Original contribution

A genomic survey of sarcomas on sun-exposed skin reveals distinctive candidate drivers and potentially targetable mutations

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1. Introduction

Malignant spindle cell neoplasms arising in chronically sun-exposed skin vary considerably in their prognosis and management, but may display significant overlap in their histologic and immunophenotypic characteristics. This spectrum of neoplasms includes tumors with vascular differentiation (angiosarcoma), smooth muscle differentiation (leiomyosarcoma [LMS]), epithelial differentiation (sarcomatoid carcinoma), and melanocytic differentiation (desmoplastic melanoma) and undifferentiated phenotypes.

Primary cutaneous angiosarcoma is a rare malignant neoplasm derived from the vascular endothelium in the skin and superficial soft tissue. Primary cutaneous angiosarcoma classically arises on chronically sun-exposed skin of the head and neck in elderly individuals. It is distinguished by clinical history from post-irradiation or lymphedema-associated angiosarcoma of the skin. It carries a poor prognosis with high rates of local recurrence and metastasis [1].

Atypical fibroxanthoma (AFX) and pleomorphic dermal sarcoma (PDS) are atypical spindle cell proliferations, typically arising on sun-exposed skin in the head and neck, that express mesenchymal markers but lack evidence of specific differentiation [2]. These tumors share similar cytomorphology. AFX and PDS are distinguished by more aggressive histologic features in PDS including significant subcutaneous extension, perineural invasion, angiolymphatic invasion, or tumor necrosis [3]. Distinction between AFX and PDS is critical as AFX has a low recurrence rate and exceedingly rare metastases, whereas PDS locally recurs up to 50% of the time and metastasizes up to 20% of the time [2].

Cutaneous LMS is a rare tumor believed to originate from the arrector pili muscle of hair follicles, in contrast to deeper LMS that arise from the vessel wall smooth muscle [4,5]. Cutaneous LMS arises on acral, sun-exposed, and post-traumatic sites. When confined to the dermis, cutaneous LMS displays a favorable course with infrequent recurrence (24%) and rare metastases, leading some to propose the alternative designation atypical smooth muscle tumor for these cases [5,6]. In contrast, LMS within the subcutis has a higher propensity for recurrence and metastasis [7].

Sarcomatoid squamous cell carcinoma (S-SCC), or spindle cell squamous cell carcinoma (SCC), is a rare variant of SCC predominantly or entirely composed of spindle cells with evidence of epithelial differentiation [5]. S-SCC can display expression of vimentin and CD10, as well as diminished expression of epithelial markers, raising a diagnostic challenge for distinction from AFX and sarcomas [8–10]. In contrast to AFX, S-SCC is associated with a more significant risk of recurrence and metastasis [5].

Soft-tissue sarcomas have been found to harbor heterogeneous genetic alterations [11] with karyotypic changes that in many cases are complex and unbalanced, hindering identification of biologically significant recurrent alterations [12]. In cutaneous tumors, this complexity is compounded by high tumor mutation burdens related to UV-associated genomic damage that raise challenges to distinguishing drivers from passenger mutations. With the exception of desmoplastic melanoma, studies of tumors on this differential diagnosis have been limited and incomplete (Table) [13–22], and to our knowledge, no study has examined these tumors in parallel. Improved understanding
of molecular alterations in these tumors may provide diagnostic and therapeutic insights. Here, we describe targeted sequencing results of a large cohort of sarcomatoid cutaneous malignancies.

