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Title:
Molecular profiling of the metaplastic spindle cell carcinoma of the breast reveals potentially targetable biomarkers

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MicroAbstract

- Spindle cell carcinoma is a rare subtype of metaplastic breast cancer, with triple-negative phenotype. Twenty-three spindle cell carcinomas were comprehensively explored for biomarkers of immuno-oncology and targeted therapies using immunohistochemistry and DNA/RNA sequencing. Spindle cell carcinomas are characterized by targetable molecular alterations in the majority of cases, but due to the lack of uniform findings, individual patient profiling is necessary.

Clinical Practice Points

- The majority of spindle cell carcinomas have triple-negative phenotype.
- Its molecular profile is similar to that of other subtypes of metaplastic breast carcinomas.
- The molecular alterations within the PIK3CA pathway along with PD-L1 expression characterize a proportion of spindle cell carcinomas and may guide targeted treatments for this rare disease.
Abstract

Introduction: Spindle cell carcinoma is a rare subtype of metaplastic breast cancer (MBC), with triple-negative (TNBC: ER-/PR-/Her2-) phenotype. It is associated with a marked resistance to conventional chemotherapy and has overall poor outcome.

Materials and Methods: Twenty-threepure spindle cell carcinomas of the breast (18 primary and 5 recurrent/metastatic) were comprehensively explored for biomarkers of immuno-oncology (I-O) and targeted therapies using immunohistochemistry and DNA/RNA sequencing.

Results: The majority (21/23) of spindle cell carcinomas were TNBC. Estrogen and androgen receptors expression above the therapeutic thresholds were detected in two cases, each. Pathogenic gene mutations were identified in 21/23 cases including \textit{PIK3CA}, \textit{TP53}, \textit{HRAS}, \textit{NF1}, and \textit{PTEN}. One case with matched pre- and post-chemotherapy samples exhibited a consistent mutational profile (\textit{PIK3CA} and \textit{HRAS} mutations) in both samples. Gene amplifications were present in five cases including one case without detectable mutations. The spindle cell carcinomas cohort had consistently low total mutational burden (all below 80\textsuperscript{th} percentile for the entire TNBC cohort). All tumors were microsatellite stable. PD-L1 expression was observed on both tumor cells (TC, in 7/21 cases), and in tumor infiltrating immune cells (IC, 2/21 cases).

Conclusions: Spindle cell carcinomas are characterized by targetable molecular alterations in the majority of cases, but due to the lack of uniform findings, individual patient profiling is necessary. Detection of individual combinations of biomarkers should improve treatment options for this rare, but aggressive disease.

Key words: Breast cancer; metaplastic carcinoma; spindle cell carcinoma; molecular profiling; immune checkpoint inhibitors; targeted therapy; mutations
**Introduction**

Metaplastic breast carcinoma (MBC) is a rare breast cancer subtype, constituting ~1% of all invasive breast cancers. Histologically, MBC is a highly heterogeneous disease, encompassing six different morphologic subtypes including spindle, squamous, chondroid, osseous, rhabdomyoid and mixed morphology. Somatic mutations in TP53, PI3K MAPK, RB1 and Wnt pathways genes have been frequently described in MBCs. MBCs are basal-like and claudin-low breast cancers with a triple-negative phenotype: Estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu negative. With rare exceptions (low-grade adenosquamous and fibromatosis-like metaplastic variants), MBCs are associated with a high recurrence/metastasis risk, chemotherapy resistance and poor outcome.

Mutational diversity is reflected in the morphologic heterogeneity of MBCs; PIK3CA mutations were detected in all morphologic variants of MBCs, excluding the chondroid variant, while TERT mutations were more prevalent in spindle cell and squamous variants.

Microarray expression based studies also revealed differences between the morphologic subtypes of MBC in regards to epithelial-mesenchymal transition (EMT)-related genes such as CDH1 and EPCAM.

PD-L1 expression in cancer and/or immune cells, as a predictor of response to immune checkpoint inhibitors, has also been described in a subset of MBCs.

Pure spindle cell variants of MBC constitute <10% of all MBCs; the spindle cell pattern is usually seen within a mixed MBC that constitutes ~70% of all MBC morphologies. In the present study, we explored a cohort of pure (>90% of invasive tumor) spindle cell MBC for the biomarkers of response to immuno-oncology (I-O) and targeted therapies.
Materials and Methods

Case selection

Twenty-three pure (>90%) spindle cell MBC identified among cases submitted to Caris Life Sciences (Phoenix, Arizona, USA) for molecular profiling were investigated in the present study. Each case underwent confirmation of the histologic diagnosis, including review of the diagnostic immunohistochemical test results performed at the referring pathology laboratory, by a board-certified pathologist at Caris Life Sciences.

