

Association of Tumor-Infiltrating Lymphocytes with Homologous Recombination Deficiency and *BRCA1/2* Status in Patients with Early Triple-Negative Breast Cancer: A Pooled Analysis



Melinda L. Telli¹, Charles Chu¹, Sunil S. Badve², Shaveta Vinayak³, Daniel P. Silver⁴, Steven J. Isakoff^{5,6}, Virginia Kaklamani⁷, William Gradishar⁸, Vered Stearns⁹, Roisin M. Connolly⁹, James M. Ford¹, Joshua J. Gruber¹, Sylvia Adams¹⁰, Judy Garber^{6,11}, Nadine Tung¹², Chris Neff¹³, Ryan Bernhisel¹³, Kirsten M. Timms¹³, and Andrea L. Richardson⁹

ABSTRACT

Purpose: Patients with triple-negative breast cancer (TNBC) with homologous recombination deficient tumors achieve significantly higher pathologic complete response (pCR) rates when treated with neoadjuvant platinum-based therapy. Tumor-infiltrating lymphocytes (TIL) are prognostic and predictive of chemotherapy benefit in early stage TNBC. The relationship between TILs, *BRCA1/2* mutation status, and homologous recombination deficiency (HRD) status in TNBC remains unclear.

Experimental Design: We performed a pooled analysis of five phase II studies that included patients with TNBC treated with neoadjuvant platinum-based chemotherapy to evaluate the association of TILs with HRD status (Myriad Genetics) and tumor *BRCA1/2* mutation status. Furthermore, the relationship between pathologic response assessed using the residual cancer burden (RCB) index and HRD status with adjustment for TILs was evaluated.

Results: Among 161 patients, stromal TIL (sTIL) density was not significantly associated with HRD status ($P = 0.107$) or tumor *BRCA1/2* mutation status ($P = 0.391$). In multivariate analyses, sTIL density [OR, 1.23; 95% confidence interval (CI), 0.94–1.61; $P = 0.139$] was not associated with pCR, but was associated with RCB 0/I status (OR 1.62; 95% CI, 1.20–2.28; $P = 0.001$). HRD was significantly associated with both pCR (OR 12.09; 95% CI, 4.11–44.29; $P = 7.82 \times 10^{-7}$) and RCB 0/I (OR 10.22; 95% CI, 4.11–28.75; $P = 1.09 \times 10^{-7}$) in these models.

Conclusions: In patients with TNBC treated with neoadjuvant platinum-based therapy, TIL density was not significantly associated with either tumor *BRCA1/2* mutation status or HRD status. In this pooled analysis, HRD and sTIL density were independently associated with treatment response, with HRD status being the strongest predictor.

Introduction

Triple-negative breast cancers (TNBC) are highly proliferative cancers that lack expression of estrogen receptors (ER) and progesterone receptors (PR), as well as overexpression or amplification of the HER2 oncogene (1). The current standard of care for the systemic treatment of newly diagnosed primary TNBC is anthracycline and

taxane-based combination chemotherapy. In operable TNBC, neoadjuvant therapy has become widely utilized given the prognostic significance of pathologic response on long-term outcomes and the opportunity to modify adjuvant therapy based on pathologic response after neoadjuvant therapy (2). Current questions of interest include whether platinum should be routinely incorporated into the curative multi-drug chemotherapy regimen for patients with newly diagnosed TNBC and whether therapies stimulating host antitumor immunity have a role in primary disease.

A subset of sporadic TNBCs share many features with germline *BRCA1* or *BRCA2* (*BRCA1/2*) mutation-associated cancers, including defective homologous recombination (HR) DNA repair (3). This HR deficiency may be driven by a number of potential mechanisms including methylation of the *BRCA1* promoter, germline mutation in non-*BRCA1/2* HR pathway genes, as well as somatic mutations in the HR pathway, including *BRCA1* or *BRCA2*. Biomarkers of homologous recombination deficiency (HRD), or “*BRCAness*,” are being investigated in breast and other cancers to determine whether they might be able to select patients for therapies targeting tumors with defective DNA repair, including platinum and PARP inhibitors. The HRD assay (Myriad Genetics) incorporates three measures of genomic instability, LOH, telomeric allelic imbalance (TAI), and large-scale state transitions (LST), to capture this biology regardless of etiology (4). Previous work from our group has shown that HR-deficient TNBC is associated with higher rates of pathologic response to neoadjuvant platinum-based therapy compared with HR nondeficient tumors (5–10).

¹Stanford University School of Medicine, Stanford, California. ²Indiana University, Indianapolis, Indiana. ³University of Washington School of Medicine, Seattle, Washington. ⁴Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania. ⁵Massachusetts General Hospital Cancer Center, Boston, Massachusetts. ⁶Harvard Medical School, Boston, Massachusetts. ⁷University of Texas Health Science Center, San Antonio, Texas. ⁸Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, Illinois. ⁹Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, Maryland. ¹⁰New York University Perlmutter Cancer Center, New York, New York. ¹¹Dana Farber Cancer Institute, Boston, Massachusetts. ¹²Beth Israel Deaconess Medical Center, Boston, Massachusetts. ¹³Myriad Genetics, Inc., Salt Lake City, Utah.

