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Changes in Triple-Negative Breast Cancer Molecular Subtypes in Patients Without Pathologic Complete Response After Neoadjuvant Systemic Chemotherapy

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abstract

PURPOSE Lehmann et al have identified four molecular subtypes of triple-negative breast cancer (TNBC)—basal-like (BL) 1, BL2, mesenchymal (M), and luminal androgen receptor—and an immunomodulatory (IM) gene expression signature modifier. Our group previously showed that the response of TNBC to neoadjuvant systemic chemotherapy (NST) differs by molecular subtype, but whether NST affects the subtype was unknown. Here, we tested the hypothesis that in patients without pathologic complete response, TNBC subtypes can change after NST. Moreover, in cases with the changed subtype, we determined whether epithelial-to-mesenchymal transition (EMT) had occurred.

MATERIALS AND METHODS From the Pan-Pacific TNBC Consortium data set containing TNBC patient samples from four countries, we examined 64 formalin-fixed, paraffin-embedded pairs of matched pre- and post-NST tumor samples. The TNBC subtype was determined using the TNBCtype-IM assay. We analyzed a partial EMT gene expression scoring metric using mRNA data.

RESULTS Of the 64 matched pairs, 36 (56%) showed a change in the TNBC subtype after NST. The most frequent change was from BL1 to M subtypes (38%). No tumors changed from M to BL1. The IM signature was positive in 14 (22%) patients before NST and eight (12.5%) patients after NST. The EMT score increased after NST in 28 (78%) of the 36 patients with the changed subtype (*v* 39% of the 28 patients without change; $P = .002254$).

CONCLUSION We report, to our knowledge, for the first time that the TNBC molecular subtype and IM signature frequently change after NST. Our results also suggest that EMT is promoted by NST. Our findings may lead to innovative adjuvant therapy strategies in TNBC cases with residual tumor after NST.

JCO Precis Oncol 6:e2000368. © 2022 by American Society of Clinical Oncology

ASSOCIATED CONTENT

Data Sharing Statement

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on January 19, 2022 and published at ascopubs.org/journal/po on March 16, 2022: DOI https://doi.org/10.1200/PO.20.00368

INTRODUCTION

Many studies have elucidated triple-negative breast cancer (TNBC) heterogeneity.[1] For example, Lehmann et al used mRNA gene expression profiling to identify TNBC molecular subtypes. Initially, they identified six subtypes: basal-like (BL) 1, BL2, mesenchymal (M), luminal androgen receptor (LAR), mesenchymal stem-like, and immunomodulatory (IM).[2] The group's subsequent study,[3] using laser capture microdissection and histopathologic quantification, reduced these subtypes to four (BL1, BL2, M, and LAR). IM status was found to be a modifier of the other TNBC molecular subtypes[4]; it was shown to be primarily driven by tumor-infiltrating lymphocytes and thus can be used to evaluate a tumor's immune status. The four TNBC subtypes can be identified in the Clinical Laboratory Improvement Amendments environment by Oncocyte Corporation (formerly Insight Genetics; Nashville, TN) using a highly modified lean version of the TNBCtype algorithm consisting of 101 genes (TNBCtype-IM).

In TNBC, about 30%-40% of patients have been shown to have a pathologic complete response (pCR) to current standard neoadjuvant systemic chemotherapy (NST). Previous studies have shown strong associations of pCR with longer overall survival and event-free survival durations[5,6]; by contrast, patients with breast cancer who did not have a pCR had significantly shorter survival durations because of higher relapse rates, especially in the TNBC subpopulation.[6,7]

CONTEXT

Key Objective

Triple-negative breast cancer (TNBC) can be classified into various molecular subtypes on the basis of the unique gene signatures. The impact of chemotherapy on molecular subtypes is unknown. We hypothesized that neoadjuvant systemic chemotherapy (NST) can change the TNBC molecular subtypes. This study evaluated TNBC patient samples using the TNBCtype-immunomodulatory assay on the basis of the TNBC molecular subtypes identified by Lehmann et al.

Knowledge Generated

We found that the TNBC molecular subtype and immunomodulatory signatures frequently change after NST. Furthermore, the post-NST residual samples showed evidence of epithelial-to-mesenchymal transition.

Relevance

To our knowledge, this is the first report of TNBC molecular subtypes changing after NST. Thus, the residual tumor's TNBC molecular subtype may assist in innovative targeted therapy strategies for patients with nonpathologic complete response after NST.

