Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Precise Immunodetection of PTEN Protein in Human Neoplasia

Rafael Pulido,[1,2] Janire Mingo,[1] Ayman Gaafar,[3] Caroline E. Nunes-Xavier,[1,4] Sandra Luna,[1] Leire Torices,[1] Javier C. Angulo,[5,6] and José I. López[1,3,7]

[1]Biocruces Bizkaia Health Research Institute, Barakaldo 48903, Spain

[2]Ikerbasque, Basque Foundation for Science, Bilbao 48011, Spain

[3]Department of Pathology, Cruces University Hospital, Barakaldo 48903, Spain

[4]Department of Tumor Biology, Institute for Cancer Research, Oslo University Hospital Radiumhospitalet, Oslo N-0310, Norway

[5]Department of Urology, University Hospital of Getafe, Getafe, Madrid 28904, Spain

[6]Clinical Department, European University of Madrid, Laureate Universities, Madrid 28904, Spain

[7]University of the Basque Country, Leioa 48940, Spain

Correspondence: rpulidomurillo@gmail.com; rafael.pulidomurillo@osakidetza.eus

PTEN is a major tumor-suppressor protein whose expression and biological activity are frequently diminished in sporadic or inherited cancers. *PTEN* gene deletion or loss-of-function mutations favor tumor cell growth and are commonly found in clinical practice. In addition, diminished PTEN protein expression is also frequently observed in tumor samples from cancer patients in the absence of *PTEN* gene alterations. This makes PTEN protein levels a potential biomarker parameter in clinical oncology, which can guide therapeutic decisions. The specific detection of PTEN protein can be achieved by using highly defined anti-PTEN monoclonal antibodies (mAbs), characterized with precision in terms of sensitivity for the detection technique, specificity for PTEN binding, and constraints of epitope recognition. This is especially relevant taking into consideration that PTEN is highly targeted by mutations and posttranslational modifications, and different PTEN protein isoforms exist. The precise characterization of anti-PTEN mAb reactivity is an important step in the validation of these reagents as diagnostic and prognostic tools in clinical oncology, including their routine use in analytical immunohistochemistry (IHC). Here, we review the current status on the use of well-defined anti-PTEN mAbs for PTEN immunodetection in the clinical context and discuss their potential usefulness and limitations for a more precise cancer diagnosis and patient benefit.

Biomarker detection constitutes one of the key parameters in precision oncology providing information to stratify cancer patients in terms of diagnosis, prognosis, and response to therapies (Salgado et al. 2017; Bode and Dong 2018). The analysis of genomic data from both tumor and normal-tissue samples is becoming one of the major sources of clinically relevant

Editors: Charis Eng, Joanne Ngeow, and Vuk Stambolic
Additional Perspectives on The PTEN Family available at www.perspectivesinmedicine.org

 doi: 10.1101/cshperspect.a036293
Cite this article as *Cold Spring Harb Perspect Med* 2019;9:a036293

information for cancer patient stratification and therapy optimization. However, other molecular variables, such as the presence of biomarker proteins in biological samples, are more informative than genetic data in some types of neoplasias (Schmidt et al. 2016; Twomey et al. 2017). Analytical immunohistochemistry (IHC), which relies on the use of sensitive monoclonal antibodies (mAbs) targeting specific biomarker proteins, is one of the most universal diagnostic techniques in modern clinical oncology. IHC provides local information at the single-cell level on the biomarker expression in the whole tumor and its microenvironment, guiding the pathologist in the diagnosis and prognosis of most solid malignancies (Leong et al. 2010; Matos et al. 2010). Power-resolution advantages of IHC include the monitoring of biomarker expression without disrupting the architecture of the tissue, which has especial relevance in tumors displaying high intratumor heterogeneity (ITH) (McGranahan and Swanton 2015; López and Angulo 2018), the opportunity of detecting specific protein posttranslational modifications that are functionally and clinically relevant (Sperinde et al. 2010; Bodo and Hsi 2011), and the possibility of monitoring the subcellular localization of the biomarker protein (Cheuk and Chan 2004). Alternative techniques to monitor the presence of cancer biomarker proteins using mAbs exist, including radioimmunoassay, enzyme-linked immunosorbent assays (ELISAs), and membrane- and bead-based protein arrays, although their implementation in the clinic is more limited (Zhang et al. 2014).

The PTEN tumor-suppressor protein plays a major and unique role in the control of cell growth and survival in all tissues, and PTEN protein is partially or totally lost in a large number of human tumors, which makes it an excellent biomarker and therapeutic target candidate in clinical oncology (McCabe et al. 2016; McLoughlin et al. 2018; Bazzichetto et al. 2019). PTEN is encoded by a single gene, but distinct PTEN protein isoforms exist, generated by alternative initiation of messenger RNA (mRNA) translation or mRNA alternative splicing (Sharrard and Maitland 2000; Malaney et al. 2017). The more abundant PTEN protein isoform contains 403 amino acids (PTEN 1–403), and constitutes the PTEN form referenced in most of the studies. Several commercial anti-PTEN mAbs are available and have been extensively used in cancer research, but their clinical use in diagnostic or predictive tests measuring PTEN presence in tumors by IHC is still pending analytical validation. This, in part, can be because, of the difficulties to standardize the IHC preanalytical and analytical conditions of mAb usage, as well as variations in the PTEN immunostaining scoring and the performance attributed to each anti-PTEN mAb in different laboratories (Eritja et al. 2015). In addition, the lack of precision in the definition of the epitopes recognized by the distinct anti-PTEN mAbs can also be a caveat in the accurate interpretation of their IHC reactivity patterns in tumor samples. In this review, we summarize the current status of the use of anti-PTEN mAbs as diagnostic and prognostic tools in human neoplasias and highlight the importance of the precise characterization of anti-PTEN mAbs in providing a more accurate assistance to precision oncology.

PTEN GENE, mRNA, AND PROTEIN EXPRESSION ALTERATIONS IN HUMAN TUMORS

The human *PTEN* gene was originally identified as a protein tyrosine phosphatase–encoding tumor-suppressor gene frequently deleted in multiple human advanced cancers, including brain, breast, and prostate cancer (Li and Sun 1997; Li et al. 1997; Steck et al. 1997). In more recent years, it has been determined that the *PTEN* gene and its protein product are recurrently altered in most human cancers because of the key role played by PTEN PI(3,4,5)P3 (PIP3) phosphatase activity in the negative regulation of the prosurvival PI3K/AKT/mTOR pathway, as well as by PTEN PIP3 phosphatase-independent tumor-suppressive functions (Worby and Dixon 2014; Pulido 2015; Lee et al. 2018). PTEN distributes in a highly regulated manner at different cell compartments, and the tumor-suppressive functional consequences of PTEN partitioning between membranes, cytoplasm, and nucleus have been well documented (Vazquez and

Devreotes 2006; Gil et al. 2007; Bassi and Stambolic 2013; Kreis et al. 2014; Bononi and Pinton 2015). PTEN functional deficiency associates with hyperactivation of the PI3K/AKT/mTOR pathway. This makes the determination of *PTEN* gene loss by molecular or FISH analysis, or the assessment of PTEN protein expression by IHC, relevant to stratify patients who could benefit from therapies based on PI3K, AKT, or mTOR inhibition by small molecule drugs (Dillon and Miller 2014; Papa and Pandolfi 2019). In this respect, the global analysis of genetically characterized human cancer cell lines revealed that genetic alterations in *PTEN* are associated with increased sensitivity to PI3K/AKT/mTOR inhibitors and decreased sensitivity to receptor-tyrosine kinase (RTK) inhibitors (Yang et al. 2013; Dillon and Miller 2014), a concept that can be extended to tumor resistance to RTK inhibitors and chemotherapeutic agents (Shoman et al. 2005; Mellinghoff et al. 2007; Steelman et al. 2008). In addition, PTEN loss has also been associated with resistance to PD-1-inhibitor T-cell-mediated immunotherapy in several cancer types (Rieth and Subramanian 2018). On the other hand, because of the positive role of nuclear PTEN in DNA-damage repair, PTEN loss sensitizes cancer cells to poly (ADP-ribose) polymerase (PARP) inhibitors by a synthetic lethality mechanism (Mendes-Pereira et al. 2009; Dedes et al. 2010; McEllin et al. 2010).

PTEN gene mutations are found in ∼5% of human tumor samples, ranking in the top group of genes most commonly mutated in human cancer (Tan et al. 2015). Importantly, a copy of the *PTEN* gene is also mutated in the germline of patients with PTEN hamartoma tumor syndrome (PHTS) (Yehia and Eng 2018), making determination of PTEN protein levels and function critical in the follow-up of their disease. From a global perspective, *PTEN* gene or PTEN protein loss is associated with cancer progression and resistance to therapies in most human tumors, although divergent findings have been reported (Table 1). For instance, in glioblastoma, ∼30% of studies revealed a positive prognostic value for *PTEN* gene or protein expression, including a study showing a positive predictive value for response to the erlotinib RTK inhibitor (Table 1; Mellinghoff et al. 2005; Montano et al. 2016). In other malignancies, such as breast cancer, most of the meta-analysis studies indicated a positive prognostic value for PTEN expression, including studies with a positive predictive value for response to trastuzumab anti-human epidermal growth receptor 2 (HER2) therapy (Table 1; Nagata et al. 2004; Wang et al. 2013; Zhang et al. 2019).

Regulation of PTEN expression, abundance, and function is exerted at multiple genetic and nongenetic levels under physiologic conditions, and aberrant PTEN alterations in cancer are caused by a wide variety of mechanisms (Hollander et al. 2011; Leslie and Foti 2011; Boosani and Agrawal 2013; Correia et al. 2014; Milella et al. 2015; Li et al. 2018b; Alvarez-Garcia et al. 2019). PTEN has been proposed as an obligate haploinsufficient tumor suppressor, in which partial loss of expression, rather than complete loss, renders maximal tumorigenicity (Berger and Pandolfi 2011). This is relevant in clinical practice because weak PTEN protein expression is often observed in tumors, as compared with normal tissues. *PTEN* gene alterations resulting in PTEN protein loss are evident and differential in distinct cancer types. For instance, advanced prostate cancers and lung cancers frequently show *PTEN* gene deletion, whereas endometrial cancers and glioblastomas often show *PTEN* mutations generating PTEN truncated proteins, including premature termination codon (PTC), frameshift small insertions, and frameshift small deletions (Fig. 1A; www.cbioportal.org; Cerami et al. 2012). Alterations in PTEN mRNA levels are also different in distinct types of cancer with prostate, ovarian, lung, and breast cancers displaying a higher frequency of PTEN mRNA down-regulation (Fig. 1B). This is the result of a combination of factors, including tissue-specific gene promoter hypermethylation, alterations in the activity of *PTEN* transcription factors, and pathologic modifications in the balance between microRNAs (miRNAs) and long noncoding RNAs targeting PTEN (Hollander et al. 2011; Boosani and Agrawal 2013; Taulli et al. 2013; Lu et al. 2016; Li et al. 2018b).

A more homogeneous global pattern of PTEN expression alteration in human neo-

Table 1. Global clinical significance of PTEN loss

Cancer type	Clinical significance of PTEN loss	Reference
Endometrial	*Low diagnostic accuracy in EH*[a]	Raffone et al. 2019a
	Association with increased risk of EC in EH	Raffone et al. 2019b
Glioblastoma	*Prognostic value only in 30% of studies*	Montano et al. 2016
Prostate	↑ GS; ↑ capsular penetration	Wang and Dai 2015
	↑ GS; ↑ recurrence	Gao et al. 2016
	↓ PFS	Xie et al. 2017
	Prognostic value	Wise et al. 2017
	Prognostic value	Jamaspishvili et al. 2018
	Prognostic and predictive (recurrence) value	Carneiro et al. 2018
Lung	↓ OS; ↓ PFS	Gu et al. 2016
	↑ Stage; ↑ LNM	Ji et al. 2018
	↑ Stage; ↑ distant metastasis; ↓ OS	Zhao et al. 2017
	↓ OS	Xiao et al. 2016
Gastric	↓ OS	Chen et al. 2014
Breast	Resistance to TZMB in recurrent or metastatic patients	Wang et al. 2013
	No predictive or prognostic value	Wang et al. 2015c
	↓ OS	Yang et al. 2016
	↑ Stage; ↑ LNM; ↓ PFS; ↓ OS	Xu et al. 2017
	↑ Stage; ↑ LNM; ↓ PFS; ↓ OS	Li et al. 2017
	Resistance to TZMB	Zhang et al. 2019
Ovarian	*No prognostic value*	Cai et al. 2014
Colorectal	Resistance to anti-EGFR; unclear prognostic value	Lo Nigro et al. 2016
	Resistance to anti-EGFR	Therkildsen et al. 2014
Renal	↓ DSS	Tang et al. 2017
	↑ Stage; ↑ distant and LNM	Que et al. 2018

Note that this is not a comprehensive list. Selected reviews and meta-analysis studies with information on the clinical significance of *PTEN* gene or PTEN protein loss are denoted.

DSS, Disease-specific survival; EC, endometrial carcinoma; EGFR, epidermal growth factor receptor; EH, endometrial hyperplasia; GS, Gleason score; LNM, lymph node metastasis; OS, overall survival; PFS, progression-free survival; TZMB, trastuzumab.

[a]Text in italics indicates studies in which the PTEN status does not show prognostic or predictive values.

plasms is observed when analyzing PTEN protein levels in tumors using IHC. Although there are important variations among different studies, most of the cancer types show, on average and independently of the anti-PTEN mAb used, total or partial loss of PTEN protein expression in 30%–50% of cases (Fig. 1C). This is in accordance with the multicenter study by Millis et al. (2016), using about 20,000 samples from diverse solid tumors, which revealed 30% of samples with PTEN protein loss as determined by IHC with the anti-PTEN 6H2.1 mAb. Interestingly, this study also documented the coexistence of PTEN loss or *PTEN* mutations with *PIK3CA* mutations in tumors, supporting the notion that PTEN PIP3 phosphatase-independent tumor-suppressive functions have clinical relevance. These findings illustrate the high incidence of PTEN protein loss in human tumors, even in the absence of *PTEN* genetic alterations, which has important clinical implications in prognostic and therapy-response prediction. Heterogeneous *PTEN* gene deletion or focal loss of PTEN protein expression in tumors is a common event in some human cancers, which makes examination of PTEN expression in neoplastic tissues by IHC very important (Garg et al. 2012; Zaldumbide et al. 2016; Yun et al. 2019). For instance, heterogeneity of PTEN immunostaining in glioblastoma tumors was associated with poor patient outcome (Idoate et al. 2014). Moreover, alterations in PTEN subcellular compartmentation, such as dynamic changes in PTEN nuclear-cytoplasmic distribution during

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

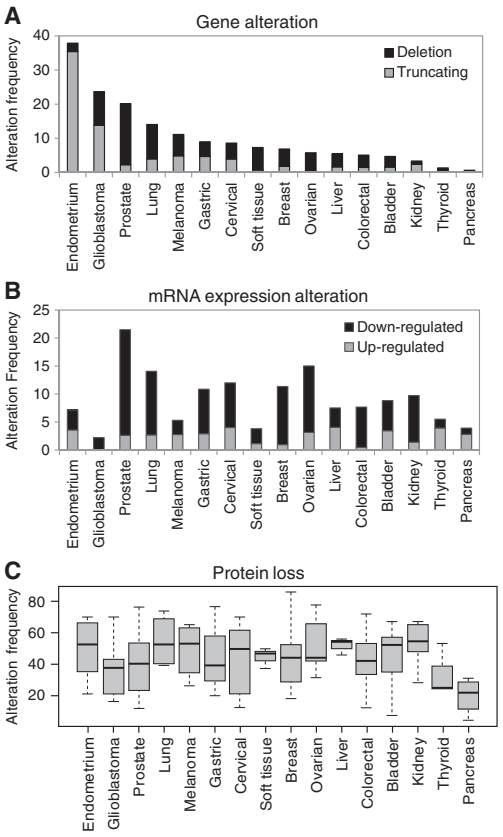

Figure 1. *PTEN* gene and protein alterations in human tumors. (*A*) Frequency of *PTEN* gene deletion in human tumors and PTEN mutations causing loss of PTEN protein (premature termination codon [PTC] mutations and frameshift small deletions or insertions). (*B*) Frequency of PTEN messenger RNA (mRNA) down- or up-regulation in human tumors (*z*-score threshold ±2). In both cases, the alteration frequencies are indicated for 16 different human cancers from data generated by the TCGA Research Network and using the cBioPortal database (cBioPortal for Cancer Genomics; Cerami et al. 2012; Gao et al. 2013). Cancer types are as follows: endometrial, uterine corpus endometrial carcinoma; gliobastoma, glioblastoma multiforme; prostate, prostate adenocarcinoma; lung, lung squamous cell carcinoma; melanoma, skin cutaneous melanoma; gastric, stomach adenocarcinoma; cervical, cervical squamous cell carcinoma; soft tissue, sarcoma; breast, breast invasive carcinoma; ovarian, ovarian serous cystadenocarcinoma; liver, liver hepatocellular carcinoma; colorectal, colorectal adenocarcinoma; bladder, bladder urothelial carcinoma; kidney, kidney renal clear cell carcinoma; thyroid, thyroid carcinoma; pancreas, pancreatic adenocarcinoma. (*C*) Boxplot of the frequency of PTEN protein loss

oncogenesis, may also have clinical relevance, which is revealed by IHC (Gil et al. 2015; Nosaka et al. 2017; Mingo et al. 2018; Mukherjee et al. 2018). Thus, detection of local PTEN protein expression in tumor samples by analytical IHC constitutes a fundamental methodology whose accuracy to assist clinical precision oncology needs to be optimized. A number of ongoing clinical trials based on PI3K/AKT/mTOR, PARP, or immune checkpoint inhibitors are using *PTEN* gene or PTEN protein loss as a stratifying patient criterion (clinicaltrials.gov). Optimization of PTEN protein IHC would benefit these trials, as well as the potential implementation of novel PTEN-dependent anticancer therapies.

