2 structural domain with an affinity for the lipid membrane, a helical structure acting as a scaffold for other structural domains, and a carboxy-terminal kinase, also known as the PI3K core [18].The p85 regulatory

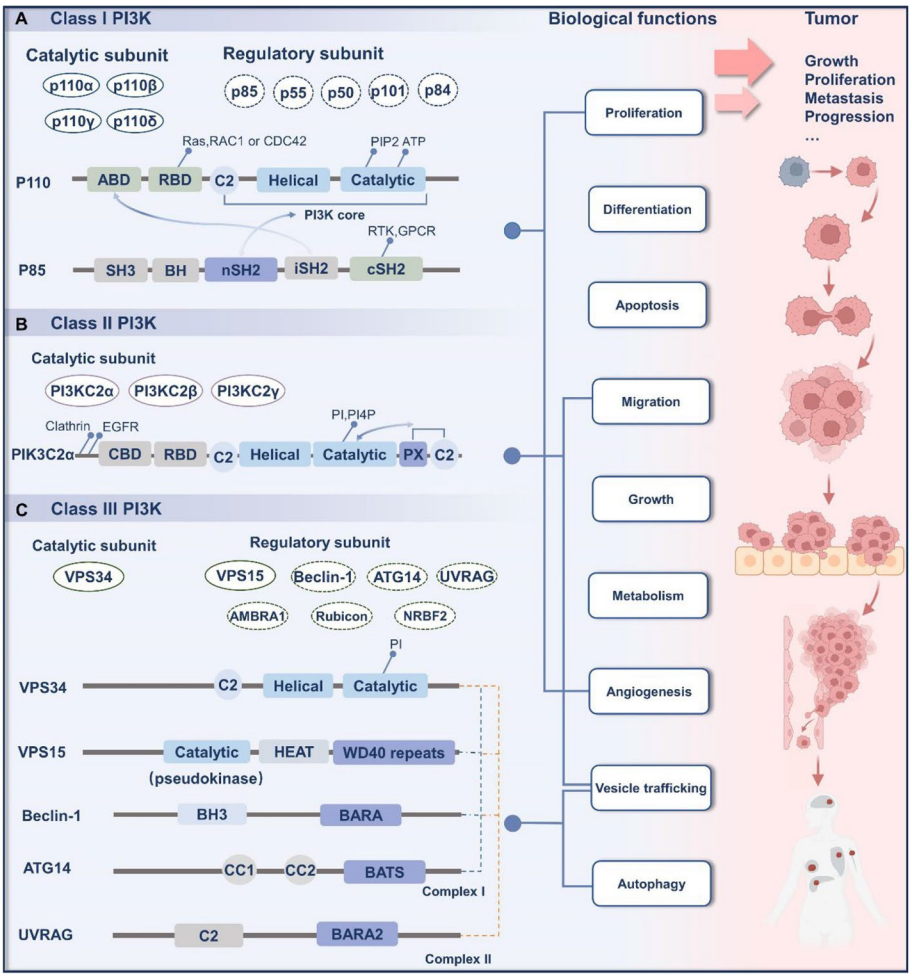


Fig. 2 An overview of PI3K family. **A** Class I PI3Ks consist of catalytic subunit and regulatory subunit, among which p85 taking inhibitory effect on p110. **B** Class II PI3Ks exist as a monomer and realize its own inhibitory properties through internal contacts. **C** Class III PI3Ks compose two complexes. Beclin 1, ATG14, and UVRAG are referred to as auxiliary subunits. Three types of PI3K have different physiological functions and regulate cellular activities together; on the other hand, abnormal PI3K signaling can promote tumor progression

subunit possesses an SH3 domain, a Bcl2-homology (BH) domain, and two SH2 domains (nSH2 at the N segment and cSH2 at the C-terminal end) interconnected via the iSH2 domain [2]. Under basal conditions, the iSH2 domain of the p85 regulatory subunit mediates the binding of the SH2 domain to the p110 catalytic subunit, exerting inhibitory effect. Upon stimulation of membrane receptors and their adaptor like RTKs and RTK adaptors, class I PI3K are recruited to the plasma membrane, where the nSH2 and cSH2 structural domains of p85 bind to phosphorylated tyrosines (YXXM motifs) in activated receptors and adaptor proteins. This binding triggers a conformational change that displaces the SH2s from the p110 catalytic subunit, thereby relieving the p85-mediated inhibition of p110, leading to PI3K activation [2, 19]. The function of the p85 subunit provided to the p110 subunit also includes stabilization and inactivating its kinase activity in the basal state [20]. Additionally, the RBD domain of the catalytic subunit enables Class I PI3K except for p110β to interact with RAS GTPases or other RAS superfamily members, among which only activated GTP-bound Ras can enhance membrane recruitment and this plasma membrane-localization relies on a lipid tail added post-translationally [21, 22]. The C-terminal kinase domain of p110α harbors ATP and PIP2 binding sites [19]. The catalytic subunit of Class IB PI3Ks, p110γ, mirrors Class IA p110, though the presence and role of the ABD domain remain unclear. Non-catalytic p84/87 and p101 share highly similar amino acids at the N- and C-termini, with the N-segment directly interacting with p110γ and the C-segment binding with Gβγ [23]. p110γ forms a heterodimer with p84/87 or p101, activated and bound to the plasma membrane by the Gβγ subunit released by the activating GPCR [24].

Class II PI3K

Class II PI3Ks represent the least understood subfamily within the broader PI3K family. Mammals contain three Class II PI3K isoforms: PI3K-C2α, PI3K-C2β, and PI3K-C2γ, while PI3K-C2γ expression is predominantly confined to the liver [5]. Class II PI3Ks lack obligate regulatory subunits and function as a monomer (Fig. 2B). Structurally, Class II PI3Ks diverge from the conserved PI3K core structure, featuring an extended N-terminal end with additional structural domains capable of protein binding. For instance, PI3K-C2α and PI3K-C2β exhibit Clathrin binding via the N-terminal region, and the N-terminal extensions of both isoforms exert inhibitory effects on the enzyme's catalytic activity [25–27]. Additionally, the N-terminal region of PI3K-C2β facilitates its binding and interaction with EGF receptors [28]. Class II PI3Ks also incorporate an extra C2 structural domain and a PX structural domain in the C-terminal region. In PI3K-C2α, the C-terminal extension of the PX-C2 structural domain stabilizes the enzyme and suppresses basal activity when folded back into the core structural domain. Upon the PX-C2 structural domain aiding the binding of PI3K-C2α to the plasma membrane containing PI(4,5)P2,the inhibitory contact of PI3K-C2α is relieved, restoring activity [29]. Regarding PI3K-C2β, protein hydrolysis of the C-terminus regulates lipid kinase activity in the nucleus, and deletion of the C2 structural domain heightens lipid kinase activity, implying a negative regulation of the catalytic structural domain [30]. Furthermore, mutants of PI3K-C2β with the inhibitory C2 structural domain deleted show an enhancement in the growth factor-independent AKT/PKB pathway [31].

Class III PI3K

VPS34 stands as the sole isoform within Class III PI3K, exhibiting conservation from yeast to humans and representing the most primitive PI3K. In mammals, VPS34 assembles into two heterotetrameric core complexes, denoted as complex I and complex II. These complexes share the VPS34, VPS15, and Beclin1 structures, forming an overall V-shaped structure. Both complexes include the VPS38/UVRAG subunit, with complex I featuring the ATG14/ATG14L subunit and complex II hosting the VPS38/UVRAG subunit (Fig. 2C). Quantitative immunoprecipitation has revealed the presence of additional VPS34 subcomplexes in cells [32]. VPS34 comprises a PI3K catalytic core, an N-segment C2 structural domain, a helical structural domain, and a C-terminal kinase structural domain. The latter two domains play a pivotal role in VPS34-catalyzed lipid activity [14]. VPS15 encompasses three structural domains: a kinase/pseudokinase structural domain, a helical structural domain, and a WD40 structural domain.

The interdependence of VPS34 and VPS15 is vital for the integrity of VPS14. Each structural domain of VPS15 interacts with at least one structural domain of VPS34, with the N-terminal pseudokinase structural domain of VPS15 binding to the C-terminal kinase structure of VPS34. This interaction restricts the activation loop, inhibiting the basal activity of VPS34. Additionally, the effects of VPS15 on VPS34 contribute to enzyme stabilization and membrane recruitment [33]. Beclin-1 comprises an N-segment unstructured region, a BH3 structural domain, two coiled-coil structural domains, and a C-terminal BARA structure [34]. The BH3 structural domain binds to VPS34-VPS15, while the BARA structural domain interacts with the BATS structural domain of ATG14 in complex I or the BARA2 structural domain of UVRAG in complex II [35]. ATG14 features an N-terminally extended convoluted helical structural domain and a C-terminal BATS structural domain. The latter is involved in sensing membrane curvature and contributes to ATG14's targeting of highly curved PI3P-rich membranes. The BATS structural domain plays a key role in the kinase activity of complex I [36]. UVRAG includes an N-terminal C2 structural domain, two coiled-coil structural domains, and a C-terminal BARA-associated structural domain (BARA2). The kinase activity of complex II relies on the Beclin-1 BARA structural domain [37].

Similar to class I PI3Ks, class III PI3Ks undergo regulation by additional subunits such as AMBRA1, Rubicon, and NRBF2. NRBF2 predominantly binds to complex I, Rubicon to complex II, and AMBRA1 transiently interacts with both complex I and complex II [37]. Essential for the full activity of Beclin-1, AMBRA1 promotes increased VPS34 activity, coordination, and enhancement of autophagy, among other functions [38]. As a negative regulator of autophagy, Rubicon specifically inhibits complex II, with its RUN domain playing a critical role in autophagosome maturation and degradation [39, 40]. NRBF2 has been reported as a positive regulator of autophagy initiation in complex I. However, conflicting experiments suggest its negative regulatory role. Intriguingly, NRBF2 may also participate in autophagy initiation independently of ULK1 and complex I [41]. In complex II, the UVRAG C2 domain prevents NRBF2 from binding [42].

Oncogenic and oncostatic roles of PI3K family

The PI3K family plays a critical role in modulating numerous pivotal cellular processes, with a significant influence stemming from its participation in the phosphatidylinositol cycle (Fig. 3). The phosphatidylinositol products generated within this cycle act as docking sites that recruit various downstream targets.

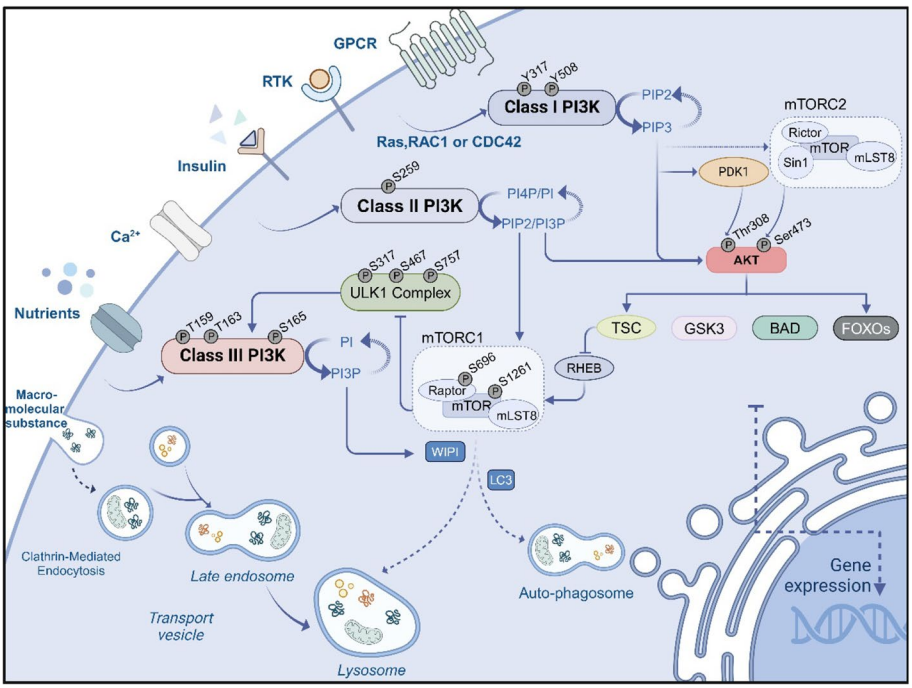


Fig. 3 The crucial signaling pathways of PI3K family in cancer. Three classes of PI3K respond to different upstream molecules and regulate distinct downstream molecules. By participating in the phosphatidylinositol cycle, class I PI3K produces PIP3, which recruits downstream molecules with PH domains to regulate cellular activities such as growth and metabolism. Class II PI3K can activates the AKT pathway by synthesizing PIP2. Additionally, Class II PI3K and class III PI3K synthesize PI3P, which recruits effector proteins with the structural domains of FYVE and PX to regulate membrane transport and autophagy

This recruitment facilitates downstream signaling, effectively translating the initial PI3K activation into a cascade of cellular responses. This mechanism underscores the central role of PI3K in regulating complex biological processes through signal transduction pathways [43]. Abnormalities in PI3K can disrupt this cyclic equilibrium, leading to cellular dysfunction. Aberrant activation of the PI3K signaling pathway is commonly observed in various types of cancers. These deviations are often accompanied by genetic alterations, which serve as primary mechanisms underlying carcinogenesis. This link highlights the critical impact of PI3K pathway dysregulation on cancer development, emphasizing the importance of targeting this pathway in cancer therapy. Additionally, abnormalities in the regulators of the phosphatidylinositol cycle can lead to widespread dysregulation of the entire signaling pathway. Key genetic abnormalities, which are often critical for clinical management, also serve as potential biomarkers. These markers are instrumental in guiding the clinical effectiveness of PI3K inhibitors and serve as potential therapeutic target for the development of novel therapies, thereby enhancing the precision of cancer treatment strategies.

Class I PI3K

Class I PI3Ks play a pivotal part in cell signaling and are activated through various pathways, including RTK signaling, G protein-coupled receptors (GPCR), and monomeric small GTPases (Ras for αδγ/RAC1 or CDC42 for β) [12, 21, 22]. Upon activation, phosphatidylinositol(4,5)-bisphosphate (PIP2) is phosphorylated into phosphatidylinositol (3,4,5)-trisphosphate (PIP3) by Class I PI3Ks. PIP3 further recruits proteins containing pleckstrin homology (PH) structure to the cell membrane, such as AKT and PDK1 [44]. Mammals express three AKT isoforms(AKT1/PKB-α, AKT2/PKB-β, and AKT3/PKB-γ)sharing a common structure and activation mechanism but exhibiting distinct roles in normal cell physiology and cancer pathogenesis [45]. For instance, in breast cancer, AKT1 supports the proliferation and survival of cancer cells with anti-metastatic effects, while AKT2 primarily promotes migration, invasion, and chemotaxis [46]. Upon recruitment, AKT undergoes phosphorylation and activation by mTORC2 and PDK1 at serine 473 and threonine 308, respectively.

Activated AKT subsequently phosphorylates downstream substrates. GSK3β is an early AKT direct substrate, which is induced N-terminal serine phosphorylation

by AKT, undergoing autoinhibitory phosphorylation. GSK3β influences various downstream targets related to glycogen metabolism, cell cycle, apoptosis, DNA repairing etc. [47–50]. Thus activated AKT forms pseudo-substrate sequence through phosphorylation to inhibit the binding of GSK3 to its substrates like glycogen synthase, positively regulating these targets via AKT signaling [51]. Meanwhile, there are evidences the related GSK downstream regulations provide negative feedback suppression of PI3K-AKT pathway. Knockdown of *GSK3β* results in decreased AKT phosphorylation [47].

The mTOR complexes can be categorized into mTORC1 and mTORC2. The mTORC1 complex includes mTOR, Raptor, and mLST8, while mTORC2 consists of mTOR, Rictor, mLST8, sin1, plus two endogenous inhibitors of the complex, PRAS40 and DEPTOR [52].

mTORC1 stands as a key downstream regulator after PI3K activation of AKT. AKT directly inhibits the TSC complex through multisite phosphorylation, which is the direct regulator of Rheb activation [53]. Rheb, a kind of GTPases, is capable of direct reaction to the kinase domain of mTOR、mLST8 and Raptor through shifting conformations, leading to intense mTORC1 activity [54]. In addition, undergoing activated mTORC1 through Rheb together with AKT direct phosphorylation, the inhibitory subunit PRAS40 is dissociated from mTORC1 resulting in more robust activation [55].

Regarding mTORC2, sin1 interacts with PIP3 through the PH structural domain, relieving the inhibitory effect on mTOR kinase. This translocate mTORC2 to the plasma membrane for substrate phosphorylation, ultimately contributing to AKT's full activation [56]. The mTOR complex plays a pivotal role in cellular regulation by responding to integrated signals from nutrients and growth factors. It modulates cell growth, proliferation, and survival through the oversight of key metabolic pathways, including glucose metabolism, lipid metabolism, mitochondrial function, protein synthesis, and nucleotide synthesis [57].

Forkhead box Os (FOXOs) contain transcription factor superfamily, among which FOXO1, FOXO3, FOXO4, and FOXO6 are direct downstream targets of AKT. These transcription factors remain conserved and strict localization, showcasing key signaling proteins in growth factor signaling. AKT participates in their interactions with the 14–3-3 protein through phosphorylation, regulating the localization to the cytoplasm and nucleus. In addition, phosphorylation of AKT can leads to the ubiquitin proteasome pathway dependent degradation of FOXOs [58]. AKT phosphorylation of FOXO is integral in regulating cellular functions like cell differentiation, apoptosis, metabolism, and proliferation. This phosphorylation of FOXO has duality, acting as both a tumor suppressor and promoter in cancer [59].