Thus, the approach to non-pCR patients is very important. Postoperative adjuvant therapy for non-pCR TNBC has proven to be effective in several clinical trials.[8] However, to establish the optimal adjuvant treatment, we propose that it is important to know the TNBC molecular subtype of the residual tumor.

In this study, we investigated TNBC molecular subtypes before and after NST in patients without pCR to test the hypothesis that TNBC subtypes can change after NST. Moreover, in cases with the changed subtype, we determined whether epithelial-to-mesenchymal transition (EMT) had occurred because EMT is a malignant phenotype constituting the first step in the potential metastatic process of residual tumors.

MATERIALS AND METHODS

Patients and Samples: Pan-Pacific TNBC Consortium Data Set

Four institutions participated in the study: Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX; Department of Medical Oncology, Chulalongkorn University, Bangkok, Thailand; Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; and Department of Breast Surgical Oncology, Showa University, Tokyo, Japan. We retrospectively collected patients' samples and clinical data using the following criteria: (1) Patients had pathologically diagnosed stage I to III TNBC and received NST and subsequent surgery between January 2009 and December 2014. (2) Tumors had triple-negative status as determined by immunohistochemistry (IHC) or fluorescence in situ hybridization. Estrogen receptor and progesterone receptor status was considered negative if $< 1\%$ of cells stained positively IHC. Human epidermal growth factor receptor 2 status was considered negative if (a) the IHC result was 0 to +1 or (b) the IHC result was +2 and fluorescence in situ hybridization results were negative. (3) Patients did not have pCR after NST, and both baseline (pretreatment) formalin-fixed, paraffin-embedded (FFPE) core-needle biopsy specimens and FFPE resection specimens (residual disease) were available. (4) Clinical data (clinical and pathologic stage and NST regimens) were available. All samples were subjected to centralized review, and the presence of tumor in the samples was confirmed by pathologists from MD Anderson and Showa University. We collected the following data from the medical records: patient age, clinical stage, treatment regimen, nuclear grade, and pathologic information for the residual tumor. The median follow-up from diagnosis was 41 (range 7-133) months.

The study was approved by the ethics committees at Showa University (number: 2125) and The University of Texas MD Anderson Cancer Center (number: PA14-0544). A waiver of informed consent was granted on the basis of the study's retrospective nature.

TNBC Subtype Classification (TNBCtype-IM Assay)

The pre- and post-NST pairs of TNBC samples that met our selection criteria were classified by the TNBC molecular subtype by Oncocyte Corporation Transcriptome libraries, which were constructed using a TruSeq RNA Exome Library Prep Kit (Illumina, San Diego, CA) using 100 ng of total RNA extracted from FFPE tissue sections according to the manufacturer's recommendations. Libraries were sequenced on an Illumina NextSeq 500 with 150 paired-end cycles and a mean of 25 million reads per sample. Transcripts were aligned to the human reference assembly GRCh37 (Ensembl) using the STAR application (v. 020201). Assembly and expression quantification were performed using Cufflinks tools (v. 2.2.1). The resulting FPKM data for each sample were compiled and analyzed with the TNBCtype-IM algorithm.[4] Samples that were unclassified were labeled UNS, indicating that the patient's TNBC expression pattern did not correlate with a specific subtype or IM modifier contained within the TNBCtype-IM

assay; these samples could express a unique and unknown signature. Additional information about TNBCtype-IM assay can be found in the Data Supplement.

EMT Score

We next evaluated whether EMT was accelerated in residual tumor after primary systemic chemotherapy. We used the previously described EMT scoring metric developed by George et al.[9] This metric quantifies the EMT spectrum; it was developed via an iterative method that ranks candidate gene products on the basis of their ability to resolve NCI-60 cell line samples with regard to their respective EMT status. The EMT scoring metric was applied to transcriptomic data to quantify the extent of EMT-ness on a scale of 0 (fully epithelial [E]) to 2 (fully M). This scoring method, on the basis of a set of EMT-relevant predictor transcripts and a set of normalizers for cross-platform application, categorizes samples into E, M, or hybrid E/M phenotypes on the basis of an ordered triple Si = (P_E, P_E/M, P_M), which represents the probability of group membership for each of the phenotypes. These probabilities are then projected on a scale of 0-2: E samples are assigned values close to 0; M samples, close to 2; and maximally hybrid E/M samples, close to 1.

