REVIEW

An Update on the Molecular Pathology of Metaplastic Breast Cancer

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Abstract: Metaplastic breast cancer (MpBC) is a fascinating morphologic sub-type of breast cancer, characterised by intra-tumoural heterogeneity. By definition, these tumors show regions of metaplasia that can present as spindle, squamous, chondroid or even osseous differentiation. MpBC are typically triple-negative, and are therefore not targetable with hormone therapy or anti-HER2 therapies, leaving only chemotherapeutics for management. MpBC are known for their aggressive course and poor response to chemotherapy. We review herein the pathology and molecular landscape of MpBC and discuss opportunities for targetted therapies as well as immunotherapies.

Keywords: metaplastic, triple-negative breast cancer, precision oncology

Introduction

Metaplastic breast cancer (MpBC) is a unique histologic subtype of breast cancer, defined by characteristic intra-tumoural heterogeneity. Although rare, MpBC accounts for significant morbidity and mortality, and has a poor prognosis. MpBC tend not to respond well to systemic chemotherapies, and together with emerging data on the genomic landscape of MpBC, there is scope for applying precision oncology in the management strategies of MpBC. We focus herein on the molecular pathology of MpBC and the current status and potential of targeted therapies to manage MpBC.

MpBC Pathology and Presentation

The clinical features of MpBC are similar to other high-grade cancers of no special type (NST), however, they often present at a more advanced stage. They tend to be large in size, with dimensions ranging from 1.2 to >10 cm and often present as a palpable breast mass, with ill-defined borders on mammography, ultrasonography, and magnetic resonance imaging. MpBC represents 0.2–1% of all breast cancers – the rates vary due to the differing definitions and classification systems used over time.

MpBC do not have any distinctive macroscopic features, with the tumor varying from well-circumscribed to having an irregular border. Microscopically, they comprise a heterogenous group with differing outcomes. In the absence of sufficient molecular and outcome data, the current WHO Classification of Tumours of the Breast[1,2] has maintained a descriptive morphological classification system, based on the type of the metaplastic elements present. MpBC are classified monophasic (when there is only one metaplastic component) or biphasic (with two or more metaplastic components

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such as squamous and spindle, or mixed metaplastic and non-metaplastic components – such as spindle and invasive carcinoma NST). Further, MpBC can also be classified into epithelial-only carcinomas (with low-grade adenosquamous carcinoma or pure squamous cell carcinoma), pure (monophasic) sarcomatoid (spindle cell or matrix-producing) carcinomas, and biphasic epithelial and sarcomatoid carcinomas.

Current Histopathological Classification

The current WHO classification includes (i) adenosquamous carcinoma – mostly low grade but can be high grade rarely and (ii) pure squamous cell carcinomas (iii) pure spindle cell carcinoma (iv) fibromatosis-like metaplastic carcinoma, (iv) metaplastic carcinoma with mesenchymal differentiation that includes chondroid (myxoid/cartilaginous), osseous (bone), rhabdomyoid (muscle) and neuroglial, and (v) mixed metaplastic carcinoma – where the mix may be multiple metaplastic elements or a mixture of epithelial and mesenchymal elements. Examples of the heterologous elements are shown in Figure 1. The detailed morphology of the subtypes is beyond the scope of this review and the reader is directed to the WHO Tumour Classification of the Breast 5th Ed (2019).[2]