In addition to PtdIns-mediated AKT activation, the structural subunits of class I PI3K play roles in cellular function regulation. Increasing evidence suggests kinase-independent effects for p110β and p110γ. For instance, p110β ablation in mouse liver impaired insulin sensitivity and glucose homeostasis independently of AKT phosphorylation levels, indicating a non-kinase-dependent role in insulin metabolism [60]. The p110γ knockout mouse cardiac model demonstrated protein interaction-dependent non-kinase activity, decreasing cAMP levels by binding to PDE3B and negatively regulating cardiac contractility [61]. While p85 and p110 typically function as exclusive chaperones in the cell, the free regulatory subunit p85 can also operate independently of p110. Its roles involve bridging protein functions, localizing signaling proteins, coordinating functions, mediating cytoskeletal changes, and facilitating endocytotic transport [62]. Additionally, p85 induces TNFα expression, exerting pro-apoptotic effects and triggering p53-mediated apoptosis [63]. In insulin signaling, p85 has both positive and negative regulatory effects on the insulin response [64, 65]. The BH structural domain (GAP structural domain) of p85 exhibits conserved sequence homology with a group of RhoGAPs, involved in catalyzing the hydrolysis of small GTPase-bound GTP (Rho/Rac/CDC42), interacting with GTP-bound forms of CDC42 and Rac1, and participating in cytoskeletal changes that drive cell migration and cytoplasmic division [66–68].

Among class I PI3Ks, mutations in the *PIK3CA* gene are most frequently observed in cancer patients, particularly in exons 4, 9, and 20(Fig. 4A). These mutations include H1047R, which accounts for 35% of cases, E545K at 17%, E542K at 11%, N345K at 6%, and H1047L at 4%. Collectively, these specific alterations comprise 73% of all known *PIK3CA* mutation types within the database [69]. The mutation's location significantly impacts the protein's structural and functional integrity. Specifically, mutations E542 and E545 are situated in the helical domain, while H1047R and H1047L, found in the kinase domain. Mutations situated in helical domain diminish the inhibitory effect of p85 on p110α also indirectly promote the direct interaction between p110α and IRS1. Kinase domain mutations stimulate lipid kinase activity by inducing the metamorphic movement necessary for catalysis at the lipid membrane, enhancing the interaction between p110α and the lipid membrane(Fig. 4B) [70–72]. HR-positive/HER2-negative ($HR^+/HER2^-$) metaplastic BC patients with *PIK3CA* mutations exhibit resistance to chemotherapy, whereas tubular BC with *PIK3CA* mutations shows sensitivity to PI3K inhibitors [73]. *PIK3CA*

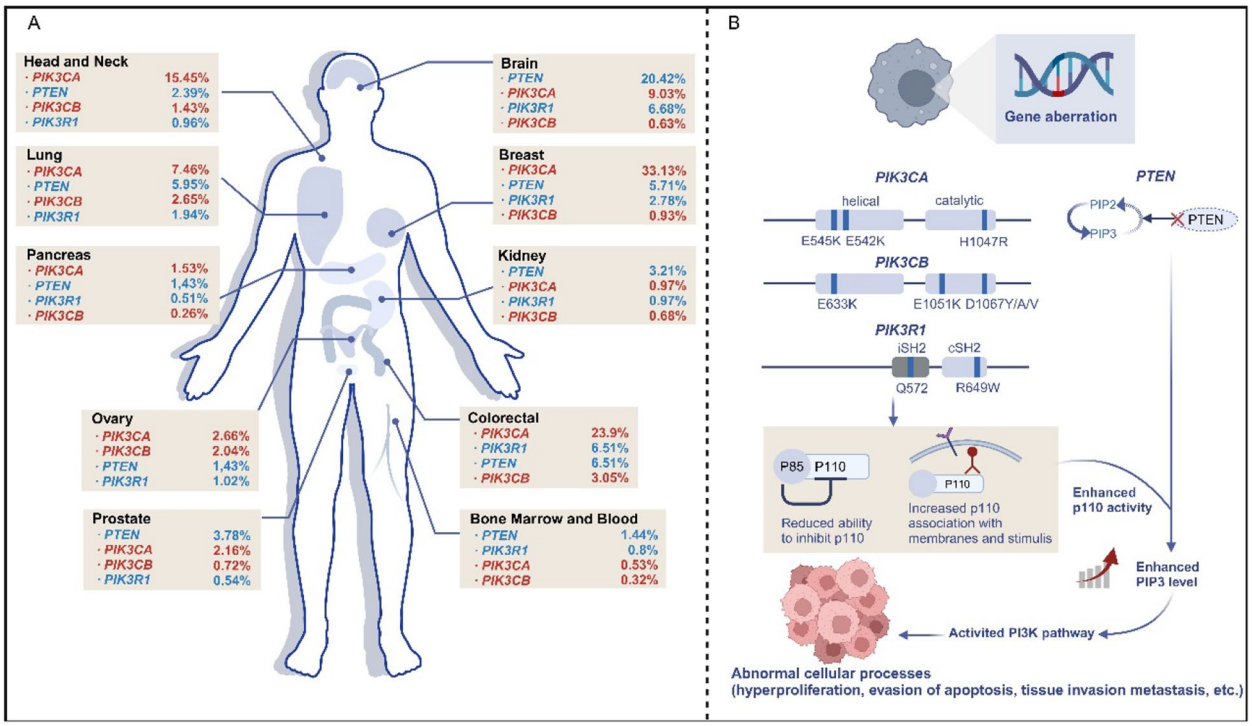


Fig. 4 Frequent Mutated Genes of PI3K signaling pathway. **A** PI3K mutations in different cancers, with *PIK3CA* and *PIK3CB* as oncogenes and *PIK3R1* and *PTEN* as tumor suppressors. **B** Different genetic alterations affecting its own function, thus altering the activation of the PI3K pathway, ultimately leading to tumorigenesis

mutations are also interconnected with poor prognosis and reduced survival [74].

PIK3CB is considered responsible for driving cancer cell proliferation and tumorigenesis, especially in *PTEN*-deficient tumors [75].The *PIK3CB* tumor-associated mutation E633K is first identified in $HER2^+$ BC, and its mutants in the helical domain will cause a conformational change in the ABD-RBD linker, increasing membrane binding of p110β, leading to p110β activation that regulates tumor growth and proliferation [76, 77]. The E1051K mutation and the D1067Y/A/V mutation occur in the kinase domain, with E1051K being a gain-of-function mutation driving PI3K signaling for tumor cell growth and migration [78]. The latter is associated with drug efficacy, modulating sensitivity to multiple drug treatments [79].

PIK3CD mutations are typically associated with immunodeficiency, and their role in cancer remains to be explored. Moreover, *PIK3CG* is associated with chromosome band 7q22, frequently missing in myeloid malignancies, the *PIK3CG* gene is considered a candidate myeloid tumor suppressor gene TSG [80].

The regulatory subunit p85 functions independently of p110, and mutations in p85 are observed in various cancers. Mutations in the *PIK3R1* gene typically manifest as a reduction or deletion of the iSH2 domain, preventing its interaction with the C2 domain of p110. This leads to attenuated p85-dependent inhibition of p110, facilitating enhanced PI3K activity and conversion [81]. *PIK3R1* is recognized as a tumor suppressor, and the oncogenic Q572 truncation of *PIK3R1* disrupts the inhibitory potency of all p85, resulting in more hyperactivation of p110α. In contrast, the R649W mutation in the cSH2 domain decreases sensitivity to receptor tyrosine kinase activation [82]. Moreover, *PIK3R1* is associated with immunodeficiency, and functionally acquired mutations in both *PIK3R1* and *PIK3CD* contribute to a combined immunodeficiency syndrome known as PI3Kδ syndrome or APDS [83]. Interestingly, *PIK3R2* is a pro-oncogenic factor, which increases with the development of the tumor stage. Overexpression of *PIK3R2* induces metastasis, while its deletion causes tumor regression and invasion reduction [84].

In addition to mutations directly in PI3K, abnormalities in downstream signaling pathways, such as *AKT*, can also exert similar oncogenic effects. For example, the *AKT1* E17K mutation, which is prevalent in various cancers including breast, colorectal, and ovarian cancers. This mutation is often found to be mutually exclusive with *PIK3CA* mutations and complete PTEN protein

loss, suggesting different, yet overlapping pathways of oncogenesis. The *AKT1* E17K mutation alters the pleckstrin homology domain of *AKT1*, enhancing its affinity for phospholipids and constitutively activating the kinase, thereby promoting cancer cell survival, proliferation, and growth [85]. *AKT2* mutations are associated with hypo-insulinemia and hypoglycemia due to abnormal activation of insulin signaling. Meanwhile, *AKT2* mutations also occur in ovarian, breast, gastric, and lung cancers, leading to the facilitation of tumor invasion and metastasis [86–88]. *AKT3*, less studied but impactful in ER-negative breast cancer and TNBC, exhibits anti-metastatic, pro-proliferative, and pro-carcinogenic effects, with mutations seen in melanoma cells [46, 89].

Furthermore, mutations in regulators of the PI3K pathway can also induce malignancy. PTEN, a crucial regulator at the cellular level, dephosphorylates PIP3 and PI(3,4)P2, transforming them back into PI(4,5)P2 and PI4P, respectively, can terminates PI3K-PIP3 signaling and AKT activation [90].The loss of *PTEN* due to genetic or epigenetic alterations usually is the primary cause of aberrant PI3K signaling [91]. The absence or reduced expression of *PTEN* is often associated with a poorer prognosis in HR^+/ $HER2^-$ or $HER2^+$BC [89]. Specifically, cancer cells lacking PTEN have shown a dependence on p110β, underscoring the significant impact of PTEN on the regulation and balance of PI3K pathway activities [75].

Class II PI3K

Stimulated by hormones, growth factors, chemokines, cytokines, phospholipids, and calcium, Class II PI3K recognize substrates PI and phosphatidylinositol 4-phosphate (PI4P), synthesizing phosphatidylinositol 3-phosphate (PI3P) and phosphatidylinositol (3,4)-bisphosphate (PI(3,4)P2), respectively. PI3P synthesis is primarily associated with PI3K2α and PI3K2β, while all three Class II PI3K isoforms catalyze the synthesis of PI(3,4)P2 [32].

Class II PI3Ks are downstream signaling molecules for cell surface receptors that govern intracellular membrane dynamics, membrane traffic, and diverse cellular processes including cell migration, insulin signaling, glucose metabolism, channel regulation, and cellular dynamics [16]. A distinctive feature is its association with clathrin: binding to the N-terminal extension and recruiting PI3K-C2α from the plasma membrane and the TGN, thus facilitating catalytic activity on phosphorylated inosine substrates [25]. PI3K-C2α deficiency hampers endothelial cell signaling and vascular barrier effects, emphasizing its crucial role in angiogenesis and barrier integrity [92]. In terms of signaling, PI3K-C2α responds to insulin by producing PI(3, 4)P2, selectively activating the PKB/AKT pathway [93]. An experiments with male heterozygous mice inactivated by a PI3K-C2α mutation results in leptin resistance, age-dependent insulin resistance, and obesity, revealing a dependent role in systemic glucose homeostasis [94]. PI3K-C2β can bind to clathrin and Raptor in mTORC1 through the N-terminal extension, acting as a negative regulator of mTORC1 [27]. In hepatocytes, the inactivation of PI3KC2β enhances class I PI3K-dependent AKT signaling upon insulin stimulation [95]. Meanwhile, PI3KC2γ significantly contribute to long-term AKT2 activation in hepatocytes through their derived endosomal PI(3,4)P2. Its deletion specifically inhibits glycogen synthase activity, reducing AKT2 activation without affecting insulin-dependent phosphorylation of AKT1 and S6K, impacting glucose homeostasis [96].

As known, angiogenesis is instrumental in the oncopathology, and current anti-cancer therapies often rely on anti-angiogenic treatment. Therefore, inhibiting PI3KC2α can slow down tumor growth. In addition, there is direct evidence that PI3KC2α non-kinase-dependently participates in the regulation of the tumor cell cycle, and its loss can lead to spindle changes causing genetic instability. This genetic instability initially impairs tumor growth but subsequently leads to fast-growing clones and increased sensitivity to anti-microtubule drugs such as taxanes [97]. It has also been shown that PI3KC2β is regulated by mi-362-5p, as well as being able to regulate the transcription factor Slug, which is engages in cancer cell's migration and invasion, and the overexpression of PI3KC2β has been seen in diverse cancers [98–100]. PI3KC2G may serve as an anti-tumor gene. Above all, Class II PI3K's effects in cancer remains to be studied.

Class III PI3K

Class III PI3K are a distinct class within the broader PI3K family that play crucial roles in various cellular processes. Unlike other PI3K classes primarily involved in signaling pathways linked to growth, survival, and proliferation, Class III PI3K are specifically engaged in intracellular membrane trafficking and autophagy.

The primary isoform of Class III PI3K is Vps34 (vacuolar protein sorting 34). Vps34 is unique among PI3Ks because it exclusively phosphorylates phosphatidylinositol to produce phosphatidylinositol 3-phosphate (PI3P). This product, PI3P, is pivotal in the formation of early endosomal structures and autophagosomes, marking cellular compartments that are crucial for protein sorting, recycling, and degradation processes. The activation of VPS34 is triggered by stimuli such as amino acids and glucose.

Vps34 functions within two distinct complexes: Complex I and Complex II. Complex I is primarily associated with autophagy and the production of PI3P, whereas Complex II plays a pivotal role in the endocytosis pathway, overseeing endosomal maturation and facilitating

the fusion of autophagosomes with late endosomes or lysosomes [14]. PI3P produced by Vps34 selectively binds and recruits effector proteins that contain FYVE and PX domains, facilitating their re-localization. This interaction is crucial for governing membrane docking and the formation of internal vesicles necessary for various cellular processes, including cycling, autophagy pathways, and fusion [101, 102]. Through these mechanisms, Vps34 regulates both autophagy and endo-lysosomal sortin. When PI3P binds the WIPI protein, downstream molecules are recruited to localize the autophagy marker LC3 to the expanded autophagosome membrane, an essential step for autophagosome formation [103]. Moreover, class III PI3Ks participate in regulating various signaling pathways. Upstream regulators of VPS34 include mTORC1 and the ULK1 complex. The ULK1 complex boosts the activity of the ATG14-containing VPS34 complex, regulating autophagy by phosphorylating Beclin1 Ser15 and directly phosphorylating ATG14 Ser29 [32, 104]. Under conditions of starvation relief (insulin or amino acid supplementation), VPS34 activity increases, positively regulating mTORC1 [105].

Class III PI3K take a dual part in tumorigenesis by regulating autophagy. Autophagy refers to the process of transporting cytoplasmic material of endogenous or exogenous origin to the lysosome for degradation, enabling the degradation and recycling of cellular components. While autophagy was originally identified as a cell survival mechanism, it plays emerging roles in mediating cell death called autophagy cell death (ACD), which defined as cell death with autophagic flux elevated and stoppable cell death under autophagy inhibition, unaccompanied the involvement of other types of programmed cell death [106].

In the initial stage of carcinogenesis, decreased autophagy levels lead to mitochondrial dysfunction, increased reactive oxygen species production, and DNA mutations, and affect cell signaling to promote tumor transformation [107]. These complex modulations exert the anti-tumor effect through autophagy, namely the eradication of cancer cells through ACD. On the other hand, autophagy can help cancer cells resist the adverse effects of metabolism and treatment, and is prominent in supporting tumor survival [108]. In conclusion, autophagy plays a multifaceted regulatory role in cancer, and class III PI3K are direct regulators.

Targeting PI3K family with small-molecule inhibitors in cancer

Class I PI3K inhibitors currently undergoing clinical trials for cancer therapy

Until January 1, 2024, the FDA has approved 80 small-molecule protein kinase inhibitors, 69 of which are utilized for treating oncological diseases [109]. These inhibitors have emerged as pivotal drugs in cancer treatment. For the sake of PI3K's essential role in cancer, its mutations, overexpression, and other genetic alterations have made it a promising drug target for cancer therapy. As mentioned earlier, class I PI3Ks in the PI3K family are most relevant to cancer, particularly PI3Kα and β. In contrast, PI3Kγ and δ are restricted to the hematopoietic system and mainly targeted for inflammatory and autoimmune diseases. The FDA has approved five classes of PI3K inhibitors, all of which are class I PI3K inhibitors (Table 1). These include the PI3Kα inhibitor Alpelisib for treating BC and the PI3Kδ inhibitors Umbralisib, Duvelisib, Copanlisib, and Idelalisib, approved for blood disorders.

Pan-PI3K Inhibitors

Pan-PI3K inhibitors (Table 2) refer to PI3K inhibitor target the four isoforms of class I PI3K (α, β, γ, and δ). However, their use is limited due to adverse effects and off-target effects.