2. Materials and methods

2.1. Case selection

All studies were conducted under protocols previously approved by the Institutional Review Board of the University of Michigan Health System (UMHS). We identified cutaneous sarcomatoid malignancies by query of the UMHS Department of Pathology database over the period of 2002—2017 using the search terms “primary angiosarcoma,” “atypical fibroxanthoma,” “pleomorphic dermal sarcoma,” “leiomyosarcoma,” and “sarcomatoid squamous cell carcinoma,” and all available cases were retrieved. Diagnosis and adequacy were confirmed by review by a board-certified dermatopathologist (P.W.H.). AFX was defined as an atypical spindle cell proliferation on sun-damaged skin with minimal/no subcutaneous involvement; no tumor necrosis, angiolymphatic invasion, or perineural invasion; and no evidence of epithelial/melanocytic/vascular/smooth muscle differentiation by cytomorphology and selected immunostaining (Table S1); in contrast, PDS demonstrated one or more of these findings. Although size was not used as a distinguishing criterion between AFX and PDS, only 3 cases classified as AFX slightly exceeded 2 cm (Table S1). Cutaneous LMS was defined as smooth muscle tumors with atypical features (including high cellularity, nuclear atypia, or mitotic activity) centered in the dermis, with or without deeper extension to the subcutis. Cases of cutaneous angiosarcoma arising in a background of prior radiation or lymphedema were excluded. After review, 111 cases were suitable for sequencing. The final cohort included AFX (n = 21), PDS (n = 17), extracutaneous undifferentiated pleomorphic sarcoma (UPS) (n = 8), cutaneous LMS (n = 5), extracutaneous LMS from the uterus or deep soft tissue (n = 9), primary cutaneous angiosarcoma (n = 7), S-SCC (n = 24), and SCC (n = 20) (Table S1). These included two matched samples (specifically, 1 pair of sample from a single primary SCC tumor and 1 pair of sample from a primary S-SCC and late recurrence); the remaining samples were all from clinically distinct cases.

### Table  Genetic alterations in cutaneous spindle cell malignancies.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Previous reports</th>
<th>Present study</th>
<th>Novel findings</th>
<th>References</th>
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<td>Recurrent</td>
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<tr>
<td>Leiomyosarcoma, cutaneous</td>
<td>-</td>
<td>RB1, TP53</td>
<td>CNV: IGF1R, PTEN</td>
<td>[7,51,52]</td>
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<tr>
<td>Leiomyosarcoma, extracutaneous</td>
<td>TP53, CDKN2A, RB1, ATM, ATRX, EGFR, IGF1R</td>
<td>TP53</td>
<td>[11,18,46,47,51,52,57,58]</td>
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<tr>
<td>Primary cutaneous angiosarcoma</td>
<td>TP53, CDKN2A, PTPRB, PLCG1, MTR, BRAF, BRCA2</td>
<td>MYC, RAS/RAF, NF1, CDKN2A</td>
<td>None</td>
<td>MYC, CCND1</td>
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<tr>
<td>Atypical fibroxanthoma</td>
<td>TP53, TERT, CDKN2A</td>
<td>MYC, CDKN2A</td>
<td>MYC, CDKN2A, PIK3CA</td>
<td>CNVs: CDK6, KIT, KRAS, MDM2</td>
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<td>Pleomorphic dermal sarcoma</td>
<td>HRAS, TP53, PIK3CA, TERT</td>
<td>BRAF, KRAS, IDH1, PDGFRA, CDKN2A,  KIT</td>
<td>TP53, RAS, CDKN2A (deletion)</td>
<td>CNV: CDK6, MET, MYCN,</td>
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<td>Cutaneous sarcomatoid squamous cell</td>
<td>-</td>
<td>CDKN2A, RAS, TP53</td>
<td>CCND1</td>
<td>All novel</td>
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Abbreviations: CNV, copy number variation.
2.2. Targeted next-generation sequencing