Apocrine Status

Apocrine status was assessed using hematoxylin and eosin–stained slides (pre-NST samples only). We defined apocrine differentiation as the presence of abundant eosinophilic cytoplasm and large nuclei with prominent nucleoli. Three pathologists each independently reviewed the case slides and classified them as apocrine and non-apocrine accordingly.

E-cadherin and Vimentin Status

We assessed E-cadherin and vimentin status by IHC to evaluate intratumor heterogeneity in regard to EMT features. The analysis is described in the Data Supplement, and the results are shown in the Data Supplement.

Residual Cancer Burden Index

The residual cancer burden (RCB) index was assessed for all cases. The RCB index was developed by Symmans et al to evaluate the RCB after NST.[10,11] The index score is derived from the largest area and cellularity of residual invasive primary cancer, the number of involved lymph nodes, and the size of largest nodal metastasis. The RCB index was scored as 0 for pathologic complete response (stage yp-T0/is, ypN0), and residual disease was categorized into three RCB index classes—RCB-I (minimal), RCB-II (moderate), and RCB-III (extensive)—on the basis of predefined cut points of 1.36 and 3.28 index scores.

Statistical Analysis

Statistical analyses were performed using R software (v 3.5.1). The associations between features were analyzed using the Fisher's exact test.

RESULTS

Patients and Samples

We collected 78 paired archived pre- and post-NST samples from patients in the Pan-Pacific TNBC Consortium data set who had residual tumor after NST. Of those, eight pre-NST samples and six post-NST samples did not pass quality control (because of inadequate sample quality or insufficient sequencing coverage depth) for TNBCtype-IM classification. The remaining 64 matched pairs were analyzed.

The median patient age was 53 years, and 52% of patients were premenopausal at diagnosis (Table 1). Among the 64 patients, 20% had clinical T4 disease and 53% had lymph node metastasis. Sixty-nine percent of patients received anthracycline and taxane regimens, and 16% of patients received anthracycline alone.

Chemotherapy Impact on TNBC Subtype Classification

For the 64 matched pairs of samples, the distributions of TNBC subtypes in pre- and post-NST samples are shown in Figures 1 and 2. Compared with previous reports in TNBC (pCR and non-pCR),[12-14] there were fewer patients with the BL1 subtype and fewer IM-positive patients; this result is expected in this non-pCR population because the BL1 subtype and IM positivity are known to be predictive markers of pCR. However, the most common pre-NST subtype was still BL1, 45% (29 of 64) of patients, followed by LAR, 19% (12 of 64) of patients (Fig 1).

Of the 64 patients, 36 (56%) showed a change in the TNBC subtype after NST, and the distribution of TNBC subtypes changed (Fig 3). The most frequent change was from BL1, which was the dominant subtype before NST, to M, which was the dominant subtype after NST. By contrast, no tumors changed from M to BL1 subtypes. After NST, 14% (9 of 64) of patients were classified as the BL1 subtype (*v* 45% before NST) and 31% (20 of 64) were classified as M (*v* 16% before NST).

Figure 3 shows the distribution of post-NST subtypes for each pre-NST subtype. Among the 29 tumors that were of BL1 subtype before NST, 31% (9 of 29) did not change subtype after NST and 38% (11 of 29) converted to M subtype. Among the five tumors that were of BL2 subtype before NST, two tumors did not change subtype after NST, two tumors converted to LAR subtype, and one tumor converted to M subtype. Among the 10 tumors that were of M subtype before NST, six tumors did not change subtype after NST, one tumor converted to BL2, one tumor converted to LAR, and the other two tumors were classified as UNS. Among the 12 tumors that were of LAR subtype before NST, 59% (7 of 12) did not change subtype after NST and 33% (4 of 12) converted to BL2.