Wortmannin and PX-866 Wortmannin and LY294002 were first-generation PI3K inhibitors that block the ATP-binding cleft of lipid kinases. Wortmannin, a natural furanosteroid metabolite, acted as a covalent nonspecific inhibitor of the PI3K family. **1** (PX-866) was a semi-synthetic derivative of wortmannin, exerting more selective for PI3K kinases than mTOR [175]. It was an orally available, irreversible pan-PI3K inhibitor [176]. Multiple experiments show that *PIK3CA* mutations or *PTEN* loss predict a positive response to **1**, while oncogenic Ras was a major indicator of resistance [177]. **1** has been approved for clinical trials, demonstrating good tolerability and potential efficacy in various cancer types. In terms of co-administration therapy, **1** reversed resistance to Epidermal growth factor receptor (EGFR) inhibitors, and increased the antitumor effect of cisplatin [177, 178].

BKM120/ Buparlisib The 6-hydroxyphenyl-2-morpholino pyrimidines series first reported as pan-PI3K inhibitor, **2** (BKM120) serves as a subsequent optimizer for C6 amino heterocycle modifications [179, 180].Through reversible inhibition of ATP, **2** served as an pan-PI3K inhibitor, exhibiting preferred inhibition of *PIK3CA* oncogenic mutation [180, 181]. A phase I dose-escalation study in advanced solid tumors identified **2** a well-tolerated profile [182]. However, other trials showed limited efficacy of PI3K pathway monotherapy in metastatic BC with *PIK3CA*/*AKT*/*PTEN* alterations [183]. In combination therapy, **2** with cetuximab (anti-EGFR) had potent antitumor effects in R/M SCCHN [184].

Table 1 Characteristics of clinically approved PI3K inhibitors

No	Drug	PI3K inhibitor class	PI3K selectivity (IC_{50}: nM)				Mechanism	Approved indication	Ref
			α	β	γ	δ			
1	Copanlisib	Pan-PI3K inhibitor	0.5	3.7	6.4	0.7	Forms four hydrogen bonds with the ATP pocket	Adult patients with relapsed follicular lymphoma after at least two prior therapies	[110]
2	Alpelisib	PI3kα inhibitor	4.6	1156	250	290	Occupy the ATP pocket and forms a hydrogen bond with PI3Kα specific residue Gln859	Combination therapy with fulvestrant to treat postmenopausal women, and men, with HR^+, $HER2^-$, *PIK3CA*-mutated, advanced or metastatic breast cancer following progression on or after treatment with an endocrine-based regimen	[8]
3	Idelalisib	PI3kδ inhibitor	820	565	89	2.5	Enter the ATP pocket of PI3Kδ with "propeller-shaped" and induce a specific pocket	R/R chronic lymphocytic leukemia in combination with rituximab in patients for whom rituximab alone would be an appropriate therapy due to other co-morbidities	[111]
4	Duvelisib	PI3kδ inhibitor	1602	85	27	2.5		Adult patients with R/R chronic lymphocytic leukemia / small lymphocytic lymphoma after at least two prior therapies	[112]
5	Umbralisib	PI3kδ inhibitor (also inhibits CK1ε with IC_{50} = 180 nM)	> 1500 -fold	> 1500 -fold	> 250 -fold	6.2		Adults with R/R marginal zone lymphoma who have received ≥ 1 prior anti-CD20-based regimen, and R/R follicular lymphoma who have received ≥ 3 prior lines of systemic therapy	[113]

Co-administration of **2** and Olaparib (PARP inhibitor) observed anticancer activity in patients with BC and OC [185]. BKM120 had manifest favorable antitumor activity in Kirsten rat sarcoma (*KRAS*)-mutant OC [186].

BAY 80–6946/Copanlisib **3** (BAY 80–6946) (Fig. 5A), a pan inhibitor, targeted the α and δ isoforms more selectively [187]. Its antiproliferative activity in BC cells was higher with *PIK3CA*-activating mutations or HER2 overexpression, suggesting predictive biomarkers [187]. *PTEN* loss may also increase its sensitivity [188]. In monotherapy, a phase I clinical trial identified **3** with excellent antitumor activity [189]. Another phase II clinical trial demonstrated a 59% objective response rate in relapsed/refractory(R/R)inert lymphoma [190]. Combination therapies showed **3** in combination with gemcitabine or gemcitabine plus cisplatin was overall well-tolerated, with early signals of benefit [191]. A phase III trial confirmed that **3** combined with rituximab (anti-CD20) improves PFS in relapsed indolent non-Hodgkin's lymphoma (iNHL) [116]. **3** is currently undergoing a phase III study comparing its treatment with a placebo in relapsed iNHL [192]. **3** is the only pan inhibitor approved by the FDA for treating recurrent FL [110].

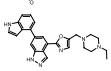
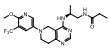
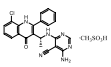
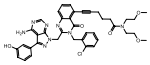
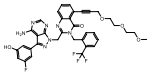
XL147/SAR245408/Pilaralisib **4** (XL147) is a reversible ATP-competitive pan-PI3K inhibitor derived from an optimized quinoxaline scaffold. Its treatment combined with blue light irradiation showed cooperative effects, showing **4** potentially as a photosensitizing reagent in photodynamic therapy for cancer [193]. The phase I study of **4** in advanced solid tumors and chronic lymphocytic leukemia (CLL) or R/R lymphoma demonstrated a favorable safety profile, pharmacodynamic effects, and antitumor activity of **4** [194–196]. However, the antitumor activity of **4** monotherapy and some combination therapies was inferior in phase II trials [117]. Another study of **4** in combination with HER3-Neutralizing Human Antibody SAR256212 for solid tumors showed that dual inhibition improves treatment efficacy [197].

GDC-0941/RG7321 /Pictilisib The development of **5** (GDC-0941) utilized the previous compound, and subsequent optimization involved retaining the morpholine moiety and replacing 3-hydroxyphenyl with 4-indazolyl to diminish glucuronidation and enhance oral bioavailability. Additionally, a sulfonylpiperazine group was introduced to improve biochemistry, cellular potency, and solubility [198]. **5** inhibited class I PI3K isoforms with similar selectivity [198]. Phase I human studies of **5** in

Table 2 Class I PI3K small-molecule inhibitors in clinical trials [8, 111–174]

No.	Drug	PI3K selectivity (IC_{50}, nM)				Diseases	Phase	NCT Number	Ref.
		α	β	γ	δ				
1	PX-866	5.5	>300	9.0	2.7	CRC, Head and neck squamous cell carcinoma (HNSCC), Prostate Cancer, Glioblastoma	II	NCT01259869	[114]
2	BKM120	52	166	262	116	BC, NSLCL, Prostate Cancer, Thymoma, CRC, Melanoma, Leukemia, Glioblastoma, Endometrial Cancer, Esophageal Cancer	III	NCT01610284	[115]
3	BAY80-6946	0.5	3.7	6.4	0.7	Lymphoma, NSCLC, T-Cell and NK-Cell Neoplasm, Thyroid Carcinoma, Endometrial Adenocarcinoma, BC, Cholangiocarcinoma, Primary Central Nervous System Lymphoma, Prostate Cancer, OC, HNSCC	III	NCT02369016	[116]
4	XL147	39	383	36	23	Endometrial Cancer, NSCLC, BC, Glioblastoma, Lymphoma	II	NCT01587040	[117]
5	GDC-0941	3	33	75	3	NSCLC, BC, HNSCC, Lymphoma, Glioblastoma	II	NCT01740336	[118]
6	CH5132799	14	129	599	36	Advanced Solid Malignancies	I	NCT01222546	[119]
7	ZSTK474	16	44	49	46	Advanced Solid Malignancies	I	NCT01682473	[120]
8	CUDC-907	5.4	19	54	39	Thyroid Neoplasms, BC, Lymphoma, Brain Tumor	II	NCT02674750	[121]
9	SF-1126	ND	ND	ND	ND	Neuroblastoma, Hepatocellular Carcinoma, Squamous Neck Cancer	II	NCT02644122	[122]
10	BEZ235	4	75	5	7	Renal Cancer, Pancreatic Neuroendocrine Tumors, Endometrial Cancer, Prostate Cancer, Perivascular Epithelioid Cell Tumors, Leukemia, BC, Glioblastoma	II	NCT01658436	[123]
11	BGT226	4	63	38	ND	BC	I/II	NCT00600275	[124]
12	LY3023414	6.07	77.6	23.8	38	Endometrial Cancer, BC, NSCLC, Prostate Cancer, Lymphoma	II	NCT02549989	[125]
13	GSK1059615	0.4	0.6	5	ND	Solid Tumours, Lymphoma	I	NCT00695448	[126, 127]
14	GSK2126458	0.019 (K_i)	0.13 (K_i)	0.06 (K_i)	0.024 (K_i)	Solid Tumours, Idiopathic Pulmonary Fibrosis	I	NCT00972686	[128]
15	XL765	39	110	9	43	NSCLC, BC, Glioblastoma, OC, Lymphoma,	II	NCT01587040	[129]
16	GDC-0980	5	27	1433	7	Endometrial Carcinoma, Lymphoma, BC, Renal Cell Carcinoma, Prostate Cancer	II	NCT01485861	[130]
17	PQR309	33	661	708	451	BC, HNSCC, Lymphoma, Advanced Solid Tumors	II	NCT03740100	[131]
18	PKI-587	0.4	6	6	8	BC, Leukemia, NSCLC, CRC, Endometrial Neoplasms,	III	NCT05501886	[132]
19	PKI-179	ND	ND	ND	ND	Advanced Solid Malignancies	I	NCT00997360	[133]
20	VS-5584	16	68	25	42	Lymphoma, Mesothelioma	I	NCT01991938	[134]
21	PF-04691502	1.2 (K_i)	2.1 (K_i)	1.6 (K_i)	1.9 (K_i)	Endometrial Neoplasms, BC	II	NCT01430585	[135]
22	GDC-0084	2 (K_i)	46 (K_i)	10 (K_i)	3 (K_i)	Lymphoma, Glioblastoma	II	NCT04906096	[136]
23	DS-7423	17	>1000	249	262	CRC, Endometrial Cancer	I	NCT01364844	[137]
24	P7170	2.2	ND	ND	ND	Advanced Refractory Solid Tumors	I	NCT01762410	[138]
25	PWT33597	19	ND	10-fold	10-fold	Advanced Malignancies	I	NCT01407380	[139]
26	BYL719	4.6	>1000	250	290	BC, Lymphangioma, Stomach Cancer, Meningioma, Endometrial Cancer, CRC, Esophageal Neoplasms, Oropharyngeal Squamous Cell Carcinoma, Melanoma, OC, HNSCC, OC	IV	NCT05631795	[8]
27	WX-037	ND				Advanced Solid Tumors	I	NCT01859351	[140]
28	GDC-0032	0.29 (K_i)	9.1 (K_i)	0.97 (K_i)	0.12 (K_i)	BC, Lymphoma, NSCLC	III	NCT02273973	[141]
29	GDC-0077	0.034 (K_i)	99.7 (K_i)	18.2 (K_i)	12.2 (K_i)	BC, NSCLC, CRC,	III	NCT05894239	[142]
30	TAK117	11.0±0.9	54-fold	36.5-fold	29.8-fold	BC, Endometrial Cancer, Lung Cancer, Endometrial Neoplasms, Gastric or Gastroesophageal Adenocarcinoma, Renal Cell Carcinoma	II	NCT02725268	[143]
31	CYH33	5.9	≥10-fold			OC, BC	II	NCT05043922	[144]
32	ASN003	ND				Melanoma, Colorectal Neoplasm	I	NCT02961283	[145]
33	RLY-2608	H1047R = 4 WT = 48 ± 17				Advanced cancers harboring PIK3CA mutations	I	NCT05216432	[146]
34	LOXO-783	ND	H1047R <5 WT>250 (EC_{50})	>250(EC_{50})		Advanced cancers harboring PIK3CA mutations	I	NCT05307705	[147]
35	STX-478	H1047R =9.4 E542K=113 E545K=71 WT=131	>9000			Advanced cancers harboring PIK3CA mutations	I/II	NCT05768139	[148]
36	GSK2636771	1000-fold	5.2	1000-fold	10-fold	Melanoma, Lymphoma, Gastric Adenocarcinoma, Hematopoietic Cell Neoplasm	II	NCT02615730	[149]
37	SAR260301	>1000	23	>1000	468	Advanced Malignancies	I	NCT01673737	[150]
38	AZD6482	86-fold	10	8-fold	108-fold	Antiplatelet Effect	I	NCT00853450	[151]
39	AZD8186	35	4	675	12	Stomach Cancer, Prostate Cancer, NSCLC, BC,	II	NCT04001569	[152]
40	KA2237	ND	19	ND	8	Lymphoma	I	NCT02679196	[153]
41	TG100-115	>1000		83	235	Myocardial Infarction	I/II	NCT00103350	[154]
42	IPI549	>2000-fold		16	>2000-fold	Urothelial Carcinoma, Bladder Cancer, BC, Renal Cell Carcinoma, NSCLC, Melanoma	II	NCT03980041	[155]

Table 2 (continued)

43	AMG319		>2000		850	18	Lymphoid Malignancy, HNSCC	II	NCT02540928	[156]
44	CAL-101		820	565	89	2.5	Leukemia, NSCLC, Lymphoma, Pancreatic Ductal Adenocarcinoma	IV	NCT02739360	[111]
45	TGR-1202		>10000(K_d)		>1000 (K_d)	6.2 (K_d)	Leukemia, Lymphoma,	III	NCT02793583	[113]
46	IPI-145		>1000	85	27	2.5	Leukemia, SCCHN, Myeloma, Lymphoma, Melanoma	III	NCT02049515	[112]
47	RP6530		>300-fold	>100-fold	33	25	Leukemia, Lymphoma, BC	II	NCT04204057	[157]
48	GS-9820		>5000	>3000	>1000	12.7	Lymphoid Malignancies	I	NCT01705847	[158]
49	INCB040093		>20000	>3000	>2000	31	Lymphoma	II	NCT02456675	[159]
50	INCB050465		>20000		>10000	1.1	Lymphoma, CRC, Endometrial Cancer, Melanoma, BC	III	NCT04551066	[160]
51	GSK2269557		>1000-fold			ND	Pulmonary Disease, APDS	II	NCT02593539	[161]
52	GSK2292767		>100-fold			10.1 (pK)	Asthma	I	NCT03045887	[161]
53	CDZ173		244	424	>2000	11	Activated PI3Kdelta Syndrome (APDS) / PASLI	III	NCT02435173	[162]
54	YY-20394		252-fold	30-fold	>1000-fold	4.6	Lymphoma	II	NCT06158386	[163]
55	ME-401		>2000-fold	30-fold	700-fold	ND	Lymphoma	III	NCT04745832	[164]
56	UCB5857		>3000	>2000	282	12	Autoimmune diseases	II	NCT02610543	[165]
57	GS-9901		750	100	190	1	Leukemia, Lymphoma	I	NCT02258555	[166]
58	BGB-10188	ND	>3000-fold			ND	Leukemia, Lymphoma	I/II	NCT04282018	[167]
59	SHC014748M		236	96	101	0.77	Lymphoma, Leukemia	II	NCT04470141	[168]
60	RV1729		16-fold	ND	2-fold	12	Asthma, COPD	I	NCT01813084	[169]
61	RV6153		>10000	>4000	28	2.5	Asthma	I	NCT02517359	[170]
62	HMPL-689		ND				Lymphoma	II	NCT05713110	[171]
63	AZD8835		6.2	431	90	5.7	BC	I	NCT02260661	[172]
64	TQ-B3525	ND	ND				Lymphoma, NSCLC, BC, Endometrial Cancer, Cervical Cancer, OC	II	NCT05284994	[173]
65	XC-302	ND	992.8	959.2	89.8	3.3	Lymphoma	II	ND	[174]

advanced solid tumors demonstrated targeted pharmacodynamic activity and antitumor efficacy, with a recommended phase II dose (RP2D) of 330 mg daily [199]. A phase Ib trial in locally recurrent or metastatic BC demonstrated a manageable safety and antitumor activity of **5** at a dose of 260 mg in combination with paclitaxel, bevacizumab (anti-VEGF), trastuzumab (anti-HER2), or letrozole [200]. Similarly, in a phase Ib study, **5** combined with various standard first-line treatment regimens in advanced NSCLC has observed safety feasibility and preliminary antitumor activity [201]. However, possibly due to toxicity limitation dose, **5** did not substantially enhance PFS rates in a phase II trial of BC [118].

CH5132799/PA-79/ Izorlisib **6** (CH5132799)was synthesized employing a structure-based drug design approach, utilizing a homology model of PI3Kα for molecular design [202]. In preclinical models, **6** either alone or in combination, exhibited sensitivity to the *PIK3CA* mutation in vitro kinase assays, in vitro tumors, and in in vivo mouse xenograft model [203]. The first-in-human study of **6** identified 48 mg twice daily as RP2D. At this dosage, the drug demonstrated favorable tolerability and clinical activity, characterized by low toxicity [119]. Moreover, it held potential for combination therapies with other targeted approaches.

ZSTK474 **7** (ZSTK474) initially categorized as a class of morpholino triazine derivatives, emerged as a promising antitumor candidate. Its identification as a PI3K inhibitor stemmed due to the resemblance of its fingerprinting profile to that of LY294002 [204]. **7** that can significantly lower mTOR inhibitory activity than its PI3K activity [205]. In vitro studies demonstrated **7**'s potent activity against both hotspot mutants (E542K, E545K, H1047R) and wild-type PI3Kα [206]. An intriguing revelation was that compared to radiation or drug therapy alone the combination of X-rays and **7** had greater therapeutic potential [207]. Currently, **7** has completed phase I clinical trial as monotherapy for the treatment of advanced solid tumors, along with an oral safety study [120].

