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Uterine PEComas: Correlation Between Melanocytic Marker Expression and *TSC* Alterations/*TFE3* Fusions

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Abstract

Uterine PEComas often present a diagnostic challenge as they share morphological and immunohistochemical features with smooth muscle tumors. Herein we evaluated a series of 19 uterine PEComas to compare the degree of melanocytic marker expression with their molecular profile. Patients ranged from 32 to 77 (median 48) years, with six tumors classified as malignant based on the modified gynecologic-specific prognostic algorithm. All patients with malignant PEComas were alive with disease or dead from disease at last follow-up, while all those of uncertain malignant potential were alive and well (median follow-up, 47 months).

Seventeen of 19 (89%) PEComas harbored either a *TSC1* or *TSC2* alteration. One of the two remaining tumors showed a *TFE3* rearrangement, but the other lacked alterations in all genes evaluated. All showed at least focal (usually strong) positivity for HMB-45, with 15/19 (79%) having > 50% expression, while the tumor lacking *TSC* or *TFE3* alterations was strongly positive in 10% of cells. Melan-A and MiTF were each positive in 15/19 (79%) tumors, but staining extent and intensity was much more variable than HMB-45. Five of six (83%) malignant PEComas also harbored alterations in *TP53, ATRX,* or *RB1*, findings not identified in any tumors of uncertain malignant potential. One malignant PEComa was microsatellite-unstable/mismatch repair protein-deficient.

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AUTHOR CONTRIBUTION STATEMENT

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ETHICS APPROVAL/CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Boards at the individual institutions.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its supplementary information files.

CONFLICT OF INTEREST STATEMENT

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

In summary, *TSC* alterations/*TFE3* fusions and diffuse (> 50%) HMB-45 expression are characteristic of uterine PEComas. In morphologically ambiguous mesenchymal neoplasms with myomelanocytic differentiation, especially those with metastatic or recurrent disease, next-generation sequencing is recommended to evaluate for *TSC* alterations, as such patients can be eligible for targeted therapy.

Keywords

PEComa; Perivascular Epithelioid Cell Tumor; Uterus; TSC; TFE3; HMB-45

INTRODUCTION

Uterine perivascular epithelioid cell tumors (PEComas) are diagnostically challenging mesenchymal neoplasms as their morphology often overlaps with smooth muscle tumors[1–5]. PEComas are characterized by the coexpression of melanocytic and smooth muscle markers, but a small subset of smooth muscle tumors may also be positive for the former, in particular, HMB-45[6–8]. Novel immunostains such as cathepsin K and PNL2 were originally speculated to aid in this diagnostic issue, but have since been found to be positive in smooth muscle tumors[2,3,9]. Recently, a study on uterine sarcomas with myomelanocytic differentiation identified four subgroups of tumors based on integration of morphological, immunohistochemical, and molecular data—malignant PEComa, sarcoma with PEComa-like features, myogenic sarcoma, and sarcoma NOS[4]. *TSC2* alterations or *TFE3* fusions were detected in 6/7 (86%) primary or recurrent tumors in the first two groups, and absent in the latter two categories (n=8). However, one malignant PEComa, as well as one of the myogenic sarcomas, lacked *TSC2* or *TFE3* alterations, but showed ≥ 50% expression for HMB-45 and positivity for MiTF; thus, it is difficult to explain their classification within the respective categories. Herein we correlate the molecular findings and degree of melanocytic marker expression in a series of 19 uterine tumors previously diagnosed as PEComa[3].

MATERIALS AND METHODS

Next-Generation Sequencing/TFE3 Fusion Status:

Formalin-fixed paraffin-embedded sections (FFPE) (n=22) or sequencing data (n=1) were available for 23/32 (72%) tumors from the prior study[3]. For the former, genomic DNA was isolated from macro-dissected sections using the QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Extraction was successful in 18/22 (82%) tumors while the remaining four failed to meet quality control measures and were excluded from analysis. Next-generation sequencing was performed using the targeted, hybrid capture 1,213-gene OncoPlus panel at the University of Chicago, as previously described[10,11]. Somatic mutation calling was performed across all 1,213 genes using a custom in-house bioinformatics pipeline as previously described[10]. Variant review was performed by two authors with specific expertise in this area (Z.O., L.R.) and included filters based on population variant frequencies (Exome Aggregation Consortium, http://exac.broadinstitute.org/), variant frequencies in cancer databases (COSMIC: catalogue of somatic mutations in cancer https://cancer.sanger.ac.uk/cosmic and cBioPortal https://

www.cbioportal.org/), and coding effects. Somatic variant calls were inspected using Integrated Genomics Viewer (IGV; Broad Institute, MIT Harvard, Cambridge, MA). *TFE3* fusions were previously assessed by fluorescence in-situ hybridization[3].

Melanocytic Marker Expression:

Immunohistochemistry for HMB-45, Melan-A, and MiTF was previously performed in tumors from our study[3]. Tumors were scored for both extent (percentage of positive cells) and intensity (1+ weak, 2+ moderate, 3+ strong) of each melanocytic marker. Stains were considered positive if nuclear (MiTF) or cytoplasmic (HMB-45, Melan-A) expression was noted.

