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Abstract

Pancreatic ductal adenocarcinoma (PDAC) has one of the worst prognoses among all malignancies. PI3K/AKT/mTOR signaling pathway, a main downstream effector of KRAS is involved in the regulation of key hallmarks of cancer. We here report that whole-genome analyses demonstrate the frequent involvement of aberrant activations of PI3K/AKT/mTOR pathway components in PDAC patients and critically evaluate preclinical and clinical evidence on the application of PI3K/AKT/mTOR pathway targeting agents. Combinations of these agents with chemotherapeutics or other targeted therapies, including the modulators of cyclin-dependent kinases, receptor tyrosine kinases and RAF/MEK/ERK pathway are also examined. Although human genetic studies and preclinical pharmacological investigations have provided strong evidence on the role of PI3K/AKT/mTOR pathway in PDAC, clinical studies in general have not been as promising. Patient stratification seems to be the key missing point and with the advent of biomarker-guided clinical trials, targeting PI3K/AKT/mTOR pathway could provide valuable assets for treatment of pancreatic cancer patients.

Abbreviations

CAFsCancer-associated fibroblastsCLLChronic lymphocytic leukemiaCRComplete responseCDKN2ACyclin-dependent kinase inhibitor 2ACDKsCyclin-dependent kinasesDPC4Deleted in Pancreatic Cancer-4DEPTORDEP-domain-containing mTOR-interacting proteinEGFREpithelial growth factor receptorENTHEpsin n-terminal homologyECMExtracellular matrixFLFollicular lymphomaGLIGlioma-associated oncogeneGPCRsG-protein coupled receptorsGAPGTPase activating proteinHhHedgehogHIF1αHypoxia inducible factor 1 alphaHAHyaluronic acidInsRInsulin receptorIGF-IRInsulin-like growth factor receptor 1LAMLymphangioleiomyomatosismLST8Mammalian lethal with Sec13 protein 8mSIN1Mammalian stress-activated protein kinase interacting proteinMETMesenchymal-epithelial transition tyrosine kinase receptorSMAD4Mothers against decapentaplegic homolog 4mTORMechanistic target of rapamycinmTORC1mTOR complex 1mTORC2mTOR complex 2TORKIsmTOR kinase inhibitorsORRObjective response rateOSOverall survivalPDACPancreatic ductal adenocarcinomaPNETsPancreatic neuroendocrine tumorsPSCsPancreatic stellate cellsPRPartial responsePXPhagocytic oxidasePTENPhosphatase and tensin homologPI3KPhosphoinositide 3-kinasePIP2Phosphoinositide- 4,5-bisphosphatePIP3Phosphoinositide-3,4,5-trisphosphatePHPleckstrin homologyPRAS40Proline-rich AKT substrate 40 kDaFKBP12Protein FK506 binding proteinPKBProtein kinase BRictorRapamycin-insensitive companion of mTORRaptorregulatory protein associated with mTORRheb GTPaseRas-related small G protein Ras homologue enriched in brainRTKsReceptor tyrosine kinasesTregRegulatory TSPARCSecreted protein acidic and rich in cysteineSLLSmall lymphocytic lymphomaSMOSmoothenedSREBPsterol regulatory element-binding proteinSDStable diseaseTel2Telomere maintenance 2TMETumor microenvironmentPTCH1Transmembrane receptor Patched 1TSC2Tuberous sclerosis complex 2TP53Tumor protein p53

Keywords

Personalized medicineKinase inhibitorsGastrointestinal tumorsEverolimusIdelalisib

1. Introduction

Pancreatic cancer is a highly fatal malignancy with one of the poorest prognoses among solid tumors. It is ranked as the seventh cause of cancer related mortality in the world (Siegel et al., 2021, Bray et al., 2018). In the United States, pancreatic cancer is the fourth leading cause of cancer death and it is predicted to become the second cause of mortality in 2040 (Siegel et al., 2021, Rahib et al., 2021). The 5-year survival rate for patients diagnosed with local and advanced disease are 37% and 3%, respectively. Since 80–85% of patients are diagnosed at an advanced stage, the overall 5-year survival amounts to the very low number of 10% (Society, 2020, https://www.cancer.net/cancer-types/pancreatic-cancer/statistics,).

1.1. Pathology of pancreatic cancer

Pancreas is anatomically located behind the stomach and the majority of its mass consists of the exocrine parts, which secrete digestive zymogens. Endocrine islet cells make a minor part of the pancreas, mainly secreting insulin and glucagon hormones. The majority of pancreatic cancers originate from exocrine part, giving rise to acinar cell carcinoma and pancreatic ductal adenocarcinoma (PDAC). PDAC is the most common pathology constituting more than

85% of pancreatic cancer cases (Ryan et al., 2014). Pancreatic neuroendocrine tumors (PNETs), which originate from islet cells accounting for about 10% of pancreatic neoplasms (https://www.cancer.net/cancer-types/pancreatic-cancer/statistics,, Hezel et al., 2006, Mostafa et al., 2017). This review is mainly focused on PDAC and unless expressed otherwise, all presented data are related to PDAC.

One important feature of PDAC is its dense stroma, making a peculiar microenvironment for cancer cells that is thought to be involved in several biological characteristics such as drug resistance. This distinctive dense stroma that surrounds cancer cells consists of various non-neoplastic cells such as cancer-associated fibroblasts (CAFs) and immune cells in addition to acellular matrix components including collagen, fibronectin, hyaluronic acid (HA), and matricellular proteins such as SPARC (secreted protein acidic and rich in cysteine), periostin, and tenascin C (Erkan et al., 2012a, Erkan et al., 2012b). PDAC stroma may act as a barrier of chemotherapy delivery to cancer cells being involved in drug resistance (Neesse et al., 2019, Vennin et al., 2018). Recently, some stromal components such as HA have been the target of a number of anti-stromal therapies, which however failed in the clinical setting (Hingorani et al., 2018, Ebelt et al., 2020, Hakim et al., 2019). This failure implies that attacking desmoplasia alone is not enough and although the stroma may act as a physical barrier limiting drug delivery, it might also paradoxically provide protective effects in restraining cancer growth and progression (Hakim et al., 2019).

1.2. Genetic alterations in pancreatic cancer

About 5–10% of pancreatic cancer cases are caused by germline mutations associated with familial syndromes such as Peutz-Jegher syndrome, Familial atypical multiple mole and melanoma syndrome, Lynch syndrome, Hereditary breast and ovarian cancer syndrome and Familial adenomatous polyposis syndromes (Nelson and Walsh, 2020, Peters et al., 2016, Solomon et al., 2012, Benzel and Fendrich, 2018).

On the other hand, somatic and also germ line mutations are associated with sporadic cases of PDAC. An average of nearly 60 genetic alterations per tumor, majority being point mutations, have been detected by PDAC genomes evaluations (Nelson and Walsh, 2020, Jones et al., 2008, Thillai et al., 2017). In particular, it has been well established that mutations in KRAS oncogene as well as a number of tumor suppressor genes such as CDKN2A (cyclin dependent kinase inhibitor 2 A), SMAD4 (Mothers against decapentaplegic homolog 4) and TP53 (tumor protein p53) are mechanistically related to the emergence of PDAC (Jones et al., 2008, Biankin et al., 2012, Sausen et al., 2015). KRAS mutations have been found in 92% of PDAC patients, most of them occurring in codon 12 (G12D) and less frequently in codons 13 (G13D) and 61 (Q61H) (Witkiewicz et al., 2015a). KRAS mutations lead to the constitutive activation of RAS-RAF and PI3K-AKTsignalling pathways, leading to the alterations of the cell cycle progression, survival, etc (Witkiewicz et al., 2015a, Mizrahi et al., 2020).

Largescale genomic analyses of pancreatic cancers have shown that several tumor suppressors including CDK2NA, TP53 and SMAD4 undergo inactivating mutations and hypermethylation in a large number of pancreatic tumors (Peters et al., 2016, Sausen et al., 2015, Makohon-Moore and Iacobuzio-Donahue, 2016, Chiorean and Coveler, 2015, Singh and

O'Reilly, 2020). CDKN2A (cyclin-dependent kinase inhibitor 2A) codes for two proteins named p16 and p14 from INK4 family that act as CDK4/6 inhibitors and regulate cell cycle progression (GeneCards, 2021). Loss of function mutations are found in about 90% of early-stage pancreatic cancers (Nelson and Walsh, 2020). P53, the protein product of TP53 tumor suppressor gene, is activated when DNA damage repair mechanisms fall short of fixing the impairment and leads to cell cycle arrest and apoptosis. TP53 mutations have been reported in about 66–90% of advanced pancreatic cancers (Nelson and Walsh, 2020, Makohon-Moore and Iacobuzio-Donahue, 2016, Chiorean and Coveler, 2015). Another tumor suppressor gene that is inactivated in approximately 50% of high-grade pancreatic cancers is SMAD4 (Mothers against decapentaplegic homolog 4) also known as DPC4 (Deleted in Pancreatic Cancer-4). SMAD4 serves as a mediator of TGF-β signal transduction that regulates cell cycle arrest and apoptosis (Chiorean and Coveler, 2015, Schlieman et al., 2003).

In a recent seminal study on families with BRCA1/2 (Breast cancer type 1/2 susceptibility protein) pathogenic variants, it was clearly shown that these variants significantly increase the risk of PDAC. PDAC was indeed the cancer type with the second and third highest risks associated with BRCA1 and BRCA2 pathogenic variants, with RRs of 2.36 (95% CI, 1.51–3.68) and 3.34 (95% CI, 2.21–5.06), respectively (Li et al., 2022).