PTEN PROTEIN DETECTION BY IHC USING DEFINED ANTI-PTEN mAbs

A major factor influencing the accurate determination of PTEN protein expression in tumors by IHC is the sensitivity and specificity of the mAb. In Table 2, a list is provided of commercial anti-PTEN mAbs available for the analysis of formalin-fixed paraffin-embedded (FFPE) tissues by IHC, as reported in the literature or indicated by the manufacturer. Some of these mAbs have been experimentally validated, and their IHC use conditions, efficiency, and reliability have been, in some cases, evaluated and compared (Pallares et al. 2005; Sakr et al. 2010; Sangale et al. 2011; Carvalho et al. 2014; Maiques et al. 2014; Eritja et al. 2015; Lavorato-Rocha et al. 2015; Ágoston et al. 2016; Castillo-Martin et al. 2016; Gil et al. 2016; Lotan et al. 2016a; Guedes et al. 2019). In Table 3 and Figure 2, the comparative staining of samples from prostate and bladder urothelial carcinomas with six experimentally

Figure 1. (*Continued*) in human tumors as detected by immunohistochemistry (IHC). Whiskers represent the minimum and maximum of all of the data; boxes represent the values between quartiles 1 and 3, and bands inside the boxes represent the median. Data are compilations from Tables 2–4. Note that the cancer categories in *C* have a wider coverage of cancer subtypes than the categories in *A* and *B*.

Table 2. Selected commercial anti-PTEN mAbs suitable for IHC

mAb	Isotype	Host	Immunogen[a]	Epitope[b]	Reference[c]
6H2.1	IgG	Mouse	PTEN 304–403	392–398	Perren et al. 1999
SP218	IgG	Rabbit	Carboxy-terminal PTEN peptide	394–402	Castillo-Martin et al. 2016
17.A (Ab-4)	IgM	Mouse	PTEN 2–403	392–402	Torres et al. 2001
Y184	IgG	Rabbit	Carboxy-terminal PTEN peptide	386–394	Sangale et al. 2011
138G6	IgG	Rabbit	Carboxy-terminal PTEN peptide	388–394	Bedolla et al. 2007
D4.3		Rabbit	Carboxy-terminal PTEN peptide	388–394	Schultz et al. 2010
217702	IgG1	Mouse	PTEN 2–403	392–400	Carvalho et al. 2014
28H6[d]	IgG1	Mouse	PTEN-203–403		Kimura et al. 2004
G-6	IgG1	Mouse	PTEN 1–403		Wang et al. 2015b
A2B1	IgG1	Mouse	PTEN 388–403		Depowski et al. 2001
11G8.11	IgG	Mouse	PTEN 1–403		Cascade BioScience
EPR9941-2	IgG	Rabbit	PTEN 1–403		Abcam
SP170	IgG	Rabbit	PTEN 200–300		Abcam; Sigma-Aldrich
SP227	IgG	Rabbit	PTEN 250–350		Abcam; Sigma-Aldrich
PTN-18	IgG2a	Mouse	PTEN 386–403		Sigma-Aldrich
RM265		Rabbit	Carboxy-terminal PTEN peptide		Sigma-Aldrich
H-3	IgG2b	Mouse	PTEN 2–28		Santa Cruz Biotechnology
F-1	IgG1	Mouse	PTEN 3–29		Santa Cruz Biotechnology
9E8	IgG1	Mouse	PTEN 320–400		Abbkine
2C10	IgG1	Mouse	PTEN 320–400		Abbkine
EP2138Y[e]	IgG	Rabbit	PTEN phospho-Ser380 peptide		Roy and Dittmer 2011
EP229[e]	IgG	Rabbit	PTEN phospho-Thr366 peptide		Abcam

Commercial anti-PTEN mAbs suitable for IHC, as reported or indicated by the supplier, are listed.

mAb, Monoclonal antibody; IHC, immunohistochemistry.

[a]PTEN amino acid numbering is indicated according to NP_000305.3.

[b]Precise epitope mapping is provided, indicating the residues encompassing the epitope, according to Mingo et al. (2019) and our unpublished results.

[c]The reference in which the mAb was first described (to the best of our knowledge) is indicated. When no reference is indicated, the supplier is indicated. Note that suppliers may change with time.

[d]The anti-PTEN 28H6 mAb only stains nuclear PTEN in tissues.

[e]These mAbs recognize PTEN phosphorylated at the indicated residues.

Table 3. Comparative IHC staining of anti-PTEN mAbs of FFPE samples from prostate and bladder urothelial carcinomas

	Prostate ($n = 81$)		Bladder ($n = 49$)	
mAb	Negative/positive	% Negative	Negative/positive	% Negative
6H2.1	62/19	76.5	25/24	51
SP218	50/31	62	28/21	57
17.A (Ab-4)	41/40	49	29/20	59
Y184	33/48	41	12/37	24.5
138G6	45/36	56	27/22	55
D4.3	42/39	52	33/16	67

Data are as reported in Mingo et al. (2019).

FFPE, Formalin-fixed paraffin-embedded.

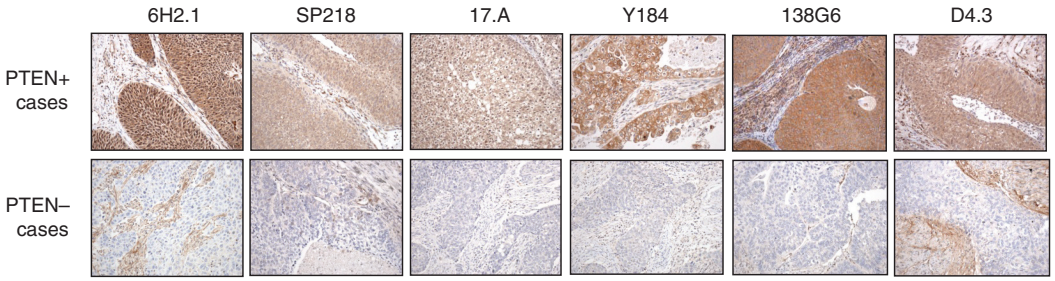

Figure 2. Immunohistochemistry (IHC) staining of formalin-fixed paraffin-embedded (FFPE) tumor tissue sections with different anti-PTEN monoclonal antibodies (mAbs). Bladder urothelial carcinoma samples, immunostained with six different anti-PTEN mAbs, are shown. For each mAb, a positive (+) and a negative (–) case are shown. Magnification, ×200.

validated anti-PTEN mAbs is shown, illustrating PTEN-positive and PTEN-negative cases. Table 4 is a compilation of IHC studies addressing the frequency of PTEN protein loss in FFPE samples from different cancer types, with indications of the specific anti-PTEN mAbs used and the clinical associations found in each study. As shown, there are differences in the frequency of usage of the distinct mAb within each cancer type, which makes it difficult to perform appropriate comparisons. There are also variations in the results obtained with the same antibody in a given cancer type, which in some cases can be a result of clinical differences in the analyzed cohorts. The clustering of studies taking into consideration the mAb used (same mAb for each cancer type) provided average values of PTEN loss between 35% and 65% of cases (Fig. 3). In spite of these extensive analyses, a consensus on which anti-PTEN mAb is more appropriate for IHC evaluation of PTEN expression in tumors does not exist yet. It is likely that this choice will depend on several factors, such as the tissue of interest and PTEN subcellular localization (cytoplasm vs. nucleus) under scrutiny. In addition, the identity of the recognized epitope in PTEN protein, and whether the detection of noncanonical PTEN isoforms is part of the study, could be important in the adequate selection of anti-PTEN mAbs as IHC biomarker tools (see below). In this respect, most of the IHC experimentally validated anti-PTEN mAbs recognize short linear epitopes at the PTEN carboxyl terminus, which reflects both the frequent usage of PTEN carboxy-terminal peptides as the immunogens and the antigenic immunodominance of the PTEN carboxyl terminus unstructured region (Fig. 4; Mingo et al. 2019). Finally, a group of anti-PTEN mAbs suitable for IHC analysis, in some cases targeting non-carboxy-terminal PTEN regions, is pending experimental evaluation and validation (Table 2).

PTEN PROTEIN IMMUNODETECTION BY OTHER TECHNIQUES

In addition to IHC, other techniques based on the use of anti-PTEN mAbs, such as immunoblot, ELISA, and flow cytometry, have been used to monitor PTEN expression in human biological samples. Some of these techniques have the advantage of a higher qualitative or quantitative resolution in terms of PTEN molecular properties, which could be important when determination of well-defined PTEN protein levels during the evolution of patient disease is desired, as could be the case in PHTS patients. Immunoblot is the standard technique to validate the specificity of anti-PTEN mAbs using cell lysates from PTEN-positive and PTEN-negative cells, and provides information on the relative molecular size of the detected PTEN proteins, which is important when addressing the expression of PTEN isoforms (Wang et al. 2015a). Immuno-

Table 4. PTEN IHC detection in human cancers using defined anti-PTEN mAbs

Cancer type	Antibody	Frequency of PTEN protein loss (%)[a]	Clinical associations of PTEN protein loss	Reference
Endometrial	6H2.1	61	—	Mutter et al. 2000
	6H2.1	68	↓ EIN versus EC	Monte et al. 2010
	6H2.1	64	↑ Endometroid versus nonendometroid	Djordjevic et al. 2012
	6H2.1	50	—	Sangale et al. 2011
	6H2.1	40.5	—	Garg et al. 2012
	6H2.1	31	—	Pallares et al. 2005
	6H2.1	55	↑ *PFS in obese patients*[b]	Westin et al. 2015
	6H2.1	24	↓ NPE versus EIN	Norimatsu et al. 2007
	17.A	39	↓ NPE versus EC	Erkanli et al. 2006
	Y184	70	—	Sangale et al. 2011
	138G6	70	—	Sangale et al. 2011
	28H6	21	—	Pallares et al. 2005
Glioblastoma	6H2.1	50	↑ Resistance to anti-EGFR	Mellinghoff et al. 2005
	6H2.1	43	—	Brown et al. 2008
	6H2.1	33	—	Kreisl et al. 2009
	6H2.1	70	↓ OS heterogeneous versus homogeneous	Idoate et al. 2014
	6H2.1	16	—	Ballester et al. 2017
	28H6	21	—	Kim et al. 2010
	28H6	41	—	Montano et al. 2011
	138G6	37.5	—	Thiessen et al. 2010
	138G6	19	—	Lv et al. 2012
Prostate	6H2.1	27	↓ PFS combined with p27 loss	Halvorsen et al. 2003
	6H2.1	39	—	Verhagen et al. 2006
	Y184	59	↑ Tumor grade	Al Bashir et al. 2019
	138G6	21.5	↓ PFS combined with high pAKT	Bedolla et al. 2007
	138G6	18	↑ PC death	Cuzick et al. 2013
	138G6	40	↓ PFS combined with ERG+	Leinonen et al. 2013
	138G6	40	↓ OS	Ferraldeschi et al. 2015
	138G6	55	—	Mehra et al. 2018
	D4.3	38	↑ Pathologic features; ↓ metastasis	Lotan et al. 2011
	D4.3	61	↓ PFS	Antonarakis et al. 2012
	D4.3	52	—	Gumuskaya et al. 2013
	D4.3	13.5	↓ PFS	Chaux et al. 2012a
	D4.3	11.5	↑ Upgrading from biopsy to rp	Lotan et al. 2015
	D4.3	25	↑ PC death combined with ERG–	Ahearn et al. 2016
	D4.3	15.5	↑ Upgrading from G6 to G7	Trock et al. 2016
	D4.3	27	↑ Upgrading from biopsy (G7) to rp (nonorgan confined disease)	Guedes et al. 2017
	D4.3	24	↓ PFS	Lotan et al. 2016b
	D4.3	55	↓ PFS combined with ERG–	Lahdensuo et al. 2016
	D4.3	22	↓ PFS	Lotan et al. 2017
	D4.3	14	↓ PFS	Lokman et al. 2018

Continued

Table 4. *Continued*

Cancer type	Antibody	Frequency of PTEN protein loss (%)[a]	Clinical associations of PTEN protein loss	Reference
	28H6	41	↓ PFS combined with ERG–	Kim et al. 2015
Lung	6H2.1	74	↑ *Well- versus poorly differentiated*; ↑ *Stages I and II versus III and IV*	Marsit et al. 2005
	138G6	41	↑ LSCC versus LAC; ↓ PFS in LAC	
	D4.3	64	↑ LSCC versus LAC; associated with smoking	Yanagawa et al. 2012
	G-6	39	↓ Well- versus poorly differentiated; ↓ stages I-II versus III-IV; ↓ OS	Hlaing et al. 2018 Wang et al. 2015b
Melanoma	6H2.1	65	—	Zhou et al. 2000
	6H2.1	44	↑ BM and ↓ OS in $BRAF^{V600}$ patients	Bucheit et al. 2014
	6H2.1	26	↑ Cadherin switch; ↓ PFS	Lade-Keller et al. 2013
	D4.3	62	↓ OS	Giles et al. 2019
Gastric	6H2.1	77	—	Bamias et al. 2010
	138G6	39	—	Tran et al. 2013
	138G6	20	—	Kim et al. 2016
Cervical	6H2.1	62	↑ Pelvic lymph node metastasis	Eijsink et al. 2010
	138G6	21	—	Tinker et al. 2013
	D4.3	50	—	Ueno et al. 2013
	28H6	12	—	El-Mansi and Williams 2006
	28H6	70	↓ CIN versus ICC	Vázquez-Ulloa et al. 2011
Soft tissue	17.A	50	—	Torres et al. 2001
	17.A	47	↓ OS	Teng et al. 2011
	138G6	37	—	Valkov et al. 2011
Breast	6H2.1	33	Association with ER– and PR–	Perren et al. 1999
	6H2.1	86	—	Yonemori et al. 2009
	6H2.1	43	↑ Resistance to TZMB; ↑ *response to lapatinib* (nuclear staining evaluation)	Dave et al. 2011
	6H2.1	27.5	Discordance between primary tumors and metastases	Gonzalez-Angulo et al. 2011
	6H2.1	55.5	↓ OS	Razis et al. 2011
	6H2.1	30	↓ *Resistance to TZMB*	Gschwantler-Kaulich et al. 2017
	17.A	50	—	Torres et al. 2001
	17.A	77	—	Panigrahi et al. 2004
	17.A	45.5	↓ Stages I and II versus stages III and IV; associated with TNB tumors	Siddiqui et al. 2016
	138G6	52	↑ Resistance to TZMB; ↓ OS	Esteva et al. 2010
	138G6	26	—	Perez et al. 2013
	138G6	18	—	Beelen et al. 2014
	D4.3	81	↑ Resistance to TZMB + anthracycline-taxane-based chemotherapy	Loibl et al. 2016
	D4.3	24	↓ Five-year survival in LNM patients	Wang et al. 2017
	D4.3	37	↑ Resistance to TZMB + lapatinib	Rimawi et al. 2018
	28H6	26	—	Bakarakos et al. 2010

Continued

Table 4. *Continued*

Cancer type	Antibody	Frequency of PTEN protein loss (%)[a]	Clinical associations of PTEN protein loss	Reference
	28H6	52	↑ Resistance to TZMB	Fabi et al. 2010
	28H6	45	—	Duman et al. 2013
	A2B1	48	↑ BC death; ↑ LNM	Depowski et al. 2001
	A2B1	32	↓ PFS	Capodanno et al. 2009
Ovarian	6H2.1	78	—	Kurose et al. 2001
	6H2.1	31	↑ *PFS in stage I and II patients and in nondifferentiated SC*	de Graeff et al. 2008
	6H2.1	37.5	—	Ho et al. 2009
	6H2.1	65.5	—	Roh et al. 2010
	6H2.1	42	↑ *PFS in high-grade SC*	Bakkar et al. 2015
	17.A	41.5	—	Wang et al. 2005
	17.A	44	↓ OS in TP53+ patients	Kolasa et al. 2006
	138G6	69	↓ PFS	Lee and Park 2009
	138G6	49	↓ OS in high-grade SC	Martins et al. 2014
Liver	138G6	54	↑ Liver function grading	Zhou and Li 2018
	28H6	56	—	Bassullu et al. 2012
	A2B1	46	↓ PFS; ↓ OS	Su et al. 2016
Colorectal	6H2.1	28	↑ MSI+ versus MSI– tumors	Zhou et al. 2002
	6H2.1	71	↑ Tumor stage	Nassif et al. 2004
	6H2.1	42	—	Goel et al. 2004
	6H2.1	45	—	Hocking et al. 2014
	6H2.1	34	↑ Tumor stage	Lin et al. 2015
	17.A	41.5	↑ Resistance to anti-EGFR + irinotecan in metastatic tumors	Loupakis et al. 2009
	138G6	12	↓ OS in LM patients	Atreya et al. 2013
	138G6	72	—	Karapetis et al. 2014
	D4.3	32.5	↓ OS in anti-EGFR therapy	Sood et al. 2012
	28H6	56	↑ LM versus nonLM patients; ↑ LM versus pt; ↓ five-year survival in LM patients	Sawai et al. 2008
	28H6	50	↓ PFS; ↓ OS	Jang et al. 2010
Bladder	D4.3	35	↑ Pathologic features	Schultz et al. 2010
	D4.3	7.5	↑ Pathologic features; ↑ LNM; ↓ OS	Rieken et al. 2017
	A2B1	52	—	Litlekalsoy et al. 2012
Renal	6H2.1	65	—	Zaldumbide et al. 2016
	138G6	28	—	Figlin et al. 2009
	D4.3	48	—	Chaux et al. 2012b
	D4.3	67	—	Chaux et al. 2013
	A2B1	54.5	—	He et al. 2007
Thyroid	6H2.1	24	—	Alvarez-Nuñez et al. 2006
	6H2.1	53	↑ LNM	Min et al. 2013
	6H2.1	24.5	Associated with follicular variant of papillary thyroid cancer	Beg et al. 2015
Pancreas	6H2.1	4	—	Perren et al. 2000
	6H2.1	18	↑ Resistance to anti-EGFR	Boeck et al. 2013

Continued

Table 4. *Continued*

Cancer type	Antibody	Frequency of PTEN protein loss (%)[a]	Clinical associations of PTEN protein loss	Reference
	6H2.1	26	↑ Recurrence/metastasis; ↓ OS	Foo et al. 2013
	138G6	31	Association with invasive carcinoma; ↓ OS	Garcia-Carracedo et al. 2013

Note that this is not a comprehensive list. Selected studies with specific information on the anti-PTEN mAb used and the percentage of cases with PTEN protein loss are denoted. Studies addressing differential expression of PTEN in cytoplasm and in the nucleus of tumor cells are not included.

BC, Breast cancer; BM, brain metastasis; CIN, cervical intraepithelial neoplasia; ER, estrogen receptor; ICC, invasive cervical carcinoma; LAC, lung adenocarcinoma; LM, liver metastasis; LNM, lymph node metastasis; LSCC, lung squamous cell carcinoma; NPE, normal proliferative endometrium; PC, prostate cancer; pCR, pathological complete response; PR, progesterone receptor; pt, primary tumor; rp, radical prostatectomy; SC, serous carcinoma; TNB, triple negative breast.