Microsatellite Instability/Mismatch Repair Protein Immunohistochemical Testing:

As part of the OncoPlus panel, a microsatellite instability (MSI) detection module using data from 336 incidentally captured homopolymers across the 1,213 captured genes was utilized as previously described[12]. Due to the impact of pre-analytic factors on the MSI-calling module and the inclusion of many FFPE blocks in this study that were > 5 years old, we required that > 90% of microsatellite loci reach the minimum sequencing depth threshold (50X coverage) for quality control and accurate MSI calling. Tumors with < 9% unstable loci were classified as microsatellite-stable, 9 to 15% as indeterminate, and > 15% as unstable. Immunohistochemistry for MSH6 and PMS2 was performed on all tumors classified as indeterminate or unstable, as well as in those without available microsatellite information. PEComas were considered mismatch repair protein-deficient if there was absence of nuclear staining in tumor cells for either MSH6 (rabbit monoclonal EPR3945, dilution 1:200; Abcam, Cambridge, MA) or PMS2 (rabbit monoclonal EPR3945, dilution 1:50; Abcam, Cambridge, MA). For all other staining patterns, the tumor was considered mismatch repair protein-proficient.

RESULTS

Clinical and Morphological Features:

Patients ranged from 32 to 77 (mean 50, median 48) years and tumors from 1 to 17 (mean 7, median 6; unknown in two) cm. Two patients had a clinical diagnosis of tuberous sclerosis. Two other patients had a history of renal angiomyolipoma or pulmonary lymphangioleiomyomatosis but never underwent further clinical or genetic evaluation. The remaining patients either had no other signs suggestive of tuberous sclerosis (n=13) or additional clinical information was not available (n=2). All tumors showed morphological features of PEComas (Supplemental File 1) and were classified as such in our prior study[3]. Based on the modified gynecologic-specific prognostic algorithm where at least three atypical features (size ≥ 5 cm, necrosis, high nuclear grade, mitoses > 1 per 50 high-power fields, vascular invasion) are required for a diagnosis of malignancy[2,3,13], 13/19 (68%) PEComas were classified as uncertain malignant potential and 6/19 (32%) as malignant (Table 1). Recurrences occurred in 6/18 (33%; unknown in one) patients with three experiencing multiple recurrences (two malignant, one uncertain malignant potential). Follow-up was available for all patients, ranging from 5 to 175 (mean 51, median 47) months, with 13/19 (68%) alive and well, 3/19 (16%) alive with disease, and 3/19 (16%)

dead of disease. All patients with a diagnosis of malignant PEComa were either alive with disease or dead of disease at last follow-up, while all with PEComas of uncertain malignant potential were alive and well; however, one patient in the latter category experienced multiple recurrences.

Next-Generation Sequencing:

Seventeen of 19 (89%) PEComas harbored either a *TSC1* or *TSC2* alteration (Figure 1a). *TSC1* alterations were detected in 9/19 (47%) tumors and included pathogenic/likely pathogenic mutations in eight and a rearrangement predicted to cause loss of function in one, with three of them also showing copy number losses. *TSC2* alterations were noted in 8/19 (42%) PEComas and included pathogenic/likely pathogenic mutations in four (with one also showing an additional variant of uncertain significance), variants of uncertain clinical significance in two, a rearrangement predicted to cause loss of function in one (also with additional copy number loss), and only copy number loss in one. For the remaining two tumors, one had a *TFE3* fusion and the other did not harbor alterations in any genes examined. *TSC1, TSC2,* and *TFE3* alterations were mutually exclusive.

ATRX and/or *TP53* mutations were detected in 4/6 (67%) malignant PEComas but were absent in all those classified as uncertain malignant potential. Additional non-recurrent pathogenic/likely pathogenic mutations included *TERT* (1/18; 6%), *ADGVR1* (1/18; 6%), *FZR1* (1/18; 6%), *KMT2D* (1/19; 5%), and *SUFU* (1/19; 5%). All variants are summarized in supplemental file 2.

Copy number losses were noted in 7/19 (37%) tumors (Figure 1b). Recurrent ones included the previously mentioned losses in *TSC1* (9q34.13) (3/19; 16%) and *TSC2* (16p13.3) (2/19; 11%), as well as *FANCA* (16q24.3) (2/19; 11%), *FGFR3* (4p16.3) (2/19; 11%), *NOTCH1* (9q34.3) (2/19; 11%), and *MYCN* (2p24.3) (2/19; 11%). Similar to the *ATRX* and *TP53* mutations described above, deletion of *TP53* (17p13.1) (case 9b) and *RB1* (13q14.2) (case 16) were only observed in malignant PEComas. Overall, 5/6 (83%) malignant PEComas harbored alterations in *TP53, ATRX,* or *RB1.*

Correlation Between Melanocytic Marker Expression and Molecular Results:

All tumors, regardless of their *TSC* alteration status, showed at least focal positivity (≥ 10%) for HMB-45. HMB-45 expression ranged from 10 to 100% (mean 72%, median 75%), with most (17/19; 89%) showing strong intensity. In PEComas with *TSC* alterations, HMB-45 was positive in 15 to 100% (mean 74%, median 75%) of cells, with > 50% staining in 14/17 (82%). Melan-A was positive in 15/19 (79%) tumors, ranging from 0 to 100% (mean 19%, median 5%), with most (8/15; 53%) displaying strong intensity. In PEComas with *TSC* alterations, Melan-A was positive in 14/17 (82%), ranging from < 1 to 100% (mean 25%, median 10%), with > 50% expression seen in 4/17 (24%). MiTF was expressed in 15/19 (79%) tumors, ranging from 0 to 100% (mean 20%, median 5%) of cells, with only 2/15 (13%) showing strong intensity. In PEComas with *TSC* alterations, MiTF was expressed in 14/17 (82%), ranging from < 1 to 100% (mean 21%, median 10%), with > 50% expression in 3/17 (18%) tumors. Immunohistochemical results are summarized in Table 2.