CUDC-907/Fimepinostat **8** (CUDC-907) was an oral dual inhibitor for both PI3K and histone deacetylase (HDAC). It was formed by the binding of the HDAC inhibitor function (isohydroxamic acid) to the PI3K inhibitor core scaffold (morpholino-pyrimidine) [208]. As a single agent, **8** has exhibited a manageable side effect profile and sustained clinical efficacy in R/R diffuse large B-cell lymphoma (DLBCL) [209]. Building on this, an expanded phase I trial demonstrated that **8**,

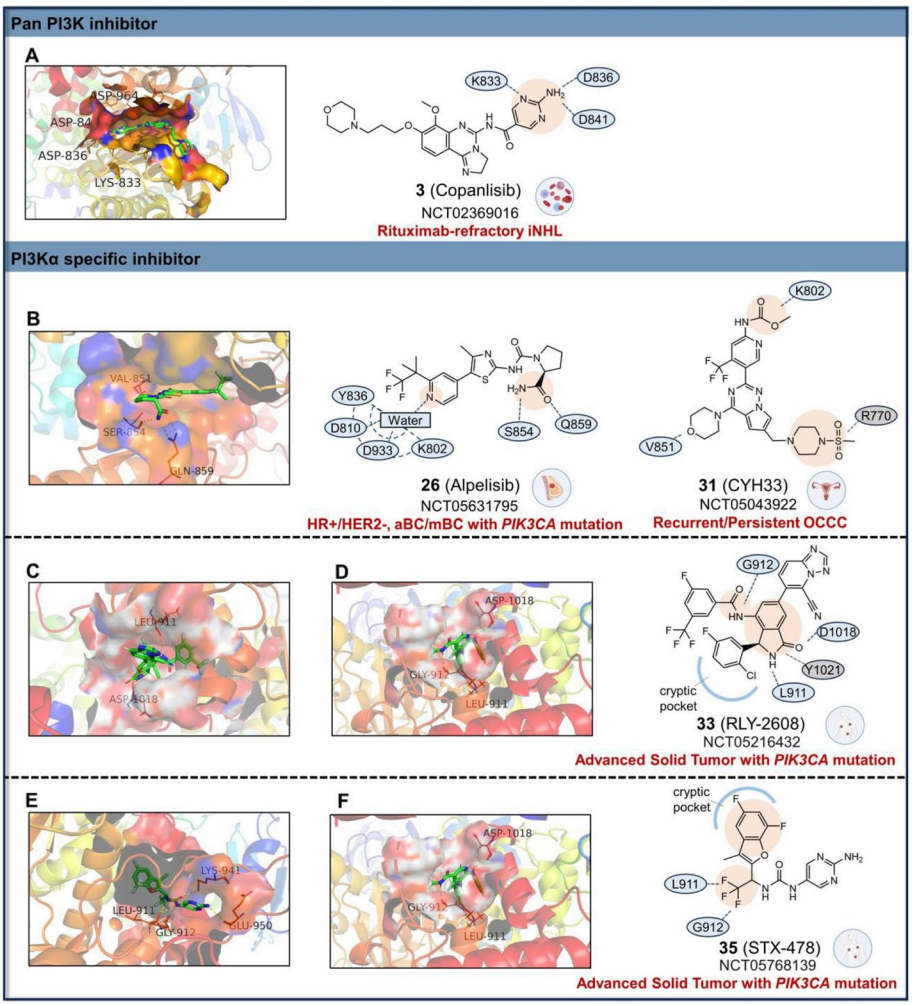


Fig. 5 The representative Class I pan PI3K inhibitors and PI3Kα specific inhibitors in clinical trials. The key interactions of the inhibitor with residues within the ATP pocket are labeled. **A** X-ray co-crystal structure of PI3Kγ in complex with 3 (PDB ID: 5G2N). **B** X-ray co-crystal structure of PI3Kα in complex with 26 (PDB ID: 4JPS). **C** X-ray co-crystal structure of WT PI3Kα in complex with 33 (PDB ID: 8TSD). **D** X-ray co-crystal structure of 33 homologous series of compounds in complex with H1047R PI3Kα (PDB ID: 8TS9). **E** X-ray co-crystal structure of WT PI3Kα in complex with 35 (PDB ID: 8TDU). **F** X-ray co-crystal structure of H1047R PI3Kα in complex with 35 (PDB ID: 8TGD)

either as monotherapy or in combination with rituximab, exhibited a similar safety profile and more promising anti-tumor activity in R/R DLBCL patients with MYC alterations [210]. Another phase II study supported MYC alteration as a predictive biomarker for response of **8** [121].Additionally, **8** has accomplished a separate phase II trial in metastatic and locally advanced thyroid cancer (NCT03002623).

Dual PI3K/mTOR inhibitors

Dual PI3K/mTOR inhibitors, specifically referring inhibitors that simultaneously target both the PI3K and mTOR families. In the PI3K pathway, mTOR inhibition contributes significantly to antitumor effects, as many of the AKT-mediated effects are mediated through mTOR targets. However, inhibiting the mTOR family in isolation may result in enhanced activation of the PI3K axis due to associated negative feedback regulation, such as p70S6K phosphorylation [211]. Dual PI3K and mTOR inhibitors can comprehensively inhibit AKT activation, exerting a broad spectrum of anti-tumor effects. However, the unpredictability of corresponding side effects and toxicity was acknowledged.

LY294002 and SF-1126 LY294002, a first-generation PI3K inhibitor, was a quercetin analog that effectively and reversibly inhibits the PI3K family in an ATP-competitive mode. However, its preclinical use was restricted due to insolubility and significant toxicity [212]. **9** (SF-1126), a

water-soluble prodrug derived from combining Arg-Gly-Asp-Ser with LY294002, binds to specific integrins in the tumor microenvironment, enhancing antitumor activity [213]. **9** has completed phase I clinical trials demonstrating good tolerability and confirmed inhibition of targeted pathways [122, 214, 215]. The phase I Expansion Study of **9** in R/R Myeloma is currently ongoing [216].

LY3023414 /Samotolisib **12** (LY3023414), exerted inhibitory impact on mTOR with IC_{50} of 165 nM. In the first-in-human phase I study of advanced cancer, **12** as single agent established a tolerable safety profile and moderate activity, identifying RP2D of 200 mg twice daily as monotherapy [217]. Results from the phase II study of combination therapy with Necitumumab and **12** in NSCLC aligned with preclinical studies, indicating safety and tolerability in patients without undue overlapping toxicity [218]. Promising combination efficacy was also observed in clinical studies of **12** with Abemaciclib (CDK4/6 inhibitor), fulvestrant, and other drugs [219, 220].However, clinical studies in combination with the crenigacestat (Notch inhibitor) were terminated due to disappointing tolerability and clinical activity [221].

GSK2126458/GSK458/Omipalisib The development strategy for **14** (GSK2126458) originated from the binding crystal structure of the thiazolidinedione ring to the ATP-binding pocket in PI3Kγ, where observed potential to accommodate a larger moiety, prompting efforts to fill the vacant space in the enzyme pocket for a more potent and selective inhibitor [222] .**14** demonstrated inhibition of mTORC1 and mTORC2 with apparent K_i values of 0.18 and 0.3 nM [222]. In the first human phase I trial, **14** exhibited a well-tolerated MTD of 2.5 mg qd, but limited single-dose activity. Its drug exposure can be evaluated by fast insulin and glucose levels [128]. The combination therapy of GSK458 with the MEK inhibitor demonstrated insufficient tolerability and limited antitumor activity [223].

XL765/SAR245409/Voxtalisib **15** (XL765) is homologous to **4**.**15** displayed potent inhibitory activity against the mTOR family, suppressing mTORC1 and mTORC2 with IC_{50} values of 150 and 910 nM [224]. In preclinical models, 2HG may serve as a potential non-invasive MRS-detectable metabolic biomarker of XL765 ‘s [223]. Phase I clinical trials in advanced solid tumors showed XL765 a manageable safety profile and efficacy with pathway signaling inhibition [225, 226]. In area of hematologic malignancy, XL765 demonstrated efficacy primarily in FL cases [227]. In combination therapies, **15** with erlotinib (EGFR inhibitor) and MEK inhibitor pimasertib, as well as letrozole lacked significant drug synergism and demonstrated limited clinical efficacy [129, 228, 229]. Conversely, in high-grade gliomas, voxtalisib together with TMZ with or without RT showed an acceptable safety profile and moderate pathway inhibition [230]. The combination of voxtalisib with rituximab in malignant lymphomas also demonstrated preliminary antitumor activity [231].

GDC-0980/Apitolisib The initial discovery of the first 2-aminopyrimidinyl-thienopyrimidine 3 and its 7-methyl analogues paved the way for subsequent optimization efforts. **16** (GDC-0980) represented the optimized compound with enhanced potency and solubility [232] .**16** has a Ki of 17 nM for mTOR kinase [233]. Multiple clinical trials have been conducted with **16**, and its phase I trial in advanced solid tumors established a MTD of 70mg qd, with observed anti-tumor activity [234]. Subsequent phase I trials demonstrated dose-dependent pharmacokinetics and targeting activity at doses ≥ 16 mg, recommending a RP2D of 40 mg daily [235]. However, another randomized phase II study showed dual-targeted **16** did not show better efficacy than the mTORC1 inhibitor everolimus [130]. GDC-0980 together with capecitabine and mFOLFOX6 + bevacizumab evaluated well toleration and favorable preliminary activity in advanced CRC [236]. Preclinical models indicated that oncogenic *PIK3CA* gene mutations and HER2 gene amplifications increased sensitivity to **16**, while hotspot mutations within *BRAF* or *RAS* were negative predictors of its potency [233, 237]. Clinical trials also suggest that **16** may have a stronger benefit in patients with mutations in the PI3K pathway [238, 239].

PKI-587/PF-05212384/ gedatolisib, PKI-179 The initial triazolopyrimidine scaffolds emerged from substituting the imidazole ring with imidazopyridine. The subsequent findings demonstrated that incorporating urea appendages to the pyrazolopyrimidine core not only enhanced potency against PI3Kα and mTOR but also increased metabolic stability [240]. In further optimization, **18** (PKI-587) with bimorpholine-1,3,5-triazine scaffold could prevent diminished potency from the metabolic oxidation of mono-morpholine, showcasing the optimal efficiency [241]. **18** (PKI-587) manifests dual inhibitor of PI3K and mTOR(IC_{50} for mTOR = 1nM) [241, 242]. Preclinical studies showcased **18** synergizes radiation therapy to increase the radiosensitivity [243]. In Early clinical studies,**18** demonstrated promising monotherapy prospects. Combination trials with paclitaxel and carboplatin, as well as other agents, demonstrated safety and antitumor activity [244, 245]. A phase III trial of Gedatolisib in advanced BC is ongoing (NCT05501886,

CTRI/2023/08/056738). *PTEN* deficiency status may correlate with treatment response [246]. To address **18**'s intravenous administration limitation, **19** (PKI-179) derived from PKI-587, exhibited excellent permeability enabling expansive administration routes [133]. **19** was previously underwent a phase I clinical trial in advanced malignant solid tumors.

VS-5584/SB2343 Poulsen et al. undertook the refinement of three scaffolds utilizing PI-103, LY294002, and ZSTK474 as reference compounds, with the purine scaffold identified for subsequent optimization. This optimization led to the development of the potent dual inhibitor **20** (VS-5584 [247]. **20** demonstrated equally potent activity against mTOR (IC_{50} = 37 nmol/L) and all class I PI3K isoforms [134]. **20** exhibited high antiproliferative efficacy across 51 cancer cell lines, encompassing both blood and solid tumors [134]. Notably, it displayed a preference for targeting cancer stem cells, impeding tumor regeneration in a small-cell lung xenograft model [248]. Preclinical models indicated that mutations in *PIK3CA* and EZH2 were associated with drug sensitivity, while *APC*, *MYCL1*, or *MYCN* were linked to drug resistance [134]. **20** is presently undergoing phase I trials in advanced non-hematologic malignancies or lymphoma (NCT01991938) and together with VS-6063 (FAK inhibitor) for the treatment of recurrent malignant mesothelioma (NCT02372227).

GDC-0084/RG766/Paxalisib **22** (GDC-0084) was a derivative resulting from the optimization of pharmacodynamic structures based on purine scaffolds, aiming to develop PI3K inhibitors with favorable blood–brain barrier permeability. **22** exhibited increased potency against mTOR (K_i = 70nM) [249]. The initial human phase I study of **22** indicated good tolerability and central nervous system permeability, with a MTD of 45 mg daily [136]. Biomarker exploration revealed that a composite biomarker generated by multiparametric MR-PET imaging could characterize **22** pharmacokinetics and predict PFS in recurrent high-grade gliomas, offering potential utility in larger clinical studies [250].

DS-7423 **23** (DS-7423) was an orally bioavailable and brain-permeable dual PI3K/mTOR inhibitor. It effectively inhibits mTOR with an IC_{50} of 34.9 nM [251]. As a single agent, **23** has exhibited preferential growth inhibition of PI3K-mutant and *PTEN*-deficient cells [251]. **23** has completed phase I clinical trial (NCT01364844), and its first-in-human trial conducted in parallel in the U.S. and Japan in advanced solid tumors demonstrated a MTD and RP2D of 240 mg/d [137].

P7170 **24** (P7170) was a small-molecule inhibitor targeting PI3K/mTOR/ALK1(IC_{50} for mTOR = 4.4 nM) [138]. In the context of NSCLC with Kras mutation, **24** had shown significant tumor suppressor effects [252]. The compound had progressed to phase I clinical study, involving advanced refractory solid tumors (NCT01762410).

PWT33597/ VDC-597 **25** (PWT33597) was originated from the pan inhibitor ZSTK47 with ameliorated PI3Kα selectivity, solubility and metabolic stability. Biochemical assays revealed that **25** effectively inhibited mTOR(IC_{50} = 14nM) [139] 0.2**5** has successfully undergone a phase I clinical trial (NCT01407380), focusing on late-stage malignant tumors.

Isoform-specific PI3K inhibitors

The four class I PI3K isoforms play distinct roles in oncology, with PI3Kα-selective inhibitors exhibiting a more pronounced therapeutic impact on genetic alterations of the *PIK3CA* gene. In contrast, *PTEN*-deficient tumors are characterized by PI3Kβ-driven growth and survival [75, 253], PI3Kβ-selective inhibitors demonstrate promising therapeutic benefits. The distributional characteristics of PI3Kδ and γ isoforms make PI3Kδ inhibitors commonly used in immune system-related tumors like lymphomas and B-cell malignancies, while PI3Kγ inhibitors find more extensive use in inflammation and are less commonly applied in oncology [254, 255]. However, the catalytic regions of class I PI3Ks share high sequence homology and the topology of their ATP-binding sites are conserved, posing a significant challenge in designing subtype-selective inhibitors.

PI3Kα inhibitor **BYL719/ Alpelisib**

The 2-aminothiazole scaffold initially emerged as a template for PI3K isoform-selective compounds. Subsequent findings revealed that incorporating (S)-pyrrolidine carboxamide molecules via a urea bond to the 2-amino group imparted alpha isoform selectivity to this class of PI3K inhibitors. **26** (BYL719) was ultimately identified for its overall favorable characterization [256].

26 (Fig. 5B) was characterized by the specificity of PI3Kα isoform. In the eutectic binding mode of **26** to PI3Kα, its pyridine nitrogen atom engaged in a network of hydrogen bonds with three water molecules and the side chains of residues Y836, D810, D933, and K802. A notable donor–acceptor hydrogen bond pair was observed between the amide group and the Q859 side

chain, indicating an interaction specific to the PI3Kα isoform [256].Preclinical models and clinical trials supported *PIK3CA* mutation as a biomarker predicting the clinical efficacy of Alpelisib [257]. **26**, along with various agents, is undergoing clinical trials in different cancers. Additionally, **26** is ongoing related phase IV clinical trials (NCT05631795).

WX-037

27(WX-037) was a class I PI3K inhibitor developed from the indole series. In preclinical studies, **27** exhibited increased sensitivity in cells and tumors with *PIK3CA* mutations or *PTEN* loss [140]. However, its phase I trial in solid tumors as a single agent and in combination with WX-554 (MEK inhibitor) was terminated for commercial reasons (NCT01859351).

GDC-0032/RG-7604/Taselisib, GDC-0077/Inavolisib

28 (GDC-0032) was developed based on a high throughput screening of a central benzoxepin scaffold [258]. **28** utilize ubiquitination to degrade target protein. PI3Kα binding to the receptor complex promotes conformational changes of the α isoform, exposing the ubiquitination site of p110α in the membrane, leading to selective degradation of p110α [144]. Notably, oncoproteins with p110α mutations are more susceptible to proteasome-mediated degradation upon **28**'s action, without affecting the level of wild-type p110α.This provided a mechanistic basis for its application in tumors with *PIK3CA* mutations [258]. Unlike **26**, there was no clear guidance on the efficacy of taselisib in *PIK3CA*-mutant solid tumors. Clinical trials had shown limited activity of taselisib as monotherapy, and the presence of *PIK3CA* mutations alone did not consistently predict taselisib activity [259–261]. However, some studies suggested that *PIK3CA* mutations make tumors more sensitive to taselisib, there was no definitive answer [262, 263]. In combination therapy, the POSEIDON phase Ib clinical trial demonstrated that taselisib can be safely used combined with endocrine therapy drugs synergistically in corresponding tumors [263–265]. However, its combination with docetaxel or paclitaxel failed to pass the safety test and were unsuitable for further development [266]. The outcome of **28** combination therapies in a randomized phase III study of BC showed no clinical benefit [141]. **29** (GDC-0077), developed through structure-based design and optimization based on **28** [267]. Identical to taselisib, **29** could induce the degradation of p110α mutants via the proteasome. This degradation was primarily mediated through RTKs recruited by p110α/p85β. Notably, low RTK activity may reduce the efficiency of degradation, and HER2-positive cancers may benefit more from this type of degradation inhibitor [268]. Inavolisib had entered a clinical phase III trial. Its combination therapy with fulvestrant has shown controlled safety and preliminary activity in phase I/Ib study involving *PIK3CA*-mutated $HR^+/HER2^-$ metastatic BC [142].