MpBC are typically, though not invariably triple-negative (TN), lacking expression of estrogen and progesterone receptors (ER/PR), and HER2. Analysis of the SEER data showed that HER2 positive MpBC had an improved overall survival compared to TN, and other MpBC including ER+/PR+/HER2-cases, which accounted for 20% of the cohort.[3] Conversely, HER2-positive metaplastic squamous cell carcinomas were recently demonstrated to have a poorer prognosis than the TN metaplastic squamous variants.[4] MpBC fit into the claudin-low and/or basal breast cancer intrinsic subtypes,[5,6] although whether or not claudin low represents an intrinsic subtype or phenotype has recently come into question.[7] A recent large meta-analysis reported that approximately three quarters of all MpBC stain positively for pan-cytokeratin biomarkers (AE1/3, MNF116) and basal cytokeratin biomarkers (34βE12, CK5/6, CK14 and CK17). GATA3, a common diagnostic marker used to identify tumours of breast origin, is expressed by only 21% of MpBC, while a novel breast marker, TRPS1, was shown to be highly expressed in 86% of MpBC, as well as non-metaplastic TNBC and BC more broadly.[8] Frequent expression of p63 was also noted, as was an absence of staining for CD34.[9] Those cases lacking cytokeratin expression were studied in more detail, and determined to be carcinomatous rather than true primary sarcomas in most cases, further evidencing the inter-tumor heterogeneity of breast cancer broadly, and MpBC specifically.[10] Indeed, a pure sarcoma
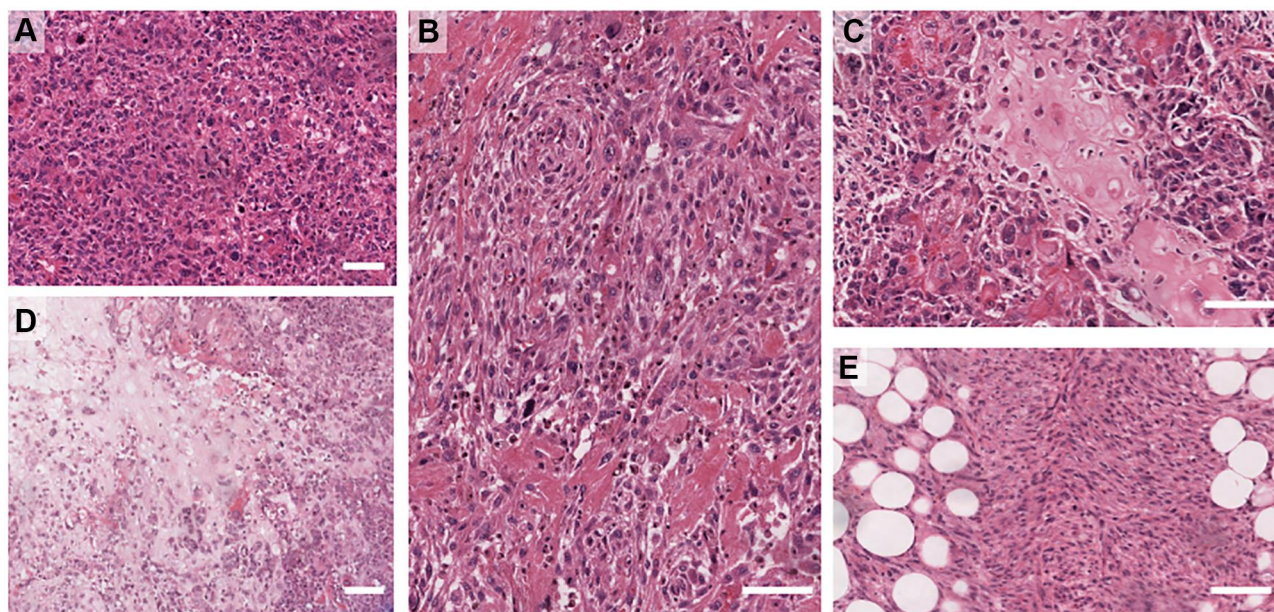

Figure 1 Examples of Metaplastic breast cancer morphologies. (**A**) High-grade, pleomorphic de-differentiated carcinoma (IBC-NST). (**B**) High-grade carcinoma with focal squamous differentiation. (**C**) Osteoid differentiation. (**D**) Chondroid differentiation. (**E**) Spindle differentiation. Scale bar is 100 μm.

of the breast is rare and is a diagnosis of exclusion, requiring extensive sampling; negative stains for p63 and a range of cytokeratins; and, a morphological examination for any evidence of epithelial differentiation.

For the adenosquamous and fibromatosis-like variants of MpBC, the grade is implicitly low, and prognostic outcome is better than for the majority of MpBC which are typically classified as high grade (grade 3) tumors. Although high histologic grading is a relatively consistent finding, its prognostic value is still uncertain.[11] A subset of MpBC tumors with extreme, bizarre cytologic pleomorphisms has been reported,[11] with a noted enrichment in the spindle phenotype.