1.3. Pharmacological therapy of pancreatic cancer

A large number of PDAC patients have metastasized at the time of diagnosis. This is largely cause by the fact that the symptoms are typically nonspecific and appear very late when the tumor is in an advanced stage. This feature in addition to inherent nature of cancer cells for early metastasis makes PDAC treatment very challenging (Mizrahi et al., 2020, Giovannetti et al., 2017).

PDAC patients, according to a four-tiered staging system based on tumor respectability, can be divided into resectable, borderline resectable, locally advanced, and metastatic categories (Bockhorn et al., 2014, Varadhachary et al., 2006). A surgical resection with post- or pre-operative chemotherapy may offer curative potential for resectable and borderline resectable patients, however, the majority of cases experience recurrent disease following surgical resection (Acher et al., 2018).

Gemcitabine-based therapies have been standard treatments for PDAC patients since a long time ago and may still be used as single therapy for non-advanced tumors (Burris et al., 1997). Currently, two standard systemic chemotherapeutic combinations including FOLFIRINOX (5-fluorouracil, folinic acid (McRee et al., 2015), irinotecan, and oxaliplatin) (Conroy et al., 2011, Conroy et al., 2018) and gemcitabine plus nab-paclitaxel (Von Hoff et al., 2013) are reported to increase the overall survival of patients with advanced PDAC (Singh and O'Reilly, 2020).

In a phase III study in 2007, an improvement in median overall survival was reported after combination therapy with gemcitabine and erlotinib, a small molecule tyrosine kinase inhibitor mainly targeting the EGFR (epithelial growth factor receptor) (Moore et al., 2007). Erlotinib then became the first FDA approved targeted therapy for treatment of locally advanced, unresectable, or metastatic pancreatic cancer. However, the median survival difference between the two arms (gemcitabine plus erlotinib vs gemcitabine plus placebo) was only 2 weeks, raising the question on whether a statistically significant difference

between different therapies is always clinically meaningful. More recently, olaparib, a PARP inhibitor, has received FDA approval for pancreatic cancer patients harboring germline BRCA1 or BRCA2 mutations, presenting the first biomarker-based targeted therapy approved for pancreatic cancer (FDA, 2019). However, recent tumor-agnostic approvals have paved the way for additional targeted therapies in advanced PDAC, such as the tropomyosin receptor kinase inhibitors larotrectinib and entrectinib, which have been FDA approved for NTRK (Neurotrophic tyrosine receptor kinase) fusion–positive cancers, having demonstrated response rates greater than 75% independent of tumor histology. Although rare (<1% of cases) NTRK gene fusions are indeed oncogenic drivers in PDAC (O'reilly and Hechtman, 2019).

Similarly, although a rare event in PDAC (about 0.8% of cases), patients with microsatellite instability-high (MSI-H) tumors can have remarkable benefit from the programmed cell death protein (PD)− 1 targeting antibody pembrolizumab, which is indeed approved for the treatment of adult and pediatric patients with unresectable or metastatic MSI-H solid tumors (Eso and Seno, 2020). These studies suggest that performing molecular profiling on PDAC can help identify potential new treatments, though randomized controlled trials are needed in order to determine whether molecular profiling in metastatic PDAC is cost-effective and whether it could improve survival. Several targeted therapies with promising outlook are under intense investigation in PDAC patients (Pecoraro et al., 2021a).

2. PI3K/AKT/mTOR signaling pathway

PI3K (phosphoinositide 3-kinase)/AKT/mTOR (mechanistic target of rapamycin kinase) pathway regulates several aspects of cellular function including survival, growth, migration and metabolism (Fig. 1) (Manning and Toker, 2017, Noorolyai et al., 2019, Hoxhaj and Manning, 2019). PI3K/AKT/mTOR is a key downstream effector pathway of RAS, and as stated earlier, RAS activation is the most prominent genetic alteration in pancreatic cancer. Although novel RAS inhibitors, such as sotorasib, have been recently approved for malignancies such as lung cancer (Skoulidis et al., 2021), these agents generally target the G12C mutant, which is rare in PDAC appearing in only about 1% of cases (Nollmann and Ruess, 2020). Therefore, targeting PI3K/AKT/mTOR, as a key downstream signaling pathway, could be considered as a more efficient therapeutic option for PDAC (Nollmann and Ruess, 2020).

PI3K is a lipid kinase that upon being activated by upstream signals received from a variety of RTKs (receptor tyrosine kinases) or GPCRs (G-protein coupled receptors), catalyzes the conversion of a cell membrane phospholipid named PIP2 (phosphoinositide- 4,5-bisphosphate) to PIP3 (phosphoinositide-3,4,5-trisphosphate) (Hirsch et al., 2020) (Fig. 1). Proteins harboring PH (pleckstrin homology), PX (phagocytic oxidase) and ENTH (epsin n-terminal homology) domains are able to bind with PIP3. AKT, for example, possesses one PH domain that specifically binds to PIP3 with high affinity and re-localizes to the inner plasma membrane. The resulting conformational change of AKT leads to its phosphorylation and subsequent activation (Manning and Toker, 2017, Takeuchi et al., 1997, Stahelin, 2009). This cascade, directly or indirectly, results in the activation of several downstream molecular

targets such as mTOR, GSK3β, FOXO1, Bcl2 family proteins, etc (Takikawa and Ohki, 2017, Song et al., 2019, Shariati and Meric-Bernstam, 2019).

On the other hand, PTEN (phosphatase and tensin homolog) acts as an important negative regulator that controls intracellular levels of PIP3. PTEN gene is an important tumor suppressor that is mutated in several types of cancer (Jamaspishvili et al., 2018, Alfieri et al., 2017). It was initially classified as a protein tyrosine phosphatase, but later it was revealed to be mostly a lipid phosphatase mainly engaging with PIP3 and also with non-enzymatic actions functioning as a scaffold protein (Lee et al., 2018). PTEN specifically catalyzes the dephosphorylation of PIP3 converting it back to PIP2 (Maehama and Dixon, 1998, Hoxhaj and Manning, 2020) (Fig. 1). The back conversion of PIP3 to PIP2 acts like a brake on the progress of PI3K/AKT/mTOR pathway and plays an important role in the balance of oncogenic processes in the cell (Hoxhaj and Manning, 2020, Cantley and Neel, 1999, Falasca et al., 2011).

2.1. PI3K signaling

There are three classes of PI3Ks with structural and functional differences, which belong to the family of lipid kinases (Table 1). Class I PI3Ks catalyze the production of PIP3 from PIP2. Moreover, Class II converts PI to PIP, while class III PI3Ks catalyzes the conversion of PIP to PIP2 (Fig. 1) (Fruman et al., 2017, Gulluni et al., 2019, Gozzelino et al., 2020).

Class I PI3Ks, divided into IA and IB subsets, are composed of a catalytic subunit of 110 kDa.

(p110) and a regulatory subunit of 85 kDa (p85). Class IA and IB PI3Ks are activated by RTKs and GPCRs, respectively, but both convert PIP2 into PIP3 upon activation (Fig. 1). The catalytic subunit of class IA has three p110α, β or δ variants, encoded by separate genes named PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha), PIK3CB (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta) and PIK3CD (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta), respectively. There are also three variants of the p85 regulatory subunit including p85α, p85β, and p55γ (Table 1) (Thillai et al., 2017, Fruman et al., 2017, Cantley, 2002, Fruman and Rommel, 2014). The p85 subunits contain SH2 and SH3 domains which bind preferentially to phosphorylated tyrosine residues (Fruman et al., 2017, Songyang et al., 1993, Yoakim et al., 1994). One catalytic subunit p110γ, encoded by PIK3CG, and two regulatory subunits p101 and p87/p84 comprise the class IB PI3Ks (Thillai et al., 2017, Fayard et al., 2010, Jiang et al., 2020, Vanhaesebroeck et al., 2012). Class II and III have different structures and functions from class I possessing also kinase-independent roles (Martini et al., 2018, Gulluni et al., 2017). PIK3C2A, PIK3C2B and PIK3C2G are three monomeric isoforms of class II PI3K, that unlike classes I and III, have no regulatory subunits (Jean and Kiger, 2014). Class II play various roles in cellular processes such as migration, glucose transport, insulin signaling, channel regulation, endocytosis and exocytosis (Mazza and Maffucci, 2011, Falasca and Maffucci, 2012). Class III composed of a Vps34 catalytic subunit and a Vps15/p150 regulatory subunit. It is involved in regulation of endocytic trafficking, phagocytosis, cytokinesis and nutrient sensing mechanisms (Backer, 2016).

2.2. AKT signaling

AKT, also known as protein kinase B (PKB), is a serine/threonine protein kinase of AGC kinase family that plays a key role in multiple cellular processes such as survival, proliferation and inhibition of apoptosis (Fig. 2) (Manning and Toker, 2017). AKT has three isoforms encoded by three different AKT1, AKT2 and AKT3 genes (Manning and Toker, 2017, Manning and Cantley, 2007). All three AKT isoforms possess three evolutionarily conserved domains including an N-terminal pleckstrin homology (PH) domain, a C-terminal regulatory domain (AGC kinase C terminal) and a central kinase catalytic domain between the two regulatory domains (Manning and Toker, 2017, Mundi et al., 2016, Feng et al., 2004). The PH domain binds to the phosphoinositides such as PIP3 at the plasma membrane with high affinity leading to AKT conformational change, and subsequent phosphorylation and activation. Indeed, the conformational changes increase AKT phosphorylation on two conserved Thr308 and Ser473 residues by PDK1 and mTORC2, respectively (Feng et al., 2004, Carmona et al., 2016, Brown and Banerji, 2017, Scheid and Woodgett, 2003).

