

Pathological Response in a Triple-Negative Breast Cancer Cohort Treated with Neoadjuvant Carboplatin and Docetaxel According to Lehmann's Refined Classification



Isabel Echavarría¹, Sara López-Tarruella¹, Antoni Picornell¹, Jose Ángel García-Saenz², Yolanda Jerez¹, Katherine Hoadley³, Henry L. Gómez⁴, Fernando Moreno², María Del Monte-Millan¹, Iván Márquez-Rodas¹, Enrique Alvarez¹, Rocío Ramos-Medina¹, Javier Gayarre¹, Tatiana Massarrah¹, Inmaculada Ocaña¹, María Cebollero⁵, Hugo Fuentes⁴, Agusti Barnadas⁶, Ana Isabel Ballesteros⁷, Uriel Bohn⁸, Charles M. Perou⁹, and Miguel Martín¹⁰

Abstract

Purpose: Triple-negative breast cancer (TNBC) requires the identification of reliable predictors of response to neoadjuvant chemotherapy (NACT). For this purpose, we aimed to evaluate the performance of the TNBCtype-4 classifier in a cohort of patients with TNBC treated with neoadjuvant carboplatin and docetaxel (TCb).

Methods: Patients with TNBC were accrued in a nonrandomized trial of neoadjuvant carboplatin AUC 6 and docetaxel 75 mg/m² for six cycles. Response was evaluated in terms of pathologic complete response (pCR, ypT0/is ypN0) and residual cancer burden by Symmans and colleagues. Lehmann's subtyping was performed using the TNBCtype online tool from RNAseq data, and germline sequencing of a panel of seven DNA damage repair genes was conducted.

Results: Ninety-four out of the 121 patients enrolled in the trial had RNAseq available. The overall pCR rate was 44.7%.

Lehmann subtype distribution was 34.0% BL1, 20.2% BL2, 23.4% M, 14.9% LAR, and 7.4% were classified as ER+. Response to NACT with TCb was significantly associated with Lehmann subtype ($P = 0.027$), even in multivariate analysis including tumor size and nodal involvement, with BL1 patients achieving the highest pCR rate (65.6%), followed by BL2 (47.4%), M (36.4%), and LAR (21.4%). BL1 was associated with a significant younger age at diagnosis and higher ki67 values. Among our 10 germline mutation carriers, 30% were BL1, 40% were BL2, and 30% were M.

Conclusions: TNBCtype-4 is associated with significantly different pCR rates for the different subtypes, with BL1 and LAR displaying the best and worse responses to NACT, respectively. *Clin Cancer Res*; 24(8): 1845–52. ©2018 AACR.

¹Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), CiberOnc, Madrid, Spain. ²Hospital Universitario Clínico San Carlos, Madrid, Spain. ³Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. ⁴Medical Oncology, Instituto Nacional de Enfermedades Neoplásicas - INEN, Lima, Peru. ⁵Department of Pathology, Hospital General Universitario Gregorio Marañón, Madrid, Spain. ⁶Medical Oncology Department, Hospital de Sant Pau, Barcelona, Spain. ⁷Medical Oncology, Hospital Universitario de La Princesa, Madrid, Spain. ⁸Medical Oncology, Hospital de Gran Canaria Dr. Negrin, Las Palmas, Spain. ⁹Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. ¹⁰Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Universidad Complutense, CiberOnc, GEICAM, Madrid, Spain.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Clinical trial information: NCT01560663

Corresponding Authors: Miguel Martín, Hospital General Universitario Gregorio Marañón, C/Dr. Esquerdo 46, Madrid 28007, Spain. E-mail: mmartin@geicam.org; and Isabel Echavarría, iechavariadg@gmail.com.

doi: 10.1158/1078-0432.CCR-17-1912

©2018 American Association for Cancer Research.

Introduction

Triple-negative breast cancer (TNBC), defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and HER-2 overexpression, represents approximately 15% of breast cancers, has no targeted therapies available yet, and is associated with an unfavorable prognosis. Many patients with early-stage TNBC are now treated with neoadjuvant chemotherapy (NACT), as presurgery treatment enables a higher rate of breast-conserving surgery, an early exposure of micrometastatic disease to systemic therapy, and, mainly, an *in vivo* test of the tumor sensitivity to chemotherapy. In addition, it has been consistently shown that pathologic response to NACT is strongly correlated with prognosis in TNBC. Patients obtaining a pathologic complete response (pCR, defined as noninvasive residual disease in breast and lymph nodes) have a high likelihood of cure, whereas those with significant residual disease have a dismal prognosis (1, 2).