With respect to the TNM classification system of cancer stage, MpBC present with a larger tumor size (TNM), with reports indicating that ~60% of MpBC have tumors between 2 and 5 cm (T2;[12]). As for triple-negative breast cancers more broadly, lymph node (LN; the N of TNM) positivity is not a prominent feature, with LN metastasis documented in about 24% of patients. Distant metastasis (TNM) occurs with or without LN spread in MpBC, and spread to the lungs and brain has been reported.[13]

MpBC – A Stem-Like Cancer with Treatment Implications

The innate plasticity of MpBC has led to suggestions that it is a stem-cell like breast cancer, and a wealth of data show that MpBC express classic stem cell markers. It is presently considered that there exist three categories of breast cancer stem cell (CSC): an ALDH+ epithelial-like CSC; CD44+/CD24− mesenchymal-like CSCs; and, a hybrid epithelial/mesenchymal-like ALDH+/CD44+/CD24− (reviewed in detail in[14]). The work of Zhang et al[15] demonstrated the increased expression of classic stem cell markers ALDH1 and CD44/CD24 ratios in a series of MpBC, much like the above-noted hybrid CSC state, and also expression of characteristic epithelial to mesenchymal transition (EMT) markers (increased ZEB1 and loss of E-cadherin). This expression of stem-like markers was also supported by Gerhard et al,[16] with most of their series showing positivity for CD44 and loss of CD24, as well as an enrichment for vimentin and loss of the claudins and E-cadherin. Given that cancer stem cells have well-documented chemoresistance,[17] it is unsurprising that MpBC, with their enrichment of both stem-like markers and the hallmarks of EMT,[5,18] also respond

poorly to chemotherapeutics. Notably, MpBC have a high frequency of *PIK3CA* mutations (see below) and these mutations correlate with poor response to neoadjuvant chemotherapy in breast cancer subtypes broadly,[19] and this holds true in the metastatic setting.[20] Drugs targeting the PI3K/AKT axis are emerging in the clinic, may be appropriate for MpBC, and are discussed further below.

MpBC Molecular Landscape

As shown in Table 1, the research community has yet to robustly elucidate a molecular landscape for MpBC, most likely due to the extensive sample heterogeneity. There is limited concordance between studies on the mutations present, however this is likely influenced by the sequencing platform (exome vs panel), and also the subtype composition of the cohorts.

PI-3 Kinase and Ras signaling pathway mutations have been shown to be early events in MpBC pathogenesis.[21] Mutation frequencies reported for MpBC range from 26%-75% for *TP53*, and 23%-70% for *PIK3CA* (Table 1) and this is supported by a recent meta-analysis of 14 studies encompassing 539 cases.[22] Other than *TP53* and *PIK3CA*, the most frequently identified mutations across multiple cohorts occur in *PTEN, NF1, HRAS, PIK3R1*. Emerging data support that the various morphologic elements feature subtly different mutation profiles, with for example, a lack of *PIK3CA* mutations found in those MpBC with chondroid differentiation.[23] Chondroid tumors were also shown to lack mutations in *TERT* promoters.[21] *TERT* promoter mutations were enriched in the spindle and squamous type tumors, while *TP53* mutations were less likely to be in spindled tumors than other MpBC types.[21] An increase in mutations in Wnt pathway genes has been reported for MpBCs,[23] with *WISP3/CCN6* mutations more frequently seen in the epithelial components, and 3/7 *CTNNB1* mutations present only in the spindle compartment of the tumor.[24]

In spite of the private mutations in the different morphological components as noted above, evidence supports that the different histologies have a shared origin, and following a detailed exome sequencing study, Avigdor et al postulated that methylation and/or non-coding changes may also regulate the phenotypic differentiation.[25] To clarify the outstanding elements of the genomic landscape of MpBC, a concerted effort must be made to standardize sequencing approaches on an adequately powered cohort of well-annotated MpBC.

Table 1 Genetic Alterations Identified Across MpBC Cohorts and Morphologies

Ref	Cohort (n)	Morphologies											
		Sqam	Spin	Chon	Oss	Spin/Squam	Spin/Mes	Spin/Chon	Mes	Myo	Angio	mixed	LGASC
Afkhami et al 2019[49]	19	8	6			3	2		2				10
Bataillon et al[50]	10												
Beca et al 2020[51]	3				3								
Edenfield et al 2017[52]	25	2	5	18									
Hayes et al 2008[24]	36	16	12	6	2								
Joneja et al 2017[53]	57	18	20	16						5	1	12	
Krings et al 2018[21]	28	5	5	10		5						3	
McCart Reed 2019[11]	30	10	3						1			16	
Ng et al 2017[23]	35	9	10	16									
Ross et al 2015[54]	20	9	2	9									
Tray et al 2018[55]	192												
Vranic et al 2020[56]	23		23										
Zhai et al 2019[57]	18	4	9	2	1	3		2					
Dave et al 2017[58]	40												

Abbreviations: N/A, not available; NS, not specified; angio, angiosarcomatous; chon, chondroid; LGASC, low grade adenosquamous carcinoma; mes, mesenchymal; myo, myoepithelial; oss, osseous; spin, spindle; squam, squamous.

