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PTEN loss in the continuum of common cancers, rare syndromes and mouse models

M. Christine Hollander, **Gideon M. Blumenthal**, **Phillip A. Dennis**

Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, 37 Convent Drive Room 1118B, Bethesda, Maryland 20892, USA.

Abstract

PTEN is among the most frequently inactivated tumour suppressor genes in sporadic cancer. PTEN has dual protein and lipid phosphatase activity, and its tumour suppressor activity is dependent on its lipid phosphatase activity, which negatively regulates the PI3K–AKT–mTOR pathway[1,2]. Germline mutations in *PTEN* have been described in a variety of rare syndromes that are collectively known as the PTEN hamartoma tumour syndromes (PHTS). Cowden syndrome is the best-described syndrome within PHTS, with approximately 80% of patients having germline *PTEN* mutations[3]. Patients with Cowden syndrome have an increased incidence of cancers of the breast, thyroid and endometrium, which correspond to sporadic tumour types that commonly exhibit somatic *PTEN* inactivation. *Pten* deletion in mice leads to Cowden syndrome-like phenotypes, and tissue-specific *Pten* deletion has provided clues to the role of *PTEN* mutation and loss in specific tumour types. Studying PTEN in the continuum of rare syndromes, common cancers and mouse models provides insight into the role of PTEN in tumorigenesis and will inform targeted drug development.

The tumour suppressor *PTEN* was first identified in 1997 by deletion mapping of brain, breast and prostate cancers[4,5]. Shortly thereafter, germline *PTEN* mutations were linked to Cowden syndrome[6] and other proliferative syndromes[7]. The term PTEN hamartoma tumour syndrome (PHTS) is now used to unify these seemingly disparate clinical syndromes into one entity (see the PHTS GeneReview on the US National Library of Medicine website; see Further information). Patients with PHTS are a rare but ideal population to study PTEN biology and targeted drug development, as loss of PTEN function seems to be driving many of the phenotypic features of this syndrome. As is common in most tumours, sporadic (non-hereditary) tumours with somatic *PTEN* alteration also carry other genetic alterations, making the role of PTEN more ambiguous. As discussed below, mouse models have shown that *Pten* deletion alone is sufficient to cause tumorigenesis in certain tissues but not in others. However, even when deletion of PTEN alone has minimal effects, it frequently contributes to tumorigenesis in the context of other genetic alterations. Efforts to compensate for loss of *Pten* by inhibiting the PI3K–AKT–mTOR pathway through genetic or pharmacological means can be investigated in genetically defined mouse models. PHTS

Correspondence to P.A.D. pdennis@nih.gov.

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provides a defined population for clinical trials of pathway-targeted therapies. This Review focuses on tumours types that occur in Cowden syndrome, that exhibit somatic *PTEN* alterations and that develop in mouse models engineered to lose *Pten*. The intersection of these three groups provides strong evidence for the functional importance of PTEN alteration in specific tumour types.

PTEN biology

The *PTEN* gene spans 105 kb and includes nine exons on chromosome 10q23. Tumour suppressor function requires both the phosphatase domain and the C2 or lipid membrane-binding domain (FIG. 1), and mutations have been reported throughout the protein. The lipid phosphatase activity of PTEN dephosphorylates the 3-phosphoinositide products of PI3K. 3-phosphoinositides can activate important survival kinases, such as phosphoinositide-dependent kinase 1 (PDK1; encoded by *PDPK1*) and AKT, as well as other proteins that are not kinases (FIG. 1). PTEN therefore negatively regulates the AKT pathway, leading to decreased phosphorylation of AKT substrates such as tuberous sclerosis 2 (TSC2) and PRAS40 (encoded by *AKT1S1*) that control mTOR activity, p27 (encoded by *CDKN1B*), p21 (encoded by *CDKN1A*), glycogen synthase kinase 3 (GSK3A and GSK3B), BCL-2-associated agonist of cell death (BAD), apoptosis signal regulating kinase 1 (MAP3K5; also known as ASK1), WT1 regulator PAWR (also known as PAR4) and CHK1, as well as members of the fork-head transcription factor family (for example, FOXO1, FOXO3 and FOXO4)[8] and others. Changes in phosphorylation alter the activity and/or localization of these proteins, which in turn affects processes such as cell cycle progression, metabolism, migration, apoptosis, transcription and translation.

Although the lipid phosphatase activity of PTEN is important for its tumour suppressor functions, other functions of PTEN may also prove to be important. For example, several studies have demonstrated that PTEN protein phosphatase activity is important for its functions in cell cycle arrest and inhibition of cell invasion *in vitro*[9–13]. The lipid phosphatase activity of PTEN is thought to mostly occur at the cell membrane, but PTEN has also demonstrated nuclear functions. The binding of PTEN to centromere protein C1 (CENP-C1) is required for centrosome stability, and its nuclear localization is required for DNA double-strand break (DSB) repair that is mediated by DNA repair protein RAD51 (REF. 14). PTEN also regulates the tumour suppressor function of anaphase-promoting complex (APC) and its regulator E-cadherin (encoded by *CDH1*) in the nucleus, independently of its lipid phosphatase activity[15]. Altered APC–CDH1 activity has been implicated in multiple tumour types[16].

PTEN mutations and cancer.

Germline mutations resulting in the loss of PTEN function or in reduced levels of PTEN are found in approximately 80% of patients with Cowden syndrome[3], and PTEN deletion, mutation or alteration occurs in many sporadic tumours[17]. The Sanger Institute maintains a database of *PTEN* mutations with 1,904 annotated mutations for 30 tumour types (see the Catalogue of Somatic Mutations in Cancer (COSMIC) website; see Further information). From this database, it is clear that in sporadic tumours, mutations, small insertions and

deletions occur throughout the length of *PTEN*, although there are higher frequency mutations, known as mutation hotspots, at specific amino acids. However, mutations at these hotspots are not specific for a particular type of cancer. For example, more than 250 different *PTEN* mutations have been described for endometrial tumours, but 19% of the 632 reported mutations correspond to Arg130 within the phosphatase catalytic site. Mutations in Arg130 occur in other tumour types (such as 4% of central nervous system (CNS) tumours), but they are most frequent in endometrial and ovarian tumours (19%). Mutant *PTEN* was reported in 18% of CNS tumours, with the highest frequency (6% of *PTEN* mutations) corresponding to Arg.

