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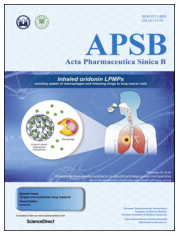

REVIEW

Class I phosphatidylinositol 3-kinase inhibitors for cancer therapy

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KEY WORDS

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PI3K inhibitor;
Drug candidate;
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PI3K/mTOR selectivity;
Anticancer

Abstract The phosphatidylinositol 3-kinase (PI3K) pathway is frequently activated in human cancers. Class I PI3Ks are lipid kinases that phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP2) at the 3-OH of the inositol ring to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3), which in turn activates Akt and the downstream effectors like mammalian target of rapamycin (mTOR) to play key roles in carcinogenesis. Therefore, PI3K has become an important anticancer drug target, and currently there is very high interest in the pharmaceutical development of PI3K inhibitors. Idelalisib has been approved in USA and Europe as the first-in-class PI3K inhibitor for cancer therapy. Dozens of other PI3K inhibitors including BKM120 and ZSTK474 are being evaluated in clinical trials. Multifaceted studies on these PI3K inhibitors are being performed, such as single and combinational efficacy, resistance, biomarkers, etc. This review provides an introduction to PI3K and summarizes key advances in the development of PI3K inhibitors.

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1. Introduction

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases that phosphorylate the 3-OH of the inositol ring of phosphoinositides[1,2]. Class I PI3Ks (generally called PI3Ks) are lipid kinases that phosphorylate phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 serves as a second messenger that plays important roles in fundamental cellular responses such as cell growth, survival, migration and metabolism[3,4]. As a catalytic antagonist of PI3K, phosphatase and tensin homolog deleted on chromosome ten (PTEN) dephosphorylates PIP3 to PIP2 (Fig. 1). Since frequent gain-of-function mutations of PI3Ks and loss-of-function mutations of PTEN in human cancers suggest that PI3Ks are closely involved in tumorigenesis[5], inhibitors targeting PI3Ks are expected to be promising anticancer drug candidates. In the past decade, dozens of PI3K inhibitors have been developed as potential chemotherapeutic drugs. Many of these have successfully entered clinical trials. In particular, idelalisib (CAL-101) has been approved in the USA and Europe as the first-in-class PI3K inhibitor for cancer therapy.

In this review, we introduce PI3Ks and briefly describe the development of some representative PI3K inhibitors in clinical trials.

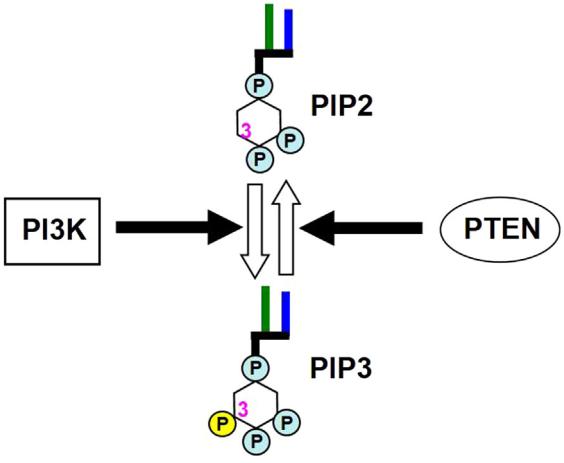


Figure 1 Schematic structures of PI3K and PTEN, and the related lipid reactions they catalyze. PI3K phosphorylates PIP2 at 3-OH to generate PIP3. As a counterpart of PI3K, PTEN dephosphorylates PIP3 to produce PIP2. PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate.

2. PI3K, critical element that is involved in carcinogenesis

PI3Ks belong to a family of lipid kinases that phosphorylate the 3-OH group of phosphoinositides[1,6,7]. Based on their primary structures and *in vitro* substrate specificity, PI3Ks are classified into three classes[8,9]. Class I PI3Ks, which preferentially catalyze the phosphorylation of PIP2 to generate PIP3, are heterodimeric kinases as complexes of a catalytic subunit p110 with a regulatory subunit p85, p101, or p84. Members of this class are generally called PI3Ks because they have been investigated far more than the other two classes. PI3K-related kinases (PIKKs), which sometimes are termed Class IV PI3Ks, are protein kinases with a similar structure to the catalytic subunits of PI3Ks. Examples of PIKKs include mTOR and DNA-dependent protein kinase (DNA-PK), which are known to be involved in protein synthesis or DNA repair[10]. Class I PI3Ks are further divided into subclasses IA and IB based on their regulatory subunit and upstream activator[7]. Class IA PI3Ks are mainly activated by various receptor tyrosine kinases (RTKs) and RAS[11]. There are three isoforms in Class IA including PI3Kα, PI3Kβ, and PI3Kδ, with the respective p110 catalytic subunit bound to the p85 regulatory subunit. Class IB PI3Kγ, which consists of catalytic subunit p110γ and a regulatory subunit p101 or p84, is mainly activated by G-protein-coupled receptors (GPCRs) such as chemokine receptors[12–14]. While the PI3Kα and PI3Kβ are expressed ubiquitously, PI3Kδ and PI3Kγ are mainly in hemopoietic cells[15]. In particular, PI3Kα is known to play an important role in tumorigenesis because a high frequency of gain-of-function mutations and amplification of *PIK3CA*, which encodes p110α; this isoform has been found in human cancers[16–20]. Additionally, PI3Kα was found to be involved in insulin signaling and glucose metabolism[21]. PI3Kβ was reported to activate platelets, suggesting a role in the development of thrombotic diseases[22]. Recently, various reports showed that PI3Kβ predominantly contributed to PIP3 production in PTEN negative cancers, suggesting the key role of PI3Kβ in tumorigenesis with PTEN inactivation[23,24]. PI3Kδ and/or γ inactivation leads to a severely impaired immune system[25,26], and blocks the recruitment of neutrophils to the sites of inflammation[27,28], suggesting that these two isoforms are involved in the immune system and inflammation. As the counterpart of PI3K, PTEN is also closely involved in cancer since frequent loss-of-function mutations were found in various human cancers[29]. In addition, *PI3K* mutation and PTEN inactivation were reported to cause resistance to cancer therapies targeting the RTKs[30]. Thus, PI3K is thought to be an attractive target for cancer chemotherapy.

