

Genomic Profiling of Advanced-Stage, Metaplastic Breast Carcinoma by Next-Generation Sequencing Reveals Frequent, Targetable Genomic Abnormalities and Potential New Treatment Options

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• **Context.**—Metastatic metaplastic breast carcinoma (MPBC) is an uncommon, but aggressive, tumor resistant to conventional chemotherapy.

Objective.—To learn whether next-generation sequencing could identify potential targets of therapy for patients with relapsed and metastatic MPBC.

Design.—Hybridization capture of 3769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer was applied to a minimum of 50 ng of DNA extracted from 20 MPBC formalin-fixed, paraffin-embedded specimens and sequenced to high uniform coverage.

Results.—The 20 patients with MPBC had a median age of 62 years (range, 42–86 years). There were 9 squamous (45%), 9 chondroid (45%), and 2 spindle cell (10%) MPBCs, all of which were high grade. Ninety-three genomic alterations were identified, (range, 1–11) with 19 of the 20 cases (95%) harboring an alteration that could potentially lead to a targeted treatment option. The most-

common alterations were in *TP53* (n = 69; 75%), *PIK3CA* (n = 37; 40%), *MYC* (n = 28; 30%), *MLL2* (n = 28; 30%), *PTEN* (n = 23; 25%), *CDKN2A/B* (n = 19; 20%), *CCND3* (n = 14; 15%), *CCNE1* (n = 9; 10%), *EGFR* (n = 9; 10%), and *KDM6A* (n = 9; 10%); *AKT3*, *CCND1*, *CCND2*, *CDK4*, *FBXW7*, *FGFR1*, *HRAS*, *NF1*, *PIK3R1*, and *SRC* were each altered in a single case. All 16 MPBCs (100%) that were negative for *ERBB2* (HER2) overexpression by immunohistochemistry and/or *ERBB2* (HER2) amplification by fluorescence in situ hybridization were also uniformly (100%) negative for *ERBB2* amplification by next-generation sequencing-based copy-number assessment.

Conclusions.—Our results indicate that genomic profiling using next-generation sequencing can identify clinically meaningful alterations that have the potential to guide targeted treatment decisions in most patients with metastatic MPBC.

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Metaplastic carcinoma of the breast (MPBC) is an uncommon, malignant tumor with a variable histologic appearance that differs substantially from classic invasive ductal adenocarcinoma and invasive lobular ade-

nocarcinoma.^{1–5} Metaplastic carcinoma of the breast typically presents at a more-advanced stage than either invasive ductal or lobular adenocarcinoma cases and, in general, has a worse prognosis.^{6,7} On routine biomarker testing for estrogen receptor (ER) and/or progesterone receptor (PR) status, most MPBCs are negative.⁸ Similarly, virtually all MPBC cases are negative for HER2 overexpression and amplification by either immunohistochemistry (IHC) and/or fluorescence in situ hybridization.^{8,9} These findings combined with messenger RNA (mRNA) profiling studies indicate that most MPBCs are so-called triple-negative breast cancers (TNBCs) that cluster with the basaloid-phenotype cancers in the breast cancer molecular-portraits system.^{10–12} However, although the TNBC and basaloid types of breast cancer are classically associated with responsiveness to cytotoxic chemotherapy,^{13,14} metastatic MPBC is generally regarded as a chemoresistant, highly aggressive form of the disease.^{15,16} Given the poor prognosis and reduced response to treatment for MPBC, this study was performed to evaluate potential, targeted treatment opportunities for patients with MPBC.