Germline PTEN mutations in PHTS are found throughout most of the *PTEN* coding region, with the exception of exon 9, which encodes the carboxy-terminal 63 amino acids[18]; 40% occur within exon 5, which encodes the phosphatase domain[18]. In sporadic tumours, only 2% of reported sporadic *PTEN* mutations occur within exon 9 and 27% occur within exon 5. Correlations between specific *PTEN* mutations and disease severity in PHTS have been suggested[3,19]. However, larger data sets and more detailed functional mapping of PTEN will certainly allow more informed models. Allelic or total deletion of PTEN is a frequent occurrence in cancers such as breast and prostate cancer, and melanoma and glioma (see the Tumorscape website; see Further information). A subset of patients with Cowden syndrome carries germline mutations in the *PTEN* promoter or in potential splice donor and acceptor sites[20]. Splicing alterations can lead to exon skipping that alters PTEN function, but promoter methylation has been shown to decrease apparently normal PTEN[21]. In mice, decreasing PTEN dosage correlates with increasing tumour susceptibility[22,23]. This suggests that reduced levels of normal PTEN are insufficient for its tumour suppressor function and raises the possibility that regulation of PTEN activity could be an important driving mechanism for cancer.

PTEN dosage.

There are multiple mechanisms for the regulation of PTEN, including transcription, mRNA stability, microRNA (miRNA) targeting, translation and protein stability. *PTEN* is transcriptionally silenced by promoter methylation in endometrial, gastric, lung, thyroid, breast and ovarian tumours, as well as glioblastoma[24–30]. In glioma, lung and prostate cancer, PTEN expression is decreased by overexpression of miRNA 21 (miR-21), miR-25a, miR-22 or the miR-106b–25 cluster[31–33]. PTEN can also be post-translationally regulated by phosphorylation, ubiquitylation, oxidation, acetylation, proteosomal degradation and subcellular localization (reviewed in REFS 34,35). Although many of these post-translational changes in PTEN have been shown to alter various cellular phenotypes *in vitro*, most have not been validated as key regulators of PTEN in human cancer or mouse models. PTEN amino acids Lys13 and Lys289 are monoubiquitylated, which leads to nuclear import *in vitro*, and Lys289 mutations have been observed in Cowden syndrome and associated with nuclear exclusion[36]. No Lys289 mutations have been reported in sporadic cancers, although Lys13 mutation was found in four of 632 endometrial cancers (see the COSMIC database; see Further information).

Cancers classically associated with PHTS

Germline *PTEN* mutation in Cowden syndrome can lead to decreased or absent expression or activity of the mutant allele. Initial efforts to model Cowden syndrome in mice used genetic deletion of a single allele of *Pten*, as loss of both alleles is embryonic lethal. These *Pten* heterozygous (*Pten*$^{+/-}$) mice recapitulated some of the neoplastic phenotypes observed in patients with Cowden syndrome, such as breast and endometrial tumours and intestinal polyps[37–39]. However, the genetic background of *Pten*$^{+/-}$ mice is a strong determinant of susceptibility to specific tumour types (BOX 1). Some strains exhibit tumour types that are not typically associated with Cowden syndrome, such as prostate and adrenal tumours and lymphoma[40], whereas other strains show a reduced incidence of tumours types that are normally associated with Cowden syndrome, such as breast and endometrial tumours[41]. Decreasing PTEN dosage has been shown to correlate with increasing tumour formation in mice, supporting the value of *Pten*$^{+/-}$ mice as models for Cowden syndrome.

Somatic *PTEN* alteration is common in many sporadic tumour types[42], some of which also occur with germline *PTEN* alteration in Cowden syndrome (TABLE 1). This suggests that *PTEN* alteration may be an aetiological factor in these tumour types. Various tissue-specific and/or inducible homozygous deletions of *Pten* have been generated in mice to model sporadic PTEN loss in tumorigenesis. In the endometrium[43], mammary gland[44] and prostate[45], and in T cells[46], homozygous deletion of *Pten* led to rapid tumour formation in the targeted tissue. Tumours took longer to develop after *Pten* deletion in the liver[47], bladder[48] and lung[49]. By contrast, when *Pten* was deleted in pancreatic β-cells[50] or the intestine[51], no malignant tumours developed, although intestinal polyps were common, as observed in Cowden syndrome. Loss of other tumour suppressors or the activation of oncogenes can nonetheless combine with *PTEN* loss to cause cancer in these organs. The following sections describe the intersection of PHTS, sporadic cancer and mouse models to delineate the role of PTEN alteration in specific cancers.

Breast cancer.

Female patients with Cowden syndrome have a high risk (an estimated 25–50% risk) of developing breast cancer over the course of their lifetime, and male patients with Cowden syndrome are also thought to be at an increased risk[52]. PTEN loss can also occur in other populations at a high risk of breast cancer, such as those that carry germline mutations in *BRCA1* in which *PTEN* deletions have been described[53], and can also occur in those at an indeterminate risk. For example, despite the fact that less than 5% of sporadic breast tumours harbour *PTEN* mutations, loss of PTEN immunoreactivity is observed in nearly 40%[54]. This highlights the importance of immunohistochemistry methodology in determining PTEN status[55]. Moreover, about 40% display loss of heterozygosity (LOH) at 10q23 (REF. 56), and aberrant promoter methylation was identified in nearly 50% of tumours[25]. As *PTEN* loss and *ERBB2* mutations both activate the AKT signalling pathway, perhaps it is not surprising that many tumours that exhibit loss of *PTEN* are also oestrogen receptor (ER)-positive and ERBB2-negative[54].

Pten$^{+/-}$ mice can develop mammary tumours at high frequencies depending on their genetic background[39]. Deletion of both *Pten* alleles in the mammary epithelium leads to altered

mammary development and high-frequency, early-onset tumours in mice[44]. Loss of a single *Pten* allele accelerated tumorigenesis in a Wnt-induced mammary tumour model, and most tumours lost the remaining *Pten* allele[57]. Similar results were observed when breast-specific *Pten* deletion was coupled with overexpression of *Erbb2* (REF. 58). In two other models, subtle decreases in PTEN expression increased the risk of tumour formation in the absence of any other introduced mutations[22,23]. These mouse studies suggest that decreased PTEN expression leads to an increased risk of breast tumour formation. Attenuated PTEN expression by gene mutation, LOH or promoter methylation may indeed be a driving alteration in breast cancer, making PTEN signalling pathways or pathways downstream of PTEN potential targets for breast cancer therapy.

Endometrial cancer.

