

1 Review

## 2 The PTEN-PI3K axis in cancer

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12 **Abstract:** The PI3K-AKT-mTOR signal transduction pathway regulates a variety of biological  
13 processes including cell growth, cell cycle progression and proliferation, cellular metabolism and  
14 cytoskeleton reorganization. Fine-tuning of the PI3K pathway signaling output is essential for the  
15 maintenance of tissue homeostasis and uncontrolled activation of this cascade leads to a number of  
16 human pathologies including cancer. Inactivation of the tumour suppressor phosphatase PTEN  
17 and/or activating mutations in the proto-typical lipid kinase PI3K have emerged as some of the  
18 most frequent events associated with human cancer and as a result the PI3K pathway has become a  
19 highly sought-after target for cancer therapies. In this review we summarize the essential role of the  
20 PTEN-PI3K axis in controlling cellular behaviors by modulating activation of key proto-oncogenic  
21 molecular nodes and functional targets. Further, we highlight important functional redundancies  
22 and peculiarities of these two critical enzymes that over the last few decades have become a central  
23 part of the cancer research field and have instructed hundreds of pre-clinical and clinical trials to  
24 better cancer treatments.

25 **Keywords:** PTEN; PI3K; cancer predisposition syndromes; targeted therapies; mouse models of  
26 human cancer.

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### 28 1. The PI3K pathway: molecular hubs and biological functions

29 The phosphatidylinositol 3-kinase, PI3K is the upstream lipid kinase of the PI3K-AKT-mTOR  
30 signal transduction pathway. Structural and functional specificities identify different classes of  
31 PI3Ks with class IA PI3Ks existing as heterodimers of a p85 type of regulatory subunit and a p110  
32 multi-isoform catalytic subunit: p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$  [1]. All p110-isoforms contribute to Class  
33 IA PI3Ks signaling but the p110 $\alpha$ -encoding gene, *PIK3CA*, is the only isoform frequently mutated in  
34 cancer [2, 3].

35 In physiological conditions, receptor tyrosine kinases (RTKs) and G-protein coupled receptors  
36 (GPCRs) activate PI3K which then catalyzes the phosphorylation of the lipid substrate  
37 phosphatidylinositol (4,5)-bisphosphate (PIP2) to generate phosphatidylinositol  
38 (3,4,5)-trisphosphate (PIP3) [4]. PIP3 is an essential second messenger that amplifies the PI3K signal  
39 by recruiting proteins containing lipid-binding domains such as the serine/threonine AGC kinase  
40 AKT and the phosphoinositide-dependent protein kinase-1, PDK1. At the membrane, PDK1 directly  
41 phosphorylates and activates AKT on Thr 308 which in turn can engage and activates over 100  
42 reported effector targets including the glycogen synthase kinase 3 (GSK3), the Forkhead Box O  
43 (FoxO) proteins, and the mammalian (or mechanistic) target of rapamycin complex 1 (mTORC1)  
44 thus controlling multiple pro-survival cellular processes [5, 6]. Maximal AKT activation however  
45 requires an additional phosphorylation event on Ser 473 which is catalyzed by the mTOR complex2  
46 (mTORC2) which can also be regulated by PIP3 [7].

47 The AKT control of mTORC1 functional status occurs through multiple molecular mechanisms  
48 one of which involves inhibition of the tumour suppressive tuberous sclerosis complex (TSC). Upon  
49 growth factors stimulation, AKT phosphorylation of the tuberous sclerosis complex 2 (TSC2) within  
50 the TSC, releases an inhibitory switch that allows the Ras-related GTPase Rheb to be GTP loaded and  
51 to activate mTORC1 on lysosomes [8]. At the lysosomes, mTORC1 engages with members of the  
52 Ragulator complex and in the presence of nutrient availability activates anabolic processes such as  
53 lipid and nucleotide synthesis, protein translation and ribosomal biogenesis and sustains cell growth  
54 and proliferation [6]. Thus, by transducing signals from the upstream cytoplasmic membrane  
55 complexes to the endocellular organelles, the PI3K pathway and its molecular hubs establish an  
56 essential temporal and spatial signaling network that arms cells with the necessary biomolecules to  
57 allow cellular growth during embryonic development and in adulthood for tissue growth and  
58 regeneration.

