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Therapeutic targeting of cancers with loss of PTEN function

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Abstract

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is one of the most frequently disrupted tumor suppressors in cancer. The lipid phosphatase activity of PTEN antagonizes the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway to repress tumor cell growth and survival. In the nucleus, PTEN promotes chromosome stability and DNA repair. Consequently, loss of PTEN function increases genomic instability. PTEN deficiency is caused by inherited germline mutations, somatic mutations, epigenetic and transcriptional silencing, post-translational modifications, and protein-protein interactions. Given the high frequency of PTEN deficiency across cancer subtypes, therapeutic approaches that exploit PTEN loss-of-function could provide effective treatment strategies. Herein, we discuss therapeutic strategies aimed at cancers with loss of PTEN function, and the challenges involved in treating patients afflicted with such cancers. We review preclinical and clinical findings, and highlight novel strategies under development to target PTEN-deficient cancers.

Keywords

Phosphatase; cancer; tumor; targeted therapy; tumor suppressor; PI3K; mTOR; synthetic lethal

Introduction

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is as a well-known tumor suppressor that has both phosphatase-dependent and -independent roles. It was first identified in 1997 as a phosphatase that is mutated or lost in several cancers [1, 2]. We now know that lesions in the *PTEN* gene, located on chromosome 10q23, occur at a significant rate in the majority of human tumor subtypes, and this locus is thought to have the highest preference for loss in humans [3].

The best-characterized tumor suppressive role of PTEN is as a lipid phosphatase that antagonizes phosphatidylinositol 3-kinase (PI3K) signaling [4]. PI3K is a critical node in a major signaling pathway that regulates cancer cell growth, survival, and metabolism (Fig. 1). When activated, PI3K phosphorylates the 3’ (D3) position on the inositol ring of

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phosphatidylinositol (4,5)-bisphosphate (PIP_2), which is present on the inner leaflet of the plasma membrane, to produce phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). PIP_3 serves as a second messenger and binds proteins containing pleckstrin homology (PH) domains. The recruitment of PH domain-containing proteins such as AKT to the plasma membrane facilitates their activation, and triggers downstream signaling cascades. Cytoplasmic PTEN negatively regulates this pathway by dephosphorylating PIP_3 at its D3 position, thereby inhibiting downstream kinase activation and preventing cancer cell growth and survival (Fig. 1 and ref. [5]). Two recent studies have found that there is a translational variant(s) long form of PTEN secreted from cell that can enter neighboring cells. Like cytoplasmic PTEN, secreted PTEN has lipid phosphatase activity and antagonizes PI3K signaling in target cells [6, 7].

PTEN has also been reported to exhibit protein phosphatase activity. *In vitro* studies showed that PTEN dephosphorylates tyrosine, serine, and threonine residues on phosphopeptides [8]. PTEN interacts with and dephosphorylates focal adhesion kinase and Shc [9, 10]. The protein phosphatase activity of PTEN also reduces cyclin D1 levels, preventing cell cycle progression [11]. Using a new bioassay to measure PTEN function in living tissue, it was recently shown that PTEN auto-dephosphorylates serine and/or threonine residues in its own C-terminal region; this event(s) appears to promote its lipid phosphatase activity [12, 13]. The protein phosphatase activity of PTEN also regulates secretion of hepatitis C virus particles in liver, possibly via regulation of cholesterol metabolism [14].

While cytoplasmic PTEN is primarily involved in regulating PI3K/PIP_3 signaling, nuclear PTEN exhibits phosphatase-independent tumor suppressive functions, including regulation of chromosome stability, DNA repair, and apoptosis (Fig. 1; reviewed in refs. [15, 16]). Despite the fact that PTEN lacks a canonical nuclear localization sequence, ubiquitination in its C-terminal region may promote its nuclear import [17]. Studies in PTEN-null mouse embryonic fibroblasts revealed that 1) nuclear PTEN interacts with Centromere-Specific Binding Protein (CENP-C), an essential component for centromere stability, and 2) PTEN is crucial for the induction of RAD51, which regulates DNA double-strand break repair [18]. Nuclear PTEN binds to the anaphase-promoting complex or cyclosome (APC/C), and heightens the association of APC/C with the co-activator CDC20 homologue 1 (CDH1) [19]. In so doing, PTEN increases the chromosome-stabilizing activity of the APC/C-CDH1 complex [19]. Nuclear PTEN may also promote apoptosis [15]. Human glioblastoma cells with predominantly nuclear PTEN were more likely to have condensed nuclei in response to apoptosis induction, compared to cells with primarily cytoplasmic PTEN [20]. Hence, intracellular localization plays an important role(s) in the regulation of PTEN function(s) [16]. These various phosphatase-dependent and -independent functions of PTEN contribute to tumor suppression, and highlight the complexity of strategies to therapeutically target PTEN-deficient cancers.

Mechanisms of functional loss of PTEN

Loss of PTEN function is a major determinant that affects tumor development across tissues. PTEN function and expression are modulated by germline and somatic *PTEN* mutations,

genomic deletion, epigenetic and transcriptional silencing, post-transcriptional regulation, post-translational regulation, and protein-protein interactions [3].

Inherited germline mutations

Patients with PTEN Hamartoma Tumor Syndrome (PHTS), which is rare in the general population, have germline mutations throughout much of the *PTEN* coding region [21]. PHTS includes the previously named Cowden Syndrome and Bannayan-Riley-Ruvalcaba Syndrome, and may include some individuals with Proteus Syndrome, Proteus-like Syndrome, and Autism Spectrum Disorder with Macrocephaly [22]. In PHTS, exon 5 encoding the PTEN phosphatase domain accounts for approximately 40% of germline mutations [21]. Some patients with Cowden Syndrome harbor germline mutations in the *PTEN* promoter, or in possibly splice donor and acceptor sites [23]. All types of germline mutations found in Cowden Syndrome lead to loss of expression or activity of PTEN [24]. PHTS patients have an increased lifetime risk of developing cancer [25–27].

Somatic Mutations

Missense, nonsense, insertion, and deletion mutations occur throughout *PTEN* and contribute to loss of PTEN expression and/or function. Although the distribution of these mutations is mostly sporadic, several mutational hotspots have been identified at amino acids Arg130, Arg173 and Arg233 (Fig. 2 and ref. [3]). However, *PTEN* mutations are not limited to a specific cancer subtype. Genomic sequence data from The Cancer Genome Atlas (TCGA) shows that mutations in PTEN occur across a wide range of cancers, with uterine cancer and glioblastoma multiforme having the highest percentages of *PTEN* mutations and homozygous loss (Table 1).

