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Short communication

Metaplastic breast cancers: Genomic profiling, mutational burden and tumor-infiltrating lymphocytes

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ABSTRACT

Metaplastic breast cancer (MPBC) is a rare subtype that accounts for <1% of all breast cancers. Although these are typically "triple negative," they are relatively chemotherapy-refractory compared to conventional triple negative invasive breast cancers with more aggressive features and an overall poor prognosis. MPBC is a heterogeneous group of tumors that are enriched for *TP53* and *PIK3CA* mutations, and have been found to have high PD-L1 expression though the mechanisms underlying its immunogenicity remain unclear. We perform comprehensive genomic profiling in the largest MPBC dataset (n = 192) to date and assess for other potential biomarkers of immune response.

1. Introduction

Metaplastic breast cancers (MPBC) comprise a group of rare tumors that represent 0.2–5% of all breast cancers [1]. These include histologically distinct subtypes that are composed of both adenocarcinoma and metaplastic (either mesenchymal or squamous) elements (Fig. 1) [2]. MPBC are genetically heterogeneous and enriched for several mutations which vary depending upon their histologic subtypes. *TP53* is the most common somatic mutation seen in as high as 50–75% of cases and has been associated with tumor progression and invasive disease [3–7]. Mutations in *PIK3CA* and *PTEN* are also frequently seen, suggesting an essential role of the PI3K/AKT/mTOR pathway in MPBC tumorigenesis [3,4,8]. Although most MPBC are triple negative and lack expression of the estrogen, progesterone and human epidermal growth factor receptor (HER2) receptors, they are typically more aggressive than conventional triple negative breast cancers (TNBC) with a median overall survival (OS) of eight months in the metastatic setting, implying that novel approaches are urgently needed [9,10].

Immune cell infiltration of early TNBC is associated with better survival [11], which raises the possibility that harnessing the anti-cancer immune response may improve outcomes in metastatic TNBC. Programmed cell death-1 (PD-1)/Programmed death-ligand 1 (PD-L1) inhibitors have shown activity in PD-L1+ metastatic TNBC [12]. As most MPBC are triple negative, immunotherapy may be an effective approach. Furthermore, frequent overexpression of PD-L1 was recently demonstrated in primary MPBC, with tumor PD-L1 expression observed in 33/72 (46%) cases [6]. Among the mechanisms by which tumor cells can regulate PD-L1 expression are oncogenic alterations such as the PTEN/PI3K and the

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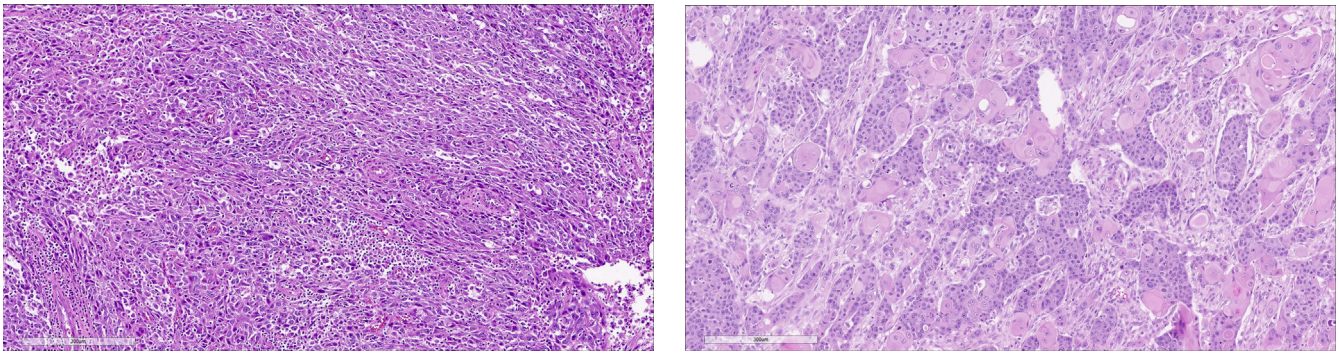

Fig. 1. Representative H and E stains of two metaplastic breast carcinomas. Panel A: Undifferentiated carcinoma with spindle and giant cells at 10×, with moderate tumor-infiltrating lymphocytes (TIL). Panel B: Squamous cell carcinoma at 10×, no TIL.