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Table 1. Genomic Alterations in 20 Cases of Advanced-Stage Metaplastic Breast Carcinoma

Case No.	Percentage of Tumor by Microscopy/Tumor Purity ^a	Coverage Depth, ×	Age, y	Sample Used for NGS	Subtype	Tumor Grade	Tumor Stage	Clinical ER/PR/HER2 Status	Genomic Alterations, Mutant Allele Frequency (%); Copy No.
B01	70/30	711	62	Breast	Squamous	3	4	ER-/PR-/HER2-	PTEN loss; 0 RB1 splice site 607+2T>A (19) TP53 Y220S (26) CREBBP K1086* (18) PTEN R14fs*10 (24) TP53 V173fs*7 (33) TNFRSF14 loss; 0 PIK3CA H1047R (4) TP53 K320* (5) MCL1 amplification; 7 ARID1A R1722* (4) NFKBIA amplification; 6 PTPN11 S502L (2) PIK3R1 Y580fs*19 (42) CCND2 amplification; 24 CDKN2A loss; 0 FGF23 amplification; 24 FANCA C1410fs*6 (12) MYC amplification; 8 CDK4 amplification; 15 MYC amplification; 9 TP53 R248Q (61) CCND1 amplification; 7 TP53 C277F (84) LRP1B S229* (41) MLL2 V1554fs*48 (40) FGF19 amplification; 7 FGF3 amplification; 7 FGF4 amplification; 7 PTEN K327fs*16 (63) TP53 N131del (75) KDM6A Q692fs*37 (56) RB1 splice site 1421+1G>C (64) CDKN2A/B loss; 0 HRAS Q61H (25) MLL R2075H (23) MSH2 loss; 0 TNFAIP3 V377M (24) MLL2 P2354fs*30 (23) EGFR amplification; 7 PTEN loss; 0 CCND3 amplification; 8 MYC amplification; 7 TP53 R273C (66) MYST3 amplification; 7
B02	80/30	749	63	Lymph node	Squamous	3	3		
B03	60/20	906	49	Breast	Squamous	3	2	ER+/PR+/HER2-	
B04	60/70	877	73	Breast	Spindle	3	3		
B05	70/40	926	71	Breast	Spindle	3	4	ER-/PR-/HER2-	
B06	60/84	1123	53	Lung	Chondroid	3	4	ER-/PR-/HER2-	
B07	30/58	1234	61	Chest wall	Chondroid	3	4	ER-/PR-/HER2-	
B08	80/85	1214	67	Breast	Chondroid	3	4	ER-/PR-/HER2-	
B09	70/80	1073	59	Brain	Squamous	3	4	ER-/PR-/HER2-	
B10	80/40	1020	57	Chest wall	Chondroid	3	4	ER-/PR-/HER2-	
B11	50/49	472	53	Breast	Squamous	3	4		

Table 1. Continued

Case No.	Percentage of Tumor by Microscopy/Tumor Purity ^a	Coverage Depth, ×	Age, y	Sample Used for NGS	Subtype	Tumor Grade	Tumor Stage	Clinical ER/PR/HER2 Status	Genomic Alterations, Mutant Allele Frequency (%); Copy No.
B12	70/76	735	66	Breast	Chondroid	3	4	ER-/PR+/HER2-	PIK3CA H1047R (45) PTEN loss; 0 ATR R177Q (62) CDKN2A/B loss; 0 TP53 E285K (47) PRKDC V3312M (46) PIK3CA H1047R (8) TP53 P64fs*85 (33) RB1 D156fs*19 (17) MED12 G44D (14) PIK3CA E545K (48) CCND3 amplification; 7 CCNE1 amplification; 8 MYC amplification; 13 TP53 S94* (58) MLL2 L4426fs*6 (10) PIK3CA E542K (59) TP53 R273C (78) MLL2 Q3499* (77)
B13	60/39	934	86	Breast	Squamous	3	4	ER-/PR-/HER2-	
B14	70/20	870	69	Breast	Squamous	3	4	ER-/PR-/HER2-	EGFR amplification; 30 PIK3CA H1047R (76) PI3KCA amplification; 16)
B15	80/18	684	64	Lung	Chondroid	3	4	ER-/PR-/HER2-	CDKN2A/B loss; 0 SOX2 amplification; 7) TP53 splice site 814_919+192del (34) AKT3 amplification; 8) FBXW7 Q508* (28) NF1 V891fs*10 (21) TP53 splice site 559+1G>T (45) KDM6A N1130fs*8 (19) LRP1B deletion, exons 4–21 (not determined) PRKDC P1159S SF3B1 K666T (27) MLL2 F2739fs*18 (27) PALB2 W1038* (21) PIK3CA E545K (45) MCL1 amplification; 7 CCNE1 amplification; 9 TP53 S94* (59) MYC amplification; 7 CCND3 amplification; 7 MLL2 L4426fs*6 (8)
B16	80/60	608	42	Breast	Chondroid	3	4	ER-/PR-/HER2-	
B17	40/60	607	60	Breast	Chondroid	3	4	ER-/PR-/HER2-	
B18	70/30	598	43	Breast	Squamous	3	4	ER-/PR-/HER2-	
B19	70/30	593	71	Breast	Squamous	3	4	ER-/PR-/HER2-	