Epigenetic, transcriptional, and post-transcriptional silencing

PTEN loss-of-function can also result from epigenetic and transcriptional silencing. Several studies have shown that CpG islands in the *PTEN* promoter are hypermethylated in cancer, leading to silencing of *PTEN* transcription [45]. This hypermethylation has been observed in breast, colorectal, endometrial, gastric, hematological, liver, lung, skin (melanoma), and prostate cancers, and glioma [46–55]. Transcription of *PTEN* can be repressed by the epigenetic repressor complex Mi-2/NuRD that contains a chromatin-remodeling ATPase and a histone deacetylase (HDAC). This repression occurs when the transcription factor Sal-Like Protein 4 (SALL4) binds to the *PTEN* promoter and recruits Mi-2/NuRD [56]. *PTEN* transcription can also be repressed by the transcription factors NF-κB, c-JUN, and BM1 [57–59].

The p53 tumor suppressive transcription factor promotes *PTEN* expression. The oncogenic transcription factors Inhibitor of DNA-binding 1 (ID1) and SNAIL can repress transcription of *PTEN* by binding to its promoter region and preventing p53 binding [53, 60]. The ubiquitous transcription factor Specificity Protein 1 (Sp1) can also inhibit *PTEN* expression: acetylated Sp1 binds to the *PTEN* promoter and recruits HDAC1 to repress *PTEN* transcription [61]. Accordingly, Sp1 overexpression upregulated PI3K pathway activation (assessed by AKT phosphorylation), and promoted migration and invasion of human salivary adenoid cystic cancer cells [61].

MicroRNAs (miRNAs), have been shown to repress translation of *PTEN* mRNA by interacting with the 3' untranslated region (reviewed in ref. [45]). Usually such miRNAs are specific to a particular cancer subtype; however, miR-21 represses PTEN expression in many cancer subtypes and metabolic diseases [45]. Intriguingly, it was proposed that miR-21 represses PTEN expression by increasing the expression of other miRNAs known to repress PTEN [62]. The transcription factor transforming growth factor beta (TGF-β), which inhibits PTEN expression in some models, upregulates miR-21 expression [63].

Post-translational regulation

Post-translational modifications including phosphorylation, acetylation, oxidation, and ubiquitylation have been shown to cause loss of PTEN function. The phosphatase activity of PTEN can be inhibited by phosphorylation of several serine and threonine resides in its C-terminal tail [64, 65], which may be driven by the kinase CK2 [66]. While such phosphorylation stabilizes PTEN, it reduces PTEN localization to the plasma membrane, thereby limiting its interaction with PIP_3 [65].

PTEN can be also inhibited by oxidation and acetylation. PTEN contains a residue characteristic of protein tyrosine phosphatases termed a catalytic cysteine nucleophile which is prone to oxidation at Cys124 [67]. Reactive oxygen species can contribute to the formation of a disulfide bond between Cys71 and Cys124 in PTEN to inhibit its catalytic activity [68, 69]. Also, peroxiredoxin 1 (PRDX1) and thioredoxin-interacting protein (TXNIP) are involved in the oxidation and subsequent inactivation of PTEN [70, 71]. p300/CREB-binding protein (CBP)-associated factor (PCAF) and CBP are thought to acetylate PTEN at Lys125–128 and Lys402, respectively, to inhibit its phosphatase activity [72].

PTEN ubiquitylation and subsequent degradation are controlled by the ubiquitin E3 ligase NEDD4 [73]. PTEN monoubiquitination at Lys13 and Lys289 promotes its nuclear localization and suppresses its phosphatase activity [17].

Protein-protein interactions

Several proteins have been shown to interact with PTEN to repress its tumor suppressive functions [3]. Parkinson Protein 7 (PARK7, DJ-1) binds PTEN under conditions of oxidative stress, and this interaction is associated with increased AKT activation and poor clinical outcome in different cancer subtypes [74]. PIP_3-dependent Rac Exchange Factor 2a (P-REX2a), Shank-Interacting Protein-Like 1 (SIPL1) and α-Mannosidase 2C1 (MAN2C1) have also been shown to bind PTEN and inhibit its phosphatase activity, leading to increased activation of AKT [75–77]. These proteins make attractive therapeutic targets to reverse loss of PTEN phosphatase activity in cancer cells.

Other PTEN-binding proteins promote PTEN stability, thereby increasing the potential for antagonism of PI3K signaling. The membrane-localized proteins E-cadherin and MAGI-2, which are lost in some cancers, promote PTEN stability [78, 79]. The p85 subunit of PI3K binds PTEN to promote stability. The genes encoding p85 isoforms (*PIK3R1*, *PIK3R2*) are frequently mutated in endometrial cancer, and some mutations destabilize PTEN and promote PI3K pathway activation [80].

Interplay between PTEN and p53 has led to the suggestion that PTEN can have tumor promoting effects in cells expressing mutant p53. Nuclear PTEN binds p53 in a phosphatase-independent manner to promote p53 stabilization, thus promoting *PTEN* transcription [81]. PTEN complexes with p300/CBP acetyltransferase to promote p53 acetylation in response to DNA damage, and p53 acetylation enhances PTEN-p53 interaction [82]. In cells expressing wild-type p53, PTEN inhibits cell proliferation and increases apoptosis. In contrast, PTEN promotes proliferation and suppresses apoptosis in cells expressing mutant p53. PTEN knockdown in mutant p53-expressing cells decreases proliferation and tumor growth in mice [83]. Thus, p53 status should be considered when PTEN may be involved in a pathway of therapeutic interest.

Challenges in determining tumor PTEN status

Loss-of-function mutations in *PTEN* occur in only a fraction of PTEN-deficient tumors. As described above, PTEN expression may be lost by many non-genomic mechanisms. It is therefore necessary to determine PTEN status in tumors by both protein quantification and DNA sequencing, as neither method alone will provide comprehensive information. Ideally, tumor PTEN phosphatase activity would also be quantified, although current technologies may not permit routine clinical implementation of such an assay.