2.3. mTOR signaling

mTOR is a protein kinase from the PI3K-related kinase family, heavily involved in cell growth, proliferation and survival mainly by enhancing anabolic processes such as protein synthesis on the one hand and suppressing catabolic processes including autophagy on the other (Hua et al., 2019, Kim and Guan, 2019, Mossmann et al., 2018). mTOR is evolutionarily conserved and serves as the catalytic subunit of two distinct multi-protein complexes termed mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) that have sophisticated functions in detecting nutrient availability and regulation of cell metabolism (Betz and Hall, 2013). These complex functions cause mTOR dysregulations to be involved in several diseases including diabetes, neurodegeneration and cancer (Mossmann et al., 2018, Liu and Sabatini, 2020).mTOR protein together with Raptor (regulatory protein associated with mTOR), and mLST8 (mammalian lethal with Sec13 protein 8, also known as GβL), proline-rich AKT substrate 40 kDa (PRAS40) and DEP-domain-containing mTOR-interacting protein (DEPTOR) and Tel2 protein form the mTORC1 complex (Fig. 3). On the other hand, mTORC2 complex is composed of six different proteins including mTOR, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase interacting protein (mSIN1), PRAS40, mLST8, DEPTOR and Tel2 (Kim and Guan, 2019, Mossmann et al., 2018, Jacinto et al., 2006, Hara et al., 2002, Laplante and Sabatini, 2009). Telomere maintenance 2 (Tel2) is important for mTOR stability as well as assembly of the mTOR complexes to maintain their activities (Fig. 3) (Kaizuka et al., 2010).

mTORC1 mainly functions as a downstream effector of AKT, while mTORC2 acts as an upstream regulator of AKT, both contributing to nutrients metabolism such as lipogenesis and glucose metabolism (Hoxhaj and Manning, 2020) (Fig. 1). mTORC1 controls cell growth and metabolism through organization of protein anabolism, while mTORC2 mostly promotes cell survival and regulates apoptosis (Laplante and Sabatini, 2015, Saxton and Sabatini, 2017).

mTORC1 activation by AKT is done through phosphorylation and inhibition of TSC2 (tuberous sclerosis complex 2) protein. TSC2 functions as a GTPase activating protein (GAP), which inhibits the Rheb GTPase (Ras-related small G protein Ras homologue enriched in brain), an activator of mTORC1 (Kim and Guan, 2019). The activated mTORC1 enhances HIF1α and MYC genes expression. HIF1α protein play an important role in induction of aerobic glycolysis in tumor cells and may help the cell to adapt to nutrient and oxygen fluctuations (Hudson et al., 2002, Düvel et al., 2010). MYC protein, on the one hand, promotes aerobic glycolysis through induction of important glucose transporters and most glycolytic enzymes expression (Csibi et al., 2014, Stine et al., 2015). On the other hand, MYC together with SREBP (sterol regulatory element-binding protein) induce lipogenesis and lead to cancer cell growth (Gouw et al., 2019).

mTORC2 via the tyrosine kinase activity of mTOR is able to activate type I insulin-like growth factor receptor (IGF-IR) and insulin receptor (InsR) and therefore contribute in glucose metabolism (Yin et al., 2016, Hua et al., 2019). Overall, mTORC1 mostly adjusts cell growth and metabolism, while mTORC2 regulates proliferation and survival by phosphorylating several members of the AGC (PKA/PKG/PKC) family of protein kinases such as AKT (Fig. 1) (Jacinto et al., 2004, Sarbassov et al., 2004).

3. PI3K/AKT/mTOR pathway alterations in pancreatic cancer

Recognition of specific molecular alterations is a crucial factor for guiding biomarker-based targeted therapies and finding the best therapeutic strategies for individual cancer patients. As mentioned above, PI3K/AKT/mTOR pathway aberrant activation leading to cell proliferation, growth, inhibition of apoptosis, etc. have been found in many cancers (Martini et al., 2018, Janku et al., 2018, Xing et al., 2019, De Santis et al., 2019). It is estimated that amplifications, activating mutations or loss of regulating pathways of PI3K/AKT/mTOR components are present in a considerable number of several cancer types (Janku et al., 2018, Yuan and Cantley, 2008, Myers et al., 2020, Martini et al., 2014). Furthermore, as discussed earlier, a number of hereditary disorders caused by germline mutations in PI3K/AKT/mTOR pathway are associated with an increased risk of developing different cancer types including PDAC (Engelman, 2009, BurrisIII, 2013, Polivka and Janku, 2014).

We have recently shown that an adaptor protein, p130Cas, functions as an important downstream effector of KRAS that drives PI3K activity leading to acinar to ductal metaplasia, a crucial feature of tumorigenesis in PDAC (Costamagna et al., 2021). This study may also offer a rationale for the notion that high expression of p130Cas indicates a potential benefit from PI3K/AKT/mTOR pathway targeting (Costamagna et al., 2021).

Many studies have demonstrated the association between the PI3K/AKT/mTOR pathway alterations and emergence of PDAC (Yuan and Cantley, 2008, Sun et al., 2011, Liu et al., 2009, Edling et al., 2010, Murthy et al., 2018). We have shown that phospho-AKT may serve as a prognostic biomarker in PDAC (Massihnia et al., 2017). Genomic alterations analysis via next generation sequencing have detected PI3K/AKT/mTOR pathway aberrations in patients suffering from pancreatic cancer with a frequency of 19%, including PI3K mutations (3.7%) and AKT amplifications (2.8%) (Pishvaian et al., 2018). Most of these aberrations have been

listed as actionable molecular alterations, defined as genomic biomarkers that may predict the benefit from a specific targeted therapy (Pishvaian et al., 2020).

Analysis of The Cancer Genome Atlas (TCGA) database for cancer genomics shows that PIK3CA, KRAS and PTEN alterations constitute the six top ranked aberrations in all cancers. PIK3CA mutations are one of the most frequently mutated genes in pancreatic cancer with a rate of 2.81%. Additionally, TCGA data demonstrate that patients with pancreatic cancer harbor mTOR, PTEN, AKT1 and AKT2 mutations with 1.69%, 1.12%, 0.56% and 0.56% frequencies, respectively (https://portal.gdc.cancer.gov/).

On the other hand, independent studies have reported variable numbers for PIK3CA mutations occurring in about 11.1% (Schönleben et al., 2008), 11.7% (Weiss et al., 2013), 3% (Heestand and Kurzrock, 2015), 2.3% (Jiang et al., 2020) and 1.0% (Janku et al., 2013) of pancreatic cancer cases. Similarly, different values of AKT aberration in pancreatic cancer have been reported. AKT1 aberration in 2.2% (Jiang et al., 2020) of pancreatic cancer cases have been shown. Furthermore, AKT2 amplification has been observed in about 10% (Cheng et al., 1996), 20% (Ruggeri et al., 1998), 32% (Altomare et al., 2002) and 3% (Jiang et al., 2020) of pancreatic cancer cases.

In addition, it has been reported that pancreatic cancer emergence, recurrence, metastasis and shorter survival is correlated with low expression of PTEN, a negative regulator of AKT pathway. The loss of PTEN has been reported in about 70% (Ying et al., 2011) and 25.6% (Foo et al., 2013) of PDAC cases.

Collectively, aforementioned molecular alterations strongly suggest that PI3K/AKT/mTOR targeting agents may provide a remarkable therapeutic opportunity for pancreatic cancer patients.

4. PI3K/AKT/mTOR inhibitors

There are several general classes of agents targeting the PI3K/AKT/mTOR pathway. PI3K inhibitors include pan-class I and isoform-selective agents. Inhibitors of AKT, mTOR, as well as dual PI3K/mTOR inhibitors constitute other members of this group of diverse agents (Table 2 and Fig. 4) (Zhang et al., 2020, Duan et al., 2020, Thorpe et al., 2015, Meng et al., 2021).

4.1. Pan-PI3K inhibitors

Pan-PI3K inhibitors are ATP-competitive agents that act on all catalytic isoforms of class I PI3K, which are expected to have potential utility in the treatment of various leukemias as well as solid tumors. However, severe side effects and toxicities associated with these inhibitors impose some limitations on their clinical application (Yang et al., 2019). In September 2017, copanlisib was approved for relapsed follicular lymphoma (FL) who has received two or more systemic therapies (Markham, 2017).

4.2. Isoform-specific PI3K inhibitors

Isoform-specific inhibitors, a new generation of PI3K inhibitors, which target specific isoforms of PI3K, may offer superior efficacy with less off-target effects and toxicity compared to pan-

PI3K inhibitors (Fig. 4) (Yang et al., 2019). Several isoform-specific agents such as alpelisib, serabelisib, taselisib and linperlisib have been extensively examined in preclinical and clinical cancer studies, including breast (Mayer et al., 2017, Williams et al., 2020, Dent et al., 2021), lung (Langer et al., 2019, Krop et al., 2022), pancreatic (Soares et al., 2018), ovarian (Starks et al., 2021), and hematological malignancies (Jiang et al., 2021, Li et al., 2021) (Table 2).