DNA and RNA profiling was performed using StrataNGS panels, which use multiplex polymerase chain reaction (PCR) to evaluate mutations and copy number alterations in cancer genes (Table S2). All samples were evaluated using a basic panel (V1) detecting mutations, copy number alterations, and/or fusions across 73 oncogenes (Table S2); samples with adequate quality and quantity of DNA were also evaluated using an expanded panel (V2) including 15 additional genes (TERT promoter mutations and tumor suppressor alterations) (Table S2). Hematoxylin and eosin—stained sections were used as a guide for dissection from a minimum of 4 formalin-fixed, paraffin-embedded (FFPE) 10-μm sections to obtain a minimal tumor purity of 60%. DNA extraction, Strata-based next-generation sequencing, and data analysis were performed as previously described [23–26]. DNA was extracted using the Siemens Tissue Preparation System (Siemens Medical Solutions USA, Inc., Malvern, PA). Bar-coded libraries were generated from 8 ng of DNA or RNA per sample using the Ion Ampliseq library kit 2.0 (ThermoFisher, 168 Third Avenue, Waltham, MA 02451, USA) with the DNA or RNA component of the StrataNGS panel. Libraries were prepared using the Ion 540 Chef Kit (Thermo Fisher Scientific, Waltham, MA) on the Ion Chef. Sequencing of multiplexed templates was performed using the Ion Torrent S5 on Ion 540 chips using the Ion 540 Chip Kit (Thermo Fisher Scientific, Waltham, MA 02451, USA), with alignment by Torrent Mapping Alignment Suite 5.8 (ThermoFisher, 168 Third Avenue, Waltham, MA 02451, USA), with the DNA or RNA component of the StrataNGS panel. Libraries were prepared using the Ion 540 Chef Kit (Thermo Fisher Scientific, Waltham, MA) on the Ion Chef. Sequencing of multiplexed templates was performed using the Ion Torrent S5 on Ion 540 chips using the Ion 540 Chip Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer’s instructions. Data analysis was performed using in-house developed, previously validated pipelines using Torrent Suite 5.8 (ThermoFisher, 168 Third Avenue, Waltham, MA 02451, USA), with alignment by Torrent Mapping Alignment Program and variant calling using the Torrent Variant Caller plugin. Annotated variants were filtered to remove synonymous or noncoding variants, poorly supported calls/sequencing artifacts, and germ line alterations. Variants present in the ESP6500 or phase I 1000G data set, the Exome Sequencing Project (ESP6500), or the Exome Aggregation Consortium at greater than 0.1% were filtered out as likely germ line alterations [27–29]. Potential driving alterations were prioritized using the COSMIC Database, Oncomine, and targeted literature searches. We have previously confirmed that these pipelines and filtering criteria identify variants that pass PCR validation with higher than 95% accuracy [23–26,30,31]. UV pattern mutations were defined as C>T transitions occurring at dipyrimidine sites or CC>TT dinucleotide substitutions.

2.3. Statistical analysis

Statistical analysis was performed using the chi-square test, using GraphPad Prism version 8.0 software (2365 Northside Dr. Suite 560 San Diego, CA 92108), with significance defined as p < 0.05. For comparison of outcomes, cases with less than 6 months of uneventful follow-up were excluded.

3. Results

Our cohort consisted of 74 cutaneous sarcomatoid lesions and 37 malignant counterparts (extracutaneous or nonsarcomatoid subtypes). The majority of cutaneous sarcomatoid lesions were located on the head and neck of elderly patients (Table S1). Notable exceptions included an AFX arising on the lower extremity in the background of xeroderma pigmentosum; a primary cutaneous angiosarcoma arising on the lower extremity (with a history of orthopedic surgery to a distal joint and no lymphedema); a S-SCC arising on the lower extremity in the background of epidermolysis bullosa; and a predilection for cutaneous LMS in our cohort to involve the extremities (Table S1). One pseudoangiomatous AFX was included (Table S1).

We detected oncogenic mutations and/or copy number variations (CNVs) in cancer-relevant genes in all groups of tumors (Figs. 1–3). Oncogenic fusions were not detected in any tumor. Although our approach does not provide formal mutation signature analysis, the detection of multiple UV-pattern mutations in a given tumor was specific (but not completely sensitive) for cutaneous origin (Table S3). TP53 mutations were the most frequent tumor suppressor inactivation event across all tumors, accounting for 96 of 196 total prioritized mutations (49%). TP53 mutations were highly recurrent in all diagnostic categories, with the exception of primary cutaneous angiosarcoma (Figs. 1–3).