Pre-NST IM signature positivity strongly correlated with the BL1 subtype; 86% of IM signature–positive samples belonged to the BL1 group (Data Supplement). Fourteen

TABLE 1. Patient Characteristics at Diagnosis

Characteristic	Categories	All (N = 64)	Pre-NST TNBCtype-IM Result, No. (%)					P
			BL1 (n = 29)	BL2 (n = 5)	M (n = 10)	LAR (n = 12)	UNS (n = 8)	
Age, years	Median	53	47	49	51.5	59	54.5	.653
Menopausal status	Premenopausal	33	16 (55)	4 (80)	5 (50)	4 (33)	4 (50)	.361
	Postmenopausal	31	13 (45)	1 (20)	5 (50)	8 (67)	4 (50)	
Clinical T classification	T1	5	3 (10)	0 (0)	1 (10)	1 (8.5)	0 (0)	.594
	T2	39	18 (62)	4 (80)	4 (40)	10 (83)	3 (37.5)	
	T3	7	4 (14)	0 (0)	2 (20)	1 (8.5)	0 (0)	
	T4	13	4 (14)	1 (20)	3 (30)	0 (0)	5 (62.5)	
Clinical N status	Positive	34	19 (66)	2 (40)	4 (40)	3 (25)	6 (75)	.097
	Negative	30	10 (34)	3 (60)	6 (60)	9 (75)	2 (25)	
Stage	I	5	3 (10)	0 (0)	1 (10)	1 (8.5)	0 (0)	.536
	II	36	15 (52)	4 (80)	6 (60)	10 (83)	1 (12.5)	
	III	23	11 (38)	1 (20)	3 (30)	1 (8.5)	7 (87.5)	
Nuclear grade	1	2	0 (0)	0 (0)	1 (10)	1 (8.5)	0 (0)	.205
	2	16	10 (34)	0 (0)	1 (10)	2 (17)	3 (37.5)	
	3	44	18 (63)	5 (100)	8 (80)	8 (66)	5 (62.5)	
	Unknown	2	1 (3)	0 (0)	0 (0)	1 (8.5)	0 (0)	
Histology	Invasive ductal	55	26 (90)	5 (100)	8 (80)	9 (75)	7 (87.5)	.529
	Others	9	3 (10)	0 (0)	2 (20)	3 (25)	1 (12.5)	
Apocrine status (H&E)	Positive	16	6 (21)	0 (0)	1 (10)	9 (75)	0 (0)	< .001
	Negative	48	23 (79)	5 (100)	9 (90)	3 (25)	8 (100)	
Primary systemic therapy	A + T	44	20 (69)	3 (60)	9 (90)	7 (58)	5 (62.5)	.763
	A alone	10	4 (14)	1 (20)	1 (10)	1 (8.5)	3 (37.5)	
	T alone	7	3 (10)	1 (20)	0 (0)	3 (25)	0 (0)	
	Others	3	2 (7)	0 (0)	0 (0)	1 (8.5)	0 (0)	
Surgery	Mastectomy	41	19 (66)	3 (60)	4 (40)	7 (58)	8 (100)	.558
	Partial resection	23	10 (34)	2 (40)	6 (60)	5 (42)	0 (0)	
Pathologic T classification	T0	4	2 (7)	0 (0)	1 (10)	0 (0)	1 (12.5)	.673
	T1	24	11 (38)	2 (40)	2 (20)	6 (50)	3 (37.5)	
	T2	27	13 (45)	2 (40)	5 (50)	6 (50)	1 (12.5)	
	T3	3	1 (3)	1 (20)	0 (0)	0 (0)	1 (12.5)	
	T4	6	2 (7)	0 (0)	2 (20)	0 (0)	2 (25)	
Pathologic N status	Positive	26	13 (45)	1 (20)	2 (20)	6 (50)	4 (50)	.376
	Negative	38	16 (55)	4 (80)	8 (80)	6 (50)	4 (50)	
Pathologic stage	I	19	10 (34)	2 (40)	1 (10)	4 (33)	2 (25)	.47
	II	32	12 (42)	3 (60)	7 (70)	8 (67)	2 (25)	
	III	12	6 (21)	0 (0)	2 (20)	0 (0)	4 (50)	
	IV	1	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)	
Vascular invasion	Positive	6	2 (7)	1 (20)	0 (0)	1 (8.5)	2 (25)	.572
	Negative	46	21 (72)	3 (60)	8 (80)	10 (83)	4 (50)	
	Unknown	12	6 (21)	1 (20)	2 (20)	1 (8.5)	2 (25)	
Lymphatic invasion	Positive	14	6 (21)	2 (40)	2 (20)	2 (17)	2 (25)	.799
	Negative	45	20 (69)	3 (60)	8 (80)	10 (83)	4 (50)	
	Unknown	5	3 (10)	0 (0)	0 (0)	0 (0)	2 (25)	