Corresponding Author: Melinda L. Telli, Stanford University, 875 Blake Wilbur Drive, MC 5826, Stanford, CA 94305-5826. Phone: 650-724-9533; Fax: 650-498-4696; E-mail: mtelli@stanford.edu

Clin Cancer Res 2020;26:2704–10

doi: 10.1158/1078-0432.CCR-19-0664

©2019 American Association for Cancer Research.

Translational Relevance

Increased tumor-infiltrating lymphocytes (TIL) are prognostic and predictive of therapy response in patients with early-stage triple-negative breast cancer (TNBC). Likewise, germline mutations in *BRCA1* and *BRCA2*, as well as measures of genomic instability, including the homologous recombination deficiency (HRD) assay, are also significantly associated with response to neoadjuvant chemotherapy, especially platinum-based therapy. The relationship between TILs, HRD, and *BRCA1/2* mutation status in early-stage TNBC has been unclear. In this pooled analysis of five neoadjuvant clinical trials of platinum-based therapy in TNBC, we found that stromal TIL density was not significantly associated with HRD status or tumor *BRCA1/2* mutation status. In a multivariate model, HRD and TIL density were independently associated with therapy response, with HRD being the strongest independent predictor. Our data suggest that these biomarkers should potentially be considered independently in future clinical trials.

There is increasing evidence that host antitumor immunity is critical for the formation and progression of cancer, as well as the effectiveness of standard-of-care therapies. In particular, the density of tumor-infiltrating lymphocytes (TIL) is strongly associated with both prognosis and prediction of neoadjuvant chemotherapy response in patients with TNBC (11–17). Interestingly, recent data demonstrated that HR-deficient and *BRCA1/2*-mutant high-grade serous ovarian cancers are enriched for immune cell infiltration (18). It was hypothesized that increased TIL infiltration may result from an increased production of neoantigens by these tumors, providing the rationale for combinatorial treatment strategies. To further evaluate the relationship between HRD, *BRCA1/2* mutations, and TIL density in early-stage TNBC, we performed a pooled analysis of five phase II neoadjuvant clinical trials of platinum-based chemotherapy where HRD status, *BRCA1/2* mutation status, and tumor for TIL scoring were available.

Materials and Methods

Data were pooled from five prospective phase II clinical trials of neoadjuvant platinum-based therapy where HRD status and tumor *BRCA1/2* mutation status were available. The eligibility criteria for inclusion in this pooled analysis included patients with stage I–III disease, TNBC status, treatment with 4–6 cycles of platinum-based neoadjuvant chemotherapy, available HRD status, available pathologic response data, and available hematoxylin and eosin (H&E) tumor section adequate for TIL assessment.

Description of the clinical studies

Full details of the study designs and efficacy endpoints of PrECOG 0105 (NCT00813956), cisplatin-1 (NCT00148694), cisplatin-2 (NCT00580333), and NCT01372579 and TBCRC 008 (NCT00616967) have been reported previously (5, 7–10). The trials included in this analysis are summarized in **Table 1**. These studies were approved by the institutional review boards at each respective institution and were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject.

The single arm phase II PrECOG 0105 study enrolled patients with stage I–IIIA (T ≥ 1cm) triple-negative (ER/PR ≤ 5%, HER2-negative)

Table 1. Trials included in analysis.

Trial	N	Weeks of therapy	Neoadjuvant regimen
PrECOG 0105	67	12–18	Carboplatin, gemcitabine, and iniparib
Cisplatin-1	18	12	Cisplatin
Cisplatin-2	32	12	Cisplatin and bevacizumab
NCT01372579	26	12	Carboplatin and eribulin
TBCRC 008	18	12	Carboplatin, nab-paclitaxel, ± vorinostat

or *BRCA1/2* germline mutation-associated breast cancer (5). A total of 93 patients were enrolled in this study and were treated with neoadjuvant carboplatin intravenously at an AUC of 2 on days 1 and 8, gemcitabine 1,000 mg/m² i.v. on days 1 and 8, and iniparib 5.6 mg/kg i.v. on days 1, 4, 8, and 11 every 21 days for 4–6 cycles. Sixty-seven patients who met the inclusion criteria were entered into the pooled analysis

Cisplatin-1 is a single arm, phase II study that enrolled 29 patients with stage I–III (T > 1.5 cm) TNBC (ER/PR < 1%, HER2-negative; ref. 7). Prior to surgery, patients received four cycles of cisplatin 75 mg/m² i.v. every 21 days. Eighteen patients who met the inclusion criteria were entered into the pooled analysis.

Cisplatin-2 is a single arm, phase II trial that enrolled 51 patients with stage I–III TNBC (ER/PR < 1%, HER2-negative) who were treated with neoadjuvant cisplatin 75 mg/m² i.v. every 3 weeks for four cycles and bevacizumab 15 mg/kg every 3 weeks for three cycles prior to definitive surgery (8). Thirty-two patients who met the inclusion criteria were entered into the pooled analysis.

NCT01372579 is a single arm, phase II trial that enrolled 30 patients with stage I–III (ER/PR < 1%, HER2-negative) TNBC (9). There was no minimum tumor size requirement. Patients received carboplatin AUC 6 i.v. every 21 days and eribulin 1.4 mg/m² days 1 and 8 every 21 days for four cycles. Twenty-six patients who met the inclusion criteria were entered into the pooled analysis.