To date, three isoform-selective PI3K inhibitors have been approved by the FDA (Table 2). Alpelisib (BYL719, NVP-BYL719) is a PI3Kα-specific inhibitor, approved for use in combination with endocrine therapy fulvestrant for hormone receptor (HR)-positive, human epidermal growth factor receptor-2 (HER2)-negative breast cancer with PIK3CA mutations (Markham, 2019). Duvelisib, the dual inhibitor of PI3Kδ/PI3Kγ, has received the FDA approval for relapsed or refractory chronic lymphocytic leukemia (CLL)/ small lymphocytic lymphoma (SLL) (Rodrigues et al., 2019). In addition, idelalisib (CAL101, GS1101), a specific inhibitor of the δ isoform, in combination with rituximab, was approved for the treatment of relapsed CLL or as monotherapy for relapsed follicular B-cell non-Hodgkin's lymphoma or relapsed SLL (Miller et al., 2015).

4.3. AKT inhibitors

Inhibiting AKT as the key effector node in the PI3K/AKT/mTOR pathway is an attractive therapeutic strategy and multiple AKT inhibitors have so far been developed or are being tested in clinical trials (Iida et al., 2020, Uko et al., 2020). Most AKT targeting agents in clinical development inhibit all 3 AKT isoforms AKT 1, 2, and 3, and are therefore termed as pan-AKT inhibitors (Fig. 4). Small molecule AKT inhibitors can be mainly divided into two classes; allosteric inhibitors and ATP competitive. Allosteric inhibitors such as perifosine, MK-2206 and vevorisertib interact with the PH domain of AKT, thereby preventing its crucial interaction with PIP3 which is anchored to plasma membrane. ATP competitive inhibitors including, capivasertib, uprosertib, and afuresertib bind to the ATP binding site in the kinase domain of the active AKT conformation (Brown and Banerji, 2017, Kang and Chau, 2020).

4.4. mTOR inhibitors

4.4.1. First-generation mTOR inhibitors (allosteric inhibitors)

Rapamycin (Rapamune, sirolimus) is a macrolide compound produced by Streptomyces hygroscopicus that forms a complex with the intracellular protein FK506 binding protein (FKBP12). The complex of rapamycin and FKBP12 binds to the FKBP-rapamycin binding (FRB) domain in the C-terminus of mTOR, becoming able to inhibit the catalytic activity of mTOR, specifically in mTORC1, but not in mTORC2 (Iriana et al., 2016). Rapamycin was first developed as an anti-fungal and immunosuppressant agent and later gained attention as a potential cytostatic compound. The clinical development of rapamycin as an anticancer agent was limited due to its poor water solubility and chemical stability. Everolimus (RAD001/Afinitor), temsirolimus (toricel), and ridaforolimus (MK-8669), also known as rapalogs, are semi-synthetic rapamycin derivatives, which display improved pharmacokinetic and pharmacodynamics properties compared with rapamycin (Martelli et al., 2018). Rapalogs have undergone clinical trials for various malignancies and have been clinically approved for the treatment of certain types of cancers (Table 2) (Conciatori et al., 2018, Popova and Jücker, 2021); everolimus was approved for HR–positive, HER2-negative, advanced breast cancer

based on results from the Breast Cancer Trials of Oral Everolimus-2 (BOLERO-2) study (Baselga et al., 2012), renal cell carcinoma (RCC) (Coppin, 2010), progressive neuroendocrine tumors of pancreatic origin (PNET) (Yao et al., 2011), and progressive, well-differentiated non-functional, neuroendocrine tumors (NET) of gastrointestinal (GI) or lung origin (Yao et al., 2016), as well as subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TSC) (Krueger et al., 2010), and renal angiomyolipoma (Capal and Franz, 2016). Sirolimus was approved as the first and only treatment for lymphangioleiomyomatosis (LAM) based on the Multicenter International LAM Efficacy of Sirolimus (MILES) trial (McCormack et al., 2011), while temsirolimus has been approved as the first-line treatment of metastatic RCC (Kwitkowski et al., 2010).

However, the inhibition of mTORC1 may lead to the activation of compensatory bypass pathways including up-regulation of PI3K/AKT and MEK/ERK as well as aberrant activation of several receptor tyrosine kinases (RTKs), such as IGF-1 receptor (IGF-1R), insulin receptor, and HER2, which could result in acquired resistance to rapamycin and its analogues (Martelli et al., 2018, Bergholz and Zhao, 2021).

4.4.2. Second-generation mTOR inhibitors (ATP competitive inhibitors)

A number of potent ATP-competitive inhibitors specific for the mTOR kinase active site, also called selective mTOR kinase inhibitors (TORKIs), have been developed to hamper the activity of both mTORC1 and mTORC2, thus preventing the feedback activation of oncogenic pathways, including PI3K/AKT by mTORC2, and avoiding the reported resistance to rapalogs (Popova and Jücker, 2021, Chiarini et al., 2019) (Fig. 4). Some of TORKIs, including vistusertib, sapanisertib, and onatasertib are already used in clinical studies for different types of cancers (Morscher et al., 2021, Voss et al., 2020, Koca et al., 2021, Al-Kali et al., 2019, Wolin et al., 2019, MacDonald et al., 2019); however, they are still not approved by the FDA.

4.4.3. Third-generation mTOR inhibitors (RapaLink-1)

Recently, it was found that tumors harboring mutations in mTOR would be resistant to treatment with TORKI. Therefore, Rapalink-1, a third-generation mTOR inhibitor, was developed from linking rapamycin with MLN0128, a second-generation mTOR inhibitor. In consequence, Rapalink-1 simultaneously acts as an allosteric inhibitor of mTORC1 via the FRB domain, and also blocks the ATP-binding pocket of the active site of mTOR (Xu et al., 2020). It has been shown that this new class of mTOR-targeted agents provides a promising treatment strategy for future therapy of cancer patients (Fan et al., 2017, Rodrik-Outmezguine et al., 2016, La Manna et al., 2020).

4.5. PI3K/mTOR dual inhibitors

A strategy for overcoming rapalogs-induced activation of upstream molecules could be the use of PI3K/mTOR dual inhibitors (Mayer and Arteaga, 2016). Dual PI3K-mTOR blockade inhibits structurally similar PI3K and downstream mTOR kinase domains by binding to ATP binding sites of these enzymes. Therefore, targeting critical nodes of the same pathway could likely lead to better anticancer activity and also could overcome drug resistance upon single inhibitors treatment (Tarantelli et al., 2020, LoRusso, 2016). Several compounds in this class, including dactolisib, voxtalisib, and samotolisib have been or are being tested in preclinical

and clinical settings (Table 2) (Brown et al., 2018, Liu et al., 2019, Salazar et al., 2018, Schötz et al., 2020, Bendell et al., 2018, Rubinstein et al., 2020).

5. Targeting PI3K/AKT/mTOR pathway in pancreatic cancer

Although a large number of small molecule inhibitors targeting the PI3K/AKT/mTOR pathway have been evaluated in the preclinical setting (Liu et al., 2021, Brown et al., 2020, Wang et al., 2020, Awasthi et al., 2019, Weisner et al., 2019, Rumman et al., 2016, Ning et al., 2017, Ahn et al., 2018, Mao et al., 2018, Xu et al., 2019) (Supplemental Table 1), only a limited number of them have found their way to clinical studies as single agent therapies for pancreatic cancer (Javle et al., 2010, Devarakonda et al., 2021, Doi et al., 2017, Ando et al., 2019) (Table 3).

As for PI3K inhibitors, several compounds such as LY294002 (Mao et al., 2018), HS-173 (Rumman et al., 2016), gedatolisib (Venkatesan et al., 2010) have shown their effectiveness in cellular and animal models of pancreatic cancer. Moreover, in phase I clinical trials, two PI3K inhibitors, copanlisib and alpelisib, previously approved for follicular lymphoma and breast cancer, respectively (Markham, 2017, Markham, 2019) showed evidence of disease control with a manageable safety profile among Japanese patients with advanced solid tumors including pancreatic cancer (Doi et al., 2017, Ando et al., 2019).

Among AKT inhibitors, perifosine and MK-2206 have been the most studied compounds aimed for pancreatic cancer targeted therapy. Perifosine (also KRX-0401) is the first lipid-based AKT inhibitor that entered clinical development. It is an alkyl phospholipid, which localizes in the plasma membrane and inhibits AKT activation by interfering with the interaction between AKT and phospholipids such as PIP3 (Brown and Banerji, 2017, Zitzmann et al., 2012, Hideshima et al., 2006). Several studies have reported the antiproliferative effects of perifosine against pancreatic cancer cells as monotherapy or in combination with chemotherapeutic drugs (Massihnia et al., 2017) and also against neuroendocrine tumor cells (Zitzmann et al., 2012). However, two small phases II clinical trials with perifosine in PDAC patients were halted prematurely, because of safety reasons and the lack of evidence on the efficacy (Marsh et al., 2007, Hedley et al., 2005). MK-2206 is another allosteric pan-AKT inhibitor that has shown promising results in preclinical in vitro and in vivo studies (Massihnia et al., 2017, Wang et al., 2020, Awasthi et al., 2019, Awasthi et al., 2012). However, when further evaluated, this agent has yielded disappointing results in human studies (Chung et al., 2017, Murphy et al., 2020).

mTOR inhibitors have also been evaluated as monotherapy for PDAC. In an early phase II trial, different dosing schedules of everolimus did not show any significant improvement in efficacy and survival in 33 gemcitabine-refractory metastatic patients. Stable disease was the best response observed in 21% of patients, while no patient experienced complete response (CR) or partial response (PR) (Wolpin et al., 2009). Likewise, no clinically relevant antitumor effect was found in another phase II study, in which everolimus or temsirolimus were used in PDAC patients (Javle et al., 2010). Following these studies, and others (Kim et al., 2017, Tabernero et al., 2008), evaluating mTOR inhibition as monotherapy in PDAC with disappointing results,

several investigators have pursued combination treatments with other cytotoxic or targeted agents, which are discussed in detail in the following sections.