The lifetime risk of endometrial cancer for patients with Cowden syndrome is estimated to be 5–10%[52,59], and 35–50% of sporadic endometrial carcinomas have *PTEN* mutations (TABLE 1). Mutations in PTEN are also observed in endometrial hyperplasia, which is thought to be a precursor lesion for endometrial carcinoma[60–62]. Many endometrial tumours have short insertion or deletion frameshift mutations that are typical of microsatellite instability. In particular, *PTEN* frameshift mutations are observed in endometrial carcinomas that are associated with hereditary non-polyposis colon cancer syndrome (HNPCC)[63]. In addition, polymorphisms in DNA mismatch repair genes affect the risk of endometrial tumours[64], suggesting that the alterations in *PTEN* that contribute to endometrial tumours can arise as a result of compromised DNA repair mechanisms. In endometrial tumours, activation of AKT is associated with loss of PTEN[65].

In mice, loss of *Pten* is sufficient to cause endometrial carcinogenesis. Depending on strain background, *Pten*$^{+/-}$ mice can develop endometrial hyperplasia with high penetrance, which in some cases can progress to endometrial carcinoma as the mice age[39]. In this model, most malignant tumours lose the remaining *Pten* allele[39], leading to AKT activation and subsequent ERα phosphorylation and activation[66]. Consequently, ER antagonists can substantially decrease hyperplasic lesions and tumour formation in these mice[66]. Likewise, inhibition of mTOR, downstream of PTEN–AKT, can prevent the progression of endometrial hyperplasia[67].

The role of DNA repair in the maintenance of *PTEN* integrity is also highlighted in mouse models of endometrial cancer. Familial mutations in the DNA mismatch repair gene *MLH1* underlie HNPCC, and deletion of *Mlh1* in *Pten*$^{+/-}$ mice accelerated endometrial carcinoma formation[68]. *Mlh1* deletion was associated with earlier LOH for the remaining *Pten* allele[68], suggesting that *Pten* may be particularly susceptible to disruptions in DNA repair.

Thyroid cancer.

Thyroid tumours were one of the first tumour types to be associated with Cowden syndrome[69]. Subsequently, about 25% of benign thyroid adenomas and several sporadic malignant thyroid tumour types were found to have *PTEN* LOH, with *PTEN* mutations occurring less frequently[70,71]. Complete loss of PTEN expression occurs in less than 10% of thyroid tumours, but occurs at a higher frequency in the anaplastic subtype[72]. A more recent

study found methylation of the *PTEN* promoter in more than 50% of thyroid tumours of various histologies, particularly follicular carcinoma, and loss of PTEN immunoreactivity correlated significantly with promoter methylation[24]. In addition, *PTEN* is rearranged in most papillary thyroid carcinomas, and in a subset of normal thyroid samples, leading to putative non-functional PTEN[73].

Despite the high prevalence of *PTEN* alterations in human tumours, *Pten*$^{+/-}$ mice only develop thyroid lesions with late onset and low frequency[74]. However, homozygous deletion of *Pten* in mouse thyroid cells led to the development of goiters and benign follicular adenomas in female mice[75]. Decreased gene dosage of PTEN may nonetheless promote thyroid carcinogenesis, because hemizygous deletion of *Pten* accelerated thyroid adenocarcinoma formation that was induced by a dominant-negative mutant thyroid hormone receptor-β, and increased metastases to the lung[76]. In addition, hemizygous deletion of *Pten* also cooperated with loss of p27 to accelerate thyroid tumorigenesis[74]. These data suggest that *Pten* mutation alone may not drive thyroid carcinogenesis in mice, but can contribute to the malignant phenotype in the setting of other genetic alterations.

Central nervous system tumours.

PTEN loss is observed in benign and malignant brain tumours. Lhermitte–Duclos disease is a rare benign tumour (a dysplastic gangliocytoma of the cerebellum) that frequently occurs in patients with Cowden syndrome and is associated with a high rate of morbidity[52]. *PTEN* LOH occurs in more than 70% of glioblastomas, with mutation of the remaining *PTEN* allele found in 44%[77]. Decreased PTEN expression is characteristic of tumour progression, as lower grade gliomas express higher levels of PTEN than glioblastomas[78,79]. Independently of tumour grade, higher PTEN expression levels significantly correlated with increased overall survival[78]. miR-26a, which targets *PTEN* mRNA for degradation, is amplified in glioma and often associated with *PTEN* LOH, suggesting that in this tumour type, multiple mechanisms may coexist to attenuate PTEN expression[31].

Pten$^{+/-}$ mice do not develop brain tumours, but homozygous deletion of *Pten* in mouse brain resulted in abnormalities that resembled those occurring in patients with Lhermitte–Duclos disease[80,81]. Deletion was associated with an increase in neural stem cells[82] (BOX 2). Deletion of *Pten* alone in adult mouse glial cells does not lead to glioma formation, but *Pten* deletion can contribute to rapid glioma formation in the context of additional genetic alterations. For example, *Pten* deletion accelerated high-grade malignant astrocytoma formation in the presence of activated HRAS1 (REF. 83), and *Pten* hemizygosity accelerated astrocytoma formation by SV40 T antigen[84]. Heterozygous deletion of *Pten* also accelerated glioblastoma formation that is induced by brain-specific heterozygous or homozygous deletion of *Trp53* (REFS 85,86) or heterozygous deletion of both *Trp53* and neurofibromatosis 1 (*Nf1*)[87]. Deletion of *Pten* accelerated glioma progression that is induced by overexpression of platelet-derived growth factor (PDFG). Overexpression of miR-26a also accelerated PDGF-induced glioma and decreased survival. This effect was dependent on PTEN, validating the *Pten*-targeting role of miR-26a in glioma[31].

Pten loss in non-PHTS-associated cancers

Prostate.

Prostate tumours have not been associated with Cowden syndrome, perhaps owing to their high incidence in the general population. One of the early cytogenetic abnormalities identified in prostate cancer was the deletion of chromosome 10q[88], and nearly a decade later frequent *PTEN* loss in primary prostate cancer was mapped to this region[89]. Prostate cancer is the most common malignancy in men, and the role of PTEN in prostate tumorigenesis and tumour progression has been extensively studied in mice.

Pten$^{+/-}$ mice develop prostate tumours from 9 months of age[74]. Homozygous deletion of *Pten* in the mouse prostate led to prostatic intraepithelial neoplasia (PIN) lesions at 6 weeks of age that progressed to invasive and metastatic prostate carcinoma within a few weeks[45]. In this model, prostate tumours responded to androgen ablation, which prolonged survival. However, highly proliferative prostate tumours were observed in these mice at necropsy, suggesting that this is a faithful model of disease progression in humans, in which androgen-independent tumours arise after androgen-ablation therapy[90].