Caris Life Sciences de-identified all reports and remnant spindle cell carcinoma samples provided by the referring laboratories. Given that the remnant tissues from previous samplings with no associated identifiers were used, this research was compliant with 45 CFR 46.101(b). Therefore, the present study was deemed exempt from Institutional Review Board approval and consent requirements were waived.

Immunohistochemistry (IHC)

IHC assays included ER, PR, AR, HER-2/neu, PD-L1, and pNTRK. In selected cases, PTEN, cKit and E-cadherin stains were done (the list of antibodies, clones and thresholds for positivity are provided in the Supplemental Table 1).

Next-generation sequencing (NGS)

The samples were profiled using massively parallel sequencing (NGS) of exons from 592 genes (SureSelect XT, Agilent, Santa Clara, CA and the NextSeq instrument, Illumina, San Diego, CA) \(^ {18} \).

The tumor mutational burden (TMB) was assessed by calculating the number of nonsynonymous missense mutations, excluding common germline variants, in one megabase of
DNA. TMB was considered high if ≥11 mutations/megabase (muts/Mb) were detected. The estimated threshold was based on a cohort of 603 TNBC cases using an 80th percentile cutoff value as recently suggested by Samstein RM et al. Microsatellite instability (MSI) was calculated from the NGS data by direct analysis of short tandem repeat tracts in the target regions of sequenced genes. The count only included alterations that resulted in increases or decreases in the number of repeats; high microsatellite instability (MSI-H) was defined as ≥46 altered microsatellite loci. This threshold was established by comparing NGS with the PCR-based microsatellite fragments analysis results from ~2100 samples.

Copy number variations (CNVs) were explored by comparing the depth of detected NGS sequence reads to reads from a diploid control. Genes having ≥ six copies were considered amplified.

The ArcherDx FusionPlex Assay (ArcherDX, Boulder, CO) was used for the gene fusion assessment. The gene fusions panel (n=54) is available here:

Results

Clinicopathologic characteristics of the cohort

Clinicopathologic data are summarized in Table 1.

The study included 23 spindle cell MBCs of which 18 were primary (17 from the breast and one from axilla) and five were recurrent/metastatic cases.

All patients were female with a mean age of 60.2 years (range, 30-83 years). With the exception of one case, all were grade 3 carcinomas (Nottingham modification of Bloom-Richardson system), and the majority (21/23) were triple negative. ER and AR (two cases each) expressions above the therapeutic thresholds of 1% and 10% respectively were rarely observed. HER-2/neu was uniformly negative in all cases (0%) (Table 1).

Genomic profile of spindle cell carcinomas

Genomic alterations were detected in 22/23 cases: Twenty-one cases had pathogenic mutations while one case (#11) that was devoid of any detectable pathogenic mutation harbored multiple gene amplifications including KDR (VEGFR2), KIT, PDGFRA, FIP1L1, and CHIC2. Only one case (#15) harbored no detectable genomic alterations (Table 1).

Mutations most frequently affected PIK3CA (10/23, one case was ER+), TP53 (6/23), HRAS and NF1 (4/23 each), and PTEN (3/23) (Supplemental Table 2).

Two cases exhibited evidence of epithelial to mesenchymal transition (EMT). The first case (#19, Table 1) was apocrine ductal carcinoma in situ (apocrine DCIS) transitioning into spindle cell carcinoma. Upon separate microdissection analyses, both in-situ and invasive components harbored identical mutational profiles (PTEN p.E242fs and HRAS p.Q61K mutations). EMT was further evidenced by the loss of E-cadherin and beta-catenin expression in
the invasive spindle cell component; however, no mutations were detected in the CDH1 or CTNNB1 genes, suggesting possible epigenetic silencing\textsuperscript{22}. AR was positive in an apocrine DCIS, but not an invasive spindle cell component. In the second case (#21, Table 1), a morphologic transition from ductal carcinoma NOS to spindle cell carcinoma was observed. The tumor also harbored a PTEN mutation (c.1027-1G>A) and additional PIK3CA (p.E542K) and CDH1 gene mutations (p.E243K, likely pathogenic without E-cadherin protein loss) in both components.

One case with available matched pre- and post-chemotherapy samples exhibited a consistent mutational profile (PIK3CA and HRAS mutations) in both samples. Similarly, another matched case (primary breast and metastatic sample from the lung) had identical mutational profiles at both sites (PIK3CA and KDM6A mutations).