Ras–MAPK pathways which have been shown to contribute to immune evasion in breast cancer [13,14]. MPBC are enriched for *PIK3CA* mutations when compared with TNBC, which may account for the significantly increased tumoral PD-L1 expression. Other causes of tumoral PD-L1 expression such as genomic amplification of 9p24.1 described in TNBCs could also contribute [15]. PD-L1 expression can also be upregulated by the induction of epithelial to mesenchymal transition (EMT) [16] which is a typical feature of MPBC [17]. Tumor-infiltrating lymphocytes (TIL) have also been demonstrated in MPBC including strongly PD-1 positive TIL [6], suggestive of an immunogenic cancer phenotype which may result from higher mutation frequency and gene copy number variance of MPBC compared with other subtypes.

The limited treatment options and poor prognosis of MPBC along with the high PD-L1 expression and TIL presence provide the rationale to study immunotherapies in this subtype. Encouragingly, in a recent case report, a patient with widely metastatic MPBC which by immunohistochemistry showed PD-L1 expression of 100% in tumor cells had a dramatic response to *anti*-PD-1 immunotherapy in an ongoing clinical trial (clinical trials.gov: NCT02752685) [18].

However, further information on genomic alterations including PD-L1 locus amplification, microsatellite instability (MSI) status, tumor mutation burden (TMB) and associated TIL infiltration as a marker of a pre-existing adaptive immune response in a larger set of patients is critical to the rational design of combination therapies. Here we perform comprehensive genomic profiling (CGP) on the largest MPBC dataset to date, along with histopathologic assessment of TIL in a subset, to assess potential biomarkers of immunotherapy response for MPBC, TMB, MSI and amplification of 9p24.1 (contains PD-L1 (CD274) and PD-L2 gene loci).

2. Methods

192 cases of MPBC were identified from 13,391 locally aggressive, relapsed and metastatic breast cancer samples using the Foundation Medicine database from January 1, 2010 to July 31, 2017. Hybrid capture-based CGP was performed using the FoundationOne assay, with DNA extracted from 40 μm of formalin-fixed, paraffin-embedded (FFPE) tissue sections and adaptor ligation-based libraries to a mean coverage depth of >650× for up to 315 cancer-related genes. Results were assessed for frequency of genomic alterations (GA) including base substitutions, insertions/deletions, select rearrangements, and copy number changes. Gene amplification was defined as 6 or more copies. TMB was calculated as the total number of somatic, coding point mutations and indels per megabase (Mb) in 1.1 Mb of sequenced tumor DNA. For this study, patient cases were classified as TMB-high (≥15 mutations/Mb) or TMB-low (<15 mutations/Mb). MSI status was assessed using an algorithm based on sequencing results. A total of 114 loci were analyzed for length variability and compiled into an overall MSI score ranging from MSI-high (MSI-H) representing patients with DNA mismatch repair deficiency (dMMR), MSI-ambiguous/unknown, or MSI-stable (MSS). We explored the association of histopathologically enumerated TIL and TMB. TIL were assessed on archived Hematoxylin and eosin stained tumor sections and enumerated per guidelines by the TIL Working Group [19] in a subset of MPBC with the highest TMB and compared with low TMB cases with the Wilcoxon rank-sum test.

