8-2020

Molecular Profiling of the Metaplastic Spindle Cell Carcinoma of the Breast Reveals Potentially Targetable Biomarkers

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Citation
Clinical Breast Cancer (2020) 20(4):326-331.e.1

Original Study

Molecular Profiling of the Metaplastic Spindle Cell Carcinoma of the Breast Reveals Potentially Targetable Biomarkers

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Abstract

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, owing to the lack of uniform findings, individual patient profiling is necessary.

Introduction: Spindle cell carcinoma is a rare subtype of metaplastic breast cancer, with triple-negative (TNBC: estrogen receptor-negative/progesterone receptor-negative/human epidermal growth factor receptor 2-negative) phenotype. It is associated with a marked resistance to conventional chemotherapy and has an overall poor outcome. **Materials and Methods:** Twenty-three pure spindle cell carcinomas of the breast (18 primary and 5 recurrent/metastatic) were comprehensively explored for biomarkers of immuno-oncology and targeted therapies using immunohistochemistry and DNA/RNA sequencing. **Results:** The majority (21/23) of spindle cell carcinomas were TNBC. Estrogen and androgen receptor expression above the therapeutic thresholds were detected in 2 cases each. Pathogenic gene mutations were identified in 21 of 23 cases, including *PIK3CA*, *TP53*, *HRAS*, *NF1*, and *PTEN*. One case with matched pre- and post-chemotherapy samples exhibited a consistent mutational profile (*PIK3CA* and *HRAS* mutations) in both samples. Gene amplifications were present in 5 cases, including 1 case without detectable mutations. The spindle cell carcinomas cohort had consistently low total mutational burden (all below the 80th percentile for the entire TNBC cohort). All tumors were microsatellite stable. Programmed death-ligand 1 expression was observed on both tumor cells (in 7/21 cases), and in tumor-infiltrating immune cells (2/21 cases). **Conclusions:** Spindle cell carcinomas are characterized by targetable molecular alterations in the majority of cases, but owing 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.

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Keywords: Immune checkpoint inhibitors, Immune therapy, Metaplastic carcinoma, Mutations, Targeted therapy

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Submitted: Dec 20, 2019; Revised: Feb 15, 2020; Accepted: Feb 18, 2020; Epub: Feb 27, 2020

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Introduction

Metaplastic breast carcinoma (MBC) is a rare breast cancer subtype, constituting ~1% of all invasive breast cancers.[1] Histologically, MBC is a highly heterogeneous disease, encompassing 6 different morphologic subtypes, including spindle, squamous, chondroid, osseous, rhabdomyoid, and mixed morphology.[1] Somatic mutations in TP53, PI3K MAPK, RB1, and Wnt pathways genes have been frequently described in MBCs.[2-11] MBCs are basal-like and claudin-low breast cancers with a triple-negative phenotype: estrogen receptor (ER), progesterone receptor (PR), and human

https://doi.org/10.1016/j.clbc.2020.02.008

epidermal growth factor receptor 2 (HER2/neu)-negative.[7,9,12-14] 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.[15]

Mutational diversity is reflected in the morphologic heterogeneity of MBCs; *PIK3CA* mutations were detected in all morphologic variants of MBCs, excluding the chondroid variant,[5,6,11] whereas *TERT* mutations were more prevalent in spindle cell and squamous variants.[5] 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*.[7]

Programmed death-ligand 1 (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.[3,9,11,16,17]

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, AZ) 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 (Z.G.) 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, androgen receptor (AR), HER2/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 Supplemental Table 1 [in the online version]).

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 et al.[19] 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 polymerase chain reaction-based microsatellite fragments analysis results from ~2100 samples.[18,20,21]

Copy number variations were explored by comparing the depth of detected NGS sequence reads to reads from a diploid control. Genes having ≥ 6 copies were considered amplified.[18]

The ArcherDx FusionPlex Assay (ArcherDX, Boulder, CO) was used for the gene fusion assessment. The gene fusion panel (n = 54) is available here: https://www.carismolecularintelligence.com/wp-content/uploads/2017/03/TN0276-v14_Profile-Menu.pdf.

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 5 were recurrent/metastatic cases.

All patients were female, with a mean age of 60.2 years (range, 30-83 years). With the exception of 1 case, all were grade 3 carcinomas (Nottingham modification of Bloom-Richardson system), and the majority (21/23) were triple negative. ER and AR (2 cases each) expressions above the therapeutic thresholds of 1% and 10%, respectively, were rarely observed. HER2/neu was uniformly negative in all cases (0%) (Table 1).