TBCRC 008 is a multicenter phase II study that enrolled 62 patients with clinical stage I–III HER2-negative breast cancer (10). Patients with any ER/PR status were included in the original study but stratified on the basis of hormone receptor status (ER and PR < 1% vs. ER or PR > 1%). Participants received 12 weeks of preoperative carboplatin AUC 2 i.v. weekly, nab-paclitaxel 100 mg/m² weekly with vorinostat 400 mg orally daily, days 1–3 of every 7-day cycle or placebo. Eighteen patients with TNBC who met the inclusion criteria were entered into the pooled analysis.

HRD testing

The HRD assay utilizes next-generation sequencing on DNA extracted from formalin-fixed, paraffin-embedded or frozen tumor tissue (4). The methods of DNA extraction are described previously (4). Genome-wide SNP data were generated from a custom Agilent SureSelect XT capture followed by sequencing on an Illumina HiSeq2500. SNP data were analyzed using an algorithm that determines the most likely allele-specific copy number at each SNP location after accounting for contamination of the tumor sample with non-tumor DNA. Tumor sequence data for *BRCA1* and *BRCA2* were analyzed for the presence of variants from wild-type sequence. Variants were classified as deleterious or suspected deleterious based on previously described criteria (19). Read coverage across each exon was used to detect large rearrangements.

The HRD score is the sum of three biomarkers of genomic instability, which includes LOH, TAI, and LSTs. HRD scores ranged between 0 and 100. Tumors with a high HRD score (≥ 42) or a tumor *BRCA1/2* (*tBRCA*) mutation were considered HR deficient. Tumors with a HRD score of <42 and no *BRCA1/2* mutation were defined as HR nondeficient. Germline *BRCA1/2* status was not available for all trials and tumor *BRCA1/2* status was determined using next-generation sequencing of the primary breast tumor. A high binary HRD score is defined as being 42 or greater, whereas a low binary HRD score is less than 42. The threshold of 42 was selected on the basis of the 5th percentile of HRD scores in tumors with known *BRCA1/2* mutation or methylation status (4).

All patients had a core biopsy at baseline, on which HRD status was assessed. All HRD assays were performed at Myriad Genetics, Inc and processed in the HRD research laboratory. Samples that did not yield a passing HRD score were excluded from this analysis.

TIL scoring

Histopathologic TIL evaluation was performed on digitized pretreatment core biopsy H&E-stained sections. Intratumoral TILs (iTIL) and stromal TILs (sTIL) were scored. Density of iTILs was scored in deciles and defined as the percentage of lymphocytic cells within tumor nests having direct tumor cell contact with no intervening stroma. Density of sTILs was also scored in deciles and defined as the percentage of lymphocytic cells in the tumor stroma. In accordance with the international TIL working group guidelines (20), an expert breast cancer pathologist (S.S. Badve) scored these sections blinded to outcome data. TILs were scored only on those patients who met the criteria for inclusion in this pooled analysis.

Pathologic response assessment

In all five trials, pathologic complete response (pCR) was defined as having no invasive disease in either the breast or axillary nodes with noninvasive breast residuals allowed (ypT0/is ypN0). In addition, pathologic response was assessed using the residual cancer burden (RCB) index (21). The RCB index is a validated measure of relapse-free survival in patients with breast cancer treated with neoadjuvant chemotherapy. RCB 0 indicates complete pathologic response (ypT0/is ypN0); RCB I, minimal residual disease; RCB II, moderate residual disease; and RCB III, extensive residual disease. As prior studies have shown that for TNBC RCB I carries the same favorable prognosis as RCB 0, responders were defined as RCB 0/I and non-responders as RCB II/III (21, 22). Patients who experienced progressive disease during neoadjuvant therapy were categorized as having no pCR and an RCB category of III.

Statistical analysis

The primary objective of this retrospective pooled analysis was to quantify iTILs and sTILs in pretreatment core biopsy breast tumor samples and evaluate the association of iTILs and sTILs with HRD status. Secondary objectives included evaluation of the association of iTILs and sTILs with *tBRCA* mutation status and binary HRD score in *BRCA* mutation-negative patients. In addition, we assessed the relationship between pCR and RCB 0/I with iTILs or sTILs while adjusting for HRD status. Finally, the relationships between pCR and RCB 0/I and HRD status while adjusting for iTILs, sTILs, age at diagnosis, clinical stage, intended duration of neoadjuvant therapy, and clinical trial were evaluated.

The evaluable analysis set includes all stage I–III patients with TNBC who have a nonmissing HRD status and at least one nonmissing TIL count. The evaluable set was used for demographics and other

Table 2. Patient characteristics ($N = 161$).