59 Because of its essential role in controlling multiple pro-survival processes, activation of the  
60 PI3K signal is tightly modulated by negative regulators that act by ensuring the timely inhibition of  
61 the pathway and by preventing excessive growth. A critical upstream member of the cascade is the  
62 tumour suppressor PTEN, a dual-specificity lipid and protein phosphatase that efficiently  
63 dephosphorylates the 3'-group of PIP3 and therefore terminates propagation of the signal to AKT  
64 and other PIP3-effector targets [9]. The presence and activation of PTEN at the cytoplasmic  
65 membrane is crucial to guarantee a controlled transduction of the PI3K signal which is then  
66 transmitted as a healthy message to the cell. Consequently, removal of this upstream break  
67 unleashes a continuous positive signal that translates into uncontrolled overgrowth [10]. Similarly,  
68 constitutive activation of the pathway achieved through acquisition of oncogenic *PIK3CA* mutations  
69 overcomes the negative regulation imposed by PTEN with ensuing tumourigenesis [4]. Thus, PTEN  
70 and PI3K define a key functional axis that in a coordinated fashion modulates the activation status of  
71 multiple proto-oncogenic signals that can be scavenged during tumourigenesis and are frequently  
72 exploited by cancerous cells for survival.

## 73 2. The tumours suppressor PTEN

### 74 2.1: Mutations, lipid function and *in vivo* studies.

75 PTEN, phosphatase and tensin homologue deleted on Chromosome 10, is one of the most  
76 frequently mutated tumour suppressor genes in human cancer [10]. PTEN is expressed early during  
77 embryogenesis and ubiquitously throughout adulthood, and its functional loss can have dramatic  
78 consequences to cellular and organismal homeostasis [11-13]. PTEN mutations occur in somatic and  
79 hereditary tumour syndromes and both conditions lead to cellular overgrowth with potential cancer  
80 progression overtime [14]. Germline *PTEN* mutations are associated with a number of tumour  
81 predisposition syndromes known as PTEN Hamartoma Tumours Syndromes, (PHTS) whereby  
82 affected patients develop disorganized and hyperplastic cellular overgrowths known as  
83 hamartomas that affect various tissues including thyroid, breast, skin and brain, and can present  
84 neurodevelopmental disorders [15]. In somatic cancers such as endometrial, breast, prostate cancer  
85 and glioblastoma, PTEN inactivation encompasses an array of mutations which include missense  
86 and nonsense mutations, mono or bi-allelic deletion of the genomic locus or silencing through  
87 promoter methylation, and also targeting by oncogenic microRNAs [9, 10].

88 Initial structural and functional studies in the late nineties highlighted how, despite the  
89 predicted role as a novel protein tyrosine phosphatase, PTEN catalytic activity presented a  
90 surprising high affinity toward phospho-lipid substrates with the second messenger PIP3 identified  
91 as the candidate of choice [16-18]. Thereafter, a number of studies confirmed that reductions in  
92 PTEN levels, or PTEN activity, not only induced PIP3 accumulation but also associated with  
93 activation of the proto-oncogene AKT, thus establishing a key connection between a novel tumour  
94 suppressor and a functional target, the PI3K pathway [19].

95 The role of PTEN as a key tumour suppressor has been demonstrated and validated in multiple  
96 animal models and *in vitro* settings. In mice, constitutive *Pten* inactivation through either

97 mono-allelic genomic loss or heterozygous expression of loss-of-function *Pten* mutations leads to  
98 tumorigenesis in multiple epithelial tissues including the mammary gland, prostate, thyroid and  
99 adrenal glands [11-13, 20, 21]. In addition, conditional *Pten* inactivation through Cre-Lox systems  
100 confirmed that disruption of *Pten* function is tumour promoting in a cell autonomous fashion and  
101 that across the different tissues, the mammary gland is exquisitely sensitive to variations in the  
102 levels of this essential tumour suppressor [22-25]. Consistent with this, induced systemic *Pten*  
103 overexpression in the mouse triggers a tumour suppressive and cancer protective state through  
104 healthy metabolism, which indicates that pharmacologic strategies able to increase levels or  
105 expression or activity of wild-type PTEN should be exploited as novel treatment modalities for  
106 cancer prevention and therapy [26].