Genomic Profile of Spindle Cell Carcinomas

Genomic alterations were detected in 22 of 23 cases: Twenty-one cases had pathogenic mutations, whereas 1 case (#11) that was devoid of any detectable pathogenic mutation harbored multiple gene amplifications including *KDR (VEGFR2), KIT, PDGFRA, FIP1L1*, and *CHIC2*. Only 1 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) (see Supplemental Table 2 in the online version).

Two cases exhibited evidence of EMT. The first case (#19) (Table 1) was apocrine ductal carcinoma in situ 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.[22] AR was positive in an apocrine ductal carcinoma in situ, but not an invasive spindle cell component. In the second case (#21) (Table 1), a morphologic transition from ductal carcinoma not otherwise specified (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

Table 1 Molecular Profiling Features of the Spindle Cell Carcinoma Cohort

Case	Site (Grade)	TNM Stage (AJCC)	Steroid Receptors' Status (%)	PD-L1 Status (%)	Mutational Profile[a] (NGS)	Copy Number Variations (NGS)
#1	Primary (3)	Unknown	Negative	Negative	*BRAF*	None
#2	Primary (3)	Unknown	ER^+ (1%)	Negative	*TP53*	
#3	Primary (3)	pT2NoMx	Negative	Positive (TC+)	*PIK3CA, HRAS*	
#4	Primary (3)	Unknown	Negative	Negative	*KDM6A*	
#5	Primary (axilla) (3)	pT3NoMx	AR^+ (10%)	Negative	*TP53, PIK3CA, NF1*	*MLLT1*
#6	Primary (recurrent) (3)	rpT3NoMx	Negative	Negative	*TP53, NF1*	
#7	Primary (3)	pT3NoMx	Negative	Negative	*NF1*	
#8	Primary (3)	pT2NoMx	Negative	Negative	*NF1, PIK3R1, BRIP1*	
#9	Primary (3)	Unknown	AR^+ (15%)	Positive (TC)	*TP53, RB1, PTEN*	
#10	Primary (recurrent) (3)	Unknown	Negative	n/a	*TP53*	*CYP2D6*
#11	Primary (3)	pT3NxMx	Negative	n/a	None	*KDR (VEGFR2), KIT*[b], *PDGFRA, FIP1L1, CHIC2*
#12	Metastatic (3)	M1	Negative	Positive (TC)	*TP53*	
#13	Primary (1)	pT3NoMx	ER^+ (10%)	Positive (TC)	*PIK3CA*	*FGF4, FGF3, FGF19, CCND1*
#14	Primary (postneoadjuvant) (3)	ypT4NoMx	Negative	Positive (TC)	*PIK3CA*	None
#15	Primary (3)	pT2NoMx	Negative	Negative	None	None
#16	Metastatic (3)	M1	Negative	Negative	*KRAS*	
#17	Primary (3)	Unknown	Negative	Negative	*PIK3CA*	
#18	Primary (3)	pT4bNxMx	Negative	Positive (TC)	*PIK3CA, HRAS*	
#19	Primary (3)	pT2NoMx	Negative	Negative	*HRAS, PTEN*	None
#20	Primary (postneoadjuvant, matched)[c] (3)	ypT1cNoMx	Negative	Negative	*PIK3CA, HRAS*	*AKT2, CCND1, FGF3, FGF4, FGFR3, NTRK1*[b]
#21	Primary (3)	pT2N1aMx	Negative	Positive (IC)	*PIK3CA, PTEN, CDH1 E243K*	None
#22	Primary (3)	Unknown	Negative	Positive (IC)	*PIK3CA E545K; NF2 V219fs*	None
#23	Primary and meta (matched) (3)	M1	Negative	Positive (100% TC)	*PIK3CA Q546K, KDM6A E1381*	None

Abbreviations: AJCC = American Joint Committee on Cancer; AR = androgen receptor; ER = estrogen receptor; IC = immune cells; n/a = not available; NGS = next-generation sequencing; PD-L1 = programmed death-ligand 1; PR = progesterone receptor; TC = tumor cells.
[a]Only pathogenic mutations are listed.
[b]Both cases were further tested by immunohistochemistry (CD117 and panTRK antibodies) and were negative.
[c]Matched core and surgical biopsy were tested; this cancer was treated with neoadjuvant chemotherapy but the tumor was chemoresistant.

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 5 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 [in the online version]).