Primary cutaneous angiosarcoma cases demonstrated recurrent gains in MYC (43%) and CCND1 (29%). In a subset of primary cutaneous angiosarcoma cases, we detected potentially actionable driver events novel to this tumor, including activating mutations in MAP2K1 (13%) and copy gains in FGFR1 (14%) (Table and S1). One case lacking MYC amplification displayed UV-pattern mutations in TP53 and CDKN2A (Fig. 1).

Mutations in RB1 were relatively restricted to cutaneous LMS (50%) compared with all other tumor types including extracutaneous LMS. We also identified copy number gains in oncogenes such as IGFR1 (22%). To our knowledge, somatic mutations in RB1 and copy gains in IGFR1 are previously unrecognized in cutaneous LMS. We also confirmed previously described copy loss of the tumor suppressor PTEN and mutations in TP53. In contrast to cutaneous LMS, extracutaneous LMS was associated with RB1 deletion rather than mutation and a higher rate of oncogene copy gains (Table and S1, Figs. 1–3).

In AFX, PIK3CA mutations were one of the most consistently detected oncogenic drivers (19%) and consistently arose from UV-pattern mutations. We detected CNVs and mutations to our knowledge not previously described in AFX, including copy number gains in the oncogenes CDK6, KRAS, and MDM2, as well as a premature stop mutation in the tumor suppressor ATM and a mutation in PTEN. In addition, we confirmed previously described alterations, most notably mutations in CDKN2A and TP53.
Of these, TP53 and CDKN2A mutations displayed a UV-mutation pattern and were recurrent in our cohort of AFX cases at a frequency similar to SCC (Figs. 1–3).

In PDS cases, RAS activation was more frequent than in AFX, accounting for 11% of mutational events. CDKN2A deletions were also more frequent in PDS than in AFX and significantly more frequent than in S-SCC (p < 0.001). Conversely, CDKN2A mutations were not detected in PDS, in contrast to AFX and S-SCC. However, the pattern of multiple TP53 mutations in a single tumor was observed in several PDS cases, similar to SCC and AFX but unlike extracutaneous UPS. We also found novel CNVs and mutations, including copy number gains in oncogenes CDK6, MET, and MYCN, and potentially targetable oncogenic mutations in IDH2, MAP2K1, and NRAS. In addition, we identified copy number gains and mutations previously reported in PDS (Table). Of these, TERT mutations displayed a UV pattern and were significantly enriched in PDS relative to AFX (p = 0.04) in our cohort (Fig. 1).

S-SCC, like PDS and conventional SCC, displayed recurrent RAS gene—activating mutations (44% across all Ras family genes). S-SCC was similar to conventional SCC with regard to TP53 and CDKN2A mutation rates (Fig. 3). We also detected additional CNVs and mutations (ATM, BRAF) that have been described in conventional SCC. S-SCC demonstrated lower rates of FGFR amplification and higher rates of RAS mutation than SCC, but neither trend reached statistical significance. Activating mutations in JAK1 (p.R724H) and copy gain of FGFR1 were detected that are potentially actionable driver events.

Clinical follow-up data were available for 26 tumors from 25 unique patients; of which, 15 had an uneventful course and
10 experienced disease recurrence/progression (local recurrence, metastasis, or death from disease) (Table S4). Of these, 22 tumors (from 21 unique patients) were evaluated by the full NGS panel including tumor suppressor mutations. Mutations in BRCA1/2 were infrequent and observed in 3 of 8 (38%) unique cases with progression (including primary cutaneous angiosarcoma, S-SCC, and PDS) and were not detected in cases with an uneventful course.

Fig. 2  Relative frequency of aberrations in selected genes across sarcomatoid neoplasms. A, Cutaneous LMS (C-LMS) are enriched for RB1 mutations, whereas RB1 deletions are recurrent in extracutaneous (EC)-UPS and EC-LMS. B, FGFR family copy number gains are detected in a subset of conventional cutaneous SCC, but are less frequent in S-SCC and other cutaneous sarcomatoid malignancies. C, CDKN2A mutation is a feature of SCC, S-SCC, and AFX, whereas deletion is more frequent in PDS. D, RAS mutations are recurrent drivers in S-SCC, SCC, and PDS, whereas PIK3CA mutations are highly frequent in AFX. SCC, squamous cell carcinoma; S-SCC, sarcomatoid squamous cell carcinoma; AFX, atypical fibroxanthoma; PDS, pleomorphic dermal sarcoma; UPS, undifferentiated pleomorphic sarcoma; LMS, leiomyosarcoma.