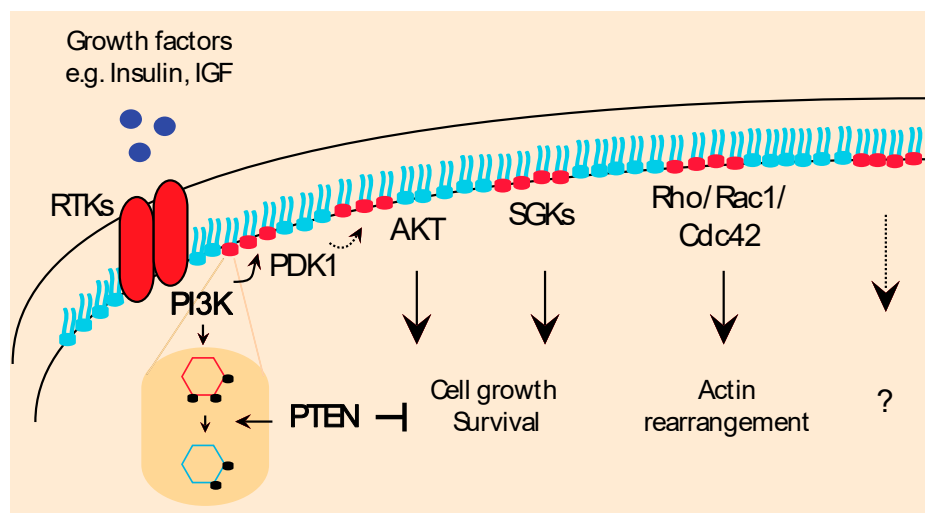
107 *Pten* inactivation frequently correlates with an active status of the PI3K pathway commonly  
108 measured by monitoring the levels of AKT phosphorylation which also suggests that AKT may be  
109 an important PTEN functional target [5]. In agreement with this, tissue-specific AKT deletion was  
110 shown to rescue the *Pten*-loss driven tumorigenesis in multiple tissues, particularly effective in  
111 endometrium and prostatic gland and interestingly, this occurred with distinct contribution of the  
112 various AKT isoforms [27, 28]. Notably, despite the high degree of structural homology, AKT1,  
113 AKT2, and AKT3 present distinct expression patterns, unique post-translational modifications and  
114 display diverse intracellular localizations suggesting that mechanisms regulating expression profiles  
115 and function of these distinct AKT isoforms can affect in various, and sometimes opposite ways, the  
116 PTEN loss-driven tumorigenesis and also add a novel layer of complexity to our understanding of  
117 the PI3K signaling network [5, 29, 30].

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## 119 2.2: *PIP3 is more than AKT*

120 PI3K activation or loss of PTEN equally leads to accumulation of the second messenger PIP3.  
121 While AKT has been demonstrated to execute many of the PIP3 biological functions, recent reports  
122 have also highlighted how PIP3 does not necessarily equals AKT activation but that additional  
123 context-dependent functional targets can better mediate the signal of this critical PI3K product [31,  
124 32].

125 In an important paper from the Garraway lab, *PIK3CA* mutations or *PTEN* inactivation were  
126 shown to differentially correlate with AKT phosphorylation levels in a number of cancer cell lines  
127 and tumour samples. Importantly, however, loss of PTEN better correlated with high levels of AKT  
128 phosphorylation than did *PIK3CA* mutations. Moreover, mutant PI3K samples with low levels of  
129 AKT phosphorylation were instead associated with activation of the PDK1-SGK3 signaling pathway  
130 whose activation supported cell viability more efficiently than AKT [32]. Additionally, independent  
131 studies have shown that PIP3-binding proteins such as P-REX1 and the Rho/Rac/Cdc24 family  
132 members are all implicated in supporting metabolic reprogramming, cytoskeleton remodeling, cell  
133 growth and cell division in an AKT-independent fashion [33, 34]. Thus, PIP3 can activate a number  
134 of parallel signaling pathways that independently function to promote growth and survival and are  
135 therefore implicated in the pathogenesis of cancer, beyond AKT. This can have important  
136 therapeutic implication particularly for *PIK3CA* and/or *PTEN* mutant cancers, **Figure 1**.



