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Review Series

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The Journal of Clinical Investigation

PTEN-opathies: from biological insights to evidence-based precision medicine

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Introduction

The tumor suppressor gene phosphatase and tensin homolog (*PTEN*; OMIM 601728) was originally recognized as being mutated somatically in multiple sporadic cancers (1, 2), as well as mutated in the germline of patients with Cowden syndrome (CS; OMIM 158350), a hereditary overgrowth and cancer predisposition disorder (3, 4). PTEN is a dual-specificity phosphatase at two levels. First, PTEN has been shown to dephosphorylate protein substrates on serine/threonine and tyrosine residues, thus acting as a dual-specificity protein phosphatase (5). One example is the tyrosine dephosphorylation of focal adhesion kinase (FAK) to inhibit cell spreading (6). Second, PTEN also dephosphorylates phosphatidylinositol 3,4,5-trisphosphate (PIP3) to phosphatidylinositol 4,5-bisphosphate (PIP2) — hence, PTEN is also a dual-specificity phosphatase in the sense that it dephosphorylates lipid substrates in addition to protein substrates (7). As a lipid phosphatase, PTEN canonically negatively regulates the phosphatidylinositol 3-kinase (PI3K) signaling cascade, thereby dampening downstream protein kinase B (PKB/AKT) signaling (7, 8). Left unchecked, such as through *PTEN* mutation or inactivation, elevated PIP3 levels cause constitutive activation of AKT with subsequent downstream cascades resulting in, e.g., upregulation of mammalian target of rapamycin (mTOR) signaling (9). This ultimately leads to cell survival, growth, proliferation, and decreased apoptosis (10–12). Notably, AKT represents only one of many PIP3-binding proteins regulated by the PI3K/PTEN axis (13, 14). Although originally believed to be an exclusively cytoplasmic phosphatase, PTEN is now known to also function within the nucleus, contributing to cell cycle regulation, DNA double-strand break repair, genomic stability, and chromatin remodeling (15–20). Therefore, although PTEN exerts much of its function as a lipid phosphatase counteracting the PI3K/AKT/mTOR signaling pathway, PTEN also exerts protein phosphatase–dependent and pan-phosphatase-independent activities within both the cytoplasm and the nucleus (ref. 21 and Figure 1).

Germline *PTEN* mutations have been identified in patients with different clinical syndromes, and that subset is termed *PTEN* hamartoma tumor syndrome (PHTS) (22). Besides *PTEN* mutation–positive CS, PHTS also encompasses individuals with Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome (PS), and Proteus-like syndrome who have *PTEN* mutations (22–26). BRRS (OMIM 153480) is a rare congenital disorder classically characterized by macrocephaly in combination with intestinal hamartomatous polyposis, vascular malformations, lipomas, and genital freckling (27, 28). PS (OMIM 176920) is a rare, complex, and highly variable disorder characterized by progressive, postnatal overgrowth of multiple tissues derived from different cell lineages (29). Relatedly, germline and somatic mosaic mutations in other genes encoding components of the PI3K/AKT/mTOR signaling pathway downstream of PTEN predispose patients to partially overlapping sets of clinical manifestations reminiscent of PHTS. These overgrowth syndromes are known as the PTEN-opathies (ref. 30 and Figure 2). A subset of individuals with the PTEN-opathies harbor germline mutations in components of the PTEN signaling cascade (Table 1), predisposing these individuals to overgrowth and/or cancer in different organs. Postzygotic somatic mosaic mutations in PTEN pathway genes cause overgrowth disorders restricted to the tissues where the mutations occurred. One example is PS, in which a somatic mosaic activating *AKT1* mutation (p.Glu17Lys) has been identified in more than 90% of individuals meeting clinical

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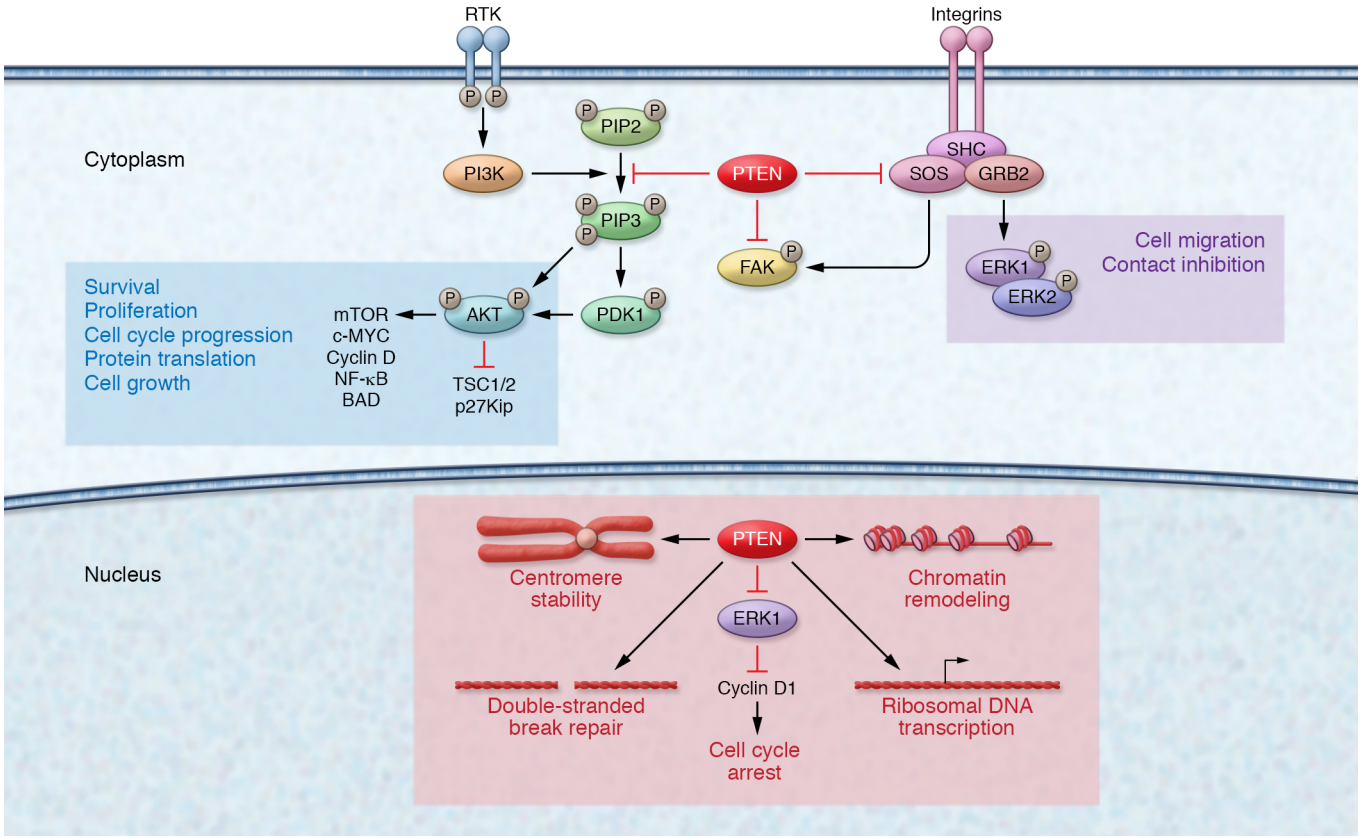