Methods to determine PTEN status by IHC were historically difficult and continue to be refined, which may contribute to conflicting results between studies. These issues are likely due to antibody and scoring variability. The most commonly used control for PTEN positivity is adjacent normal cells (*i.e.*, vascular endothelium) within a tumor section, which should be PTEN-positive. If the PTEN IHC signal is weaker in tumor cells compared to normal cells, the tumor is usually scored as “PTEN-deficient,” and tumors with malignant cells with no IHC signal are considered “PTEN-null.” This scoring system is subjective, prone to variability, and difficult to implement into routine clinical practice. Also, there are reports of PTEN heterogeneity by IHC within tumors [84, 85], which complicates scoring and interpretation. Reverse-phase protein array (RPPA) analysis, which is analogous to quantitative high-throughput dot-blotting, of lysates from 306 breast tumors showed that PTEN levels are strongly, inversely correlated with levels of activated AKT [86]. Efforts are underway to employ RPPA in routine clinical use; this method may be useful to accurately determine PTEN protein content, and is amenable to multiplex analysis of a panel of proteins and post-translational modifications [87].

Further confounding is the fact that tumors exhibited genetic heterogeneity. Biopsies of seven regions from a primary tumor and one from a liver metastasis within a patient with renal cell carcinoma were analyzed by DNA (exome) sequencing. A *PTEN* splice site mutation was detected in 3/6 regions of the primary tumor, and a *PTEN* missense mutation was detected in a fourth region of the primary tumor; the other two regions of the primary tumor, and the metastasis, were *PTEN*-wild-type [88]. In another study of multiple biopsies from 134 prostate cancers, *PTEN* exhibited genetic heterogeneity in 6 cases (5%), whereby one or both *PTEN* alleles were lost in different regions of a tumor [89]. Given that *PTEN* is often silenced at the transcriptional level, it is possible that the remaining intact *PTEN* allele in hemizygous prostate tumors was transcriptionally silenced. It should be considered that

different regions within a tumor may exhibit convergent phenotypic evolution (*i.e.*, different patterns of mutations to achieve the same phenotype). Hence, determination of *PTEN* status may not be as important as determination of the resultant phenotype (*e.g.*, PHTS, Tuberous Sclerosis, and Progeria exhibit similar phenotypes; described below), which may be more amenable to therapeutic intervention, and capture a larger patient population.

Therapeutically targeting loss of PTEN phosphatase activity

Loss of PTEN lipid phosphatase activity leads to PIP_3 accumulation at the plasma membrane, which activates the AKT/mTOR pathway to drive cell growth, proliferation, and survival [5]. As such, therapeutics targeting several nodes of this pathway are under development.

Pre-clinical findings on treatment of cancers with loss of PTEN phosphatase activity

Mouse models with genetic loss of PTEN have proven valuable in the testing and development of therapies to target loss of PTEN phosphatase activity. *Pten* heterozygous mice (*Pten*$^{+/-}$) exhibit several of the phenotypes displayed by patients with Cowden's Syndrome, including increased susceptibility to endometrial and breast cancers [90]. *Pten*$^{+/-}$ mice treated with the pharmacological inhibitor of mTORC1 Temsirolimus (CCI-779) had reduced or no development of several tumor subtypes [91]. Another rapamycin analogue (Everolimus, Afinitor, RAD001) retarded the development of endometrial hyperplasia, and rapamycin decreased growth of prostate tumors in *Pten*$^{+/-}$ mice [92, 93]. In mice with prostate-specific deletion of *Pten*, heterozygous deletion of components of the TORC1/2 complexes (*Mtor, Mlst8*) increased lifespan [94]. In addition, deletion of *Akt1* in prostate-specific *Pten*$^{+/-}$ mice prevented endometrial and prostate tumor growth [95]. These findings suggest that inhibitors of the AKT/mTOR pathway may be effective in preventing and controlling growth of PTEN-deficient tumors. Indeed, genetic lesions in *PTEN* are associated with increased sensitivity to Temsirolimus (allosteric mTORC1 inhibitor), AZD6482 (PI3K/p110β inhibitor), MK-2206 (allosteric AKT inhibitor), and 17-AAG (HSP90 chaperone inhibitor that induces degradation of many proteins including HER2 and AKT) (Fig. 3).

In addition to promoting tumorigenesis, loss of PTEN drives resistance to anti-cancer therapeutics. Knockdown of *PTEN* in estrogen receptor α (ER)-positive breast cancer cells conferred resistance to the anti-estrogens tamoxifen and fulvestrant, and to estrogen deprivation [4]. *PTEN* knockdown increased ER transcriptional activity, Insulin-like Growth Factor-1 Receptor (IGF-1R) and ErbB3 receptor tyrosine kinase activity, AKT activation, and cell proliferation [4]. Another study showed that *PTEN* knockdown in non-small cell lung cancer cells induced resistance to the EGFR inhibitor Gefitinib, but not to the VEGFR/EGFR dual inhibitor Vandetanib [96]. Genetic *PTEN* aberrations are associated with resistance to Bosutinib (BCR/Abl and Src inhibitor), BMS-754807 (IGF-1R/InsR and c-Met inhibitor), and OSI-906 (IGF-1R/InsR inhibitor) (Fig. 3). These data suggest that targeted agents acting at the level of PI3K or downstream may be most effective for treatment of PTEN-deficient cancers, while agents targeting signaling nodes upstream of PI3K will be less useful.

Most recently, PTEN loss was shown to reduce the sensitivity of melanoma cells to the γ-secretase inhibitor (GSI) RO4929097 [97]. GSIs are used to treat patients with melanoma because of their ability to prevent cleavage and activation of the Notch receptor, which is usually de-regulated in melanoma. RO4929097 was only effective at inducing senescence and apoptosis in human melanoma cell lines with wild-type PTEN. PTEN rescue in *PTEN*-null or -mutant melanoma cells restored sensitivity to RO4929097.

Post-translational modifications in PTEN also confer resistance to targeted therapies. PTEN phosphorylation at Tyr240, mediated by Src and Fibroblast Growth Factor Receptor (FGFR) 2 and 3 was shown to confer resistance to EGFR inhibitors in glioblastoma multiforme (GBM) *in vitro* and *in vivo* [98]. Although this modification in PTEN does not abrogate its ability to repress PI3K signaling, it occurs frequently in human GBM tumors and has been linked to shortened survival [98]. To corroborate their findings, the authors showed that a phospho-inhibiting Tyr240Phe mutation in *PTEN* enhanced sensitivity of xenograft-derived GBM cells to EGFR inhibitors [98].