TAK-117/MLN1117/INK1117/Serabelisib

30 (TAK-117), developed by Takeda, had entered clinical phase II trials, and its first-in-human phase I study in advanced malignancies revealed the limiting potential of monotherapy. Intermittent dosing, rather than continuous dosing, has been suggested to support combinations with other antitumor agents [269]. Another phase I study in advanced solid tumors demonstrated that the combination of **30** with the sapanisertib (mTOR inhibitor) and paclitaxel was well-tolerated and manifested initial significant efficacy in patients with abnormalities in the PI3K pathway [270]. However, this combination was poorly tolerated and less effective than everolimus (mTOR inhibitor) in advanced kidney cancer in another randomized phase II trial [143].

CYH33

31 (CYH33) belongs to a class of pyrrolobenzotriazine analogs developed through structural modifications of the lead compound, which originated from the scaffold hopping strategy of PI-103 [271]. The design process was inspired by the segments of GDC-0941 and BKM120. The binding mode of **31** (CYH33) was not only based on typical ATP competitive inhibitors, but also enhances its interaction with proteins due to its morpholine ring formed a hydrogen-bonding interaction with Val851, anchoring the central scaffold in a position similar to the covalent ligand **68** (CNX-1351) resulting in high protein affinity [271]. **31** had entered phase II clinical trials, following the first human phase Ia study in patients with solid tumors. With recommended MTD and RP2D of 40 mg qd, **31** observed noted antitumor efficacy in solid tumors with *PIK3CA* mutations [144]. Ongoing clinical trials are seeking combination therapies, including **31** in combination with olaparib in advanced solid tumors (NCT04586335) and together with endocrine therapy for advanced HR + and HER2- BC (NCT04856371).

ASN003

32 (ASN003) was an inhibitor targeting both the RAS-RAF and PI3K pathways [272]. Preclinical studies with **32** have demonstrated favorable antiproliferative activity in

tumor models characterized by mutations in the B-RAF and PI3K pathways [272]. **32** has progressed to phase I clinical trial development. The phase I pharmacokinetics/pharmacodynamics (PK/PD) study in advanced solid tumors has reported pleasant tolerability and systemic exposure.

RLY-2608

Varkaris et al. utilized CryoEM and MD simulations to unveil dynamic distinctions in the tail conformation of WT and H1047R PI3Kα [146]. Following this, a drug screen, utilizing free energy in a DNA-encoded library and perturbation calculations, coupled with X-ray structures of compounds bound to WT and H1047R, identified **33** (RLY-2608). **33** was a first-in-class allosteric mutant-selective inhibitor of PI3Kα. The inhibition of PI3Kα H1047R by **33** was not ATP-competitive and its inhibition was not only 12-fold more selective for PI3Kα H1047R than for WT, but it was also highly selective for PI3K isoforms [146].The improved affinity may result from more efficient filling of the core and pocket space, more extensive Y1021 interactions, stronger D1018 backbone hydrogen bond formation, and improved stacking of the hydrophobic pockets where phenyl resides, whereas the mutation selectivity was explained by the energetic coupling of tail detachment and cryptic pocket(Fig. 5C and D) [146]. In WT p110α, the burial of the C-terminal tail constrains the conformations thus preventing ligand to enter the cryptic pocket, while the H1047R mutation was conducive to the detachment state of the C-terminal tail and deranged corresponding conformations, increasing sampling of a ligand-accessible cryptic site. **33** overcame the constraints of orthosteric PI3Kα inhibitors like most prominently drawback hyperglycemia. It exhibited anti-tail and helical structural domain mutation activity in cells and inhibited tumor growth in *PIK3CA* mutant xenograft model with negligible impact on insulin [146]. In addition, **33** produced objective tumor responses in two patients with advanced HR^+BC diagnosed with *PIK3CA* mutations, while no WT PI3Kα-related toxicity observed [146]. **33** had entered clinical trials and is enrolling in its first-in-human study as a single agent or combination therapy with Fulvestrant for the treatment of advanced solid tumors (NCT05216432).

LOXO-783/LOX-22783

34 (LOX-22783) is a potent mutation-selective inhibitor targeting the H1047R mutation of PI3Kα [147]. **34** demonstrates remarkable antitumor effects in H1047R-driven preclinical models of BC without inducing hyperglycemia or other common toxicities associated with broader PI3K inhibitors [273]. In addition to its efficacy and safety, **34** exhibits high oral bioavailability and the ability to cross the blood–brain barrier, expanding its potential use in treating cancers within the central nervous system. The compound shows promise not only when used alone but also in combination with standard-of-care treatments. In both HR^+ and triple-negative breast cancer (TNBC) harboring PI3Kα H1047R mutations, **34** synergistically enhances the anti-tumor effects of existing therapies. Currently, **34** and its combination therapy are undergoing evaluation in a phase 1 trial (PIKASSO-01, NCT05307705).

STX-478

A comprehensive hit-finding strategy identified **35** (STX-478) as a mutation-selective variant PI3Kα inhibitor. **35** selectively inhibit all mutant forms of the PI3Kα kinase domain, including the common variant H1047R, with selectivity over WT PI3Kαby 14-fold, and weakly against E542K and E545K helical structural domain mutants [148]. Through co-crystal binding modeling, it was revealed that **35** conflicts with residues F937 and L938, leading to the re-localization of both residues, contributing to the conformational transition of residues 936–940 and creating space for **35** to occupy the cryptic allosteric site of action. The binding modeling also showed the urea forms a bifurcated hydrogen bond with the L911 and a suboptimal hydrogen bond with the G912(Fig. 5E and F) [148]. Although X-ray crystallography did not display differential binding to WT and mutant PI3Kα, the results from Surface Plasmon Resonance assays indicate superior accessibility and binding affinity in the mutant form [148]. **35** alone exerted excellent antitumor efficacy in preclinical models of tumors harboring PI3Kα mutations without insulin resistance, and it achieves durable and stable tumor regression in combination with fulvestrant and/or cyclin-dependent kinase (CDK) 4/6 inhibitors [274]. **35** is undergoing phase I/II clinical trials as a monotherapy or in combination therapy (NCT05768139).

PI3Kβ inhibitor GSK2636771

36 (GSK2636771) was developed by the structural optimization of **69** (TGX-221) [275]. Crystallographic studies of PI3Kβ revealed that the binding site Y778, a specific tyrosine residue of p110β, played a crucial role in inhibitor binding(Fig. 6) [276]. **36** had progressed to phase II clinical trials for advanced tumors with *PTEN* loss. The initial single-agent study recommended a dose of 400 mg qd, demonstrating a manageable safety profile and target-related clinical benefit with a genomic association

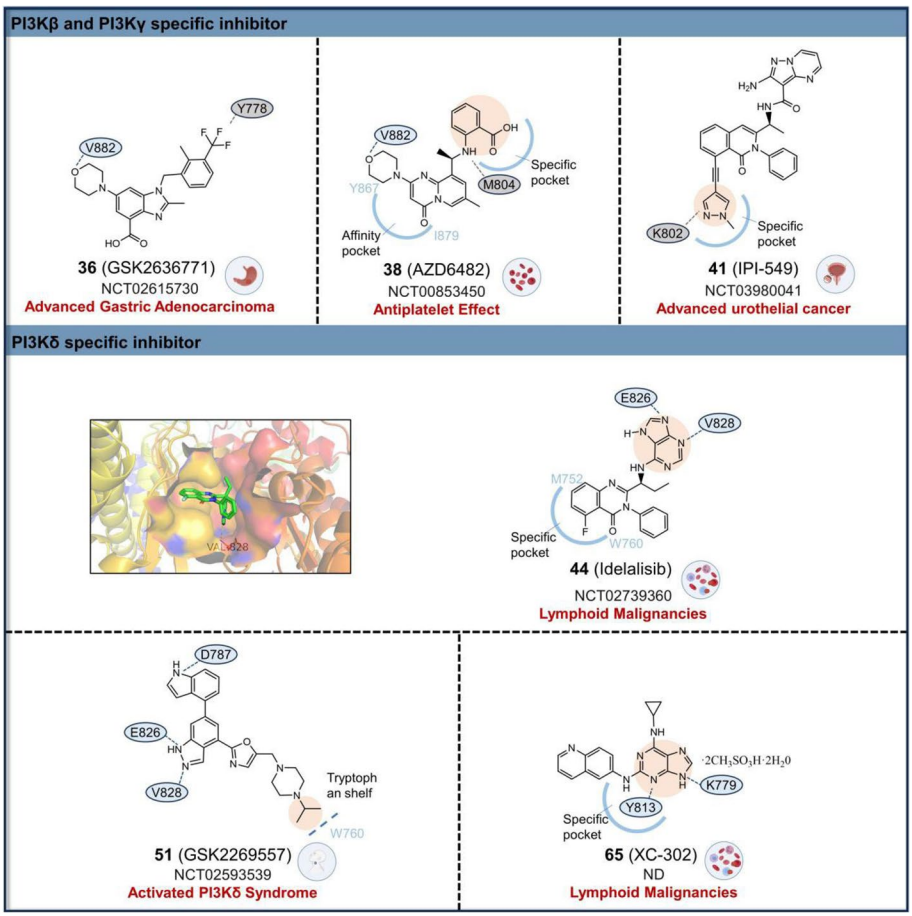


Fig. 6 The representative PI3K isoform specific inhibitors in clinical trials. X-ray co-crystal structure of PI3Kδ in complex with 44 (PDB ID: 4XE0)

to *PIK3CB* [275]. Combination therapies, such as **36** with Enzalutamide, pembrolizumab (anti-PD1) and paclitaxel demonstrated tolerability but limited antitumor activity. Clinical trial data suggests an association between *PTEN* loss and clinical benefit from **36** [275, 277]. Additionally, preclinical studies have proposed a germline PIK3R1 variant (M326I) as a potential genetic biomarker, in combination with *PTEN* loss, to predict **36** efficacy [278].

AZD6482/KIN193

38 (AZD6482) shares a chemical structure similar to **69** (TGX-221). characterized as a PI3Kβ inhibitor [151]. The pyrimidinone core of **38** was positioned tightly against the side chains of Y867 and I879 in the 'affinity pocket' of PI3Kγ. The anthranilic side chain induces the so-called PI3Kγ 'specificity' pocket, which was absent in the apo enzyme, mainly by shifting the side chain of M804. This structural interaction contributed to the high potency of **38** against PI3Kγ [151]. **38** had demonstrated anti-tumor efficacy in various human preclinical models and exhibited potent inhibition in *PTEN*-deficient cancer cells. Apart from its inhibitory effect on the PI3K pathway, **38** had been found to possess systemic antiplatelet effects, suggesting its potential as an antiplatelet target [151]. As of the provided information, **38** is undergoing phase I clinical trials, with a primary focus on its antiplatelet properties.

AZD8186

39 (AZD8186),developed through the optimization to improve solubility and metabolic stability, exhibited a preference for inhibiting the PI3Kβ and δ isoforms [279]. Beyond regulating the PI3K pathway, **39** also inhibited enzymes in the cholesterol biosynthesis pathway and alters metabolites in *PTEN*-deficient models of various tumors [280]. **39** had progressed to phase II clinical studies, and its phase I study in advanced solid tumors had shown an acceptable safety and tolerability profile, both as a single agent and along with acetate/prednisone or vistusertib (mTOR inhibitor) [152]. However, a phase Ib/

II study in metastatic renal cell carcinoma with *PTEN* deletion or abnormal *PTEN*/*PIK3CB* genes did not show additional benefit with the combination therapy of **39** and paclitaxel [281]. It was noted that, apart from the effect of *PTEN* deficiency on the potency of PI3Kβ inhibitors, *PIK3CB* activating mutations have the potential to be biomarkers for predicting susceptibility to **39** [281].

KA2237

40 (KA2237) was an orally active and selective p110β/δ inhibitor [282]. Clinical studies of **40** in patients with lymphoma have been conducted, and preliminary results from its first-human phase I study recommend RP2D of 200 mg daily. In R/R B-cell lymphoma, **40** had demonstrated manageable toxicity and promising single-agent clinical activity [153].

PI3Kγ inhibitor **IPI-549/Eganelisib**

The identification of **42** (IPI-549) was aided by the success of **46** (IPI-145), subsequent work found that alkyne substitution was able to differentiate between γ and δ isoforms at non-conserved residues (Lys802 of PI3K-γ, Thr750 of PI3K-δ) and that less hydrophobic moieties were able to maintain a good selectivity for PI3Kγ [283]. **42** had been demonstrated to modulate the tumor immune microenvironment, enhancing antitumor immunity [284]. **42** 's monotherapy and combination therapy with the PD-1 inhibitor nivolumab showed good tolerability and early clinical activity [285]. Preliminary analysis of a phase II trial in patients with advanced urothelial carcinoma indicated that the combination of **42** with nivolumab improved overall response rate (ORR) and PFS compared to nivolumab monotherapy [155]. Additionally, a preliminary phase II study of **42** in combination with atezolizumab (anti-PD1) and nab-paclitaxel for the treatment of TNBC demonstrated manageable toxicity and promising antitumor activity [286].

PI3Kδ inhibitor **CAL-101 /GS-1101/Zydelig/ Idelalisib**

44(CAL-101) became the first FDA-approved PI3K inhibitor. The X-ray binding structure of **44** to p110δ revealed a "propeller-shaped" conformation, inducing a conformational change in the ATP-binding pocket and forming a specific hydrophobic pocket between Trp760 and Met752, conferring selectivity to PI3Kδ [287]. **44**'s monotherapy had shown modest anti-tumor activity in various lymphoid cancers. Its role in B-cell malignancies shifted the focus of PI3Kδ inhibitors from immune disorders to hematologic cancers. Clinical trials combining **44** with the bruton tyrosine kinase(BTK) inhibitor tirabrutinib and the anti-CD20 antibody ofatumumab demonstrated synergistic antitumor effects [288, 289]. However, due to serious hepatotoxicity, pneumonia, infections, and other adverse effects, the FDA issued a black box warning and halted six clinical trials of combination therapy in 2016.

TGR-1202/RP5264/ Umbralisib

45 (TGR-1202) shared a core structure with **44** and a similar overall structure to the casein kinase 1 epsilon (CK1ε) inhibitor PF4800567. Thus,**45** was a dual inhibitor targeting both PI3Kδ and CK1ε [290]. **45** had demonstrated the ability to overcome the typical adverse effects associated with immune-mediated reactions to **44** and **46**. Additionally, **45** had been shown to improve the quantity and function of CLL-T regulatory cells in comparison to other PI3K inhibitors [291]. It had demonstrated favorable tolerability and exhibited potent anti-tumor efficacy in diverse clinical trials for leukemia. Clinical studies had also explored **45** in combination with other agents, such as ibrutinib (BTK inhibitor), ublituximab (anti-CD20) or Obinutuzumab (anti-CD20) plus chlorambucil, demonstrating tolerability and promising therapeutic outcomes.

IPI-145/INK1197/ Duvelisib

46 (IPI-145) was structurally similar to **44**. However, **46** differed in its subtype binding affinity, targeting the inhibition of both PI3Kδ and PI3Kγ [292]. **46** had shown antitumor ability in hematologic cancers without toxic effects on normal B cells. The dual blockade of PI3Kδ and PI3Kγ by **46** also enabled the regulation of various cellular activities and tumor immunity [293]. Its monotherapy demonstrated acceptable tolerability in a phase II study in refractory iNHL [294]. Additionally, combination therapies of **46** with bendamustine, rituximab, and fludarabine plus cyclophosphamide plus rituximab also showed acceptable tolerability, supporting further efficacy studies [112].

RP6530/Tenalisib

47 (RP6530) was a compound that has shown high potency against both PI3Kδ and PI3Kγ enzymes [295]. **47** had progressed to phase II clinical studies, where its single-agent activity has shown favorable clinical responses and low toxicity in advanced or R/R hematologic malignancies [157, 296]. Furthermore, combination therapy with Romidepsin (HDAC inhibitor) had demonstrated a favorable safety profile and antitumor activity in R/R TCL [297].

GS-9820/Acalisib

Clinical trials of **48** (GS-9820) were initially conducted in R/R lymphoid malignancies, and it had been revealed preliminary clinical activity in this phase IB study [158]. However, it's noted that **48** exhibited similar toxicity to other PI3Kδ inhibitors like **44**.

INCB040093/Dezapelisib

49 (INCB040093) had progressed to phase I clinical trials, and initial findings from a phase I study in R/R B-cell lymphoma indicate tolerability and antitumor activity when used as monotherapy as well as combined with JAK1 inhibitor itacitinib [159].