[a]PTEN loss includes partial loss (weak or focal expression) or total loss (absence of expression) of PTEN protein detection.

[b]Text in italics indicates studies in which the indicated association with PTEN loss is in contradiction with PTEN tumor-suppressor function. Empty lines indicate no clinical associations reported in the study.

blot has been successfully used to semiquantitatively monitor the PTEN protein levels in PHTS patient-derived lymphoblast cell lines, and its use has been proposed as a predictor of *PTEN* germline mutations (Ngeow et al. 2012). Other membrane-based antibody approaches, including reverse phase protein array (RPPA), have been used in high-throughput monitoring of parallel expression of PTEN protein and other biomarkers in human cancer cell lines and tumor clinical samples (Stemke-Hale et al. 2008; Calderaro et al. 2014; Wiegand et al. 2014; Aslan et al. 2018). Flow cytometry allows single-cell quantitative analysis of molecular markers from cells in solution, although in the case of intracellular markers, such as PTEN protein, flow cytometry requires cell fixation and permeabilization. Flow cytometry has been mainly applied to monitor PTEN protein expression in hemopoietic cells (Yang et al. 2007; Woolley and Salmena 2016; Wu and Song 2018). Finally, examples of ELISA as a technique to monitor PTEN concentration from patient samples include its use in the determination of circulating PTEN protein levels in serum from cancer or diabetic patients (Li et al. 2015b; Razavi et al. 2017; Wu and Song 2018). These tests could provide diagnostic or predictive information, but further standardization and analytical and clinical validation are required.

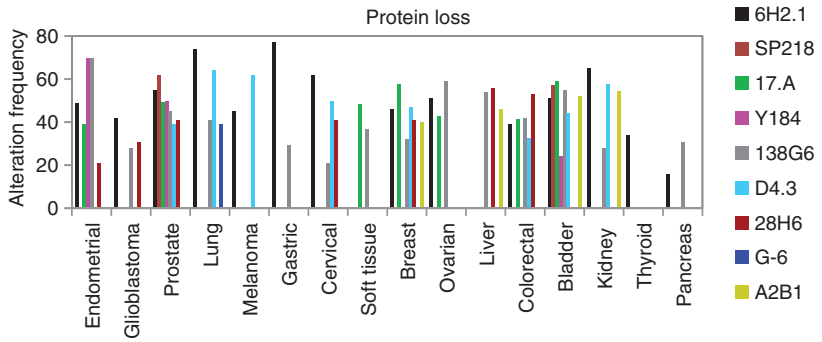

Figure 3. Frequency of PTEN protein loss in human tumors, as detected by IHC using defined anti-PTEN mAbs. Data are compilations from Tables 3 and 4 and are represented as the average of samples with PTEN protein loss clustered by cancer type and the anti-PTEN mAb used. Note that the distinct mAbs have not been used in all cancer types.

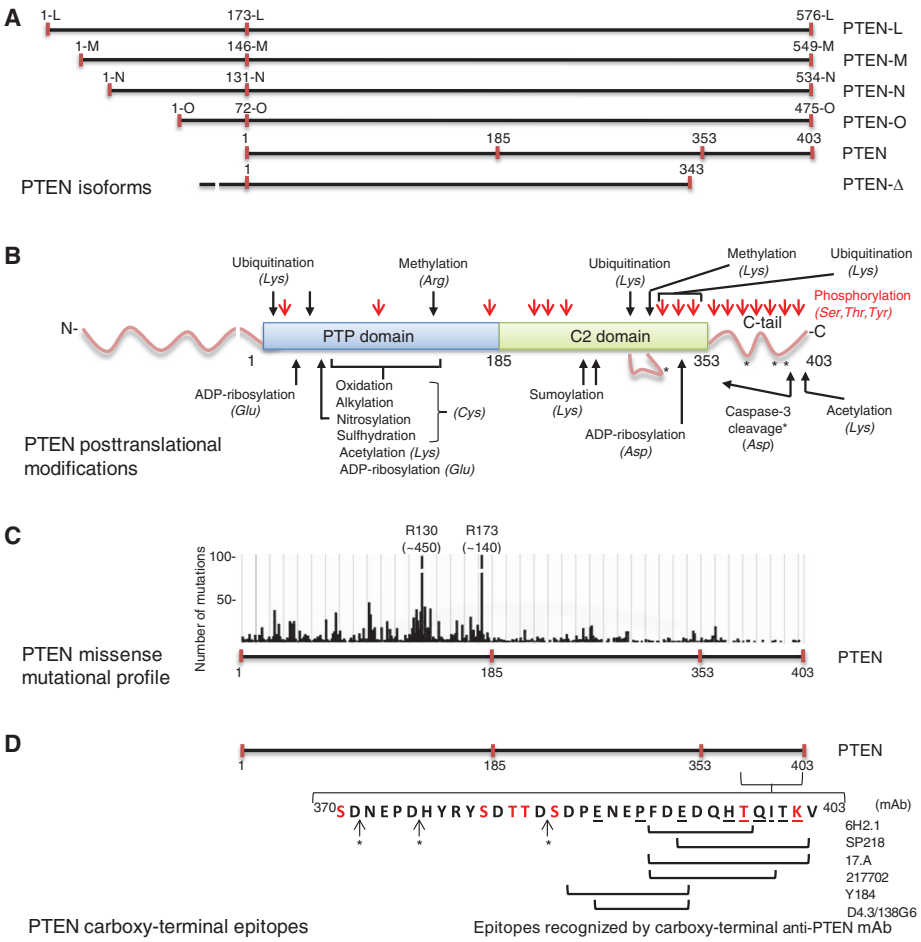

Figure 4. Variability of PTEN amino acid composition and posttranslational modifications in relation with PTEN carboxy-terminal epitopes. (*A*) Schematic depiction of PTEN isoforms. PTEN long isoforms (PTEN-L, PTEN-M, PTEN-N, and PTEN-O), generated by alternative translation initiation, are shown in the *upper* part. Amino acid numbering and nomenclature are according to Pulido et al. (2014) and Tzani et al. (2016). PTEN-Δ isoform (PTEN 1-343-Ser), generated by alternative splicing, is shown in the *bottom*. PTEN (1–403) is shown in the *middle* with indication of the residues flanking the PTP and C2 domains and the carboxy-terminal intrinsically disordered region (C-tail). (*B*) Schematic of PTEN posttranslational modifications. The distinct posttranslational modifications undergone in the different PTEN domains are denoted with indications of the identity of the residues modified as reported for PTEN (1–403; see Table 5 for more information). The disordered region in the C2 domain (residues 286–310) is shown as a line loop. Note that the existence of these modifications in the PTEN long isoforms has not been reported. (*C*) Number of PTEN missense mutations along PTEN protein found in human tumor samples as annotated in the COSMIC database (Catalogue of Somatic Mutations in Cancer, Wellcome Trust Sanger Institute; Forbes et al. 2017). Note that the *y*-axis is scaled to allow visualization of low-frequency mutations. The total number of missense mutations for residues R130 and R173 is in parentheses. (*D*) Delimitation of PTEN carboxy-terminal epitopes recognized by the indicated commercial anti-PTEN mAb. PTEN carboxy-terminal amino acid sequence (residues 370–403) is indicated with a one-letter code. Amino acids in red are subjected to phosphorylation or acetylation (K402). Amino acids underlined are targeted by disease-associated mutations (see Table 6 for more information). *, caspase-3 cleavage sites as shown in *B*. Epitope mapping is from Mingo et al. (2019) and our unpublished observations.

PRECISE DEFINITION OF THE REACTIVITY OF ANTI-PTEN mAbs

Several PTEN protein variants are generated by the alternative initiation of PTEN mRNA translation, resulting in PTEN proteins with amino-terminal extensions in their amino acid sequence and distinct subcellular localizations (Fig. 4A; Hopkins and Parsons 2014; Pulido et al. 2014; Tzani et al. 2016; Malaney et al. 2017). Although a variety of functions have been proposed for some of these amino-terminal-extended PTEN variants, little is known about their expression in human cancer and their specific tumor-suppressive roles (Hopkins et al. 2013; Liang et al. 2014, 2017; Wang et al. 2015a,b, 2018; Cao et al. 2018; Li et al. 2018a; Jochner et al. 2019). The longer PTEN variant, PTEN-L (573 amino acids; also described as PTEN-α), can be secreted to the extracellular environment and internalized to acceptor cells, where it can execute growth-restrictive functions (Hopkins et al. 2013; Wang et al. 2015a). In addition, PTEN-L is also found located in mitochondria, where it regulates mitophagy and mitochondrial function (Liang et al. 2014; Li et al. 2018a; Wang et al. 2018). The generation, characterization, and validation of anti-PTEN mAbs that specifically recognize the distinct amino-terminal-extended PTEN isoforms are necessary to monitor the expression and function of these longer PTEN forms during oncogenic processes. Alternative splicing of the unique PTEN precursor mRNA renders a PTEN isoform lacking the carboxy-terminal PTEN amino acids encoded in exon 9 (PTEN-Δ, PTEN 1-343-Ser; Sharrard and Maitland 2000). Distinct to other PTEN aberrant nonfunctional isoforms generated by alternative splicing (Agrawal et al. 2005; Agrawal and Eng 2006; Sarquis et al. 2006), PTEN-Δ may behave as a functional PTEN protein, although its specific involvement in human oncogenesis has not been disclosed (Breuksch et al. 2018). As mentioned above, most of the anti-PTEN mAbs currently used recognize carboxy-terminal PTEN epitopes (Table 2; Fig. 4; Mingo et al. 2019), which precludes the detection of PTEN-Δ isoforms using these reagents, regardless of PTEN mRNA translational initiation.

PTEN protein is heavily targeted by diverse regulatory posttranslational modifications, including phosphorylation, ubiquitination, sumoylation, and proteolysis, among others (Fig. 4B; Table 5; Aronchik et al. 2014; Correia et al. 2014; Xu et al. 2014; Kim et al. 2017; Lee et al. 2018). Phosphorylation of the PTEN carboxy-terminal region regulates PTEN protein stability, nuclear-cytoplasmic shuttling, and function. These modifications affect not only PTEN function, but also the antigenicity of the PTEN modified protein. For instance, the PTEN regulatory carboxy-terminal tail is proteolytically cleaved by caspase-3 during apoptosis (Torres et al. 2003; Singh et al. 2013), which generates PTEN truncated proteins with reduced stability and displaying enhanced membrane binding and nuclear accumulation (Georgescu et al. 1999; Gil et al. 2006), but lacking the immunodominant carboxy-terminal region. As occurs with the PTEN-Δ isoforms, this precludes the use of most of the current anti-PTEN mAbs to detect the PTEN caspase-3 cleaved forms. In addition, the PTEN carboxy-terminal tail is enriched in Ser/Thr residues that can be phosphorylated by multiple protein kinases, including CK2, CK1, GSK3β, and LKB1, among others (Fig. 4B; Table 5; Hopkins et al. 2014; Fragoso and Barata 2015; Lee et al. 2018). These phosphorylations regulate PTEN inter- and intramolecular interactions that result in the fine-tuning of PTEN subcellular location and activity in cells, which is highly relevant in the context of PTEN protein immunodetection in clinical samples. A model is proposed in which carboxy-terminal phosphorylated PTEN adopts a stable compact conformation with reduced catalytic activity and impaired membrane binding and nuclear accumulation (Vazquez et al. 2000, 2001; Gil et al. 2007; Odriozola et al. 2007; Rahdar et al. 2009; Bolduc et al. 2013; Chia et al. 2015; Masson et al. 2016). In line with this model, direct inhibition of CK2 has been proposed as a feasible approach to reconstitute PTEN function in human cancer (Shehata et al. 2010; Barata 2011). Our PTEN epitope mapping analysis suggests that most of PTEN carboxy-terminal phosphorylations do not affect the binding of anti-PTEN carboxy-terminal mAbs to PTEN. One

Table 5. PTEN amino acids posttranslationally modified

Amino acid[a]	Posttranslational modification	Reference[b]
Lys13	Ubiquitination	Trotman et al. 2007
Tyr27	Phosphorylation	Liu et al. 2014
Glu40	ADP-ribosylation	Li et al. 2015a
Lys66	Ubiquitination	Gupta and Leslie 2016
Cys71	Oxidation	Lee et al. 2002
	Alkylation	Covey et al. 2010
	Nitrosylation	Kwak et al. 2010
	Sulfhydration	Ohno et al. 2015
Cys83	Nitrosylation	Kwak et al. 2010
Ser113	Phosphorylation	Chen et al. 2015
Cys124	Oxidation	Lee et al. 2002
	Alkylation	Covey et al. 2010
	Nitrosylation	Kwak et al. 2010
	Sulfhydration	Ohno et al. 2015
Lys125, Lys128	Acetylation	Okumura et al. 2006
Glu150	ADP-ribosylation	Li et al. 2015a
Arg159	Methylation	Feng et al. 2019
Lys 163	Acetylation	Meng et al. 2016
Tyr174	Phosphorylation	Liu et al. 2014
Ser229, Thr232	Phosphorylation	Li et al. 2005
Tyr240	Phosphorylation	Fenton et al. 2012
Lys254, Lys266	Sumoylation	Huang et al. 2012
Lys289	Ubiquitination	Trotman et al. 2007
Asp301	Cleavage	Torres et al. 2003
Lys313	Methylation	Nakakido et al. 2015
Thr319, Thr 321	Phosphorylation	Li et al. 2005
Asp326	ADP-ribosylation	Li et al. 2015a
Lys327, Lys330	Ubiquitination	Lee et al. 2013
Tyr336	Phosphorylation	Yim et al. 2009
Lys 342, Lys344	Ubiquitination	Lee et al. 2013
Ser362, Thr366	Phosphorylation	Al-Khouri et al. 2005
Ser370	Phosphorylation	Torres and Pulido 2001
Asp371, Asp375	Cleavage	Torres et al. 2003
Ser380, Thr382 Thr383	Phosphorylation	Torres and Pulido 2001
Asp384	Cleavage	Torres et al. 2003
Ser385	Phosphorylation	Torres and Pulido 2001
Thr398	Phosphorylation	Bassi et al. 2013
Lys402	Acetylation	Ikenoue et al. 2008

[a]Amino acids are indicated by the three-letter code.

[b]The reference in which the posttranslational modification was first described (to the best of our knowledge) is indicated. Additional potential posttranslationally modified residues, as identified by proteomic studies, can be found at www.phosphosite.org.

exception is PTEN Thr398 phosphorylation by the ataxia telangiectasia-mutated (ATM) kinase during the DNA-damage response, a process that favors PTEN nuclear exclusion (Bassi et al. 2013). Phosphorylation of PTEN at Thr398 is likely to impede the binding of the anti-PTEN mAbs recognizing the more carboxy-terminally located PTEN epitopes (Mingo et al. 2019). Thus, in cells exposed to DNA-damaging chemotherapy drugs, the recognition of PTEN by some anti-PTEN carboxy-terminal mAbs could be reduced. Phospho-specific anti-PTEN mAbs

(antiphospho PTEN) targeting PTEN carboxy-terminal phosphorylated residues exist, including some mAbs suitable for IHC (Table 2), which could provide relevant complementary information to the PTEN protein expression status in clinical samples. For instance, a positive correlation was found in Kaposi's sarcoma tumor specimens between antiphospho PTEN (Ser380) and antiphospho Akt (Thr308) IHC staining, suggesting the Ser 380 phosphorylation-mediated inactivation of PTEN in these tumors (Roy and Dittmer 2011). However, the effective use of antiphospho PTEN mAbs in the clinical setting is still limited, mainly because of the multiplicity of clustered PTEN carboxy-terminal phosphorylation sites, which hampers the accurate detection of the PTEN phosphorylated residues, and to the complexity of PTEN phosphorylation/dephosphorylation events in different tissues. Together, these observations point out the necessity of validation of anti-PTEN mAbs recognizing non-carboxy-terminal PTEN regions, which could be used in PTEN immunodetection analyses in parallel with the currently used anti-PTEN mAbs (Andrés-Pons et al. 2005).

Regulated mono- and polyubiquitination occurs in a variety of PTEN Lys residues, controlling PTEN protein stability, dimerization, subcellular localization, and tumor-suppressive functions (Leslie et al. 2016; Lee et al. 2018, 2019). This may have an important impact in the clinic because pharmacological reactivation of PTEN in cancer treatment has been proposed using direct inhibitors of E3 ubiquitin ligases targeting PTEN for degradative or nondegradative ubiquitination (Aronchik et al. 2014; Lee et al. 2019). In addition, ubiquitination constitutes another important source of antigenic variability in several regions of PTEN protein, which should be considered in the case of anti-PTEN mAbs recognizing these amino acid regions. Finally, the amino-terminal extensions of PTEN long isoforms present potential post-translational modification motifs, including abundant phosphorylation and *O*-glycosylation motifs, and PTEN-L binds to the carbohydrate-binding protein concanavalin A (Hopkins et al. 2013; Malaney et al. 2013). It will be important to precisely characterize the posttranslational modifications of PTEN long isoforms, and relate these modifications with the function and antigenic properties of these PTEN proteoforms.

In addition to mutations causing PTEN protein loss (Fig. 1A), the *PTEN* gene is frequently targeted in tumors and PHTS patients by missense mutations resulting in single amino acid substitutions, which have variable consequences in PTEN protein stability, subcellular localization, and function (Rodríguez-Escudero et al. 2011; Gil et al. 2015; Leslie and Longy 2016). The distribution of PTEN somatic missense mutations along the PTEN protein is shown in Figure 4C, showing some of the hotspot mutations at the PTP domain. Note that the frequency of missense mutations in the distinct PTEN domains follows the pattern PTP domain >C2 domain >carboxy-terminal tail. These mutations have the potential to affect PTEN tumor-suppressor functions, and they may also introduce antigenic modifications in PTEN protein, which can affect the binding of anti-PTEN mAbs and the interpretation of PTEN immunodetection analyses, especially when the mutation has no clear pathologic effect. This is shown in Table 6 for mutations targeting the PTEN carboxy-terminal tail and the currently used anti-PTEN carboxyl terminus mAbs. As shown, most of the disease-associated mutations at the PTEN carboxyl terminus affect the binding of distinct anti-PTEN mAbs in concordance with their antigen specificity (Table 6; Fig. 4D), although these mutations were found not to affect the inhibitory function of PTEN on the PI3K/AKT pathway (Mingo et al. 2019). The low relative frequency of somatic or inherited mutations in the *PTEN* gene segment encoding the PTEN carboxy-terminal tail makes this region a good recognition target for reliable anti-PTEN mAbs in clinical practice, although the physiologic or pathologic conditions in which the PTEN carboxy-terminal epitopes are lost (including PTEN alternative splicing, caspase-3 cleavage, posttranslational modifications, and targeting by carboxy-terminal PTC mutations) needs to be addressed in the interpretation of the results.