Table 1. Continued

Case No.	Percentage of Tumor by Microscopy/Tumor Purity ^a	Coverage Depth, ×	Age, y	Sample Used for NGS	Subtype	Tumor Grade	Tumor Stage	Clinical ER/PR/HER2 Status	Genomic Alterations, Mutant Allele Frequency (%); Copy No.
B20	30/58	733		Breast	Chondroid	3	4	ER-/PR-/HER2-	FGFR1 amplification; 13 SRC amplification; 9 MYC amplification; 8 TP53 R273C (84) LRP1B splice (27) C17orf39 amplification; 12

Abbreviation: NGS, next generation sequencing.

^a Calculated estimate of the percentage of DNA derived from tumor DNA based on aneuploidy.

MATERIALS AND METHODS

Hybridization capture of 3769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer was applied to a minimum of 50 ng of DNA extracted from 20 formalin-fixed, paraffin-embedded cases of relapsed and metastatic MPBC that had previously been treated with systemic therapies.¹⁷ There were no early stage or untreated MPBC cases included in this study. The 20 cases represented all (100%) of the MPBC samples received at Foundation Medicine, Inc (Cambridge, Massachusetts), for next-generation sequencing between January 1, 2013, and February 1, 2014. The 20 MPBC samples were sequenced to high, uniform coverage (average ×833, with >99% of exons covered at greater than ×100). All MPBC cases were reviewed by 2 pathologists (J.S.R. and S.B.) and were subdivided into histologic subtypes: predominantly spindle cell (sarcomatoid), predominantly squamous, predominantly chondroid, and mixed. Formalin-fixed, paraffin-embedded specimens were sequenced to high (average ×833), uniform coverage, as previously described.¹⁷ Genomic alterations (base substitutions, small insertions/deletions [indels], select rearrangements, and copy-number alterations) were determined. *Potentially actionable alterations* were defined as those linked to anticancer drugs on the market or in registered clinical trials. Local site permissions to use clinical samples and their accompanying medical records and pathology reports were used for this study. No additional research was performed on the tumor samples beyond the DNA sequencing.