Uterine carcinosarcoma (UCS) are considered the metaplastic cancers of the gynaecological tract, and a recent study performed a comparative analysis of 57 UCS with 35 MpBC.[26] Genetic differences unique to the UCS were reported, with a significant enrichment for mutations in *FBXW7* and *PPP2R1A*, and *HER2* amplifications, while shared genomic features included alterations in *TP53, PIK3CA, PTEN* and EMT-related Wnt and Notch signalling components. Interestingly, unlike the UCS, almost half of the profiled MpBC had a dominant homologous recombination deficiency (HRD; signature 3) signature, and these same cases showed other features of a HRD including large scale transitions, and allelic imbalance extending to the telomeres.

Precision Oncology for MpBC

In the absence of indications for hormone and anti-HER2 therapies, and given their typically large size at presentation, MpBC are managed with chemotherapeutics in addition to surgery (with/without radiation). However, early

NS	Platform	Hypothesis or Discovery?	TP53 (n,%)	PIK3CA (n,%)	Other alterations in at least 2 cases (n, %)
	Onco48 Ion AmpliSeq	H	13/19, 68	8/19, 42	PTEN (3/19, 16)
	Ion Ampliseq Cancer Hotspot panel v2	H	0/10, 0	7/10, 70	
	Agilent SureSelect Human All Exon v4	D	3/3, 100	1/3, 33.3	OSMR (2/3, 67)
	Ion Ampliseq Cancer Hotspot panel v2	H	16/25, 64	12/25, 48	ERBB4 (9/25, 36); FLT3 (15/25, 60)
	Sanger Sequencing	H	N/A	N/A	CTNNB1 (7/27, 26); WISP3/CCN6 (5/27, 18.5); APC (2/27; 7)
3	Illumina TruSeq Amplicon cancer panel	H	32/57, 65	13/57, 23	HRAS (3/57, 5)
	Custom hybrid capture	H	18/28, 64	9/28, 32	TERT (7/28, 25); HRAS (2/28, 7); KRAS (2/28, 7); NF1 (2/28, 7)
	Illumina Nextera Rapid Capture Exome	D	21/30, 70	10/30, 33	PTEN (7/30, 23), and NF1 (4/30, 13)
	Agilent SureSelect Human All Exon v4	D	24/35, 69	10/35, 29	PTEN (4/35, 11); ARID1A (4/35, 11), KMT2C (4/35, 11); PIK3R1 (4/35, 11)
	FoundationOne	H	15/20, 75	8/25, 40	CDKN2A/B (4/20, 20); CCND3 (3/20; 15), CCNE1 (2/20, 10); EGFR (2/20, 10).
	FoundationOne	H	125/192, 65	67/192, 35	
	SureSelect XT, 592 genes, Agilent	H	6/23, 26	10/23, 43	PTEN (3/23, 13); NF1 (4/19, 17); HRAS (4/23, 17)
	Ion Ampliseq Cancer Hotspot panel v2	H	10/18, 56	7/18, 39	PTEN (3/18, 17)
40	CAST PCR	H	N/A	N/A	RPL39 (39/40, 98)

studies showed that systemic therapy was less effective in MpBC[12] and this data has held true over time and is supported by the overall poor outcomes of MpBC patients.[27] In fact, while 90% of diagnoses of MpBC are for localized disease, half of these patients will progress to advanced BC over time.[28,29] Treatment in the neoadjuvant setting appears to afford little advantage, with a 10–17% pathological complete response rate reported[30–33] for American studies, while studies in Japan and Turkey reported no complete responders.[34,35] It is clear that

efficacious treatments for MpBC are an unmet clinical need, and while some clinical trials specifically for MpBC are being initiated, the potential for novel therapeutic interventions must be capitalized upon.