(Continued on following page)

TABLE 1. Patient Characteristics at Diagnosis (Continued)

Characteristic	Categories	All (N = 64)	Pre-NST TNBCtype-IM Result, No. (%)					P
			BL1 (n = 29)	BL2 (n = 5)	M (n = 10)	LAR (n = 12)	UNS (n = 8)	
Adjuvant chemotherapy	Yes	11	4 (14)	1 (20)	2 (20)	1 (8.5)	3 (37.5)	.79
	No	53	25 (86)	4 (80)	8 (80)	11 (91.5)	5 (62.5)	
Adjuvant radiation	Yes	49	22 (76)	5 (100)	6 (60)	8 (67)	8 (100)	.445
	No	15	7 (24)	0 (0)	4 (40)	4 (33)	0 (0)	
Institution location	Japan	26	10 (34)	2 (40)	4 (40)	10 (83)	0 (0)	.109
	United States	15	8 (28)	0 (0)	3 (30)	0 (0)	4 (50)	
	Thailand	16	8 (28)	2 (40)	1 (10)	1 (8.5)	4 (50)	
	Korea	7	3 (10)	1 (20)	2 (20)	1 (8.5)	0 (0)	

NOTE. Data represent No. of patients unless otherwise specified. Abbreviations: A, anthracycline; BL, basal-like; H&E, hematoxylin and eosin; IM, immunomodulatory; LAR, luminal androgen receptor; M, mesenchymal; T, taxane; TNBC, triple-negative breast cancer; UNS, unstable.

(22%) patients had a positive IM signature in their pre-NST samples, and eight (12.5%) had a positive IM signature in their post-NST samples. Nine patients converted to IM-negative status, and three patients acquired IM-positive status after NST (Data Supplement).

To assess how morphological findings correlate with molecular subtypes, we examined hematoxylin and eosin–stained pre-NST tumor samples. Apocrine differentiation correlated with the LAR subtype; 75% (9 of 12) of LAR patients showed apocrine differentiation. In addition, 21% (6 of 29) of BL1 patients showed apocrine differentiation (Table 1).

Chemotherapy Impact on EMT Score

An increased EMT score after NST was seen in 78% (28 of 36) of patients whose subtype changed, compared with only 39% (11 of 28) of patients whose subtype did not change ($P = .00225$; Table 2). Among the patients with a subtype change from BL1 to M, 82% (9 of 11) of patients had increased EMT scores and one patient had a decreased EMT score (Data Supplement).

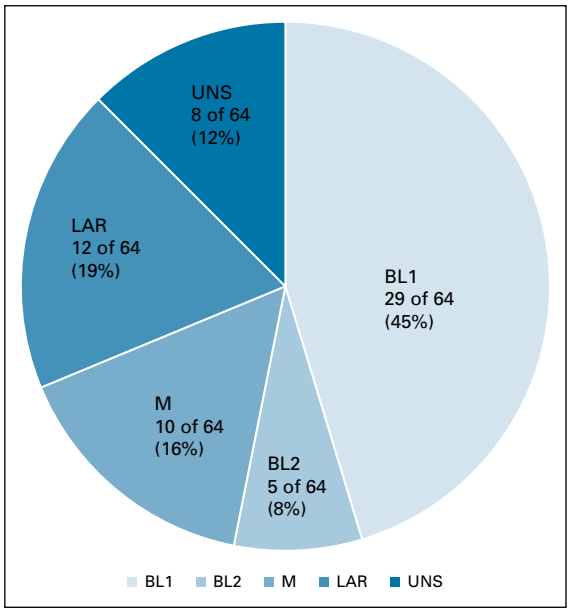

FIG 1. Distribution of triple-negative breast cancer subtypes before NST (core needle samples) for patients who did not have pathologic complete response (n = 64). BL, basal-like; LAR, luminal androgen receptor; M, mesenchymal; UNS, unstable.

Correlation Between the Subtype Change and the RCB Score

RCB was evaluable for all samples. Subtype changes were observed regardless of the residual tumor volume, but were more pronounced in tumors with a low residual tumor volume (Table 3).

Correlation Between the EMT Score and the RCB Score

We also determined the correlation between the EMT score and the RCB score to eliminate the possibility that in tumors with a low residual tumor volume, more stromal components were included in the analysis, which may increase EMT. There was no statistically significant difference between these scores (Data Supplement).