INCB050465/IBI-36/ Parsaclisib

The discovery of **50** (INCB050465) was built upon the structure of **49** with the aim of improving potency, pharmacokinetic characteristics, and toxicity [298] 0.5**0** had demonstrated promise in overcoming the hepatotoxicity associated with the purine moiety in first-generation PI3Kδ inhibitors [299]. **50** is currently in phase III clinical trial development, and phase I/II studies in R/R B-cell malignancies had indicated well-tolerated, rapid, and preliminary anti-tumor activity [300, 301]. However, a phase II study in DLBCL was terminated due to futility [160]. Combination studies of **50** with itacitinib or R-ICE regimen showed safe tolerability but limited clinical activity [300, 302]. Additionally, combining **50** with pembrolizumab in a phase Ib trial for patients with advanced solid tumors exhibited potential for further exploration [303].

YY-20394/ Linperlisib

The clinical development of **54** (YY-20394) had progressed to phase II studies focusing on non-solid tumors as well as hematologic malignancy [304, 305]. Results thus far indicated a manageable safety profile and favorable efficacy. Moreover, findings from a single-arm phase 1b/2 trial underscored the safety and efficacy of **54** when administered in combination with the gemcitabine and oxaliplatin chemotherapy regimen, particularly in patients grappling with R/R DLBL [163].

ME-401/PWT-143/ Zandelisib

55 (ME-401) exhibited remarkable selectivity for PI3Kδ, attributing to its extended occupancy of p110δ and the capacity to accumulate, forming the foundation for its exceptional clinical potency [306]. **55** garnered FDA fast-track approval in 2020, earning recognition for its potential in treating adult patients with R/R FL who have undergone a minimum of two prior systemic therapies. Furthermore, **55**, whether administered as monotherapy or in combination with the BTK inhibitor zanubrutinib and rituximab, has demonstrated commendable tolerability [307].

BGB-10188

58 (BGB-10188) had shown promising antitumor effects in preclinical models of different types of B-cell lymphomas and had an improved safety profile [167]. **58** is currently in phase I/II clinical trials with monotherapy and combination therapy with Zanubrutinib and Tislelizumab (anti-PD1) being conducted in various R/R mature B-cell malignancies (NCT04282018).

SHC014748M

59 (SHC014748M) had entered phase II clinical trials and its monotherapy had demonstrated favorable safety and clinical efficacy in phase I studies in R/R indolent B-cell malignancies [168]. Phase II trials of **59** in Peripheral T Cell Lymphoma, FL, and Marginal Zone Lymphoma (MZL) are ongoing (NCT04470141, NCT04431089).

HMPL-689/ Amdizalisib

Having progressed to phase II clinical trials, **62** (HMPL-689)had exhibited promising outcomes in terms of monotherapy in R/R B-cell lymphoma, showcasing both manageable toxicity and preliminary clinical activity [308]. Furthermore, **62** is currently undergoing evaluation in a phase II trial that explores its combination with Tazemetostat (EZH2 inhibitor) in R/R lymphoma (NCT05713110).

AZD8835

The development of **63** (AZD8835) was initiated with the optimization of compounds from the aminopyridine and aminopyrazine series, aiming to enhance kinase selectivity [172]. In terms of class I PI3K kinase selectivity, **63** emerged as a dual-targeted inhibitor, showing significant potency against PI3Kα and PI3Kδ, with a 15 to 75-fold higher potency than PI3Kγ and β [172]. **63** has completed a phase I clinical trial in advanced solid tumors (NCT02260661).

TQ-B3525

64 (TQ-B3525), a PI3Kδ/α inhibitor, showed higher anti-PI3Kα/δ than **2** in preclinical studies [173]. **64**

had progressed to phase II clinical trials with studies focused on patients with various types of lymphoma, NSCLC, ovarian and BC (NCT04615468, NCT05284994, NCT04836663, NCT04355520). A phase I study in advanced malignancies showed that **64** was well tolerated and had promising antitumor activity in R/R lymphoma [173].

XC-302/Puqutinib

65(Puqutinib), a PI3Kδ inhibitor resulting from high-throughput screening of compound libraries, exhibited selective inhibition of PI3Kδ [174]. **65** interacted with both the specific and affinity pockets of the PI3Kδ active site. Notably, it differed from **44** as its purine moiety forms hydrogen bonds at Tyr813 and Lys779, instead of interactions at Glu826 and Val828 [309]. **65** had entered clinical trials in China, manifesting substantial safety, tolerability, and efficacy against hematologic malignancies in phase I trials. Currently advancing into phase II clinical trials in China, **65** holds promise as a potential therapeutic agent for hematologic malignancies.

Current status of PI3K inhibitors for cancer therapy

Despite the extensive research and development of PI3K inhibitors, drug-related toxicity observed during the clinical treatment of patients has emerged as a significant obstacle in their development. This toxicity primarily results from the targeting of different PI3K isoforms by these inhibitors, incorporating both on-target and off-target effects (See below, Fig. 7A). Pan-inhibitors, which block all class I PI3K isoforms, are limited by a broad spectrum of off-target effects. Dual PI3K/mTOR inhibitors even exhibit broader toxicity. **10** (BEZ235) and its analog **11** (BGT226) had ended up with discontinued further studies due to significant toxicity and unsatisfactory antitumor effects, same as **17** (PQR309) and **21** (PF-04691502). In the case of isoform-selective inhibitors, targeting PI3Kα often leads to toxicity primarily caused by GSK3 downstream of AKT, resulting in manifestations such as hyperglycemia and hyperinsulinemia [310]. Another prevalent side effect is rash, associated with the involvement of the PI3K pathway in cellular processes in epidermal cells [310]. Additionally, diarrhea and stomatitis are also common side effects of PI3Kα inhibitors. For PI3Kδ inhibitors, side effects mainly arise from autoimmune toxicity, with gastrointestinal toxicity, myelosuppression, and opportunistic infections being common toxic manifestations [311]. For these distinctive toxicities, the first PI3Kδ inhibitor, **43** (AMG319) eventually terminated further clinical development. The development of **44** currently also faces the same challenges. In addition to target-related side effects, hepatotoxicity, nausea, diarrhea, and other general side effects may also occur. It's worth noting that while side effects of PI3K inhibitors are common, they are generally reversible and tend to subside when the dose is reduced, the treatment is slowed, or the treatment is discontinued.

Furthermore, drug resistance remains a significant obstacle in the use of targeted antitumor drugs, with its mechanisms broadly categorized into endogenous resistance and acquired resistance. Endogenous resistance refers to the inherent properties of the tumor that preclude effective responses to treatment from the outset. These properties may include genetic, epigenetic, or microenvironmental factors that naturally limit the efficacy of the drug. On the other hand, acquired resistance develops over the course of treatment and is characterized by a gradual diminishment in therapeutic effects or even a complete lack of clinical response after an initial period of effectiveness. This type of resistance can arise due to various mechanisms, such as mutations in the target protein, activation of alternative signaling pathways, or adaptive responses within the tumor microenvironment. PI3K inhibitor treatment for tumors also faces challenges related to drug resistance, which is often attributed to feedback loops, compensatory parallel signaling, and the downstream activation of pathways associated with PI3K, which reinstates PI3K-associated effects. Genetic mutations in PI3K itself also contribute to drug resistance. The side effects of targeting p110α, such as increased blood glucose and insulin levels, can impair the therapeutic response to PI3K inhibitors by reactivating PI3K signaling through the glucose-insulin feedback pathway [312]. This insulin feedback can be mitigated through ketogenic diets or Sodium-glucose cotransporter-2 (SGLT2) inhibitors [312]. FOXO, a substrate phosphorylated by AKT, regulates upstream RTKs and other reactants in the PI3K pathway. Inhibition of FOXO phosphorylation, a consequence of sustained PI3K/AKT signaling inhibition, allows FOXO to activate RTKs and downstream AKT molecules, leading to de-repression of the PI3K/AKT pathway [313]. The RAS-RAF-MEK-ERK pathway, as a cross-signaling pathway of the PI3K signaling pathway, synergistically promotes tumor growth and interacts with PI3K [314]. Activation of the RAS-RAF-MEK-ERK pathway results in resistance due to the weakened inhibitory effect of PI3K. Mutated *RAS* genes, for instance, activate both the RAF-ERK and PI3K pathways, reducing tumor sensitivity to PI3K inhibitors, as well as MEK inhibition or *ERK* knockdown can counteract the inhibitory effects of PI3K inhibitors on tumors [314, 315]. Similar cross-reactive signaling pathways include NF-κB signaling and Wnt/β-catenin signaling [314]. Abnormalities in the

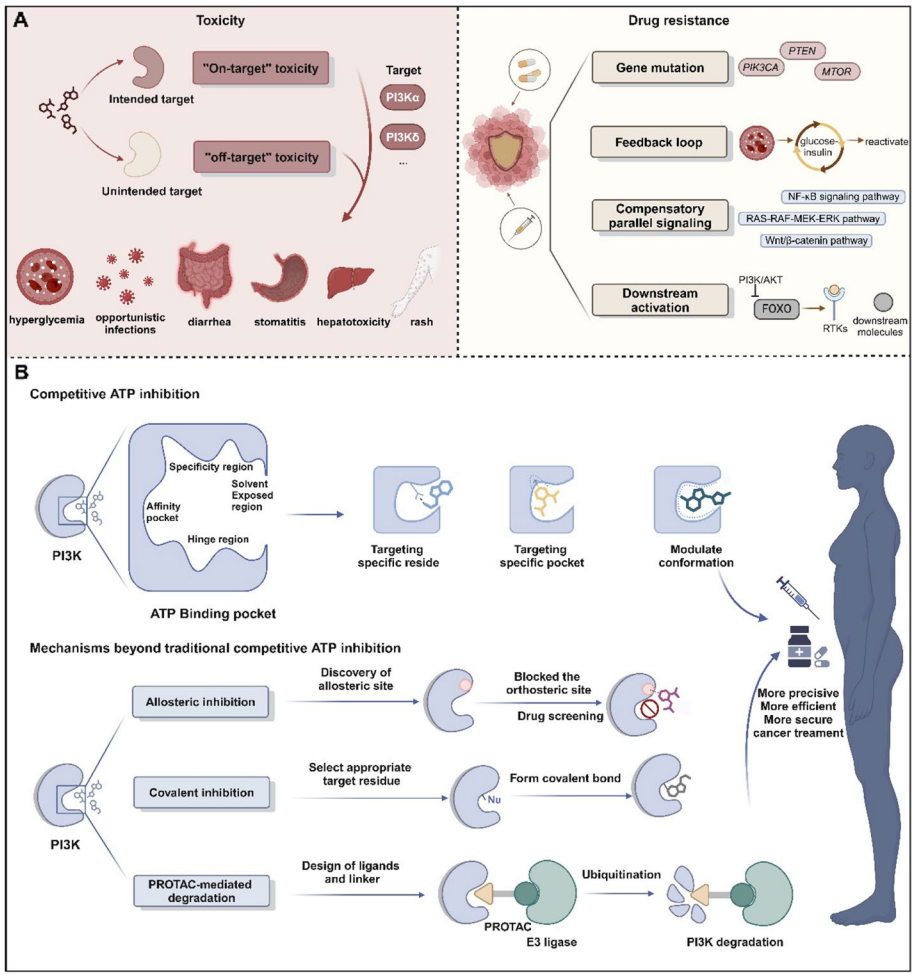


Fig. 7 Clinical Applications review and Future Prospects. **A** Toxicity and Resistance dilemma for Clinical Applications. **B** Future directions of PI3K inhibitor design

PI3K gene itself can also lead to drug resistance, such as acquired amplification and mutations of *PIK3CA*, which not only significantly upregulate the PI3K signal transduction itself, but also lead to resistance to selective PI3K inhibitors [73]. *PTEN*, acting as a tumor suppressor, is frequently absent in various cancers. *PTEN* deficiency leads to resistance to PI3Kα inhibitors, and sustained inhibition of PI3Kα in turn results in various genomic alterations like *PTEN* loss [10]. On the other hand, *PTEN* loss induces PI3Kβ dependence, and in preclinical models of prostate cancer with *PTEN* loss, PI3Kβ inhibitors combined with PI3Kα inhibitors exhibit superior tumor suppression [316]. Similar PI3K signaling pathway molecular mutations that can lead to drug resistance include mTOR. The effectiveness of monotherapy with PI3K inhibitors is impeded by the compensatory activation of alternative signaling pathways. PI3K combination therapy gradually become future mainstream, and clinical Insights into these resistance mechanisms will guide clinical combination therapies. Various PI3K inhibitors are explored in combination with EGFR inhibitors, MEK inhibitors, multikinase inhibitors, Notch inhibitors, Checkpoint kinase 1 inhibitors, Polo-like kinase 1 inhibitors, RAS/ERK signaling inhibitors, CDK4/6 inhibitors, and other targeted therapeutic agents. The exploration of combination therapies remains an ongoing area of investigation.

In the evaluation of pharmacodynamic biomarkers, the relationship between genetic mutations in the PI3K pathway and the clinical efficacy of inhibitors warrants further investigation. Frequent mutations and amplifications of *PIK3CA* in PI3K itself lead to pathway activation. Clinical trials have revealed diverse forms and numbers of mutations in *PIK3CA*. Tumor cells carrying multiple clonal *PIK3CA* mutations (cis-*PIK3CA* mutations) can individually hyperactivate the PI3K pathway and exhibit increased sensitivity to p110α inhibition. On the other hand, subclonal multiple *PIK3CA* mutations

may require additional mutations from covariant signaling pathway genes, including alterations in RTK pathway genes, to adequately drive tumor growth and proliferation via PI3K and parallel pathways. Consequently, inhibiting PI3K alone may not be sufficient to fully attenuate tumor growth and proliferation. This updated understanding of *PIK3CA* gene mutations informs the use of PI3K inhibitors and suggests more rational combination therapies [141, 317]. In addition to genomic changes, plasma metabolomics emerges as a useful tool for assessing biomarker modulation in early clinical studies, where changes in circulating metabolites can reveal associations with mutations [318]. PI3K inhibitors with selectivities exhibit variability in downstream phosphorylated proteins, and phosphoproteomics can reflect the differential roles that PI3K inhibitors play in tumor therapy and drug resistance [319]. The inclusion of pharmacodynamic biomarkers and functional imaging monitoring biomarkers also holds significant value.

Designing strategies for targeting class I PI3K with small-molecule inhibitors

As modern technology becomes more up-to-date and iterative, the development of PI3K inhibitors has become more accessible. Experimental high-throughput screening, computer-aided virtual screening, assay-based structure–activity relationship (SAR), and computational-aided SAR are anticipated to persist as integral methods in future inhibitor development. For example, in silico docking techniques can be applied to all stages from drug screening to preclinical and clinical stages, like drug virtually screening and ADME-Tox properties evaluation. Centered on CADD techniques, it utilizes ligand-receptor interactions, structure optimization and synthesis to realize rapid drug discovery [320]. In silico technology has the advantage of being efficient, fast and economical, becoming an important part of the drug discovery process.

In the realm of research and development technology, beyond the conventional X-ray crystallography, an array of emerging technologies, including cryoEM, molecular modeling techniques, 3D-QSAR, and molecular dynamics, is gaining prominence. These innovative techniques offer diverse perspectives on inhibitor-target interactions patterns between inhibitors and targets, enriching the tool for in-depth comprehension of the mechanisms.

In terms of the class I PI3K inhibitor's mechanism, the majority manifest reversible competitive inhibition by binding to the ATP pocket of the protein (Fig. 7B). The pivotal regions within the PI3K ATP-binding site encompass four areas: the hinge region, specificity region, affinity region (hydrophobic region I), and solvent-exposed region (hydrophobic region II or ribose-binding region). Key considerations for designing potent PI3K inhibitors based on the ATP binding pocket include: 1. Maintaining substituents for binding to Val residues in the hinge region 2. Incorporating heterocyclic cores that do not compromise target potency 3. Ensuring the presence of a hydrogen bond donor/acceptor on the heterocyclic substituent or side chain to form a hydrogen bond with the affinity region or in conjunction with a water molecule 4. Extending the side chain to the solvent-exposed region to establish additional interactions with surrounding amino acids [321, 322]. Most of the new PI3K inhibitors of the last five years have been based on structural modifications of previous PI3K inhibitors by the mechanism of ATP inhibition, with the main scaffolds including quinazoline, quinoline, triazine, thienopyrimidine, imidazopyridine, pyridopyrimidine or other core structures. These improvements usually lie in the utilization of improved structures to enhance biological activity and metabolic profiling, or designing groups capable of targeting specific residues or pockets to improve selectivity. Strategies such as group substitution, core substitution, HipHop, and scaffold jumping based on bioisterism will also continue in inhibitor optimization.

Targeting specific residues and pockets of the ATP-binding pocket

In the realm of subtype-selective inhibitors, challenges arise due to the high sequence homology between the catalytic structural domains and the conserved ATP-binding site (Fig. 8). Strategies for developing subtype-selective inhibitors have concentrated on inducing specific pockets and targeting non-conserved residues in the ATP-binding pocket. Interestingly, the specific binding pocket is typically associated with helical inhibitors, a common feature in PI3Kδ inhibitors. Furthermore, most selective PI3Kβ and γ inhibitors also exhibit helical characteristics. These helical inhibitors share similar chemical structures, characterized by a bicyclic heterocyclic aromatic core with a six-membered aryl group directly attached to it. Additionally, a hinge binder (HB) is connected via a short spacer group, often containing an amino linker and a chiral carbon. However, **66** (PI-103) adopted a flat conformation within the ATP pocket but also induced a conformational change in the side chain of Lys802, creating a spacious cavity that can accommodate various large substituents which were used to guide the structural modification of subsequent compounds like **5** and **67** (Compound Vib) [321, 323].