Variable		Statistic
Clinical characteristics		
Age	Mean (SD)	49.4 (10.83)
	Range	24–78
Clinical stage	I	12 (7.5%)
	II	126 (78.3%)
	III	23 (14.3%)
Intended therapy duration	12 weeks	104 (64.6%)
	18 weeks	57 (35.4%)
Trial	PrECOG	67 (41.6%)
	Other	94 (58.4%)
Clinical endpoints		
pCR	No	110 (68.3%)
	Yes	51 (31.7%)
RCB	0/I	75 (46.6%)
	II/III	84 (52.2%)
	Missing	2 (1.2%)
HR deficiency		
<i>tBRCA</i> mutation status	Negative	122 (75.8%)
	Positive	34 (21.1%)
	Missing	5 (3.1%)
Binary HRD score	Low (HRD < 42)	62 (38.5%)
	High (HRD ≥ 42)	95 (59.0%)
	Missing	4 (2.5%)
HRD status	Nondeficient	61 (37.9%)
	Deficient	100 (62.1%)
TIL density		
iTIL	$<1\%$	135 (83.9%)
	10%	22 (13.7%)
	20%	4 (2.5%)
sTIL	$<1\%$	37 (23.0%)
	10%	60 (37.3%)
	20%	24 (14.9%)
	30%	21 (13.0%)
	40%	6 (3.7%)
	$\geq 50\%$	13 (8.1%)

baseline characteristics and for all statistical analyses. A subset of the evaluable set included *BRCA1/2*-negative tumors. This set was utilized for secondary analysis. Missing data were not imputed in this study. All *P* values are two-sided and statistical significance was set at 0.05. Statistical analysis was performed using SAS.

In the primary analysis, the distribution of iTIL and sTIL counts that were quantified, were investigated. As the counts were not normally distributed, the Mann–Whitney test was used to evaluate the association of iTIL or sTIL densities in decile form with HRD status, *BRCA1/2* mutation status, and binary HRD score. In the secondary analyses, multivariate logistic regression was utilized to assess the association of the binary responses pCR and RCB 0/I with independent variables like iTILs, sTILs, HRD status, clinical stage, age at diagnosis, clinical trial, and intended duration of neoadjuvant therapy. The OR was reported with a 95% confidence interval (CI).

Results

Patient characteristics

A total of 161 patients from five clinical trials met criteria for inclusion. The patient demographics and clinicopathologic data are shown in **Table 2**. The average patient age was 49.4 years. Twelve patients (7.50%) were clinical stage I, 126 patients (78.3%) were stage II, and 23 patients (14.3%) were stage III.

Regarding clinical endpoints, pCR was achieved in 51 (31.7%) patients and RCB 0/I was achieved in 75 (46.6%) patients. Among the 161 patients, 100 patients (62.1%) were HR deficient, which includes patient's with an HRD score ≥ 42 or a tumor mutation in *BRCA1/2*. Within this group, 34 patients (21.1%) had a tumor mutation in *BRCA1/2*. Furthermore, 62 (38.5%) patients from the entire group had a low binary HRD score (HRD score < 42), whereas 95 (59.0%) had a high binary score (HRD score ≥ 42). The majority of patients had an iTIL density of $< 1\%$ (83.9%) and 64 (39.8%) had a sTIL density $\geq 20\%$.

Association between TILs, HRD, and *tBRCA1/2* status

iTIL and sTIL densities were not significantly associated with HRD status ($P = 0.369$ and $P = 0.107$, respectively). Furthermore, iTIL and sTIL density were not associated with *tBRCA1/2* mutation status ($P = 0.312$ and $P = 0.391$). Among *tBRCA1/2*-negative patients, binary HRD score (≥ 42 vs. < 42) was not associated with iTIL or sTIL density ($P = 0.536$ and $P = 0.228$, respectively).

Association of TILs and pathologic response

Elevated sTIL density was not associated with pCR ($P = 0.107$), but was significantly associated with RCB 0/I ($P = 0.002$) among patients treated with platinum-based neoadjuvant chemotherapy, as shown in Fig. 1.

In univariate analysis, HR-deficient patients were more likely to achieve pCR (OR 9.54; 95% CI, 3.82–29.12; $P = 9.47 \times 10^{-8}$) and RCB 0/I (OR 9.10; 95% CI, 4.25–21.09; $P = 1.36 \times 10^{-9}$) compared with HR nondeficient patients (Table 3). Furthermore, tumors with *tBRCA1/2* mutations were also more likely to achieve pCR (OR 2.30; 95% CI, 1.05–5.04; $P = 0.038$) and RCB 0/I (OR 2.83; 95% CI, 1.29–6.53; $P = 0.009$) compared with *tBRCA1/2* wild-type tumors. This association

was also seen in the *tBRCA1/2*-negative patients with a high binary HRD score for both pCR (OR 8.78; 95% CI, 3.34–27.78; $P = 2.52 \times 10^{-6}$) and RCB 0/I (OR 8.20; 95% CI, 3.61–20.08; $P = 1.57 \times 10^{-7}$).

In regards to TILs, iTIL density was not associated with either pCR or RCB 0/I in univariate analyses. Patients with elevated sTIL densities were more likely to achieve a RCB 0/I outcome (OR 1.34; 95% CI, 1.11–1.66; $P = 0.002$) compared with lower sTIL densities, but sTIL density was not significantly associated with pCR. Other factors of significance in univariate analyses for pCR included age at diagnosis and trial. Significant factors for RCB 0/I in univariate analyses included age at diagnosis and clinical stage.