Figure 1. Cytoplasmic and nuclear PTEN signaling. In the cytoplasm, PTEN canonically functions to regulate the PI3K/AKT/mTOR signaling pathway. Under growth factor stimulation, PI3K is activated and catalyzes the phosphorylation of PIP2 to PIP3. PIP3 recruits PDK1 to the plasma membrane, which then contributes to the activation of AKT. AKT regulates a myriad of downstream cellular processes such as cell growth, proliferation, and decreased apoptosis. The lipid phosphatase activity of PTEN counteracts PI3K by dephosphorylating PIP3 to PIP2, thereby dampening AKT activation. In the nucleus, PTEN plays a vital role in maintaining genomic stability, chromosomal architecture, cell cycle control, and the regulation of ribosome biogenesis within nucleoli.

diagnostic criteria (31). Finally, somatic mutations in components of the PTEN signaling cascade occurring in postnatal somatic tissue can drive a vast array of sporadic cancers (32–35).

Overgrowth syndromes are important to diagnose, not only for timely disease management, but also because several of these conditions are associated with elevated risks of cancer. Here, we utilize the PTEN-opathies, particularly PHTS, as a model to examine how perturbation of the PTEN signaling pathway leads to a spectrum of heterogeneous clinical phenotypes. We discuss the genetic, functional, and mechanistic insights that put forth why some organs overgrow but never turn malignant while others develop malignancies. Importantly, the elucidation of the underlying mechanisms is of clinical importance since it promotes the implementation of evidence-based medical management and preventative and therapeutic approaches.

PTEN dysfunction and cancer

The identification of germline *PTEN* mutations allowed for the comprehensive elucidation of component cancers and associated

Table 1. Germline mutation frequencies of PTEN pathway genes in the PTEN-opathies

Syndrome (OMIM)	Gene	Germline mutation frequency	References
Cowden syndrome, CS (158350)	*PTEN*	25%–85%	41, 60, 83
	AKT1	2%	167
	PIK3CA	9%	167
Bannayan-Riley-Ruvalcaba syndrome, BRRS (153480)	*PTEN*	60%	83, 84, 168, 169
Macrocephaly–autism spectrum disorder, macro-ASD (605309)	*PTEN*	10%–20%	71, 108–111
Proteus and Proteus-like syndromes, PS (176920)	*PTEN*	7%–67%	23–25, 44, 170
Megalencephaly–capillary malformation syndrome, MCAP (602501)	*PIK3CA*	8%	171
Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome, MPPH (603387)	*PIK3R2*	Up to 41%	172
	AKT3	Up to 29%	172

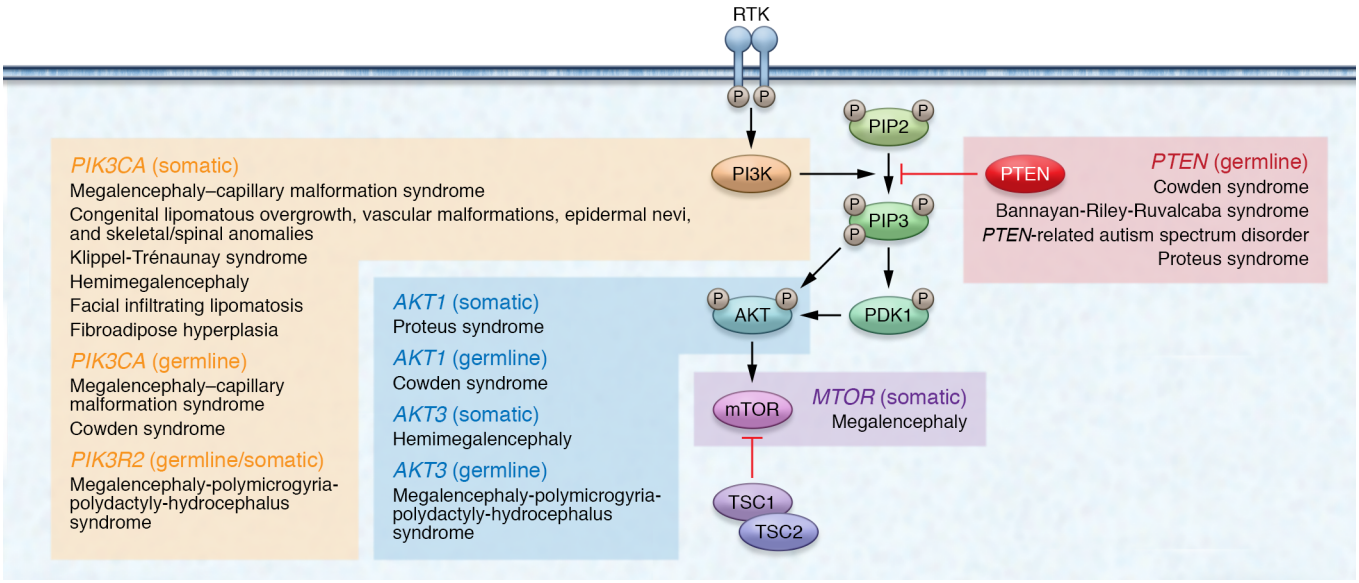

Figure 2. The classic PTEN pathway and associated PTEN-opathies. The PTEN-opathies encompass a spectrum of disorders with mutations within genes encoding proteins belonging to the PTEN pathway. *PIK3CA*-related overgrowth spectrum (PROS) includes distinct clinical entities with phenotypic overlap among the different syndromes. These overgrowth disorders are typically associated with postzygotic somatic mosaic *PIK3CA* mutations in affected tissues and are characterized by segmental overgrowth affecting the body (e.g., CLOVES syndrome, fibroadipose hyperplasia) or the brain (e.g., megalencephaly–capillary malformation syndrome [MCAP], hemimegalencephaly). *PIK3CA* encodes the catalytic p110α subunit protein of PI3K. Similarly to PTEN dysfunction, PIK3CA activation results in phosphorylation and activation of AKT, ultimately resulting in overgrowth-promoting downstream effects within the PI3K/AKT/mTOR signaling pathway downstream of PTEN. Expectedly, these syndromes show clinical phenotypic overlap with PHTS, including megalencephaly, vascular malformations, overgrowth, and neurocognitive deficits.