RESULTS

The 20 patients with MPBC had a median age of 62 years (range, 42–86 years). There were 9 predominantly squamous (45%), 9 predominantly chondroid (45%), and 2 predominantly spindle cell (sarcomatoid) (10%) MPBCs, all of which were originally diagnosed as high-grade tumors (Table 1). All MPBCs (20 of 20; 100%) had pure MPBC histology, and none of the cases (0 of 20; 0%) in this series had foci of either classic invasive ductal carcinoma or invasive lobular carcinoma admixed with the metaplastic carcinoma areas. All of the MPBCs (20 of 20; 100%) were advanced stage: 1 MPBC (5%) was stage II, 2 (10%) were stage III, and 17 (85%) were stage IV. Because this study included only patients with relapsed and metastatic disease, there were no well-differentiated or stage-I tumors included in this patient cohort. Of the 16 cases (80%) with available biomarker results, 6% (n = 1) were ER⁺, 12% (n = 2) were PR⁺, and 100% (n = 16) were HER2⁻ by either IHC or fluorescence in situ hybridization testing. Thus, 87% of the MPBCs were TNBC and 13% were either ER⁺ or PR⁺ positive. The targeted next-generation sequencing assay used in this study identified 93 genomic alterations in the 20 MPBC, with at least one alteration identified in all cases (mean [SD], 4.65 [2.08] per tumor). Nineteen (95%) of the 20 MPBC cases harbored 36 alterations that could potentially lead to a targeted therapeutic treatment option (mean [SD], 1.8 [1.08] per tumor).^{18,19} The most-common, biologically relevant alterations were alterations in *TP53* (n = 15; 75%), *MYC* (n = 6; 30%), *MLL2* (n = 6; 30%), and *KDM6A* (n = 2; 10%). The most-common, potentially targetable alterations were mutations, amplifications, and homozygous deletions of *PIK3CA* (n = 8; 40%), *PTEN* (n = 5; 25%), *CDKN2A/B* (n = 4; 20%), *CCND3* (n = 3; 15%), *CCNE1* (n = 2; 10%), and *EGFR* (n = 2; 10%), with *AKT3*, *CCND1*, *CCND2*, *CDK4*, *FBXW7*, *FGFR1*, *HRAS*, *NF1*, *PIK3R1*, and *SRC* altered in a single case (Figure 1; Table 1). The actionable alterations discovered in the MPBC have potential for a variety of targeted therapies, including inhibitors of mTOR, cyclin-dependent kinase inhibitors,