Clinical findings on PTEN status and response to RTK/PI3K pathway-targeted therapeutics

Several drugs are currently in clinical trials for the treatment of patients with PTEN-deficient cancers (Table 2). Drug sensitivity information gleaned from preclinical studies has been partially validated in the clinic. The observation that PTEN deficiency is associated with resistance to RTK inhibitors *in vitro* makes mechanistic sense: if a PI3K antagonist (PTEN) is defective, then less PI3K activation from RTKs should be required to drive PIP_3 signaling. However, whether PTEN loss is associated with resistance to RTK-directed therapies in cancer patients remains a matter of debate. Mature clinical data exist concerning the relationship between PTEN status and response to the anti-HER2 therapeutic antibody Trastuzumab in patients with breast cancers that overexpress the HER2 RTK protooncogene. First, we must consider the putative mechanisms of Trastuzumab anti-tumor action: Trastuzumab binds to the extracellular domain of HER2 on the cell surface, disrupts HER2-HER3 heterodimers (which activate PI3K) [99], inhibits cleavage of the HER2 extracellular domain (which removes the Trastuzumab-binding epitope from HER2) [100], and induces antibody-dependent cellular cytotoxicity [101, 102]. Several groups found that PTEN loss was generally associated with poor response to Trastuzumab therapy, whether this agent was administered in the neoadjuvant, adjuvant, or metastatic settings [103–105]. Oddly, PTEN loss was associated with improved response (assessed by pathological complete response) to neoadjuvant treatment with the EGFR/HER2 kinase inhibitor lapatinib followed by Trastuzumab [104]. Contrary to these smaller studies, results from a recent phase III trial (NCCTG N9831) with 1,201 patients treated with adjuvant chemotherapy plus Trastuzumab (sequential or concurrent) revealed that PTEN status did not have an impact on disease-free-survival [106]. Therefore, the role of PTEN status in sensitivity to HER2-directed therapy in HER2+ breast cancer remains unclear.

If PTEN loss confers resistance to Trastuzumab, adding a drug that blocks a signaling node downstream of PI3K may abrogate the effects of PTEN loss. In a phase I/II clinical study, patients with metastatic breast cancer that progressed on Trastuzumab were treated with Trastuzumab plus the TORC1 inhibitor Everolimus. Patients with PTEN-deficient tumors

had decreased overall survival compared to those with PTEN-normal tumors, and progression-free survival was not affected by PTEN status [152]. In two phase I trials, patients with metastatic breast cancer that had progressed on Trastuzumab were treated with Trastuzumab plus Everolimus, with or without Vinorelbine chemotherapy. These studies did not report PTEN status of tumors, but encouraging anti-tumor activity was observed [153, 154]. These data indicate that PTEN deficiency may not sensitize tumors to TORC1 inhibition, but a fraction of patients with PTEN-deficient, HER2+, Trastuzumab-resistant breast cancer may benefit from the combination of Trastuzumab and Everolimus.

Several clinical studies are ongoing to evaluate the benefit of PI3K/AKT/mTOR pathway inhibitors in patients with advanced HER2+ breast cancer. Most trials thus far with PI3K inhibitors only include patients with tumors harboring *PIK3CA* mutations or PTEN deficiency. However, early data suggest that PTEN and *PIK3CA* status are not associated with response to the PI3K/mTOR dual inhibitor BEZ235 in patients with HER2+, metastatic, Trastuzumab-resistant breast cancer, or to the PI3K inhibitor Buparlisib (BKM120) in patients with advanced ER+ breast cancer [155, 156]. Notably, all of the aforementioned trials have been conducted with patients with advanced breast cancer. The benefit of PI3K/AKT/mTOR pathway inhibitors in early-stage breast cancer is being tested in ongoing studies [*e.g.*, NeoPHOEBE, NCT01816594, testing neoadjuvant Trastuzumab, Buparlisib (BKM120), and paclitaxel].

EGFR is a major oncogenic driver in colorectal cancer (CRC) and squamous cell carcinomas of the head and neck (HNSCC), and patients with EGFR-expressing tumors are often treated with an EGFR antibody-based regimen (*i.e.*, Cetuximab or Panitumumab). PTEN deficiency has been linked with Cetuximab resistance in preclinical studies in CRC [157]. In a meta-analysis of five small clinical studies, PTEN loss in tumors was associated with decreased objective response, shorter progression-free survival, and shorter overall survival in patients with primary or metastatic CRC treated with Cetuximab-based therapy [158–163]. While clinical studies suggest that PTEN deficiency is associated with poor disease outcome following EGFR antibody therapy for CRC, PTEN loss/mutation often co-exists with mutations in *BRAF* and *KRAS*, two oncogenes that play significant roles in determining drug sensitivity/resistance; so mutational overlap confounds interpretation of the effects of PTEN loss. Results from early-phase clinical studies show encouraging anti-tumor activity of the combination of an EGFR antibody and a TORC1 inhibitor (Everolimus or Temsirolimus) [164, 165]. Ongoing phase I/II studies testing combinations of EGFR antibodies and PI3K/AKT/mTOR pathway inhibitors (*e.g.*, NCT01256385, NCT00522665, NCT01283334, NCT01816984, NCT01602315, NCT01252628, NCT01719380) will provide larger numbers of patients to assess the role of PTEN status in therapeutic response.

While PTEN deficiency has been associated with increased sensitivity to PI3K pathway inhibitors in preclinical studies in select cancer subtypes [139, 166–171], early clinical data from patients receiving single-agent therapies give mixed results. A phase I study with the PI3K inhibitor Buparlisib (BKM120) in patients with advanced solid tumors showed no association between PTEN status and clinical response [108]; these data imply that selection of patients for Buparlisib therapy based on PTEN status is not beneficial, and that some PTEN-deficient tumors will respond to Buparlisib. In contrast, PTEN deficiency may

sensitize tumors to TORC1 inhibition. In a phase II study with patients with metastatic castration-resistant prostate cancer treated with Everolimus, *PTEN* loss (determined by FISH) was associated with improved response and longer progression-free survival [172]. Patients with advanced pancreatic neuroendocrine tumors treated with standard-of-care plus Everolimus exhibited significantly increased progression-free survival compared to placebo/standard-of-care [173]; while the association between PTEN status and clinical benefit remains to be determined, it is likely that at least a fraction of patients with PTEN-deficient pancreatic cancers benefited from Everolimus.

A body of evidence suggests that PTEN-deficient cancer cells rely on the p110β isoform of Class IA PI3K to drive phosphatidylinositol signaling [174–177]. PI3K/p110β is thought to be activated primarily by G protein-coupled receptors (GPCRs). In contrast, PTEN-wild-type cells seem to engage the p110α or p110δ isoforms, depending on tumor subtype (p110δ is thought to be more important in hematologic malignancies). As such, clinical testing of p110β-specific inhibitors in patients with PTEN-deficient cancers is ongoing (*e.g.*, NCT01458067).