Mutations in BRCA1/2 were infrequent and observed in 3 of 8 (38%) unique cases with progression (including primary cutaneous angiosarcoma, S-SCC, and PDS) and were not detected in cases with an uneventful course. HRAS mutations

Fig. 3  Distinctive patterns of genomic alterations in sarcomatoid neoplasms. Prioritized variants with a recurrence rate of 0.20 or higher in each tumor type are shown. RAS, combined frequency of HRAS, KRAS, and NRAS mutations; FGFR, combined frequency of FGFR1-4 copy gains; SCC, squamous cell carcinoma; S-SCC, sarcomatoid squamous cell carcinoma; AFX, atypical fibroxanthoma; PDS, pleomorphic dermal sarcoma; UPS, undifferentiated pleomorphic sarcoma; LMS, leiomyosarcoma.
were restricted to cases with progression, although the sample size was very small (2/8 [25%]).

4. Discussion

In this genomic survey of cutaneous sarcomatoid malignancies, we find that the tumors within this differential diagnosis harbor distinct patterns of genetic alterations. **TP53** is highly recurrent among most tumor groups. In contrast, primary cutaneous angiosarcoma displayed frequent **MYC** amplifications and **CCND1** gains in our cohort. Other relatively specific mutations might also be informative in diagnostically challenging cases. For example, **RB1** mutations were relatively restricted to cutaneous tumors, and **CDKN2A** mutations were more frequent in AFX (as compared with PDS, which displayed **CDKN2A** deletions). Driver fusions were absent across all diagnostic categories.

Our observations raise the possibility that some genetic alterations could be prognostically informative, although definitive conclusions were limited owing to the sample size. For example, **BRCA1/2** mutations were specific to tumors associated with disease progression, and furthermore, they were not detected in AFX, suggesting worse prognosis with these mutations. **BRCA** mutations have been associated with susceptibility to poly ADP ribose polymerase (PARP) inhibitor therapy in other tumor types [32], raising the possibility that mutation status of the **BRCA1/2** genes may aid in therapeutic planning and monitoring of these tumors.

UV signature mutations, characterized by C>T transitions at dipyrimidine sites and CC>TT dinucleotide substitutions, predominate in many cutaneous malignancies including melanoma, basal cell carcinoma, SCC, and virus-negative Merkel cell carcinoma [33,34]. Our approach provides limited mutational coverage and hence does not allow for rigorous mutation signature analysis. With this caveat, we detected multiple UV-pattern mutations in a subset of cutaneous tumors across all diagnostic categories, supporting the presence of UV photodamage in many or all of these tumors. In contrast, the extracutaneous tumors we examined displayed 0–1 UV-pattern mutations; we predict that UV pattern mutations in these tumors arose owing to other mechanisms, such as C>T deamination events (half of which will occur at dipyrimidine sites by random chance) [33].