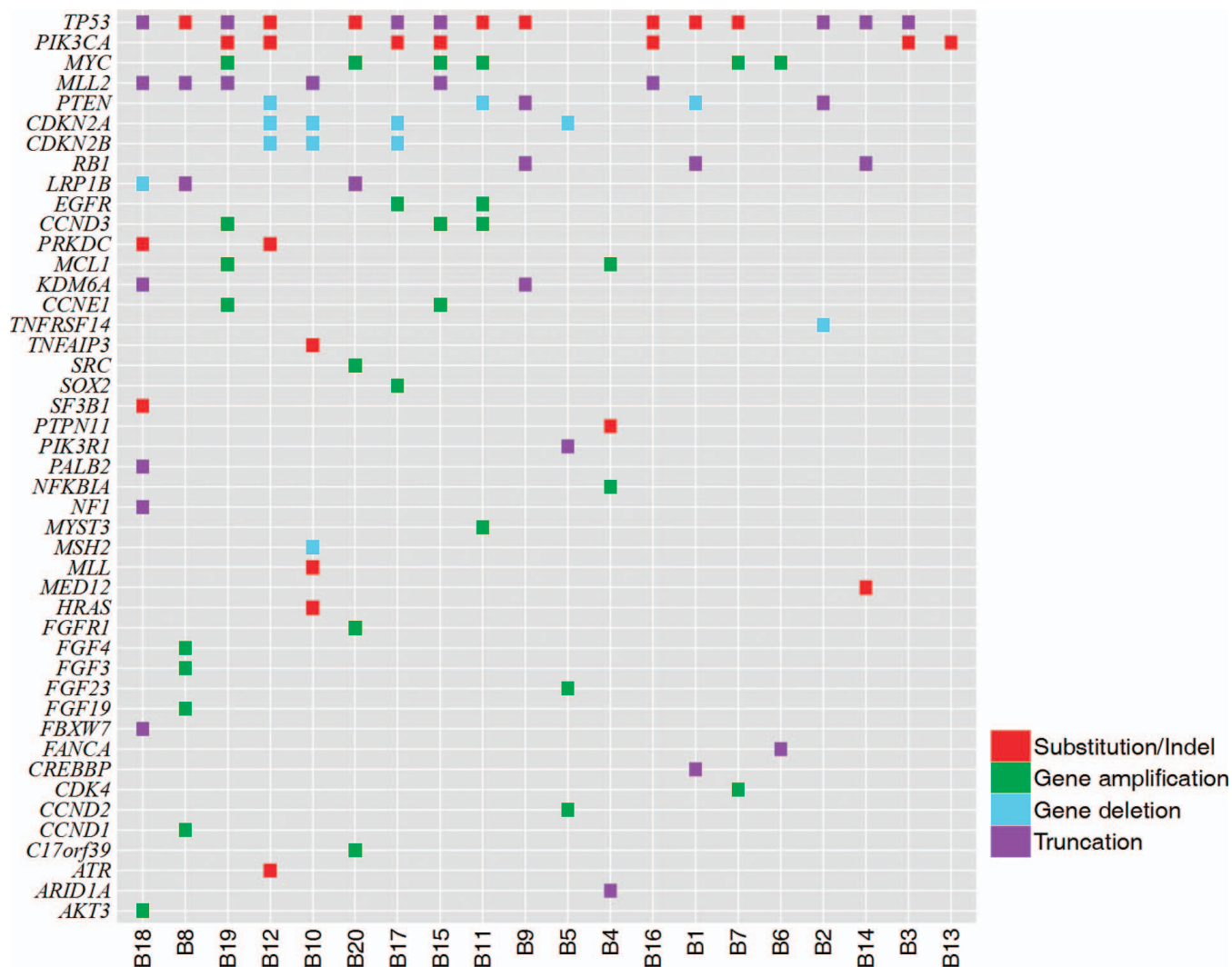


Figure 1. Tile plot of genomic alterations detected by genomic profiling of 20 cases of advanced-stage metaplastic breast carcinoma.

MEK, EGFR, FGFR1, and SRC. The results of routine HER2 testing were available for 16 patients (80%) with MPBC and all (100%) were negative for HER2 overexpression, as determined by IHC and/or negative for HER2 amplification as determined by fluorescence in situ hybridization at outside laboratories. All 16 (100%) of those cases were also negative for *ERBB2* copy-number gain (amplification) by the next-generation sequencing assay.

COMMENT

Molecular studies of MPBC to date have predominantly used IHC, fluorescence in situ hybridization, and mRNA transcriptional profiling techniques and have demonstrated that MPBC clusters with the TNBC/basaloid phenotypes.^{20–22} Metaplastic carcinomas of the breast have further been associated with the so-called claudin-low subtype of the basaloid phenotype in the molecular-portraits system.²³ In addition, mRNA profiling has linked the various histologic appearances of MPBC to various expression of a diverse group of genes.^{24–25} Studies²⁶ of *TP53* have demonstrated that *TP53* mutation is present in both the epithelial and mesenchymal components in MPBC with mixed histologies at a similar frequency (~75%). In the

current study, the 20 MPBCs showed a histologically uniform pattern in that none of the tumors featured a mixture of differentiated invasive ductal adenocarcinoma with metaplastic spindle cell (sarcomatoid), squamous, or chondroid foci. Although the numbers of cases in each category is small, we identified no specific pattern of altered cancer genes that were associated with the sarcomatoid (spindle cell), squamous, or chondroid histologic subtypes. Similarly, when the MPBC cases are categorized into pure epithelial (0 cases; 0%), pure mesenchymal (7 cases; 35%), and mixed epithelial and mesenchymal (13 cases; 65%) groups, no significant differences in the genomic alterations between groups was observed. Instead, similar to prior profiling studies, we identified a heterogeneous combination of alterations, yielding a relatively unique mutation profile for each tumor despite apparent histologic similarities. Additionally, the current study also confirms 2 noteworthy findings from previously published MPBC transcriptional profiling studies: *EGFR* amplification in the absence of *EGFR* mutations^{27–28} and alterations in the Wnt signaling pathway without mutations in *CTNNB1*.^{29–31}