PI3K pathway is closely involved in survival, growth, invasion of cancer cells and tumor angiogenesis. As shown in Fig. 2, after activation by RTK, GPCR or RAS, PI3K phosphorylates PIP2 to produce PIP3; this reaction is reversed by PTEN. PIP3 binds the pleckstrin homology (PH)-domain-containing protein kinases such as Akt and PDK1, to activate and recruit them to the plasma membrane. After recruitment by PIP3, Akt is activated by PDK1 and mTOR complex 2 (mTORC2)[3]. Activation of Akt promotes cell cycle progression by regulating glycogen synthesis kinase 3β (GSK3β) and the downstream cyclin D1. Akt also acts to maintain cell survival through inhibition of Bcl2-antagonist of cell death (BAD). Furthermore, Akt promotes cell growth by phosphorylation of the downstream mTOR complex 1 (mTORC1)[31], which translates mRNAs to protein *via* the p70S6K-S6 and 4E-BP1-eIF4E pathways[32]. In addition, hypoxia-inducible factor 1α (HIF-1α) was reported to be up-regulated downstream of mTORC1, and therefore promotes tumor angiogenesis by transcribing vascular endothelial growth factor (VEGF)[33]. By activating NF-κB and inducing secretion of matrix metalloproteinase (MMP), Akt also promotes cell invasion[34]. However, phosphorylation of S6K negatively regulates insulin receptor substrate (IRS), leading to a negative feedback loop[35–37]. Therefore, inhibition of mTORC1 may activate upstream proteins such as PI3K and Akt[38], and consequently reduces the inhibitory potency.

Development of novel PI3K inhibitors attracted a great deal of attention from both academia and industry, while classic PI3K inhibitors LY294002 and wortmannin did not reach clinical trials due to the toxicity and poor druggability. Among the PI3K inhibitors under active development, some are PI3K isoform specific inhibitors like idelalisib (CAL-101) and IPI-145, but most are pan-PI3K inhibitors (Table 1). Besides, some exhibit

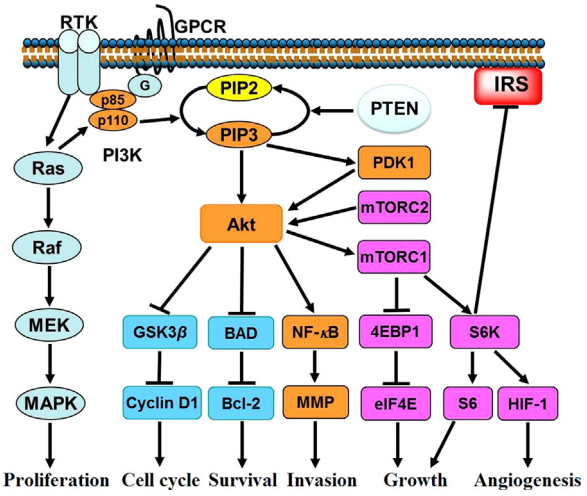


Figure 2 PI3K/Akt/mTOR pathway involved in tumorigenesis and metastasis. After activation by RTKs, GPCR or RAS, PI3K catalyzes the phosphorylation of PIP2 to generate PIP3, which binds and recruits Akt and PDK1. Akt can be activated by PDK1 and mTORC2, after recruitment by PIP3. By increasing the level of cyclin D1, Akt promotes the cell cycle progression. Akt also acts to maintain cell survival by phosphorylation of BAD and release of the anti-apoptotic protein Bcl-2. Furthermore, Akt regulates cell growth by phosphorylation of the downstream mTORC1, which promotes translation of mRNAs to synthesize protein *via* p70S6K-S6 and 4E-BP1-eIF4E pathways. In addition, HIF-1α is up-regulated downstream of mTORC1, leading to angiogenesis. By activating NF-κB and inducing secretion of MMP, Akt promotes cell invasion. However, the mTORC1/S6K cascade negatively regulates IRS, which leads to a feedback loop. 4E-BP1, 4E-binding protein 1; GPCR, G protein-coupled receptor; GSK3β, glycogen synthesis kinase 3β; HIF-1, hypoxia-inducible factor 1; IRS, insulin receptor substrate; p70S6K, p70S6 kinase; PDK1, 3-phosphoinositide-dependent protein kinase 1; RTK, receptor tyrosine kinase.

selectivity over mTOR, such as BKM120, GDC-0941 and ZSTK474[7,39], whereas others show no obvious selectivity, such as NVP-BEZ235, GDC-0980 and GSK2126458[7,40] (Table 1).