DISCUSSION

To our knowledge, this is the first report that NST can change the molecular subtype of the residual tumor in TNBC. We found that the TNBC molecular subtype and IM signature frequently changed after NST. In addition, we showed that EMT was promoted by chemotherapy as measured by a partial EMT gene expression scoring metric. Our findings may lead to innovative adjuvant therapy strategies in TNBCs that do not achieve pCR after NST. Moreover, although the number of samples was small, we demonstrated that there are occasions in which chemotherapy may induce an IM signature, which could expand treatment options with immunotherapies for non-pCR patients.

Evidence has shown that cancer treatments affect the tumor biology and can lead to acquired epigenetic changes and mutations, some of which cause resistance.[15-17]

The risk of relapse is higher in patients with pathologic residual invasive disease after NST than in patients with

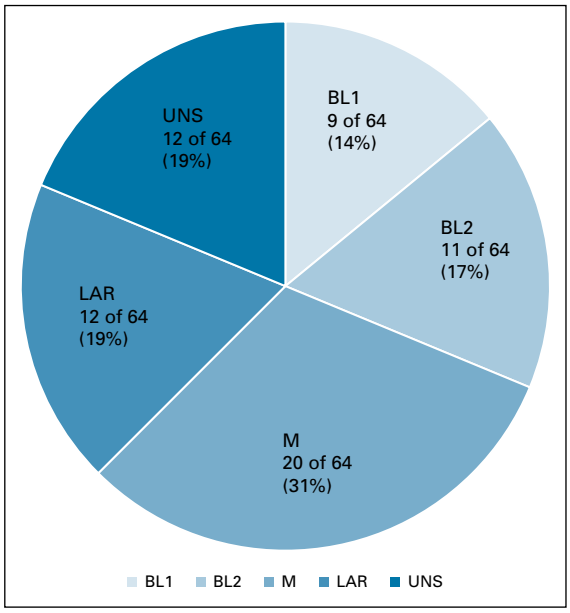

FIG 2. Distribution of triple-negative breast cancer subtypes after NST (surgical samples) for patients who did not have pathologic complete response (n = 64). BL, basal-like; LAR, luminal androgen receptor; M, mesenchymal; UNS, unstable.

pCR.[6] Although it has been unclear whether there is a survival benefit from postoperative systemic chemotherapy after NST, several studies recently reported that adjuvant therapy for the non-pCR population increased disease-free and overall survival.[8,18] According to these results, adjuvant therapies are promising treatment options for patients with breast cancer who have residual tumor after NST. To seek optimal adjuvant treatments, we must evaluate the biology of the residual tumor and elucidate the effect of NST. Our study is a valuable first step in revealing the effect of NST in inducing molecular subtype changes in more than 50% of patients; mRNA profiling analysis after NST may suggest the optimal adjuvant treatment for the individual patient. Moreover, this study showed that unlike intrinsic subtypes,[19] TNBCtype-IM dynamically captured changes in molecular biology through temporal mRNA profiling.

On the other hand, intratumor heterogeneity is attracting attention because of the development of the technique of genome-wide profiling, which distinguishes single tumor cells and circulating tumor cells.[20-22] Intratumor heterogeneity in breast cancers has been confirmed genetically and epigenetically in recent genome profiling reports.[22] Thus, the possibility exists that pre-NST samples may not represent the characteristics of the tumor as a whole because of the small region of tumor tissue that is obtained through a core needle biopsy.

RCB was evaluable for all samples. As we expected, a low RCB-I was more common in patients with subtype change (9 of 36 [25%]) than in patients without subtype change (1 of 28 [3.5%]; Table 3). However, focusing on RCB-II and RCB-III, which reflect a moderate or extensive amount of residual tumor, 50% (27 of 54) changed subtype and the most frequent change was from BL1 to M subtypes (37%). Thus, the subtype changed even in cases in which the effect of chemotherapy was poor and high RCB remained.