Table 6. Reactivity of anti-PTEN mAbs with carboxy-terminal PTEN amino-acid substitution variants associated to disease

		mAb					
Mutation	Cancer type/disease	6H2.1	SP218	17.A	Y184	138G6	D4.3
WT		+	+	+	+	+	+
E388Q	Renal carcinoma[a]	+	+	+	+/−	+/−	+/−
P391L	PHTS[b]	+	+	+	+/−	+/−	+/−
P391H	PHTS[b]	+	+	+	+/−	+/−	+/−
P391S	ASD[c]	+	+	+	−	+	+/−
E394K	Urothelial carcinoma[d]	+	+/−	−	−	+/−	+/−
H397Y	Glioma[a]						
	Stomach carcinoma[a]	−	−	+/−	+	+	+
T398S	Glioma[a]						
	Ovarian carcinoma[a]	+	+	+/−	+	+	+
Q399H	Lung carcinoma[a]	+	+	−	+	+	+
I400V	PHTS[b]	+	+	+	+	+	+
T401I	Glioma[a]						
	Leiomyosarcoma[a]	+	−	+/−	+	+	+
K402E	PHTS[b]	+	+	−	+	+	+
K402N	PHTS[b]	+	+	−	+	+	+

Binding results are from Mingo et al. (2019) and our unpublished observations.
+, Binding equivalent to PTEN wild type (WT); +/−, diminished binding; –, no binding.
[a]COSMIC (Wellcome Trust Sanger Institute; Forbes et al. 2017).
[b]ClinVar (NCBI; Landrum et al. 2016).
[c]HGMD Professional (Stenson et al. 2014).
[d]cBioPortal (Cerami et al. 2012).

As a summary, when searching for optimal non-carboxy-terminal PTEN amino acid sequences as epitopic regions to obtain an anti-PTEN mAb, it would be ideal to choose PTEN protein peptides with low mutational load and posttranslational modifications. In this regard, the PTEN PTP domain is more heavily targeted by mutations than the C2 domain (Fig. 4C), which makes the PTEN C2 domain a good target for the development of clinically robust anti-PTEN mAbs. Moreover, the PTEN PTP domain displays 48% amino acid identity with the PTP domains from the testis-specific proteins TPTE and TPIP (Walker et al. 2001; Tapparel et al. 2003), which could favor cross-reactivity of mAbs raised against PTEN PTP domain peptides. The rational design of anti-PTEN mAbs recognizing PTEN precise epitopes, with potential use in clinical oncology, requires detailed knowledge about the antigenicity and specificity of the chosen immunogen peptide, its isoform-dependent location in the PTEN polypeptide chain, and its targeting by posttranslational modifications or mutations.

CONCLUDING REMARKS

The decrease in the amount of PTEN tumor-suppressor protein in tissues, independently of the acute functional regulation of its catalysis, is known to have pathological consequences. This makes the mAb-based determination of PTEN protein expression levels in patients and human tumors a relevant undertaking in clinical trials and routine clinical practice in the near future, including routine analytical IHC in clinical pathology. It would be important to set up a consensus on the use of specific anti-PTEN mAbs in clinical practice and how to score the expression and subcellular localization of PTEN protein in the analysis of tumor samples. The complexity in the physiologic regulation of PTEN function, including the abundance of PTEN posttranslational modifications, the existence of distinct

PTEN isoforms, and the high frequency of *PTEN* gene mutations in tumors, are issues that need to be considered in the interpretation of PTEN expression results obtained with specific anti-PTEN mAbs. The use in parallel of more than one anti-PTEN mAb, recognizing different PTEN epitopes, could help to obtain relevant information in this regard. An important requirement for the clinical validation of anti-PTEN mAbs, and their optimal use as prognostic and predictive informative tools in clinical oncology, is the precise definition of their reactivity in terms of epitope specificity and epitope recognition constraints. The availability of a well-defined panel of anti-PTEN mAbs recognizing different PTEN regions, suitable to monitor with accuracy the expression of PTEN proteins in biological samples and tumor tissue sections, will be helpful for the efficacy of precision cancer therapies dependent on PTEN tumor-suppressor function.

ACKNOWLEDGMENTS

This work was partially supported by Grants SAF2013-48812-R (to R.P.) and SAF2016-79847-R (to R.P. and J.I.L.) from Ministerio de Economía y Competitividad (Spain and Fondo Europeo de Desarrollo Regional), Grant 2013111011 from Gobierno Vasco, Departamento de Salud (Basque Country, Spain), and 239813 from The Norwegian Research Council, Norway (to C.E.N-X.). J.M. is the recipient of a predoctoral fellowship (PRE_2014_1_285) from Gobierno Vasco, Departamento de Educación (Basque Country, Spain). L.T. is the recipient of an oncology predoctoral fellowship from Asociación Española Contra el Cáncer (AECC, Junta Provincial de Bizkaia, Spain). We thank Ikerbasque, the Basque Foundation for Science, for their help and support.

REFERENCES

Ágoston EI, Micsik T, Ács B, Fekete K, Hahn O, Baranyai Z, Dede K, Bodoky G, Bursics A, Kulka J, et al. 2016. In depth evaluation of the prognostic and predictive utility of PTEN immunohistochemistry in colorectal carcinomas: Performance of three antibodies with emphasis on intracellular and intratumoral heterogeneity. *Diagn Pathol* **11:** 61. doi:10.1186/s13000-016-0508-0

Agrawal S, Eng C. 2006. Differential expression of novel naturally occurring splice variants of PTEN and their functional consequences in Cowden syndrome and sporadic breast cancer. *Hum Mol Genet* **15:** 777–787. doi:10.1093/hmg/ddi492

Agrawal S, Pilarski R, Eng C. 2005. Different splicing defects lead to differential effects downstream of the lipid and protein phosphatase activities of PTEN. *Hum Mol Genet* **14:** 2459–2468. doi:10.1093/hmg/ddi246

Ahearn TU, Pettersson A, Ebot EM, Gerke T, Graff RE, Morais CL, Hicks JL, Wilson KM, Rider JR, Sesso HD, et al. 2016. A prospective investigation of PTEN loss and ERG expression in lethal prostate cancer. *J Natl Cancer Inst* **108:** djv346. doi:10.1093/jnci/djv346

Al Bashir S, Alzoubi A, Alfaqih MA, Kheirallah K, Smairat A, Haddad H, Al-Dwairy A, Fawwaz BAB, Alzoubi M, Trpkov K. 2019. PTEN loss in a prostate cancer cohort from Jordan. *Appl Immunohistochem Mol Morphol* doi:10.1097/PAI.0000000000000732

Al-Khouri AM, Ma Y, Togo SH, Williams S, Mustelin T. 2005. Cooperative phosphorylation of the tumor suppressor phosphatase and tensin homologue (PTEN) by casein kinases and glycogen synthase kinase 3β. *J Biol Chem* **280:** 35195–35202. doi:10.1074/jbc.M503045200

Alvarez-Garcia V, Tawil Y, Wise HM, Leslie NR. 2019. Mechanisms of PTEN loss in cancer: It's all about diversity. *Semin Cancer Biol*. doi:10.1016/j.semcancer.2019.02.001

Alvarez-Nuñez F, Bussaglia E, Mauricio D, Ybarra J, Vilar M, Lerma E, de Leiva A, Matias-Guiu X, Thyroid Neoplasia Study Group. 2006. PTEN promoter methylation in sporadic thyroid carcinomas. *Thyroid* **16:** 17–23. doi:10.1089/thy.2006.16.17

Andrés-Pons A, Valiente M, Torres J, Gil A, Roglá I, Ripoll F, Cervera J, Pulido R. 2005. Functional definition of relevant epitopes on the tumor suppressor PTEN protein. *Cancer Lett* **223:** 303–312. doi:10.1016/j.canlet.2004.09.047

Antonarakis ES, Keizman D, Zhang Z, Gurel B, Lotan TL, Hicks JL, Fedor HL, Carducci MA, De Marzo AM, Eisenberger MA. 2012. An immunohistochemical signature comprising PTEN, MYC, and Ki67 predicts progression in prostate cancer patients receiving adjuvant docetaxel after prostatectomy. *Cancer* **118:** 6063–6071. doi:10.1002/cncr.27689

Aronchik I, Kundu A, Quirit JG, Firestone GL. 2014. The antiproliferative response of indole-3-carbinol in human melanoma cells is triggered by an interaction with NEDD4-1 and disruption of wild-type PTEN degradation. *Mol Cancer Res* **12:** 1621–1634. doi:10.1158/1541-7786.MCR-14-0018

Aslan O, Cremona M, Morgan C, Cheung LW, Mills GB, Hennessy BT. 2018. Preclinical evaluation and reverse phase protein array-based profiling of PI3K and MEK inhibitors in endometrial carcinoma in vitro. *BMC Cancer* **18:** 168. doi:10.1186/s12885-018-4035-0

Atreya CE, Sangale Z, Xu N, Matli MR, Tikishvili E, Welbourn W, Stone S, Shokat KM, Warren RS. 2013. PTEN expression is consistent in colorectal cancer primaries and metastases and associates with patient survival. *Cancer Med* **2:** 496–506. doi:10.1002/cam4.97

Bakarakos P, Theohari I, Nomikos A, Mylona E, Papadimitriou C, Dimopoulos AM, Nakopoulou L. 2010. Immunohistochemical study of PTEN and phosphorylated mTOR proteins in familial and sporadic invasive breast carcinomas. *Histopathology* **56:** 876–882. doi:10.1111/j.1365-2559.2010.03570.x

Bakkar RM, Xie SS, Urbauer DL, Djordjevic B, Vu K, Broaddus RR. 2015. Intact PTEN expression by immunohistochemistry is associated with decreased survival in advanced stage ovarian/primary peritoneal high-grade serous carcinoma. *Int J Gynecol Pathol* **34:** 497–506. doi:10.1097/PGP.0000000000000205

Ballester LY, Fuller GN, Powell SZ, Sulman EP, Patel KP, Luthra R, Routbort MJ. 2017. Retrospective analysis of molecular and immunohistochemical characterization of 381 primary brain tumors. *J Neuropathol Exp Neurol* **76:** 179–188.

Bamias A, Karina M, Papakostas P, Kostopoulos I, Bobos M, Vourli G, Samantas E, Christodoulou C, Pentheroudakis G, Pectasides D, et al. 2010. A randomized phase III study of adjuvant platinum/docetaxel chemotherapy with or without radiation therapy in patients with gastric cancer. *Cancer Chemother Pharmacol* **65:** 1009–1021. doi:10.1007/s00280-010-1256-6

Barata JT. 2011. The impact of PTEN regulation by CK2 on PI3K-dependent signaling and leukemia cell survival. *Adv Enzyme Regul* **51:** 37–49. doi:10.1016/j.advenzreg.2010.09.012

Bassi C, Stambolic V. 2013. PTEN, here, there, everywhere. *Cell Death Differ* **20:** 1595–1596. doi:10.1038/cdd.2013.127

Bassi C, Ho J, Srikumar T, Dowling RJ, Gorrini C, Miller SJ, Mak TW, Neel BG, Raught B, Stambolic V. 2013. Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. *Science* **341:** 395–399. doi:10.1126/science.1236188

Bassullu N, Turkmen I, Dayangac M, Yagiz Korkmaz P, Yasar R, Akyildiz M, Yaprak O, Tokat Y, Yuzer Y, Bulbul Dogusoy G. 2012. The predictive and prognostic significance of c-erb-B2, EGFR, PTEN, mTOR, PI3K, p27, and ERCC1 expression in hepatocellular carcinoma. *Hepat Mon* **12:** e7492. doi:10.5812/hepatmon.7492

Bazzichetto C, Conciatori F, Pallocca M, Falcone I, Fanciulli M, Cognetti F, Milella M, Ciuffreda L. 2019. PTEN as a prognostic/predictive biomarker in cancer: An unfulfilled promise? *Cancers* **11:** E435. doi:10.3390/cancers11040435

Bedolla R, Prihoda TJ, Kreisberg JI, Malik SN, Krishnegowda NK, Troyer DA, Ghosh PM. 2007. Determining risk of biochemical recurrence in prostate cancer by immunohistochemical detection of PTEN expression and Akt activation. *Clin Cancer Res* **13:** 3860–3867. doi:10.1158/1078-0432.CCR-07-0091

Beelen K, Opdam M, Severson TM, Koornstra RH, Vincent AD, Wesseling J, Muris JJ, Berns EM, Vermorken JB, van Diest PJ, et al. 2014. PIK3CA mutations, phosphatase and tensin homolog, human epidermal growth factor receptor 2, and insulin-like growth factor 1 receptor and adjuvant tamoxifen resistance in postmenopausal breast cancer patients. *Breast Cancer Res* **16:** R13. doi:10.1186/bcr3606

Beg S, Siraj AK, Jehan Z, Prabakaran S, Al-Sobhi SS, Al-Dawish M, Al-Dayel F, Al-Kuraya KS. 2015. PTEN loss is associated with follicular variant of Middle Eastern papillary thyroid carcinoma. *Br J Cancer* **112:** 1938–1943. doi:10.1038/bjc.2015.169

Berger AH, Pandolfi PP. 2011. Haplo-insufficiency: a driving force in cancer. *J Pathol* **223:** 137–146. doi:10.1002/path.2800

Bode AM, Dong Z. 2018. Recent advances in precision oncology research. *NPJ Precis Oncol* **2:** 11. doi:10.1038/s41698-018-0055-0

Bodo J, Hsi ED. 2011. Phosphoproteins and the dawn of functional phenotyping. *Pathobiology* **78:** 115–121. doi:10.1159/000296015

Boeck S, Jung A, Laubender RP, Neumann J, Egg R, Goritschan C, Vehling-Kaiser U, Winkelmann C, Fischer von Weikersthal L, Clemens MR, et al. 2013. EGFR pathway biomarkers in erlotinib-treated patients with advanced pancreatic cancer: Translational results from the randomised, crossover phase 3 trial AIO-PK0104. *Br J Cancer* **108:** 469–476. doi:10.1038/bjc.2012.495

Bolduc D, Rahdar M, Tu-Sekine B, Sivakumaren SC, Raben D, Amzel LM, Devreotes P, Gabelli SB, Cole P. 2013. Phosphorylation-mediated PTEN conformational closure and deactivation revealed with protein semisynthesis. *Elife* **2:** e00691. doi:10.7554/eLife.00691

Bononi A, Pinton P. 2015. Study of PTEN subcellular localization. *Methods* **77-78:** 92–103. doi:10.1016/j.ymeth.2014.10.002

Boosani CS, Agrawal DK. 2013. PTEN modulators: A patent review. *Expert Opin Ther Pat* **23:** 569–580. doi:10.1517/13543776.2013.768985

Breuksch I, Welter J, Bauer HK, Enklaar T, Frees S, Thüroff JW, Hasenburg A, Prawitt D, Brenner W. 2018. In renal cell carcinoma the PTEN splice variant PTEN-Δ shows similar function as the tumor suppressor PTEN itself. *Cell Commun Signal* **16:** 35. doi:10.1186/s12964-018-0247-9

Brown PD, Krishnan S, Sarkaria JN, Wu W, Jaeckle KA, Uhm JH, Geoffroy FJ, Arusell R, Kitange G, Jenkins RB, et al. 2008. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. *J Clin Oncol* **26:** 5603–5609. doi:10.1200/JCO.2008.18.0612

Bucheit AD, Chen G, Siroy A, Tetzlaff M, Broaddus R, Milton D, Fox P, Bassett R, Hwu P, Gershenwald JE, et al. 2014. Complete loss of PTEN protein expression correlates with shorter time to brain metastasis and survival in stage IIIB/C melanoma patients with BRAFV600 mutations. *Clin Cancer Res* **20:** 5527–5536. doi:10.1158/1078-0432.CCR-14-1027

Cai J, Xu L, Tang H, Yang Q, Yi X, Fang Y, Zhu Y, Wang Z. 2014. The role of the PTEN/PI3K/Akt pathway on prognosis in epithelial ovarian cancer: A meta-analysis. *Oncologist* **19:** 528–535. doi:10.1634/theoncologist.2013-0333

Calderaro J, Rebouissou S, de Koning L, Masmoudi A, Herault A, Dubois T, Maille P, Soyeux P, Sibony M, de la Taille A, et al. 2014. PI3K/AKT pathway activation in bladder carcinogenesis. *Int J Cancer* **134:** 1776–1784. doi:10.1002/ijc.28518

Cao Y, Wang H, Yang L, Zhang Z, Li C, Yuan X, Bu L, Chen L, Chen Y, Li CM, et al. 2018. PTEN-L promotes type I interferon responses and antiviral immunity. *Cell Mol Immunol* **15:** 48–57. doi:10.1038/cmi.2017.102

Capodanno A, Camerini A, Orlandini C, Baldini E, Resta ML, Bevilacqua G, Collecchi P. 2009. Dysregulated PI3K/Akt/PTEN pathway is a marker of a short disease-free survival in node-negative breast carcinoma. *Hum Pathol* **40:** 1408–1417. doi:10.1016/j.humpath.2009.02.005

Carneiro A, Barbosa ARG, Takemura LS, Kayano PP, Moran NKS, Chen CK, Wroclawski ML, Lemos GC, da Cunha IW, Obara MT, et al. 2018. The role of immunohistochemical analysis as a tool for the diagnosis, prognostic evaluation and treatment of prostate cancer: A systematic review of the literature. *Front Oncol* **8:** 377. doi:10.3389/fonc.2018.00377

Carvalho KC, Maia BM, Omae SV, Rocha AA, Covizzi LP, Vassallo J, Rocha RM, Soares FA. 2014. Best practice for PTEN gene and protein assessment in anatomic pathology. *Acta Histochem* **116:** 25–31. doi:10.1016/j.acthis.2013.04.013

Castillo-Martin M, Thin TH, Collazo Lorduy A, Cordon-Cardo C. 2016. Immunopathologic assessment of PTEN expression. *Methods Mol Biol* **1388:** 23–37. doi:10.1007/978-1-4939-3299-3_3

Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, et al. 2012. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov* **2:** 401–404. doi:10.1158/2159-8290.CD-12-0095

Chaux A, Peskoe SB, Gonzalez-Roibon N, Schultz L, Albadine R, Hicks J, De Marzo AM, Platz EA, Netto GJ. 2012a. Loss of PTEN expression is associated with increased risk of recurrence after prostatectomy for clinically localized prostate cancer. *Mod Pathol* **25:** 1543–1549. doi:10.1038/modpathol.2012.104