lifetime risks (36). Three independent studies revealed elevated risks for breast, thyroid, endometrial, kidney, and colon cancers and melanoma in PHTS (36–38). Similarly to other hereditary cancer syndromes, the risk for bilateral and multifocal cancer is elevated (22). Relatedly, individuals with PHTS have a 7-fold increased risk of developing second malignant primary neoplasms (39). Collectively, these cancer risk assessment studies inform clinical surveillance recommendations and medical management of individuals with germline *PTEN* mutations (36), with the aim of detecting malignancies at the earliest, most manageable stages (Table 2).

PTEN comprises nine exons canonically encoding a 403–amino acid protein (1, 40). Broadly, *PTEN* mutations could impact the abundance of PTEN protein, resulting in haploinsufficiency; result in reduced or lost phosphatase activity; act in a dominant-negative manner; and/or result in aberrant localization and function (21). The germline mutation spectrum in PHTS is broad, with mutations affecting all nine exons of *PTEN* (refs. 36, 39, 41, 42, and Figure 3). Approximately two-thirds of germline *PTEN* mutations occur in exons 5, 7, and 8 (41). Interestingly, up to 40% of all germline *PTEN* mutations are located in exon 5, encoding the core catalytic motif, although this exon represents only 20% of the coding sequence (41, 43, 44). Relatedly, two distinct Alu elements have been reported in two unrelated CS patients with identical break points within exon 5, suggesting that this exon is a possible retrotransposition hotspot (45). Mutations within the core catalytic motif typically abrogate pan-phosphatase (lipid and protein) activity, such as mutations affecting p.Cys124, but rarely, mutations such as p.Gly129Glu result in abrogation of lipid phosphatase activity only (4, 46, 47). Interestingly, several *PTEN* mutations retain partial or even complete catalytic activity (48), suggesting alternative mechanisms for compromised PTEN function. For example, catalytically active mutant PTEN p.Lys289Glu is characterized by a nuclear import defect due to loss of monoubiquitination at p.Lys289 (49). Nuclear PTEN is thought to be protected from polyubiquitination and subsequent proteasome-mediated degradation in the cytoplasm; therefore, it is able to dampen AKT signaling and induce p53-independent apoptosis (49). In support of these observations, nuclear exclusion of PTEN has been associated with more aggressive, advanced-stage cancers (50–54). Relatedly, the N-terminal phosphatase domain contains two ATP-binding motifs, critical for regulating PTEN exit from the nucleus (55). Expectedly, ATP-binding motif mutants (e.g., p.Lys62Arg, p.Tyr65Cys, p.Lys125Glu) do not bind ATP efficiently, resulting in nuclear PTEN mislocalization. This subsequently leads to increased cellular proliferation, reduced/abrogated apoptosis, and increased anchorage-independent growth (56, 57). PTEN has also been shown to be SUMOylated at Lys266 within the C2 domain, which facilitates PTEN binding to the plasma membrane through electrostatic interactions and subsequent suppression of PI3K/AKT signaling, both in vitro and in vivo (58). Additionally, germline *PTEN* mutations have been observed at Lys254 (Figure 3), a residue that is also SUMOylated to enhance PTEN nuclear import to then function in DNA repair (59). Therefore, mutations at Lys254 result in nuclear exclusion of PTEN and compromised DNA repair mechanisms.

Aside from intragenic mutations, approximately 10% of CS patients harbor germline *PTEN* promoter mutations (60). Patho-

Table 2. Component cancer risks, clinical surveillance, and management recommendations for PHTS

	Population risk (SEER)	Lifetime risk in PHTS[A]	Screening/surgical guidelines[B]	Age to start	Frequency
Breast (female)	12%	67%–85%	Breast awareness and self-exam: report changes to health care provider	18	Consistent
			Clinical breast exam	25[C]	Every 6–12 months
			Mammogram with consideration of tomosynthesis and breast MRI with contrast	30–35[C]	Every 12 months
			Discuss mastectomy	Personalized	As needed
Thyroid	1%	6%–38%	Thyroid ultrasound	Time of PHTS diagnosis, including childhood	Every 12 months
Kidney	1.6%	2%–34%	Consider renal ultrasound	40	Every 1–2 years
Endometrium	2.6%	21%–28%	Encourage patient education and prompt response to symptoms (e.g., abnormal bleeding)	Not applicable	Not applicable
			Consider screening via endometrial biopsy	Not applicable	Every 1–2 years
			Transvaginal ultrasound in postmenopausal women at the clinician's discretion	Not applicable	As needed
			Discuss hysterectomy with completion of childbearing	Personalized	As needed
Colon	5%	9%–17%	Colonoscopy	35[C] unless symptomatic	Every 5 years or more frequently depending on whether patient is symptomatic or polyps are found
Dermatologic[D]	2%	2%–6%	Dermatologic exam	Personalized	Clinician's recommendation
Developmental	NA	NA	Consider psychomotor assessment in children	Time of PHTS diagnosis	Clinician's recommendation
			Brain MRI if symptomatic	Time of PHTS diagnosis	Clinician's recommendation

[A]Cancer lifetime risks calculated to age 70 by Tan et al. (36) and Bubien et al. (37), and to age 60 by Nieuwenhuis et al. (38). Cancer risk percentage ranges reflect lowest and highest frequencies reported in all three studies. [B]Annual comprehensive physical examination starting at age 18 years or 5 years before the youngest age of diagnosis of a component cancer in the family (whichever comes first), with particular attention to thyroid examination. Encourage patient education regarding the signs and symptoms of cancer. [C]Cancer screening should begin 5–10 years before the earliest known component cancer in the family or according to the ages listed in the table, whichever comes first. [D]Lifetime cancer risk estimates of skin cutaneous melanoma. SEER, surveillance, epidemiology, and end results; PHTS, *PTEN* hamartoma tumor syndrome. Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Genetic/Familial High-Risk Assessment: Breast and Ovarian V.1.2019.

genic promoter mutations result in decreased *PTEN* transcription and translation, the latter due to altered mRNA secondary structure (60, 61). More recently, some unsuspected *PTEN* intronic variants were shown to result in pathogenic exon skipping, alternative splicing, or the use of cryptic splice sites (62). These splicing changes correlate with significantly lower PTEN protein levels and elevated p-AKT in patients with splicing changes compared with those without aberrant splicing. Finally, large *PTEN* deletions occur in approximately 3% to 10% of PHTS patients and can be found over the entire coding sequence (41, 42, 60).