Confirming our hypothesis, certain TNBC subtypes were found to have changed after NST. In fact, more patients had the M and BL2 subtypes after NST. Consistent with our previous knowledge of populations with pCR, in which

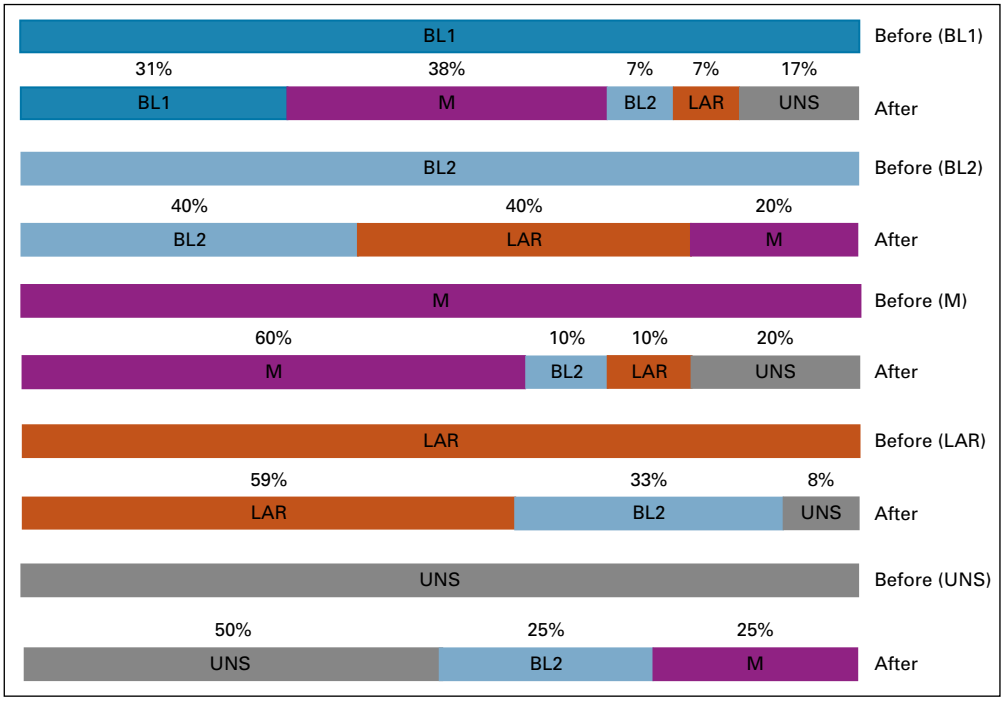

FIG 3. Post-NST triple-negative breast cancer subtypes for patients in each pre-NST subtype group. BL, basal-like; LAR, luminal androgen receptor; M, mesenchymal; UNS, unstable.

TABLE 2. Correlation Between the Subtype Change and the EMT Score

Status After NST	Increased EMT Score	Decreased or Equal EMT Score	*P*
Same subtype (n = 28)	11	17	.00225
Change in subtype (n = 36)	28	8	

Abbreviation: EMT, epithelial-to-mesenchymal transition.

TABLE 3. Correlation Between the Subtype Change and the RCB Score

Status After NST	RCB-I (n = 10)	RCB-II (n = 39)	RCB-III (n = 15)	*P*
Same subtype (n = 28)	1	17	10	.019
Change in subtype (n = 36)	9	22	5	

Status After NST	RCB-I (n = 10)	RCB-II/RCB-III (n = 54)	*P*
Same subtype (n = 28)	1	27	.034
Change in subtype (n = 36)	9	27	

Abbreviation: RCB, residual cancer burden.

80% of patients had the BL1 subtype,[23] the non-pCR population was under-represented in the BL1 subtype (45%) and had fewer IM-positive samples because the BL1 subtype and IM positivity are predictive markers for achieving pCR.[12,13,23,24]

The most frequent subtype change was from the BL1 to the M subtype (38% after NST), and no tumors did the opposite. This subtype is characterized by genes involved in motility, the extracellular matrix, cell differentiation pathways, and EMT.[23] This subtype is also enriched in gene expression related to cell motility (the Rho pathway), cellular differentiation, and growth pathways (the anaplastic lymphoma kinase pathway, transforming growth factor beta signaling, and the Wnt/β-catenin pathway). This finding suggests that either chemotherapy accelerates the development of M features and EMT or there was heterogeneity of cell types within tumors and selective resistance and outgrowth of M cells during NST.