Pten$^{+/-}$ mice have been crossed with various other strains of genetically engineered mouse (GEM) models that represent the genetic or phenotypic changes that are observed in human prostate cancer. In many cases, concurrent *Pten* hemizygosity coupled with deletions in other genes accelerates tumorigenesis. For example, concurrent deletion of *Cdkn1b*, which is often lost in human prostate tumours, accelerated prostate tumorigenesis[74]. Concurrent deletion of the transcription factor *Nkx3.1* decreased survival, increased metastasis and resulted in tumours with androgen independence, which is associated with a poor prognosis in patients with prostate cancer[91]. A *Tmprss2–Erg* translocation, which was recently described in human prostate tumours[92], in mice can cooperate with *Pten* hemizygosity to accelerate invasive prostate adenocarcinoma[93,94]. Heterozygous deletion of *Pten* also accelerated prostate tumorigenesis and decreased survival in the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model[95]. The use of *Pten* hypomorphic alleles demonstrated that decreasing PTEN levels correlate with increased progression of prostate tumours in the mouse[96], suggesting that *Pten* may be haploinsufficient for prostate tumorigenesis and/or prostate tumour progression.

Melanoma.

Despite the fact that melanomas have not been associated with Cowden syndrome, sporadic melanomas frequently have a loss of *PTEN* through LOH, deletion and mutation[97]. *PTEN* can also be epigenetically silenced in melanoma, as decreased *PTEN* transcript levels were associated with *PTEN* promoter methylation[98]. *PTEN* methylation also correlated with decreased survival[99]. In another study, low PTEN expression was associated with melanoma ulceration, which is characteristic of aggressive tumours, but did not significantly correlate with overall survival[100]. A link between DNA damage and PTEN mutation in melanoma has been suggested by Wang *et al.*[101], who showed that more than 50% of the melanomas from patients with xeroderma pigmentosum showed *PTEN* mutation types that are typically associated with ultraviolet radiation exposure[101].

In mice, *Pten* deletion in pigmented mouse cells does not lead to the development of spontaneous melanoma, despite an increase in the number of dermal melanocytes. However, in this model, topical carcinogen treatment led to melanoma formation in nearly 50% of the mice within 20 weeks[102]. In conjunction with *Cdkn2a* (encoding p14ARF) deletion, nearly 10% of *Pten*$^{+/-}$ mice developed spontaneous melanoma[103]. Simultaneous activation of BRAF and deletion of *Pten* in melanocytes leads to early onset spontaneous melanomas, with metastasis to the lymph nodes and lung[104]. Notably, the mTOR inhibitor rapamycin increased survival in these mice by more than twofold[104]. These mouse studies indicate that *Pten* is probably not a driving mutation in melanoma, but can contribute to a malignant phenotype in the presence of other genetic alterations.

Lung cancer.

Lung cancer has rarely been described in Cowden syndrome[105] and somatic *PTEN* mutations occur at a low frequency in small-cell lung cancer (SCLC)[106] and non-small-cell lung cancer (NSCLC)[107]. However, other mechanisms to diminish PTEN function may be more important in lung cancer. For example, 24% of early NSCLC samples lack PTEN expression, which correlated with *PTEN* promoter methylation[30]. In a later study, *PTEN* protein expression was reduced or lost in 74% of lung tumours, and was associated with low or aberrant *TP53* staining[108]. Levels of miR-21 were upregulated in lung tumours compared with normal lung tissue in 74% of cases and were correlated with decreased levels of *PTEN* mRNA and advanced tumour stage[32].

PTEN function may determine treatment outcome in lung cancer. Mutant epidermal growth factor receptor (*EGFR*) is a frequent driving mutation in lung cancer in never-smokers[109], whose tumours initially respond to treatment with EGFR inhibitors. However, resistant tumours emerge through multiple mechanisms, one of which might be homozygous deletion of *PTEN*[110]. Regardless of *EGFR* status, *PTEN* promoter methylation is significantly associated with poor outcome in surgically treated early stage lung cancer[111].

Pten$^{+/-}$ mice have not been reported to develop lung tumours. However, lung-specific homozygous deletion of *Pten* in alveolar type II cells led to lung adenocarcinoma in 87% of mice at 40–70 weeks of age, and increased both the number and size of urethane-induced lung adenomas[49]. Lung-specific homozygous deletion in bronchiole epithelium cells did not produce tumours in mice, but accelerated tumours driven by mutant *Kras*, and dramatically decreased survival[112].

Pancreatic cancer.

Pancreatic cancer is not associated with Cowden syndrome, and mutations in *PTEN* are rare in sporadic cancers. However, pancreatic tumours frequently have altered localization of PTEN, suggesting that subcellular sequestration of PTEN may decrease its function[113]. In mice, homozygous deletion of *Pten* in the pancreas leads to metaplasia, which progresses to carcinoma in about 20% of mice[114]. *Pten* deletion in pancreatic β-cells only, does not lead to tumour formation[50]. However, co-deletion of *Smad4*, the common mediator of signal transduction by transforming growth factor-β (TGFβ), does lead to tumour formation, which

is accompanied by increased active AKT and mTOR signalling[115]. These results suggest that PTEN might contribute to pancreatic cancer.

Studies of human cancer and mouse models suggest that alterations in *PTEN* might have some role in pancreatic tumours[113–115], liver tumours[47,116–119], bladder tumours[48,120–122], adrenal pheochromocytomas[123], leukaemia[124,125] and lymphoma[40,46,126–128]. However, in most cases the available human data do not support *PTEN* as a major factor in these tumour types. Supporting data are included in TABLE 1.

Drug development for PTEN-deficient disorders

Mouse models of tumorigenesis and diseases such as Cowden syndrome can not only help to discern cause–effect and mutation–disease relationships, but can also be used for preclinical testing and to validate targets for cancer therapy and prevention. For example, deletion of *Akt1* in *Pten*-heterozygous mice prevents endometrial and prostate tumorigenesis, and heterozygous deletion of *Mtor* or *Mlst8* (a component of both mTOR TORC1 and TORC2 complexes) prolongs the life of mice with prostate tumours that are associated with prostate-specific deletion of *Pten*[129,130]. A hypomorphic mutation in *Pdpk1* (REF. 131) and a pharmacological inhibition of mTOR[132] both prevent the formation of multiple tumour types in $Pten^{+/-}$ mice. These data suggest that inhibitors of pathway components such as AKT1, mTOR or PDK1 might be developed for cancer prevention in or the treatment of patients with germline or tumour-specific *PTEN* mutations. Inhibitors of mTOR, such as rapamycin (also known as sirolimus) and its analogues, temsirolimus and everolimus, can prevent tumorigenesis in multiple mouse models of cancer. For example, everolimus reduced the progression of endometrial hyperplasia, and sirolimus reversed premalignant lesions and/or decreased proliferation in prostate tumours in $Pten^{+/-}$ mice[67,133]. Metformin, an activator of AMP-activated protein kinase (AMPK) that leads to inactivation of mTOR, delayed tumour onset in $Pten^{+/-}$ mice[134].