Two tumors did not show any *TSC* alterations. One of them (case 15) was composed of an alveolar to nested growth of epithelioid cells with clear cytoplasm. It harbored a *PSF-TFE3* fusion and was strongly and diffusely (100%) HMB-45 positive, but negative for Melan-A and MiTF. The other one (case 2; Figure 2) was comprised of sheets and nests of predominantly epithelioid cells (70%), with cytoplasm ranging from clear to eosinophilic and granular to rhabdoid, as well as a perivascular/radial distribution of tumor cells. It showed strong, but focal (10%) expression for HMB-45 and Melan-A, and strong and diffuse (80%) MiTF staining.

One tumor (case 9; Figure 3) was biphasic, being comprised of two morphologically and immunohistochemically distinct areas. Approximately 40% of the tumor (case 9a) cells grew in sheets, nests, and trabeculae and were markedly pleomorphic, associated with brisk mitoses and tumor cell necrosis. This component was strongly and diffusely (100%) positive for HMB-45, moderately and diffusely (60%) positive for Melan-A, and negative for MiTF and all myogenic markers (data not shown). The remaining 60% (case 9b) was composed of fascicles of moderately atypical spindle cells with appreciable mitoses and tumor cell necrosis. It showed strong, but very rare (< 1%) HMB-45 expression, was negative for Melan-A and MiTF, and strongly and diffusely positive for smooth muscle actin, desmin, and caldesmon (data not shown). Both components harbored the same *ATRX* and *ADGRV1* mutations, but a *TSC1* mutation was detected only in the epithelioid component, and multiple copy number losses (including *TSC1* and *TP53,* among others) only in the spindled component.

Microsatellite Instability/Mismatch Repair Protein Immunohistochemical Testing:

One PEComa (case 12) was microsatellite-unstable by next-generation sequencing, while the remaining tumors analyzed by sequencing were either stable (n=8), indeterminate (n=1), or unable to be determined based on insufficient sequencing coverage of microsatellite loci (< 90% of loci at 50X coverage) (n=8). The microsatellite-indeterminate PEComa and those without microsatellite information (n=9) were mismatch repair protein-proficient, while the one microsatellite-unstable tumor was mismatch repair protein-deficient (loss of MLH1 and PMS2, retained MSH2 and MSH6).

DISCUSSION

Even before the first series on uterine PEComas in 2002[1], there has been speculation to whether PEComas are a distinct entity or related to smooth muscle tumors, likely an epithelioid variant with melanocytic differentiation[6–8,14–16]. It is generally accepted that in the appropriate morphological setting, the presence of any expression for at least two melanocytic markers (or one melanocytic marker and cathepsin K), a diagnosis of PEComa can be rendered[2]. However, as there is often morphological overlap between PEComas and smooth muscle tumors, the question arises whether the extent of melanocytic marker expression is imperative in differentiating between the two entities. Cathepsin K was originally used in the differential diagnosis of renal cell carcinoma and epithelioid angiomyolipoma[17], but its use has major limitations in the distinction between PEComa and smooth muscle tumors[3,9]. Herein, we further explored this issue by comparing the genomic

findings with the degree of melanocytic marker expression in a series of 19 PEComas previously published[3].

All tumors in this cohort fulfilled the immunohistochemical criteria used for PEComas (18 with positivity for at least two melanocytic markers and one positive for HMB-45 and cathepsin K). Since the latter PEComa (case 15) harbored a *PSF-TFE3* fusion, it is not surprising that both Melan-A and MiTF were negative, as has been described in *TFE3* translocation-associated PEComas[18,19]. Of the three melanocytic markers, only HMB-45 was strongly positive in all but two tumors, with just four showing < 50% expression. One of these four neoplasms (case 2) showed 10% HMB-45 expression and did not harbor a *TSC/TFE3* alteration, while the remaining three with 15%, 35%, and 40% HMB-45 expression showed *TSC* alterations. We did not find Melan-A or MiTF to be particularly helpful as no correlation was obvious between *TSC* alterations and their expression pattern.

This naturally leads to the question as to whether case 2 is truly a PEComa versus a smooth muscle tumor with aberrant melanocytic marker expression. Several potential interpretations can be postulated. The presence of a different TSC dysregulation mechanism (epigenetic modification, miRNA, etc), a structural *TSC* alteration not detected by the assay, or alterations in a mTOR pathway-associated gene not covered by the assay may argue for this tumor still being a true PEComa. For instance, two *TSC* wild-type PEComas have recently been shown to harbor mutations in *FLCN*, a gene encoding for a protein involved in the mTOR pathway[20]. Although this gene was covered by our platform, it is still possible that there might be another mTOR pathway-associated gene that was not covered by our assay. Finally, although the morphology and immunoprofile was not typical of a *TFE3* translocation-associated PEComa, it is possible that this tumor could harbor a *TFE3* fusion that was not detected by FISH or a novel fusion in uterine PEComas yet to be identified. On the other hand, it may be postulated that this tumor may not be a PEComa, but perhaps an epithelioid smooth muscle neoplasm with PEComa-like morphological features and focal melanocytic marker expression. Myogenic markers, including desmin, caldesmon, and smooth muscle actin, were strongly and diffusely positive (data not shown), but this finding is also observed in PEComas[1–3]. We cannot further classify this mesenchymal tumor with certainty, but would favor it to be at most of uncertain malignant potential based on its morphology and wild-type status for genes commonly altered in malignancy (i.e. *TP53, CDKN2A/2B, RB1, ATRX*, etc). Thus, one could argue that due to the expression of three melanocytic markers, this tumor could still be part of the PEComa family.