Chaux A, Schultz L, Albadine R, Hicks J, Kim JJ, Allaf ME, Carducci MA, Rodriguez R, Hammers HJ, Argani P, et al. 2012b. Immunoexpression status and prognostic value of mammalian target of rapamycin and hypoxia-induced pathway members in papillary cell renal cell carcinomas. *Hum Pathol* **43:** 2129–2137. doi:10.1016/j.humpath.2012.01.009

Chaux A, Albadine R, Schultz L, Hicks J, Carducci MA, Argani P, Allaf M, Netto GJ. 2013. Dysregulation of the mammalian target of rapamycin pathway in chromophobe renal cell carcinomas. *Hum Pathol* **44:** 2323–2330. doi:10.1016/j.humpath.2013.05.014

Chen J, Li T, Liu Q, Jiao H, Yang W, Liu X, Huo Z. 2014. Clinical and prognostic significance of HIF-1α, PTEN, CD44v6, and survivin for gastric cancer: A meta-analysis. *PLoS ONE* **9:** e91842. doi:10.1371/journal.pone.0091842

Chen JH, Zhang P, Chen WD, Li DD, Wu XQ, Deng R, Jiao L, Li X, Ji J, Feng GK, et al. 2015. ATM-mediated PTEN phosphorylation promotes PTEN nuclear translocation and autophagy in response to DNA-damaging agents in cancer cells. *Autophagy* **11:** 239–252. doi:10.1080/15548627.2015.1009767

Cheuk W, Chan JK. 2004. Subcellular localization of immunohistochemical signals: Knowledge of the ultrastructural or biologic features of the antigens helps predict the signal localization and proper interpretation of immunostains. *Int J Surg Pathol* **12:** 185–206. doi:10.1177/106689690401200301

Chia YC, Catimel B, Lio DS, Ang CS, Peng B, Wu H, Zhu HJ, Cheng HC. 2015. The C-terminal tail inhibitory phosphorylation sites of PTEN regulate its intrinsic catalytic activity and the kinetics of its binding to phosphatidylinositol-4,5-bisphosphate. *Arch Biochem Biophys* **587:** 48–60. doi:10.1016/j.abb.2015.10.004

Correia NC, Gírio A, Antunes I, Martins LR, Barata JT. 2014. The multiple layers of non-genetic regulation of PTEN tumour suppressor activity. *Eur J Cancer* **50:** 216–225. doi:10.1016/j.ejca.2013.08.017

Covey TM, Edes K, Coombs GS, Virshup DM, Fitzpatrick FA. 2010. Alkylation of the tumor suppressor PTEN activates Akt and β-catenin signaling: A mechanism linking inflammation and oxidative stress with cancer. *PLoS ONE* **5:** e13545. doi:10.1371/journal.pone.0013545

Cuzick J, Yang ZH, Fisher G, Tikishvili E, Stone S, Lanchbury JS, Camacho N, Merson S, Brewer D, Cooper CS, et al. 2013. Prognostic value of PTEN loss in men with conservatively managed localised prostate cancer. *Br J Cancer* **108:** 2582–2589. doi:10.1038/bjc.2013.248

Dave B, Migliaccio I, Gutierrez MC, Wu MF, Chamness GC, Wong H, Narasanna A, Chakrabarty A, Hilsenbeck SG, Huang J, et al. 2011. Loss of phosphatase and tensin homolog or phosphoinositol-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2-overexpressing locally advanced breast cancers. *J Clin Oncol* **29:** 166–173. doi:10.1200/JCO.2009.27.7814

Dedes KJ, Wetterskog D, Mendes-Pereira AM, Natrajan R, Lambros MB, Geyer FC, Vatcheva R, Savage K, Mackay A, Lord CJ, et al. 2010. PTEN deficiency in endometrioid endometrial adenocarcinomas predicts sensitivity to PARP inhibitors. *Sci Transl Med* **2:** 53ra75. doi:10.1126/scitranslmed.3001538

de Graeff P, Crijns AP, Ten Hoor KA, Klip HG, Hollema H, Oien K, Bartlett JM, Wisman GB, de Bock GH, de Vries EG, et al. 2008. The ErbB signalling pathway: Protein expression and prognostic value in epithelial ovarian cancer. *Br J Cancer* **99:** 341–349. doi:10.1038/sj.bjc.6604471

Depowski PL, Rosenthal SI, Ross JS. 2001. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. *Mod Pathol* **14:** 672–676. doi:10.1038/modpathol.3880371

Dillon LM, Miller TW. 2014. Therapeutic targeting of cancers with loss of PTEN function. *Curr Drug Targets* **15:** 65–79. doi:10.2174/1389450114666140106100909

Djordjevic B, Hennessy BT, Li J, Barkoh BA, Luthra R, Mills GB, Broaddus RR. 2012. Clinical assessment of PTEN loss in endometrial carcinoma: Immunohistochemistry outperforms gene sequencing. *Mod Pathol* **25:** 699–708. doi:10.1038/modpathol.2011.208

Duman BB, Sahin B, Acikalin A, Ergin M, Zorludemir S. 2013. PTEN, Akt, MAPK, p53 and p95 expression to predict trastuzumab resistance in HER2 positive breast cancer. *J BUON* **18:** 44–50.

Eijsink JJ, Noordhuis MG, ten Hoor KA, Kok M, Hollema H, de Bock GH, Nijman HW, Schuuring E, Wisman GB, van der Zee AG. 2010. The epidermal growth factor receptor pathway in relation to pelvic lymph node metastasis and survival in early-stage cervical cancer. *Hum Pathol* **41:** 1735–1741. doi:10.1016/j.humpath.2010.04.017

El-Mansi MT, Williams AR. 2006. Evaluation of *PTEN* expression in cervical adenocarcinoma by tissue microarray.

Int J Gynecol Cancer **16:** 1254–1260. doi:10.1136/ijgc-00009577-200605000-00046

Eritja N, Santacana M, Maiques O, Gonzalez-Tallada X, Dolcet X, Matias-Guiu X. 2015. Modeling glands with PTEN deficient cells and microscopic methods for assessing PTEN loss: Endometrial cancer as a model. *Methods* **77-78:** 31–40. doi:10.1016/j.ymeth.2014.11.001

Erkanli S, Kayaselcuk F, Kuscu E, Bagis T, Bolat F, Haberal A, Demirhan B. 2006. Expression of survivin, PTEN and p27 in normal, hyperplastic, and carcinomatous endometrium. *Int J Gynecol Cancer* **16:** 1412–1418. doi:10.1136/ijgc-00009577-200605000-00071

Esteva FJ, Guo H, Zhang S, Santa-Maria C, Stone S, Lanchbury JS, Sahin AA, Hortobagyi GN, Yu D. 2010. PTEN, PIK3CA, p-AKT, and p-p70S6K status: Association with trastuzumab response and survival in patients with HER2-positive metastatic breast cancer. *Am J Pathol* **177:** 1647–1656. doi:10.2353/ajpath.2010.090885

Fabi A, Metro G, Di Benedetto A, Nisticò C, Vici P, Melucci E, Antoniani B, Perracchio L, Sperduti I, Milella M, et al. 2010. Clinical significance of PTEN and p-Akt co-expression in HER2-positive metastatic breast cancer patients treated with trastuzumab-based therapies. *Oncology* **78:** 141–149. doi:10.1159/000312656

Feng J, Dang Y, Zhang W, Zhao X, Zhang C, Hou Z, Jin Y, McNutt MA, Marks AR, Yin Y. 2019. PTEN arginine methylation by PRMT6 suppresses PI3K-AKT signaling and modulates pre-mRNA splicing. *Proc Natl Acad Sci* **116:** 6868–6877. doi:10.1073/pnas.1811028116

Fenton TR, Nathanson D, Ponte de Albuquerque C, Kuga D, Iwanami A, Dang J, Yang H, Tanaka K, Oba-Shinjo SM, Uno M, et al. 2012. Resistance to EGF receptor inhibitors in glioblastoma mediated by phosphorylation of the PTEN tumor suppressor at tyrosine 240. *Proc Natl Acad Sci* **109:** 14164–14169. doi:10.1073/pnas.1211962109

Ferraldeschi R, Nava Rodrigues D, Riisnaes R, Miranda S, Figueiredo I, Rescigno P, Ravi P, Pezaro C, Omlin A, Lorente D, et al. 2015. PTEN protein loss and clinical outcome from castration-resistant prostate cancer treated with abiraterone acetate. *Eur Urol* **67:** 795–802. doi:10.1016/j.eururo.2014.10.027

Figlin RA, de Souza P, McDermott D, Dutcher JP, Berkenblit A, Thiele A, Krygowski M, Strahs A, Feingold J, Boni J, et al. 2009. Analysis of PTEN and HIF-1α and correlation with efficacy in patients with advanced renal cell carcinoma treated with temsirolimus versus interferon-α. *Cancer* **115:** 3651–3660. doi:10.1002/cncr.24438

Foo WC, Rashid A, Wang H, Katz MH, Lee JE, Pisters PW, Wolff RA, Abbruzzese JL, Fleming JB, Wang H. 2013. Loss of phosphatase and tensin homolog expression is associated with recurrence and poor prognosis in patients with pancreatic ductal adenocarcinoma. *Hum Pathol* **44:** 1024–1030. doi:10.1016/j.humpath.2012.09.001

Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, Cole CG, Ward S, Dawson E, Ponting L, et al. 2017. COSMIC: Somatic cancer genetics at high-resolution. *Nucleic Acids Res* **45:** D777–D783. doi:10.1093/nar/gkw1121

Fragoso R, Barata JT. 2015. Kinases, tails and more: Regulation of PTEN function by phosphorylation. *Methods* **77-78:** 75–81. doi:10.1016/j.ymeth.2014.10.015

Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, et al. 2013. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science Sign* **6:** pl1.

Gao T, Mei Y, Sun H, Nie Z, Liu X, Wang S. 2016. The association of phosphatase and tensin homolog (PTEN) deletion and prostate cancer risk: A meta-analysis. *Biomed Pharmacother* **83:** 114–121. doi:10.1016/j.biopha.2016.06.020

Garcia-Carracedo D, Turk AT, Fine SA, Akhavan N, Tweel BC, Parsons R, Chabot JA, Allendorf JD, Genkinger JM, Remotti HE, et al. 2013. Loss of PTEN expression is associated with poor prognosis in patients with intraductal papillary mucinous neoplasms of the pancreas. *Clin Cancer Res* **19:** 6830–6841. doi:10.1158/1078-0432.CCR-13-0624

Garg K, Broaddus RR, Soslow RA, Urbauer DL, Levine DA, Djordjevic B. 2012. Pathologic scoring of PTEN immunohistochemistry in endometrial carcinoma is highly reproducible. *Int J Gynecol Pathol* **31:** 48–56. doi:10.1097/PGP.0b013e3182230d00

Georgescu MM, Kirsch KH, Akagi T, Shishido T, Hanafusa H. 1999. The tumor-suppressor activity of PTEN is regulated by its carboxyl-terminal region. *Proc Natl Acad Sci* **96:** 10182–10187. doi:10.1073/pnas.96.18.10182

Gil A, Andrés-Pons A, Fernández E, Valiente M, Torres J, Cervera J, Pulido R. 2006. Nuclear localization of PTEN by a Ran-dependent mechanism enhances apoptosis: Involvement of an N-terminal nuclear localization domain and multiple nuclear exclusion motifs. *Mol Biol Cell* **17:** 4002–4013. doi:10.1091/mbc.e06-05-0380

Gil A, Andrés-Pons A, Pulido R. 2007. Nuclear PTEN: A tale of many tails. *Cell Death Differ* **14:** 395–399. doi:10.1038/sj.cdd.4402073

Gil A, Rodríguez-Escudero I, Stumpf M, Molina M, Cid VJ, Pulido R. 2015. A functional dissection of PTEN N-terminus: Implications in PTEN subcellular targeting and tumor suppressor activity. *PLoS ONE* **10:** e0119287. doi:10.1371/journal.pone.0119287

Gil A, López JI, Pulido R. 2016. Assessing PTEN subcellular localization. *Methods Mol Biol* **1388:** 169–186. doi:10.1007/978-1-4939-3299-3_12

Giles KM, Rosenbaum BE, Berger M, Izsak A, Li Y, Illa Bochaca I, Vega-Saenz de Miera E, Wang J, Darvishian F, Zhong H, et al. 2019. Revisiting the clinical and biologic relevance of partial PTEN loss in melanoma. *J Invest Dermatol* **139:** 430–438. doi:10.1016/j.jid.2018.07.031

Goel A, Arnold CN, Niedzwiecki D, Carethers JM, Dowell JM, Wasserman L, Compton C, Mayer RJ, Bertagnolli MM, Boland CR. 2004. Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. *Cancer Res* **64:** 3014–3021. doi:10.1158/0008-5472.CAN-2401-2

Gonzalez-Angulo AM, Ferrer-Lozano J, Stemke-Hale K, Sahin A, Liu S, Barrera JA, Burgues O, Lluch AM, Chen H, Hortobagyi GN, et al. 2011. PI3K pathway mutations and PTEN levels in primary and metastatic breast cancer. *Mol Cancer Ther* **10:** 1093–1101. doi:10.1158/1535-7163.MCT-10-1089

Gschwantler-Kaulich D, Tan YY, Fuchs EM, Hudelist G, Köstler WJ, Reiner A, Leser C, Salama M, Attems J, Deutschmann C, et al. 2017. PTEN expression as a predictor for the response to trastuzumab-based therapy in Her-2 overexpressing metastatic breast cancer. *PLoS ONE* **12:** e0172911. doi:10.1371/journal.pone.0172911

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Gu J, Ou W, Huang L, Wu J, Li S, Xu J, Feng J, Liu B, Zhou Y. 2016. PTEN expression is associated with the outcome of lung cancer: Evidence from a meta-analysis. *Minerva Med* **107:** 342–351.

Guedes LB, Tosoian JJ, Hicks J, Ross AE, Lotan TL. 2017. PTEN loss in Gleason score 3 + 4 = 7 prostate biopsies is associated with nonorgan confined disease at radical prostatectomy. *J Urol* **197:** 1054–1059. doi:10.1016/j.juro.2016.09.084

Guedes LB, Morais CL, Fedor H, Hicks J, Gurel B, Melamed J, Lee P, Gopalan A, Knudsen BS, True LD, et al. 2019. Effect of preanalytic variables on an automated PTEN immunohistochemistry assay for prostate cancer. *Arch Pathol Lab Med* **143:** 338–348. doi:10.5858/arpa.2018-0068-OA

Gumuskaya B, Gurel B, Fedor H, Tan HL, Weier CA, Hicks JL, Haffner MC, Lotan TL, De Marzo AM. 2013. Assessing the order of critical alterations in prostate cancer development and progression by IHC: Further evidence that PTEN loss occurs subsequent to ERG gene fusion. *Prostate Cancer Prostatic Dis* **16:** 209–215. doi:10.1038/pcan.2013.8

Gupta A, Leslie NR. 2016. Controlling PTEN (phosphatase and tensin homolog) stability: A dominant role for lysine 66. *J Biol Chem* **291:** 18465–18473. doi:10.1074/jbc.M116.727750

Halvorsen OJ, Haukaas SA, Akslen LA. 2003. Combined loss of PTEN and p27 expression is associated with tumor cell proliferation by Ki-67 and increased risk of recurrent disease in localized prostate cancer. *Clin Cancer Res* **9:** 1474–1479.

He L, Fan C, Gillis A, Feng X, Sanatani M, Hotte S, Kapoor A, Tang D. 2007. Co-existence of high levels of the PTEN protein with enhanced Akt activation in renal cell carcinoma. *Biochim Biophys Acta* **1772:** 1134–1142. doi:10.1016/j.bbadis.2007.07.001

Hlaing AM, Furusato B, Udo E, Kitamura Y, Souda M, Masutani M, Fukuoka J. 2018. Expression of phosphatase and tensin homolog and programmed cell death ligand 1 in adenosquamous carcinoma of the lung. *Biochem Biophys Res Commun* **503:** 2764–2769. doi:10.1016/j.bbrc.2018.08.037

Ho CM, Lin MC, Huang SH, Huang CJ, Lai HC, Chien TY, Chang SF. 2009. PTEN promoter methylation and LOH of 10q22-23 locus in PTEN expression of ovarian clear cell adenocarcinomas. *Gynecol Oncol* **112:** 307–313. doi:10.1016/j.ygyno.2008.09.040

Hocking C, Hardingham JE, Broadbridge V, Wrin J, Townsend AR, Tebbutt N, Cooper J, Ruszkiewicz A, Lee C, Price TJ. 2014. Can we accurately report PTEN status in advanced colorectal cancer? *BMC Cancer* **14:** 128. doi:10.1186/1471-2407-14-128

Hollander MC, Blumenthal GM, Dennis PA. 2011. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. *Nat Rev Cancer* **11:** 289–301. doi:10.1038/nrc3037

Hopkins BD, Parsons RE. 2014. Molecular pathways: Intercellular PTEN and the potential of PTEN restoration therapy. *Clin Cancer Res* **20:** 5379–5383. doi:10.1158/1078-0432.CCR-13-2661

Hopkins BD, Fine B, Steinbach N, Dendy M, Rapp Z, Shaw J, Pappas K, Yu JS, Hodakoski C, Mense S, et al. 2013. A secreted PTEN phosphatase that enters cells to alter signaling and survival. *Science* **341:** 399–402. doi:10.1126/science.1234907

Hopkins BD, Hodakoski C, Barrows D, Mense SM, Parsons RE. 2014. PTEN function: The long and the short of it. *Trends Biochem Sci* **39:** 183–190. doi:10.1016/j.tibs.2014.02.006

Huang J, Yan J, Zhang J, Zhu S, Wang Y, Shi T, Zhu C, Chen C, Liu X, Cheng J, et al. 2012. SUMO1 modification of PTEN regulates tumorigenesis by controlling its association with the plasma membrane. *Nat Commun* **3:** 911. doi:10.1038/ncomms1919

Idoate MA, Echeveste J, Diez-Valle R, Lozano MD, Aristu J. 2014. Biological and clinical significance of the intratumour heterogeneity of PTEN protein expression and the corresponding molecular abnormalities of the *PTEN* gene in glioblastomas. *Neuropathol Appl Neurobiol* **40:** 736–746. doi:10.1111/nan.12117

Ikenoue T, Inoki K, Zhao B, Guan KL. 2008. PTEN acetylation modulates its interaction with PDZ domain. *Cancer Res* **68:** 6908–6912. doi:10.1158/0008-5472.CAN-08-1107