Several compounds that have been designed to inhibit the PI3K–AKT–mTOR pathway in cancer are in clinical development, including newer mTOR inhibitors that target the ATP-binding domain. Some of these have cross-reactivity with class I PI3Ks and other proteins with PI3K domains (TABLE 2). These pathway inhibitors may be useful in the prevention of malignancy or in treating existing tumours. Patients with germline mutations of *PTEN* could be an ideal population to test these inhibitors, as pathway activation is a feature of both benign and malignant tumours in Cowden syndrome. Easily accessible benign tumours in the skin and gastrointestinal tract of patients with Cowden syndrome could provide *in vivo* evidence of target modulation and be a reliable surrogate for cancer cells.

Of all of the pathway inhibitors in development, inhibitors of the TORC1 complex, such as sirolimus and its analogues, are the most developed and have established safety profiles that are most relevant for rare syndromes. For example, sirolimus was tested in a Phase II trial of patients with tuberous sclerosis, which, like Cowden syndrome, is a highly morbid familial syndrome in which the loss of a tumour suppressor gene leads to mTOR activation[135]. In patients with tuberous sclerosis, prolonged use of sirolimus seemed to be safe and showed preliminary efficacy in shrinking angiomyolipomas and improving pulmonary function[135].

Treatment with everolimus similarly caused a sustained decrease in subependymal giant-cell astrocytomas (SEGAs) in patients with tuberous sclerosis[136]. A case report also showed that sirolimus decreased tumour burden in a child with Proteus syndrome and a germline *PTEN* mutation[137]. Sirolimus is currently being tested in patients with Cowden syndrome (clinical trial number:).

In cancer, temsirolimus and everolimus are approved for the treatment of advanced renal cell carcinoma, and are being tested as single agents, and in combination, in various other malignancies. The activity of rapamycin analogues as single agents in common cancers has been modest, however, which could be related to feedback activation of AKT through insulin receptor substrate 1 (IRS1) or through direct phosphorylation at Ser473 by TORC2 (REF. 138) (FIG. 2). Feedback activation of AKT has been observed in *PTEN*-null glioblastoma biopsy samples from patients treated with sirolimus, and was associated with a shorter time to disease progression. Nonetheless, the modest results of clinical trials with TORC1 inhibitors in cancers in which *PTEN* inactivation is common suggest that the inhibition of TORC1 alone is insufficient to induce meaningful tumour regression[139,140].

The next generation of pathway inhibitors includes dual PI3K–mTOR inhibitors, PI3K inhibitors, AKT inhibitors and mTOR complex catalytic site inhibitors (reviewed in REFS 141–143). These compounds may better compensate for the loss of *PTEN* by targeting more upstream components of the pathway and may circumvent feedback AKT activation. However, these agents are likely to be more toxic than the pure TORC1 inhibitors and are also likely to be less useful for cancer prevention in patients with rare syndromes.

Trial design considerations for PHTS and PTEN-deficient cancers.

Given the rarity of Cowden syndrome, cancer prevention trials pose a challenge. Pilot studies using pathway inhibitors that focus on tissues at risk for malignant transformation are more feasible. For example, a trial evaluating the effects of a pathway inhibitor on endometrial hyperplasia or fibrocystic changes of the breast in patients with Cowden syndrome would be a useful proof-of-concept, but this would require multiple biopsies, which might be objectionable to patients with Cowden syndrome who do not have cancer. Molecular imaging to assess tumour metabolism using fluorodeoxyglucose (FDG)–positron emission tomography (PET) or tumour cell proliferation using deoxyfluorothymidine (FLT)–PET might be useful surrogates for patients with Cowden syndrome who are unwilling or unable to undergo biopsies. Trials in patients with Cowden syndrome could also test pathway inhibitors as a means of ameliorating the severe but non-malignant manifestations of the disease, such as Lhermitte–Duclos disease, in which improvement in neurological function could be measured clinically. Selecting objective and reliable clinical end points for these studies is challenging, but pharmacodynamic end points and assays that are validated in trials of patients with Cowden syndrome could be applied to general oncology trials.

The location of *PTEN* mutations or relevant epigenetic modifications may assist the choice of therapy for *PTEN*-deficient malignancies. For example, if mutations occur in the C-terminal PEST domain and spare the phosphatase domain, treatment with a proteasome inhibitor might rescue PTEN from degradation. Moreover, treatment with statins might

increase the expression of PTEN through peroxisome proliferator-activated receptor-γ (PPARG)-mediated promoter activation[144], and demethylating agents or histone deacetylase inhibitors might reverse epigenetic silencing. Three recent studies suggest that PTEN is required for homologous recombination, which could be exploited therapeutically. In one mouse study, T cell-specific *Pten* deletion resulted in lymphomas with T cell receptor (*Tcr*)–*Myc* translocations resulting from aberrant *Tcr* recombination[145]. In PTEN-deficient endometrial cancer cell lines, decreased homologous recombination underlies sensitivity to polyadenosine diphosphate ribose polymerase (PARP) inhibitors[146]. *Pten* deletion decreased homologous recombination in mouse astrocytes through the downregulation of the DNA repair protein RAD51. These studies raise the possibility that PARP inhibitors may have efficacy for PTEN-deficient tumours[147], owing to generalized defects in homologous recombination.

As PTEN loss mediates resistance to targeted therapies against receptor tyrosine kinases, combinations of PI3K or AKT inhibitors with cell surface receptor inhibitors might be effective. For example, acquired resistance to EGFR tyrosine kinase inhibitors in lung cancer and trastuzumab in *ERBB2*-amplified breast cancer are associated with *Pten* loss and/or maintenance of AKT activation[110,148,149]. Inhibition of multiple nodes of the signalling cascade may effectively overcome acquired resistance. Alternatively, targeting of parallel networks by targeting the PI3K–AKT pathway and the MEK–ERK (MAPK1, MAPK3 and MAPK1) pathway may also overcome acquired resistance, have antitumour activity and ultimately accelerate the development of these agents to treat patients with germline or somatic loss of PTEN.