One tumor (case 9) was quite unusual in that it was clearly biphasic with morphologically and immunophenotypically distinct spindled and epithelioid areas. Macro-dissection of these components revealed identical *ATRX* and *ADGRV1* mutations in both, with a *TSC1* mutation detected in the epithelioid areas and copy number losses in multiple genes in the spindled areas. Based on molecular data, we can infer this is a clonally-related neoplasm rather than a collision tumor, with two pathways of clonal evolution. One pathway where a *TSC1* mutation was acquired likely resulted in a PEComa-like morphology and immunoprofile, and the second, with chromosomal instability with multiple copy number losses, led to a leiomyosarcoma-like morphology and immunophenotype. This neoplasm offers insight into whether PEComas and smooth muscle tumors are separate or related

entities as it highlights that this tumor likely arose from the same progenitor cell and underwent divergent differentiation. Whether this concept is universal for all PEComas merits further study, especially those investigating epigenetic modifications/cell of origin.

Our study demonstrated several similarities with a recent series integrating morphological, immunohistochemical, and molecular features of 15 uterine mesenchymal tumors with myomelanocytic differentiation[4]. In that series, histologically ambivalent tumors were examined, while we typified neoplasms morphologically and immunohistochemically consistent with PEComa. Furthermore, they only sequenced 8/15 (53%) primary tumors (with the remainder studied being recurrences) whereas we only evaluated primary tumors. They identified a *TSC2* alteration (n=5) or *TFE3* fusion (n=1) in 6/7 (86%) malignant PEComas and PEComa-like sarcomas, that parallels our findings with 18/19 (95%) tumors having *TSC* or *TFE3* alterations. Of note, none of their 15 tumors harbored *TSC1* alterations, a finding we observed in 9/19 (47%) tumors, and has been previously been described in a subset of PEComas[20–22]. Those with characteristic PEComa genomic alterations were all positive for HMB-45 (two “positive”, “focal”, 15% strong, 20% strong, 100% strong; percentages and intensity not provided in tumors characterized as “positive” or “focal”), which aligns with our findings. One of their tumors (UMT01) classified as lung recurrence of a malignant PEComa showed moderate to strong HMB-45 expression in 70% of cells, lacked *TSC* or *TFE3* alterations, but harbored mutations in *TP53, RB1,* and *BRD4*[4]. As previously reported[20,23] and also confirmed in our study (discussed in detail below), *TSC*-altered PEComas may show concurrent *TP53* mutations. However, in contrast to our series, UMT01 lacked *TSC* alterations, but showed > 50% HMB-45 staining. The several interpretations speculated for our case 2 can be applied to this tumor. Another sarcoma and its recurrence from their study (UMT06 and UMT06-R) showed 5% and 20% strong HMB-45 expression, respectively[4]. The primary tumor harbored mutations in *TP53* and *ATRX*, whereas the recurrence had the same *ATRX* mutation as well as novel *TSC2* and *TERT* mutations. The change in the HMB-45 staining pattern is similar to a HMB-45 negative uterine leiomyosarcoma reported by Silva et al. that acquired variable positivity for HMB-45 in the recurrences[7]. Thus, it can be speculated that as only 5% of cells were HMB-45 positive in the primary tumor, a *TSC2*-mutated subclone was likely present that became the predominant component in the recurrence. This tumor might be analogous to our case 9 with both PEComa and LMS-like components, with the exception that its PEComa-like clone was a smaller interspersed component rather than the two morphologically and immunohistochemically distinct foci we observed.

None of the sarcomas from the other two groups described by Selenica et al. (myogenic sarcoma and sarcoma NOS) harbored *TSC* or *TFE3* alterations, and all except one (UMT02) showed limited (≤ 15%) HMB-45 expression. However, in UMT02 tumor, 50% of cells were strongly HMB-45 positive, with alterations noted in *TP53* and *MED12*. As *MED12* mutations have been described in 11% to 21% of leiomyosarcomas[24,25], and to our knowledge not in PEComas, this tumor likely represents a leiomyosarcoma with significant HMB-45 expression. As 5/6 (83%) of their tumors in the myogenic sarcoma category showed ≤ 15% HMB-45 expression, it is highly plausible that case 2 in this series represents a leiomyoma with aberrant (10%) HMB-45 expression. Of note, *MED12* is covered by our platform and was not altered in this tumor.

The question of whether *TSC* alterations are specific to PEComas or if they may occur in morphological mimickers must be considered. Previously, *TSC2* alterations in uterine mesenchymal neoplasms have been reported in a leiomyoma with bizarre nuclei[26], two leiomyosarcomas[27,28], and two "high-grade non-leiomyosarcoma sarcomas"[24]. Scanned slides without immunohistochemical data are available for the tumor reported as uterine leiomyosarcoma by The Cancer Genome Atlas, but in our opinion does not morphologically resemble leiomyosarcoma[28]. None of the other tumors with reported *TSC2* alterations have any morphological or immunohistochemical information available for review. Although it is entirely plausible that *TSC* alterations may be detected in rare smooth muscle tumors, it appears that they are most prevalent in PEComas, and hence can help establish the diagnosis in morphologically and immunohistochemically ambiguous neoplasms. Currently, this distinction is largely academic and thus an extensive molecular evaluation is not necessary in most situations. However, it is important to note that in a patient with metastatic or recurrent disease if a *TSC* alteration is identified, she may be eligible for targeted therapy with MTOR inhibitors[29–32]. A proposed algorithm for the molecular work-up of a uterine mesenchymal neoplasm with immunohistochemical evidence of myomelanocytic differentiation is provided (Figure 4).