To obtain PI3Kα specificity, the residues at the position where PI3Kα Gln859 is located are not conserved (aspartic acid for β, lysine for γ, and asparagine for δ) [324]. Another specific residue in PI3Kα, Arg770, has a longer structure than residues at the same position in

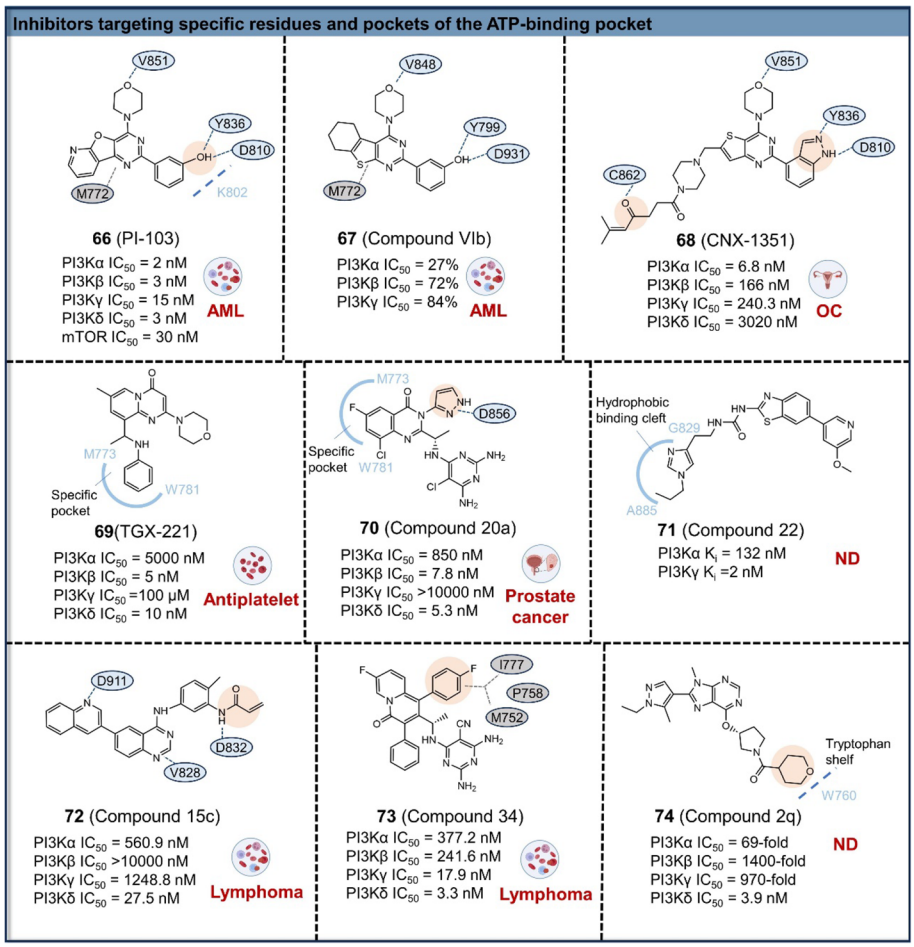


Fig. 8 Preclinical representative Class I PI3K inhibitors targeting specific residues and pockets of the ATP-binding pocket

other isoforms, making it more likely to interact with hydrogen-bonded ligands [324]. In addition, the specific residue Cys862 targeted by covalent inhibitors is also present in PI3Kα [325]. Cys862, proximal to the small-molecule binding site and specific to PI3Kα, was identified as a promising target for PI3Kα specificity. This led Nacht et al. to develop **68** (CNX-1351) as a selective covalent inhibitor of PI3Kα.This selective inhibitor covalently modifies cysteine862 (Cys862) of PI3Kα, an amino acid specific to the α-isome [325].

Correspondingly in PI3Kβ, **69** (TGX-221) adopted a propeller shape embedded in an ATP pocket and induced a p-loop conformational change through the movement of the Met773 residue, which resulted in the formation of a small hydrophobic pocket confined by Met773 and Trp781 where the aniline moiety located, explaining its superior PI3Kβ selectivity [326]. **69**'s binding mode offered structural guidance for targeting PI3Kβ. In addition to **36** targeting specific residue Tyr778, in an effort to enhance selectivity through interaction with the PI3Kβ-specific residue Asp856, Perreault et al. devised a PI3K inhibitor based on **44** with a propeller-shaped structure. **70** (Compound 20a), not only inducing specific pockets of PI3Kβ but also with a pyrazole moiety aimed at interacting with Asp856, stood out as a potent PI3Kβ/δ-selective inhibitor [327, 328].

To target PI3Kγ, **42** utilized the interaction of non-conserved residue Lys802 showing superior PI3Kγ selectivity. Similarly, Glu829, Glu814, and Ala885 in the semi-hydrophobic binding cleft can be targeted for designing selective inhibitors [255].**71** (Compound 22) selectively inhibited PI3Kγ with IC_{50} of 2nM, 66-fold that of PI3Kα. Its binding pattern to PI3Kγ revealed the presence of specific residues (Gly829 and Ala885) in the hydrophobic binding cleft of PI3Kγ that were different from those of other class I PI3Ks, which enhanced PI3Kγ selectivity by reducing unfavorable interactions [329].

In PI3Kδ, commonly propeller-shaped compounds selectively bind to the gatekeeper catalytic sites Thr750 and Trp760. The small size of Thr750 allows these compounds to pass through the pocket opening, binding to residues at the rear of the ATP-binding pocket of the δ isoform. In other isoforms, Trp760 is replaced at the same position by Arg1170 (α), Arg539 (β), and Thr750 is replaced by Lys529 (β) and Lys802 (γ), resulting in differences in selectivity [330]. Differently, with the aid of acrylamide fragment, **72** (Compound 15c) formed a hydrogen bond with another non-conserved residue Asp832 at the entrance of the PI3Kδ binding pocket, and use ligand's interaction with Asp911 established an additional bond, anchoring itself within the protein cavity [331]. Despite of propeller-shaped compounds usually composed of three blades like CAL-101, Shukla employed a pharmacophoric expansion strategy, introducing another arm to create a series of innovative "four-blade propeller" compounds. The newly introduced C-3 phenyl ring of **73** (Compound 34) made additional hydrophobic interaction with I777, M752, and P758 [332]. However, filling the space between Trp760 and Met752 alone may not be sufficient for achieving optimal PI3K isoform selectivity. **74** (Compound 2q) 's selectivity is partially situated in the "tryptophan shelf," exploiting differences in the dynamics of the G-loop-equivalent between isoforms—an essential factor for isoform selectivity [333].

In summary, inhibitor selectivity is intricately linked to the overall binding mode, involving complex interactions within various regions of ATP and influenced by protein and inhibitor conformation. Future drug discovery endeavors could benefit from exploring the steric structure of inhibitors at interaction sites within the ATP pocket.

Modulating conformation in ATP-binding pocket

Conformational modulation has emerged as a strategy for designing isoform selectivity (Fig. 9). Such as using atropisomerism-induced conformational changes to lock the conformation in PI3Kβ inhibitors. As representative, **75** ((P)-14) occupied specificity pocket between Met773 and Trp781, and its atropisomerism was introduced by restricting the free rotation of the C-N bond at the two ring junctions through a C5 quinoline substitution and double substitution of the C2-position of the benzimidazole [334]. For PI3Kγ inhibitors, conformational changes in the "DFG" sequence caused by alkyl tail could influence selectivity. Specifically, **76** (AZ2), with an additional alkyl tail, exhibited optimal PI3Kγ activity. Through X-ray crystal structure analysis of **76** with PI3Kγ, it was revealed that its N-alkyl tail is oriented perpendicular to the isoindolinone core, extending deep into the ATP-binding pocket near Asp787 and Met788. This induced a noticeable movement in Asp787, leading to a conformational change in the 'DFG' motif (residues 911–913). Significantly, a distinct pocket named the 'alkyl tail pocket' opened to accommodate these alkyl tail analogs. This marked the first instance of an orthosteric inhibitor causing conformational and dynamic changes in the p110γ structural domain over a substantial area, laying the groundwork for subsequent PI3Kγ inhibitor development [335]. The successor **77** (Compound 4) was expanding on the structural attributes of **39** and **76**, equipped with the isoindolinone segment of **76** with a bicyclic hinge-binding motif, allowing the N-alkyl tail of the isoindolinone to extend deeper into the "alkyl pocket," thereby enhancing PI3Kγ selectivity [336]. **78**(Compound 56) also belonged to this series, exhibited favorable drug-like properties [337]. The macrocyclic strategies involving conformational restriction through spatial site-blocking represent a pioneering class of compounds that offer advantages such as influencing the targeting potency of immobilized compounds through macrocyclization, modulating target/non-target selectivity, etc. For instance, **79** (MCX-83) was based on the structures of pyridazine and di-fluoro-substituted phenyl fragments of GSK-2126458 as linkers in the design of MCX, demonstrating superior potency against PI3K and mTOR, along with a favorable pharmacokinetic profile.

Exploring mechanisms beyond traditional competitive ATP inhibition

80 (PIK-75) exhibited a unique property by inhibiting PI3Kα through a mixed inhibitory mechanism, involving both non-competitive inhibition of ATP and competitive inhibition of the substrate PI. It also had distinctive bind interactions between non-conserved residues Ser773 and Gln859 in PI3Kα leading to the specific selectivity (Fig. 10). Similar to **80** (PIK-75), the hybrid inhibitors **81** (J-32) and **82** (A-66S) demonstrated unique characteristics. Additionally, if the kinase-independent function of p110β and p110 signaling involves targeting the Gβγ subunit downstream of GPCRs, inhibitors designed to disrupt the Gβγ binding site in p110β may exhibit a distinct clinical tumor profile [338]. Furthermore, given the common occurrence of PI3Kα mutations in cancer, recent research has focused on developing inhibitors selective for both PI3Kα mutants and the wild type. However, the development of PI3Kα mutant-selective inhibitors poses additional challenges due to the lack of changes in the active site of p110α in common mutations. The allosteric inhibitor in terms of **33** employed non-ATP competitive inhibition for PI3Kα H1047R,which was designed based on the discovery that the origin of mutation selectivity as the difference in a cryptic pocket between the mutant

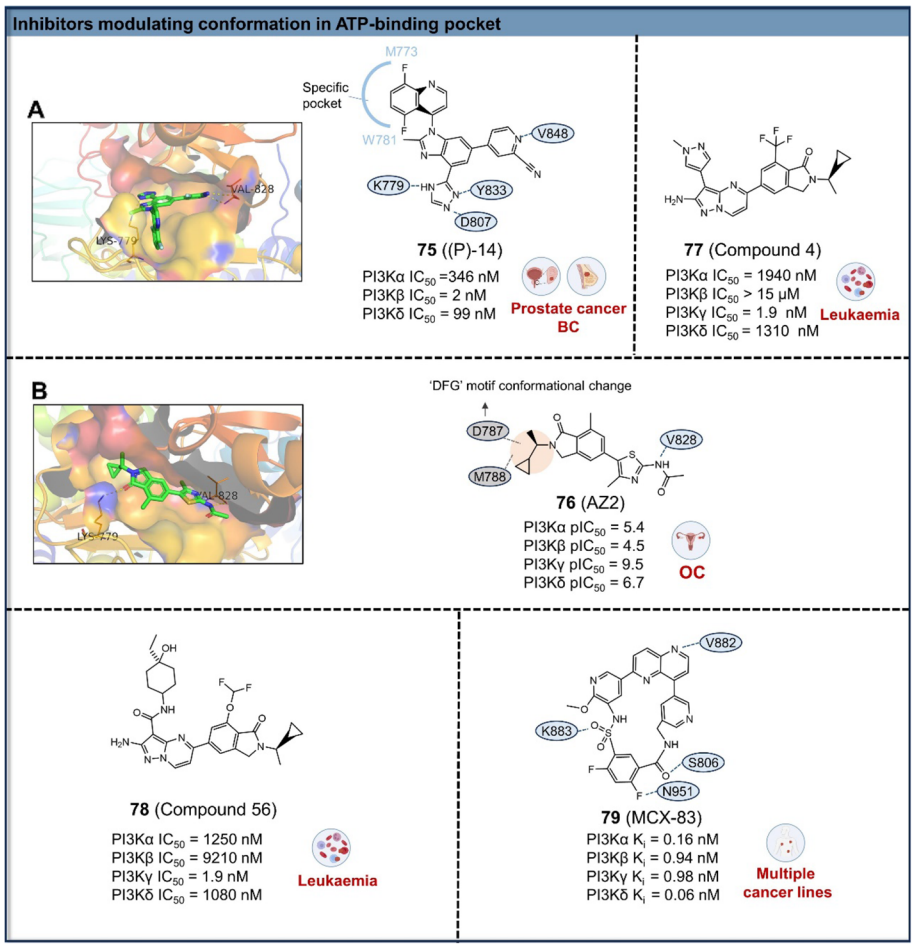


Fig. 9 Preclinical representative Class I PI3K inhibitors modulating conformation in ATP-binding pocket. **A** X-ray co-crystal structure of PI3Kβ in complex with 75 (PDB ID: 6DGT). **B** X-ray co-crystal structure of PI3Kγ in complex with 76 (PDB ID: 6FTN)

and wild type [146]. Based on this variability, it may be possible to develop more allosteric inhibitors targeting this cryptic pocket. Covalent inhibitors constitute a subset of PI3K inhibitors. Despite the initial generation PI3K inhibitor wortmannin having intolerable toxic side effects, covalent inhibitors offer unique advantages for future development. **83** (Neolymphostin A), a derivative closely related to lymphostin, served as a covalent kinase inhibitor targeting PI3K/mTOR.**83** showed identical mechanism to wortmannin but greater "drug-like" attributes, featuring heightened water solubility and a combination of hydrogen bond donors and acceptors [339]. From a series of ester-selective covalent inhibitors targeting the conserved residue Lys779 in PI3Kδ, **84** (Compound 4) demonstrated a > 20-fold selectivity window with minimal off-target binding [340]. Combining covalent strategies and precision targeting, future development are directed towards subtype-specific covalent inhibitors, which can comprehensively and durably inhibit the target and its downstream oncogenic signaling [325]. This approach reduces the frequency of doses and minimizes off-target effects, thereby improving the therapeutic index by accurately targeting specific pathways and avoiding toxicity associated with pan-inhibitors. An additional advantage of the covalent strategy lies in the companion covalent probe, employed to assess PI3Kα occupancy in vivo before clinical use [341]. This serves as a powerful translational tool for evaluating clinical PK/PD. In addition, PROTACs are emerging as a promising approach for promoting protein-specific degradation by recruiting ubiquitin molecules. Notably, these PROTACs may selectively mediate the degradation of oncoproteins mutated in *PIK3CA* without affecting the levels of wild-type *PIK3CA*. Unlike traditional small molecule drugs, the pharmacological mechanism of PROTAC involves target protein degradation, obviating the necessity for a highly precise target binding site [342]. Consequently, PROTACs exhibit a longer-lasting and more potent inhibitory effect with reduced susceptibility to drug resistance. This selective degradation offers valuable

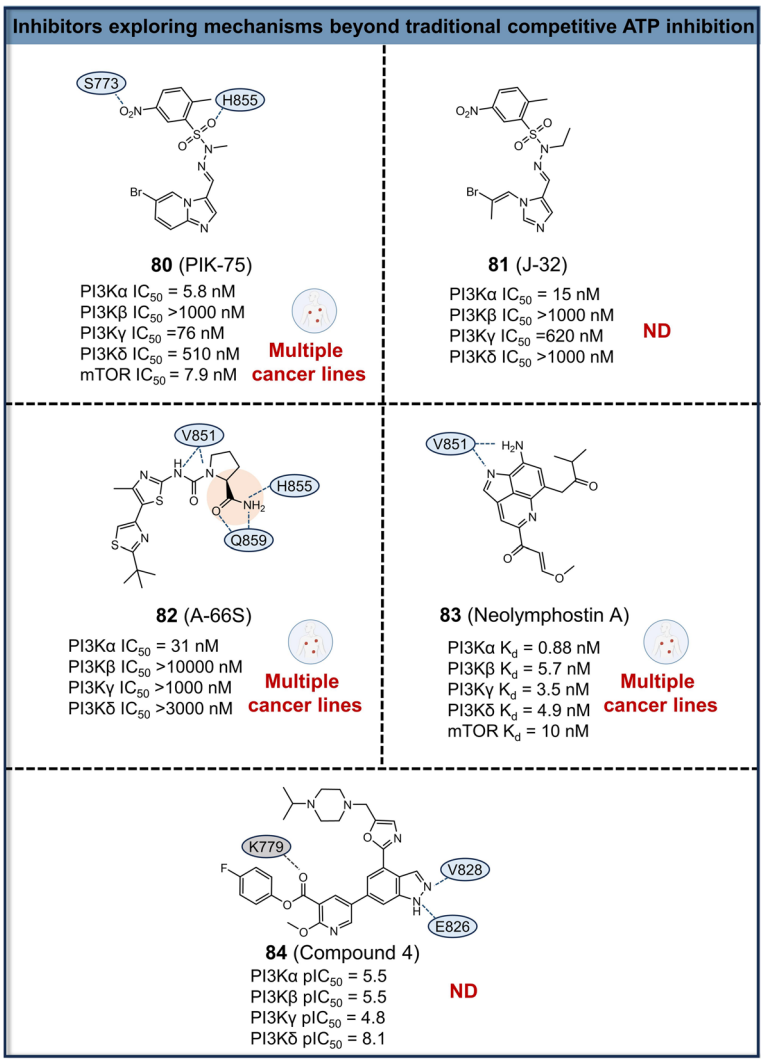


Fig. 10 Preclinical representative Class I PI3K inhibitors exploring mechanisms beyond traditional competitive ATP inhibition

insights for the development of *PIK3CA* mutant-selective inhibitors.