3. PI3K inhibitors approved or in clinical trials

3.1. Idelalisib

Idelalisib was originally developed by Calistoga as CAL-101, but later transited to Gilead. Like the analog IC87114 which has been used as a chemical tool[41], idelalisib also specifically targets PI3Kδ, with more than 30-fold selectivity over other PI3K isoforms[42]. In preclinical studies, idelalisib induces apoptosis in chronic lymphocytic leukemia (CLL), independently of patient genomic features[43]. In contrast, idelalisib does not exhibit cytotoxicity toward T cells or natural killer cells[43]. A phase I study in healthy volunteers demonstrated favorable pharmacokinetics with no lymphocyte count change and no notable toxicity during one week of oral administration. In CLL patients, a dose of 150 mg every 12 h was found to be optimal for the phase II study based on the tolerability and pharmacokinetics. In a phase II study, 84% of CLL patients achieved a reduction in lymph node and spleen size of no less than 50%. Median progression-free survival was 15.8 months, with median overall survival (OS) not reached[44]. Combination studies with other therapies have been investigated. For patients receiving idelalisib with rituximab or bendamustine, over 90% patients achieved a decrease in lymph node size of 50% or greater[45]. In a phase III trial of idelalisib (150 mg, twice a day) in combination with rituximab (n = 110) in heavily, pretreated patients (median of 3 prior therapies) with relapsed CLL, an overall response rate of 81% and overall survival of 91% at 12 months were obtained. The incidence of grade 3 or higher adverse events included neutropenia (34%), thrombocytopenia (10%), anemia (5%), elevation in transaminases (5%), and diarrhea (4%)[46]. Collectively, favorable efficacy and mild toxicity of idelalisib was observed in clinical studies. In addition, a population pharmacokinetic model has been established for idelalisib and its inactive metabolite from the data of phase I or II studies[47]. Idelalisib was approved in 2014 for therapy of relapsed CLL in combination with rituximab, and for monotherapy of relapsed follicular lymphoma (FL) or relapsed small lymphocytic lymphoma (SLL), as the first PI3K inhibitor licensed for cancer treatment.

3.2. IPI-145

IPI-145 was originally developed by Intellikine with the name of INK-1197. With a structure similar to idelalisib, IPI-145 also preferentially inhibits the PI3Kδ isoform with an IC_{50} of 3 nmol/L, but inhibits PI3Kγ potently as well with an IC_{50} of 27 nmol/L[48]. IPI-145 sensitizes B-cell receptor (BCR)–stimulated and/or stromal co-cultured primary CLL cells to apoptosis. This compound also potently inhibits cytokine-induced proliferation of CLL cells, without obvious cytotoxicity to normal B- and T-lymphocytes, suggesting the favorable efficacy on CLL[49]. Clinical results demonstrated the efficacy on indolent non-Hodgkin's lymphoma (NHL) and CLL[49]. The rate of nodal response exceeds 70% for treatment of CLL with oral IPI-145[50]. In addition, IPI-145 shows promise as an agent for the treatment of autoimmune and inflammatory diseases[48]. Presently, IPI-145 is in phase III clinical trials for therapy of NHL, CLL and follicular lymphoma.

3.3. NVP-BEZ235

NVP-BEZ235 was developed by Novartis as a PI3K inhibitor with IC_{50} values of 4, 76, 5 and 7 nmol/L for PI3Kα, β, δ and γ, respectively[51]. This agent is described as a dual PI3K/mTOR inhibitor since it also potently inhibits mTOR[51,52]. We reported that DNA-PK was inhibited by NVP-BEZ235 with a similar potency to its PI3Kα inhibition[53]. NVP-BEZ235 potently inhibited growth of a panel of cancer cells, induced cell cycle arrest at G1 phase[51,54], and blocked tumor angiogenesis[55]. Interestingly, NVP-BEZ235 exhibited equal efficacy on HER2-positive breast cancer BT474 cells with p110α mutations compared to those cells with wild type p110α[54], suggesting the potential application to patients with p110α mutations which are often resistant to Herceptin (a humanized monoclonal HER-2 antibody). NVP-BEZ235 is now undergoing evaluation in phase II clinical trials for the treatment of bladder cancer, pancreatic cancer as well as perivascular epithelioid cell tumors.

3.4. BKM120

Also developed by Novartis, BKM120 is a pan-PI3K inhibitor that targets all four isoforms of class I PI3K, but with more than 10-fold selectivity over mTOR[39]. Evaluation in a panel of 353 cancer cell lines showed a preferential inhibition of tumor cell lines with

Table 1 Selected PI3K inhibitors approved or in clinical trials.