Multivariate logistic regression was used to assess whether sTIL or iTIL density and HRD status were significant predictors of pCR and RCB 0/I after adjusting for clinical covariates (Table 4). In this model, HR-deficient status remained a significant predictor of both pCR (OR 12.09; 95% CI, 4.11–44.29; $P = 7.82 \times 10^{-7}$) and RCB 0/I (OR 10.22; 95% CI, 4.11–28.75; $P = 1.09 \times 10^{-7}$). In the multivariate model, neither iTIL (OR 0.75; 95% CI, 0.26–2.14; $P = 0.585$) nor sTIL density (OR 1.23; 95% CI, 0.94–1.61; $P = 0.139$) showed an association with pCR. Furthermore, iTIL was not associated with RCB 0/I (OR 0.53; 95% CI, 0.17–1.63; $P = 0.260$), but sTIL was (OR 1.62; 95% CI, 1.20–2.28; $P = 0.001$). In the multivariate analysis, age at diagnosis and intended duration of therapy were not independently predictive for pCR or RCB 0/I. Clinical stage was an independent predictor for RCB 0/I only. Clinical trial was an independent predictor for pCR only.

Discussion

In this cohort of 161 patients pooled from five phase II neoadjuvant clinical trials of platinum-based therapy, the densities of iTIL and sTIL were not significantly associated with HRD status, *tBRCA1/2* mutation

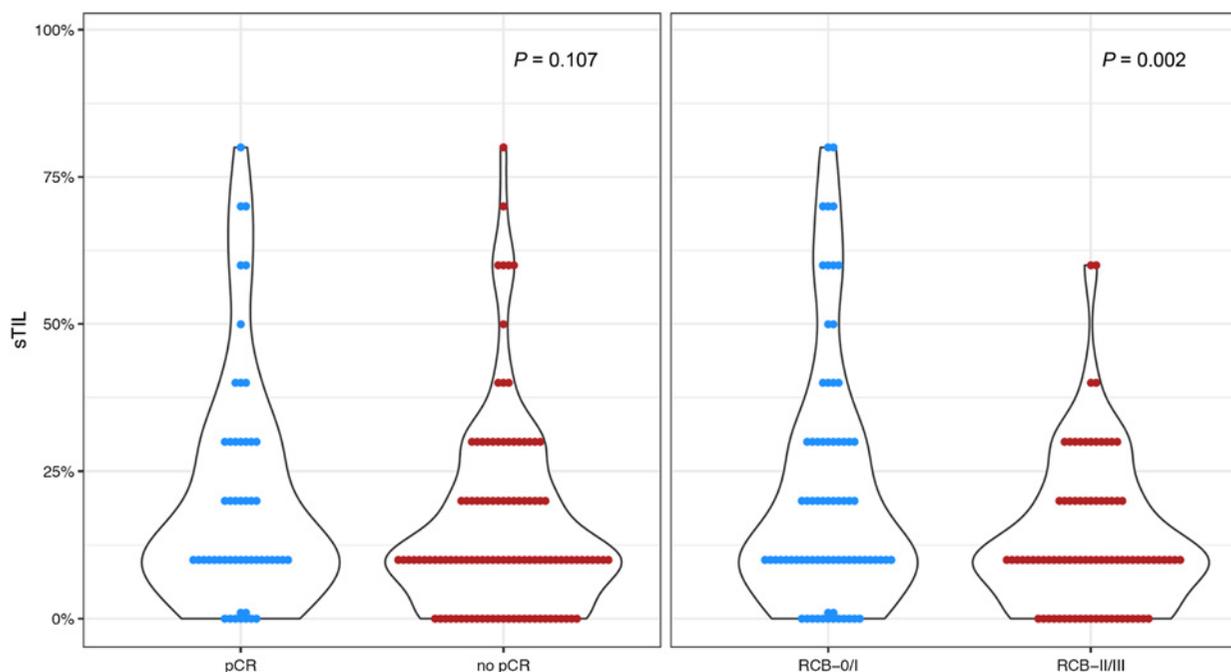


Figure 1.

Association of sTIL density and pathologic response. Violin plots show the distribution of sTIL density by chemotherapy response (pCR vs. no pCR and RCB 0/I vs. RCB II/III). The width of each violin plot is proportional to estimated density of sTILs and points are overlaid to identify the distribution seen in our data. P values are from univariate logistic regression and test the association between sTILs and response.

Table 3. Univariate logistic regression analysis for pathologic response.

Variable		pCR		RCB O/I	
		OR (95% CI)	P	OR (95% CI)	P
HR deficiency					
HRD status	Nondeficient	1	9.47×10^{-8}	1	1.36×10^{-9}
	Deficient	9.54 (3.82–29.12)		9.10 (4.25–21.09)	
tBRCA mutation status	Negative	1	0.038	1	0.009
	Positive	2.30 (1.05–5.04)		2.83 (1.29–6.53)	
Binary HRD score	Low	1	2.52×10^{-6}	1	1.57×10^{-7}
	High	8.78 (3.34–27.78)		8.20 (3.61–20.08)	
TIL density					
iTIL		1.40 (0.67–2.85)	0.355	1.63 (0.81–3.44)	0.174
sTIL		1.16 (0.97–1.40)	0.107	1.34 (1.11–1.66)	0.002
Clinical characteristics					
Age		0.95 (0.92–0.98)	0.002	0.95 (0.92–0.98)	0.002
Clinical stage	I	3.24 (0.97–11.57)	0.130	6.16 (1.54–41.16)	0.025
	II	1		1	
	III	0.82 (0.28–2.14)		0.85 (0.33–2.12)	
Intended therapy duration	12 Weeks	1	0.300	1	0.063
	18 Weeks	1.44 (0.72–2.85)		1.86 (0.97–3.62)	
Trial	PrECOG 0105	1	0.032	1	0.120
	NCT00148694	0.38 (0.08–1.31)		0.30 (0.09–0.90)	
	NCT00580333	0.35 (0.11–0.98)		0.47 (0.19–1.10)	
	NCT01372579	1.40 (0.55–3.54)		0.57 (0.23–1.43)	
	TBCRC 008	1.91 (0.66–5.56)		1.12 (0.38–3.42)	