None of the tested spindle cell carcinomas (n=9) exhibited pNTRK positivity by IHC including a case with NTRK1 gene amplification (Table 1). No NTRK gene fusions or any other fusions were detected in any of the successfully tested cases (n=14).

Gene amplifications were detected in five of 12 evaluable cases. Two spindle cell carcinomas harbored CCND1 (encodes cyclin D1 protein) gene amplification. Both cases also had multiple gene amplifications within the fibroblast growth factors family (FGF3, FGF4, FGF19 and fibroblast growth factor receptor 3 (FGFR3) (Table 1 and Supplemental Table 3).

**Immuno-Oncology (I-O) biomarkers in spindle cell carcinomas**

The spindle cell carcinomas consistently expressed a low TMB of between 3 and 10 muts/Mb. Additionally, all spindle cell carcinomas were microsatellite stable (MSS).
One third of the spindle cell carcinomas expressed PD-L1 above the 1% threshold in cancer cells (7/21) (Figure 1, Case#18, upper images); three exhibited diffuse PD-L1 expression in cancer cells (50-100% cancer cell positive, Figure 1A-B). In contrast, PD-L1 expression in immune cells was observed in only two cases, both were triple-negative (Figure 1, case#21, lower images).
Discussion

Recent studies have identified mutations in the TP53, PI3K MAPK, RB1 and Wnt pathways as the most frequent somatic mutations in MBCs. Our data confirm that spindle cell MBC shares similar molecular features with other morphologic subtypes of MBCs. PIK3CA mutations are particularly relevant since the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) classified them as strong predictors of response to PIK3CA inhibitors (level IA) (Supplemental Table 2). Furthermore, the FDA recently approved the PIK3CA inhibitor Piqray (alpelisib) for the treatment of ER-positive and PIK3CA-mutated, advanced or metastatic breast cancer following progression on, or after an, endocrine-based regimen. One of the PIK3CA-mutated spindle cell carcinomas from our series was ER-positive. In addition, several clinical trials and case studies have revealed promising effects of PIK3CA/mTOR inhibitors in patients with advanced/metastatic MBC that harbor mutations in the PI3K pathway. Basho et al. demonstrated that mTOR inhibitors (temsirolimus or everolimus) combined with doxorubicin and bevacizumab were more effective in the treatment of MBC than in non-MBC. Similarly, Moulder et al. showed the effectiveness of mTOR inhibitors (temsirolimus) in the treatment of MBC. In short, the presence of PIK3CA, PIK3RI and PTEN mutations in ~60% of spindle cell MBC may be a potential therapeutic guide for a substantial proportion of these carcinomas.

Mutations in HRAS were observed in 17% of the spindle cell MBCs, three of which had a coincident PIK3CA mutation. HRAS mutations have been well described in other breast cancer subtypes including MBCs. Interestingly, co-occurring HRAS and PIK3CA mutations have recently been recognized as driver mutations in both benign and malignant adenomyoepitheliomas of the breast. In cell culture models, the HRAS p.Q61R mutation...
appears to drive neoplastic transformation of breast cancer cells followed by reduced E-cadherin expression, increased myoepithelial differentiation and activation of the Akt/PIK3CA pathway. These features, commonly seen in MBC, underlie the phenotypic similarities between the two entities. In our cohort, we clearly demonstrated the EMT in two cases (#19 and 21).

Our study also revealed *NF1* gene mutations in a proportion of spindle cell carcinomas. *NF1* germline mutations are responsible for neurofibromatosis type 1 (OMIM#162200) while somatic *NF1* mutations have been described in various cancers including breast cancer. Several previous studies have identified *NF1* mutations in MBC including germline mutations in patients with neurofibromatosis type 1. Our findings provide further evidence of a role for the *NF1* gene in a subset of MBC.

Recently, the FDA approved I-O therapy with atezolizumab for TNBC containing ≥ 1% PD-L1 positive immune cells (IC) in the tumor biopsy, based on the IMpassion130 clinical trial (NCT02425891). We found that one third of spindle cell MBC expressed PD-L1; however, it was predominantly expressed in the neoplastic, tumor cell (TC) component. This finding was in line with our previous study of MBC and a study by Dill et al. Only two cases in the current study clearly expressed PD-L1 solely in the immune cell (IC) component of the tumor above the companion diagnostics threshold of 1%. For atezolizumab the predictive PD-L1 expression is found in immune cells (in tumors expressing ≥1% area occupied by PD-L1+ IC), not in TC expressing PD-L1. This is in contrast to a case study of Adams et al. who revealed an impressive clinical response in a patient with TC PD-L1+ (22c3 clone) advanced MBC treated by combined anti-PD-1 therapy with pembrolizumab and nab-paclitaxel. Similarly, Al Sayed et al. reported a complete response to the combination of a novel anti-PD-L1 antibody, durvalumab, with
paclitaxel in a patient with chemoresistant, metastatic MBC whose neoplastic cells overexpressed PD-L1 \(^{41}\).