Perspectives and conclusions

The comparison of sporadic tumours carrying PTEN alteration, tumours that occur with germline *PTEN* mutation in Cowden syndrome, and tumours that develop in *Pten*-deficient GEM strains provides evidence that the development of many different tumour types seems to be driven by the loss of PTEN function. mTOR inhibitors have been approved for the treatment of advanced renal cell carcinoma and SEGA that is associated with tuberous sclerosis. Upstream pathway inhibitors of PI3K and AKT are in clinical development, both in combination with traditional chemotherapy and with inhibitors of parallel pathways such as MEK–ERK. This is a reasonable approach as *PTEN* mutations and subsequent activation of the AKT–mTOR pathway provide survival signals that are associated with resistance to therapy. However, one key question that remains to be answered is whether tumours that develop as a consequence of PTEN attenuation are addicted to that signal. Given that *PTEN* alteration is so prevalent in many human tumour types, validating PTEN as a target during different stages of tumorigenesis is crucial to validating any downstream targets. Mouse models could be used to show whether re-expression of *Pten* in *Pten*-deficient tumours leads to tumour regression, as is the case for *Trp53*-null lymphomas and sarcomas upon *Trp53* re-expression[150], or whether it is context-dependent as is the case for the reconstitution of *Trp53* in lung tumours[151,152]. Identification of novel PTEN functions and crucial signalling events downstream of PTEN could provide additional targets and new therapeutic approaches.

It is becoming clear that PTEN may have many important functions, any or all of which might contribute to its tumour suppressor activity. *Pten* deletion clearly contributes to tumorigenesis in multiple tissues in mice. The continued characterization of specific human *PTEN* mutations is driving the discovery of novel PTEN functions that might correlate with specific tumour risk in Cowden syndrome and might have implications for sporadic tumours.

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This Review is dedicated to T.S., a dear patient with Cowden syndrome. The authors remain devoted to the study and cure of Cowden syndrome in her honour and the honour of others who wrestle with the consequences of disease caused by the loss of PTEN.

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At a glance

- PTEN hamartoma tumour syndrome (PHTS) is a group of syndromes characterized by benign growths and a high risk for cancers of the breast, endometrium and thyroid. Cowden syndrome is the best characterized of these and 85% of patients have germline *PTEN* mutations. The range of abnormalities in patients with PHTS varies from patient to patient.
- Somatic *PTEN* mutations and deletions, and inactivation of *PTEN* by methylation or microRNA silencing, are common in multiple tumour types. These include the classical PHTS-associated tumours like breast, endometrium and thyroid, but also tumours of the central nervous system, prostate, lung, pancreas, liver and adrenal glands, as well as melanoma, leukaemia and lymphoma.
- Mouse models of Cowden syndrome, in which a single allele of *Pten* is deleted or mutated, exhibit characteristic Cowden syndrome phenotypes. Tumour types are very much dependent on the genetic background of the mice suggesting that there may be genetic risk factors for PHTS penetrance in humans.
- Tissue-specific deletion of *Pten* in mice can lead to rapid, slow or no tumours, depending on the tissue type. In some cases, tissue-specific *Pten* deletion can cooperate with other genetic alterations to enhance tumorigenesis. These mouse models have validated mutation or loss of *PTEN* as an aetiological factor in similar human tumours.
- PTEN is a lipid phosphatase that acts as a negative regulator of the PI3K–AKT–mTOR pathway, which is an important regulator of cell growth and survival. As such, pharmacological inhibition of this pathway may be exploited for therapy of tumours with altered *PTEN*, or for tumour prevention in patients with PHTS.

Box 1 |

What determines tumour risk in Cowden syndrome?

A limited number of mouse studies suggest that both the type of germline *Pten* mutation and the genetic background can affect risk for specific tumour types. Comparison of three different Cowden syndrome-specific *Pten* mutations in the same mouse strain indicated that specific *Pten* mutations may contribute to risk for specific tumour types[153]. In this study, specific mutations altered the relative frequency of uterus, prostate, thyroid and mammary neoplasms but did not alter the range of tumour types. These types of studies may help to stratify *PTEN* mutations in patients with Cowden syndrome in order to identify those at the highest risk for specific tumour types. Conversely, studies using *Pten* $^{+/-}$ and *Pten*$\Delta^{5/+}$ (deletion of exon 5) mice indicate that genetic background is also a very strong determinant of tumour susceptibility in mice[153]. Given the diversity of the human genome, identification of risk factors that contribute to tumour susceptibility in Cowden syndrome might help to predict the risk of specific tumours in this population. For example, polymorphisms in caspase 8 have been identified as risk factors for breast and ovarian cancers in tumour-prone *BRCA1* mutation carriers[154]. Naturally occuring polymorphisms within *PTEN* itself are found at a disproportionately high rate in patients with Cowden syndrome, even in the absence of apparent *PTEN* mutation, suggesting that certain *PTEN* haplotypes might function as risk-modifying factors[20]. However, given the number of different *PTEN* mutations in Cowden syndrome that may also affect risk even large genome-wide association studies (GWAS) might have trouble detecting additional risk loci. Identification of risk-modifying loci in inbred mouse models for Cowden syndrome could inform more targeted searches for human risk factors. In addition, risk factors for Cowden syndrome tumours might also prove to be risk factors for PTEN-mutant sporadic tumours. However, in Cowden syndrome, PTEN alteration in non-tumour cell types, such as stroma, endothelial and immune cells, may also contribute to increased tumour risk[46,155,156] possibly exacerbating other general risk factors.

Box 2 |

The role of PTEN in the maintenance of tissue and cancer stem cells

The fact that loss of *PTEN* can cause or contribute to tumorigenesis in several tissues suggests that PTEN might control tumour-initiating cells. In fact, *Pten* deletion can increase the self-renewal capacity of normal stem cells and increase the number of putative tumour-initiating cells. In neural stem cells, *Pten* deletion increases self-renewal capacity[157], which was further augmented by co-deletion of *Trp53* (REF. 86). *Pten* deletion in the adult subependymal zone also increased neural stem cell self-renewal, leading to enhanced olfactory bulb mass and enhanced olfactory function[158]. Increased stem and progenitor cells have been reported in *Pten*-deficient prostate, lung, intestinal and pancreatic tissues before tumour formation[49,114,159–161]. In both haematopoietic cells and melanocytes, *Pten* deletion leads to normal stem cell exhaustion[102,162,163], but paradoxically, in haematopoietic cancer stem cells, *Pten* deletion leads to unlimited expansion[162,164]. Although still an emerging concept, the role of tumour-initiating cells and control by PTEN is an area of intense investigation.