The addition of platinum salts to standard anthracycline-taxane neoadjuvant regimens has demonstrated a significant increase in pCR rates in TNBC, reaching above 50% (3, 4),

Translational Relevance

Although gene expression profiling has provided a better understanding of TNBC, there still is a need for the identification of predictors of response to chemotherapy in this subset of patients. Our study brings innovative data on the predictive value of Lehmann's refined classification tool (TNBCtype-4) in a homogeneous cohort of patients treated with a platinum-containing, anthracycline-free, neoadjuvant regimen, carboplatin and docetaxel. This study shows a meaningful differential response to neoadjuvant chemotherapy among the different subtypes, with BL1 and LAR displaying the highest and lowest pCR rates, respectively (65.6% vs. 21.4%). This robust association, together with the novel data on the subtype distribution within BRCA-mutated TNBC, provides a new evidence of TNBC heterogeneous biology, which may enable a future selection of therapies in these patients.

although at the expense of significantly higher toxicity. For instance, the combination of carboplatin and weekly paclitaxel, followed by dose-dense doxorubicin and cyclophosphamide, reached pCR rates of 54% (ypT0/is ypN0) in the CALGB40603 trial, whereas the combination without carboplatin achieved a pCR rate of 41% (3). In a similar manner, neoadjuvant treatment with paclitaxel, non-pegylated liposomal doxorubicin and bevacizumab, with or without carboplatin, achieved pCR rates of 53.2% and 36.9%, respectively ($P = 0.005$). In contrast, classical regimens based on anthracyclines and taxanes have shown pCR rates of around 35% to 40% (5). Neoadjuvant regimens based on taxanes and platinum salts, without anthracyclines, such as the combination of docetaxel and carboplatin, reach pCR rates of around 50% (6, 7). However, comparison among different studies could be biased by different patient population and stage of disease.

Nevertheless, about half of the patients will not achieve a pCR, and a significant proportion of these patients will relapse despite NACT. Thus, TNBC requires a reliable predictor of response to NACT that will enable the selection of patients for whom conventional chemotherapy is insufficient, and direct them to clinical trials with new drugs or new therapeutic approaches.

Intrinsic subtype by gene expression profiling provided a new insight into breast cancer, classifying these tumors into four subtypes (8, 9). Although TNBC and basal-like were initially considered equivalent, TNBC is in fact a highly heterogeneous disease, and only 70% to 80% of TNBC are classified as PAM50 basal-like subtype (10). Other gene expression-based classifiers of TNBC have arisen in recent years, with some sharing features and subtypes between them, but without complete overlap (11, 12). The Lehmann classification (TNBCtype) has become one of the most studied (13). Initially composed of six subtypes, further analysis revealed that the mesenchymal stem-like (MSL) and immunomodulatory (IM) subtypes were more a reflection of the tumor microenvironment rather than of the own tumor cells, and, therefore, the classification was simplified into four TNBC subtypes: basal-like 1 (BL1), basal-like 2 (BL2), mesenchymal (M), and luminal androgen receptor (LAR; ref. 13). A correlation of Lehmann subtypes with pathologic response to NACT based on anthracyclines and taxanes was previously observed with the BL1 group having the highest pCR rate (14). The presence of a subset of

TNBC tumors that bear features of ER+ breast cancer has been known for long, with an overexpression of hormone-regulated pathways, and in special androgen signaling (15, 16). There are signs of antitumor activity with androgen blockade in patients with expression of androgen receptor in IHC staining and predictive signatures of androgen blockade efficacy are being developed (17, 18). In the gene expression level, all new TNBC classifications have identified this LAR subtype, that corresponds with PAM50 nonbasal tumors (11–14).

In this study, we aimed to evaluate the predictive value of Lehmann subtyping in a TNBC cohort treated with neoadjuvant carboplatin and docetaxel. In addition, we analyzed its correlation with the PAM50 intrinsic subtype classification.

Patients and Methods

Patients and treatment

An *ad hoc* study of predictive biomarkers was conducted within a prospective, nonrandomized trial evaluating the clinical efficacy of neoadjuvant carboplatin and docetaxel previously published (6). Patients with newly diagnosed TNBC were accrued from seven institutions across Spain and Peru. Eligibility criteria included a pathologically confirmed diagnosis of invasive breast cancer, age over 18, stage I to III disease and no prior chemotherapy treatment for any malignancies. TNBC was defined as the absence of expression of estrogen and progesterone receptor (ER and PR < 1%) and HER2 status was defined as negative using the ASCO/CAP guidelines (19, 20). IHC for ER, PR, and Ki67 was determined by local review. Patients received six cycles of carboplatin AUC 6 and docetaxel 75 mg/m² every 3 weeks followed by surgery. G-CSF support was used following individual institution guidelines. Postoperative radiotherapy was indicated following clinical practice guidelines and adjuvant treatment in case of residual disease was left at the physician discretion.

The study was registered at clinicaltrials.gov (NCT 01560663) and was approved by the Ethical Board at all the participating institutions. All patients signed a written informed consent.

Assessment of response

Pathologic complete response was defined as the absence of invasive tumor in the breast and axillary lymph nodes (ypT0/is ypN0) and residual disease was assessed by Symmans residual cancer burden calculator (21). Assessment of response was done by local pathology.

Genomic profiling

Extraction of nucleic acids was done centrally at the Translational Oncology Laboratory (LAOT) at the Hospital Gregorio Marañón (Madrid, Spain).

RNA was extracted from formalin-fixed paraffin-embedded (FFPE) core biopsies prior to treatment initiation from the breast tumor, using the RNasy FFPE (Quiagen, Germany). RNA quantification and quality control were performed on NanoDrop 2000. Paired samples from nonresponders are not available yet.