Treatment of patients with PTEN Hamartoma Tumor Syndrome (PHTS)

Germline mutations in *PTEN* predispose to PHTS. Such individuals exhibit macrocephaly, delayed mental development, skin lesions, vascular abnormalities, and cancer predisposition. The lifetime risks for cancer among PHTS individuals versus the general population are: female breast (85.2% vs. 12.4%); colorectal (9% vs. 5%); thyroid (35.2% vs. 1%); kidney (33.6% vs. 1.6%); endometrial (28.2% vs. 2.6%); melanoma (6% vs. 2%) [25–27]. A gain-of-function somatic mutation in *AKT1* was recently discovered in patients with Proteus Syndrome [178]. Individuals with Tuberous Sclerosis carry germline mutations in *TSC1* or *TSC2*, exhibit benign tumors, and have an elevated risk of developing subependymal giant cell astrocytoma [179]. These observations indicate that similar phenotypes can result from mutations in genes encoding proteins present at distinct signaling nodes, all of which converge to activate the AKT/mTOR pathway (Fig. 1). Such signaling is known to promote cell growth and proliferation, leading to tissue overgrowth and the formation of benign tumors. However, DNA replication promotes the acquisition of additional genetic lesions, PTEN deficiency can increase genomic instability, and tissue overgrowth promotes inflammation. These processes likely contribute to malignant transformation and cancer development.

A logical target for treatment of these syndromes is TORC1, which lies downstream of the signaling nodes known to be mutated/lost in afflicted individuals. Trials testing TORC1 inhibitors (Rapamycin, Everolimus) in patients with Tuberous Sclerosis have shown promising results and tumor shrinkage in many cases [180–185]. Given that the penetrance of PHTS in individuals with germline *PTEN* mutations is ~80%, preventive therapy may be beneficial prior to cancer development. This presents a challenge with the use of TORC1 inhibitors because the effects of long-term treatment are unknown, and these drugs elicit significant adverse events (*e.g.*, mucositis, rash, ulcers, fatigue, anorexia, diarrhea, nausea, arthralgia, thrombocytopenia, and immunosuppression) that will decrease quality of life. Early clinical data indicate that short-term TORC1 inhibition is beneficial in patients with

PHTS [186], and the results of recently completed phase I/II studies with TORC1 and PI3K inhibitors are pending (*e.g.*, NCT00971789, NCT00600275, NCT00620594).

Therapeutically targeting loss of PTEN nuclear/genomic activity

Nuclear PTEN is important for the regulation of genome stability, homologous recombination, and apoptosis [15, 16]. PTEN loss or disruption of nuclear import leads to severe chromosomal alterations [16]. Several recent studies have proposed that the role of PTEN in regulation of homologous recombination can be harnessed therapeutically. PTEN deficiency incites a defect in homologous recombination in tumor cells. This defect sensitizes tumor cells to inhibitors of polyadenosine diphosphate ribose polymerase (PARP), an enzyme important for repair of DNA double-strand breaks [187]. Moreover, PTEN loss in endometrial cancer cells decreases homologous recombination and sensitizes cells to PARP inhibitors [188]. In line with these findings, a mouse model with T cell-specific deletion of *Pten* develops T cell receptor (*Tcr*)-*Myc* translocations caused by a defect in *Tcr* recombination [189]. These findings suggest that PARP inhibitors may be beneficial for the treatment of tumors with loss of PTEN nuclear/genomic activity.

Since PTEN deficiency causes a defect in homologous recombination, cells rely on PARP for repair of double-strand DNA breaks. PTEN deficiency therefore sensitizes cancer cells to PARP inhibition [188, 190, 191]. PARP inhibitors are in widespread clinical testing for a variety of cancer subtypes with deficiencies in genome integrity, mainly in patients with breast or ovarian cancers harboring mutations in *BRCA1* or *BRCA2*. Such genetic lesions can co-occur with PTEN aberrations, confounding interpretation of the contribution of PTEN deficiency to response to PARP inhibitors. Similarly, prostate cancers exhibiting PTEN loss often harbor a genetic rearrangement leading to a *TMPRSS22-ERG* fusion. The TMPRSS22-ERG protein product promotes the formation of DNA double-strand breaks and interacts with PARP, thus sensitizing cells to PARP inhibition [192, 193].

Inference from early clinical results suggests that some PTEN-deficient cancers may be sensitive to PARP inhibition. In a phase I trial with the PARP inhibitor Niraparib, tumors in 7/10 patients with advanced prostate cancer exhibited clinical response [194]. Given that the majority of prostate cancers are PTEN-deficient, it is likely that a fraction of Niraparib-sensitive prostate tumors were PTEN-deficient in this study. A case report also described significant tumor response to the PARP inhibitor Olaparib in a patient with PTEN-null, *BRCA1/2*-wild-type, metastatic endometrial cancer [195]. Retrospective analysis of genetic alterations and PTEN status in ongoing studies with PARP inhibitors will help resolve the question of whether PTEN deficiency, in the absence of co-existing defects in DNA repair, is associated with sensitivity to PARP inhibition.

Novel strategies to develop therapeutics targeting PTEN-deficient cancers

Synthetic lethality occurs when aberrations in two genes cause cell death, but individually these aberrations do not. This approach can be used to discover PTEN synthetic lethal interactions in PTEN-deficient tumors [196]. A recent study by Ashworth and colleagues identified *Nemo-Like Kinase (NLK)*, *Polo-Like Kinase 4* (PLK4) and *MonoPolar Spindle 1 (MLK)* as synthetic lethal genes in PTEN-deficient cancer cells [197]. In addition, the

synthetic lethal effect of *NLK* can be abolished by knockdown of *Forkhead Box O1 (FOXO1)*, which suggests that FOXO1 mediates the effects of NLK.

Two recent reports describing cellular export of functional PTEN that can enter other cells and suppress PI3K/PIP_3 signaling suggest that PTEN itself may be therapeutic. Putz *et al*. found that PTEN is exported in exosomes in a Ndfip1-dependent manner. Ndfip1 is an adaptor for Nedd4 E3 ubiquitin ligases, and the ubiquitination site Lysine-13 of PTEN is required for PTEN export [198]. Hopkins *et al.* reported that a translational variant gives rise to a longer PTEN protein ("PTEN-Long") that is secreted from cells. This membrane-permeable lipid phosphatase enters other cells and antagonizes PI3K signaling [199]. It is therefore conceivable that PTEN may be delivered to cancer cells to treat patients with PTEN-deficient cancers.