Genomic Biomarkers and Targeted Therapy for MpBC

MpBC are characteristically triple-negative BC, thus eliminating these patients from current tailored therapeutic

Table 2 Active Trials Open to Metaplastic Breast Cancer Patients

Targeted Therapies			Phase
ARQule	ARQ751 (pan-AKT inhibitor) with fulvestrant or paclitaxel in patients with *PIK3CA/AKT/PTEN* mutations	NCT02761694	Ib
ARTEMIS	A Robust TNBC Evaluation Framework to Improve Survival- molecular profiling of treatment naïve tumour while patient undergoes NACT; targeted therapy trials recommended where appropriate	NCT02276443	Ib/II
L-NMMA	Pan-nitric oxide synthase inhibitor plus docetaxel in advanced or metastatic TNBC patients	NCT02834403	Ib/II
Women's MoonShot	Neoadjuvant Treatment with PaCT (panitumumab (anti-EGFR) carboplatin and paclitaxel) for Patients With Locally Advanced TNBC	NCT02593175	II
	Enzalutamide and Paclitaxel Before Surgery in Treating Patients With Stage I–III Androgen Receptor-Positive TNBC	NCT02689427	IIb
	Liposomal Doxorubicin, Bevacizumab (anti-VEGF), and Everolimus (mTOR inhibitor) in Patients With Locally Advanced TNBC With Tumors Predicted Insensitive to Standard Chemotherapy; A Moonshot Initiative	NCT02456857	II
Immunotherapies			Phase
DART	Dual anti-CTLA4 (nivolumab) and anti-PD1 (ipilimumab) blockade	NCT02834013	II
Morpheus-TNBC	Multiple Immunotherapy-Based Treatment Combinations in Patients With Metastatic or Inoperable Locally Advanced TNBC	NCT03424005	Ib/II
SWOG S1418	Pembrolizumab (anti-PD1) as adjuvant therapy for TNBC after neoadjuvant therapy	NCT02954874	III
	Pembrolizumab (anti-PD1) plus nab-paclitaxel for TNBC and HR+/HER2− breast cancer	NCT02752685	II
TN First-Line	Neoadjuvant Trial of Nab-Paclitaxel and MPDL3280A, a PDL-1 Inhibitor in Patients With TNBC	NCT02530489	II
PAveMenT	Palbociclib and Avelumab in Metastatic AR+ TNBC	NCT04360941	Ib

options of hormone therapy and anti-HER2 therapy. This triple-negativity, does however make them eligible for a multitude of trials currently recruiting, including those assessing benefit of immune checkpoint inhibitors; a non-exhaustive list of open trials is presented in Table 2.

MpBC show frequent alterations in the PI3K/AKT/mTOR pathway making them candidates for targeted therapies such as everolimus, temsirolimus, and alpelisib. In a Phase I intervention, a 42% rate of partial/complete remission was reported for a combination of temsirolimus and bevacizumab (HIF inhibitor).[36] A 25% response rate (complete/partial response) was achieved in MpBC treated with temsirolimus/everolimus in combination with standard chemotherapy and a 21% objective response rate was also reported for the regimen of doxorubicin, bevacizumab and temsirolimus/everolimus,[37] however genetic analysis showed that while PI3K pathway alterations were associated with a significant improvement in objective response rate (31% vs 0%) they were not associated with an improved clinical benefit rate (44% vs 45%). Detailed analysis of this

trial data showed an improvement in overall survival for the MpBC patients, and suggests that MpBC histology is an indicator for doxorubicin with bevacizumab and everolimus/temsirolimus.[38] A lone MpBC participant in the BELLE-4 Phase II/III trial responded well to a combined therapy of paclitaxel and the PI3K inhibitor buparlisib[39] although toxicity was noted, and indeed buparlisib was subsequently discontinued from development, with a significantly higher burden of adverse effects noted for buparlisib than alpelisib in the B-YOND (hormone receptor positive, phase Ib) trial.[40] Pre-clinical data in MpBC patient derived xenograft models suggest that a combination of PI3K and MAPK inhibitors may be a potential avenue for therapy in *PIK3CA* mutated MpBC patients.[41]

CDK4/6 inhibitors (eg, ribociclib, palbociclib, abemaciclib) are now approved as standard of care for advanced, hormone receptor positive breast cancers, however this proliferation check-point may also be a useful target in TNBC, and trials are underway to determine the efficacy of this approach (reviewed in[42]), including in combination with

immune checkpoint inhibitors (PAveMenT: NCT04360941). A recent case report demonstrated a dramatic but short-term benefit from combined dabrafenib and trametinib in an advanced MpBC patient.[43] Dabrafenib and trametinib target BRAF and MEK signalling, respectively, and their application in MpBC has not previously been reported.

Although a pre-clinical study did not support the efficacy of PARP inhibitor olaparib in an MpBC-like mouse model,[44] given the recent evidence of a dominant HRD signature in almost 50% of the MpBC profiled,[26] the suggestion by Tray et al[45] that PARP inhibition for MpBC needs further study is certainly warranted. These studies together support further investigations into a range of targeted therapies and highlight their potential value in MpBC.