status, or binary HRD score. Multiple prior studies have identified TIL density as an important biomarker for both prognosis and prediction of chemotherapy benefit in early stage TNBC (11–17). *BRCA1/2* mutation status, as well as HRD status have also been found to be associated with significantly higher pCR rates to neoadjuvant chemotherapy (3, 5–10). Our data suggest that in early stage TNBC, TIL, HRD, and *tBRCA1/2* biomarker subsets may be distinct and nonoverlapping. This is an important negative finding and is in contrast to a prior study suggesting that HR-deficient and *BRCA1/2*-mutant ovarian cancers are enriched for immune cell infiltration (19). Overall, this suggests that in early stage patients with TNBC

with or without *BRCA1/2* mutations or HR deficiency, tumor immunogenicity is an independent phenomenon. This finding is important for future clinical investigations of DNA repair-targeted therapeutics and immunotherapies in primary TNBC. In addition, these data suggest that factors other than genomic instability associated with HRD deficiency are driving antitumor immunity in primary TNBC. Currently, multiple studies are ongoing to evaluate PARP inhibitors and PD-1/PD-L1 inhibitors in TNBC and *BRCA1/2* mutation-associated breast cancer with early proof-of-concept data reported from investigations in the advanced disease setting (23, 24). Efforts to better understand the biomarkers predicting these responses in

Table 4. Multivariate logistic regression analysis with pathologic response.

Variable		pCR		RCB O/I	
		OR (95% CI)	P	OR (95% CI)	P
HR deficiency					
HRD status	Nondeficient	1	7.82×10^{-7}	1	1.09×10^{-7}
	Deficient	12.09 (4.11–44.29)		10.22 (4.11–28.75)	
TIL density					
iTIL		0.75 (0.26–2.14)	0.585	0.53 (0.17–1.63)	0.260
sTIL		1.23 (0.94–1.61)	0.139	1.62 (1.20–2.28)	0.001
Clinical characteristics					
Age		0.96 (0.92–1.00)	0.054	0.97 (0.94–1.01)	0.151
Clinical stage	I	3.22 (0.71–16.62)	0.131	9.26 (1.53–84.84)	0.032
	II	1		1	
	III	0.47 (0.11–1.72)		0.70 (0.21–2.30)	
Intended duration of neoadjuvant therapy	12 weeks	1	0.263	1	0.833
	18 weeks	2.72 (0.49–22.01)		1.20 (0.20–6.70)	
Trial	PrECOG 0105	1	0.005	1	0.313
	NCT00148694	0.91 (0.10–9.57)		0.30 (0.04–2.13)	
	NCT00580333	1.07 (0.15–10.05)		0.75 (0.12–4.56)	
	NCT01372579	7.91 (1.16–77.08)		0.85 (0.12–5.58)	
	TBCRC 008	8.31 (1.16–84.58)		1.85 (0.25–13.69)	

individual patients will be important for the development of this combinatorial strategy in breast cancer and other solid tumors.

Both sTIL density and HRD status were significantly associated with favorable response (RCB 0/I) in univariate and multivariate analyses. However, sTIL density was not associated with pCR, whereas HRD status was strongly associated with an improved odds of pCR. Taken together, the lack of association of TIL density with HRD status and the positive association of sTIL density with RCB 0/I, suggests that sTIL is independently predictive of response to platinum-based therapy, albeit less predictive than HRD status. In this cohort, HRD status was a superior predictor of response to platinum-based chemotherapy in TNBC in comparison with TIL density with ORs for HRD being 6- to 10-fold higher. A recent pooled analysis from Denkert and colleagues included 906 patients with TNBC treated with neoadjuvant chemotherapy reported a pCR rate of 31% among 260 patients with low sTILs (0%–10%), 31% pCR rate among 373 patients with intermediate sTILs (11%–59%), and a pCR rate of 50% among 273 patients with high sTILs ($\geq 60\%$). In our study, sTIL density 0%–10% was observed in 60% of patients and only 8.1% had sTIL $\geq 60\%$. Given the lack of difference in neoadjuvant therapy response in the low and intermediate sTIL groups reported by Denkert and colleagues, this explains, in our assessment, the lack of association we observed between sTIL density and pCR. Why the distribution of sTIL is so much higher in their German cohort compared with what we observed in our cohort treated in the United States is unclear. To evaluate this further, we looked at an article by Adams and colleagues, which evaluated TILs in a primarily U.S. patient population and included patients with TNBC enrolled on the adjuvant Eastern Cooperative Oncology Group (ECOG) 1199 and ECOG 2197 trials. In this article, consistent with our TIL distribution finding, 12 of 481 (2.4%) of patients with TNBC had sTIL density $\geq 60\%$.