Intrinsic subtype was performed from PAM110 panels, including the PAM50 gene set, on the nCounter platform (NanoString Technologies Inc.) at the LAOT facilities. The PAM110 assay included the 50 PAM50 genes plus an additional set aimed to identify the claudin-low signature and neoangiogenesis signatures (see list of genes in Supplementary Table S2). Further details can be found at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL17071> and <https://www.ncbi.nlm.nih.gov/GSE181548> and <https://www.ncbi.nlm.nih.gov/GSE181466> GEO datasets). RNA sequencing (RNAseq) was performed at the

University of North Carolina at Chapel Hill, North Carolina. Total FFPE RNA was used to create a total RNAseq library using the Illumina TruSeq Total RNA library Prep Kit with Ribo-Zero Gold Kit. Libraries were then sequenced two per lane on a HiSeq2500 with a 48 × 7 × 48 bp configuration. Alignment with the Human Genome Sequence GRCh37 was done using MapSplice v 2.2.1 and RSEM v1.3.0. Analysis of RNAseq data was done in collaboration between the University of North Carolina and the Hospital Gregorio Marañón.

RSEM normalized data were uploaded into the TNBCtype online tool: <http://cbc.mc.vanderbilt.edu/tnbc> in order to get Lehmann's subtype distribution (22, 23). Patients were classified into four subtypes: BL1, BL2, M, and LAR.

In addition, germline DNA from the patients was extracted from whole blood samples prior to the initiation of therapy using the QIAamp the DNA Blood Midi Kit (Quiagen, Germany). A panel of 7 DNA damage repair genes (BRCA1, BRCA2, BARD1, PALB2, RAD50, RAD51C, and RAD51D) were analyzed through targeted next-generation sequencing, by Sistemas Genómicos (Valencia, Spain).

Statistical analysis

All statistical analyses were performed on R v3.2.1. Fisher exact test and chi-squared test were used for the comparison of categorical variables, Student *t* test and Kruskal–Wallis test were used for independent continuous variables, and multivariate analyses were performed with multiple logistic regressions. Confidence intervals (CIs) for categorical variables have been estimated with the Clopper–Pearson method.

Results

Patients

From 2010 to 2015, 121 patients with TNBC were accrued in seven participating institutions in Spain and Peru. RNAseq could be performed in 97 of the patients included in the cohort (80.2%). In the remaining patients, insufficient amount of RNA extracted from the core biopsies precluded an appropriate analysis. In addition, two patients were lost to follow-up with no available information about their outcomes and another patient received only one cycle of TCb due to toxicity, and was considered as not evaluable for the analysis (Fig. 1).

Baseline characteristics of the patients are summarized in Table 1. Median age at diagnosis was 51 years. There were no significant differences between global trial population and patients included in the molecular analysis both in baseline characteristics and response rates (Table 1). Almost two thirds of the patients had axillary involvement, 52.1% and 46.8% of the patients had stage II and III disease, respectively. pCR rate among the 94 available patients for molecular analysis was 44.7% and up to 56.4% achieved a pathologic good response (pCR or RCBI). Median follow-up was 35 months.

Lehmann subtype distribution

Lehmann subtype distribution was as follows: 34.0% BL1, 20.2% BL2, 23.4% M, and 14.9% LAR. Seven patients (7.4%) in our cohort were classified as ER+ with Lehmann subtyping tool and were discarded for the subtype distribution (Table 2).

In this cohort, 83% were considered Basal-like by PAM50 subtype, approximately two-thirds were classified as BL1 and BL2 by TNBCtype-4 (64.1%) and most of the remaining corresponded to M subtype (28.2%). On the contrary, only 6.2% of our non-

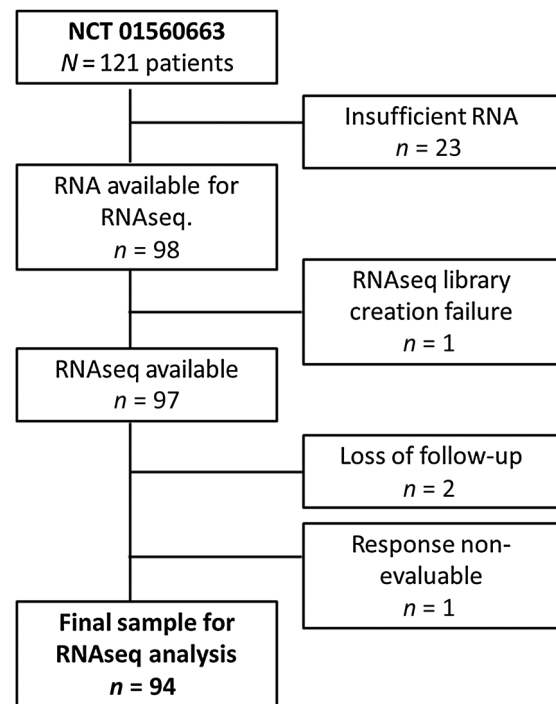


Figure 1.
Consort diagram.

basal patients by PAM50 were classified as BL1 or BL2, whereas most of these patients corresponded to LAR (68.8%).