3. Results

A total of 192 cases of MPBC (1.4% of breast cancers) are included in this study. All patients were female with a median age of 60 (range 24–86). All MPBC cases (100%) harbored a wide variety of GA involving more than 100 individual genes, the most frequent being *TP53* (65%) and *PIK3CA* (35%) (Fig. 2). There were no MSI-H cases (0/126) and amplification of 9p24.1 was observed in only one case (0.6%) (Table 1). Most MPBC had a low tumor mutation burden, with a median TMB of 2.7 mutations/Mb (range 0–39.6). Only 4/192 cases (2%) had high TMB (score 15 or greater), including 3 cases (1.6%) with TMB >20. Tumor sections were available for TIL review from 9/11 cases with the highest TMB scores (>10 mutations/Mb), as well as 11 control cases with the lowest TMB scores. TIL were more frequently observed in high versus low TMB MPBC, with median TIL percentage of 40 and 20 (range 10–80 and range 10–60), respectively, although this difference was not statistically significant (Wilcoxon rank-sum test, $p = 0.15$).

4. Discussion

This is the largest dataset of MPBC that has been genomically profiled to date. Our findings confirm that MPBC are highly enriched for *TP53* and *PIK3CA* mutations, which may have both predictive and prognostic implications. In a single institution study, patients with *PIK3CA* mutated tumors had a worse prognosis and shorter recurrence free survival than patients with *PIK3CA* wild-type tumors (RFS 33% vs. 100%, $p < 0.01$) [20]. However, when treated with liposomal doxorubicin, bevacizumab, and an mTOR inhibitor (either everolimus or temsirolimus), patients with the *PIK3CA* mutation had significant objective response rates compared to those without the mutation [21]. These findings provide a rationale for therapies targeting the PI3K/AKT/mTOR pathway [22]. *TP53* mutations were also recently associated with improved clinical outcomes in MPBC compared to wild-type [23] and predicted sensitivity to antiangiogenesis agents [24], though these findings