5.1. Targeting PI3K/AKT/mTOR pathway in tumor stroma

The tumor microenvironment (TME) of PDAC is characterized by the presence of dense desmoplastic/stromal reaction surrounding cancerous tissue that consists of cancer-associated fibroblasts (CAFs), pancreatic stellate cells (PSCs), immune cells, extracellular matrix (ECM), and soluble proteins, including growth factors and cytokines (Ligorio et al., 2019). The dynamic and complex interactions between TME components and tumor cells in PDAC may contribute to tumor progression, metastasis, and drug resistance (Firuzi et al., 2019, Che et al., 2020). Emerging evidence indicates that PI3K/AKT signaling pathway is also involved in the crosstalk between tumor cells and stromal cells, thereby promoting tumor cell aggressiveness as well as drug resistance in PDAC (Murthy et al., 2018).

In particular, CAFs have been reported to contribute to chemoresistance and increased proliferation and migration of PDAC cells (Boyd et al., 2021), providing a rational for targeting mTOR pathway in these cells, which has shown to improve the effectiveness of gemcitabine in vitro and in vivo (Duluc et al., 2015). Additionally, PI3K/AKT-mTOR pathway in immune cells has been also shown to promote pancreatic tumorigenesis and cancer progression (Pons-Tostivint et al., 2017). For example, Ali and colleagues showed that the PI3K pathway is critical for the maintenance and immunosuppressive function of regulatory T (Treg) cells and the inactivation of PI3Kδ inhibited the immunosuppressive capacity of Tregs in PDAC (Ali et al., 2014). Another study indicated that myeloid cell-specific PI3Ks isoforms drive the immunosuppressive activity of tumor-associated macrophages, so inhibition of PI3K leads to restoration of T cell-mediated antitumor immunity, reduced desmoplasia, tumor cell invasion and metastasis in animal models of PDAC (Kaneda et al., 2016).

5.2. Combination of PI3K/AKT/mTOR inhibitors and chemotherapy

Based on emerging evidence on the crucial role of PI3K/AKT/mTOR in PDAC, several agents targeting this pathway have been tested in combination with conventional chemotherapeutics in order to increase the efficacy of the later drugs in vitro and in vivo (Massihnia et al., 2017; Wang et al., 2020; Awasthi et al., 2019; Mao et al., 2018; Duong et al., 2012). Combined use of PI3K and MAPK inhibitors have been shown to enhance the response to gemcitabine/nab-paclitaxel (Awasthi et al., 2019). Another study has recently reported that MK-2206 can sensitize the human pancreatic cancer cell lines to gemcitabine by reducing cell proliferation and AKT phosphorylation (Wang et al., 2020).

Moreover, the efficacy of the combination of these inhibitors with chemotherapeutic agents has been investigated also in pancreatic cancer patients (Table 3). Several combination therapies with rapalogs and chemotherapeutic agents, including gemcitabine (Karavasilis et al., 2018, Joka et al., 2014, Costello et al., 2014), capecitabine (Kordes et al., 2015, Kordes et al., 2013), and paclitaxel (Sessa et al., 2010) have been examined in PDAC patients.

In a single-arm phase II trial of the first- and second-line treatment of PDAC, Kordes and colleagues investigated the combination of everolimus and capecitabine in 31 patients with advanced disease. PR and SD were observed in 6% and 32%, respectively, and with the

median OS of 8.9 months, suggesting that this combination therapy might enhance the efficacy of capecitabine monotherapy, especially when administered as first-line treatment (Kordes et al., 2015).

Moreover, the efficacy of the combination of mTOR inhibitors with gemcitabine, as the first-line treatment for PDAC, has been investigated in several studies (Table 3) (Joka et al., 2014, Costello et al., 2014, Babiker et al., 2019). Promising results were obtained from the small cohorts of patients with advanced pancreatic cancer enrolled in a phase I study, which showed a 78% clinical benefit rate including 65% SD and 13% PR, while there was no CR (Joka et al., 2014). These findings are similar to the outcome of another study evaluating the combination of everolimus with gemcitabine and cisplatin in patients with treatment-refractory solid tumors including pancreatic cancer; Among two patients with complete responses, one patient had recurrent pancreatic cancer (Costello et al., 2014). However, based on the results from a phase II trial by Karavasilis and collaborators, although the combination of gemcitabine with temsirolimus seemed to be feasible in patients with locally advanced or metastatic pancreatic cancer with manageable side effects, it failed to show any meaningful clinical efficacy (Karavasilis et al., 2018).

Additionally, combining mTOR inhibitor ridaforolimus with paclitaxel has been well tolerated with encouraging antitumor activity in 29 individuals with solid tumors. Two partial responses were observed; one in a patient with pancreatic cancer who had been previously treated with capecitabine and 5-fluorouracil (Sessa et al., 2010). Similarly, combination of temsirolimus and docetaxel did not meet its primary objective and appeared impractical for further development in a trial involving 26 patients with refractory solid malignancies including pancreatic cancer (23% of patients) due to dose-limiting toxicities (Amin et al., 2021).

Phase I study of idelalisib, as the first FDA approved inhibitor of PI3K, in combination with nab-paclitaxel and modified (m)FOLFOX6 in PDAC patients was prematurely terminated because of safety concerns related to the increased number of death and undesirable side effects identified in phase III clinical studies of idelalisib for hematological malignancies (NCT01980888, NCT01732913, and NCT01732926) (Borazanci et al., 2020). The efficacy of the combination of PI3K inhibitors with gemcitabine or cisplatin plus gemcitabine has been evaluated in a phase I study with 50 patients affected by advanced solid tumors, including pancreatic cancer patients. Combination treatment with cisplatin/gemcitabine has shown a favorable clinical response with an acceptable toxicity profile (Kim et al., 2018). However, data obtained from phase I study in 17 patients with advanced refractory solid tumors showed promising results in terms of partial response in only one patient with stage IV pancreatic cancer treated with the combination of PI3K inhibitor BKM120 and mFOLFOX6 (McRee et al., 2015).

5.3. Combination of PI3K/AKT/mTOR inhibitors and targeted therapies

5.3.1. Combined inhibition of PI3K/AKT/mTOR and cyclin-dependent kinases (CDKs)

Cell cycle progression is a tightly regulated process in all cell types. An aberrant cell cycle regulation pattern is indeed a hallmark of most cancers. The family of cyclin-dependent kinases (CDKs) has critical functions in cell cycle regulation that act in coordination with their cyclin partners (Wijnen et al., 2021). Various CDK/cyclin complexes phosphorylate multiple

protein targets, thus driving cell cycle progression of distinct phases (G1, S, G2 and M). Dysregulation of CDK and cyclin activity in the cell cycle is associated with uncontrolled cell proliferation in human tumors (Otto and Sicinski, 2017).

Since pancreatic cancers are frequently associated with loss-of-function mutations in tumor suppressor genes involved in cell cycle regulation such as TP53, CDKN2A and SMAD4, controlling CDKs might have potential to substantially impact PDAC progression (Wijnen et al., 2021, Pecoraro et al., 2021b, García-Reyes et al., 2018). In this regard, CDKN2A is frequently inactivated in 80–95% of PDAC cases endowing cancer cells with the capacity to avoid cell cycle suppression. CDKN2A generates several transcript variants encoding different proteins with distinct functions. The main isoform, p16INK4a, functions as inhibitor of CDK4/6 and the other isoform, p14ARF inhibits the oncogenic action of MDM2 by blocking its function in the degradation of p53 (García-Reyes et al., 2018). The active cyclin D/CDK4/6 complexes promote phosphorylation and inactivation of the tumor suppressor retinoblastoma protein (RB), which is considered as a key negative regulator of cell cycle progression. The CDK4/6-cyclin D-Rb pathway is involved in transition from G1 phase of the cell cycle to the S phase (Bai et al., 2017).

Despite the strong biological basis for involvement of CDK enzymes in cancer progression, as well as the antitumor activity of CDK4/6 inhibitors monotherapy observed in pancreatic cancer preclinical models (Chou et al., 2018, Witkiewicz et al., 2015b), inherent resistance has been reported by some investigators (Heilmann et al., 2014, Franco et al., 2014a). In this regard, since the mTOR pathway can directly affect the activity of CDKs, facilitating cell cycle progression and contributing to resistance to CDK4/6 inhibitors, mTOR inhibition might represent a potential mechanism to enhance the antitumor activity of CDK4/6 inhibitors (Fig. 5) (Lamm et al., 2019, Knudsen et al., 2019).

The study by Knudsen and colleagues has recently reported that inhibition of CDK4/6 is linked to the upregulation of cyclin expression, cyclin D1 and cyclin E contributing to acquired resistance in a panel of patient-derived xenograft (PDX) models. Combined targeting of mTOR and CDK4/6 could synergistically improve the efficacy of CDK4/6 inhibitors and overcome resistance (Knudsen et al., 2019). Similar findings were also previously observed in several CDKN2A-deficient pancreatic cancer cell lines treated with a combination of PD-0332991, a CDK4/6 small molecule inhibitor, and PI3K/mTOR inhibitors BEZ235, AZD0855 and GDC0980 (Franco et al., 2014).