Jamaspishvili T, Berman DM, Ross AE, Scher HI, De Marzo AM, Squire JA, Lotan TL. 2018. Clinical implications of PTEN loss in prostate cancer. *Nat Rev Urol* **15:** 222–234. doi:10.1038/nrurol.2018.9

Jang KS, Song YS, Jang SH, Min KW, Na W, Jang SM, Jun YJ, Lee KH, Choi D, Paik SS. 2010. Clinicopathological significance of nuclear PTEN expression in colorectal adenocarcinoma. *Histopathology* **56:** 229–239. doi:10.1111/j.1365-2559.2009.03468.x

Ji ZY, Li HF, Lei Y, Rao YW, Sun ML, Wang XW, Zhang YL. 2018. Phosphatase and tensin homolog protein may be linked to lymph node metastasis and tumor node metastasis staging in nonsmall cell lung cancer. *J Cancer Res Ther* **14:** S138–S144. doi:10.4103/0973-1482.165865

Jochner MCE, An J, Lättig-Tünnemann G, Kirchner M, Dagane A, Dittmar G, Dirnagl U, Eickholt BJ, Harms C. 2019. Unique properties of PTEN-L contribute to neuroprotection in response to ischemic-like stress. *Sci Rep* **9:** 3183. doi:10.1038/s41598-019-39438-1

Karapetis CS, Jonker D, Daneshmand M, Hanson JE, O'Callaghan CJ, Marginean C, Zalcberg JR, Simes J, Moore MJ, Tebbutt NC, et al. 2014. *PIK3CA*, *BRAF*, and PTEN status and benefit from cetuximab in the treatment of advanced colorectal cancer—Results from NCIC CTG/AGITG CO.17. *Clin Cancer Res* **20:** 744–753. doi:10.1158/1078-0432.CCR-13-0606

Kim B, Myung JK, Seo JH, Park CK, Paek SH, Kim DG, Jung HW, Park SH. 2010. The clinicopathologic values of the molecules associated with the main pathogenesis of the glioblastoma. *J Neurol Sci* **294:** 112–118. doi:10.1016/j.jns.2010.03.019

Kim SH, Kim SH, Joung JY, Lee GK, Hong EK, Kang KM, Yu A, Nam BH, Chung J, Seo HK, et al. 2015. Overexpression of ERG and wild-type PTEN are associated with favorable clinical prognosis and low biochemical recurrence in prostate cancer. *PLoS ONE* **10:** e0122498. doi:10.1371/journal.pone.0122498

Kim HS, Shin SJ, Beom SH, Jung M, Choi YY, Son T, Kim HI, Cheong JH, Hyung WJ, Noh SH, et al. 2016. Comprehensive expression profiles of gastric cancer molecular

subtypes by immunohistochemistry: Implications for individualized therapy. *Oncotarget* **7:** 44608–44620.

Kim DH, Suh J, Surh YJ, Na HK. 2017. Regulation of the tumor suppressor PTEN by natural anticancer compounds. *Ann NY Acad Sci* **1401:** 136–149. doi:10.1111/nyas.13422

Kimura F, Watanabe J, Hata H, Fujisawa T, Kamata Y, Nishimura Y, Jobo T, Kuramoto H. 2004. PTEN immunohistochemical expression is suppressed in G1 endometrioid adenocarcinoma of the uterine corpus. *J Cancer Res Clin Oncol* **130:** 161–168. doi:10.1007/s00432-003-0517-8

Kolasa IK, Rembiszewska A, Janiec-Jankowska A, Dansonka-Mieszkowska A, Lewandowska AM, Konopka B, Kupryjańczyk J. 2006. *PTEN* mutation, expression and LOH at its locus in ovarian carcinomas. Relation to *TP53*, *K-RAS* and *BRCA1* mutations. *Gynecol Oncol* **103:** 692–697. doi:10.1016/j.ygyno.2006.05.007

Kreis P, Leondaritis G, Lieberam I, Eickholt BJ. 2014. Subcellular targeting and dynamic regulation of PTEN: Implications for neuronal cells and neurological disorders. *Front Mol Neurosci* **7:** 23. doi:10.3389/fnmol.2014.00023

Kreisl TN, Lassman AB, Mischel PS, Rosen N, Scher HI, Teruya-Feldstein J, Shaffer D, Lis E, Abrey LE. 2009. A pilot study of everolimus and gefitinib in the treatment of recurrent glioblastoma (GBM). *J Neurooncol* **92:** 99–105. doi:10.1007/s11060-008-9741-z

Kurose K, Zhou XP, Araki T, Cannistra SA, Maher ER, Eng C. 2001. Frequent loss of PTEN expression is linked to elevated phosphorylated Akt levels, but not associated with p27 and cyclin D1 expression, in primary epithelial ovarian carcinomas. *Am J Pathol* **158:** 2097–2106. doi:10.1016/S0002-9440(10)64681-0

Kwak YD, Ma T, Diao S, Zhang X, Chen Y, Hsu J, Lipton SA, Masliah E, Xu H, Liao FF. 2010. NO signaling and S-nitrosylation regulate PTEN inhibition in neurodegeneration. *Mol Neurodegener* **5:** 49. doi:10.1186/1750-1326-5-49

Lade-Keller J, Riber-Hansen R, Guldberg P, Schmidt H, Hamilton-Dutoit SJ, Steiniche T. 2013. E- to N-cadherin switch in melanoma is associated with decreased expression of phosphatase and tensin homolog and cancer progression. *Br J Dermatol* **169:** 618–628. doi:10.1111/bjd.12426

Lahdensuo K, Erickson A, Saarinen I, Seikkula H, Lundin J, Lundin M, Nordling S, Butzow A, Vasarainen H, Bostrom PJ, et al. 2016. Loss of PTEN expression in ERG-negative prostate cancer predicts secondary therapies and leads to shorter disease-specific survival time after radical prostatectomy. *Mod Pathol* **29:** 1565–1574. doi:10.1038/modpathol.2016.154

Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Hoover J, et al. 2016. ClinVar: Public archive of interpretations of clinically relevant variants. *Nucleic Acids Res* **44:** D862–D868. doi:10.1093/nar/gkv1222

Lavorato-Rocha AM, Anjos LG, Cunha IW, Vassallo J, Soares FA, Rocha RM. 2015. Immunohistochemical assessment of PTEN in vulvar cancer: Best practices for tissue staining, evaluation, and clinical association. *Methods* **77–78:** 20–24. doi:10.1016/j.ymeth.2014.12.017

Lee YK, Park NH. 2009. Prognostic value and clinicopathological significance of p53 and PTEN in epithelial ovarian cancers. *Gynecol Oncol* **112:** 475–480. doi:10.1016/j.ygyno.2008.11.031

Lee SR, Yang KS, Kwon J, Lee C, Jeong W, Rhee SG. 2002. Reversible inactivation of the tumor suppressor PTEN by H_2O_2. *J Biol Chem* **277:** 20336–20342. doi:10.1074/jbc.M111899200

Lee JT, Shan J, Zhong J, Li M, Zhou B, Zhou A, Parsons R, Gu W. 2013. RFP-mediated ubiquitination of PTEN modulates its effect on AKT activation. *Cell Res* **23:** 552–564. doi:10.1038/cr.2013.27

Lee YR, Chen M, Pandolfi PP. 2018. The functions and regulation of the PTEN tumour suppressor: New modes and prospects. *Nat Rev Mol Cell Biol* **19:** 547–562.

Lee YR, Chen M, Lee JD, Zhang J, Lin SY, Fu TM, Chen H, Ishikawa T, Chiang SY, Katon J, et al. 2019. Reactivation of PTEN tumor suppressor for cancer treatment through inhibition of a MYC-WWP1 inhibitory pathway. *Science* **364:** eaau0159.

Leinonen KA, Saramaki OR, Furusato B, Kimura T, Takahashi H, Egawa S, Suzuki H, Keiger K, Ho Hahm S, Isaacs WB, et al. 2013. Loss of PTEN is associated with aggressive behavior in ERG-positive prostate cancer. *Cancer Epidemiol Biomarkers Prev* **22:** 2333–2344. doi:10.1158/1055-9965.EPI-13-0333-T

Leong TY, Cooper K, Leong AS. 2010. Immunohistology–past, present, and future. *Adv Anat Pathol* **17:** 404–418. doi:10.1097/PAP.0b013e3181f8957c

Leslie NR, Foti M. 2011. Non-genomic loss of PTEN function in cancer: Not in my genes. *Trends Pharmacol Sci* **32:** 131–140. doi:10.1016/j.tips.2010.12.005

Leslie NR, Longy M. 2016. Inherited *PTEN* mutations and the prediction of phenotype. *Semin Cell Dev Biol* **52:** 30–38. doi:10.1016/j.semcdb.2016.01.030

Leslie NR, Kriplani N, Hermida MA, Alvarez-Garcia V, Wise HM. 2016. The PTEN protein: Cellular localization and post-translational regulation. *Biochem Soc Trans* **44:** 273–278. doi:10.1042/BST20150224

Li DM, Sun H. 1997. TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor β. *Cancer Res* **57:** 2124–2129.

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, et al. 1997. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **275:** 1943–1947. doi:10.1126/science.275.5308.1943

Li Z, Dong X, Wang Z, Liu W, Deng N, Ding Y, Tang L, Hla T, Zeng R, Li L, et al. 2005. Regulation of PTEN by Rho small GTPases. *Nat Cell Biol* **7:** 399–404. doi:10.1038/ncb1236

Li N, Zhang Y, Han X, Liang K, Wang J, Feng L, Wang W, Songyang Z, Lin C, Yang L, et al. 2015a. Poly-ADP ribosylation of PTEN by tankyrases promotes PTEN degradation and tumor growth. *Genes Dev* **29:** 157–170. doi:10.1101/gad.251785.114

Li YY, Xiao R, Li CP, Huangfu J, Mao JF. 2015b. Increased plasma levels of FABP4 and PTEN is associated with more severe insulin resistance in women with gestational diabetes mellitus. *Med Sci Monit* **21:** 426–431. doi:10.12659/MSM.892431

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Li S, Shen Y, Wang M, Yang J, Lv M, Li P, Chen Z, Yang J. 2017. Loss of PTEN expression in breast cancer: Association with clinicopathological characteristics and prognosis. *Oncotarget* **8:** 32043–32054.

Li G, Yang J, Yang C, Zhu M, Jin Y, McNutt MA, Yin Y. 2018a. PTENα regulates mitophagy and maintains mitochondrial quality control. *Autophagy* **14:** 1742–1760. doi:10.1080/15548627.2018.1489477

Li W, Zhang T, Guo L, Huang L. 2018b. Regulation of PTEN expression by noncoding RNAs. *J Exp Clin Cancer Res* **37:** 223. doi:10.1186/s13046-018-0898-9

Liang H, He S, Yang J, Jia X, Wang P, Chen X, Zhang Z, Zou X, McNutt MA, Shen WH, et al. 2014. PTENα, a PTEN isoform translated through alternative initiation, regulates mitochondrial function and energy metabolism. *Cell Metab* **19:** 836–848. doi:10.1016/j.cmet.2014.03.023

Liang H, Chen X, Yin Q, Ruan D, Zhao X, Zhang C, McNutt MA, Yin Y. 2017. PTENβ is an alternatively translated isoform of PTEN that regulates rDNA transcription. *Nat Commun* **8:** 14771. doi:10.1038/ncomms14771

Lin PC, Lin JK, Lin HH, Lan YT, Lin CC, Yang SH, Chen WS, Liang WY, Jiang JK, Chang SC. 2015. A comprehensive analysis of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) loss in colorectal cancer. *World J Surg Oncol* **13:** 186. doi:10.1186/s12957-015-0601-y

Litlekalsoy J, Hostmark JG, Costea DE, Illemann M, Laerum OD. 2012. Time-dependent biological differences in molecular markers of high-grade urothelial cancer over 7 decades (ras proteins, pTEN, uPAR, PAI-1 and MMP-9). *Virchows Arch* **461:** 541–551. doi:10.1007/s00428-012-1323-y

Liu J, Visser-Grieve S, Boudreau J, Yeung B, Lo S, Chamberlain G, Yu F, Sun T, Papanicolaou T, Lam A, et al. 2014. Insulin activates the insulin receptor to downregulate the PTEN tumour suppressor. *Oncogene* **33:** 3878–3885. doi:10.1038/onc.2013.347

Loibl S, Darb-Esfahani S, Huober J, Klimowicz A, Furlanetto J, Lederer B, Hartmann A, Eidtmann H, Pfitzner B, Fasching PA, et al. 2016. Integrated analysis of PTEN and p4EBP1 protein expression as predictors for pCR in HER2-positive breast cancer. *Clin Cancer Res* **22:** 2675–2683. doi:10.1158/1078-0432.CCR-15-0965

Lokman U, Erickson AM, Vasarainen H, Rannikko AS, Mirtti T. 2018. PTEN loss but not ERG expression in diagnostic biopsies is associated with increased risk of progression and adverse surgical findings in men with prostate cancer on active surveillance. *Eur Urol Focus* **4:** 867–873. doi:10.1016/j.euf.2017.03.004

Lo Nigro C, Ricci V, Vivenza D, Granetto C, Fabozzi T, Miraglio E, Merlano MC. 2016. Prognostic and predictive biomarkers in metastatic colorectal cancer anti-EGFR therapy. *World J Gastroenterol* **22:** 6944–6954. doi:10.3748/wjg.v22.i30.6944

López JI, Angulo JC. 2018. Pathological bases and clinical impact of intratumor heterogeneity in clear cell renal cell carcinoma. *Curr Urol Rep* **19:** 3. doi:10.1007/s11934-018-0754-7

Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, Hicks JL, Park BH, Humphreys E, Partin AW, et al. 2011. PTEN protein loss by immunostaining: Analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clin Cancer Res* **17:** 6563–6573. doi:10.1158/1078-0432.CCR-11-1244

Lotan TL, Carvalho FL, Peskoe SB, Hicks JL, Good J, Fedor H, Humphreys E, Han M, Platz EA, Squire JA, et al. 2015. PTEN loss is associated with upgrading of prostate cancer from biopsy to radical prostatectomy. *Mod Pathol* **28:** 128–137. doi:10.1038/modpathol.2014.85

Lotan TL, Wei W, Ludkovski O, Morais CL, Guedes LB, Jamaspishvili T, Lopez K, Hawley ST, Feng Z, Fazli L, et al. 2016a. Analytic validation of a clinical-grade PTEN immunohistochemistry assay in prostate cancer by comparison with PTEN FISH. *Mod Pathol* **29:** 904–914. doi:10.1038/modpathol.2016.88

Lotan TL, Wei W, Morais CL, Hawley ST, Fazli L, Hurtado-Coll A, Troyer D, McKenney JK, Simko J, Carroll PR, et al. 2016b. PTEN loss as determined by clinical-grade immunohistochemistry assay is associated with worse recurrence-free survival in prostate cancer. *Eur Urol Focus* **2:** 180–188. doi:10.1016/j.euf.2015.07.005

Lotan TL, Heumann A, Rico SD, Hicks J, Lecksell K, Koop C, Sauter G, Schlomm T, Simon R. 2017. PTEN loss detection in prostate cancer: Comparison of PTEN immunohistochemistry and PTEN FISH in a large retrospective prostatectomy cohort. *Oncotarget* **8:** 65566–65576.

Loupakis F, Pollina L, Stasi I, Ruzzo A, Scartozzi M, Santini D, Masi G, Graziano F, Cremolini C, Rulli E, et al. 2009. PTEN expression and *KRAS* mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. *J Clin Oncol* **27:** 2622–2629. doi:10.1200/JCO.2008.20.2796

Lu YM, Cheng F, Teng LS. 2016. The association between phosphatase and tensin homolog hypermethylation and patients with breast cancer, a meta-analysis and literature review. *Sci Rep* **6:** 32723. doi:10.1038/srep32723

Lv S, Teugels E, Sadones J, De Brakeleer S, Duerinck J, Du Four S, Michotte A, De Grève J, Neyns B. 2012. Correlation of EGFR, IDH1 and PTEN status with the outcome of patients with recurrent glioblastoma treated in a phase II clinical trial with the EGFR-blocking monoclonal antibody cetuximab. *Int J Oncol* **41:** 1029–1035. doi:10.3892/ijo.2012.1539

Maiques O, Santacana M, Valls J, Pallares J, Mirantes C, Gatius S, Garcia Dios DA, Amant F, Pedersen HC, Dolcet X, et al. 2014. Optimal protocol for PTEN immunostaining; role of analytical and preanalytical variables in PTEN staining in normal and neoplastic endometrial, breast, and prostatic tissues. *Hum Pathol* **45:** 522–532. doi:10.1016/j.humpath.2013.10.018

Malaney P, Uversky VN, Davé V. 2013. The PTEN long N-tail is intrinsically disordered: Increased viability for PTEN therapy. *Mol Biosyst* **9:** 2877–2888. doi:10.1039/c3mb70267g

Malaney P, Uversky VN, Davé V. 2017. PTEN proteoforms in biology and disease. *Cell Mol Life Sci* **74:** 2783–2794. doi:10.1007/s00018-017-2500-6

Marsit CJ, Zheng S, Aldape K, Hinds PW, Nelson HH, Wiencke JK, Kelsey KT. 2005. PTEN expression in non-small-cell lung cancer: Evaluating its relation to tumor characteristics, allelic loss, and epigenetic alteration. *Hum Pathol* **36:** 768–776. doi:10.1016/j.humpath.2005.05.006

Martins FC, Santiago I, Trinh A, Xian J, Guo A, Sayal K, Jimenez-Linan M, Deen S, Driver K, Mack M, et al. 2014. Combined image and genomic analysis of high-grade serous ovarian cancer reveals PTEN loss as a common driver event and prognostic classifier. *Genome Biol* **15:** 526. doi:10.1186/s13059-014-0526-8

Masson GR, Perisic O, Burke JE, Williams RL. 2016. The intrinsically disordered tails of PTEN and PTEN-L have distinct roles in regulating substrate specificity and membrane activity. *Biochem J* **473:** 135–144. doi:10.1042/BJ20150931

Matos LL, Trufelli DC, de Matos MG, da Silva Pinhal MA. 2010. Immunohistochemistry as an important tool in biomarkers detection and clinical practice. *Biomark Insights* **5:** 9–20. doi:10.4137/BMI.S2185

McCabe N, Kennedy RD, Prise KM. 2016. The role of PTEN as a cancer biomarker. *Oncoscience* **3:** 54–55.