Genomic drivers of angiosarcoma are incompletely understood, especially in the context of primary cutaneous angiosarcoma. Amplification and overexpression of the **MYC** gene, a transcription factor involved in the regulation of cellular proliferation, differentiation, and apoptosis, is highly recurrent in post-irradiation angiosarcomas, but studies have been mixed regarding the presence of this amplification in primary cutaneous angiosarcoma [17,35,36]. **PTRB1** and **PLCG1** mutations are recurrent in angiosarcomas, but may be infrequent in primary cutaneous angiosarcoma [37–39]. One recent large study found that **MYC** amplifications occurred in some cases of primary cutaneous angiosarcoma (40%) [17] and were therefore less frequent than in post-irradiation angiosarcoma (>90%) [40–42]. Additional molecular data on primary cutaneous angiosarcoma were recently reported by a whole-exome sequencing study that included 12 angiosarcomas of the head/neck/face/scalp; of which, 9 occurred in the absence of prior radiation for other cancers. This group had a high tumor mutation burden with UV signature mutations and **TP53** alterations in a majority (67%), as well as mutation or copy gain affecting the RAS/RAF/MAPK pathway in some cases [37]. A single case from that study had concurrent **CCND1** and **MYC** gains, similar to tumors in our cohort. A targeted sequencing study by Murali et al. [38] included 9 primary cutaneous angiosarcomas arising in the setting of chronic sun damage and found that the majority (67%) demonstrated mutation or copy gain affecting the RAS/RAF/MAPK pathway, as well as **CDKN2A** loss. Although **TP53** mutations were detected in this study, the specific frequency for primary cutaneous angiosarcoma was not reported [38]. In a case report, a primary cutaneous angiosarcoma arising in a background of xeroderma pigmentosum was found to harbor DNA polymerase (**POLE**) mutation and genomic photodamage [43]. Across all of these studies, **TP53** mutation and **MYC** gain were mutually exclusive with a rare exception, similar to our cohort. Therefore, our findings further support the possibility that primary cutaneous angiosarcoma may be divided into predominantly nonoverlapping **TP53** mutant and **MYC** amplified subgroups; of which, the latter was more prominent in our cohort. Furthermore, another subset of primary cutaneous angiosarcoma may be **MYC/TP53** wild-type tumors and associated with various other potential drivers such as **MTOR** activation.

AFX and PDS are reported to share several chromosomal alterations. However, PDS harbors a higher frequency of alterations than AFX, which may contribute to its more aggressive behavior. Griewank et al. [14] evaluated 27 cases of AFX and 34 cases of PDS and demonstrated that **TERT** promoter mutations were detected in 25 (93%) of AFX and 26 (76%) of PDS cases. These mutations were associated with a UV pattern. **TERT** promoter mutations result in increased telomerase expression, therefore allowing cells to proliferate continuously without apoptosis or senescence. Our findings suggest that **TERT** promoter mutations may not be as highly recurrent in AFX and PDS as previously suspected **TP53** alterations have been identified as the potential driver in most cases of PDS [44]. Similar to our findings, mutations in **RAS** have been identified in up to one-third of PDS cases—in contrast to AFX, in which **RAS**-activating mutations are not generally seen [15,44]. In agreement with a previous study, we find that deep deletion of **CDKN2A** is more frequently detected in PDS [45]. Of note, **CDKN2A** copy loss appears to have
limited specificity for distinguishing PDS from AFX, whereas CDKN2A mutations were restricted to AFX. Overall, although CDKN2A inactivation is a common event across these tumors, the type of CDKN2A alteration in PDS is more similar to extracutaneous UPS than AFX. Although our targeted approach does not provide genome-wide copy number data, we hypothesize that chromosomal numerical alterations (including CDKN2A deletion) might overall play a more significant role relative to mutations in PDS as compared with AFX.

Previous studies have demonstrated that LMSs have complex karyotypes [18,46]. Extracutaneous LMS cases harbor frequent copy number alterations that predominate over mutations [47]. Although there is substantial karyotypic heterogeneity and complexity, recurring patterns of copy number alterations suggest a primary role for dysregulation of the MDM2-p53 and the P16-CDK4-RB1 pathways, arising from inactivation of the tumor suppressor genes TP53, RB1, and less frequently CDKN2A [p16] [11]. In line with this, a minority of extracutaneous LMS harbors mutations in TP53 and RB1 [48–50]. There are few mutational studies of cutaneous LMS, and to our knowledge, all were associated with background germline mutations. In two case reports with mutational analysis, cutaneous LMS was associated with a germline fumarate hydratase mutation and a TP53 mutation [51,52]. In another case, a cutaneous LMS with clear cell morphology was associated with germline RB1 mutation and loss of Rb protein expression [7]. Although our cohort is small, our findings raise the possibility that genomic mutations, especially in TP53 and RB1, might play a more consistent and significant role in cutaneous LMS than in extracutaneous LMS. This might be analogous to patterns of CDKN2A inactivation in AFX compared with PDS/UPS. A limitation of our study is that LMS cases in our cohort, although centered in the dermis, had significant involvement of the subcutis (with the possible exception of one case lost to follow-up before complete excision, for which subcutaneous involvement could not be assessed). Therefore, although we predict that our cases are of pilar smooth muscle origin, we acknowledge the possibility that some tumors in our cohort might originate from vascular smooth muscles, with potential implications for differences in tumor biology. Similarly, it is possible that purely dermal LMS might display molecular differences from cutaneous LMS with subcutaneous extension (analogous to differences between AFX and PDS) that would not be revealed by this study.