Aside from *TSC* alterations, only two other recurrent genetic aberrations were identified in this study, *TP53* (3/19; 16%) and *ATRX* (2/19; 11%). *TP53* and/or *ATRX* mutations were only detected in malignant PEComas (4/6; 67%), while another malignant PEComa showed a *RB1* loss. The tumor that harbored both *TP53* and *ATRX* mutations strongly expressed HMB-45 in 40% of cells, whereas the two with *TP53* mutations (including one with a *TSC2* variant of uncertain clinical significance), the one with an *ATRX* mutation, and the one with *RB1* loss showed strong and diffuse HMB-45 positivity. Given the presence of a *TSC* variant and strong HMB-45 expression in ≥ 40% of cells, we believe that the diagnosis of malignant PEComa, as opposed to leiomyosarcoma with aberrant melanocytic marker expression is justified. Similarly, *TP53, ATRX,* and *RB1* alterations have been described in a small subset of *TSC*-altered PEComas from diverse sites, most of which were classified as malignant, and analogous to our PEComas, showed variable amounts of HMB-45 expression[4,20,23]. Although the number of PEComas evaluated by next-generation sequencing is limited, the presence (or lack thereof) of an alteration in one or more of these genes might be a helpful adjunct to the morphological-based algorithm in predicting behavior; thus, additional studies are needed to corroborate this finding.

Brief discussion is warranted regarding the patient who suffered from multiple recurrences of her uncertain malignant potential PEComa. This tumor lacked a *TSC* mutation or rearrangement, but instead harbored a *TSC2* deletion as well as a *TERT* promoter mutation. *TERT* promoter mutations are recurrent in multiple cancer types[33]. It is well recognized that they likely contribute to tumor biology and aggressive clinical behavior, including those in the gynecologic tract[34–38]. Whether this mutation is also characteristic for PEComas of uncertain malignant potential that recur merits further investigation.

By next-generation sequencing microsatellite testing, one tumor (case 12) was microsatellite-unstable and showed loss of MLH1 and PMS2. As there were no alterations in *MLH1* or *PMS2* by next-generation sequencing, we favor this abnormality to be secondary

to *MLH1* promoter methylation. To our knowledge, this is the second mismatch repair protein-deficient/microsatellite unstable uterine PEComa[39], a finding which is important to note and warrants further study, as such patients can be eligible for targeted therapy with PD-L1 inhibitors[40].

In summary, we identified *TSC* or *TFE3* alterations in most (18/19; 95%) uterine tumors morphologically and immunohistochemically compatible with PEComa, and 14/17 (82%) had > 50% HMB-45 expression. We also recognized that most malignant PEComas harbor alterations in *TP53, ATRX,* or *RB1*, and rare PEComas are mismatch repair protein deficient/microsatellite unstable. Finally, detection of identical mutations (*ATRX, ADGRV1*) in a morphologically and immunohistochemically biphasic tumor that subsequently acquired different alterations (*TSC1* mutation versus single copy deletions) in the different components, concludes this is a clonal neoplasm that underwent two pathways of evolution. Further studies are necessary to explore this finding and ultimately determine the relationship between PEComas and smooth muscle tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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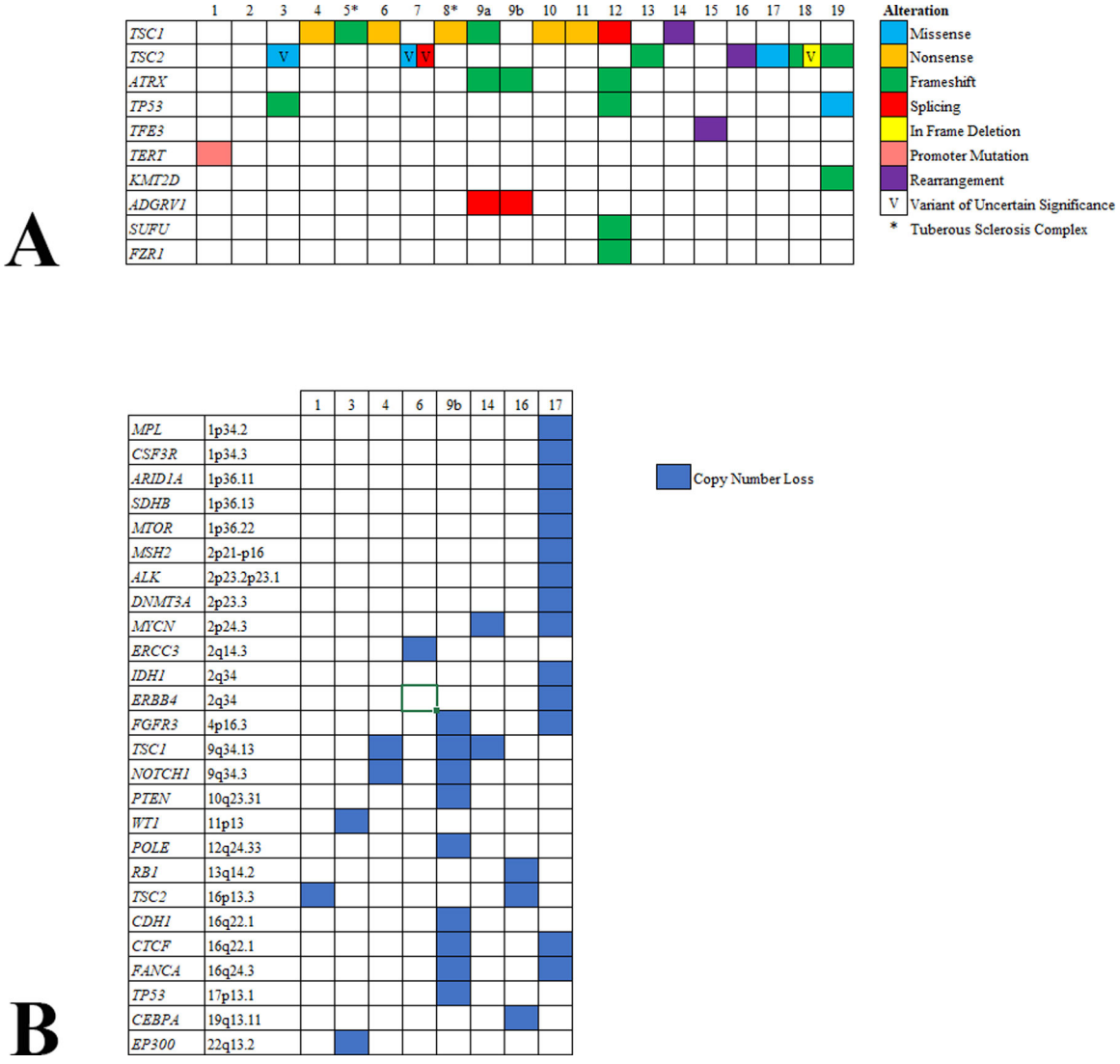