Interestingly, PTEN encodes at least two proteins by means of noncanonical translation initiation. The first identified isoform represents a longer PTEN protein, named PTEN-Long (PTEN-L, also known as PTENα), that contains 173 additional amino acids at the amino-terminus due to the usage of an alternative CUG translation initiation site upstream of the canonical AUG sequence (63). Additionally, PTEN-L can be secreted to enter other cells directly, and can be detected in human serum and plasma. PTEN-L has also been shown to interact with canonical PTEN to regulate mitochondrial function and energy production (64). More recently, another N-terminal extended PTEN isoform, named PTENβ, has been identified (65). PTENβ translation is initiated from an AUU codon upstream of the AUG initiation codon for canonical PTEN. This isoform specifically localizes in cell nucleoli, and regulates ribosomal DNA (rDNA) transcription and cellular proliferation. As these newly identified PTEN protein isoforms are characterized by distinct subcellular localizations and biological functions, further studies are warranted to better understand how these isoforms contribute to carcinogenesis. Importantly, since PTEN-L and PTENβ share the canonical PTEN sequence, mutations that impact canonical *PTEN* would be expected to impact these isoforms as well. However, mutations within the N-terminal extended regions of PTEN-L and PTENβ can have downstream effects independent of canonical PTEN. An intriguing hypothe-

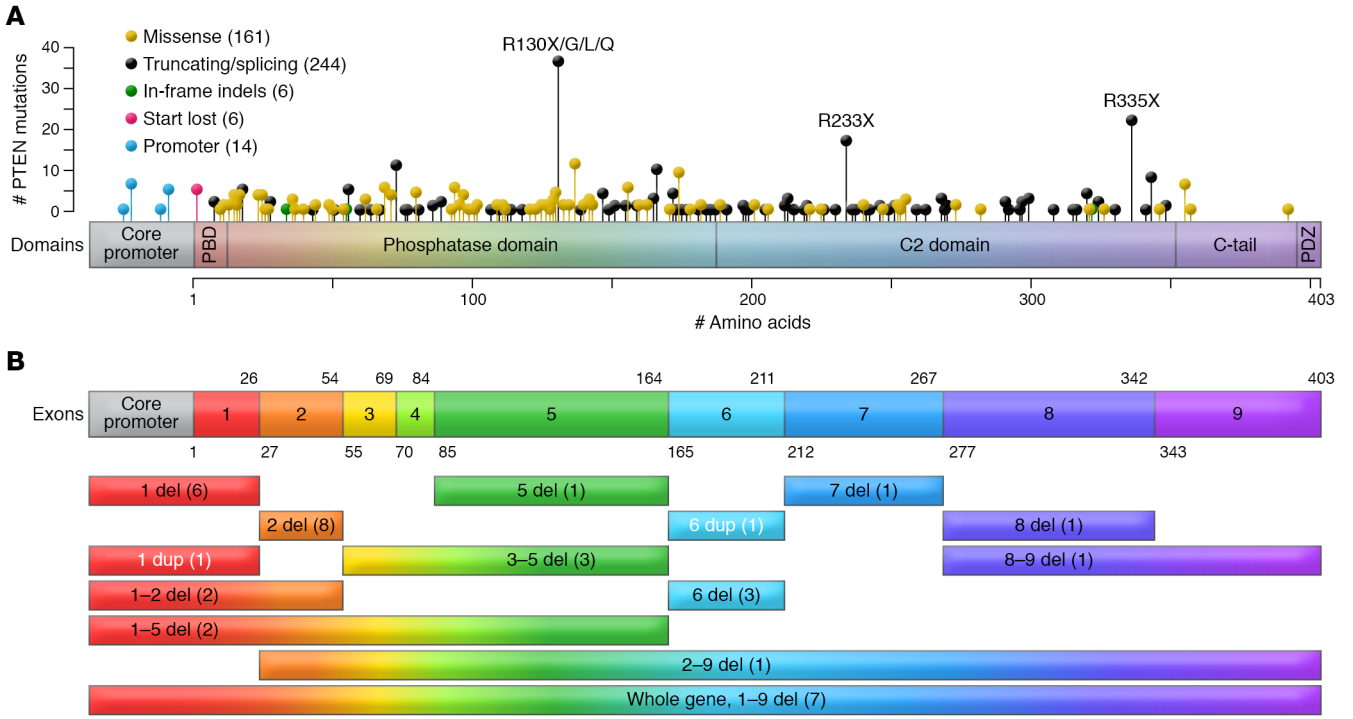

Figure 3. PTEN structure and germline mutation spectrum in PHTS. (**A**) *PTEN* germline mutation spectrum from 431 PHTS patients. PTEN is canonically a 403–amino acid protein. Different types of mutations are depicted in the lollipop plot overlaying the PTEN protein structure. The frequency of mutations correlates with the heights of the vertical lines representing each lollipop. PTEN comprises a PIP2-binding domain (PBD), a phosphatase domain, a C2 domain, and a C-terminal tail including a PDZ-binding domain. The active site is included within amino acid residues 123 and 130. (**B**) *PTEN* consists of 9 exons that encode the 403–amino acid protein. The exons are overlaid to match the protein domains in **A**. Intronic regions are not represented. The colored bars represent large deletions (abbreviated as del) and duplications (abbreviated as dup) annotated by affected exon numbers and the number of affected patients in parentheses. Figure adapted with permission from ref. 174.

sis is the tissue-specific expression of various PTEN protein isoforms, which could, in turn, predispose PHTS individuals to different phenotypes in a genotype-dependent manner. Indeed, the complex interplay among the PTEN family proteins could partly explain why a wide spectrum of clinical phenotypes are observed in the PTEN-opathies, with implications for the precise clinical management of these disorders.