To clarify the validity of this hypothesis, we evaluated EMT using a gene expression scoring metric.[9] EMT is a cellular process involving a multitude of phenotypic and morphologic changes that drive increased migratory and invasive potential.[9] It has been implicated in acceleration of metastasis, acquisition of tumor initiation potential, resistance to anoikis, refractory response to chemotherapy, and the ability to evade the immune system.[25-27] Recent studies have shown that cells need not undergo complete EMT for dissemination and that cells in one or more hybrid E/M phenotypes may be more metastatic than those that have undergone complete EMT.[28] Thus, quantification of EMT status in a given sample can indicate metastatic aggressiveness. We evaluated the EMT score to infer whether the change in subtype was acquired because of the effects of chemotherapy. Although the small number of patients prevented determining the precise relationship with the EMT score for each subtype, the increased EMT score after NST in the patients with subtype changes (P = .00225) suggests that EMT is promoted by chemotherapy in at least some of the population. This result provides support that subtype changes occurred not only because of intratumor heterogeneity.

The limitations of this study include the small number of patients; although we created a pan-Pacific data set, the number of patients with each molecular subtype was limited and the patients had varying characteristics because of the retrospective nature of the study. For example, there were several NST regimens although the majority of patients received anthracycline and taxane regimens. Because of these diverse characteristics, it was difficult to measure clinically relevant outcomes, such as overall survival and disease-free survival. A further limitation was that the institutions had different specimen storage conditions.

Our findings suggest that when considering adjuvant treatment for non-pCR patients, the biology of the residual tumor should be considered and residual tumor might have more M properties than the pretreatment TNBC. Thus, targeting M features and EMT has potential as optimal therapy for non-pCR patients. A number of EMT targets have potential. These include Ras-mitogen–activated protein kinase activation, which cooperates to promote EMT and metastasis[29] and Hedgehog signaling, which regulates EMT.[30] Phosphatidylinositol 3-kinase has also been suggested to trigger EMT.[31-33] EMT is regulated by transforming growth factor beta signaling, which promotes tumor growth, invasion, and evasion of immune surveillance.[34,35] Similarly, FGF-2 induces EMT and is another druggable target.[36-38] Inhibitors of these growth factors may have potential as targeted therapies for non-pCR patients.

In summary, we found that the TNBC molecular subtype and IM signature frequently changed in patients with residual disease after NST. In addition, we provide evidence suggesting that EMT is promoted by chemotherapy in some patients. Further investigation is necessary to determine whether the cause of the subtype change is intratumor

heterogeneity or acquired biologic changes. Our findings may lead to innovative adjuvant chemotherapy strategies in TNBCs that do not show pCR after NST. Our findings support re-evaluation of residual tumor in future clinical trials for non-PCR patients. Furthermore, since the most frequent subtype change was from the BL1 to the M subtype and there were no cases of the reverse, targeting M features and EMT might have potential for the optimal treatment of non-pCR patients. Finally, re-evaluating immune status (ie, the IM signature) may expand the opportunity to use immunotherapies. These findings warrant independent confirmation in future studies.

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DISCLAIMER

The funders did not participate in the design of the study, the analysis or interpretation of data, or the writing or approval of the manuscript. The TNBC molecular subtype sample analysis was performed by Oncocyte Corporation.

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S.K. and N.T.U. contributed equally to this work.

PRIOR PRESENTATION

Presented in part as an abstract at the American Society of Clinical Oncology Annual Meeting, Chicago, IL, June 1-5, 2018.

SUPPORT

Supported by a Grant-in-Aid for Scientific Research 16K21374 (to H.M.); the National Institutes of Health through award Nos 1R01CA205043-01A1 (to N.T.U.), R01CA123318 (to N.T.U.), and P30CA016672 (MD Anderson's Cancer Center Support Grant; used the Clinical Trials Office and Bioinformatics Shared Resource); Breast Cancer Research Foundation grants BCRF-18-164 and APR-18-006 (to N.T.U.); the Morgan Welch Inflammatory Breast Cancer Research Program and Clinic; and a State of Texas Rare and Aggressive Breast Cancer Research Program grant (to N.T.U.).

DATA SHARING STATEMENT

The RNA-seq data generated and/or analyzed during this study are publicly available.

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The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

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Research Funding: Medivation, Bayer, Amgen, Puma Biotechnology, Merck, Daiichi Sankyo, Celgene, GlaxoSmithKline, Kyowa Hakko Kirin, Bio-Path Holdings, Novartis, Sysmex, Preferred Medicine

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

We thank the study participants. Editorial assistance was provided by Sunita Patterson, Senior Scientific Editor, Research Medical Library, The University of Texas MD Anderson Cancer Center.

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