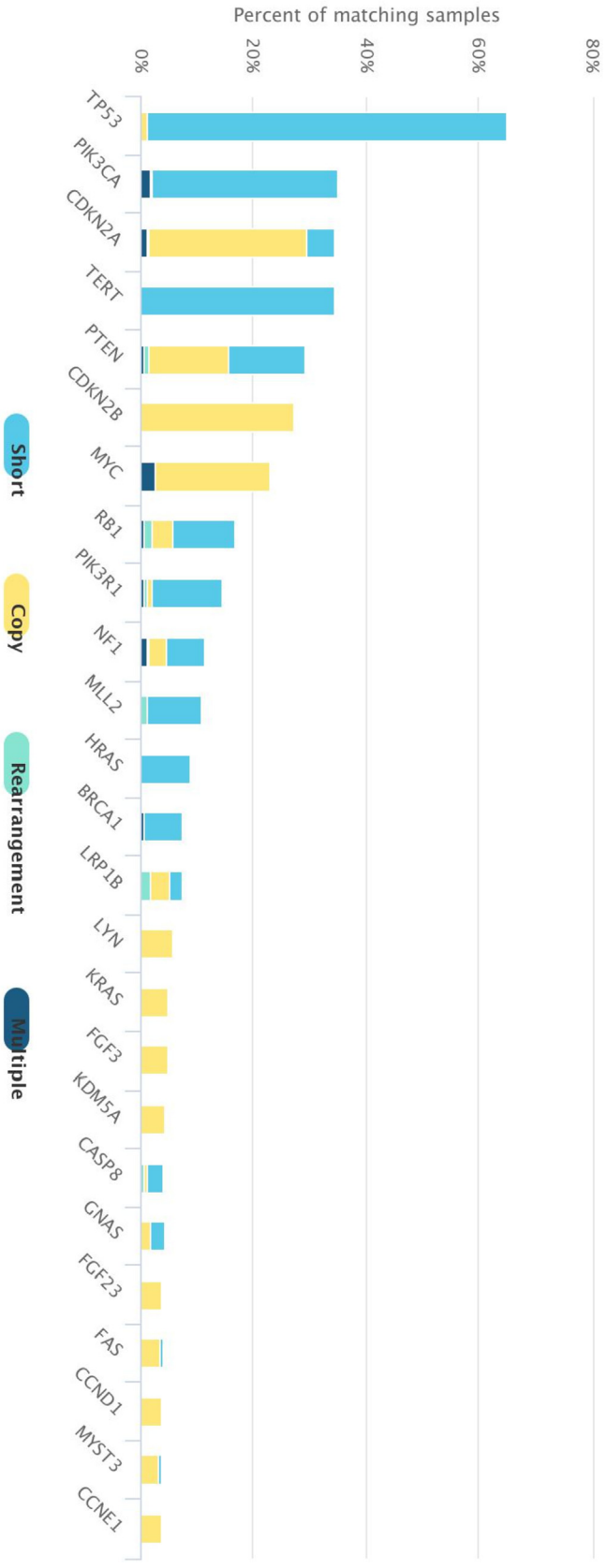

Fig. 2. Short tail distribution of genomic alterations in MPBC (n = 192) with frequencies ≥3.5%. Short: substitutions and indels.

Table 1
Frequency of selected genomic alterations.

Genomic Alteration	Frequency
TP53	125/192 (65%)
PIK3CA	67/192 (35%)
MSI-High	0/126 (0%)
9p24.1 (PD-L1) amplification	1/192 (0.5%)

need to be validated in larger studies.

Previous studies have shown that MPBC are associated with high tumoral PD-L1 expression, although our study suggests that this is not due to *PD-L1 gene* amplification, which was a rare event. Additionally, most MPBC had a low TMB and are microsatellite stable. TIL analysis in the small subset of tumors with a high TMB showed significant infiltration of lymphocytes (median of 40%, range 10–80%) but no statistically significant correlation was seen between TMB and TIL, either due to the small sample size or the finding that low TMB tumors also had significant lymphocytic infiltration (median 20%, range 10–60). TIL appear to be frequent in MPBC, confirming recent findings by Joneja et al. [6]. Altogether these findings suggest that additional processes are involved in the immunogenicity of MPBC, and further studies are needed. Correlative analyses in blood and tumor tissue are planned in an ongoing Phase II therapeutic trial, led by the National Cancer Institute (NCI) and the Southwestern Oncology Group (SWOG), the DART study (NCT02834013) which evaluates dual *anti*-CTLA-4 (ipilimumab) and *anti*-PD-1 (nivolumab) blockade in rare tumors, including a MPBC cohort (Arm 36).

Conflicts of interest

Nancy Tray: No disclosures.
Jessica Taff: No disclosures.
Baljit Singh: No disclosures.
James Suh: Former employee of Foundation Medicine, Incorporated; Stock and Other Ownership Interests: Loxo Oncology.
Nhu Ngo: Employee of Foundation Medicine, Incorporated.
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References

[1] Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ. Metaplastic carcinoma. WHO classification of tumours of the breast. fourth ed. Lyon, France: International Agency for Research on Cancer (IARC); 2012. p. 48–52.

[2] Weigelt B, Eberle C, Cowell CF, Ng CK, Reis-Filho JS. Metaplastic breast carcinoma: more than a special type. Nat Rev Canc 2014;14(3):147–8.

[3] Ng CKY, Piscuoglio S, Geyer FC, et al. The landscape of somatic genetic alterations in metaplastic breast carcinomas. Clin Canc Res 2017;23(14):3859–70.

[4] Ross JS, Badve S, Wang K, et al. Genomic profiling of advanced-stage, metaplastic breast carcinoma by next-generation sequencing reveals frequent, targetable genomic abnormalities and potential new treatment options. Arch Pathol Lab Med 2015;139(5):642–9.

[5] Piscuoglio S, Ng CKY, Geyer FC, et al. Genomic and transcriptomic heterogeneity in metaplastic carcinomas of the breast. NPJ Breast Cancer 2017;3:48.

[6] Joneja U, Vranic S, Swensen J, et al. Comprehensive profiling of metaplastic breast carcinomas reveals frequent overexpression of programmed death-ligand 1. J Clin Pathol 2017;70(3):255–9.