Of the seven patients considered ER+ with regards to ESR1 expression, 42.9% were basal-like by PAM50, 42.9% normal-like, and 14.3% HER2E.

Baseline characteristics according to Lehmann subtype

We compared baseline characteristics among each of the Lehmann subtypes (Table 3). Except for age and ki67, no significant differences in the clinical–pathologic features were found between the four subtypes. BL1 and LAR were associated with a significant younger and higher age at diagnosis, respectively (median age 41 and 67.5 years), $P < 0.01$. Patients with LAR tumors included in our cohort tended to have more locally advanced tumors than the rest of the subtypes. Indeed, BL1 and LAR tumors had a trend toward more frequent nodal involvement (78.1% and 85.7% vs. 52.6% and 57.1% for BL2 and M, respectively), although these differences did not reach statistical significance ($P = 0.18$). Significant differences in ki67 values were observed across subtypes ($P < 0.01$), BL1 tumors displayed the highest ki67 values and LAR the lowest. There were no differences in the percentage of high grade tumors (grade 3) nor in tumor size.

Response according to Lehmann subtype

Response to NACT with TCb was significantly associated with Lehmann subtype ($P = 0.027$). BL1 patients achieved the highest pCR rate (65.6%; 95% CI, 46.8–81.4%), followed by BL2 (47.4%; 95% CI, 24.4–71.1%), M (36.4%; 95% CI, 17.2–59.3%), and LAR (21.4%; 95% CI, 4.7–50.8%; Fig. 2). Patients classified as ER+ obtained a pCR rate of 14.3% (95% CI, 0.4–57.9%).

Table 1. Baseline characteristics of our cohort

	N = 121	N = 94	P
Age			
Median (range)	51.4 (28–80)	51 (28–78)	0.61
Ethnicity ^a			
Caucasian	117 (96.7%)	90 (95.7%)	1.0
Asian	2 (1.7%)	2 (2.1%)	
Hispanic	2 (1.7%)	2 (2.1%)	
Menstrual status at diagnosis			
Premenopausal	58 (47.9%)	43 (45.7%)	0.51
Perimenopausal	1 (0.8%)	1 (1.1%)	
Postmenopausal	60 (51.2%)	49 (52.1%)	
NA	2 (1.7%)	1 (1.1%)	
Tumor size by MRI (mm)			
Median, range	40 (9–180)	42 (12–180)	0.65
Axillary involvement			
N0	43 (35.5%)	29 (30.9%)	0.07
N+	78 (64.5%)	65 (69.1%)	
T stage			
T1	4 (3.3%)	4 (4.3%)	0.71
T2	66 (54.5%)	49 (52.1%)	
T3	24 (19.8%)	20 (21.3%)	
T4	27 (22.3%)	21 (22.3%)	
AJCC TNM			
I	1 (0.8%)	1 (1.1%)	0.52
II	66 (54.5%)	49 (52.1%)	
III	54 (44.6%)	44 (46.8%)	
Ki67			
Median, range	70% (3–100%)	70% (3–100%)	0.95
<50%	38 (31.4%)	35 (37.2%)	
Histological grade			
G1–G2	31 (25.6%)	22 (23.2%)	0.35
G3	87 (71.9%)	70 (74.5%)	
NA	3 (2.5%)	2 (2.1%)	
Response data			
pCR	57 (47.1%)	42 (44.7%)	0.38
RD	64 (52.9%)	52 (55.3%)	

MRI, magnetic resonance imaging.

N+, node-positive; N0, node-negative; NA, not available; T stage, tumor stage according to AJCC 7th edition; AJCC, American Joint Committee on Cancer; G, grade. Fisher exact test has been used for categorical variables, Mann–Whitney test for comparison of two means (age, tumor size, and ki67).

^aHispanic definition refers to individuals from Latin American ancestry.

When compared to BL1, LAR and M subtypes had significant lower pCR rates, with an OR of achieving a pCR of 0.14 (95% CI, 0.32–0.64) and 0.30 (95% CI, 0.09–0.95), respectively ($P < 0.01$ and $P = 0.037$). This significant association was maintained when multivariate analysis including tumor size and nodal involvement were performed for both M and LAR subtypes ($P = 0.015$ and 0.008), and BL2 showed a trend towards a worse response too ($P = 0.075$).

In accordance with these results, RCB considered as a continuous variable, was significantly different among all four subtypes ($P = 0.004$).

Lehmann subtypes among BRCA/non-BRCA carriers

Mutational profiling of homologous repair genes was available for 85 of our patients (90.4%). Among our 10 germline

mutation carriers (eight in BRCA1, one in BRCA2, and one in BARD), 30% ($n = 3$) were BL1, 40% ($n = 4$) were BL2 and 30% ($n = 3$) were M. pCR rates among BRCA carriers were similar to those obtained in the general TNBC population [66.6% ($n = 2$) for BL1, 50% ($n = 2$) for BL2 and 33.3% ($n = 1$) for M; $P = 1.00$; Supplementary Table S1).

Discussion

In this study we evaluated the distribution of TNBCtype-4 subtypes according to the classification of Lehmann and colleagues and its association with response to NACT based on carboplatin and docetaxel (24).