PTEN dysfunction in PHTS offers important biological insights in the context of common sporadic cancers. Indeed, *PTEN* is one of the most frequently somatically mutated genes in cancer (66–68). The experimental data, in turn, offer insights regarding how germline *PTEN* mutations cause the clinical manifestations observed in PHTS. Cell survival, growth, apoptosis, migration, and genomic instability represent processes that influence cell fate and reflect overgrowth and cancer-related phenotypes. In time, it became evident that PTEN is also critical for normal development and physiology (11, 69). These findings help explain the occurrence of neurodevelopmental disorders such as megalencephaly, autism spectrum disorder (ASD), and developmental delay in individuals with PHTS (70–72). Importantly, germline *PTEN* mutations have been reported in previously undiagnosed individuals with isolated PHTS-related phenotypes, indicating that the syndrome is indeed underdiagnosed (71, 73–79). Certainly, utilizing knowledge about PHTS pathogenesis aids in establishing a molecular diagnosis, itself critical both for understanding the pathomechanisms behind and for subsequent medical management of the observed phenotypes.

Finally, because it is technically challenging to functionally interrogate all germline and somatic *PTEN* mutations, research efforts have focused on devising high-throughput methods to evaluate pathogenicity. Surprisingly, several residues within the catalytic pocket are shown to be tolerant to mutations, with solvent exposure playing a critical role in dictating tolerance (80). Moreover, several uncharacterized *PTEN* variants result in decreased PTEN thermodynamic stability and abundance, thus expanding the list of potentially functional variants (81). Collectively, such efforts foster evidence-based, functionally relevant classification of *PTEN* mutations into more clinically actionable categories. Predictably, meta-analysis of outputs coupled with clinical phenotypic correlations will likely yield more robust classifications. Such analyses culminated in a recently completed effort through ClinGen's *PTEN* Variant Curation Expert Panel (82).

Genotype-phenotype correlations and modifiers of cancer risks

As with other inherited cancer syndromes, while it is possible to risk-assess increased organ-specific cancer probabilities, it is still impossible to predict at an individual level who will go on to develop any particular component cancer during his or her lifetime. Hence, multiple studies have attempted to find predictive *PTEN* genotype-

phenotype correlations. Earlier studies revealed an association between *PTEN* germline mutations and malignant breast disease (83, 84). Missense mutations and mutations within and 5′ of the phosphatase core motif appear to be associated with multiorgan manifestations, serving as a surrogate of disease severity (83). Other groups did not detect such genotype-phenotype correlations (85), likely because their sample size of studied PHTS patients is small ($n = 13$), compared with the 44 families and 43 probands of the preceding studies (83, 84). More recently, germline *PTEN* frameshift mutations have been found to be overrepresented, but not absolute, in thyroid cancer (86), nonsense mutations overrepresented in colorectal cancer (36), promoter mutations overrepresented in breast cancer (36), and missense mutations overrepresented in individuals with ASD (87). Interestingly, a theoretical computational approach revealed global 3-dimensional PTEN structural instability and inactive conformation in cancer-associated *PTEN* mutations, whereas ASD-associated *PTEN* mutations revealed localized destabilization contributing to partial opening of the active site (88). Such effects cannot be extrapolated from PTEN's secondary structure alone and indeed provide an important dimension to consider for assessing *PTEN* genotype–PHTS phenotype associations. Additionally, studies have shown that ASD-associated mutations tend to retain higher PTEN activity relative to non–ASD-associated mutations (80, 89, 90). Whether assayed in vivo in yeast or in vitro in mammalian stable or primary cell lines, partial hypomorphic PTEN lipid phosphatase activity is retained in individuals with ASD, versus total loss of PTEN lipid phosphatase activity in individuals with more severe PHTS-related phenotypes (80, 89–91). Relatedly, it is predicted that *PTEN* mutations that result in the accumulation of stable inactive PTEN protein would lead to more severe PHTS-related developmental phenotypes and malignancies (90). Finally, imbalances in PTEN subcellular localization could impact PHTS phenotypic manifestations. A murine model of germline-mislocalized cytoplasm-predominant PTEN exhibits macrocephaly and a neurocognitive profile reminiscent of high-functioning ASD (92, 93). Intriguingly, germline-mislocalized nuclear-predominant PTEN can exist in patients with either cancer or ASD (57, 94). In this context, it is tempting to speculate whether mutant nuclear PTEN plays distinct roles in the affected tissues related to the latter disparate phenotypes.

The lack of absolute *PTEN* genotype-phenotype correlations suggests that additional factors act as phenotypic modifiers in PHTS. A proof-of-principle study showed that approximately 6% of *PTEN* mutation–positive CS/CS-like individuals also harbor germline variants in genes encoding three of the four subunits of mitochondrial complex II (*SDHB*, *SDHC*, *SDHD*), which were originally discovered as alternative susceptibility genes in *PTEN* wild-type CS/CS-like patients (95, 96). Individuals carrying *SDHx* variants show an increased risk of breast and thyroid cancers that surpasses the risks mediated by mutant *PTEN* alone (95). Interestingly, while individuals with *SDHx* variants alone show the highest prevalence of thyroid cancer, the coexistence of a *PTEN* mutation was associated with a 77% snapshot prevalence of breast cancer, as compared with 32% with *PTEN* mutations alone and 57% with *SDHx* variants alone. Although the prevalence of thyroid cancer was not significantly elevated in individuals with both *PTEN* mutations and *SDHx* variants, the histology was papillary for all tumors versus the notable enrichment in follicular thyroid tumors in individuals with only *PTEN* mutations. Mechanistically, *SDHx* variants result in ROS-mediated stabilization of HIF-1α, destabilization and decreased protein expression of p53 due to defective interaction with NQO1, and resistance to apoptosis (95). These data also reveal how mitochondrial dysfunction leads to tumorigenesis subsequent to elevated flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD^+), the cofactor and product of NQO1 enzymatic catalysis, respectively. Subsequent studies showed that *SDHD* p.G12S and p.H50R variants directly lead to impaired PTEN subcellular localization and function through SRC-induced oxidation, accompanied by apoptosis resistance and induction of cellular migration (97). Importantly, the selective SRC inhibitor bosutinib could rescue these tumorigenic phenotypes only when wild-type PTEN was present. Similarly, *SDHD* p.G12S and p.H50R variants result in reduced autophagy in a PTEN-dependent manner (98). From a clinical perspective, these data provide mechanistic insights that could explain the increased prevalence of thyroid cancer in CS patients with *SDHx* variants alone compared with those with *PTEN* mutations alone, as well as the seemingly paradoxical decreased prevalence of thyroid cancer in the setting of coexisting *PTEN* mutations and *SDHx* variants.