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**Figure 1.** The PTEN-PI3K axis modulates activation of multiple pro-survival signal transduction pathways. In physiological conditions, growth factors stimulate PI3K which once active phosphorylates the phospholipid substrate PIP<sub>2</sub> to generate the second messenger PIP<sub>3</sub>. PIP<sub>3</sub> recruits and activates a number of functional targets such as PDK1, AKT isoforms, the SGK signaling pathways and the Rho family of GTPases and promotes a plethora of biological effects to sustain cell growth, proliferation and cytoskeleton modification. The lipid phosphatase PTEN converts PIP<sub>3</sub> to PIP<sub>2</sub> and terminates propagation of the growth signal to maintain normal cellular and tissue homeostasis.

### 147 3. The proto-oncogene PI3K and PTEN

#### 148 3.1: *PIK3CA* Mutations and functions vis a vis *PTEN* regulation.

149 The family of lipid kinases PI3Ks encompasses three classes of enzymes and eight different  
150 isoforms which display overlapping as well as distinct biological functions [1]. Because of their  
151 implication to cancer, here we will focus mostly on the role of class IA PI3Ks and their cross talk with  
152 the tumour suppressor PTEN. For details on functions and regulation of additional classes of PI3Ks  
153 we refer the reader to accompanying reviews within this book.

154 Class IA PI3Ks phosphorylates the 3'-hydroxyl position of the PI(4,5)P<sub>2</sub> to generate the second  
155 messenger PIP<sub>3</sub>. Of the three isoforms belonging to this class of kinases, *PIK3CA* is the only gene  
156 ubiquitously expressed and frequently mutated in human cancer [2, 35]. *PIK3CA* amplification or  
157 clustering of somatic mutations in either the helical domain (e.g. G545L) or the kinase domain of the  
158 p110 $\alpha$  subunit (e.g. H1047R) occurs in up to 30% of colon, brain, breast and gastric cancers [2]. *In*  
159 *vitro* and structural studies have suggested that these cancer-associated mutations affect p110 $\alpha$   
160 activity through two possible mechanisms [36]. They can either induce a release from the p85  
161 inhibitory subunit thus conferring p110 $\alpha$  an increased baseline catalytic activity, as observed with  
162 mutations in the helical domain; or they can impose a conformational change in the activation loop,  
163 as described for mutations in the H1047 site [37, 38]. Regardless, the final outcome is an increased  
164 p110 $\alpha$  activation which promotes tumourigenesis and multipotency [39-41].

165 Upon growth factors stimulation, the p85:p110 heterodimer is recruited to the membrane  
166 where binding of the p85 regulatory subunit to phosphorylated tyrosine residues on RTKs or  
167 adaptor molecules derepresses the inhibitory switch on p110, and allows p110 activation and PIP<sub>3</sub>  
168 production [36]. Accumulation of PIP<sub>3</sub> is tightly controlled by the PTEN lipid phosphatase activity  
169 and recent reports have described a direct binding between PTEN and p85 $\alpha$ , one of the five p85  
170 isoforms expressed in mammals [42]. The proposed model suggests that in a homodimeric  
171 conformation, p85 $\alpha$  is able to directly bind an unphosphorylated PTEN and enhance PTEN stability,  
172 membrane recruitment and lipid phosphatase activity toward PIP<sub>3</sub> [43, 44]. Consequently, this

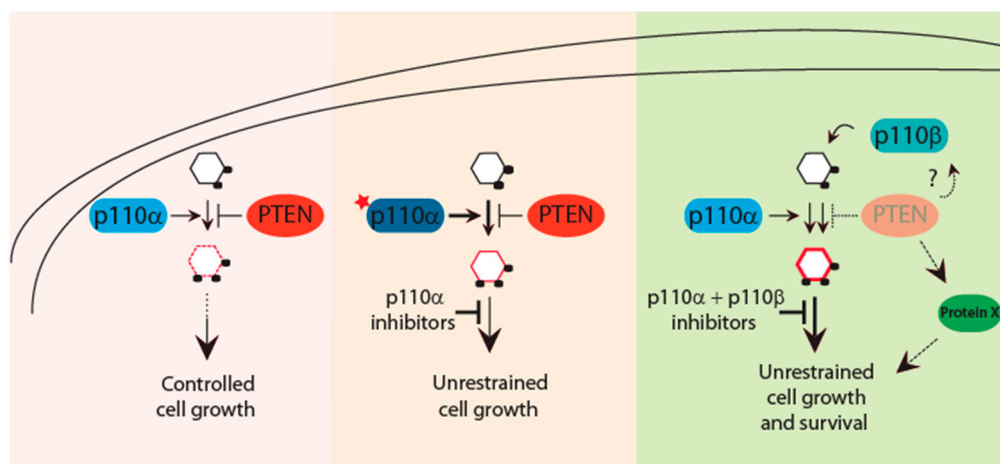
173 would establish a negative feedback loop whereby mechanisms promoting PIP3 generation can also  
174 initiate a protective response that terminates its function. Interestingly, we and others have  
175 demonstrated that PTEN exists as an unphosphorylated homodimer and that in this active  
176 conformation PTEN dephosphorylates PIP3 [20, 45]. Thus, a fine equilibrium of homo (PTEN:PTEN  
177 and p85 $\alpha$ :p85 $\alpha$ ) and hetero-dimers (p85 $\alpha$ :p110 and p85 $\alpha$ :PTEN) characterizes the formation of a  
178 functional molecular complex, also known as the PTEN associated complex (PAC), that as a  
179 consequence of diverse post-translational events such as phosphorylation and ubiquitination,  
180 assembles at the membrane and efficiently regulates PIP3 production and hydrolysis.