I-O Biomarkers in Spindle Cell Carcinomas

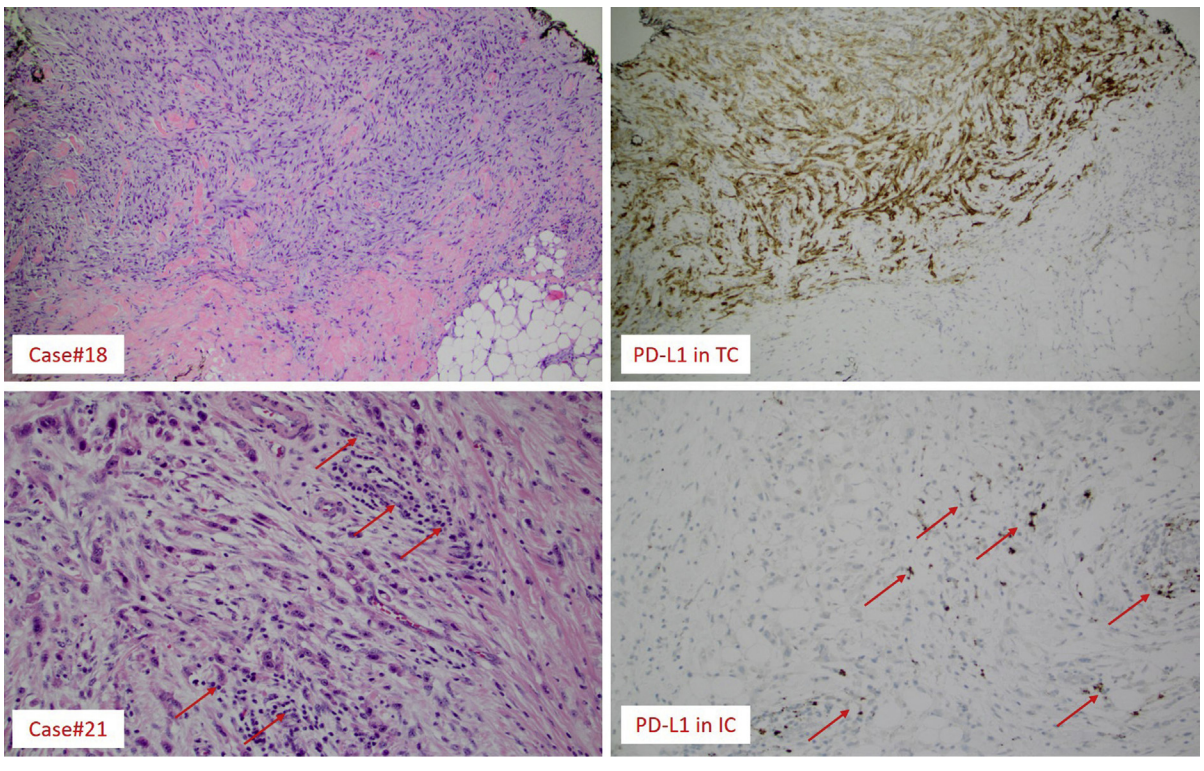
The spindle cell carcinomas consistently expressed a low TMB of between 3 and 10 muts/Mb. Additionally, all spindle cell carcinomas were microsatellite stable (MSS).

One-third (7/21) of the spindle cell carcinomas expressed PD-L1 above the 1% threshold in cancer cells (Figure 1, Case #18, upper images); 3 exhibited diffuse PD-L1 expression in cancer cells (50%-100% cancer cell positive) (Figure 1A and B). In contrast, PD-L1 expression in immune cells was observed in only 2 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.[2-11] Our data confirm that spindle cell MBC shares similar molecular features with other morphologic subtypes of MBCs.[6,9-11,23] *PIK3CA* mutations are particularly relevant because the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) classified them as strong predictors of response to PIK3CA inhibitors (level IA) (see Supplemental Table 2 in the online version).[24,25] Furthermore, the United States Food and Drug Administration (FDA) recently approved the PIK3CA inhibitor

Figure 1 Two Triple-negative Spindle Cell Carcinomas With PD-L1 Positivity: Case #18 (Upper 2 Figures) With Diffuse (70%) PD-L1 Expression in Cancer Cells (TCs); Case #21 (Lower 2 Figures) Showing PD-L1 Positivity at 1% in ICs (Red Arrows). The Left-sided Images Represent Hematoxylin and Eosin Stained Slides; Both Cases Were Tested With VENTANA PD-L1 (SP142) Assay, a United States Food and Drug Administration-Approved Test



Abbreviations: IC = immune cells; PD-L1 = programmed death-ligand 1; TC = tumor cells.