[7] Krings G, Chen YY. Genomic profiling of metaplastic breast carcinomas reveals genetic heterogeneity and relationship to ductal carcinoma. Mod Pathol : Official J United States Can Acad Pathol Inc Nov 2018;31(11):1661–74.

[8] Moulder S, Helgason T, Janku F, et al. Inhibition of the phosphoinositide 3-kinase pathway for the treatment of patients with metastatic metaplastic breast cancer. Ann Oncol 2015;26(7):1346–52.

[9] Aydiner A, Sen F, Tambas M, et al. Metaplastic breast carcinoma versus triple-negative breast cancer: survival and response to treatment. Medicine 2015;94(52). e2341.

[10] Rayson D, Adjei AA, Suman VJ, Wold LE, Ingle JN. Metaplastic breast cancer: prognosis and response to systemic therapy. Ann Oncol 1999;10(4):413–9.

[11] Adams S, Gray RJ, Demaria S, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two Phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol : Official J Am Soc Clin Oncol 20 Sep 2014;32(27):2959–66.

[12] Schmid P, Adams S, Rugo HS, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 29 Nov 2018;379(22):2108–21.

[13] Mittendorf EA, Philips AV, Meric-Bernstam F, et al. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res 2014;2(4):361–70.

[14] Loi S, Dushyanthen S, Beavis PA, et al. RAS/MAPK activation is associated with reduced tumor-infiltrating lymphocytes in triple-negative breast cancer: therapeutic cooperation between MEK and PD-1/PD-L1 immune checkpoint inhibitors. Clin Canc Res : Official J Am Assoc Canc Res 2016;22(6):1499–509.

[15] Barrett MT, Anderson KS, Lenkiewicz E, et al. Genomic amplification of 9p24.1 targeting JAK2, PD-L1, and PD-L2 is enriched in high-risk triple negative breast cancer. Oncotarget 2015;6(28):26483–93.

[16] Alsuliman A, Colak D, Al-Harazi O, et al. Bidirectional crosstalk between PD-L1 expression and epithelial to mesenchymal transition: significance in claudin-low breast cancer cells. Mol Canc 2015;14:149.

[17] Zhang Y, Toy KA, Kleer CG. Metaplastic breast carcinomas are enriched in markers of tumor-initiating cells and epithelial to mesenchymal transition. Mod Pathol : Official j United States Can Acad Pathol Inc 2012;25(2):178–84.

[18] Adams S. Dramatic response of metaplastic breast cancer to chemo-immunotherapy. NPJ Breast Cancer 2017;3:8.

[19] Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26(2):259–71.

[20] Afkhami M, Amanam IU, Aoun PA, et al. Genomic profiling of metaplastic breast cancer: a single center experience. J Clin Oncol 2017;35(15_suppl). e12041-e12041.

[21] Basho RK, Gilcrease M, Murthy RK, et al. Targeting the PI3K/AKT/mTOR pathway for the treatment of mesenchymal triple-negative breast cancer: evidence from a Phase 1 trial of mTOR inhibition in combination with liposomal doxorubicin and bevacizumab. JAMA Oncol 2017;3(4):509–15.

[22] Moulder S, Moroney J, Helgason T, et al. Responses to liposomal Doxorubicin, bevacizumab, and temsirolimus in metaplastic carcinoma of the breast: biologic rationale and implications for stem-cell research in breast cancer. J Clin Oncol : Official J Am Soc Clin Oncol 2011;29(19). e572–575.

[23] Vallecillo LB, Chang JT, Chen K, et al. Whole exome sequencing of metaplastic breast cancer (MpBC): effect of mutation status on survival. J Clin Oncol 2017;35(15_suppl). 1090-1090.

[24] Wheler JJ, Janku F, Naing A, et al. TP53 alterations correlate with response to VEGF/VEGFR inhibitors: implications for targeted therapeutics. Mol Canc Therapeut 2016;15(10):2475–85.