Other therapeutic strategies have been proposed to target tumors with PTEN loss. It was suggested that statins may be used to increase PTEN expression since they promote the activity of the transcription factor peroxisome proliferator-activated receptor-gamma (PPARγ), known to upregulate *PTEN* mRNA levels [200]. Since *PTEN* can be silenced by methylation, demethylating agents may reverse epigenetic silencing of *PTEN* [24, 201]. Furthermore, it was proposed that the mRNA of the *PTEN* pseudogene *PTENP1* can be used to sequester miRNAs that repress *PTEN* translation [202]. Finally, therapies that target P-REX2a and SIPL1 may be useful to derepress PTEN phosphatase activity.

Summary

The recent development of targeted anti-cancer therapeutics has been focused primarily on oncogenes and tumor promoters, most commonly in the form of kinase inhibitors. Designing anti-cancer therapeutics directed at loss of tumor suppressors has traditionally been more difficult. Even now, most of the strategies to target PTEN-deficient cancers utilize inhibitors of kinases that lie at the level of PI3K or downstream. With the relatively recent implementation of synthetic lethal screens in cancer drug target studies, novel therapeutic angles may be uncovered that will expand our thinking of PTEN deficiency beyond the PI3K pathway and genome instability. Retrospective analysis of PTEN status in tumors from patients participating in ongoing clinical trials will provide much needed support for the idea that PTEN deficiency modulates drug sensitivity and resistance.

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Abbreviations

PI3K	phosphatidylinositol 3-kinase
RTK	receptor tyrosine kinase
miRNA	microRNA

PARP	polyadenosine diphosphate ribose polymerase
PIP_3	phosphatidylinositol (3,4,5)-trisphosphate
PHTS	PTEN Hamartoma Tumor Syndrome
PLK	polo-like kinase
HDAC	histone deacetylase
FISH	fluorescence *in situ* hybridization

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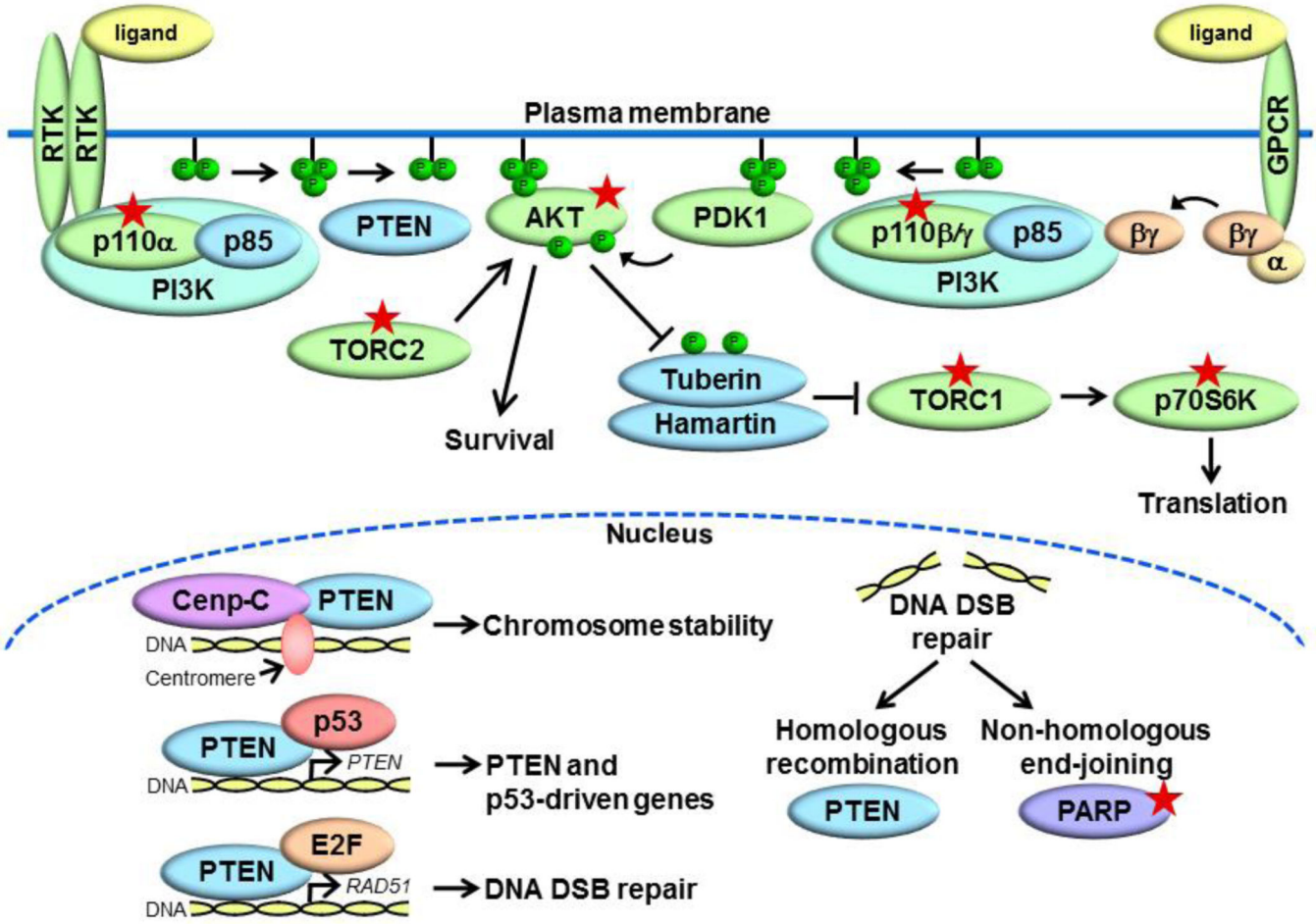

Fig. 1. PTEN exhibits tumor suppressive functions in the cytoplasm and nucleus

The phosphatidylinositol 3-kinase (PI3K) pathway regulates cancer cell growth and survival. This pathway is activated by ligand binding to receptor tyrosine kinases (RTKs) and/or G protein coupled receptors (GPCRs). PI3K is then recruited to the membrane where it phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP_2) to produce phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), leading to activation of several signaling cascades including AKT/mTORC1. Cytoplasmic PTEN negatively regulates this pathway by dephosphorylating PIP_3 at its D3 position. Nuclear PTEN promotes chromosome stability and regulates DNA double-strand break repair. Red star indicates a potential therapeutic target for which a drug(s) is in development.