Owing to the strategy of combining PI3K inhibitors with other targeted drugs, the development of dual-target inhibitors has also become the future direction of PI3K inhibitor development. The development of dual-target inhibitors can be approached through both vertical (involving two targets in the same pathway) and horizontal (involving two targets in different pathways) directions. This review specifically concentrates on PI3K/mTOR dual-target inhibitors, emphasizing the former approach. Alternatively, employing the development strategy of lateral dual-target inhibitors, inspired by **8**, future inhibitor development can combine PI3K with other inhibitors targeting tumor-related targets. This association aims to achieve dual-target, dual-mechanism antitumor effects through the pharmacodynamic groups of the two classes of inhibitors. Importantly, the use of a single dual-targeted inhibitor, rather than combining two individual drugs targeting each pathway separately, may offer several advantages, including a favorable pharmacokinetic profile, reduced toxicity, enhanced patient compliance, and ultimately more effective clinical development compared to combination therapy. Moreover, since **13** (GSK1059615) and **37** (SAR260301) were terminated due to poor pharmacokinetic characterization, applications like specific chemical modification, prodrug strategy and improved drug delivery technology are also directions in drug design.

Class II and Class III inhibitors in preclinical studies

Class II PI3K inhibitors

Until recently, the biological role of Class II PI3Ks remained elusive. However, growing interest has shifted

towards exploring the potential development of these families and their inhibitors. The roles played by the three isoforms of Class II PI3K in cancer are still relatively poorly understood. Nevertheless, elevated expression of the Class II PI3K family is observed across a broad spectrum of tumors. In these instances, PI3KC2α might be implicated in tumorigenesis and development by contributing to angiogenesis. PI3KC2β, on the other hand, influences cancer cell growth, proliferation, and metastasis through its regulatory effects on the cell cycle. The gene encoding PI3KC2γ, identified as *PIK3C2G*, predominantly functions as a tumor suppressor gene [343]. Small molecule inhibitors targeting Class II PI3K are also briefly discussed here (Table 3).

MIPS-21335

Given the absence of an existing PI3KC2α-selective inhibitor, the development of **85** (MIPS-21335) was inspired by the off-target effect observed with the class I PI3K inhibitor PIK-90 [344]. **85** had the enhanced specificity for PI3KC2α (IC_{50} = 7nM) and are currently under investigation in the field of anti-thrombosis.

PTCOIN3

PTCOINs represent a series of small-molecule inhibitors designed to target PI3KC2α [345]. Among them, **86** (PITCOIN3) demonstrated remarkable specificity for PI3KC2α with very high potency more than > 10,000 times over other family members. **86**'s pteridone scaffolder occupied adenine binding pockets, while the two adjacent arms extending outward and gave the inhibitor a propeller-like conformation. In the bind mode of PI3K2Cα, the pteridinone scaffold of **86** formed a single hydrogen bond with V1187 located in the kinase hinge region, and the phenyl sulfonamide group had hydrophobic interactions with P1188 and E1131, which contributed to the improved selectivity (Fig. 11). As of now, PTCOINs are exclusively under investigation in the antithrombotic field.

PI-701, 702

87 (PI-701) and **88** (PI-702) stood out as the first selective inhibitors of PI3KC2β, displaying moderate potency against PI3KC2β (IC_{50} = 0.5–0.6 μM), while other PI3K isozymes are inhibited at concentrations higher than 10 μM. In tumor models with elevated PI3KC2β expression, **87** significantly reduced cell proliferation and survival [346].

Compound 26

89 (Compound 26) originated from a core structure of **4**, belonging to the same developmental series as **87** and **88**. **89** exhibited an IC_{50} of 0.34μM for PI3KC2γ, which is more than 80 times higher than that of PI3KC2β). Despite of the absence of crystal structures for class II PI3Ks, it was hypothesized that the reduction in potency of class I inhibitors in **89** was due to the change from quinoxaline to pyrazine [347]. However, there is currently no available data on the biology of PI3KC2γ for **89**.

Class III PI3K inhibitors

Within class III PI3Ks, Vps34, forming the core structure with Vps15 and Beclin-1, engages with various subunits to create diverse complexes. These complexes play crucial roles in membrane endosomal trafficking processes, endosome-lysosome maturation, and autophagy. The involvement of class III PI3Ks in these cellular processes underscores their significance in regulating cancer cell activities. Specifically, they influence tumor growth, proliferation, migration, invasion, and metastasis by modulating autophagy. Autophagy, being a recent research focus, is recognized for its complex regulatory role in tumor cells and the microenvironment. Beyond autophagy, class III PI3Ks also can induce oncogenic transformation and enhance tumor progression through other mechanisms. The class III PI3K inhibitors are briefly summarized below (Table 3).

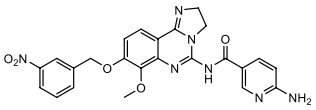
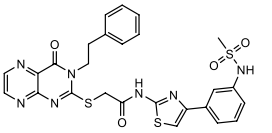
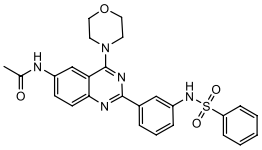
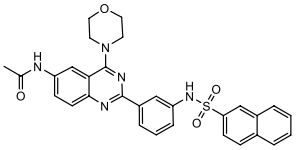
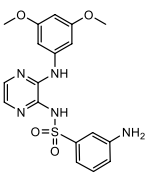
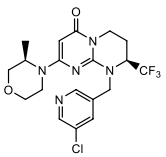
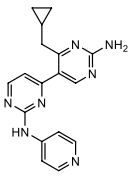
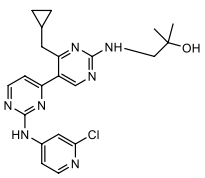
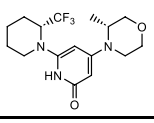
SAR405

90 (SAR405) characterized as the first potent and specific Vps34 inhibitor [348]. Notably, it exhibited inhibitory activity against other class I and class II PI3Ks with an activity below 10μM. The morpholine part of **90** played a role in its fine selectivity for protein kinases, while the methyl substitution of the morpholine took advantage of the larger lumen of the Vps34 ATP-binding pocket compared to other PI3Ks. **90** demonstrated the ability to target both late endosome-lysosome compartments and prevent autophagy. Moreover, it exhibited synergistic effects with everolimus, cisplatin, dual FGFR inhibitors, PD-L1/PD-1 blockers, and class I PI3K or HER2 inhibitors, showcasing its potential in combination therapies against tumors [352–356].

PIK-III

81 (PIK-III) was identified through high-throughput screening and subsequent medicinal chemistry optimization [357]. **81** formed two hydrogenbond interactions between I685, and interactions between aminopyrimidine moiety and the side chains of D671 and D644 are connected with solvent-mediated hydrogen-bonding network. F612 is of vital importance in VPS34 binding selectivity, allowing the cyclopropyl group fit into the hydrophobic cavity and form an optimal interaction with the hinge, while F612 is replaced in PI3Kα. Moreover, **81** had the ability to impede autophagy by regulating NCOA4, leading to

Table 3 The representative Class II and Class III PI3K selectively small-molecule inhibitors [344–351]

No.	Target	Compound	Selectivity (IC_{50}: nM)					Ref.
			Target	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ	
85	PI3KC2α	MIPS-21335 	7	140	386	ND	742	[343]
86		PITCOIN3 	126	>10000				[344]
87	PI3KC2β	PI-701 	528	>10000				[345]
88		PI-702 	632	>10000				[345]
89	PI3KC2γ	Compound 26 	340	ND				[346]
90	VPS34	SAR405 	12	>10000				[347]
91		PIK-III 	18	>3000	>9000	>3000	>1000	[348]
92		VPS34-IN1 	25	>40-fold				[349]
93		SB02024 	1.1(K_d)	>4000 (K_d)	>1000 (K_d)	>10000 (K_d)	>8000 (K_d)	[350]

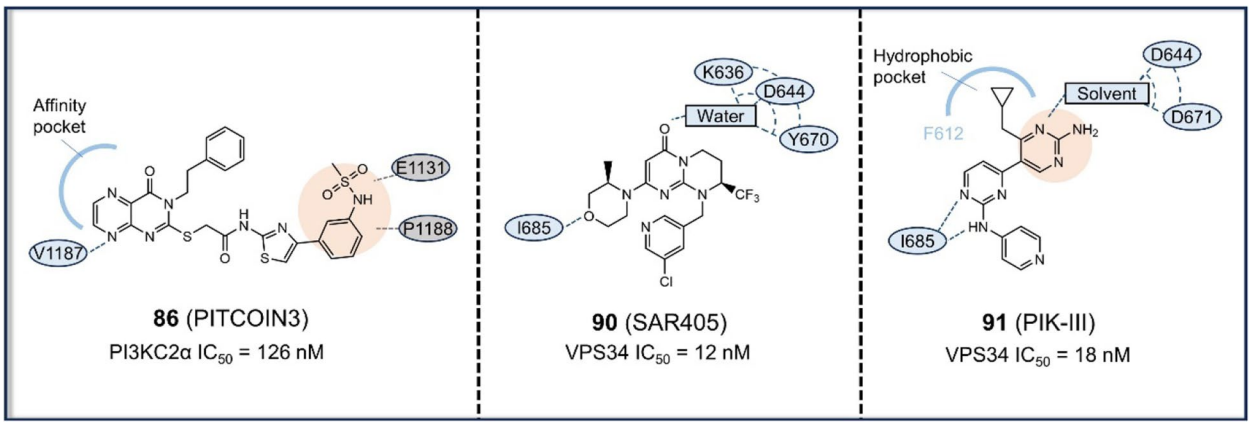


Fig. 11 The representative Class II and III PI3K inhibitors

anti-tumor effects in leukemia and colorectal cancer(CRC) [349, 358].

VPS34-IN1

82 (VPS34-IN1) demonstrated an impressive IC_{50} of 25 nM for Vps34. Notably, its inhibitory impact was minimal on class I and II PI3K [359]. **82** exhibited significant antitumor effects as monotherapy, particularly in BC and leukemia [350, 360].

SB02024

83 (SB02024) emerged as an exceptionally potent VPS34 inhibitor, identified through high-throughput screening of anticancer drug libraries [351]. In preclinical models, **83** demonstrated effective inhibition of autophagy and reduces cell viability. Moreover, it enhanced the sensitivity of BC cells to Sunitinib and Erlotinib. Beyond BC, **83** showed promise in synergizing with PD-L1/PD-1 therapies, offering potential treatment avenues for melanoma and CRC [351, 354].

Concluding remarks and future perspectives

The PI3K signaling pathway holds a crucial physiological function in cellular processes, and its hyperactivation in cancer presents numerous targets for targeted cancer therapeutics. The three classes of PI3K, particularly class I PI3K, play distinct pathogenic roles in oncology. While significant research and development efforts for class I PI3K inhibitors have traditionally focused on targeting the p110α and p110δ isoforms, with widespread applications in treating various solid tumors and hematologic malignancies, there is an increasing recognition of the unique roles that the PI3Kβ and PI3Kγ isoforms play in oncology.

Although the clinical application and research of p110γ and p110β are typically associated with inflammatory diseases, increasing evidence suggests their synergistic potential with other targeted drugs in treating tumors. Class II and class III PI3Ks have gained attention for their roles in cancers in recent years. It's noteworthy that many Class II inhibitors are often assessed in the context of Class I inhibitors, where the inhibition of Class II PI3K is recognized as off-target effect.

The future development of PI3K inhibitors should prioritize precision by focusing on the design of inhibitors tailored to specific structural variations and binding sites of the target proteins. This approach is essential for mitigating off-target toxicity. Optimization of molecular structures is critical for minimizing adverse reactions, underscoring the necessity of a refined approach in the development of these inhibitors. One of the promising strategies to reduce toxicity is the exploration of allosteric inhibitors and PROTACs (Proteolysis Targeting Chimeras). These novel approaches aim to selectively target specific mutant phenotypes of PI3K, thereby sparing normal PI3K signaling in non-target tissues. This selective targeting holds significant potential for enhancing the efficacy and safety of PI3K inhibitors, presenting a compelling avenue for research that demands further investigation. This avenue warrants further exploration to enhance the precision of PI3K inhibitor development.

Combining PI3K inhibitors with other drugs presents an avenue for potentially greater efficacy than monotherapy. Targeted drug combinations can be explored both inter-pathway (targeting drugs in parallel but interconnected signaling pathways) and intra-pathway (targeting drugs downstream of PI3K), including the ERK, CDK4/6 signaling pathways, and mTOR targets. Further investigation into combinations involving PI3K inhibitors, and other antitumor agents such as chemotherapeutic agents, immunotherapies, and photonics (radiation therapy) is essential for future studies. However, the management of toxicity in such combination therapies becomes crucial for their widespread application. Moreover, apart from

the medicinal chemistry optimization of the drug itself, optimization of pharmaceutical formulation processes offers another avenue for enhancing in vivo efficacy. This can involve loading the drug delivery system to enable PI3K inhibitors to improve solubility, tissue targeting, and other pharmacological aspects. For PI3K pathway gene mutations, particularly *PIK3CA* among the most frequent oncogenes in cancer, its role as a biomarker is still under exploration. A deeper understanding of the specific mutations in the *PIK3CA* gene may provide a more precise explanation of the relationship between mutation and phenotype.

In conclusion, this review offers an insightful overview of the structural and roles of PI3Ks in cancer. It summarizes small-molecule inhibitors targeting PI3Ks within the past 5 years, discusses future strategies for developing targeted small-molecule inhibitors, and their application in cancer therapies, and provides unique insights into the development and application of small-molecule drugs targeting PI3Ks for cancer treatment.

Abbreviations

ACD	Autophagic cell death
BC	Breast cancer
BH	Bcl2-homology
BTK	Bruton tyrosine kinase
CDK4/6	Cyclin-dependent kinase 4/6
CLL	Chronic lymphocytic leukemia
CRC	Colorectal cancer
DLBCL	Diffuse large B-cell lymphoma
EGFR	Epidermal growth factor receptor
EIC_{50}	Median effect concentration
ERK	Extracellular-signal regulated kinase
FDA	Food and Drug Administration
FL	Follicular lymphoma
FOXO3	Forkhead box O3
GSK3β	Glycogen synthase kinase 3 beta
TNF	Tumor necrosis factor
HER2	Human epidermal growth factor receptor 2
HL	Hodgkin's lymphoma
HNSCC	Head and neck squamous cell carcinoma
HR	Hormone receptor
HR + /HER2-	HR-positive/HER2-negative
IC50	Half-maximal inhibitory concentration
iNHL	Indolent non-Hodgkin lymphoma
K_d	Dissociation constant
K_i	Inhibition constant
KRAS	Kirsten rat sarcoma
MEK/MAPK	Mitogen-activated protein kinase
MTD	Maximum tolerated dose
mTORC1	Mammalian target of rapamycin complex 1
mTORC2	Mammalian target of rapamycin complex 2
NSCLC	Non-small-cell lung cancer
OC	Ovarian cancer
ORR	Overall response rate
PDK1	3-Phosphoinositide-dependent protein kinase-1
PFS	Progression-free survival
PH	Pleckstrin homology
PI3K	Phosphatidylinositol-3-kinase
pIC_{50}	Negative of the log of the IC50
PK/PD	Pharmacokinetics/pharmacodynamics
PtdIns(3,4)P2/ PI(3,4)P2	Phosphatidylinositol(3,4)-bisphosphate
PtdIns(3,4,5) P3/PIP3	Phosphatidylinositol(3,4,5)-trisphosphate
PtdIns(4,5) P2/PIP2	Phosphatidylinositol(4,5)-bisphosphate
PtdIns/PI	Phosphatidylinositides
PtdIns3P/PI3P	Phosphatidylinositol 3-phosphate
PtdIns4P/PI4P	Phosphatidylinositol 4-phosphate
PTEN	Phosphatase and tensin homolog
R/R	Relapsed/refractory
RBD	Ras-binding domain
RP2D	Recommended phase 2 dose
RTK	Receptor tyrosine kinase
SAR	Structure-activity relationship
SGLT2	Sodium-glucose cotransporter-2
SH2	Src-homology 2
SH3	Src-homology 3
TNBC	Triple-negative breast cancer
TSC	Tuberous sclerosis proteins
WT	Wild type

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Authors' contributions
Leilei Fu, Gu He and Jin Zhang conceived the project and supervised the project. Hongyao Li and Xiang Wen wrote the manuscript. Hongyao Li and Zhichao Fan made the Figures and the Tables. Yueting Ren polished the language. Leilei Fu, Gu He and Jin Zhang proofread and revised the manuscript. All authors approved the final manuscript.

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Declarations

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The authors declare no competing interests.

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