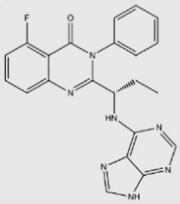
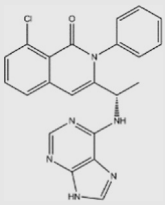
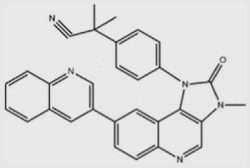
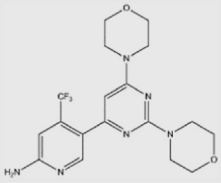
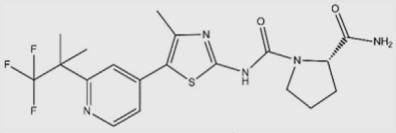
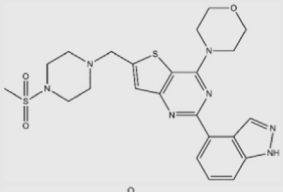
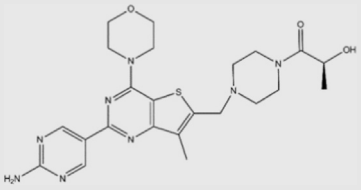
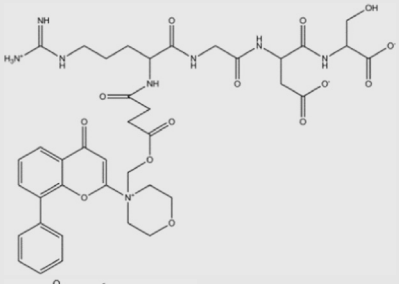
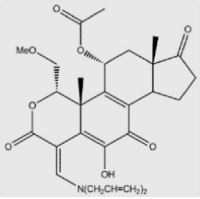
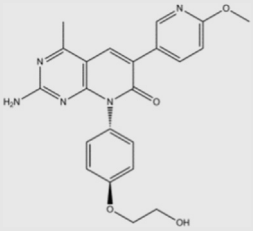
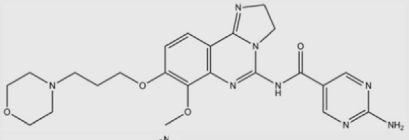
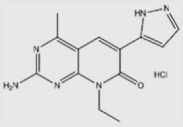
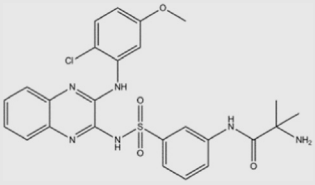
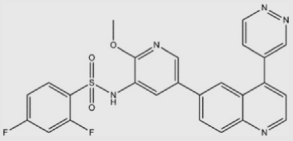
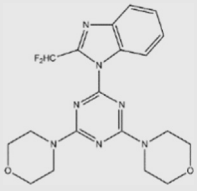
Inhibitor	Structure	IC_{50} (nmol/L)				Isoform specificity	Selectivity over mTOR	Organization (development status)
		p110α	p110β	p110δ	p110γ			
Idelalisib		820	565	2.5	89	p110δ specific	unknown	Gilead (launched)
IPI-145		1602	85	3	27	PI3Kδ, γ specific	unknown	Infinity Pharmaceuticals (phase III)
NVP-BEZ235		4	76	5	7	pan	No	Novartis (phase I)
BKM-120		52	166	116	262	pan	Yes	Novartis (phase III)
BYL-719		5	1200	290	250	PI3Kα specific	Yes	Novartis (phase III)
GDC-0941		3	33	3	75	pan	Yes	Genentech (phase I)
GDC-0980		5	27	7	14	pan	No	Genentech (phase II)
SF1126		NA	NA	NA	NA	pan	No	SignalRx (phase I)
PX-866		6	>300	3	9	pan	unknown	Oncothyreon (phase II)

Table 1 (*continued*)

Inhibitor	Structure	IC_{50} (nmol/L)				Isoform specificity	Selectivity over mTOR	Organization (development status)
		p110α	p110β	p110δ	p110γ			
PF-04691502		1.8[a]	2.1[a]	1.6[a]	1.9[a]	pan	No	Pfizer (phase I)
BAY-80-6946		0.5	3.7	6.4	0.7	pan	Yes	Bayer (phase III)
XL-765		39	113	43	9	pan	No	Sanofi (phase I/II)
XL-147		39	383	36	23	pan	Yes	Sanofi (phase I)
GSK2126458		0.019	0.13	0.024	0.06	pan	No	GlaxoSmithKline (phase I)
ZSTK474		16	44	5	49	pan	Yes	Zenyaku (phase I/II)

NA, not available.
[a]K_i.