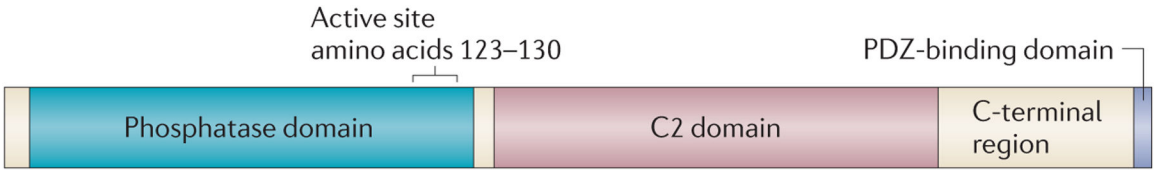

Figure 1 |. Schematic of the PTEN protein.

PTEN contains two key domains that are required for its tumour suppressor function; the phosphatase (catalytic) domain (amino acids 14–185)[165] with an active site included within the residues 123 and 130 (REF. 166), and the C2 (lipid membrane-binding) domain (amino acids 190–350)[167]. The importance of other domains such as the PDZ-binding domain (in grey; amino acids 401–403)[168], which binds proteins containing PDZ domains, and the carboxy-terminal region (amino acids 351–400), which contains PEST sequences and may contribute to protein stability and activity[169], is less defined in the tumour suppressor functions of PTEN.

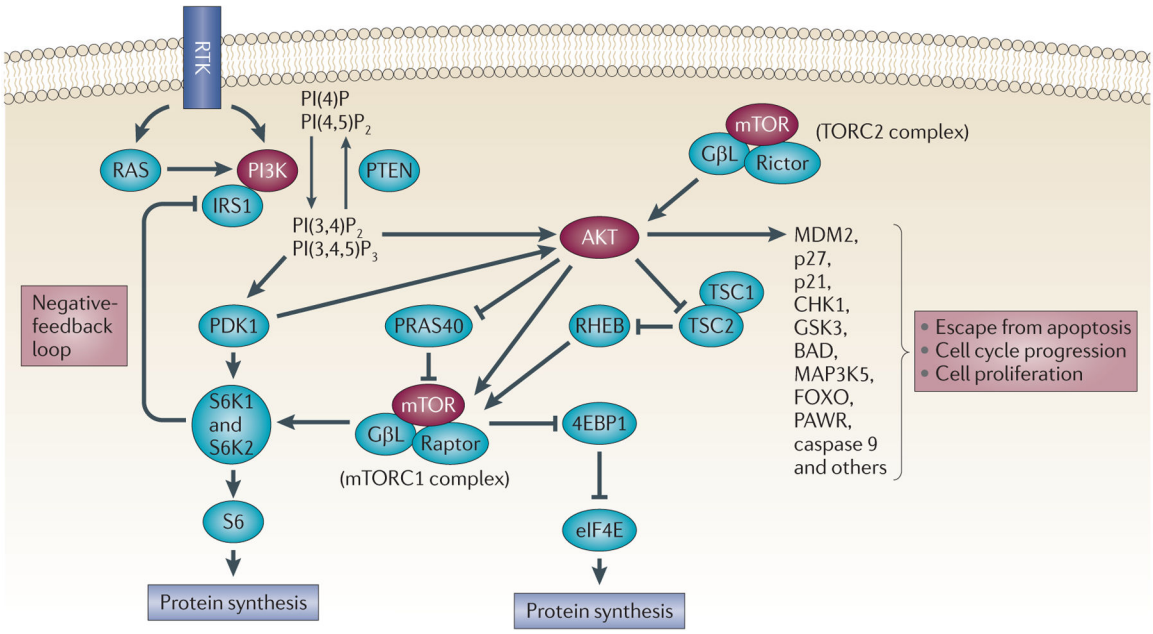

Figure 2 |. Canonical PTEN–PI3K–AKT–mTOR pathway.

PTEN opposes PI3K function, leading to inactivation of AKT crucial downstream target[1]. When PTEN activity is decreased or absent, products of PI3K activate AKT through the activation of its upstream kinase phosphoinositide-dependent kinase 1 (PDK1; encoded by *PDPK1*)[170]. Other upstream regulators of the pathway include receptor tyrosine kinases (RTKs) such as ERBB2 and epidermal growth factor receptor (EGFR) that are important in breast and lung cancer, respectively (reviewed in REF. 171). Important downstream targets of AKT (such as p27, p21, FOXO and PAWR (also known as PAR4)) are involved in multiple functions that are crucial for tumour cell growth and survival (reviewed in REF. 8). mTOR activity is also increased when PTEN activity is lost, and mTOR itself has important targets, including AKT, as well as proteins required for protein translation such as ribosomal protein S6 kinase (S6K; encoded by *RPS6KB1* and *TPS6KB2*) and eukaryotic initiation factor 4E binding protein (4EBP1; encoded by *EIF4EBP1*)[172]. mTOR exists in two different protein complexes, TORC1 and TORC2 (REF. 173). Inhibitors of TORC1 by drugs such as rapamycin can activate AKT by deactivating a negative-feedback loop mediated by S6K and insulin receptor substrate 1 (IRS1)[174,175]. Proteins that can be targeted by drugs (as outlined in TABLE 2) are indicated in red. BAD, BCL-2-associated agonist of cell death; GSK3, glycogen synthase kinase 3; MAP3K5, apoptosis signal regulator kinase 1.

Table 1 |

Summary of evidence for *PTEN* and *Pten* alteration in specific cancers, by tissue