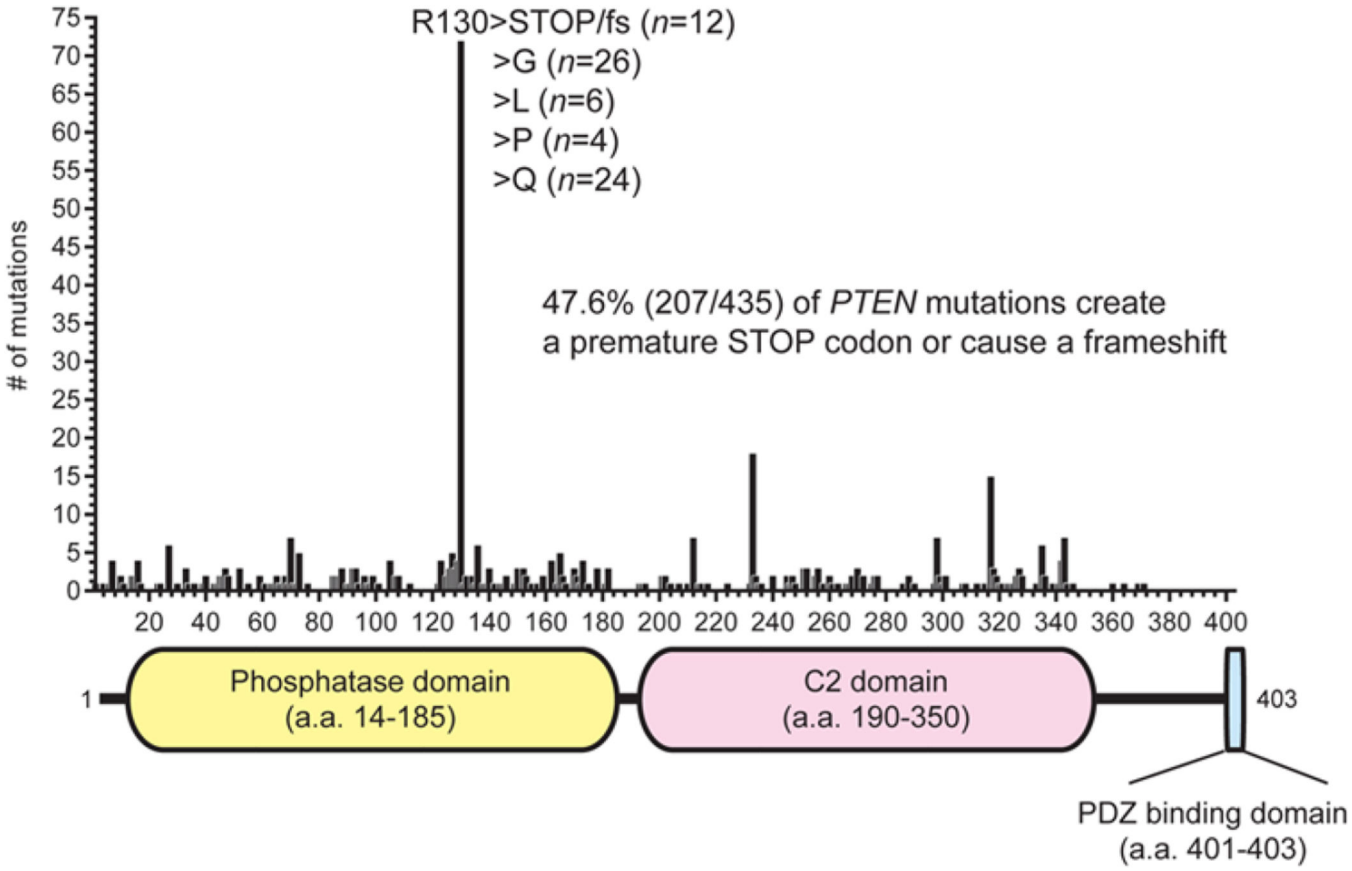

Fig. 2. Location and frequency of somatic mutations in *PTEN*

Somatic mutations in *PTEN* occur throughout the coding region. 47.6% of these mutations form a premature STOP codon or cause a frameshift mutation leading to a truncated PTEN protein. Data was obtained from The Cancer Genome Atlas (TCGA) via the cBio Portal for Cancer Genomics [29, 44]. Domain mapping was obtained from UniProt [203].

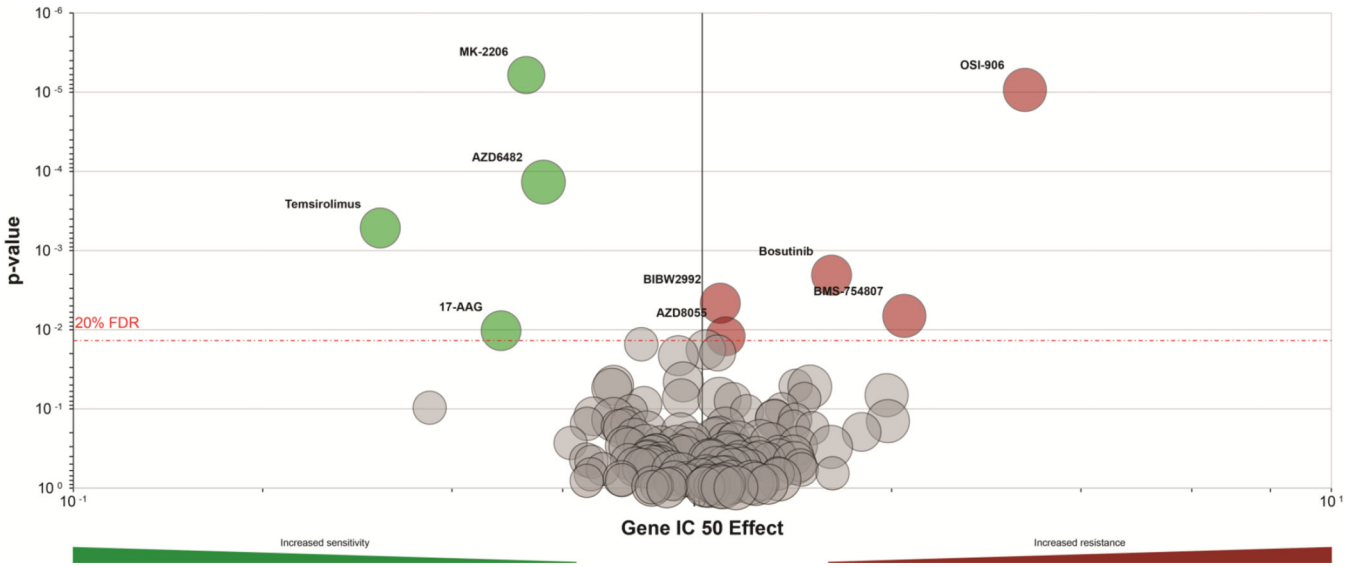

Fig. 3. Genetic lesions in *PTEN* are associated with increased sensitivity to PI3K, AKT, and mTOR inhibitors, and decreased sensitivity to RTK inhibitors