PIK3CA mutation for BKM120. Our multifaceted study on BKM120 and other PI3K inhibitors using various biochemical and cell-based assays suggest that BKM120 might have a unique mechanism, possibly independent of PI3K inhibition[56]. Potent antitumor effects *in vivo* were indicated in tumor bearing models. In addition, BKM120 blocks VEGF-induced neovascularization *in vivo*, suggesting antiangiogenic activity[39]. Combinational use of BKM120 with cisplatin exhibits enhanced efficacy[57]. A phase I clinical study in Japan showed that BKM120 had a manageable safety profile, and could be rapidly absorbed in a dose-proportional manner[58]. Clinical efficacy and tolerability of BKM120 were also evaluated in postmenopausal women with estrogen receptor–positive metastatic breast cancer, in combination with fulvestrant using daily or intermittent schedules (days 1–5 each week). Compared with daily dosing, intermittent use of BKM120 led to less frequent adverse effects. The maximum tolerated dose of BKM120 was defined as 100 mg daily. The clinical benefit rate was 58.6%, and the median progression-free survival was 12.4 months for all evaluable 29 patients[59]. While the response was not relevant to *PIK3CA* mutation, patients with PTEN negative, progesterone receptor (PgR) expression, or *TP53* mutation exhibited resistance to BKM120[59]. BKM120 is now under study for breast cancer therapy in phase III clinical trials. It also holds promise for the treatment of head and neck squamous cell carcinoma (HNSCC), non-small cell lung carcinoma (NSCLC), lymphoma and glioblastoma multiforme[60].

3.5. *BYL719*

BYL719 is another PI3K inhibitor developed by Novartis. Different from either NVP-BEZ235 or BKM-120, BYL719 is a PI3Kα specific inhibitor with an IC_{50} of 5 nmol/L[61]. *In vivo* results showed that BYL719 dose- and time-dependently inhibited the PI3K signaling, and exhibited robust antitumor efficacy with good

tolerability. PIK3CA was indicated to be the foremost predictive biomarker for sensitivity to BYL719[62]. A combination study reported that BYL719 could enhance the effect of protein kinase C inhibitor in G-protein mutant uveal melanoma cells[63]. Presently, BYL719 is under evaluation for breast cancer therapy in phase III clinical trial, and phase II study for treatment of NSCLC and HNSCC are ongoing.

3.6. GDC-0941

GDC-0941 is developed by Genentech *via* structural modification of PI-103[64], which was reported to have unfavorable pharmacokinetic property[65]. GDC-0941 inhibited PI3Kα, β, δ and γ with IC_{50} as 3, 33, 3 and 75 nmol/L, respectively. Weaker inhibition against mTOR with IC_{50} of 580 nmol/L suggested the PI3K inhibitory selectivity. GDC-0941 arrested cell cycle at G1 phase[66], and induced apoptosis in a subset of cancer cells[66]. As an orally administered agent, GDC-0941 exhibited significant *in vivo* antitumor efficacy on human cancer xenografts[64,66,67]. Furthermore, combination of GDC-0941 with trastuzumab, petuzumab, or chemotherapeutic drug docetaxel enhanced their antitumor efficacy[67]. GDC0941 is presently under evaluation for treatment of solid tumors and NHL in phase I clinical trials.

3.7. GDC-0980

GDC-0980 is also a PI3K inhibitor developed by Genentech. In contrast to GDC-0941, GDC-0980 also potently inhibits mTOR and therefore is described as a dual PI3K/mTOR inhibitor[40]. *In vitro* and *in vivo* results indicated that GDC-0980 exhibited potent growth inhibition against breast, prostate, and lung cancer but relatively less activity on melanoma and pancreatic cancers with *KRAS* and *BRAF* mutations[40]. Combinational use of GDC-0980 enhanced the antitumor activity of docetaxel[40]. Salphati et al. investigated the absorption and disposition of GDC-0980, and suggested 55 mg once daily might be a clinically efficacious dose. In phase I trials, modest but durable activity was shown in patients with advanced solid tumors[68]. GDC-0980 is now under evaluation in phase II clinical trials for therapy of kidney, prostate, endometrial and breast cancers[68].

3.8. SF1126

SF1126 is a prodrug of LY294002 by conjugation with Arg-Gly-Asp-Ser (RGDS), aiming to increase solubility and to target tumors *via* binding to specific integrins in the tumor microenvironment. Compared with the parent drug LY294002, SF1126 showed higher distribution in tumor tissues, and therefore more favorable *in vivo* antitumor efficacy without severe toxicity[69]. In addition, SF1126 exhibited anti-angiogenic activity, which might be attributed to the RGDS part since it targets the angiogenic integrins a$\nu\beta$3 and a5β1[69]. Moreover, combination of SF1126 with taxotere led to dramatic regression of PC3 tumors, superior to monotherapy using either taxotere or SF1126[69]. SF1126 is under investigation in phase I clinical trials for therapy of solid cancer, and relapsed or refractory neuroblastoma.

3.9. PX-866

PX-866 is a structure-modified compound based on classic PI3K inhibitor wortmannin, which failed to enter clinical trial due to the bad stability and liver toxicity[70]. PX-866 overcomes such defects and exhibits remarkable *in vivo* antitumor efficacy *via* both oral and i.v. administrations[70,71]. In contrast to most of the other novel PI3K inhibitors in clinical trials, PX-866 is an irreversible PI3K inhibitor. Interestingly, PX-866 was reported to show similar effect on tumors with *RAS* mutation which were regarded to be resistant to PI3K inhibitors, compared to those with wild type RAS[72]. PX-866 is presently evaluated for treatment of glioblastoma multiforme in phase II clinical trial.