The TNBCtype distribution in our cohort is very similar to the one described by Lehmann and colleagues with their last

Table 2. Lehmann subtype distribution according to PAM50 intrinsic subtype

		All N (%)	PAM50 intrinsic subtype	
			Basal N (%)	Nonbasal N (%)
Lehmann TNBC type	BL1	32 (34.0%)	32 (41.0%)	0 (0%)
	BL2	19 (20.2%)	18 (23.1%)	1 (6.2%)
	M	22 (23.4%)	22 (28.2%)	0 (0%)
	LAR	14 (14.90%)	3 (3.8%)	11 (68.8%)
	ER+	7 (7.4%)	3 (3.8%)	4 (25%)

N, number of patients.

Table 3. Baseline characteristics among Lehmann subtypes

	BL1	BL2	M	LAR	P
N	32	19	22	14	
Age median	41	51	50.5	67.5	<0.001
T size (median)	48	40	40	58.5	0.40
N + (%)	78.1%	52.6%	63.6%	85.7%	0.13
Median Ki67	80%	60%	70%	40%	<0.001
G3 (%)	84.4%	63.2%	72.7%	64.3%	0.17

N, number of patients; T size, tumor size; N+, nodal involvement; G3, grade 3. Tests for P values: Age, T, and Ki, Kruskal-Wallis; N and G, Fisher exact test.

modified classification (24). BL1 was the most frequent subtype, and the combination of BL1 and BL2 reached around 50% to 55% of the samples in both their cohort and ours. Most of the data available on TNBC subtype classification and response to neoadjuvant chemotherapy is based on the former Lehmann's classification into six different subtypes (13). For instance, according to the original classification, genomic profiling of 350 TNBC revealed that 15% and 6% were BL1 and BL2, 20% IM, 8% LAR, 17% M, and 7% MSL (25). Similar distribution was observed in TNBC included in the GECAM/2006-03 trial (26). It is worth noting that up to 7% of our patients with TNBC were classified as ER+ with regards to ESR1 gene expression, with no specific correlation with PAM50 intrinsic subtype. Prat and colleagues had 17% of their patients classified as ER+, with a similar PAM50 distribution among this group than in our cohort (25).

Regarding the distribution of the TNBCtype in PAM50 basal versus nonbasal TNBC, we found a distinct distribution pattern between both groups. PAM50 basal-like were enriched in basal subtypes, whereas LAR was the main component of the nonbasal tumors, in accordance to previously described data (25, 27).

We found a significant association of the TNBCtype-4 classification with pCR following neoadjuvant carboplatin and docetaxel ($P = 0.027$), with BL1 displaying the highest pCR rates (65.6%). Although differential response with TNBCtype has shown inconsistent results across different cohorts of patients, the benefit in terms of pCR for BL1 has been invariably described (25). It is noteworthy that our BL1 patients exhibited higher pCR rates than those described previously (65.6% vs. 40–55% with different combinations; refs. 13, 22). BL1, in addition, seems to

display the best disease free-survival at 10 years, with a global DFS of 60% (24).

Although the TNBCtype classification seems to bring homogeneous data with regards to pCR and long-term outcome for BL1, LAR and BL2 subtypes harbor contradictory data across different studies for long-term survival and pCR rates respectively, although no formal analyses have been conducted. For instance, initial data suggested that BL2 tumors might be a group with a special chemoresistance, as described by Masuda and colleagues, who found no pCR within this group of patients, in a cohort of patients treated with anthracyclines and taxanes (14). However, our BL2 patients achieved a pCR rate of 47.4%, the second highest rate among our cohort. This finding is supported by other studies that found pCR rates among BL2 of around 35% to 40% (26). As for LAR tumors, while they have been invariably associated with low response rate to NACT, data regarding long-term survival has been contradictory, displaying the best and worst outcome in different studies (24, 14, 27).

This study may shed some light to the tailoring strategies of neoadjuvant treatments in TNBC, because we could hypothesize that the use of TCb might enable the omission of anthracyclines in specific subsets of patients with TNBC. In fact, because BL1 subtype is associated with a significantly younger age at diagnosis and the best pCR rate and 10 year-DFS, this subset of patients could presumably be spared from the anthracycline long-term cardiac toxicity. In addition, the CRE-ATE-X trial recently demonstrated a significant increase in DFS (3y-DFS rate: 69.8% vs. 56.1%; HR = 0.58; 95% CI, 0.39–0.87) and OS (3y-OS rate: 78.8% vs 70.3%; HR = 0.52; 95% CI,