Figure 1.

Pathogenic/likely pathogenic mutations (A) and copy number alterations (B) detected in uterine PEComas.

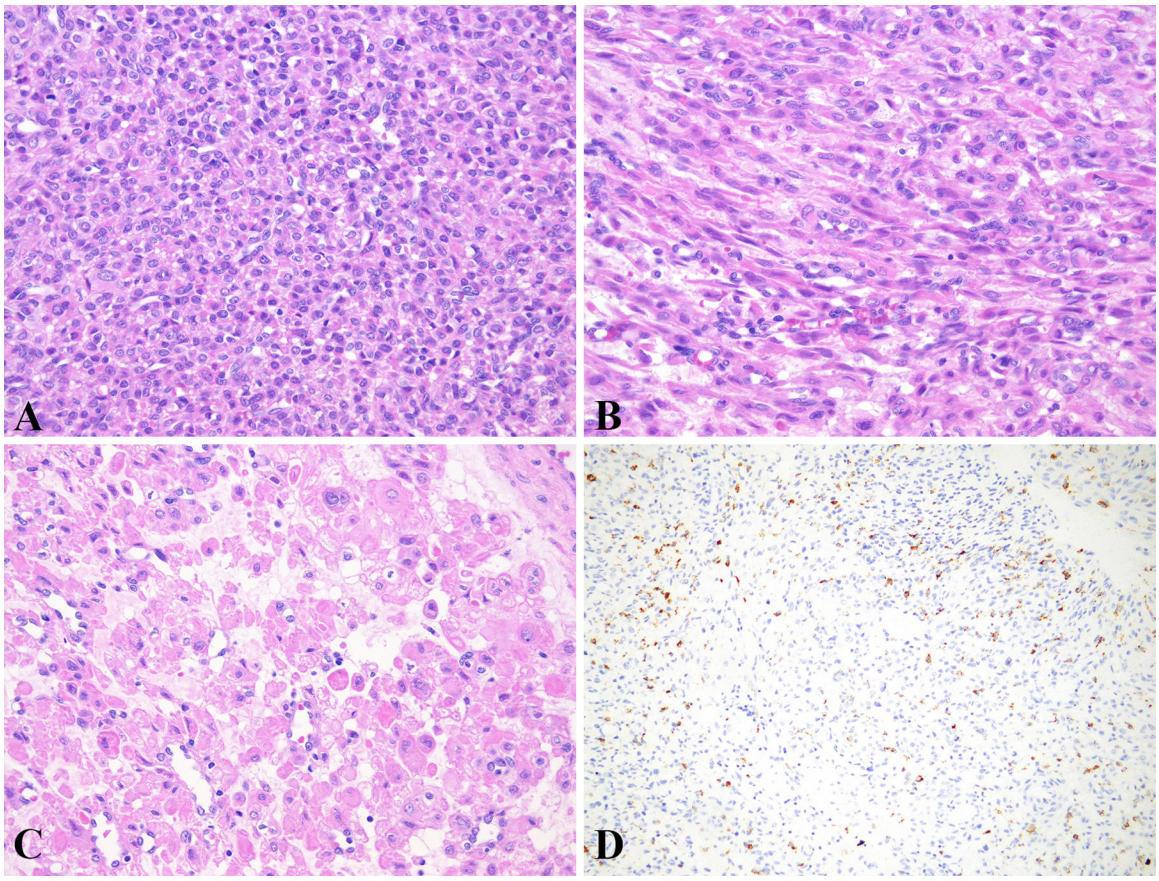

Figure 2.