3.10. PF-04691502

PF-04691502 was developed by Pfizer as an orally active, dual inhibitor of PI3K and mTOR[73]. *In vitro*, PF-04691502 inhibits proliferation of U87 (*PTEN* null) and SKOV3 (*PIK3CA* mutation) at sub-micromolar concentrations. Favorable antitumor efficacy was also confirmed in xenograft models of the above two cancer cells, as well as gefitinib- and erlotinib-resistant NSCLC cells[73]. Moreover, PF-04691502 can enhance TP53/p73 expression in human HNSCC xenograft with wild type TP53 and significantly inhibit tumor growth[74]. Phase I results indicated that oral administration of PF-04691502 was tolerable at 8 mg once daily[75]. PF-04691502 is now under evaluation in phase I clinical trials.

3.11. BAY 80-6946

BAY 80-6946 is a highly-selective pan-PI3K inhibitor developed by Bayer[76]. *In vitro*, BAY 80-6946 showed superior antitumor activity in breast cancer cell lines with PIK3CA and/or HER2 overexpression, compared to those with wild-type PIK3CA but without HER2. Distinct from most of the other novel PI3K inhibitors in clinical trials, BAY 80-6946 is administered intravenously. *In vivo*, BAY 80-6946 induced 100% complete tumor regression when dosed as a single agent every second day in rats bearing *HER2*-amplified and *PIK3CA*-mutant KPL4 breast tumors[76]. BAY 80-6946 is being evaluated in phase III clinical trial for therapy of B-cell lymphoma.

3.12. XL-765

Originally developed by Exelixis as a PI3K/mTOR inhibitor, XL-765 inhibits PI3Kα, β, δ and γ with IC_{50} values of 39, 383, 36 and 23 nmol/L, respectively, and also shows inhibition against mTOR with an IC_{50} of 157 nmol/L[77]. XL765 inhibits the phosphorylation of Akt, p70S6K, and S6 both *in vitro* and *in vivo* dose-dependently[78]. Besides, combination with autophagy inhibitor chloroquine showed efficacy on pancreatic adenocarcinoma[79], and co-treatment with temozolomide enhanced the activity of the latter on invasive pituitary adenoma xenograft model[80]. Presently, XL765 is under evaluation in phase I/II clinical trials for therapy of patients with malignant neoplasms[81].

3.13. XL147

Also first developed by Exelixis, XL-147 exhibits a comparable activity to XL-765 in terms of PI3K inhibition, but does not inhibit mTOR even at 10 μmol/L[77,82]. Like XL765, XL147 also showed potent antitumor efficacies in xenograft models of various human cancer cells, *via* blockade of PI3K/Akt pathway[78,83]. However, XL147 is less active than XL765 in hormone-insensitive prostate cancer models, which was attributed to its weak mTOR

inhibition[84]. Preliminary result in phase I clinical trial already showed that both XL147 and XL765 are tolerable[81,85]. XL147 is now under evaluation in phase I clinical trials for therapy of solid tumors as well as lymphoma.

3.14. GSK2126458

GSK2126458 was developed by GlaxoSmithKline as dual PI3K/mTOR inhibitor[86]. Durable responses were observed in its first-in-human phase I study in patients with solid tumors like sarcoma, kidney, breast, endometrial, oropharyngeal and bladder cancer[87]. Fasting insulin and glucose levels were indicated to act as pharmacodynamics biomarkers while *PIK3CA* mutation showed not predictive of response[87]. Additionally, combination with the MEK (MAPK/ERK kinase) inhibitor AZD6244 showed favorable efficacy on castration-resistant prostate cancer *via* blocking both the RAS/RAF/MEK/ERK and PI3K/Akt/mTOR pathways simultaneously[88]. Presently, GSK2126458 is undergoing phase I clinical trials for therapy of solid tumors, idiopathic pulmonary fibrosis and lymphoma.

3.15. ZSTK474

ZSTK474 is an *S*-triazine derivative synthesized by Zenyaku as an anticancer drug candidate together with more than 1500 other analogs[89]. It was identified as a PI3K inhibitor by Compare Analysis using the JFCR39 cancer cell line panel coupled with a drug-activity database[90,91]. ZSTK474 competed with ATP