There were several limitations to our study. First, as a pooled analysis there is inherent heterogeneity among the different studies pooled in terms of cohort and treatment variables. In particular, patients did not all receive the same chemotherapy regimen, as some received platinum monotherapy, while others were treated with multi-drug combinations. Importantly, no patients received anthracycline-based neoadjuvant therapy. Patients treated with taxane-containing therapy had a higher odds of pCR in our multivariate model, but this difference was nonsignificant when looking at RCB 0/I as an endpoint. Germline *BRCA1/2* mutation status was not uniformly available and for this analysis *BRCA1/2* mutation data were obtained from tumor sequencing. While the overwhelming majority of *BRCA1/2* mutations found on tumor sequencing are germline in origin, we could not determine that in our study. Overall, 34 of 161 (21.1%) patients included had tumor mutations in *BRCA1/2* in this analysis and the small sample could have reduced the power to see an association with TIL density. Regarding sample size, this pooled analysis included 161 patients of among 224 patients enrolled in these studies with TNBC (72%). While the reason for exclusion were many, the most common reasons included lack of available response data, receipt of <4 cycles of therapy, lack of adequate tumor remaining for HRD and TIL analysis particularly with the older studies, and no HRD status available. We considered the possibility that high TIL infiltration may result in high nontumor DNA content, in making it more likely that these cases would result in failed HRD assays. As the HRD assay is able to be calculated down to 20%–30% tumor content, there would need to be a large infiltration of TILs for HRD analysis to fail. While we did not have TIL data available on the excluded cases in this analysis, in PrECOG 0105, we did have TIL scoring for all patients treated with six cycles of therapy from a prior analysis. Among 23 patients excluded from this

analysis with prior TIL assessment, 2 patients were excluded on the basis of ER-positive disease, 2 excluded due to receipt of <4 cycles of therapy, 1 excluded because of being lost to follow-up with no response data (never had surgery), 3 excluded because of inability to score TIL, and 15 excluded for no HRD data (no remaining tissue available, insufficient tumor to attempt DNA extraction, inadequate DNA yield following extraction, and insufficient tumor DNA content). Among 20 excluded cases with TIL data, 8 had sTIL 0%–10%, 8 had sTIL 11%–59%, and 4 had sTIL $\geq 60\%$. This, along with the overall very low frequency of high TIL cases observed in the pooled analysis by Adams and colleagues, suggests that this possibility is unlikely to have introduced significant confounding in our results. Despite these limitations, this study has strengths as well. The cohort was highly representative of early stage TNBC seen in practice in the United States, the overall sample size was large, and all patients were prospectively treated on a clinical trial with pathologic response as the primary endpoint. As such, the cohort was very well characterized and TILs were independently scored by an expert pathologist blinded to the outcome data.

In summary, we observed that TIL density, while associated with response to neoadjuvant platinum-based chemotherapy, was not associated with tumor HRD or *BRCA1/2* mutation status in early stage patients with TNBC. Furthermore, tumor HRD status was associated with a much higher odds of favorable pathologic response than TIL density in this pooled analysis of five prospective trials. Given the disparate results we observed compared with those observed in high-grade serous ovarian cancer, further evaluation of the overlap of these biomarkers in independent datasets of early stage patients with TNBC is needed. In addition, our study reports a relatively low frequency of high TIL tumors (sTIL density $\geq 60\%$) among patients included in this pooled analysis. These data, along with that of other United States-treated cohorts, suggests the frequency of high TIL triple-negative tumors may vary geographically with much higher frequencies reported in Western Europe. Further study of these geographic differences in the degree of TIL infiltration of primary TNBC are required and may have implications for current management and future investigational approaches.

Disclosure of Potential Conflicts of Interest

M.L. Telli is an employee/paid consultant for Tesaro, Vertex, AstraZeneca, PharmaMar, G1 Therapeutics, Celldex, Merck, Immunomedics, Aduro, Genentech/Roche, Celgene, Pfizer, Daiichi Sankyo, and AbbVie. S. Vinayak reports receiving other commercial research support from Oncosec, and is an advisory board member/unpaid consultant for Oncosec and Tesaro. D.P. Silver and A.L. Richardson are listed as co-inventors on a patent on telomeric allelic imbalance, which is owned by the Dana-Farber Cancer Institute and Partners Healthcare and licensed to Myriad Genetics. S.J. Isakoff is an employee/paid consultant for Abbvie, Hengrui, Immunomedics, Mylan, Myriad, Puma, Oncopep, and Genentech, and reports receiving commercial research grants from Abbvie, AstraZeneca, Genentech, Merck, Oncopep, and Pharmamar. V. Kaklamani reports receiving commercial research grants from Eisai, and speakers bureau honoraria from Eisai, Pfizer, Novartis, Genentech, Puma, and Celgene. V. Stearns reports receiving commercial research grants from Abbvie, Biocept, Pfizer, Novartis, Medimmune, and Puma, and other remuneration from Immunomedics. J.J. Gruber reports receiving commercial research grants from Curis, Inc. and Guardant. J. Garber reports receiving commercial research grants from AstraZeneca. N. Tung reports receiving commercial research grants from AstraZeneca and Myriad Genetics. R. Bernhisel is an employee/paid consultant for Myriad Genetics. K.M. Timms is an employee/paid consultant for and holds ownership interest (including patents) in Myriad Genetics. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M.L. Telli, S.S. Badve, S. Vinayak, V. Kaklamani, R.M. Connolly, J.M. Ford, S. Adams, A.L. Richardson