McEllin B, Camacho CV, Mukherjee B, Hahm B, Tomimatsu N, Bachoo RM, Burma S. 2010. PTEN loss compromises homologous recombination repair in astrocytes: Implications for glioblastoma therapy with temozolomide or poly(ADP-ribose) polymerase inhibitors. *Cancer Res* **70:** 5457–5464. doi:10.1158/0008-5472.CAN-09-4295

McGranahan N, Swanton C. 2015. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. *Cancer Cell* **27:** 15–26. doi:10.1016/j.ccell.2014.12.001

McLoughlin NM, Mueller C, Grossmann TN. 2018. The therapeutic potential of PTEN modulation: Targeting strategies from gene to protein. *Cell Chem Biol* **25:** 19–29.

Mehra R, Salami SS, Lonigro R, Bhalla R, Siddiqui J, Cao X, Spratt DE, Palapattu GS, Palanisamy N, Wei JT, et al. 2018. Association of ERG/PTEN status with biochemical recurrence after radical prostatectomy for clinically localized prostate cancer. *Med Oncol* **35:** 152. doi:10.1007/s12032-018-1212-6

Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ, et al. 2005. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* **353:** 2012–2024. doi:10.1056/NEJMoa051918

Mellinghoff IK, Cloughesy TF, Mischel PS. 2007. PTEN-mediated resistance to epidermal growth factor receptor kinase inhibitors. *Clin Cancer Res* **13:** 378–381. doi:10.1158/1078-0432.CCR-06-1992

Mendes-Pereira AM, Martin SA, Brough R, McCarthy A, Taylor JR, Kim JS, Waldman T, Lord CJ, Ashworth A. 2009. Synthetic lethal targeting of *PTEN* mutant cells with PARP inhibitors. *EMBO Mol Med* **1:** 315–322. doi:10.1002/emmm.200900041

Meng Z, Jia LF, Gan YH. 2016. PTEN activation through K163 acetylation by inhibiting HDAC6 contributes to tumour inhibition. *Oncogene* **35:** 2333–2344. doi:10.1038/onc.2015.293

Milella M, Falcone I, Conciatori F, Cesta Incani U, Del Curatolo A, Inzerilli N, Nuzzo CCMA, Vaccaro V, Vari S, Cognetti F, et al. 2015. PTEN: Multiple functions in human malignant tumors. *Front Oncol* **5:** 24.

Millis SZ, Ikeda S, Reddy S, Gatalica Z, Kurzrock R. 2016. Landscape of phosphatidylinositol-3-kinase pathway alterations across 19 784 diverse solid tumors. *JAMA Oncol* **2:** 1565–1573. doi:10.1001/jamaoncol.2016.0891

Min HS, Lee C, Jung KC. 2013. Correlation of immunohistochemical markers and BRAF mutation status with histological variants of papillary thyroid carcinoma in the Korean population. *J Korean Med Sci* **28:** 534–541. doi:10.3346/jkms.2013.28.4.534

Mingo J, Rodríguez-Escudero I, Luna S, Fernández-Acero T, Amo L, Jonasson AR, Zori RT, López JI, Molina M, Cid VJ, et al. 2018. A pathogenic role for germline PTEN variants which accumulate into the nucleus. *Eur J Hum Genet* **26:** 1180–1187. doi:10.1038/s41431-018-0155-x

Mingo J, Luna S, Gaafar A, Nunes-Xavier CE, Torices L, Mosteiro L, Ruiz R, Guerra I, Llarena R, Angulo JC, et al. 2019. Precise definition of PTEN C-terminal epitopes and its implications in clinical oncology. *NPJ Precis Oncol* **3:** 11. doi:10.1038/s41698-019-0083-4

Montano N, Cenci T, Martini M, D'Alessandris QG, Pelacchi F, Ricci-Vitiani L, Maira G, De Maria R, Larocca LM, Pallini R. 2011. Expression of EGFRvIII in glioblastoma: Prognostic significance revisited. *Neoplasia* **13:** 1113–1121. doi:10.1593/neo.111338

Montano N, D'Alessandris QG, Izzo A, Fernandez E, Pallini R. 2016. Biomarkers for glioblastoma multiforme: Status quo. *J Clin Transl Res* **2:** 3–10.

Monte NM, Webster KA, Neuberg D, Dressler GR, Mutter GL. 2010. Joint loss of PAX2 and PTEN expression in endometrial precancers and cancer. *Cancer Res* **70:** 6225–6232. doi:10.1158/0008-5472.CAN-10-0149

Mukherjee A, Patterson AL, George JW, Carpenter TJ, Madaj ZB, Hostetter G, Risinger JI, Teixeira JM. 2018. Nuclear PTEN localization contributes to DNA damage response in endometrial adenocarcinoma and could have a diagnostic benefit for therapeutic management of the disease. *Mol Cancer Ther* **17:** 1995–2003. doi:10.1158/1535-7163.MCT-17-1255

Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, Weng LP, Eng C. 2000. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. *J Natl Cancer Inst* **92:** 924–930. doi:10.1093/jnci/92.11.924

Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, et al. 2004. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* **6:** 117–127. doi:10.1016/j.ccr.2004.06.022

Nakakido M, Deng Z, Suzuki T, Dohmae N, Nakamura Y, Hamamoto R. 2015. Dysregulation of AKT pathway by SMYD2-mediated lysine methylation on PTEN. *Neoplasia* **17:** 367–373. doi:10.1016/j.neo.2015.03.002

Nassif NT, Lobo GP, Wu X, Henderson CJ, Morrison CD, Eng C, Jalaludin B, Segelov E. 2004. PTEN mutations are common in sporadic microsatellite stable colorectal cancer. *Oncogene* **23:** 617–628. doi:10.1038/sj.onc.1207059

Ngeow J, He X, Mester JL, Lei J, Romigh T, Orloff MS, Milas M, Eng C. 2012. Utility of PTEN protein dosage in predicting for underlying germline *PTEN* mutations among patients presenting with thyroid cancer and Cowden-like phenotypes. *J Clin Endocrinol Metab* **97:** E2320–E2327. doi:10.1210/jc.2012-2944

Norimatsu Y, Moriya T, Kobayashi TK, Sakurai T, Shimizu K, Tsukayama C, Ohno E. 2007. Immunohistochemical expression of PTEN and β-catenin for endometrial intraepi-

thelial neoplasia in Japanese women. *Ann Diagn Pathol* **11:** 103–108. doi:10.1016/j.anndiagpath.2006.06.009

Nosaka R, Yamasaki F, Saito T, Takayasu T, Kolakshyapati M, Amatya VJ, Takeshima Y, Sugiyama K, Kurisu K. 2017. Role for loss of nuclear PTEN in a harbinger of brain metastases. *J Clin Neurosci* **44:** 148–154. doi:10.1016/j.jocn.2017.06.004

Odriozola L, Singh G, Hoang T, Chan AM. 2007. Regulation of PTEN activity by its carboxyl-terminal autoinhibitory domain. *J Biol Chem* **282:** 23306–23315. doi:10.1074/jbc.M611240200

Ohno K, Okuda K, Uehara T. 2015. Endogenous S-sulfhydration of PTEN helps protect against modification by nitric oxide. *Biochem Biophys Res Commun* **456:** 245–249. doi:10.1016/j.bbrc.2014.11.066

Okumura K, Mendoza M, Bachoo RM, DePinho RA, Cavenee WK, Furnari FB. 2006. PCAF modulates PTEN activity. *J Biol Chem* **281:** 26562–26568. doi:10.1074/jbc.M605391200

Pallares J, Bussaglia E, Martínez-Guitarte JL, Dolcet X, Llobet D, Rue M, Sanchez-Verde L, Palacios J, Prat J, Matias-Guiu X. 2005. Immunohistochemical analysis of PTEN in endometrial carcinoma: A tissue microarray study with a comparison of four commercial antibodies in correlation with molecular abnormalities. *Mod Pathol* **18:** 719–727. doi:10.1038/modpathol.3800347

Panigrahi AR, Pinder SE, Chan SY, Paish EC, Robertson JF, Ellis IO. 2004. The role of PTEN and its signalling pathways, including AKT, in breast cancer; an assessment of relationships with other prognostic factors and with outcome. *J Pathol* **204:** 93–100. doi:10.1002/path.1611

Papa A, Pandolfi PP. 2019. The PTEN–PI3K axis in cancer. *Biomolecules* **9:** 153. doi:10.3390/biom9040153

Perez EA, Dueck AC, McCullough AE, Chen B, Geiger XJ, Jenkins RB, Lingle WL, Davidson NE, Martino S, Kaufman PA, et al. 2013. Impact of PTEN protein expression on benefit from adjuvant trastuzumab in early-stage human epidermal growth factor receptor 2-positive breast cancer in the North Central Cancer Treatment Group N9831 trial. *J Clin Oncol* **31:** 2115–2122. doi:10.1200/JCO.2012.42.2642

Perren A, Weng LP, Boag AH, Ziebold U, Thakore K, Dahia PL, Komminoth P, Lees JA, Mulligan LM, Mutter GL, et al. 1999. Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. *Am J Pathol* **155:** 1253–1260. doi:10.1016/S0002-9440(10)65227-3

Perren A, Komminoth P, Saremaslani P, Matter C, Feurer S, Lees JA, Heitz PU, Eng C. 2000. Mutation and expression analyses reveal differential subcellular compartmentalization of PTEN in endocrine pancreatic tumors compared to normal islet cells. *Am J Pathol* **157:** 1097–1103. doi:10.1016/S0002-9440(10)64624-X

Pulido R. 2015. PTEN: A yin-yang master regulator protein in health and disease. *Methods* **77-78:** 3–10. doi:10.1016/j.ymeth.2015.02.009

Pulido R, Baker SJ, Barata JT, Carracedo A, Cid VJ, Chin-Sang ID, Dave V, den Hertog J, Devreotes P, Eickholt BJ, et al. 2014. A unified nomenclature and amino acid numbering for human PTEN. *Sci Signal* **7:** pe15. doi:10.1126/scisignal.2005560

Que WC, Qiu HQ, Cheng Y, Liu MB, Wu CY. 2018. PTEN in kidney cancer: A review and meta-analysis. *Clin Chim Acta* **480:** 92–98. doi:10.1016/j.cca.2018.01.031

Raffone A, Travaglino A, Saccone G, Campanino MR, Mollo A, De Placido G, Insabato L, Zullo F. 2019a. Loss of PTEN expression as diagnostic marker of endometrial precancer: A systematic review and meta-analysis. *Acta Obstet Gynecol Scand* **98:** 275–286. doi:10.1111/aogs.13513

Raffone A, Travaglino A, Saccone G, Viggiani M, Giampaolino P, Insabato L, Mollo A, De Placido G, Zullo F. 2019b. PTEN expression in endometrial hyperplasia and risk of cancer: A systematic review and meta-analysis. *Arch Gynecol Obstet* **299:** 1511–1524. doi:10.1007/s00404-019-05123-x

Rahdar M, Inoue T, Meyer T, Zhang J, Vazquez F, Devreotes PN. 2009. A phosphorylation-dependent intramolecular interaction regulates the membrane association and activity of the tumor suppressor PTEN. *Proc Natl Acad Sci* **106:** 480–485. doi:10.1073/pnas.0811212106

Razavi SA, Modarressi MH, Yaghmaei P, Tavangar SM, Hedayati M. 2017. Circulating levels of PTEN and KLLN in papillary thyroid carcinoma: Can they be considered as novel diagnostic biomarkers? *Endocrine* **57:** 428–435. doi:10.1007/s12020-017-1368-4

Razis E, Bobos M, Kotoula V, Eleftheraki AG, Kalofonos HP, Pavlakis K, Papakostas P, Aravantinos G, Rigakos G, Efstratiou I, et al. 2011. Evaluation of the association of PIK3CA mutations and PTEN loss with efficacy of trastuzumab therapy in metastatic breast cancer. *Breast Cancer Res Treat* **128:** 447–456. doi:10.1007/s10549-011-1572-5

Rieken M, Shariat SF, Karam JA, Foerster B, Khani F, Gust K, Abufaraj M, Wood CG, Weizer AZ, Raman JD, et al. 2017. Frequency and prognostic value of PTEN loss in patients with upper tract urothelial carcinoma treated with radical nephroureterectomy. *J Urol* **198:** 1269–1277. doi:10.1016/j.juro.2017.06.096

Rieth J, Subramanian S. 2018. Mechanisms of intrinsic tumor resistance to immunotherapy. *Int J Mol Sci* **19:** E1340. doi:10.3390/ijms19051340

Rimawi MF, De Angelis C, Contreras A, Pareja F, Geyer FC, Burke KA, Herrera S, Wang T, Mayer IA, Forero A, et al. 2018. Low PTEN levels and *PIK3CA* mutations predict resistance to neoadjuvant lapatinib and trastuzumab without chemotherapy in patients with HER2 over-expressing breast cancer. *Breast Cancer Res Treat* **167:** 731–740. doi:10.1007/s10549-017-4533-9

Rodríguez-Escudero I, Oliver MD, Andrés-Pons A, Molina M, Cid VJ, Pulido R. 2011. A comprehensive functional analysis of *PTEN* mutations: Implications in tumor- and autism-related syndromes. *Hum Mol Genet* **20:** 4132–4142. doi:10.1093/hmg/ddr337

Roh MH, Yassin Y, Miron A, Mehra KK, Mehrad M, Monte NM, Mutter GL, Nucci MR, Ning G, McKeon FD, et al. 2010. High-grade fimbrial-ovarian carcinomas are unified by altered p53, PTEN and PAX2 expression. *Mod Pathol* **23:** 1316–1324. doi:10.1038/modpathol.2010.119

Roy D, Dittmer DP. 2011. Phosphatase and tensin homolog on chromosome 10 is phosphorylated in primary effusion lymphoma and Kaposi's sarcoma. *Am J Pathol* **179:** 2108–2119. doi:10.1016/j.ajpath.2011.06.017

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Sakr RA, Barbashina V, Morrogh M, Chandarlapaty S, Andrade VP, Arroyo CD, Olvera N, King TA. 2010. Protocol for PTEN expression by immunohistochemistry in formalin-fixed paraffin-embedded human breast carcinoma. *Appl Immunohistochem Mol Morphol* **18:** 371–374. doi:10.1097/PAI.0b013e3181d50bd5

Salgado R, Moore H, Martens JWM, Lively T, Malik S, McDermott U, Michiels S, Moscow JA, Tejpar S, McKee T, et al. 2017. Societal challenges of precision medicine: Bringing order to chaos. *Eur J Cancer* **84:** 325–334. doi:10.1016/j.ejca.2017.07.028

Sangale Z, Prass C, Carlson A, Tikishvili E, Degrado J, Lanchbury J, Stone S. 2011. A robust immunohistochemical assay for detecting PTEN expression in human tumors. *Appl Immunohistochem Mol Morphol* **19:** 173–183. doi:10.1097/PAI.0b013e3181f1da13

Sarquis MS, Agrawal S, Shen L, Pilarski R, Zhou XP, Eng C. 2006. Distinct expression profiles for PTEN transcript and its splice variants in Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome. *Am J Hum Genet* **79:** 23–30. doi:10.1086/504392

Sawai H, Yasuda A, Ochi N, Ma J, Matsuo Y, Wakasugi T, Takahashi H, Funahashi H, Sato M, Takeyama H. 2008. Loss of PTEN expression is associated with colorectal cancer liver metastasis and poor patient survival. *BMC Gastroenterol* **8:** 56. doi:10.1186/1471-230X-8-56

Schmidt KT, Chau CH, Price DK, Figg WD. 2016. Precision oncology medicine: The clinical relevance of patient-specific biomarkers used to optimize cancer treatment. *J Clin Pharmacol* **56:** 1484–1499. doi:10.1002/jcph.765

Schultz L, Albadine R, Hicks J, Jadallah S, DeMarzo AM, Chen YB, Nielsen ME, Gonzalgo ML, Sidransky D, Schoenberg M, et al. 2010. Expression status and prognostic significance of mammalian target of rapamycin pathway members in urothelial carcinoma of urinary bladder after cystectomy. *Cancer* **116:** 5517–5526. doi:10.1002/cncr.25502

Sharrard RM, Maitland NJ. 2000. Alternative splicing of the human *PTEN/MMAC1/TEP1* gene. *Biochim Biophys Acta* **1494:** 282–285. doi:10.1016/S0167-4781(00)00210-4

Shehata M, Schnabl S, Demirtas D, Hilgarth M, Hubmann R, Ponath E, Badrnya S, Lehner C, Hoelbl A, Duechler M, et al. 2010. Reconstitution of PTEN activity by CK2 inhibitors and interference with the PI3-K/Akt cascade counteract the antiapoptotic effect of human stromal cells in chronic lymphocytic leukemia. *Blood* **116:** 2513–2521. doi:10.1182/blood-2009-10-248054

Shoman N, Klassen S, McFadden A, Bickis MG, Torlakovic E, Chibbar R. 2005. Reduced PTEN expression predicts relapse in patients with breast carcinoma treated by tamoxifen. *Mod Pathol* **18:** 250–259. doi:10.1038/modpathol.3800296

Siddiqui S, Akhter N, Deo SV, Shukla NK, Husain SA. 2016. A study on promoter methylation of PTEN in sporadic breast cancer patients from North India. *Breast Cancer* **23:** 922–931. doi:10.1007/s12282-015-0665-0

Singh M, Chaudhry P, Fabi F, Asselin E. 2013. Cisplatin-induced caspase activation mediates PTEN cleavage in ovarian cancer cells: A potential mechanism of chemoresistance. *BMC Cancer* **13:** 233. doi:10.1186/1471-2407-13-233

Sood A, McClain D, Maitra R, Basu-Mallick A, Seetharam R, Kaubisch A, Rajdev L, Mariadason JM, Tanaka K, Goel S. 2012. PTEN gene expression and mutations in the PIK3CA gene as predictors of clinical benefit to anti-epidermal growth factor receptor antibody therapy in patients with KRAS wild-type metastatic colorectal cancer. *Clin Colorectal Cancer* **11:** 143–150. doi:10.1016/j.clcc.2011.12.001

Sperinde J, Jin X, Banerjee J, Penuel E, Saha A, Diedrich G, Huang W, Leitzel K, Weidler J, Ali SM, et al. 2010. Quantitation of p95HER2 in paraffin sections by using a p95-specific antibody and correlation with outcome in a cohort of trastuzumab-treated breast cancer patients. *Clin Cancer Res* **16:** 4226–4235. doi:10.1158/1078-0432.CCR-10-0410

Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, et al. 1997. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* **15:** 356–362. doi:10.1038/ng0497-356