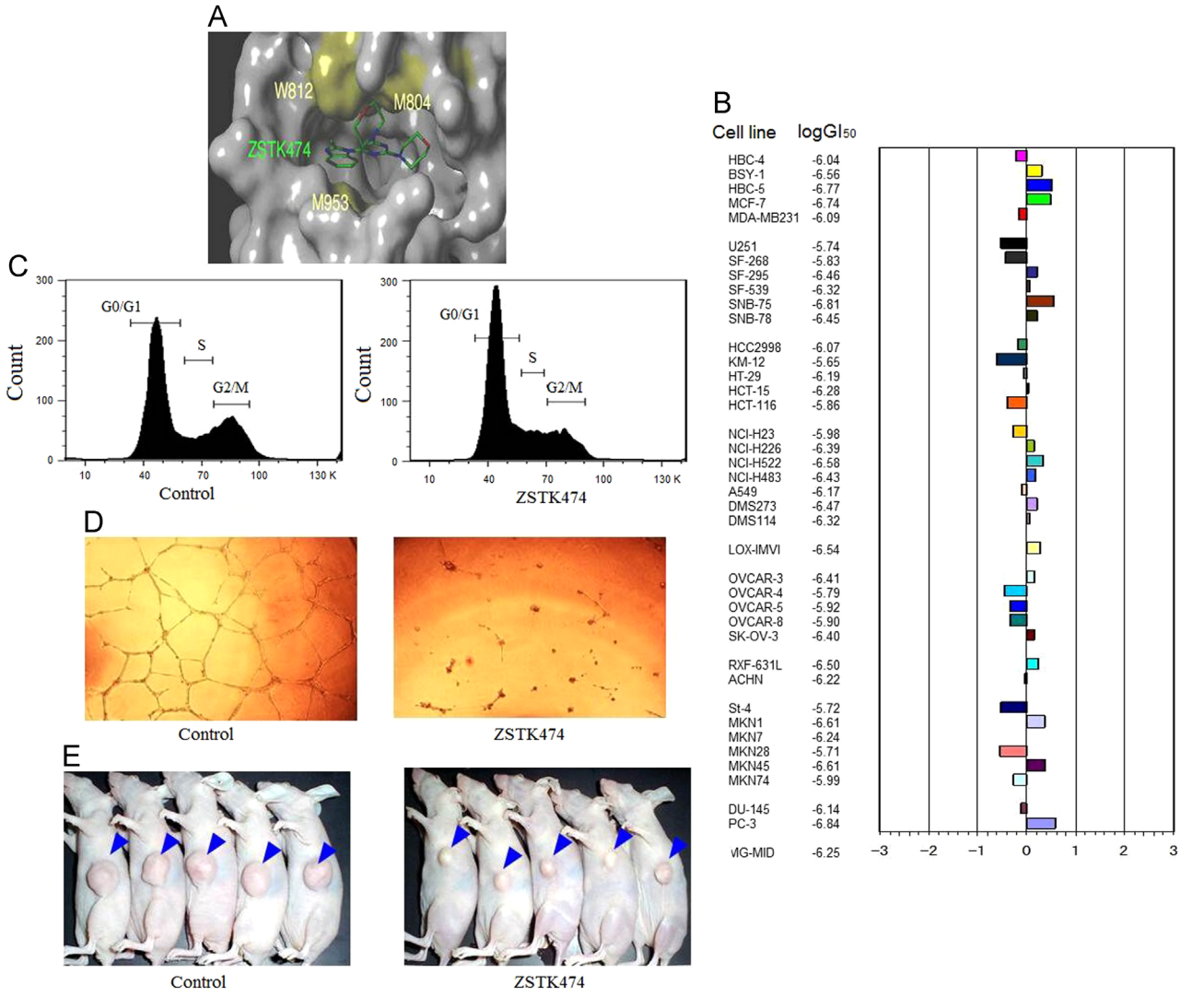


Figure 3 Antitumor activities of ZSTK474. (A) ZSTK474 inhibits PI3K by occupying the ATP binding pocket[89]. (B) Fingerprint of ZSTK474 for the JFCR39 panel. Fingerprint indicates the differential growth inhibition pattern of ZSTK474 for the cell lines in JFCR39 panel. The X-axis shows difference in logarithmic scale between the mean of $\log GI_{50}$ values for all 39 cell lines (MG-MID, expressed as 0 in the fingerprint) and the $\log GI_{50}$ for each cell line in JFCR39 panel. Columns to the right of 0 indicate the sensitivity of the cell lines to a given compound and columns to the left indicate the resistance. MG-MID is mean of $\log GI_{50}$ values for all 39 cell lines[89]. (C) ZSTK474 induces G1 arrest in breast cancer MCF-7 cells[94]. (D) *In vitro* antiangiogenic effect of ZSTK474. ZSTK474 potently blocks the *in vitro* tube formation by HUVECs[95]. (E) *In vivo* antitumor efficacy of ZSTK474. Oral administration of ZSTK474 at 400 mg/kg to WiDr xenograft daily from day 0 to 26, except for days 6, 13 and 20, leads to obvious tumor growth inhibition[90].