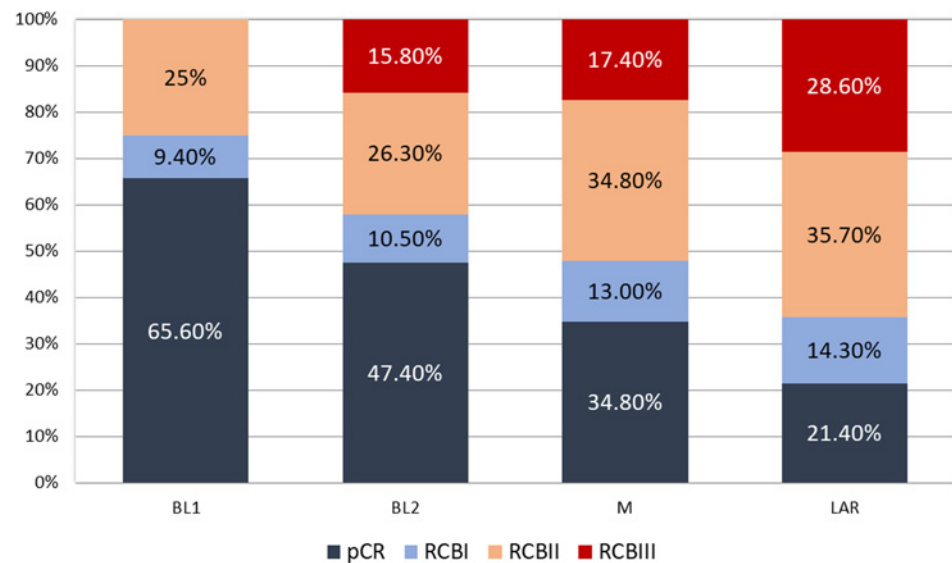


Figure 2. Symmans RCB according to Lehmann subtype. RCB, residual cancer burden.

Table 4. pCR rates according to Lehmann TNBCtype-4

	NCT 01560663	Lehmann 2016 (13)	Jovanovic 2017 (23)
<i>N</i>	94	306	48
Treatment	Tcb x 6	Anthracyclines and taxanes combinations	CDDP-Paclitaxel ± Everolimus
TNBCtype-4			
BL1	32 (34.0%)	110 (35.9%)	15 (31%)
BL2	19 (20.2%)	67 (21.9%)	3 (6%)
M	22 (23.4%)	77 (25.2%)	15 (31%)
LAR	14 (14.9%)	52 (17.0%)	4 (8%)
ER+	7 (7.4%)		
MSL			9 (19%)
pCR rates			
BL1	21 (65.6%)	46 (41.8%)	8 (53%)
BL2	9 (47.4%)	12 (17.9%)	1 (33%)
M	8 (36.4%)	29 (37.7%)	7 (47%)
LAR	3 (21.4%)	15 (28.8%)	1 (25%)
ER+	1 (14.3%)	-	-
MSL	-	-	3 (33%)

Cb, carboplatin; T, docetaxel; CDDP, cisplatin; *N*, number of patients.

0.30–0.90) among TNBC with residual disease after NACT who received adjuvant capecitabine (28). We can speculate that capecitabine should be tested earlier on in association with other drugs in patients with TNBCtype-4 not likely to achieve a pCR, such as LAR and M. Thus, performing this classification and tailoring NACT among patients with TNBC would definitely improve cost-effectiveness in this setting and optimize treatment, preventing unnecessary toxicities.

However, LAR tumors have consistently shown low response rates and pCR in other series (29), and, however inconsistent across studies, AR TNBC seem to show a better long-term prognosis, supporting that TNBC/LAR tumors have a distinct biology compared to non-LAR TNBC (14, 29–31). AR-driven TNBC represent a subset of tumors for which pCR might not be prognostic, and thus, that may display a favorable outcome despite residual disease after NACT (32, 33). This chemoresistance of AR-driven TNBC could be filled by molecularly targeted therapies directed against the androgen receptor (13), which have been evaluated both in the metastatic and early setting (17). For instance, several trials are evaluating adjuvant enzalutamide in patients with TNBC with AR+ disease (NCT02750358), as well as in the neoadjuvant setting in combination with chemotherapy (NCT02689427). Although adjuvant antiandrogen therapy in patients with residual disease following NACT seemed like an interesting option to consider, the phase III ENDEAR trial (NCT02929576) was prematurely discontinued.

In addition to the use of the former TNBCtype classification in most of the published data, there is little evidence regarding TNBCtype performance in patients treated with non-anthracycline, platinum salt-containing regimens (Table 4). A recent phase II study evaluating the addition of neoadjuvant everolimus to cisplatin and paclitaxel, performed TNBCtype in 48 of their patients, exhibiting similar trends of response to our cohort overall, although it included the MSL subtype (34).

We then analyzed the TNBCtype and PAM50 distribution among carriers of mutation in a panel of seven homologous repair genes, with all our tumors classified as basal-like by PAM50 and as BL1, BL2, and M with TNBCtype-4. We also analyzed response with regards to mutational status, with no differences in pathologic response between carriers and non-carriers. Data regarding TNBCtype distribution among BRCA

carriers are still scarce. Isakoff and colleagues described seven metastatic TNBC with BRCA mutation, and found 42.9% of BL1, 28.6% of M, and 14.3% of MSL and unstable respectively (35). Moreover, Telli et al. found 9.1% of BL1, 36.4% of IM, 18.2% M, 9.1% MSL, and 27.3% of unstable among 11 BRCA1/2 carriers within the PrECOG0105 trial (36). Nevertheless, all these data should be taken with caution due to the small sample size of all three studies.