Sheets of epithelioid cells (A) and fascicles of spindle cells (B). Non-cohesive epithelioid cells with clear to eosinophilic and granular cytoplasm. Note scattered cells with a rhabdoid appearance (C). Strong, but focal HMB-45 expression (D).

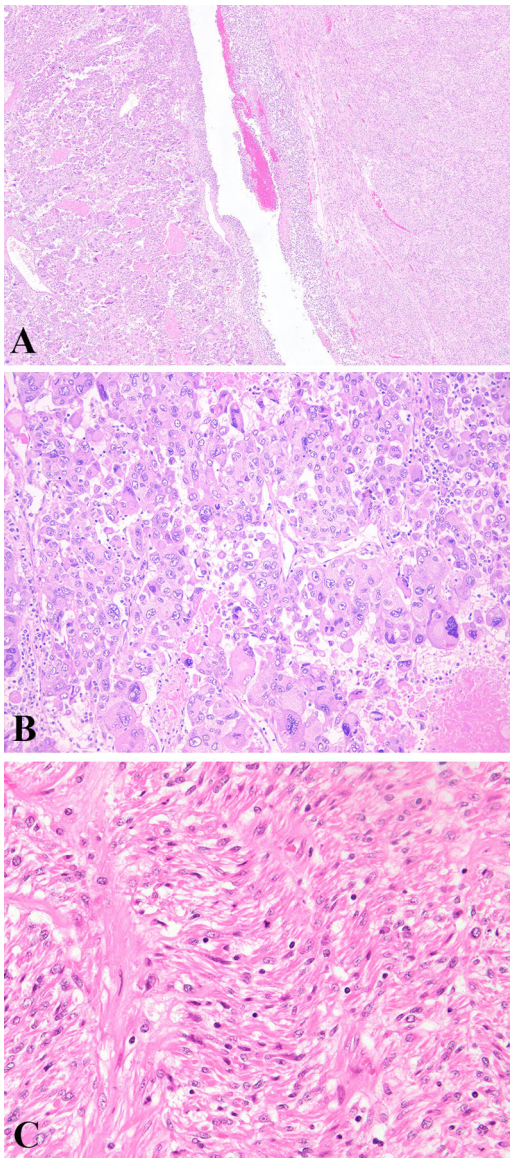

Figure 3.

Biphasic neoplasm with epithelioid (left) and spindled (right) components. Non-cohesive epithelioid cells forming vague nests with marked atypia, brisk mitoses, and tumor cell necrosis (B). Fascicles of spindle cells with moderate atypia and scattered mitoses (C).

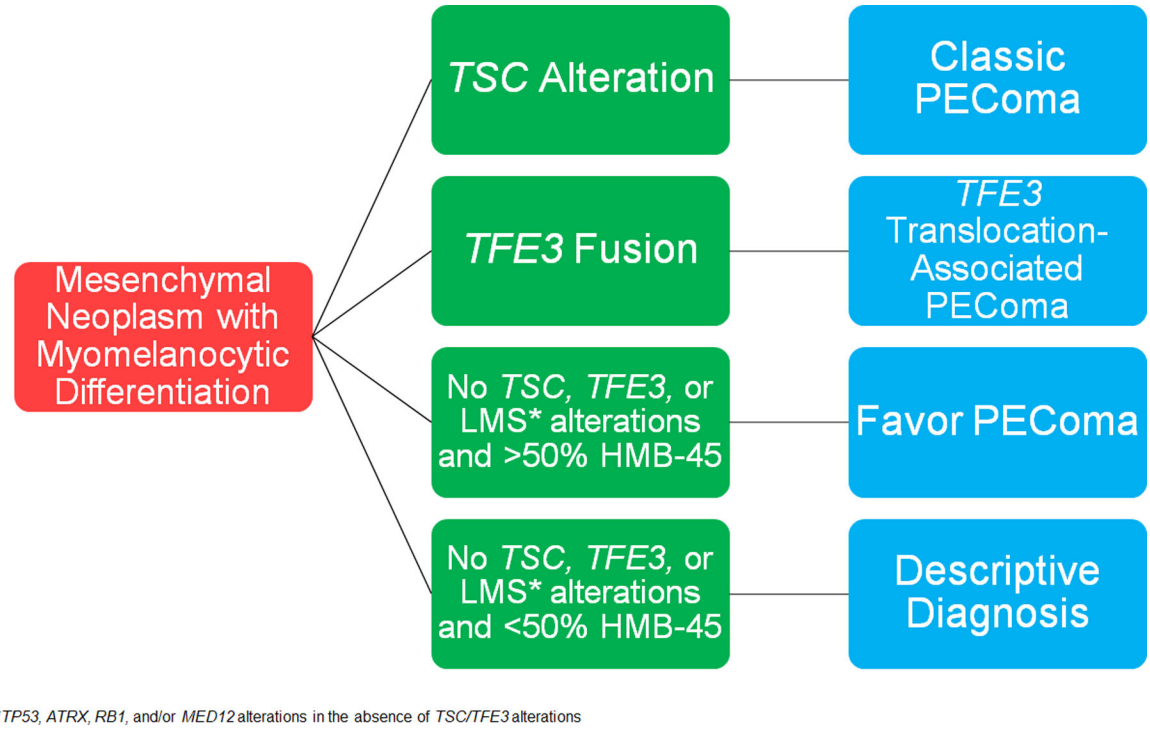

Figure 4.

Proposed algorithm for evaluation of a uterine tumor with myomelanocytic differentiation by immunohistochemistry.