A hypothesis-generating pilot study further identified microbiomic differences in fecal samples derived from *PTEN* mutation–positive patients with and without PHTS component cancers (99). Functional metagenomic analysis revealed enrichment of cancer-relevant biological processes such as folate biosynthesis, genetic information processing, and cell growth/death pathways in fecal samples from PHTS cancer patients compared with those without a cancer diagnosis. These data suggest that gut dysbiosis could also play a role as a cancer risk modifier in PHTS patients. Conceivably, with increased sample sizes and independent replication, we suspect that novel associations will be discovered and expanded beyond cancer, toward phenotypes such as ASD and non-neoplastic overgrowths. Collectively, this knowledge will be impactful for more tailored medical management of PHTS patients.

Germline predisposition – overgrowth versus cancer

The discovery of *PTEN* as the Cowden syndrome gene paved the way for understanding how its disruption contributes to disease etiology (1, 3, 4). Functional characterization further established *PTEN* as a bona fide tumor suppressor gene (Figure 1). Studies in *Drosophila* and mouse models have shown that PTEN and downstream PI3K/AKT/TOR signaling play a central role in regulating cell number and size. Hence, dysfunction of this pathway recapitulates the growth anomalies observed in the *PTEN*-related human diseases. *Drosophila* PTEN has been shown to regulate cell number and size when mutated, leading to hyperplastic overgrowth in fruit fly mutant tissue (100). Similarly to mammalian signaling pathways, *Drosophila* PTEN regulates growth by antagonizing DDP110 (the *Drosophila* homolog of PI3K), and by acting as a negative regulator of insulin receptor signaling (101–103). With this knowledge of the basic mechanistic principles, what remains elusive, however, is the ability to identify factors that regulate progression from overgrowth to malignancy in a defined set of organs.

Table 3. Preclinical studies, case reports, and clinical trials using PI3K/AKT/mTOR inhibitors for the treatment of the PTEN-opathies

Drug target	Drug	Indication (reference)	Treatments and reported outcomes
Preclinical studies of patient-derived cells			
PI3K	Wortmannin, LY294002	*PIK3CA*-related overgrowth spectrum, PROS (173)	Decreased proliferation of primary dermal fibroblasts from skin biopsies of overgrowth lesions and decreased phosphorylation of AKT and p70S6K
Pan-AKT	ARQ 092	*AKT1*-related Proteus syndrome (136)	Suppression of AKT and downstream signaling in patient-derived cells and tissues mosaic for the *AKT1* somatic gain-of-function p.Glu17Lys mutation
		PROS (137)	Decreased proliferation of primary fibroblasts with *PIK3CA* mutations, decreased phosphorylation of AKT and downstream targets, less cytotoxicity in comparison with rapamycin and wortmannin
Case reports			
mTORC1	Sirolimus (rapamycin)	*PTEN*-related Proteus syndrome (125)	Dose: 0.1 mg/kg/d, divided into 2 doses. Duration: 17+ months. Outcomes: resolution of respiratory and nutritional complications, reduction in soft-tissue masses, reduction in the size of mesenteric lymph nodes, minimal effect on the size or appearance of external subcutaneous lipomata.
		Bannayan-Riley-Ruvalcaba syndrome (126)	Dose: 0.8 mg/m^2/dose, twice daily. Duration: 12 months. Outcome: decreased size of the vascular mass.
		PTEN hamartoma tumor syndrome, PHTS (127)	Dose: 0.1 mg/kg/d. Duration: 19 months. Outcomes: transient improvement of somatic growth and reduced thymus volume, absence of clinical benefit.
		Infantile Lhermitte-Duclos disease (128)	Dose: 0.6 mg/kg/d. Duration: 12 months. Outcomes: decreased episodes of decerebrate posturing, regaining of normal appearance of the pituitary stalk, less compression of the brainstem.
PI3K	BYL719 (p110α)	PROS (138)	Dose: 50–250 mg/d. Duration: 6–18 months. Outcomes: patient-dependent with general improvement of overgrowth; decrease in size of vascular tumors, reduction of hemihypertrophy, attenuation of scoliosis.
Clinical trials			
mTORC1	Sirolimus	PHTS/Cowden syndrome, CS	Phase II clinical trial completed and results forthcoming (NCT00971789)
		PROS	Phase II clinical trial completed and results forthcoming (NCT02428296)
	Everolimus	PHTS with neurocognitive deficits	Phase I/II clinical trial currently recruiting (NCT02991807)
PI3K/mTOR	BGT226	CS patients with advanced solid malignancies	Phase II clinical trial completed and results forthcoming (NCT00600275)
	BEZ235		Phase I/II clinical trial completed and results forthcoming (NCT00620594)
Pan-AKT	ARQ 092	Patients with overgrowth diseases and/or vascular anomalies	Phase I/II clinical trial currently recruiting (NCT03094832)

The most obvious explanation for organ-specific cancer development could be that the expression of the cancer-related gene, here *PTEN*, could be limited to the tissues in which malignancies arise. However, PTEN is ubiquitously expressed in all three germ cell layers throughout development, supporting the occurrence of hamartomatous overgrowths and variable multisystem phenotypes in individuals with germline *PTEN* mutations (11, 69, 104, 105). Homozygous *Pten*-knockout mice die before birth, further supporting a critical role for PTEN in embryogenesis (11, 105–107). High-level PTEN expression has also been reported during human development in tissues known to be associated with PHTS (104). However, this does not corroborate the tendency of these organs to develop malignancies when PTEN malfunctions. For example, the strongest PTEN protein levels are observed throughout the central and peripheral nervous systems (104), even though brain cancer is not a PHTS component cancer. Nevertheless, neurodevelopmental phenotypes are observed in PHTS, including macrocephaly (about 94% of patients), ASD (108–111), and Lhermitte-Duclos disease (LDD), a pathognomonic hamartomatous overgrowth of the cerebellum (112). Immunohistochemical studies show decreased or absent PTEN expression accompanied by elevated p-AKT in the affected LDD dysplastic gangliocytoma cells (113). Interestingly, murine studies have found that even a subtle reduction in PTEN causes increased tumorigenesis in a tissue-specific manner (114). In humans, reduced PTEN protein dose in CS-derived lymphoblastoid cell lines tends to occur in conjunction with an underlying germline *PTEN* mutation and to correlate with increasing clinical phenotypic burden (41). Further investigation in CS/CS-like patients with thyroid cancer reveals that low PTEN protein levels from blood-derived lymphoblastoid cells can predict for the presence of a germline *PTEN* mutation (115). Importantly, low blood PTEN levels correlate with weak or absent PTEN staining in the affected PHTS-derived thyroid tissues. Hence, one possibility is that variable tissue-specific thresh-

olds of PTEN protein dosage could potentially influence particular PHTS-related phenotypes.

