Precise Immunodetection of PTEN Protein in Human Neoplasia

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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

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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 iso-

form 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-

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Table 1. Global clinical significance of PTEN loss

Cancer type	Clinical significance of PTEN loss	Reference
Endometrial	<i>Low diagnostic accuracy in EH^a</i>	Raffone et al. 2019a
	Association with increased risk of EC in EH	Raffone et al. 2019b
Glioblastoma	<i>Prognostic value only in 30% of studies</i>	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
	<i>No predictive or prognostic value</i>	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	<i>No prognostic value</i>	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.

^aText 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 rele-

vance. 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

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; glioblastoma, 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

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oncogenesis, may also have clinical relevance, which is revealed by IHC (Gil et al. 2015; No-saka 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.

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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	IgG	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.

^aPTEN amino acid numbering is indicated according to NP_000305.3.^bPrecise epitope mapping is provided, indicating the residues encompassing the epitope, according to Mingo et al. (2019) and our unpublished results.^cThe 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.^dThe anti-PTEN 28H6 mAb only stains nuclear PTEN in tissues.^eThese 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

mAb	Prostate (<i>n</i> = 81)		Bladder (<i>n</i> = 49)	
	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, $\times 200$.

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-

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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
Glioblastoma	28H6	21	—	Pallares et al. 2005
	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	—	Ly et al. 2012
	6H2.1	27	↓ PFS combined with p27 loss	Halvorsen et al. 2003
Prostate	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

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Table 4. Continued

Cancer type	Antibody	Frequency of PTEN protein loss (%) ^a	Clinical associations of PTEN protein loss	Reference
Lung	28H6	41	↓ PFS combined with ERG–	Kim et al. 2015
	6H2.1	74	↑ <i>Well- versus poorly differentiated; Stages I and II versus III and IV</i>	Marsit et al. 2005
	138G6	41	↑ LSCC versus LAC; ↓ PFS in LAC	Yanagawa et al. 2012
	D4.3	64	↑ LSCC versus LAC; associated with smoking	
	G-6	39	↓ <i>Well- versus poorly differentiated; stages I-II versus III-IV</i> ; ↓ OS	Hlaing et al. 2018 Wang et al. 2015b
Melanoma	6H2.1	65	—	Zhou et al. 2000
	6H2.1	44	↑ BM and ↓ OS in <i>BRAF</i> ^{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
Soft tissue	28H6	70	↓ CIN versus ICC	Vázquez-Ulloa et al. 2011
	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; ↑ <i>response to lapatinib</i> (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	↓ <i>Resistance to TZMB</i>	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

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Table 4. *Continued*

Cancer type	Antibody	Frequency of PTEN protein loss (%) ^a	Clinical associations of PTEN protein loss	Reference
Ovarian	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
	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
Liver	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
	138G6	54	↑ Liver function grading	Zhou and Li 2018
	28H6	56	—	Bassullu et al. 2012
	A2B1	46	↓ PFS; ↓ OS	Su et al. 2016
	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
Colorectal	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
	D4.3	35	↑ Pathologic features	Schultz et al. 2010
	D4.3	7.5	↑ Pathologic features; ↑ LNM; ↓ OS	Rieken et al. 2017
	A2B1	52	—	Littlekalsoy 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
	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
	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.

^aPTEN loss includes partial loss (weak or focal expression) or total loss (absence of expression) of PTEN protein detection.

^bText 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.

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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.

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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, GSK3 β , 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

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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-Khoury 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

^aAmino acids are indicated by the three-letter code.^bThe 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 im-

portant 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.

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

Mutation	Cancer type/disease	mAb					
		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.

^aCOSMIC (Wellcome Trust Sanger Institute; Forbes et al. 2017).^bClinVar (NCBI; Landrum et al. 2016).^cHGMD Professional (Stenson et al. 2014).^dcBioPortal (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.

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