### 181 3.2: p110 isoforms, targeted therapies and PTEN.

182 Cancer-associated *PIK3CA* mutations occur frequently in breast cancer and are oncogenic in  
183 mammary epithelial cells and glands [41, 46]. *In vitro* studies and a number of independently  
184 generated *in vivo* mouse models have shown that conditional expression of mutant PI3K, often the  
185 hotspot H1047R mutation induced by various mammary gland-active Cre (*e.g.* MMTV-Cre,  
186 WAP-Cre), promotes late onset mammary tumourigenesis (*i.e.* over 13 months) which is accelerated  
187 by multiple rounds of pregnancies [41, 47]. Because of the frequency of *PIK3CA* mutations and their  
188 oncogenic role *in vivo*, the lipid kinase PI3K has become a highly sought-after target for the  
189 development of novel anticancer drugs [48]. Multiple small molecules have been developed over the  
190 years with the capacity to either specifically target single p110 isoforms (isoform-specific inhibitors)  
191 or to recognize multiple, if not all p110 isoforms through the generation of isoform-sparing  
192 inhibitors or pan-PI3K inhibitors [4]. Efficient inhibition of all p110 isoforms, although desirable for  
193 cancer suppression, has however presented a number of counterproductive effects mostly associated  
194 with dose-limiting toxicities that has hindered the optimal therapeutic response to these drugs in the  
195 clinic [49]. Targeting single PI3K isoforms has therefore emerged as a safer therapeutic option  
196 because isoform specific PI3K inhibitors are better tolerated by patients and can also be more easily  
197 combined with additional targeted therapies as it is currently being tested with hormone therapies  
198 and cell cycle checkpoint inhibitors for the treatment of multiple types of breast cancer [50]. Beyond  
199 toxicity, pre-clinical and clinical studies have also shown that a key challenge for the identification of  
200 optimal therapeutic windows for PI3K inhibitors is a better patient stratification and the  
201 identification of biomarkers of response that can predict sensitivity to treatment [51]. To this end,  
202 *PIK3CA* mutant patients have been shown to display better response rates to PI3K inhibitors than  
203 patients with wild-type *PIK3CA*; in a similar way, activating AKT mutations such as the E17K can  
204 predict better response to AKT inhibitors [52]. Thus, is not just the activation of the PI3K pathway  
205 per se but rather the specific molecular mechanisms promoting the oncogenic signal that can help  
206 develop tailored treatments and more efficacious therapies for cancer management [53].