Although concomitant targeting of CDK4/6 and mTOR has shown promise in several preclinical models, disappointing results have been reported in a recent study in which ribociclib, a CDK4/6 inhibitor was combined with everolimus in a phase I study in 12 patients with chemo refractory PDAC (Weinberg et al., 2020). Nevertheless, a phase I clinical trial testing the combination of the CDK4/6 inhibitor palbociclib with the PI3K/mTOR inhibitor gedatolisib has been initiated for patients with solid tumors including pancreatic cancer (NCT03065062) (Supplementary Table 2).

5.3.2. Combined inhibition of PI3K/AKT/mTOR and Hedgehog/Glioma-associated oncogene (HH/GLI) pathway

Aberrant activation of the Hedgehog (Hh) signaling pathway, through the control of processes such as proliferation, invasiveness, tumorigenesis and also the expansion of cancer stem cells (CSCs), is associated with the progression of several human cancers including PDAC (Onishi and Katano, 2014, Saini et al., 2019). The Hh signaling includes canonical and non-canonical pathways. Canonical activation of Hh signaling is initiated by binding of HH ligands to the transmembrane receptor Patched 1 (PTCH1), resulting in the release of its suppression on the G protein-coupled receptor Smoothened (SMO). Subsequently, SMO activates the final effector of Hh signaling, the glioma-associated oncogene (GLI) transcription factor. In addition to the canonical PTCH1-SMO route, non-canonical mechanisms referring to an SMO-independent stimulation of GLI activity is also involved in tumorigenesis.

Several studies suggest that a cross-talk between PI3K/AKT/mTOR and Hh signaling could be involved in maintaining a malignant phenotype (Larsen and Møller, 2020).

In this context, it was shown that the combination of inhibitors of SMO (NVP-BEZ-235) and PI3K/mTOR (NVP-LDE-225) may be superior to monotherapy by targeting pancreatic CSCs (Sharma et al., 2015). Similarly, other in vitro and in vivo reports have revealed that dual blockade of GLI and mTOR with GANT61 and rapamycin, respectively, resulted in enhanced inhibition of tumor growth and a significant reduction in the expression of stem cell marker and sphere formation in pancreatic CSC lines (Miyazaki et al., 2016).

However, in contrast with promising preclinical results, the rationally based combination of Hh inhibitor with sirolimus was well-tolerated but lacked a significant clinical benefit in a phase I study for advanced pancreatic cancer (Carr et al., 2020). In addition, as shown by a number of failed attempts, targeting SMO receptors does not seem to be a useful therapeutic strategy in pancreatic cancer (Catenacci et al., 2015, McCleary-Wheeler et al., 2020). It is suggested that due to the importance of SMO-independent GLI1 activation in PDAC pathogenesis (Pietrobono et al., 2019), targeting GLI1 might be more effective compared to upstream mediators of HH signaling (Carr et al., 2020).

5.3.3. Combined inhibition of PI3K/AKT/mTOR and receptor tyrosine kinases (RTKs)

Several receptor tyrosine kinases such as vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor 1 receptor (IGF1R), EGFR, and mesenchymal-epithelial transition tyrosine kinase receptor (MET) are frequently dysregulated in pancreatic cancer (Moosavi et al., 2019). Targeting RTKs could be an efficient therapeutic strategy to reduce cell growth and invasion (Damghani et al., 2021, Moosavi et al., 2021a). In preclinical and clinical studies, PI3K/AKT/mTOR pathway inhibitors have been used in combination with RTKs targeting agents in order to take advantage of synergistic effects as well as to circumvent drug resistance (Brown and Toker, 2015, Conway et al., 2019).

EGFR overexpression has been reported in up to 95% of pancreatic tumors (Miller et al., 2020). EGFR inhibitors are prone to therapeutic resistance due to various mechanisms, such as the activating mutations (T790M), activation of alternative signaling pathways (e.g. IGF-1R, c-MET), and upregulation of the downstream pathways (e.g. PI3K/mTOR or RAS/ERK1/2).

Erlotinib, a small molecule EGFR inhibitor, in combination with gemcitabine has been clinically approved for the first-line treatment of patients with locally advanced, unresectable, or metastatic pancreatic cancer (Khozin et al., 2014). However, the development of resistance is a frequently encountered problem leading to the limited therapeutic benefits of this first-generation EGFR inhibitor (Chong and Jänne, 2013, Ioannou et al., 2016). Experimental evidence in pancreatic cancer indicates that PI3K/mTOR pathway activity is an established resistance mechanism to erlotinib and suggests that this resistance could be overcome by co-targeting of EGFR and PI3K (Ioannou et al., 2016, Buck et al., 2006). On the other hand, it has been reported that EGFR feedback activation can lead to acquired resistance to mTOR inhibition, which was restored by combination oferlotinib and AZD8055, a second-generation mTOR inhibitor (Wei et al., 2015).

Based on these findings, the combination of erlotinib with mTOR inhibitors has been used in clinical trials (Javle et al., 2010, Park et al., 2020) (Table 3). From an early phase II trial, the combination of erlotinib with everolimus did not show any clinical benefit in patients with advanced pancreatic cancer (Javle et al., 2010). In a phase I trial for patients with advanced solid tumors including pancreatic cancer, Park and colleagues have recently evaluated the effect of combined mTOR and EGFR targeting by using temsirolimus and erlotinib, respectively. Among 26 patients evaluable for response, 17 experienced prolonged disease stabilization, however, there were no complete or partial responses (Park et al., 2020). The predictive role of several biomarkers including PTEN, EGFR, and PIK3CA mutations were also evaluated. The results indicated that the presence of these mutations was not correlated with response to treatment (Park et al., 2020).

Cetuximab is a monoclonal antibody that binds to the extracellular domain of EGFR, interrupting the downstream signaling cascade. A clinical study using this antibody in combination with everolimus and capecitabine failed to demonstrate a survival advantage in 31 patients with pancreatic cancer. The authors suggested that a reason for the lack of efficacy with a large molecule like the monoclonal antibody cetuximab, is that the highly desmoplastic nature of pancreatic cancer with an expanded fibrotic stroma and minimal vascularization might impair an adequate drug delivery to cancer cells (Kordes et al., 2013). In addition, disappointing results have also been reported in a recent study in which cetuximab was combined with gemcitabine (Berlin et al., 2018). In another study of temsirolimus with cetuximab in patients with various solid tumors, such as pancreatic, colorectal, breast cancer and NSCLC, the combination therapy yielded modest effects, thus it was not further pursued (Hollebecque et al., 2017).

Recent evidence indicates that combined therapy with PI3K and MET pathway inhibitors represent rational therapeutic options in several malignancies (Moosavi et al., 2021b). In a phase I trial of temsirolimus with the MET inhibitor tivantinib, there was an acceptable safety profile with promising efficacy in 29 patients with solid malignancies (Kyriakopoulos et al., 2017).

5.3.4. Combined targeting of PI3K/AKT/mTOR and RAF/MEK/ERK pathways

RAS, as the upstream protein, activates both the RAF/MEK/ERK and PI3K/AKT/mTOR signaling pathways, and there are overlapping feedback activities providing crosstalk between these

signaling pathways. Inhibition of one cascade results in the activation of the other, leading to acquired resistance in cancer cells (Fig. 6). Thereby, in KRAS-mutant tumors such as pancreatic cancer, a strategy that simultaneously targets MEK and PI3K pathway seems to be a promising treatment strategy (Lee et al., 2020, Cao et al., 2019, Alagesan et al., 2015).

A study by Soares and colleagues reported that in PDAC cells, dual PI3K/mTOR kinase inhibitor, dactolisib (BEZ235), induced rapid over-activation of MEK/ERK pathway via a PI3K-independent feedback mechanism, and that drug resistance can be tackled with combination therapies by inhibition of a horizontal combined blockade strategy with the dual MEK and PI3K/mTOR inhibition (Soares et al., 2015). The potential of a combination strategy targeting the RAF/MEK/ERK together with the PI3K/AKT pathway led to synergistic inhibitory effects on tumor growth with an 80% inhibitory rate in the xenograft model with KRAS-mutant PDAC (Ning et al., 2017). However, other reports demonstrated only modest activity of combined MAPK and PI3K/AKT inhibition (Ischenko et al., 2015, Ciuffreda et al., 2017).

Based on the findings from in vitro and animal studies, several clinical trials evaluating the combination of PI3K/AKT inhibitors with MAPK/ERK inhibitors have been conducted in patients with pancreatic cancer (Chung et al., 2017, Grilley-Olson et al., 2016) (Table 3). A phase I clinical trial with a pan-PI3K/mTOR inhibitor, GSK2126458 and a MEK inhibitor, trametinib, in 69 patients with solid tumors including pancreatic cancer showed poor tolerability and limited anti-tumor activity (Grilley-Olson et al., 2016). Mutations in RAS, RAF, or PI3K were detected in 70% of patients, but no associations were found between response and mutational status (Grilley-Olson et al., 2016). Similarly, a randomized phase II trial including 137 patients with pancreatic cancer who failed gemcitabine-based therapy demonstrated that dual inhibition of AKT and MEK with MK-2206 and selumetinib, respectively, did not show any survival benefit compared to the group that received the standard of care, mFOLFOX (Chung et al., 2017). It was suggested that the failure of these drug combinations could reside in the fact that due to toxicity-related treatment delays and dose reduction in the experimental arm, only modest inhibition of each pathway was achieved in tumor tissue (Chung et al., 2017).