Steelman LS, Navolanic PM, Sokolosky ML, Taylor JR, Lehmann BD, Chappell WH, Abrams SL, Wong EW, Stadelman KM, Terrian DM, et al. 2008. Suppression of PTEN function increases breast cancer chemotherapeutic drug resistance while conferring sensitivity to mTOR inhibitors. *Oncogene* **27:** 4086–4095. doi:10.1038/onc.2008.49

Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, et al. 2008. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* **68:** 6084–6091. doi:10.1158/0008-5472.CAN-07-6854

Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. 2014. The Human Gene Mutation Database: Building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet* **133:** 1–9. doi:10.1007/s00439-013-1358-4

Su R, Nan H, Guo H, Ruan Z, Jiang L, Song Y, Nan K. 2016. Associations of components of PTEN/AKT/mTOR pathway with cancer stem cell markers and prognostic value of these biomarkers in hepatocellular carcinoma. *Hepatol Res* **46:** 1380–1391. doi:10.1111/hepr.12687

Tan H, Bao J, Zhou X. 2015. Genome-wide mutational spectra analysis reveals significant cancer-specific heterogeneity. *Sci Rep* **5:** 12566. doi:10.1038/srep12566

Tang L, Li X, Gao Y, Chen L, Gu L, Chen J, Lyu X, Zhang Y, Zhang X. 2017. Phosphatase and tensin homolog (PTEN) expression on oncologic outcome in renal cell carcinoma: A systematic review and meta-analysis. *PLoS ONE* **12:** e0179437. doi:10.1371/journal.pone.0179437

Tapparel C, Reymond A, Girardet C, Guillou L, Lyle R, Lamon C, Hutter P, Antonarakis SE. 2003. The TPTE gene family: Cellular expression, subcellular localization and alternative splicing. *Gene* **323:** 189–199. doi:10.1016/j.gene.2003.09.038

Taulli R, Loretelli C, Pandolfi PP. 2013. From pseudo-ceRNAs to circ-ceRNAs: A tale of cross-talk and competition. *Nat Struct Mol Biol* **20:** 541–543. doi:10.1038/nsmb.2580

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org

Teng HW, Wang HW, Chen WM, Chao TC, Hsieh YY, Hsih CH, Tzeng CH, Chen PC, Yen CC. 2011. Prevalence and prognostic influence of genomic changes of EGFR pathway markers in synovial sarcoma. *J Surg Oncol* **103:** 773–781. doi:10.1002/jso.21852

Therkildsen C, Bergmann TK, Henrichsen-Schnack T, Ladelund S, Nilbert M. 2014. The predictive value of *KRAS*, *NRAS*, *BRAF*, *PIK3CA* and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. *Acta Oncol* **53:** 852–864. doi:10.3109/0284186X.2014.895036

Thiessen B, Stewart C, Tsao M, Kamel-Reid S, Schaiquevich P, Mason W, Easaw J, Belanger K, Forsyth P, McIntosh L, et al. 2010. A phase I/II trial of GW572016 (lapatinib) in recurrent glioblastoma multiforme: Clinical outcomes, pharmacokinetics and molecular correlation. *Cancer Chemother Pharmacol* **65:** 353–361. doi:10.1007/s00280-009-1041-6

Tinker AV, Ellard S, Welch S, Moens F, Allo G, Tsao MS, Squire J, Tu D, Eisenhauer EA, MacKay H. 2013. Phase II study of temsirolimus (CCI-779) in women with recurrent, unresectable, locally advanced or metastatic carcinoma of the cervix. A trial of the NCIC Clinical Trials Group (NCIC CTG IND 199). *Gynecol Oncol* **130:** 269–274. doi:10.1016/j.ygyno.2013.05.008

Torres J, Pulido R. 2001. The tumor suppressor PTEN is phosphorylated by the protein kinase CK2 at its C terminus. Implications for PTEN stability to proteasome-mediated degradation. *J Biol Chem* **276:** 993–998. doi:10.1074/jbc.M009134200

Torres J, Navarro S, Roglá I, Ripoll F, Lluch A, Garcia-Conde J, Llombart-Bosch A, Cervera J, Pulido R. 2001. Heterogeneous lack of expression of the tumour suppressor PTEN protein in human neoplastic tissues. *Eur J Cancer* **37:** 114–121. doi:10.1016/S0959-8049(00)00366-X

Torres J, Rodriguez J, Myers MP, Valiente M, Graves JD, Tonks NK, Pulido R. 2003. Phosphorylation-regulated cleavage of the tumor suppressor PTEN by caspase-3: Implications for the control of protein stability and PTEN–protein interactions. *J Biol Chem* **278:** 30652–30660. doi:10.1074/jbc.M212610200

Tran TN, Brettingham-Moore K, Duong CP, Mitchell C, Clemons NJ, Phillips WA. 2013. Molecular changes in the phosphatidylinositide 3-kinase (PI3K) pathway are common in gastric cancer. *J Surg Oncol* **108:** 113–120. doi:10.1002/jso.23357

Trock BJ, Fedor H, Gurel B, Jenkins RB, Knudsen BS, Fine SW, Said JW, Carter HB, Lotan TL, De Marzo AM. 2016. PTEN loss and chromosome 8 alterations in Gleason grade 3 prostate cancer cores predicts the presence of un-sampled grade 4 tumor: Implications for active surveillance. *Mod Pathol* **29:** 764–771. doi:10.1038/modpathol.2016.63

Trotman LC, Wang X, Alimonti A, Chen Z, Teruya-Feldstein J, Yang H, Pavletich NP, Carver BS, Cordon-Cardo C, Erdjument-Bromage H, et al. 2007. Ubiquitination regulates PTEN nuclear import and tumor suppression. *Cell* **128:** 141–156. doi:10.1016/j.cell.2006.11.040

Twomey JD, Brahme NN, Zhang B. 2017. Drug-biomarker co-development in oncology—20 years and counting. *Drug Resist Updat* **30:** 48–62. doi:10.1016/j.drup.2017.02.002

Tzani I, Ivanov IP, Andreev DE, Dmitriev RI, Dean KA, Baranov PV, Atkins JF, Loughran G. 2016. Systematic analysis of the *PTEN* 5′ leader identifies a major AUU initiated proteoform. *Open Biol* **6:** 150203. doi:10.1098/rsob.150203

Ueno S, Sudo T, Oka N, Wakahashi S, Yamaguchi S, Fujiwara K, Mikami Y, Nishimura R. 2013. Absence of human papillomavirus infection and activation of PI3K-AKT pathway in cervical clear cell carcinoma. *Int J Gynecol Cancer* **23:** 1084–1091. doi:10.1097/IGC.0b013e3182981bdc

Valkov A, Kilvaer TK, Sorbye SW, Donnem T, Smeland E, Bremnes RM, Busund LT. 2011. The prognostic impact of Akt isoforms, PI3K and PTEN related to female steroid hormone receptors in soft tissue sarcomas. *J Transl Med* **9:** 200. doi:10.1186/1479-5876-9-200

Vazquez F, Devreotes P. 2006. Regulation of PTEN function as a PIP3 gatekeeper through membrane interaction. *Cell Cycle* **5:** 1523–1527. doi:10.4161/cc.5.14.3005

Vazquez F, Ramaswamy S, Nakamura N, Sellers WR. 2000. Phosphorylation of the PTEN tail regulates protein stability and function. *Mol Cell Biol* **20:** 5010–5018. doi:10.1128/MCB.20.14.5010-5018.2000

Vazquez F, Grossman SR, Takahashi Y, Rokas MV, Nakamura N, Sellers WR. 2001. Phosphorylation of the PTEN tail acts as an inhibitory switch by preventing its recruitment into a protein complex. *J Biol Chem* **276:** 48627–48630. doi:10.1074/jbc.C100556200

Vázquez-Ulloa E, Lizano M, Avilés-Salas A, Alfaro-Moreno E, Contreras-Paredes A. 2011. Abnormal distribution of hDlg and PTEN in premalignant lesions and invasive cervical cancer. *Gynecol Oncol* **122:** 663–668. doi:10.1016/j.ygyno.2011.05.017

Verhagen PC, van Duijn PW, Hermans KG, Looijenga LH, van Gurp RJ, Stoop H, van der Kwast TH, Trapman J. 2006. The *PTEN* gene in locally progressive prostate cancer is preferentially inactivated by bi-allelic gene deletion. *J Pathol* **208:** 699–707. doi:10.1002/path.1929

Walker SM, Downes CP, Leslie NR. 2001. TPIP: A novel phosphoinositide 3-phosphatase. *Biochem J* **360:** 277–283. doi:10.1042/bj3600277

Wang Y, Dai B. 2015. PTEN genomic deletion defines favorable prognostic biomarkers in localized prostate cancer: A systematic review and meta-analysis. *Int J Clin Exp Med* **8:** 5430–5437.

Wang Y, Kristensen GB, Helland A, Nesland JM, Børresen-Dale AL, Holm R. 2005. Protein expression and prognostic value of genes in the erb-b signaling pathway in advanced ovarian carcinomas. *Am J Clin Pathol* **124:** 392–401. doi:10.1309/BL7EMW66LQX6GFRP

Wang Y, Liu Y, Du Y, Yin W, Lu J. 2013. The predictive role of phosphatase and tensin homolog (PTEN) loss, phosphoinositol-3 (PI3) kinase (PIK3CA) mutation, and PI3K pathway activation in sensitivity to trastuzumab in HER2-positive breast cancer: A meta-analysis. *Curr Med Res Opin* **29:** 633–642. doi:10.1185/03007995.2013.794775

Wang H, Zhang P, Lin C, Yu Q, Wu J, Wang L, Cui Y, Wang K, Gao Z, Li H. 2015a. Relevance and therapeutic possibility of PTEN-long in renal cell carcinoma. *PLoS ONE* **10:** e114250. doi:10.1371/journal.pone.0114250

Wang J, Chen H, Liao Y, Chen N, Liu T, Zhang H, Zhang H. 2015b. Expression and clinical evidence of miR-494 and PTEN in non–small cell lung cancer. *Tumour Biol* **36:** 6965–6972. doi:10.1007/s13277-015-3416-0

Wang Y, Qu X, Shen HC, Wang K, Liu Q, Du JJ. 2015c. Predictive and prognostic biomarkers for patients treated with anti-EGFR agents in lung cancer: A systemic review and meta-analysis. *Asian Pac J Cancer Prev* **16:** 4759–4768. doi:10.7314/APJCP.2015.16.11.4759

Wang LL, Hao S, Zhang S, Guo LJ, Hu CY, Zhang G, Gao B, Zhao JJ, Jiang Y, Tian WG, et al. 2017. PTEN/PI3K/AKT protein expression is related to clinicopathological features and prognosis in breast cancer with axillary lymph node metastases. *Hum Pathol* **61:** 49–57. doi:10.1016/j.humpath.2016.07.040

Wang L, Cho YL, Tang Y, Wang J, Park JE, Wu Y, Wang C, Tong Y, Chawla R, Zhang J, et al. 2018. PTEN-L is a novel protein phosphatase for ubiquitin dephosphorylation to inhibit PINK1-Parkin-mediated mitophagy. *Cell Res* **28:** 787–802. doi:10.1038/s41422-018-0056-0

Westin SN, Ju Z, Broaddus RR, Krakstad C, Li J, Pal N, Lu KH, Coleman RL, Hennessy BT, Klempner SJ, et al. 2015. PTEN loss is a context-dependent outcome determinant in obese and non-obese endometrioid endometrial cancer patients. *Mol Oncol* **9:** 1694–1703. doi:10.1016/j.molonc.2015.04.014

Wiegand KC, Hennessy BT, Leung S, Wang Y, Ju Z, McGahren M, Kalloger SE, Finlayson S, Stemke-Hale K, Lu Y, et al. 2014. A functional proteogenomic analysis of endometrioid and clear cell carcinomas using reverse phase protein array and mutation analysis: protein expression is histotype-specific and loss of ARID1A/BAF250a is associated with AKT phosphorylation. *BMC Cancer* **14:** 120. doi:10.1186/1471-2407-14-120

Wise HM, Hermida MA, Leslie NR. 2017. Prostate cancer, PI3K, PTEN and prognosis. *Clin Sci* **131:** 197–210. doi:10.1042/CS20160026

Woolley JF, Salmena L. 2016. Measurement of PTEN by flow cytometry. *Methods Mol Biol* **1388:** 39–51. doi:10.1007/978-1-4939-3299-3_4

Worby CA, Dixon JE. 2014. Pten. *Annu Rev Biochem* **83:** 641–669. doi:10.1146/annurev-biochem-082411-113907

Wu J, Song Y. 2018. Expression and clinical significance of serum MMP-7 and PTEN levels in patients with acute myeloid leukemia. *Oncol Lett* **15:** 3447–3452.

Xiao J, Hu CP, He BX, Chen X, Lu XX, Xie MX, Li W, He SY, You SJ, Chen Q. 2016. PTEN expression is a prognostic marker for patients with non–small cell lung cancer: A systematic review and meta-analysis of the literature. *Oncotarget* **7:** 57832–57840.

Xie H, Xie B, Liu C, Wang J, Xu Y. 2017. Association of PTEN expression with biochemical recurrence in prostate cancer: Results based on previous reports. *OncoTargets Ther* **10:** 5089–5097. doi:10.2147/OTT.S132653

Xu W, Yang Z, Zhou SF, Lu N. 2014. Posttranslational regulation of phosphatase and tensin homolog (PTEN) and its functional impact on cancer behaviors. *Drug Design Dev Ther* **8:** 1745–1751. doi:10.2147/DDDT.S71061

Xu F, Zhang C, Cui J, Liu J, Li J, Jiang H. 2017. The prognostic value and potential drug target of phosphatase and tensin homolog in breast cancer patients: A meta-analysis. *Medicine* **96:** e8000. doi:10.1097/MD.0000000000008000

Yanagawa N, Leduc C, Kohler D, Saieg MA, John T, Sykes J, Yoshimoto M, Pintilie M, Squire J, Shepherd FA, et al. 2012. Loss of phosphatase and tensin homolog protein expression is an independent poor prognostic marker in lung adenocarcinoma. *J Thorac Oncol* **7:** 1513–1521. doi:10.1097/JTO.0b013e3182641d4f

Yang J, Liu J, Zheng J, Du W, He Y, Liu W, Huang S. 2007. A reappraisal by quantitative flow cytometry analysis of PTEN expression in acute leukemia. *Leukemia* **21:** 2072–2074. doi:10.1038/sj.leu.2404740

Yang W, Soares J, Greninger P, Edelman EJ, Lightfoot H, Forbes S, Bindal N, Beare D, Smith JA, Thompson IR, et al. 2013. Genomics of drug sensitivity in cancer (GDSC): A resource for therapeutic biomarker discovery in cancer cells. *Nucleic Acids Res* **41:** D955–D961. doi:10.1093/nar/gks1111

Yang ZY, Yu YY, Yuan JQ, Shen WX, Zheng DY, Chen JZ, Mao C, Tang JL. 2016. The prognostic value of phosphatase and tensin homolog negativity in breast cancer: A systematic review and meta-analysis of 32 studies with 4393 patients. *Crit Rev Oncol Hematol* **101:** 40–49. doi:10.1016/j.critrevonc.2016.01.013

Yehia L, Eng C. 2018. 65 years of the double helix: one gene, many endocrine and metabolic syndromes: PTEN-opathies and precision medicine. *Endocr Relat Cancer* **25:** T121–T140. doi:10.1530/ERC-18-0162

Yim EK, Peng G, Dai H, Hu R, Li K, Lu Y, Mills GB, Meric-Bernstam F, Hennessy BT, Craven RJ, et al. 2009. Rak functions as a tumor suppressor by regulating PTEN protein stability and function. *Cancer Cell* **15:** 304–314. doi:10.1016/j.ccr.2009.02.012

Yonemori K, Tsuta K, Shimizu C, Hatanaka Y, Hashizume K, Ono M, Kouno T, Ando M, Tamura K, Katsumata N, et al. 2009. Immunohistochemical expression of PTEN and phosphorylated Akt are not correlated with clinical outcome in breast cancer patients treated with trastuzumab-containing neo-adjuvant chemotherapy. *Med Oncol* **26:** 344–349. doi:10.1007/s12032-008-9127-2

Yun JW, Lee S, Ryu D, Park S, Park WY, Joung JG, Jeong J. 2019. Biomarkers associated with tumor heterogeneity in prostate cancer. *Transl Oncol* **12:** 43–48. doi:10.1016/j.tranon.2018.09.003

Zaldumbide L, Erramuzpe A, Guarch R, Pulido R, Cortes JM, Lopez JI. 2016. Snail heterogeneity in clear cell renal cell carcinoma. *BMC Cancer* **16:** 194. doi:10.1186/s12885-016-2237-x

Zhang X, Soori G, Dobleman TJ, Xiao GG. 2014. The application of monoclonal antibodies in cancer diagnosis. *Exp Rev Mol Diagn* **14:** 97–106. doi:10.1586/14737159.2014.866039

Zhang C, Guo Y, Li J, Tian X, Duan X. 2019. The role of the phosphatase and tensin homolog status in predicting pathological complete response to neoadjuvant anti-HER2 therapies in HER2-positive primary breast cancer: A meta-analysis. *Medicine* **98:** e14261. doi:10.1097/MD.0000000000014261

Zhao Y, Zheng R, Li J, Lin F, Liu L. 2017. Loss of phosphatase and tensin homolog expression correlates with clinicopathological features of non-small cell lung cancer pa-

Cold Spring Harbor Perspectives in Medicine
www.perspectivesinmedicine.org
CSH PERSPECTIVES

tients and its impact on survival: A systematic review and meta-analysis. *Thorac Cancer* **8:** 203–213. doi:10.1111/1759-7714.12425

Zhou J, Li X. 2018. Association of PTEN expression with liver function and inflammatory changes in patients with liver cancer after chemotherapy. *Oncol Lett* **16:** 6633–6637.

Zhou XP, Gimm O, Hampel H, Niemann T, Walker MJ, Eng C. 2000. Epigenetic PTEN silencing in malignant melanomas without *PTEN* mutation. *Am J Pathol* **157:** 1123–1128. doi:10.1016/S0002-9440(10)64627-5

Zhou XP, Loukola A, Salovaara R, Nystrom-Lahti M, Peltomäki P, de la Chapelle A, Aaltonen LA, Eng C. 2002. PTEN mutational spectra, expression levels, and subcellular localization in microsatellite stable and unstable colorectal cancers. *Am J Pathol* **161:** 439–447. doi:10.1016/S0002-9440(10)64200-9