207 Nevertheless, the efficacy of PI3K inhibitors in the clinic cannot prescind from the functional  
208 status of PTEN. Indeed, a clinical trial testing efficacy of the p110 $\alpha$ -isoform specific inhibitor  
209 alpelisib, found that 6 breast cancer patients harbouring *PIK3CA* mutations displayed initial positive  
210 response to treatment until selective pressure promoted acquisition of novel mutations in the  
211 tumour suppressor *PTEN* which associated with metastatic growth and eventually led to treatment  
212 failure [54]. Mechanistically, complete *PTEN* loss was found to overcome drug inhibition by  
213 promoting engagement of the p110 $\beta$  isoform hence inducing a compensatory reactivation of the  
214 PI3K pathway. PTEN regulation of the p110 isoforms switch has been observed and reported in  
215 multiple types of cancer and in prostate cancer p110 $\beta$  activation has been shown to primarily  
216 mediate *PTEN*-loss driven tumourigenesis [55-58]. Moreover, a few studies have also shown that in  
217 cancer, *PIK3CA* and *PTEN* mutations are not necessarily mutually exclusive but that their  
218 concomitant alterations can have biological effects and therapeutic implication [59-61]. Thus a  
219 number of critical questions remain to be addressed such as: *what are the molecular mechanisms that*  
220 *select for one versus the other p110 isoforms in a context of PTEN deficiency? And in a setting of combinatorial*  
221 *PI3K and PTEN mutations, are the distinct p110 isoforms all functionally relevant? And, are there additional*  
222 *p110-independent signaling pathways emanating the oncogenic signal driven by PTEN loss?* To date, there is  
223 no evidence of a direct binding between PTEN and any of the p110 isoforms that could help address

224 these questions and even though PTEN has been shown to bind p85 $\alpha$ , it is not known whether this  
 225 interaction is conserved across the remaining p85 isoforms. Further, PTEN itself executes a number  
 226 of tumour suppressive functions which can be mediated by lipid phosphatase-dependent and  
 227 independent activities and that have been shown to play important role in cancer suppression and  
 228 resistance to therapies, as we detail below (Figure 2)[62].



229  
 230 **Figure 2.** The PTEN-PI3K axis dictates response to PI3K-directed therapies. Finely regulated PIP3  
 231 levels are under the control of the PI3K-PTEN axis. Acquisition of *PIK3CA* mutations tilts the  
 232 physiologic balance to overcome PTEN inhibition and promote tumorigenesis. *PIK3CA* mutant  
 233 cancers are sensitive and better respond to isoform-specific PI3K inhibitors. Loss of PTEN function  
 234 has been associated with activation of multiple p110 isoforms that act with tissue-specific  
 235 dependencies. Thus, PTEN-deficient tumours may require inhibition of more than one p110 isoform  
 236 for effective therapies. Further, multiple PTEN-protein targets have been identified which can  
 237 contribute to the PTEN-driven tumorigenesis. These substrates together with PI3K inhibitors can  
 238 provide a better therapeutic option for PTEN mutant cancers.

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#### 4. PTEN beyond PI3K

241 4.1: *PIP3-independent PTEN functions*

242 Despite the many shared functional targets, loss of PTEN in preclinical settings has not  
 243 demonstrated as clear an association in terms of response to PI3K inhibitors as observed with  
 244 mutant PI3K [52]. In cell lines and xenograft studies, Tanaka et al found that *PIK3CA* mutant cells  
 245 consistently displayed high sensitivity to the class I PI3Ks inhibitor CH5132799, whereas PTEN loss  
 246 was associated with positive as well as negative response to the drug *in vitro* and *in vivo* [63].  
 247 Similarly, in a panel of 18 breast cancer cell lines tested in response to the dual PI3K and mTOR  
 248 inhibitor BEZ235, it was found that the presence of *PIK3CA* mutations and *HER2* amplification  
 249 strongly associated with growth inhibition and apoptosis in response to the drug, whereas mutant  
 250 PTEN cells were seemingly protected and displayed high phosphorylation levels of the mTORC1  
 251 target RPS6 by the ERK pathway [64]. Further, in a mouse model of prostate cancer we found that  
 252 *Pten* loss is accompanied by a profound suppression of MAPK signaling. However, *Pten* loss  
 253 potentially cooperates with inactivation of the tumour suppressor *Pml* that promotes increased  
 254 lipogenesis and progression to metastasis specifically through reactivation of the MAPK pathway  
 255 [65]. Thus, while PTEN-deficient tumours can rely on multiple p110 isoforms for growth and  
 256 survival, *in vivo* models and *in vitro* studies of drug sensitivity in response to Pan-PI3K or dual PI3K  
 257 inhibitors should take into accounts signaling consequences that may not be directly dependent on  
 258 PIP3, and that are triggered by accompanying mutations.