Additionally, Ciuffreda and colleagues demonstrated that the combination of MEK and PI3K inhibitors is effective only in preclinical models of pancreatic cancer harboring inactivating PTEN point mutations, thus explaining the disappointing results of the selumetinib/MK-2206 combination treatment in unselected PDAC patients (Ciuffreda et al., 2017).

Hence, it is suggested that PTEN status, as a predictive biomarker may identify patients who would benefit from combined therapy with AKT and MEK pathway inhibitors (Bazzichetto et al., 2019). Other combinations of PI3K/AKT/mTOR inhibitors with RAF/MEK/ERK inhibitors are being tested in clinical studies (Supplemental Table 2).

6. **Conclusions**

PI3K/AKT/mTOR pathway has crucial roles in the emergence of several hallmarks of cancer including cell proliferation, growth, and evasion of cell death among others. A number of

small molecule inhibitors of this pathway have now been approved for treatment of various malignancies including lymphomas, leukemias and solid tumors such as breast cancer and RCC. There is strong biological basis emerging from genetic studies that PI3K/AKT/mTOR pathway aberrations are frequently found in different cohorts of PDAC patients pointing out the value of these alterations as predictive biomarkers and actionable drug targets.

Furthermore, preclinical in vitro and animal studies have shown that modulators of this pathway either as single agents or in combination with chemotherapeutics or other targeted agents can considerably block the growth and invasiveness of PDAC cells.PI3K/AKT/mTOR pathway inhibitors have not been successful as single agents in PDAC patients, but few human studies have shown marginal clinical benefit for combination of these agents with chemotherapeutics and some targeted therapies such as inhibitors of RTKs.

Overall, compared to the wealth of genetic and preclinical pharmacological evidence accumulated about the important role of PI3K/AKT/mTOR pathway in PDAC, the results of human clinical trials have been below expectations. Different issues may explain these unfavorable outcomes; One point to consider is that predictive biomarkers could be extremely useful to guide the selection of individuals who may benefit from targeted therapies. For instance, our recent study, which showed the involvement of p130Cas as a crucial downstream effector of KRAS that drives acinar to ductal metaplasia through PI3K pathway, provides strong evidence on the notion that high expression of p130Cas could serve as a predictive biomarker to stratify patients that may draw benefit from PI3K/AKT/mTOR pathway targeting (Costamagna et al., 2021). Moreover, inactivating mutations of PTEN or amplifications/activating mutation of PIK3CA and AKT have been used as predictive biomarkers of response to therapy in few studies with limited success, but their potential should be more deeply evaluated in larger cohorts of patients. Biomarker-based studies in pancreatic cancer generally remain rare compared to other cancers so far and they may constitute the future of personalized medicine which will hopefully prove itself as an important asset for management of PDAC patients.

CRediT authorship contribution statement

Motahareh Mortazavi: Investigation; Writing – original draft, Writing – review & editing. Fatemeh Moosavi: Investigation, Supervision, Writing – original draft, Writing – review & editing. Miriam Martini: Writing – review & editing. Elisa Giovannetti: Writing – review & editing. Omidreza Firuzi: Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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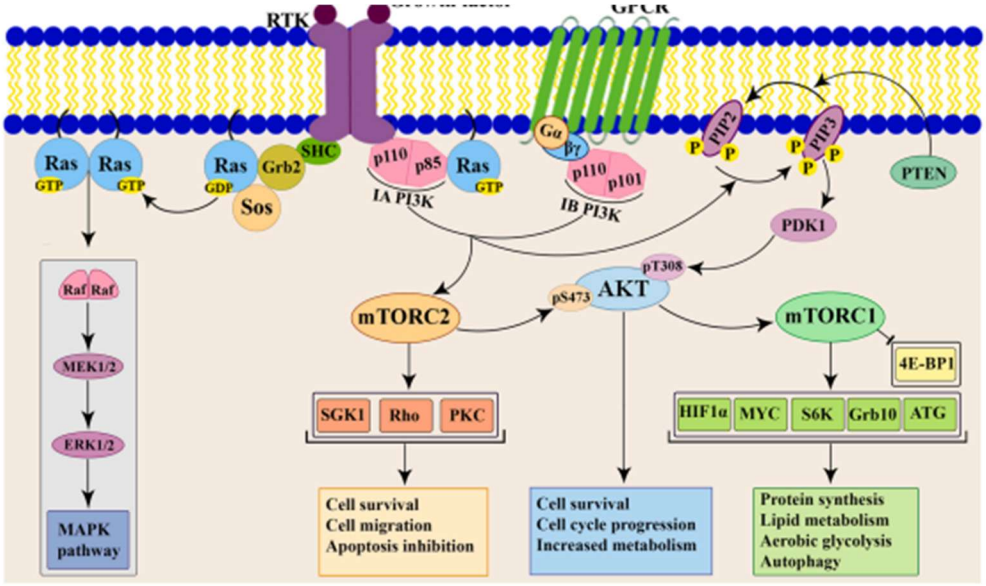
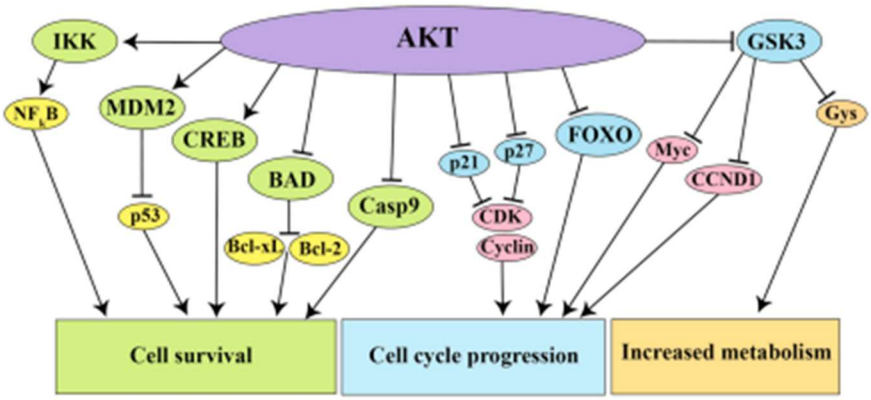


Fig. 1. PI3K/AKT/mTOR signalling pathway in normal and cancerous cells. Stimulation of different RTKs and GPCRs by extracellular signals, causes the activation of class IA and IB PI3Ks, respectively. Active PI3Ks produce PIP_3 from PIP_2 that recruits AKT to the cell membrane rendering it susceptible to phosphorylation on Thr308 and Ser473 by PDK1 and mTORC2, respectively. Meanwhile, PTEN negatively regulates AKT activation through dephosphorylation of phosphoinositides. AKT phosphorylation and activation consequently leads to the activation of mTORC1 complex consisting of multiple proteins affecting many downstream targets. Several key downstream molecules of AKT are shown in Fig. 2. Activation of KRAS also causes at the same time the initiation of the other very important parallel pathway of RAF/MEK/ERK heavily involved in tumorigenesis. Finally, these cascades result in the activation of many downstream molecular targets involved in cell proliferation, growth, survival and apoptosis evasion. GPCR: G-protein coupled receptor, Grb2: Growth factor receptor-bound protein 2, MAPK: mitogen-activated protein kinase, MEK: MAPK/ERK kinase, mTORC1: mechanistic target of rapamycin complex 1, mTORC2: mechanistic target of rapamycin complex 2, PDK1: Phosphoinositide-dependent kinase 1, PI3K: phosphoinositide 3-kinase, PIP2: phosphoinositide- 4,5-bisphosphate, PIP3: phosphoinositide-3,4,5-trisphosphate, PTEN: phosphatase and tensin homolog, RAF: Rapidly accelerated fibrosarcoma, RTK: receptor tyrosine kinase, SOS: Son of Sevenless.

Table 1
Different classes of PI3K enzymes and their subunits.

PI3K class	Subunit	Gene	Protein	Aliases
Class I	IA Catalytic	*PIK3CA*	PI3K, catalytic, α polypeptide	p110α
		PIK3CB	PI3K, catalytic, β polypeptide	p110β
		PIK3CD	PI3K, catalytic, δ polypeptide	p110δ
	IA Regulatory	*PIK3R1*	PI3K, regulatory subunit 1 (α)	p85α
		PIK3R2	PI3K, regulatory subunit 2 (β)	p85β
		PIK3R3	PI3K, regulatory subunit 3 (γ)	p55γ
	IB Catalytic	*PI3K3CG*	PI3K, catalytic, γ polypeptide	p110γ
	IB Regulatory	*PIK3R5*	PI3K, regulatory subunit 5	p101
		PIK3R6	PI3K, regulatory subunit 6	p87/p84
Class II	Catalytic	*PIK3C2A*	PI3K, class 2, α polypeptide	PI3K-C2α
		PIK3C2B	PI3K, class 2, β polypeptide	PI3K-C2β
		PIK3C2G	PI3K, class 2, γ polypeptide	PI3K-C2γ
Class III	Catalytic	*PIK3C3*	PI3K, class 3	Vps34
	Regulatory	*PIK3R4*	PI3K, regulatory subunit 4	Vps15/ p150

Class IA PI3K are activated by receptor tyrosine kinases (RTKs), while class IB are downstream to the G-protein coupled receptors (GPCRs).