Table 1:

Clinical and Morphological Features of Uterine PEComas

Case Number	Age (years)	Tuberous Sclerosis	≥ 5 cm	Necrosis	High-Nuclear Grade	Mitoses > 1 / 50 HPFs	Vascular Invasion	# of Atypical Features	Classification	Recurrences	Follow-Up
1	63	N/A	+	-	-	-	-	1	UMP	Multiple sites	NED, 175 months
2	35	-	+	-	-	+	-	2	UMP	-	NED, 57 months
3	63	-	+	+	+	+	-	4	Malignant	N/A	DOD, 5 months
4	67	N/A	+	+	+	+	+	5	Malignant	Lung	DOD, 20 months
5	36	+	-	-	-	-	-	0	UMP	-	NED, 11 months
6	35	-	-	-	-	+	-	1	UMP	-	NED, 55 months
7	77	-	-	-	-	-	-	0	UMP	-	NED, 15 months
8	44	+	-	-	-	-	-	0	UMP	-	NED, 47 months
9	62	-	+	+	+	+	-	4	Malignant	Lung, liver	AWD, 66 months
10	32	-	+	-	-	-	-	1	UMP	-	NED, 49 months
11	48	-	N/A	-	-	-	-	0	UMP	-	NED, 117 months
12	50	-	+	+	+	+	+	5	Malignant	Mediastinum	AWD, 7 months
13	55	-	-	-	-	-	-	0	UMP	-	NED, 88 months
14	43	-	-	-	-	+	-	1	UMP	-	NED, 19 months
15	36	-	N/A	-	-	+	-	1	UMP	-	NED, 47 months
16	48	-	+	+	+	+	-	4	Malignant	Vagina	DOD, 18 months
17	50	Suspicious	+	-	-	+	-	2	UMP	-	NED, 40 months
18	39	Suspicious	+	-	-	-	+	2	UMP	-	NED, 118 months
19	64	-	+	+	+	+	-	4	Malignant	Pelvis, liver	AWD, 24 months

HPF=high-power field, N/A=not available, + = present, - = absent, UMP=uncertain malignant potential, NED=no evidence of disease, DOD=dead of disease, AWD=alive with disease

Table 2:

Immunohistochemical and Molecular Features of Uterine PEComas

Case #	Classification	HMB-45 (Extent, Intensity)	Melan-A (Extent, Intensity)	MiTF (Extent, Intensity)	*TFE3* Fusion	Mutations	Copy Number Losses	MSI (% unstable loci)	MMR
1	UMP	100%, 3+	60%, 2+	10%, 1+	-	*TERT* promoter	*TSC2*	F	P
2	UMP	10%, 3+	10%, 2+	80%, 3+	-	-	-	6.6	NP
3	Malignant	100%, 3+	< 5%, 3+	0	-	*TSC2* (VUS), *TP53*	*EP300, WT1*	F	P
4	Malignant	60%, 3+	< 1%, 1+	< 5% 1+	-	*TSC1*	*TSC1, NOTCH1*	F	P
5	UMP	60%, 3+	0	< 5%. 1+	-	*TSC1*	-	5.4	NP
6	UMP	75%, 2+	20%, 2+	30%, 2+	-	*TSC1*	*ERCC3*	8.4	NP
7	UMP	55%, 2+	5%, 1+	10%, 1+	-	*TSC2* (VUS x2)	-	12.3	P
8	UMP	100%, 3+	0	5%, 1+	-	*TSC1*	-	F	P
9a	Malignant	100%, 3+	60%, 2+	0	-	*TSC2, ATRX, ADGRV1*	-	F	P
9b		< 1%, 3+	0	0	-	*ATRX, ADGRV1*	*TSC1, CDH1, CTCF, FANCA, FGFR3, TP53, NOTCH1, POLE, PTEN*	F	P
10	UMP	100%, 3+	25%, 3+	100%, 3+	-	*TSC1*	-	F	P
11	UMP	15%, 3+	< 5%, 3+	5%, 1+	-	*TSC1*	-	10.2	P
12	Malignant	40%, 3+	< 1%, 3+	< 5%, 1+	-	*TSC1, ATRX, TP53, SUFU, FZR1*	-	18.0	D
13	UMP	35%, 3+	55%, 3+	70%, 2+	-	*TSC2*	-	F	P
14	UMP	70%, 3+	< 1%, 3+	10%, 1+	-	*TSC1* rearrangement	*TSC1, MYCN*	8.0	NP
15	UMP	100%, 3+	0	0	*PSF-TFE3*	-	-	7.6	NP
16	Malignant	80%, 3+	15%, 3+	0	-	*TSC2* rearrangement	*TSC2, CEBPA, RB1*	6.0	NP
17	UMP	90%, 3+	< 1%, 2+	30%, 2+	-	*TSC2*	*FANCA, CTCF, IDH1, ERBB4, FGFR3, MYCN, DNMT3A, ALK, MSH2, MTOR, SDHB, ARID1A, CSF3R, MPL*	5.9	NP
18	UMP	70%, 3+	0	10%, 1+	-	*TSC2, TSC2* (VUS x1)	-	4.5	NP
19	Malignant	100%, 3+	100%, 3+	< 1%, 1+	-	*TSC2, TP53, KMT2D*	-	NP	P

Mod Pathol. Author manuscript; available in PMC 2022 March 31.

MSI=microsatellite instability testing, MMR=mismatch repair protein, UMP=uncertain malignant potential, - = absent, F=failed, NP=not performed, VUS=variant of uncertain clinical significance, P=proficient, D=deficient