(Fig. 3A) in inhibiting all four PI3K isoforms, with IC_{50} values of 16, 44, 5 and 49 nnol/L for PI3Kα, β, δ and γ, respectively, suggesting that it is a pan-PI3K inhibitor[92]. However, it showed far weaker inhibitory activity against mTOR and DNA-PK, compared to the PI3K inhibition[52,53,92]. Furthermore, it did not inhibit a panel of 139 protein kinases[90]. *In vitro*, ZSTK474 inhibited the growth of 39 human cancer cell lines with a mean 50% growth inhibition (GI_{50}) value of 0.32 μmol/L[90] (Fig. 3B), and blocked cell cycle progression at G1 phase in various human cancer cells (Fig. 3C)[90,93,94]. The G1 arrest effect might be attributed to downregulation of cyclin D1 and upregulation of p27[93,94]. Moreover, ZSTK474 showed anti-angiogenic effect *in vitro* and *in vivo*[95] (Fig. 3D). The *in vivo* anti-angiogenic effect was attributed to its dual inhibition mechanism: inhibition of VEGF secretion in cancer cells and direct inhibition of PI3K in endothelial cells[96]. In addition, ZSTK474 inhibited migration and invasion of prostate cancer cell PC3, and blocked the phosphorylation of Girdin and production of MMPs, suggesting the anti-metastatic activity[34]. Like other novel PI3K inhibitors such as NVP-BEZ235, BKM120 and GDC-0941, ZSTK474 inhibits both mutant PIK3CA and the wild type one[56]. While it does not induce obvious apoptosis generally, it indeed induces autophagy in MCF-7 breast cancer cells[94]. As an orally administered pan-PI3K inhibitor, ZSTK474 showed potent *in vivo* antitumor efficacy on cancer xenografts at both early and advanced stages (Fig. 3E), without obvious toxicity being observed[90,93,95]. However, acquired resistance after long term treatment of ZSTK474 has been observed, which might be attributed to over-expression of insulin-like growth factor 1 receptor (IGF1R)[95]. In addition, combination with imatinib exhibited synergic antileukemia activity on chronic myeloid leukemia K562 cells as well as adriamycin-resistant K562/A02 cells[96]. ZSTK474 is now under evaluation in phase I/II clinical trials for treatment of solid tumors.

4. Discussion

An exciting race for development of PI3K inhibitor as anticancer drug candidate between pharmaceutical companies has lasted since 2006 when ZSTK474 was first reported[90]. Currently, over a dozen of PI3K inhibitors are still in clinical trials for cancer therapy. Among them, there are PI3K isoform specific inhibitors, pan-PI3K inhibitors, dual PI3K/mTOR inhibitors, as well as a prodrug of PI3K inhibitor. Interestingly, the main companies involved in the development of PI3K inhibitors, including Novartis, Genentech, as well as Sanofi (Table 1), have various types of PI3K inhibitors evaluated in clinical trials. The reason could be to maximize chances of clinical success because a key question still remains to be answered: what is the most effective and tolerable inhibition profile of an inhibitor targeting PI3K superfamily? That is, whether an isoform-specific PI3K inhibitor is superior to that targeting all PI3K isoforms, and whether dual inhibition of PI3K and mTOR is superior to specifically targeting PI3K. The PI3Kδ-specific inhibitor idelalisib has been approved for treatment of acute lymphocytic leukemia (ALL), suggesting that at least targeting PI3Kδ is enough for lymphoma and leukemia therapy. Considering that the existence of PI3Kδ is mainly restricted to hemopoietic cells, inhibitors specifically targeting this isoform might be preferred to avoid possible metabolic side effects due to inhibition of PI3Kα and β. On the other hand, since the PI3Kα-specific inhibitor BYL719, the pan-PI3K inhibitor BKM120 and the dual PI3K/mTOR inhibitor GDC-0980 have passed phase I clinical trials, the tolerability might not be a problem for PI3K inhibitors with or without PI3Kα isoform specificity as well as PI3K/mTOR selectivity. Regarding the selectivity over mTOR, it was reported that additional inhibition of mTOR is required for the sensitivity to PI3Kα specific inhibitor BYL719 in *PIK3CA* mutant breast cancer[97], although clinical evidence is needed. To answer the question of what is the most desirable type of PI3K inhibitor, the tumor lineage and the genetic status of cancer should also be considered. As an example, while many reports suggest targeting PI3Kβ could treat PTEN negative cancers, one study showed that PI3Kβ-specific inhibitors had no effect on endometrial cancer (EEC) cell lines[98]. In any case, the more results of clinical trials are reported, the more we will know about which type of PI3K inhibitor is most favorable for specific types of cancer.

Biomarker development has become tremendously important for precision therapy of cancer. Predicative biomarker can help to identify patients who are most likely to benefit from specific treatments, thereby optimizing personalized medical treatment. While some preclinical reports indicated that *PIK3CA* mutation could serve as predictive biomarkers for treatment with various types of PI3K inhibitors[39,40,62,70], contrary conclusions have also been obtained in other reports[54,56]. Moreover, early clinical trial results do not support the conclusion as well[99].

Since PI3K inhibitors are generally cytostatic but not cytotoxic agents, their antitumor efficacy should be tumor stabilization rather than tumor regression in patients with advanced solid tumors. On the other hand, besides PI3K, Akt can be activated by other kinases like Ack1, TBK1 and DNA-PK[100]. In addition, it was reported that PI3Kα inhibition led to rebound of PIP3, which was produced by the PI3Kβ isoform[101]. Therefore, combination of PI3K inhibitors with standard chemotherapeutic drugs, radiation therapy, as well as other molecular-targeted drugs should increase efficacy. This conclusion is supported by growing evidence. For example, when combined with paclitaxel, a lower dose of BAY 80-6946 was sufficient to induce complete tumor regression in mice[76]. In contrast to a single use of PF-04691502 which failed to stop tumor progression, combination with MEK inhibitor PD-0325901 led to obvious tumor regression in a model with *KRAS* mutation[102].

A large body of scientific work has demonstrated that targeting PI3K is an important cancer therapy strategy. The ongoing clinical evaluation on PI3K inhibitors is expected to provide a new class of molecular targeted anticancer drugs.

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