In our study, two PD-L1+ (one in TC and IC, respectively) spindle cell carcinomas harbored PTEN mutations. PTEN mutations in cancer cells may induce immunosuppressive expression signatures and the lack of response to anti-PD-1 therapies \(^{42}\). Taken together, PD-L1 status in various subgroups of MBC needs to be precisely determined (cell type expressing PD-L1) in the context of additional mutational data (e.g. PTEN) and may not unequivocally predict response to I-O therapy. Other, lineage-agnostic predictive biomarkers for immune checkpoint inhibitors (TMB and MSI status) were negative (low TMB and microsatellite stable) in our series of spindle cell carcinomas, similar to the studies of Ng et al. \(^{6}\) and Tray et al. \(^{9}\). TMB and MSI status in spindle cell carcinomas are also comparable with the data from our large cohort >3000 TNBC NOS that exhibited a very low frequency of MSI-H and high TMB \(^{43}\).

Determination of the AR status in TNBC is important and positivity has been reported in various subtypes of breast cancer including both TNBC NOS and MBC \(^{2,44}\). Two spindle cell carcinomas from our cohort were also AR-positive. A phase II clinical trial by Gucalp et al. reported AR positivity at 12% among TNBC \(^{44}\). A clinical benefit rate was seen in 19% of the patients treated with the anti-AR drug bicalutamide \(^{44}\). Another study conducted on 116 TNBC revealed a significant clinical activity of enzalutamide in patients with advanced AR-positive TNBC \(^{45}\).

Although we found CCND1 and FGF family genes (FGF3, FGF4, FGF19, and FGFR1) amplified in a proportion of spindle cell carcinomas, these genes appear not to be reliable predictors of response to their respective inhibitors in breast cancer \(^{24}\). Therefore, the ESCAT
categorized these biomarkers as “Tier X”\textsuperscript{24} and their clinical relevance in spindle cell carcinomas remains unclear.

In conclusion, spindle cell carcinomas are characterized by targetable molecular alterations in the majority of cases, but due to the lack of uniform findings, individual patient profiling is necessary. Detection of individual combinations of biomarkers should improve treatment options for this rare, but aggressive disease.
Conflict of Interest

Zoran Gatalica, Phillip Stafford, Jeffrey Swensen, Joanne Xiu and David Spetzler are all employees of Caris Life Sciences. Semir Vranic has received honoraria from Caris Life Sciences. Other authors declare no conflict of interest.

Acknowledgement

The preliminary data from this study were presented at the ESMO Breast Cancer that was held in Berlin, Germany, May 2-4, 2019.

Authors’ contributions

References


Tables
## Table: Mutational Profile

<table>
<thead>
<tr>
<th>Case</th>
<th>Site (grade)</th>
<th>TNM Stage (AJCC)</th>
<th>Steroid receptors’ status (%)</th>
<th>PD-L1 status (%)</th>
<th>Mutational profile* (NGS)</th>
<th>Copy number variations (NGS)</th>
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<td>Metastatic (3)</td>
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<td>Positive (TC)</td>
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<td>Negative</td>
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<td>AKT2, CCND1, FGF3, FGF4, FGF3, NTRK1**</td>
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<td>Positive (100% TC)</td>
<td>PIK3CA Q546K, KDM6A E1381</td>
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</tbody>
</table>

---

*Only pathogenic mutations are listed.

** Both cases were further tested by immunohistochemistry (CD117 and panTRK antibodies) and were negative.
***Matched core and surgical biopsy were tested; this cancer was treated with neoadjuvant chemotherapy but the
tumor was chemoresistant.

n/a = Not available

TC = Tumor cells; IC = Immune cells

ER = Estrogen receptor; PR = Progesterone receptor; AR = Androgen receptor

NGS = Next-generation sequencing

Table 1. Molecular profiling features of the spindle cell carcinoma cohort.
Figures

Figure 1. Two triple-negative spindle cell carcinomas with PD-L1 positivity: Case#18 (upper two figures) with diffuse (70%) PD-L1 expression in cancer cells (TC); Case#21 (lower two figures) showing PD-L1 positivity at 1% in immune cells (red arrows). The left-sided images represent hematoxylin-eosin (H&E) stained slides; both cases were tested with VENTANA PD-L1 (SP142) Assay, FDA-approved test.