Given the histologic picture of MPBC with various types of mesenchymal differentiation, including the spindle cell

Table 2. Significant Targetable Genomic Alterations Discovered by Next-Generation Sequencing Assessment of 20 Cases of Advanced-Stage Metaplastic Breast Carcinoma (MPBC)

Gene (%)	Genomic Alterations			Total Targetable Genomic Alterations, No.	Potential, Targeted Therapeutic
	Loss/Homozygous Deletion, No.	Base Substitution, No.	Truncation, No.		
<i>PIK3CA</i> (40)		8		8	Everolimus Temozolomide
<i>PTEN</i> (25)	3		2	5	Everolimus Temozolomide
<i>CDKN2A/B</i> (20)	4			4	CDK 4/6 inhibitors
<i>CCND3</i> (15)				3	Nutlins
<i>CCNE1</i> (10)				2	Nutlins
<i>EGFR</i> (10)				2	Erlotinib Afatinib Gefitinib
<i>HRAS</i> (5)		1		1	Trametinib
<i>AKT3</i> (5)				1	Everolimus Temozolomide
<i>CCND1</i> (5)				1	Nutlins
<i>CCND2</i> , (5)				1	Nutlins
<i>CDK4</i> (5)				1	CDK 4/6 inhibitors
<i>FBXW7</i> (5)			1	1	Everolimus Temozolomide
<i>FGFR1</i> (5)				1	Pazopanib Ponatinib Regorafenib
<i>NF1</i> (5)			1	1	Everolimus Temozolomide
<i>PI3KR1</i> (5)			1	1	Everolimus Temozolomide
<i>SRC</i> (5)				1	Bosutinib Dasatinib

(sarcomatoid) and chondroid variants of the disease, there has been significant interest in studying genes and biologic pathways associated with the epithelial to mesenchymal transition and the cancer stem cell hypothesis^{32–37} in MPBC. Previous studies using IHC or mRNA profiling of MPBC have identified altered expression levels of epithelial to mesenchymal transition genes or directly related pathways. In particular, altered expression of *SNAIL*, a transcriptional repressor of E-cadherin (*CDH1*), has been proposed as an important epithelial-mesenchymal transition pathway gene associated with recurrence and metastasis in the disease, especially in the chondroid variant of MPBC.^{38–40} However, in this study, sequence alterations or copy-number gains or losses of these epithelial-mesenchymal transition-associated genes were not identified. In particular, no mutations or homozygous deletions of *CDH1* were identified in this study.

The disease-free and overall survival results for patients with MPBC is significantly shorter than that for non-MPBC treated with cytotoxic agents in either the metastatic or the neoadjuvant settings.^{12–14} Nineteen of the 20 MPBC cases (95%) in this series harbored 36 alterations that could potentially lead to a targeted, therapeutic treatment option (mean [SD], 1.8 [1.06] per tumor). This result is similar to a series⁴¹ of 273 routine (non-MPBC) breast cancers evaluated using the same assay, where 246 (90%) harbored at least one potentially actionable alteration. The clinically meaningful alterations that could conceivably guide targeted treatment decisions were identified in 19 of 20 (95%) of the patients in multiple, biologic pathways (Table 2). Cell-cycle alterations were common, including homozygous deletion of *CDKN2A* in 4 (20%) and amplifications of *CCND3*,

CCNE1, *CCND1*, *CCND2*, and *CDK4* in 8 (40%) of the MPBC cases, a subset of which may indicate the potential for use of cell-cycle inhibitors. An example is case B07, a chest wall relapse of a MPBC in a 61-year-old woman whose tumor demonstrated amplification of *CDK4* (17 copies), in addition to amplification of *MYC* (9 copies) and the R248Q base substitution in *TP53* (Figure 2, A through C). *CDK4* amplification has been reported⁴² in 1.5% to 15% of breast carcinomas and expression has been correlated with amplification. Recent results^{43–46} focused on targeting cell-cycle regulatory genes in clinical trials for patients with cancer, and alterations in those cell growth regulatory pathways are showing significant promise.