Targeting the MpBC Immune Microenvironment

The potential benefit of therapeutic modulation of the immune system in breast cancer is becoming increasingly clear for TNBC, as well as MpBC. A case report of a remarkable, durable response to pembrolizumab (anti-PD-1) in combination with nab-Paclitaxel in advanced, pre-treated spindled MpBC was reported in 2017.[46] A similar combination of durvalumab (anti-PD-L1) and paclitaxel was also shown to provide a sustained, complete response in a second case report of advanced MpBC, this time with squamous features.[47] In this case, 20% of tumor cells stained with medium intensity (clone SP142), and there was an absence of staining in the TILS; while in the former case, 100% of tumor cells stained positively for PD-L1 using the 22C3 clone. Indeed, there is no standardized definition criteria for PD-L1 staining at this stage, and the characterization of expression of this and other immune checkpoint markers across TNBC and MpBC has only recently emerged. As shown in Table 3, heterogeneity in percentage positivity of PD-L1 in tumor cells is reported across TNBC, with a higher rate of positivity consistently reported for MpBC. MpBC tumor cells show a range of PD-L1 expression from 17% to 80%, recording both cytoplasmic and membranous staining, and in the immune cells from 48% to 69%. Combinations of immune-checkpoint inhibitors are also being evaluated, with the DART (Dual Anti-CTLA-4 and Anti-PD-1 blockade in Rare Tumors, Table 2) trial facilitating an MpBC specific assessment.[48] Primary endpoint data confirmed clinical activity of ipilimumab combined with nivolumab and resulted in 3 cases of 17 showing a durable response, which

Table 3 PD-L1 Expression in Metaplastic Breast Cancer

Ref	Year	Cohort	PD-L1 Antibody Clone	Staining Criteria	% Cases with Positive PD-L1 Expression
Mittendorf et al[59]	2014	120 TNBC	5H1	Memb. staining in >5% tumor cells	19% tum
Joneja et al[53]	2016	297 TNBC (75 MpBC)	SP142	Tumor with ≥2+ intensity in ≥5% of the tumor cells	MpBC 46% tum; 9% TNBC tum
Beckers et al[60]	2016	161 TNBC	E1L3N	≥ 1% tum memb. or cyto. and ≥1% immune cells in the stromal compartment	64% tum memb., 80% cyto. and 93% stromal compartment
Dogukan et al[61]	2019	61 TNBC (incl 6 MpBC)	E1L3N	Memb. or cyto. staining in ≥1% tumor cells; ≥5% staining in peri-tumoral regions	38% tumor cell; 48% tumor immune microenvironment
Morgan et al[62]	2020	146 TNBC (27 MpBC)	SP263	Memb. staining in ≥1% of tumor cells; immune cells	MpBC 30% tum, 73% immune; TNBC 10% tum, 59% immune
Afkhani et al[49]	2019	14 MpBC	SP263	>1+ intensity in >1% immune cells	50% immune cells
Lien et al[63]	2020	82 MpBC	SP142	% tumor cells; immune cells as % of tumor area	17% tum; 48% immune cell
Kalaw et al[64]	2020	125 MpBC	E1L3N	≥5% tumor/immune cells displaying cyto. or memb. staining	73% tum, 69% stromal TILS
Chao et al[65]	2020	60 MpBC	E1L3N	≥ 1% memb. or cyto. Staining	50% tum, 60% TILs

Abbreviations: BC, breast cancer; ER, estrogen receptor; IBC-NST, invasive breast cancer-no special type; MpBC, metaplastic breast cancer; NACT, neo-adjuvant chemotherapy; PR, progesterone receptor; TILs, tumour infiltrating lymphocytes; TNBC, triple-negative breast cancer; Cyto, cytoplasmic; memb., membraneous; tum, tumour.

supports further investigation. It is hoped that trials such as the Morpheus-TNBC Phase 1/1b umbrella trial (Table 2, NCT03424005), will provide insights to further our understanding of the biomarkers and patient indicators for a range of immunotherapeutic interventions.

Summary

The morphologically diverse metaplastic breast cancers account for significant global morbidity and mortality, in spite of their relatively rare frequency, due to their aggressive clinical course. As more molecular pathology data emerges on the genomic underpinnings of this intriguing tumor type, we are increasingly better placed to consider MpBC for targeted therapies and immunotherapies.

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