Intriguingly, despite the fact that germline *PTEN* mutations result in component cancers within a restricted set of organs (36), *PTEN* somatic driver mutations are enriched in multiple sporadic cancer types that are not components of the PHTS spectrum, including prostate cancer, glioblastoma multiforme, and others (1). Moreover, identical germline *PTEN* mutations often result in apparently disparate phenotypes (e.g., cancer versus nonmalignant overgrowths), including in an intrafamilial manner (116). These observations suggest that additional factors act as overgrowth versus cancer phenotypic modifiers in PHTS. Indeed, while germline *PTEN* mutations predispose PHTS patients to cancer, it is the landscape of acquired somatic alterations that likely governs cancer initiation and progression. Hence, although the germline *PTEN* mutations affect all cells of PHTS patients, the tissue-restricted pattern of particular modifying factors could explain the nonrandom progression to malignancy in specific organs. Additionally, the type of germline *PTEN* mutation could also influence eventual cell fates. For example, germline *PTEN* mutations such as C-terminal deletions that result in genomic instability could prime tissues that are particularly sensitive to DNA damage for progression to malignancy (117). Finally, the immune system has been recognized as a major determinant of cancer development (118–120). PTEN loss promotes resistance to tumor immune cell infiltration through the production of inhibitory cytokines, hence resulting in immune escape (121). Interestingly, pregnant mice treated with low-dose lipopolysaccharide to induce maternal inflammation produce offspring with brain overgrowth (122). This phenotype is more pronounced in *Pten*-heterozygous mice compared with wild type, indicating evident crosstalk between genetic susceptibility and the inflamed microenvironment mediated through ROS signaling. Importantly, ROS cause oxidation and subsequent inactivation of PTEN, a mechanism observed in a subset of CS/CS-like patients (97, 123). Hence, the manifestation of a cancer phenotype does represent a complex interplay among predisposing factors, genetic and epigenetic confounders, tissue-specific signaling networks, oncogenic signaling pathways, and microenvironmental context (124).

Molecularly targeted therapeutics

Altered PI3K/AKT/mTOR signaling in the PTEN-opathies implies that PI3K, AKT, and mTOR are germane targets for therapeutic intervention (Table 3). Proof-of-principle case reports demonstrate the use of the mTORC1 inhibitor sirolimus (rapamycin) to alleviate the symptoms and overgrowth manifestations of individuals with PHTS (125–128). Indeed, sirolimus has been used in a phase II open-label clinical trial in individuals with PHTS. Additionally, a double-blind drug-placebo, crossover trial with the mTORC1 inhibitor everolimus is currently accruing PHTS patients with ASD (22). Notably, mTORC1 inhibitors have been used in patients with tuberous sclerosis complex (TSC) (129–132) and Peutz-Jeghers syndrome (PJS) (133). *TSC1/2* and *STK11/LKB1*, the susceptibility genes for TSC and PJS, respectively, are not only upstream of mTOR (9, 134) but are also downstream of PTEN signaling (135).

In addition to mTORC1 inhibition, upstream components of the PTEN signaling pathway, such as PI3K and AKT, also serve as candidates for pharmacologic inhibition in the PTEN-opathies (Table 3). As such, AKT and PIK3CA inhibitors have been used in PS and *PIK3CA*-related overgrowth spectrum (PROS) disorders (136–138). *PIK3CA* encodes the p110α catalytic subunit protein of PI3K. Preclinical studies using the allosteric pan-AKT inhibitor ARQ 092 revealed suppression of AKT and downstream signaling in cells and tissues from PS patients, mosaic for the *AKT1* somatic gain-of-function p.Glu17Lys mutation (136). ARQ 092 treatment of primary fibroblast cells from PROS patients also showed promising results; compared with sirolimus and the PI3K inhibitor wortmannin, ARQ 092 resulted in higher antiproliferative activity and lower cytotoxicity, at least in vitro (137). Relatedly, a recent proof-of-principle study demonstrated the successful usage of the PIK3CA inhibitor BYL719 (alpelisib) in a preclinical murine model of PROS and subsequently for the treatment of 19 patients with severe PROS disorders (138). Importantly, in these PTEN-opathies, all patients harbored somatic mutations; the ultimate goal from treatment is to continuously reduce progrowth signals in affected tissues with minimal toxicity toward normal wild-type cells. However, this becomes more challenging in the germline context, such as PHTS, where a high therapeutic index becomes even more critical since all cells harbor the underlying *PTEN* mutation. Moreover, constitutional PTEN pathway dysfunction would theoretically necessitate some type of chronic treatment regimen. However, lifelong mTOR and PIK3CA inhibition might not be feasible because of immunosuppressive effects, disruption of systemic glucose homeostasis, and the critical role the PTEN pathway plays in normal tissue and organ development (11, 69, 139, 140). Although isolated case reports and studies (125, 126, 128, 138) show promise for the therapeutic management of the PTEN-opathies, longitudinal studies are necessary to evaluate long-term safety and efficacy.