Tissue	*PTEN* alteration in human cancer	Neoplasms and tumours in PHTS	Tumours in *Pten* $^{+/-}$ mice	Mice: tissue-specific deletion outcome	Mice: enhanced tumours in the presence of additional alterations*	Refs
Breast	Mutation <5%, LOH 40%, promoter methylation 50% and loss of expression ~40%	25–50% lifetime risk for women	Yes	Tumours	*Wnt* or *Erbb2* transgenes	39,44,57,58
Endometrium	Mutation 35–50%	Yes	Yes	NR	*Mlh1*$^{-/-}$ accelerated *Pten* LOH in *Pten*$^{+/-}$ mice	39,60–63,68
Thyroid	Homozygous deletion <10%, promoter methylation >50%, and rearrangement in most papillary thyroid carcinomas	Yes	Late onset and low frequency	Goiter and benign follicular adenomas in females	Thyroid hormone receptor-β (*Thrb*) transgene, with metastasis	24,70–76
Prostate	Frequent LOH and miR-22 and miR-106b-25 cluster overexpression	NR	Late onset	Early onset of invasive, metastatic prostate tumours	*Cdkn1b*$^{+/-}$, *Nkx31*$^{-/-}$, *Tmprss2-Erg* fusion protein and SV40 Tag	42,74,89,91–94
Leukaemia or lymphoma	Deletion 10% of T-ALL and 27% mutation in T-ALL	NR	Lymphoma and radiation decreases latency	Early onset lymphoma and autoimmunity (T cell deletion)	NR	40,124,126–128,212
Glioma	LOH >70%, mutation 44% (coincident with LOH) and miR-26a amplification	Dysplastic gangliocytoma of the cerebellum in LD	NR	Macrocephaly, seizures and benign cerebellar abnormalities	Mutant *Hras*, SV40 Tag, *Trp53*$^{-/-}$, *Trp53*$^{+/-}$ and *Nf1*$^{+/-}$	31,77–81,83–87
Melanoma	LOH 30–60%, mutation 10–20% (metastases) and >50% frequent promoter methylation in patients with XP	NR	NR	No spontaneous melanoma but melanoma induced by carcinogen in 50%	*Braf*$^{+/-}$	97,99–103,213,214
Lung cancer	Mutation infrequent, promoter methylation frequent, miR-21 upregulation 74% and loss of PTEN 74%	Occasional	NR	Late-onset lung adenocarcinoma 87% and increased carcinogen-induced lung tumours	Mutant *Kras*	30,32,49,105–109,112
Liver	Mutation <5%, PTEN expression lost in 12% and PTEN expression lost in HepC HCC	NR	Infrequent	Fatty liver and insulin hypersensitivity	*Vhl*$^{+/-}$	116–118,215,216
Bladder	LOH 23%, homozygous deletion 6%, mutation 23% (late stage) and decreased or absent PTEN expression 53%	NR	NR	Late-onset transitional cell carcinomas in 10%	*Trp53*$^{-/-}$	48,120–122
Kidney	LOH 25%	NR	NR	NR	NR	120
Pancreas	Altered localization common	NR	NR	Metaplasia and carcinoma 20%	*Smad4*$^{-/-}$	113–115
Adrenal pheochromocytoma	LOH more common in malignant than in benign tumours	NR	Yes	NR	*Cdkn2a*$^{-/-}$	39,103,123

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Tissue	*PTEN* alteration in human cancer	Neoplasms and tumours in PHTS	Tumours in *Pten* $^{+/-}$ mice	Mice: tissue-specific deletion outcome	Mice: enhanced tumours in the presence of additional alterations*	Refs
Colon and intestine	Up to 18% mutated and up to 19% LOH depending on tumour type	Yes and benign polyps in >90%	Hyperplastic changes	NR	*Apc*$^{+/-}$	37,217–219

HepC HCC, hepatitis C-positive hepatocellular carcinoma; LD, Lhermitte–Duclos syndrome; LOH, loss of heterozygosity; miRNA, microRNA; NR, not reported; PHTS, PTEN hamartoma tumour syndromes; T-ALL, T cell acute lymphocytic leukaemia; XP, xeroderma pigmentosum.

**Pten* alteration led to decreased tumour latency or increased tumour stage in the presence of these additional alterations.

Table 2 |

Selected drugs targeting the PI3K–AKT–mTOR pathway that is activated in tumours deficient for PTEN

Drug	Target	Human trials	Human results	Mouse results
XL147	PI3K	Phase I/II	One partial response in NSCLC in Phase Ia[176]	Not reported
GDC-0941	PI3K	Phase I	One partial response in breast cancer in Phase Ia[177]	Growth inhibition but not regression in xenografts[178] and prolonged tumour regression in combination with imatinib[179]
PX-866	PI3K	Phase I/II	Best response reported: stable disease in 7 of 31 evaluable patients in Phase Ia[180]	Prevents TGFα-induced pulmonary fibrosis in mice[181]
BKM120	PI3K	Phase I/II	One partial response in triple-negative breast cancer in Phase Ia[182]	Prevents emergence of resistance to inhibitors of SMO in medulloblastoma xenografts[183]
CAL-101	PI3K (delta)	Phase I/II	Objective response rate 9 of 15 in indolent NHL, 6 of 7 mantle cell lymphoma and 4 of 17 CLL in Phase Ia[184]	Not reported
BEZ235	PI3K and mTOR (TORC1 and TORC2)	Phase I/II	Two partial responses in Cowden syndrome and breast cancer in Phase Ia[185]	Prevents emergence of resistance to inhibitors of SMO in medulloblastoma xenografts[183]
SF1126 (h)	PI3K	Phase I	Best response reported: stable disease in Phase Ia[186]	Prevents tumour growth in xenografts[187]
GDC-0980	PI3K and mTOR (TORC1 and TORC2)	Phase I	One partial response in mesothelioma in Phase Ia[188]	Not reported
XL765	PI3K and mTOR (TORC1 and TORC2)	Phase I/II	Best response reported: stable disease in Phase Ia[189]	Decreased xenograft growth and increased survival in combination with temozolomide[190]
PKI-402	PI3K and mTOR (TORC1 and TORC2)		Not reported	Xenograft tumour regression with subsequent regrowth[191]
PKI-587 (also known as PF-05212384)	PI3K and mTOR (TORC1 and TORC2)	Phase I	Not reported	Xenograft tumour regression[192]
Rapalogues (rapamycin, sirolimus, everolimus and temsirolimus)	mTOR (TORC1)	Approved	Improved overall survival and progression-free survival in RCC[193,194], improved progression-free survival in PNET[195], 75% response rate in subependymal giant-cell astrocytoma in TSC[136], 40% response rate in MCL and lower in other tumour types (reviewed in REF. 196)	Prevention of uterine and adrenal tumours in *Pten*$^{+/-}$ mice[132], prolonged survival in a mouse model of Cowden syndrome[197], decreased *Pten*$^{-/-}$ prostate tumour growth[198], prevention of lung tumours[199], anal tumours[200], lymphoma[201], bladder tumours[202], mammary tumours[203], prostate tumours[198] and regression of salivary gland tumours[204] and PNET[205]
AZD8055	mTOR	Phase I/II	Not reported	Growth inhibition or tumour regression in xenografts[206]
Perifosine	AKT	Phase III	Improved TTP and overall survival in randomized Phase II of capecitabine with or without perifosine in refractory colorectal cancer[207]	Growth inhibition and increased survival in multiple myeloma xenograft[208], growth inhibition in neuroblastoma xenograft[209]
MK-2206	AKT	Phase I/II	Best response reported: stable disease in Phase Ia[210]	Modest xenograft growth inhibition as a single agent[211]

CLL, chronic lymphoid leukaemia; MCL, mantle cell lymphoma; NHL, non-Hodgkin's lymphoma; NSCLC, non-small-cell lung cancer; PNET, pancreatic neuroendocrine tumour; RCC, renal cell carcinoma; SMO, smoothened; TGFα, tumour growth factor-α; TSC, tuberous sclerosis; TTP, time to progression.