As part of the Genomics of Drug Sensitivity in Cancer Project [204], >500 genetically characterized cell lines were profiled for sensitivity to a panel of 139 small molecules. This database (http://www.cancerrxgene.org) was queried to identify drugs with sensitivity/resistance profiles associated with *PTEN* genetic alterations. Data are presented as a volcano plot, where x-axis indicates magnitude of effect that *PTEN* alterations have on IC_{50} values in response to drug, y-axis indicates p-value from MANOVA of drug-*PTEN* interaction, size of a circle reflects number of PTEN alterations contributing to the analysis for a given drug, and red dashed line represents a Benjamini-Hochberg multiple testing correction threshold with a false discovery rate of 20%. Statistically significant *PTEN*-related sensitizing or resistance effects are indicated by green circles and red circles, respectively. *PTEN* alterations are associated with increased sensitivity to MK-2206 (AKT inhibitor), AZD6482 (PI3K/p110β inhibitor), Temsirolimus (allosteric mTORC1 inhibitor), and 17-AAG (Hsp90 inhibitor), and with increased resistance to BIBW2992 (Afatinib; EGFR/HER2 inhibitor), AZD8055 (ATP-competitive mTORC1/2 inhibitor), Bosutinib (Src and Abl inhibitor), BMS-754807 (IGF-1R/InsR inhibitor), and OSI-906 (IGF-1R/InsR inhibitor).

Table 1

Frequencies of *PTEN* genetic lesions across cancer subtypes

Genomic sequence data from The Cancer Genome Atlas (TCGA) [29] was interrogated using the cBioPortal for Cancer Genomics [44]. The percentages of tumors exhibiting *PTEN* mutations or homozygous loss are indicated, followed in parentheses by the numbers of cases containing such lesions / total number of cases analyzed.

Cancer Type	% of tumors with *PTEN* mutation or homozygous loss (# altered/total)	Reference(s)
Bladder	4.1% (4/97)	[28]
Lower Grade Glioma	5.3% (9/169)	Provisional [29]
Breast	7.47% (57/760)	[30–33]
Cervical	13.9% (5/36)	Provisional [29]
Colorectal	6.3% (14/221)	[34, 35]
Glioblastoma Multiforme	41.9% (99/236)	Provisional [29, 36]
Head & Neck SCC	2.6% (8/302)	Provisional [29]
Renal clear cell	3.4% (10/290)	Provisional [29]
Renal papillary	3% (3/100)	Provisional [29]
Lung Adenocarcinoma	3.9% (5/129)	[37, 38]
Lung SCC	11.2% (20/179)	[39]
Ovarian	7.28% (23/316)	[40]
Prostate	13.6% (14/103)	[41]
Sarcoma	2.9% (6/207)	[42]
Melanoma	12.4% (28/225)	Provisional [29]
Stomach	11.3% (13/115)	Provisional [29]
Thyroid	1.9% (6/318)	Provisional [29]
Uterine	66.3% (159/240)	[43]

Table 2

Drugs under clinical development that may be useful to treat patients with PTEN-deficient cancers

The phase of clinical development is indicated in the right column.

Drug target	Drug	Mechanism of action	Clinical Phase	Reference(s)
Class IA PI3K	BKM120	ATP-competitive	3	[107, 108]
	XL-147	ATP-competitive	2	[109]
	PX-866	ATP-competitive	2	[110]
	GDC-0941	ATP-competitive	2	[111]
	BAY80-6946	ATP-competitive	2	[112]
	CH5132799	ATP-competitive	1	[113, 114]
	GDC-0084	ATP-competitive	1	
	ZSTK474	ATP-competitive	1	[115]
p110α/γ/δ/PI3K-specific	GDC-0032	ATP-competitive	1	[116]
p110α/PI3K-specific	BYL719	ATP-competitive	2	[117, 118]
	MLN1117	ATP-competitive	1	[119]
p110β/PI3K-specific	GSK2636771	ATP-competitive	1	[120]
PI3K/PLK	Rigosertib	ATP-competitive	3	[121]
PI3K/HDAC	CUDC-907	Bifunctional	1	[122]
PI3K/mTOR	PKI-587 (PF-05212384)	ATP-competitive	2	[123, 124]
	BEZ235	ATP-competitive	2	[125]
	BGT226	ATP-competitive	2	[126, 127]
	GDC-0980	ATP-competitive	2	[128–130]
	XL-765	ATP-competitive	2	
	SF1126	ATP-competitive*	1	[131]
	LY3023414	ATP-competitive	1	
TORC1	Everolimus (RAD001)	Indirect, FKBP12-mediated	Approved	[132, 133]
	Temsirolimus (CCI-779)	Indirect, FKBP12-mediated	Approved	[134]
	Ridaforolimus (AP-23573)	Indirect, FKBP12-mediated	3	[135]
TORC1/TORC2	MLN0128	ATP-competitive	1/2	[136]
	AZD-2014	ATP-competitive	1	[137]
	CC-223	ATP-competitive	1	[138]
AKT/p70S6K/PKA	AZD-5363	ATP-competitive	1	[139]
AKT/p70S6K	LY2780301	ATP-competitive	1	[140]
AKT	GDC-0068	ATP-competitive	1	[141]
	GSK2110183	ATP-competitive	1	[142]
	MK-2206	Allosteric	2	[143]
PARP	Olaparib	Inhibits DNA repair	2	[144]
	Veliparib	Inhibits DNA repair	2	[145]
	Iniparib	Inhibits DNA repair	3	[146]

Drug target	Drug	Mechanism of action	Clinical Phase	Reference(s)
	Rucaparib	Inhibits DNA repair	2	[147]
	CEP-9722	Inhibits DNA repair	2	[148, 149]
	E7016	Inhibits DNA repair	2	[150]
	E7449	Inhibits DNA repair	2	[151]

* indicates peptide conjugate.