Our study has some notable strengths. First, it is one of the first cohorts presenting data with the new classification TNBCtype-4. Second, most of the published data comes from retrospective analysis of patients heterogeneously treated, mainly with combinations of anthracyclines and taxanes. Our cohort, in contrast, is uniformly treated with carboplatin and docetaxel, and no data has been published yet about TNBCtype performance in response prediction with this regimen. Third, we present novel data on the subtype distribution and response among BRCA-related genes mutation carriers.

However, our study has some significant limitations, such as the short follow-up available to date, that precludes survival analysis and, mainly, the small sample size and thus, the wide CI ranges obtained.

In conclusion, although confirmation by other independent series is required, Lehmann's refined classification TNBCtype-4 could help select the neoadjuvant therapy in TNBC. Patients with BL subtypes could be candidates for standard chemotherapy, while the remaining subtypes may need to be directed for new, experimental therapies.

Disclosure of Potential Conflicts of Interest

F. Moreno is a consultant/advisory board member for Pfizer and Roche. A. Barnadas reports receiving speakers bureau honoraria from Pfizer and Roche, and is a consultant/advisory board member for Lilly and Pfizer. C.M. Perou holds ownership interest (including patents) in and is a consultant/advisory board member for Bioclassifier LLC. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: S. López-Tarruella, Y. Jerez, U. Bohn, C.M. Perou, M. Martin

Development of methodology: S. López-Tarruella, A. Picornell, Y. Jerez, E. Alvarez, A. Barnadas, U. Bohn, M. Martin

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. López-Tarruella, J. Ángel García-Saenz, Y. Jerez, H.L. Gómez, F. Moreno, I. Márquez-Rodas, R. Ramos-Medina, T. Massarrah, I. Ocaña, H. Fuentes, A. Barnadas, U. Bohn, C.M. Perou, M. Martin

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): I. Echavarría, S. López-Tarruella, A. Picornell, Y. Jerez, K. Hoadley, H.L. Gómez, M. Del Monte-Millan, I. Márquez-Rodas, E. Alvarez, J. Gayarre, A. Barnadas, U. Bohn, C.M. Perou, M. Martin

Writing, review, and/or revision of the manuscript: I. Echavarría, S. López-Tarruella, A. Picornell, J. Ángel García-Saenz, Y. Jerez, K. Hoadley, H.L. Gómez, F. Moreno, M. Del Monte-Millan, I. Márquez-Rodas, E. Alvarez, R. Ramos-Medina, J. Gayarre, T. Massarrah, I. Ocaña, H. Fuentes, A. Barnadas, A.I. Ballesteros, U. Bohn, C.M. Perou, M. Martin

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Picornell, M. Del Monte-Millan, E. Alvarez, J. Gayarre, U. Bohn

Study supervision: Y. Jerez, U. Bohn, M. Martin

Other (pathologist analysis of pathological specimens): M. Cebollero

Acknowledgments

Funding for M. Martin was supported by the research grant from Instituto de Salud Carlos III (ISCIII, PI 12/02684), FEDER (RETICC-RD12/0036/0076) [Ayuda cofinanciada por el Fondo Europeo de Desarrollo Regional (FEDER). Una manera de hacer Europa" and CiberOnc]. C.M. Perou was supported by funds from the NCI Breast SPORE program P50-CA58223.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 5, 2017; revised October 9, 2017; accepted January 25, 2018; published OnlineFirst January 29, 2018.