PIK3CA mutations are important genomic alterations in the pathogenesis and progression of breast cancer.^{47–49} *PIK3CA* mutations were identified in 8 (40%) of the patients with MPBC in the current study (Figure 3, A and B), which is consistent with a previous study³⁵ that demonstrated *PIK3CA* mutations in 9 of 19 MPBCs (48%). The *PIK3CA* mutation frequency in MPBC thus appears to be greater than the 25% frequency listed for all types of breast cancer⁵⁰ and for TNBCs as a group.⁵¹ *PIK3CA* mutations have been linked to improved outcomes in breast cancer.⁵² The PI3K pathway is now widely considered a target of therapy for cancer in general⁵³ and for breast cancer, in particular.⁵⁴ Moreover, activating mutations in *PIK3CA* predict sensitivity to inhibitors of PI3K or its downstream signaling pathway (the PI3K/AKT/mTOR pathway).⁵⁵ The mTOR inhibitor everolimus has been approved for use in hormone receptor-positive, HER2-, advanced breast cancer, in combination with exemestane, and clinical trials with other mTOR inhibitors continue in breast cancer.⁵⁶ PTEN (phos-

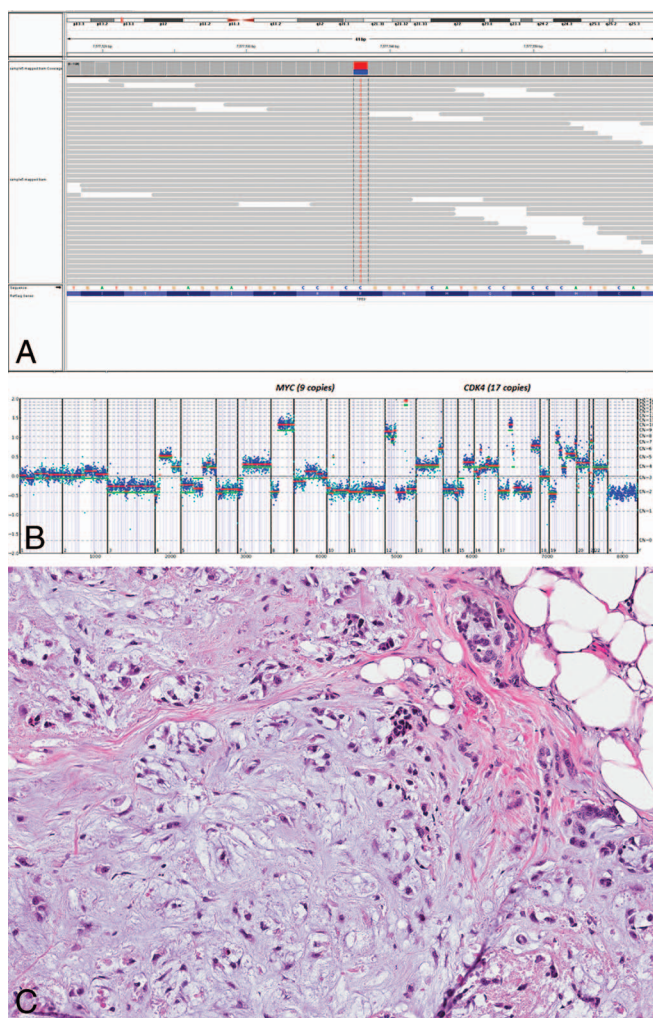


Figure 2. Chest wall recurrence of a stage-IV chondroid metaplastic breast carcinoma in a 61-year-old woman (case B07). *A*, An aTP53 R248Q base substitution mutation. *B*, The copy-number plot showing amplifications of the CDK4 (16 copies) and MYC (9 copies) genes. *C*, A hematoxylin-eosin-stained slide of the recurrent tumor showing high tumor grade and chondroid appearance (original magnification $\times 10$).