Piqray (alpelisib) for the treatment of ER^+ 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^+. In addition, several clinical trials and case studies have revealed promising effects of PIK3CA/mammalian target of rapamycin (mTOR) inhibitors in patients with advanced/metastatic MBC that harbor mutations in the PI3K pathway.[11,23,26-28] 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.[28] Similarly, Moulder et al showed the effectiveness of mTOR inhibitors (temsirolimus) in the treatment of MBC.[23] In short, the presence of *PIK3CA, PIK3R1,* and *PTEN* mutations in ~60% of spindle cell MBC may be a potential therapeutic guide for a substantial proportion of these carcinomas.[6]

Mutations in *HRAS* were observed in 17% of the spindle cell MBCs, 3 of which had a coincident *PIK3CA* mutation. *HRAS* mutations have been well described in other breast cancer subtypes including MBCs.[2,3,10,29,30] Interestingly, co-occurring *HRAS* and *PIK3CA* mutations have recently been recognized as driver mutations in both benign and malignant adenomyoepitheliomas of the breast.[31,32] 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,[32] underlie the phenotypic similarities between the 2 entities.[33] In our cohort, we clearly demonstrated the EMT in 2 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), whereas somatic *NF1* mutations have been described in various cancers including breast cancer.[4,34] Several previous studies have identified *NF1* mutations in MBC, including germline mutations in patients with neurofibromatosis type 1.[4,10,35-40] 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 (ICs) 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[3] and a study by Dill et al.[16] Only 2 cases in the current study clearly expressed PD-L1 solely in the IC component of the tumor above the companion diagnostics threshold of 1%. For atezolizumab, the predictive PD-L1 expression is found in ICs (in

tumors expressing ≥ 1% area occupied by PD-L1$^+$ IC), not in TCs 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-positive (22c3 clone) advanced MBC treated by combined anti-PD-1 therapy with pembrolizumab and nab-paclitaxel.[17] 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, 2 PD-L1-positive (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 (eg, *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 MSS) 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 of > 3000 patients with 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 patients with 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,"[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, owing 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.

Clinical Practice Points

- The majority of spindle cell carcinomas have triple negative phenotype.
- The 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.

Disclosure

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. All other authors state that they have no conflicts of interest.

Supplemental Data

Supplemental tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2020.02.008.

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Supplemental Data

Supplemental Table 1 The List of Antibodies, Clones and Thresholds for Their Positivity

Antibody	Clone (Manufacturer)	Threshold for Positivity (References)
Estrogen receptor (ER)	SP1 (Ventana)	≥1%[1]
Progesterone receptor (PR)	1E2 (Ventana)	≥1%[1]
Androgen receptor (AR)	AR27 (Leica Biosystems)	>10%[2-4]
HER-2/neu	4B5 (Ventana)	>10% at 3 + intensity[5]
PD-L1	SP142 (Ventana)	≥1% of the tumor area (TNBC) or at ≥ 1% of cancer cells for non-TNBC[6,7]
pNTRK	EPR17341 (Abcam)	≥1%[8]
PTEN	6H2.1 (Dako Agilent)	Any positivity
E-cadherin	EP700Y (Cell Marque)	Any positivity
c-Kit (CD117)	9.7 (Ventana)	Any positivity

Abbreviations: HER2 = Human epidermal growth factor receptor 2; PD-L1 = programmed death-ligand 1; TNBC = triple negative breast cancer.

Supplemental Table 2 The Frequency of Genomic Alterations in Spindle Cell Carcinomas According to the Levels of Evidence (LOE) as Recommended by the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT)

Genomic Alterations in Spindle Cell Carcinomas	% in Spindle Cell Carcinomas, n/N (%)	Level of Evidence (LOE) in Breast Cancer[a]
PIK3CA mutations	10/23 (43)	IA
PTEN loss (mutations)	3/23 (13)	IIA
NF1 mutations	4/23 (17)	IVA
PIK3R1 mutations	1/23 (4)	IVA
CDH1 mutations	1/23 (4)	IVA
TP53 mutations	6/23 (26)	IVA

IA = "Alteration-drug match is associated with improved outcome in clinical trials" (prospective randomized study).
IIA = "Alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown" (retrospective studies).
IVA = "Pre-clinical evidence of actionability" (in vitro and in vivo models).
[a]Based on the ESCAT (ESMO Scale for Clinical Actionability of Molecular Targets) evidence tier (references: *Ann Oncol* 2018; 29:1895-902; *Ann Oncol* 2019; 30:365-73).

Supplemental Table 3 A List of Genomic Alterations in Spindle Cell Carcinomas for Which Clinical Data Indicate That They Are Not Actionable

Common Genomic Alterations in Spindle Cell Carcinomas	% in Spindle Cell Carcinomas, n/N (%)	Tier X Alterations[a]
CCND1 amplification	2/12 (17)	Not actionable
FGF genes amplification	2/12 (17)	Not actionable

[a]Tier X indicates genomic alterations that are not clinically proven as targetable.

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