AKT
IKK
GSK3
NF$_k$B
MDM2
CREB
BAD
Casp9
p21
p27
FOXO
Myc
CCND1
Gys
p53
Bcl-xL
Bcl-2
CDK
Cyclin
Cell survival
Cell cycle progression
Increased metabolism

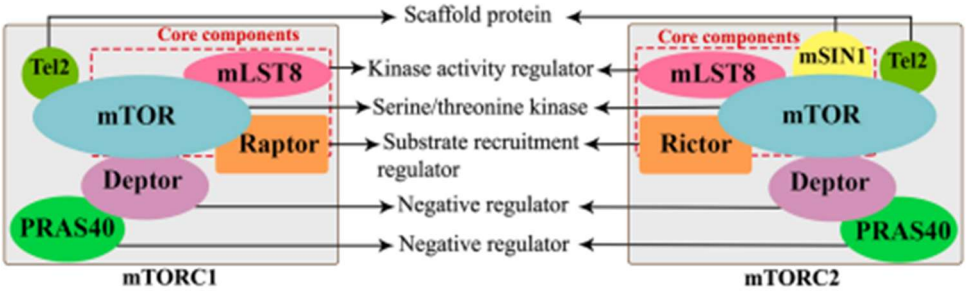

Scaffold protein
Core components
Tel2
mLST8
mTOR
Raptor
Deptor
PRAS40
Kinase activity regulator
Serine/threonine kinase
Substrate recruitment regulator
Negative regulator
Negative regulator
mTORC1
Core components
mSIN1
Tel2
mLST8
mTOR
Rictor
Deptor
PRAS40
mTORC2

Table 2
List of PI3K/AKT/mTOR pathway inhibitors being studied in clinical experiments.

Classification	Drugs	Target	Class	Approval status
Pan-PI3K inhibitor	Buparlisib (Synonyms: BKM120, NVP-BKM120)	Class I PI3K	ATP competitive	–
	Copanlisib (Synonyms: BAY 80–6946)	Class I PI3K (predominantly PI3Kα and PI3Kδ)	ATP competitive	FDA approved for patients with relapsed follicular lymphoma
	MEN1611 (Synonyms: CH5132799, PA 799)	Class I PI3K (preferentially PI3Kα)	ATP competitive	–
	Pictilisib (Synonyms: GDC-0941, RG7621)	Class I PI3K	ATP competitive	–
	Pilaralisib (Synonyms: XL147, SAR245408)	Class I PI3K	ATP competitive	–
	Sonolisib (Synonyms: PX866)	Class I PI3K	Allosteric	–
	ZSTK474 (Synonyms: KIN001–167)	Class I PI3K	ATP competitive	–
Isoform-specific PI3K inhibitors	Acalisib(Synonyms: GS-9820)	p110δ	NIA*	–
	Alpelisib (Synonyms: BYL719, NVP-BYL719)	p110α	ATP competitive	FDA approved for hormone receptor (HR)-positive, human epidermal growth factor receptor-2 (HER2)-negative breast cancer patients with a PIK3CA mutation
	AMG 319 (Synonyms: ACP-319)	p110δ	NIA	–
	AZD8186	p110β (PI3Kβ), p110δ	NIA	–
	Duvelisib (Synonyms: IPI145, INK1197)	p110δ, P110γ	ATP competitive	FDA approved for patients with relapsed or refractory chronic lymphocytic leukaemia (CLL)/ small lymphocytic lymphoma (SLL)
	Eganelisib (Synonyms: IPI 549)	p110γ	NIA	–
	GSK2636771	p110β	NIA	–
	Idelalisib (Synonyms: CAL101, GS1101)	p110δ	ATP competitive	FDA approved for patients with relapsed follicular B-cell non-Hodgkin lymphoma or relapsed SLL
	Linperlisib (Synonyms: YY20394)	p110δ	NIA	–
	Parsaclisib (Synonyms: INCB050465, IBI376)	p110δ	NIA	–
	SAR260301	p110β	ATP competitive	
	Serabelisib (Synonyms: MLN1117, INK1117, TAK-117)	p110α	ATP competitive	–
	Taselisib (Synonyms: GDC0032, RG7604, RO5537381)	p110α	ATP competitive	–
	Tenalisib (RP6530)	p110δ, P110γ	NIA	
AKT inhibitor	Afuresertib (Synonyms: GSK2110183)	AKT1, AKT2, AKT3	ATP competitive	–
	BAY1125976	AKT1, AKT2	Allosteric	–
	Borussertib	AKT1, AKT2	Allosteric	
	Capivasertib (Synonyms: AZD5363)	AKT1, AKT, AKT3	ATP competitive	–
	Ipatasertib (Synonyms: RG-7440, GDC0068)	AKT1, AKT, AKT3	ATP competitive	–
	LY2780301	Dual AKT1, 2, 3 and p70S6K	ATP competitive	–
	MK-2206	AKT1, AKT2	Allosteric	–
	MSC2363318A (Synonyms: M2698)	Dual AKT1, 2, 3 and p70S6K	ATP competitive	–
	Perifosine (Synonyms: KRX0401, NSC 639966)	AKT1, AKT, AKT3	Allosteric	–
	Triciribine (Synonyms: NSC 154020, TCN)	AKT1, AKT, AKT3	ATP competitive	–
	Uprosertib (Synonyms: GSK2141795)	AKT1, AKT, AKT3	ATP competitive	–
	Vevorisertib (Synonyms: ARQ 751)	AKT1, AKT, AKT3	Allosteric	–
mTOR inhibitors	DS-3078a	mTORC1/ mTORC2	ATP competitive	–

Table 2 (*continued*)

Classification	Drugs	Target	Class	Approval status
	Everolimus (Synonyms: RAD001, RAD666, SDZRAD)	mTORC1	Allosteric	FDA approved for patients with advanced HR–positive, HER2-negative, breast cancer; renal cell carcinoma (RCC); progressive neuroendocrine tumors of pancreatic origin (PNET); progressive, well-differentiated non-functional, neuroendocrine tumors (NET) of gastrointestinal (GI) or lung origin; subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TSC); renal angiomyolipoma
	Onatasertib (Synonyms: ATG-008, CC-223)	mTORC1 / mTORC2	ATP competitive	–
	OSI-027 (Synonyms: ASP7486)	mTORC1 / mTORC2	ATP competitive	–
	Rapamycin (Synonyms: Sirolimus, SILA 9268 A, AY-22989, WY-090217, RAPA)	mTORC1	Allosteric	FDA approved for patients with lymphangioleiomyomatosis (LAM)
	Ridaforolimus (Synonyms: Deforolimus, AP23573, MK-8669)	mTORC1	Allosteric	–
	Sapanisertib (Synonyms: INK128, MLN0128, TAK-228)	mTORC1 / mTORC2	ATP competitive	–
	Temsirolimus (Synonyms: CCI 779, WAY-CCI 779)	mTORC1	Allosteric	FDA approved for patients with metastatic RCC
	Vistusertib (Synonyms: AZD2014)	mTORC1 / mTORC2	ATP competitive	–
Dual PI3K/mTOR inhibitors	Apitolisib (Synonyms: GDC0980, RG-7422, GNE 390)	Dual PI3K/mTOR	ATP competitive	–
	BGT226 (Synonyms: NVP-BGT226)	Dual PI3K/mTOR	ATP competitive	–
	Bimiralisib (Synonyms: PQR309	Dual PI3K/mTOR	ATP competitive	–
	Dactolisib (Synonyms: NVP-BEZ235, BEZ-235)	Dual PI3K/mTOR	ATP competitive	–
	DS-7423	Dual PI3K/mTOR	ATP competitive	–
	Gedatolisib (Synonyms: PF-05212384, PKI-587	Dual PI3K/mTOR	ATP competitive	–
	Omipalisib (Synonyms: GSK458, GSK2126458)	Dual PI3K/mTOR	ATP competitive	–
	Paxalisib (Synonyms: GDC-0084, RG7666)	Dual PI3K/mTOR	ATP competitive	–
	PWT33597 (Synonyms: VDC-597)	Dual PI3K/mTOR	ATP competitive	–
	Samotolisib (Synonyms: LY3023414)	Dual PI3K/mTOR	ATP-competitive	–
	Voxtalisib (Synonyms: XL-765, SAR245409)	Dual PI3K/mTOR	ATP-competitive	–
	VS-5584 (Synonyms: SB 2343)	Dual PI3K/mTOR	ATP-competitive	–
Other inhibitors	CC-115	mTOR/ DNA-dependent protein kinase (DNA-PK)	ATP-competitive	–
	Cenisertib (Synonyms: R763, AS703569)	AKT/FLT3/VEGFR2/LYN/ BTK/KIT/Aurora-kinase-A/B	ATP-competitive	–
	Fimepinostat (Synonyms: CUDC-907)	PI3K/pan histone deacetylase (HDAC) inhibitor	Inhibition of PI3K and HDAC activity	–
	Regosertib (Synonyms: ON 01910)	PI3Kand polo-like kinase 1 (PLK1)	Allosteric	–
	Umbralisib (Synonyms: RP-5264 TGR-1202)	Dual PI3Kδ/casein kinase-1ε	ATP-competitive	FDA approved for patients with relapsed or refractory marginal zone lymphoma (MZL) and relapsed or refractory follicular lymphoma (FL)

*No information available