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260 4.2: *PTEN lipid and protein phosphatase activity side-by-side*

261 Early studies in 1997 identified PTEN as a novel tumour suppressor belonging to the  
262 superfamily of tyrosine receptor phosphatases and, more specifically, PTEN was described as a dual  
263 specificity protein-phosphatase with activity towards phospho-serine and threonine and  
264 phospho-tyrosine peptides [66]. Ever since, multiple phospho-proteins have been identified and  
265 proposed as direct PTEN targets but the contribution of these functional targets to the  
266 well-established PTEN biologic function has remained contentious, reviewed in [9].

267 In trying to address this issue through an *in vivo* approach, we and others generated Pten  
268 knock-in (KI) mice harbouring loss-of-function PTEN mutations known to differentially affect the  
269 distinct PTEN catalytic activities [20, 21]. We selected the cancer-associated Pten G129E mutation  
270 which was shown to affect PTEN lipid function while leaving PTEN protein phosphatase activity  
271 intact; and in a second model we targeted the *Pten* exon5 to generate mice expressing the Pten C124S  
272 mutation which lack lipid and protein phosphatase activity and renders Pten phosphatase dead [66].  
273 The Leone lab chose to model the Pten G129E mutation and the Pten C124R mutation with similar  
274 effect on Pten catalytic activity [21]. In these studies we found that homozygous expression of both  
275 mutations was lethal in uterus and homozygous KI mice died at similar stage during embryonic  
276 development thus suggesting that Pten activity towards phospho-lipid substrates is essential for  
277 proper embryogenesis. In adulthood, heterozygous expression of either Pten mutations promoted  
278 tumour development in a number of tissues with no obvious difference between the two Pten KI  
279 models in terms of tumour onset and disease progression. However, through careful analysis, we  
280 also discovered that Pten C124S/+ mice presented twice as many adenomas of the pituitary gland  
281 than PtenG129E/+ mice and that Pten C124S/+ male mice developed complete testicular atrophy by  
282 10 months of age which was not found in Pten G129E/+ mice. Further, loss of Pten lipid function  
283 induced formation of pre-malignant hepatic fatty nodules which were rarely found in Pten C124S/+  
284 mice suggesting that loss of PTEN protein phosphatase activity could somehow rescue the liver  
285 phenotype [20]. Thus, through these studies we conclude that although the PTEN lipid phosphatase  
286 activity is responsible for many of the PTEN loss-driven phenotypes, consistent with the critical role  
287 of PI3K and PIP3, but that in a tissue specific manner, or under particular physiologic conditions  
288 such as stress, aging, or cell regeneration, PTEN protein phosphatase activity becomes more  
289 apparent and in that, it modulates mechanisms of survival and growth. Consistent with this  
290 hypothesis, Zhang et al reported that loss of PTEN contributed to resistance to Trastuzumab  
291 treatment in ERBB2-overexpressing breast cancer cell lines and xenograft models by directly  
292 dephosphorylating and activating the proto-oncogene SRC on tyrosine 416 [67]. Thus, upon  
293 drug-targeting, PTEN lipid and protein phosphatase activities both impact response to treatments  
294 and synergistically act to coordinate cell survival. This also indicates that the functional relevance of  
295 PTEN protein phosphatase activity may be revealed under specific biological conditions and that  
296 validated PTEN protein targets should also be taken into consideration, in addition to PI3K  
297 inhibitors, for the treatment of PTEN-driven diseases including cancer. Generation of Pten KI mice  
298 with specific loss of PTEN protein phosphatase activity only and intact lipid function as observed  
299 with the PTEN Y138L mutation will further help to thoroughly address this issue.

## 300 6. Conclusions

301 In this review, we have summarized key functions and regulatory mechanisms associated with  
302 the PTEN-PI3K axis in cancer. We have dissected the functional crosstalk of PTEN and PI3K with  
303 additional components of the PI3K pathway and highlighted the important role of established as  
304 well as novel mediators of the pro-survival PIP3 signal. Further, we have integrated the  
305 conventional role of PTEN as a master regulator of PIP3 levels and proposed a novel concept in  
306 favour of a PIP3-independent role of the PTEN catalytic functions in response to therapies and  
307 beyond, and hope that through future studies these, as well as additional theories and hypotheses,  
308 will lead us to a better and more comprehensive understanding of the PI3K signaling network  
309 towards effective combinatorial treatments for cancer.

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- 316 “The authors declare no conflict of interest.”



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