Sarcomatoid carcinomas are rare, and few mutational studies have characterized their genomic aberrations. In one targeted next-generation sequencing on fifteen esophageal sarcomatoid carcinomas, TP53 alterations were identified in all patients, similar to our cohort [53]. A large sequencing study of cutaneous SCC included one case of S-SCC that harbored TP53, CDKN2A, and HRAS mutations [54]. One study of cutaneous carcinosarcomas described TP53 mutations in two tumors with SCC and undifferentiated sarcoma components [55] that might have been classified as S-SCC by our criteria; unlike our results, this study did not identify further similarities to SCC such as CDKN2A inactivation. In contrast to S-SCC, cutaneous conventional SCC has been well characterized and exhibits heterogeneous genomic alterations [19]. Li et al. [19] identified TP53, CDKN2A, and NOTCH1/2 as the most recurrent gene alterations in SCC. They also found 45% of samples harbored oncogenic alterations that activated the RAS/RTK/P13K pathway, which correlated with a worse prognosis [19]. Additional loss of heterozygosity at 3p, 2q, 8p, and 13 and gains of 3q and 8q have been noted in SCC. Missero and Antonini [56] and Ha et al. [20] reported that p63, a member of the TP53 gene family expressed in the basal proliferative layer of the epidermis, is overexpressed in cutaneous SCC and cutaneous S-SCC. p63 overexpression might cooperate with RAS/ MAP kinase signaling to promote the acceleration of skin tumor formation and proliferation [56]. Importantly, we show that S-SCC has close similarity to conventional SCC with regard to highly recurrent drivers and hence may be susceptible to similar therapeutic approaches. We cannot exclude the possibility that S-SCC might harbor additional significant alterations not evaluated by our targeted approach; therefore, it would be interesting to further validate these observations by more comprehensive sequencing approaches.

Our study has several important limitations. To evaluate small lesions from archival FFPE samples, we used a focused targeted panel approach. Hence, we cannot comment on mutations in genes not covered by the panel (including PTHR1, PLCG1, NOTCH family, and FAT family genes); genome-wide copy number alterations and rearrangements; tumor mutation burden; or mutational signatures. The somatic nature of mutations was deduced by computational filtering rather than comparison with matched normal tissue. Therefore, our approach will not reliably nominate somatic mutations that overlap with reported germline changes. In addition, we acknowledge that our approach is not suited to characterization of germline events. Notably, for most patients, sufficient clinical history was available to allow for reasonable exclusion or inclusion of an inherited tumor syndrome; this information, combined with rigorous filtering against germline databases, renders it unlikely that deleterious germline mutations were misattributed as somatic mutations by our approach. Our targeted approach does not detect fusions not included on the gene panel or those with unexpected exon/gene partners. Finally, owing to rarity, some groups in our cohort were of relatively small size, limiting statistical comparisons.

This study expands the spectrum of known targetable driver events in these cutaneous sarcomatoid malignancies and establishes recurrent patterns of tumor suppressor inactivation and oncogene activation specific to tumor differentiation. Although some cutaneous sarcomas are similar to their extracutaneous counterparts,
others display significant molecular differences, supporting distinct genetic pathways of tumorigenesis. Our findings suggest that molecular profiling can be a useful ancillary study in diagnostically challenging cutaneous sarcomatoid neoplasms and may also be helpful in identifying potential therapeutic susceptibilities in clinically aggressive cases.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2020.06.003.

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