Another major caveat to molecular targeting of the PI3K/AKT/mTOR pathway is feedback activation of collateral oncogenic signaling pathways, causing resistance. This led to the investigation of combinatorial therapies that would, in theory, effectively target the growth-promoting signals without loss of feedback controls. Indeed, inhibiting mTORC1 has been shown to result in feedback activation of upstream signaling components such as AKT through insulin receptor substrate 1 (IRS1) or through direct phosphorylation at Ser473 by mTORC2 (141). However, experimental studies show promise in that the rebound upregulation of AKT during mTORC1 inhibition can be abrogated by pretreatment or cotreatment with resveratrol, at least in vitro (142). Moreover, PI3K inhibition can result in therapeutic resistance in *PIK3CA*-mutant cell lines due to a rebound insulin-dependent feedback mechanism (140), or failure to suppress CDK4/6 as evidenced through persistent RB phosphorylation (143). In these contexts, the combination of various PI3K inhibitors with antiglycemic therapies or CDK4/6 inhibitors, respectively, results in the attenuation of the progrowth feedback signaling cascades, hence overcoming resistance. Interestingly, NVP-BEZ235, a dual PI3K/mTOR inhibitor, has been shown to selectively inhibit the growth of a subset of androgen receptor–positive (AR^+) breast cancer cell lines (144). AR is positively correlated with PTEN expression in breast cancer, owing to direct *PTEN* transcription that is mediated by an androgen response element in the *PTEN* promoter

(145, 146). Mechanistically, the beneficial effect of AR activation in combination with PI3K/AKT/mTOR inhibition in AR^+/ER^+ breast cancers can be explained, at least partially, through PTEN upregulation and MYC suppression (144). Interestingly, the converse phenomenon has been extensively studied in prostate cancer and has been shown to be context-dependent (147–149). As such, *PTEN*-deficient prostate cancer cells have decreased AR transcription, and PI3K pathway inhibition activates AR signaling by alleviating the feedback inhibition on HER2/3 kinases (148). Therefore, the crosstalk between PTEN and AR signaling will likely be genotype- and context-dependent.

Although most therapeutic strategies are aimed at attenuating downstream oncogenic signaling consequent to PTEN dysfunction, strategies to enhance PTEN levels and/or activity represent promising therapeutic modalities. This is particularly pertinent for the cell-permeable PTEN-L (63) that would theoretically allow the restoration of PTEN levels in the context of PTEN haploinsufficiency. Moreover, PTEN expression and/or activity could also be enhanced through modulating negative and positive regulators of PTEN (e.g., transcription factors, miRNAs, protein ubiquitination machinery, etc.). Certainly, these approaches are context-dependent with respect to baseline endogenous PTEN levels and activity, tissue specificity, and the requisite of establishing long-term effects, among many other factors. Importantly, restoring wild-type PTEN in the context of a stable mutant PTEN protein could worsen the condition owing to dominant-negative effects (150). Another plausible approach is through gene editing of mutant *PTEN* alleles to restore or even enhance PTEN function (e.g., increased phosphatase activity or recruitment to the plasma membrane) (151). While gene editing poses many challenges, including off-target effects and activation of adaptive immune responses (152, 153), recent advances show promise in mitigating these outcomes (154–156). Undoubtedly, gene editing will be exceptionally challenging in the germline setting where the whole organism is targeted for editing.

Finally, given the broad spectrum and diverse functional consequences of germline *PTEN* mutations, targeting the PI3K/AKT/mTOR signaling pathway may not be effective in all contexts, e.g., when disease-associated *PTEN* mutations impact lipid phosphatase–independent functions. One possible approach could be to target the vulnerabilities caused by patient-specific germline *PTEN* mutations. For example, because PTEN plays a vital role in maintaining genomic integrity in the nucleus, it is possible to therapeutically use PARP inhibitors (157). Moreover, patients with germline *PTEN* mutations within the C-terminal region could benefit from proteasome inhibitors to mitigate PTEN degradation (158). Indeed, treatment with the proteasome inhibitor MG-132 can restore both nonsense and missense mutant PTEN protein levels in vitro (159). However, restoring missense mutant PTEN levels in the presence of the wild-type allele can also result in dominant-negative effects, and could worsen the condition (150). Predictably, in the era of precision medicine, cancer prevention is key and determining context-specific therapeutic indices will be vital for the effective management of the PTEN-opathies.

Immunotherapy

The PI3K/AKT/mTOR pathway is an important regulator of immunity (160, 161). Since PTEN is a master regulator of this pathway, it is therefore not surprising that PTEN disruption can result in immune dysregulation. The latter is germane in carcinogenesis, whereby immune surveillance, evasion of immune recognition, and a chronically inflamed microenvironment represent major immune hallmarks of cancer (118). Additionally, PI3K/AKT/mTOR pathway activation has been shown to modulate responses to immunotherapy. Loss of PTEN in the sporadic context has been associated with resistance to anti–PD-1 therapy in melanoma (121), a CS component cancer, and more recently in a case study of metastatic uterine leiomyosarcoma (162). Interestingly, activation of the PI3K/AKT/mTOR pathway has been shown to drive expression of PD-1/PD-L1 in a subset of solid tumors, causing immunoresistance (163–165). Indeed, because PTEN seems to be a major immunotherapeutic response predictor, multiple questions arise regarding the promising utility of immunotherapeutic agents in individuals with germline *PTEN* mutations and cancer. Studies have shown that a subset of individuals with PHTS have autoimmune phenotypes as well as B and T cell–related immune dysfunctions (161, 166). Importantly, reduction in peripheral lymphocyte numbers in comparison with control subjects, including decreased $CD4^+$ cell numbers and hence absolute $FOXP3^+$ Treg numbers, would suggest that these individuals will have a different response to immunotherapy compared with individuals with normal immune systems.

Perspective

The PTEN-opathies represent a paradigm whereby one pathway appears etiologic for a wide spectrum of clinically distinct phenotypes. The recognition and characterization of the PTEN-opathies allow for significant advances in understanding how clinical phenotypic manifestations result from underlying molecular and cellular processes to then guide risk assessment, therapeutics, and preventative strategies. Preclinical studies and clinical trials show promise for the treatment of a subset of the PTEN-opathies. However, this becomes more complex in the germline context, where a high therapeutic index is mandatory, yet exceptionally challenging. Indeed, individuals with germline *PTEN* mutations have a lifelong predisposition to PHTS-related signs and symptoms, necessitating prolonged treatments that could impact normal growth and development and cause nontargeted cytotoxicity. One of the most serious complications of the PTEN-opathies, particularly PHTS, is the increased lifetime risk for cancer. Although *PTEN*-enabled organ-specific cancer risk estimates and management guidelines are part of the routine clinical armamentarium of precision care, it remains virtually impossible to absolutely predict which individual (versus a probability) will develop which component malignancy. Nonmalignant component phenotypes of PHTS, such as ASD and severe vascular malformations, can be chronically debilitating and affect quality of life for patients and their families. Intriguingly, identical germline *PTEN* mutations are observed in patients with these seemingly disparate phenotypes (e.g., cancer versus ASD), indicating that additional factors may act as phenotypic modifiers in PHTS. Hence, future studies elucidating absolute modifiers of disease manifestations and associated signaling networks will be key to define more precise and effective preventative and therapeutic strategies for the individual at risk.

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