References

- Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* 2014;384:164–72.
- Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res* 2007;13:2329–34.
- Sikov WM, Berry DA, Perou CM, Singh B, Cirincione CT, Tolane SM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). *J Clin Oncol* 2015;33:13–21.
- von Minckwitz G, Schneeweiss A, Loibl S, Salat C, Denkert C, Rezai M, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBC 66): a randomised phase 2 trial. *Lancet Oncol* 2014;15:747–56.
- von Minckwitz G, Untch M, Nüesch E, Loibl S, Kaufmann M, Kümmel S, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo-adjuvant chemotherapy trials. *Breast Cancer Res Treat* 2011;125:145–56.
- Sharma P, López-Tarruella S, García-Saenz JA, Ward C, Connor CS, Gómez HL, et al. Efficacy of neoadjuvant carboplatin plus docetaxel in triple-negative breast cancer: combined analysis of two cohorts. *Clin Cancer Res* 2017;23:649–57.
- Kern P, Kalisch A, von Minckwitz G, Pütter C, Kolberg H-C, Pott D, et al. Neoadjuvant, anthracycline-free chemotherapy with carboplatin and docetaxel in triple-negative, early-stage breast cancer: a multicentric analysis of rates of pathologic complete response and survival. *J Chemother* 2016;28:210–7.
- Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* 2000;406:747–52.
- Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001;98:10869–74.
- Prat A, Adamo B, Cheang MCU, Anders CK, Carey LA, Perou CM. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. *Oncologist* 2013;18:123–33.
- Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua SAW, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. *Clin Cancer Res Off J Am Assoc Cancer Res* 2015;21:1688–98.
- Jézéquel P, Loussouarn D, Guérin-Charbonnel C, Campion L, Vanier A, Gouraud W, et al. Gene-expression molecular subtyping of triple-negative breast cancer tumours: importance of immune response. *Breast Cancer Res* 2015;17:43.
- Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and pre-clinical models for selection of targeted therapies. *J Clin Invest* 2011;121:2750–67.
- Masuda H, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, Meric-Bernstam F, et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. *Clin Cancer Res* 2013;19:5533–40.
- Farmer P, Bonnefoi H, Becette V, Tubiana-Hulin M, Fumoleau P, Larsimont D, et al. Identification of molecular apocrine breast tumours by microarray analysis. *Oncogene* 2005;24:4660–71.
- Doane AS, Danso M, Lal P, Donaton M, Zhang L, Hudis C, et al. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. *Oncogene* 2006;25:3994–4008.
- Gucalp A, Tolane SM, Isakoff SJ, Ingle JN, Liu MC, Carey LA, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. *Clin Cancer Res* 2013;19:5505–12.
- Parker JS, Peterson AC, Tudor IC, Hoffman J, Uppal H. A novel biomarker to predict sensitivity to enzalutamide (ENZA) in TNBC. *J Clin Oncol* 2015;33:1083–1083.
- Hammond MEH, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American society of clinical oncology/college of american pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol* 2010;28:2784–95.
- Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: american society of clinical oncology/college of american pathologists clinical practice guideline update. *J Clin Oncol* 2013;31:3997–4013.
- Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, et al. Measurement of residual cancer burden to predict survival after neoadjuvant chemotherapy. *J Clin Oncol* 2007;25:4414–22.
- Chen X, Li J, Gray WH, Lehmann BD, Bauer JA, Shyr Y, et al. TNBCtype: a subtyping tool for triple-negative breast cancer. *Cancer Inform* 2012;11:147–56.
- Ring BZ, Hout DR, Morris SW, Lawrence K, Schweitzer BL, Bailey DB, et al. Generation of an algorithm based on minimal gene sets to clinically subtype triple negative breast cancer patients. *BMC Cancer* 2016;16:143.
- Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, et al. Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. Sapino A, editor. *PLOS ONE* 2016;11:e0157368.
- Prat A, Fan C, Fernández A, Hoadley KA, Martinello R, Vidal M, et al. Response and survival of breast cancer intrinsic subtypes following multi-agent neoadjuvant chemotherapy. *BMC Med* 2015;13:303.
- Chica-Parrado MR, Santonja A, Lluich-Hernandez A, Albanell J, Sanchez-Muñoz A, Chacón I, et al. Luminal androgen receptor role and pathological complete response rate to neoadjuvant chemotherapy in triple negative breast cancer. *Ann Oncol* 2016;24(suppl 6):vi56-57.
- Mayer IA, Abramson VG, Lehmann BD, Pietersen JA. New strategies for triple-negative breast cancer—deciphering the heterogeneity. *Clin Cancer Res* 2014;20:782–90.
- Masuda N, Lee S-J, Ohtani S, Im Y-H, Lee E-S, Yokota I, et al. Adjuvant capecitabine for breast cancer after preoperative chemotherapy. *N Engl J Med* 2017;376:2147–59.

29. Asano Y, Kashiwagi S, Onoda N, Kurata K, Morisaki T, Noda S, et al. Clinical verification of sensitivity to preoperative chemotherapy in cases of androgen receptor-expressing positive breast cancer. *Br J Cancer* 2016;114:14–20.
30. Rampurwala M, Wisinski KB, O'Regan R. Role of the androgen receptor in triple-negative breast cancer. *Clin Adv Hematol Oncol HO* 2016; 14:186–93.
31. Gasparini P, Fassan M, Cascione L, Guler G, Balci S, Irkkan C, et al. Androgen receptor status is a prognostic marker in non-basal triple negative breast cancers and determines novel therapeutic options. *PLoS One* 2014;9:e88525.
32. Loibl S, Müller BM, von Minckwitz G, Schwabe M, Roller M, Darb-Esfahani S, et al. Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy. *Breast Cancer Res Treat* 2011;130: 477–87.
33. Yu K-D, Zhu R, Zhan M, Rodriguez AA, Yang W, Wong S, et al. Identification of prognosis-relevant subgroups in patients with chemoresistant triple-negative breast cancer. *Clin Cancer Res* 2013;19:2723–33.
34. Jovanovic B, Mayer IA, Mayer EL, Abramson VG, Bardia A, Sanders M, et al. A randomized phase II neoadjuvant study of cisplatin, paclitaxel with or without everolimus in patients with stage II/III triple-negative breast cancer (TNBC). *Clin Cancer Res* 2017;23:4035–4045
35. Isakoff SJ, Mayer EL, He L, Traina TA, Carey LA, Krag KJ, et al. TBCRC009: a multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. *J Clin Oncol* 2015;33:1902–9.
36. Telli ML, Jensen KC, Vinayak S, Kurian AW, Lipson JA, Flaherty PJ, et al. Phase II study of gemcitabine, carboplatin, and iniparib as neoadjuvant therapy for triple-negative and BRCA1/2 mutation-associated breast cancer with assessment of a tumor-based measure of genomic instability: PrECOG 0105. *J Clin Oncol* 2015;33:1895–901.