phatase and tensin homolog) functions as a tumor suppressor by negatively regulating the PI3K/Akt/mTOR pathway.⁵⁷ Mutations in *PTEN* have been reported⁵⁰ in 5% of breast cancers but have not been previously analyzed, to our knowledge, specifically in metaplastic breast carcinoma. In this study, 5 of the 20 MPBC cases (25%) harbored *PTEN* alterations, including 3 homozygous deletions and 2 truncating mutations, which could potentially benefit from PI3K inhibitors in at least a subset of these typically difficult-to-treat breast cancers.

In summary, MPBC is a rare, aggressive subtype of breast cancer, which remains a significant clinical problem given the high propensity to present at advanced stages and progress rapidly in a chemoresistant fashion. This study identified at least one clinically meaningful alteration that could potentially lead to a targeted treatment option in most patients. However, the future effect of the cost of the next-generation sequencing testing and potential use of targeting agents for metastatic MPBC must be weighed against the potential for increasing disease-free and overall survival in this disease. Moreover, the further development of person-

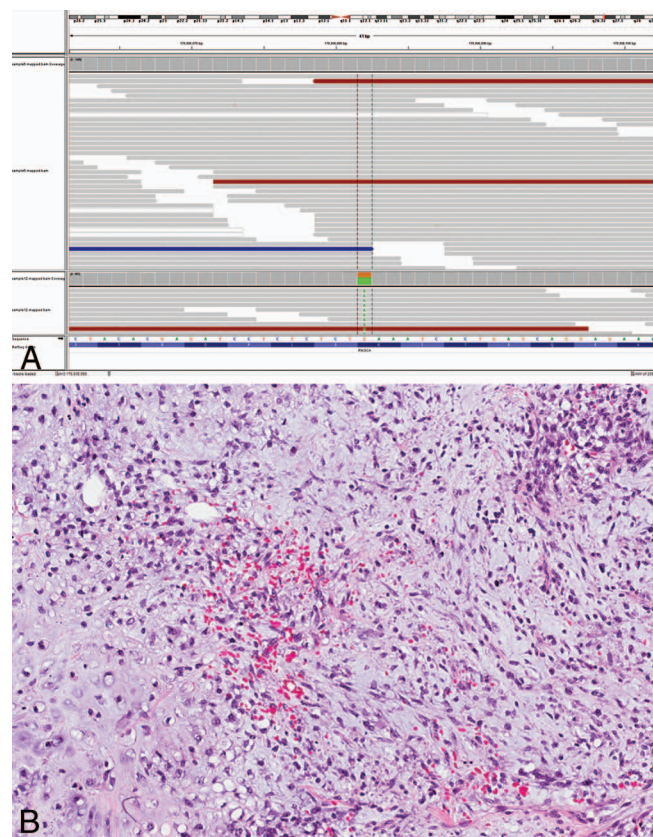


Figure 3. A high-grade, stage-IV, triple-negative metaplastic breast carcinoma with chondroid differentiation in a 60-year-old woman (case B16). *A*, An E542K base substitution in the PIK3CA gene. Genomic profiling also revealed TP53 R273C and MLL2 Q3499* mutations. *B*, A hematoxylin-eosin-stained section of the primary metaplastic breast carcinoma showing chondroid differentiation (original magnification $\times 10$).

alized oncology for MPBC and all types of breast cancer will require the continued development of mechanisms driven clinical trials, such as the emerging umbrella and basket trials, which are based on the use of biomarkers to select patients for both single-agent and multiagent, novel treatments. Given the poor prognosis and limited treatment options for patients with metastatic MPBC, genomic profiling using next-generation sequencing has the potential to identify new treatment paradigms and to fulfill an unmet clinical need in this disease.

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