Development of methodology: M.L. Telli, S.S. Badve, D.P. Silver, C. Neff, K.M. Timms, A.L. Richardson

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.L. Telli, S.S. Badve, S.J. Isakoff, V. Kaklamani, W. Gradishar, V. Stearns, R.M. Connolly, J.M. Ford, J. Garber, N. Tung, C. Neff, K.M. Timms, A.L. Richardson

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.L. Telli, S.S. Badve, S. Vinayak, D.P. Silver, S.J. Isakoff, W. Gradishar, J.M. Ford, S. Adams, N. Tung, R. Bernhisel, K.M. Timms

Writing, review, and/or revision of the manuscript: M.L. Telli, C. Chu, S.S. Badve, S. Vinayak, D.P. Silver, S.J. Isakoff, V. Kaklamani, W. Gradishar, V. Stearns, R.M. Connolly, J.M. Ford, J.J. Gruber, S. Adams, J. Garber, N. Tung, K.M. Timms, A.L. Richardson

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Neff

Study supervision: V. Stearns, K.M. Timms

Acknowledgments

Myriad Genetics performed HRD testing on a research basis for this analysis. Research reported in this article was supported by Susan G Komen for the Cure (grant 150062 to M.L. Telli), Breast Cancer Research Foundation (BCRF-18-137 to A.L. Richardson), National Center For Advancing Translational Sciences of the NIH under Award Number UL1TR003142 (to M.L. Telli and J.M. Ford), Stanford Cancer Institute (to M.L. Telli and J.M. Ford), and the Translational Breast Cancer Research Consortium (to V. Stearns and R.M. Connolly).

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 May 30, 2019; revised October 10, 2019; accepted November 25, 2019; published first December 3, 2019.

References

- Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007;13:4429–34.
- 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.
- Telli ML, Stover DG, Loi S, Aparicio S, Carey LA, Domchek SM, et al. Homologous recombination deficiency and host anti-tumor immunity in triple-negative breast cancer. *Breast Cancer Res Treat* 2018;171:21–31.
- Timms KM, Abkevich V, Hughes E, Neff C, Reid J, Morris B, et al. Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. *Breast Cancer Res* 2014;16:475.
- Telli ML, Jensen KC, Vinayak S, Kurian AW, Lipsos JA, Flaherty PJ, et al. A 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.
- Telli ML, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, et al. Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. *Clin Cancer Res* 2016;22:3764–73.
- Silver DP, Richardson AL, Eklund AC, Wang ZC, Szallasi Z, Li Q, et al. Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. *J Clin Oncol* 2010;28:1145–53.
- Ryan PD, Tung NM, Isakoff SJ, Golshan M, Richardson A, Corben AD, et al. Neoadjuvant cisplatin and bevacizumab in triple negative breast cancer (TNBC): safety and efficacy. *J Clin Oncol* 27:15s, 2009 (suppl; abstr 551).
- Kaklamani VG, Jeruss JS, Hughes E, Siziopikou K, Timms KM, Gutin A, et al. Phase II neoadjuvant clinical trial of carboplatin and eribulin in women with triple negative early-stage breast cancer (NCT01372579). *Breast Cancer Res Treat* 2015;151:629–38.
- Connolly RM, Leal JP, Goetz MP, Zhang Z, Zhou XC, Jacobs LK, et al. TBCRC 008: early change in 18F-FDG uptake on PET predicts response to preoperative systemic therapy in human epidermal growth factor receptor 2-negative primary operable breast cancer. *J Nucl Med* 2015;56:31–7.
- Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J Clin Oncol* 2014;32:2959–66.
- Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol* 2010;28:105–13.
- Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. *J Clin Oncol* 2015;33:983–91.
- Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. *Ann Oncol* 2014;25:1544–50.
- Loi S, MacCallum P. Host antitumor immunity plays a role in the survival of patients with newly diagnosed triple-negative breast cancer. *J Clin Oncol* 2014;32:2935–7.
- Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. *J Clin Oncol* 2013;31:860–7.
- Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumor-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol* 2018;19:40–50.
- Strickland KC, Howitt BE, Shukla SA, Rodig S, Ritterhouse LL, Liu JF, et al. Association and prognostic significance of BRCA1/2-mutation status with neointigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. *Oncotarget* 2016;7:13587–98.
- Eggington JM, Bowles KR, Moyes K, Manley S, Esterling L, Sizemore S, et al. A comprehensive laboratory-based program for classification of variants of uncertain significance in hereditary cancer genes. *Clin Genet* 2014;86:229–37.
- Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruner G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol* 2015;26:259–71.
- Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. *J Clin Oncol* 2007;25:4414–22.
- Symmans WF, Wei C, Gould R, Yu X, Zhang Y, Liu M, et al. Long-term prognostic risk after neoadjuvant chemotherapy associated with residual cancer burden and breast cancer subtype. *J Clin Oncol* 2017;35:1049–60.
- Vinayak S, Tolaney SM, Schwartzberg LS, Miita MM, Anne-Lee McCann G, Tan AR, et al. TOPACIO/Keynote-162: niraparib + pembrolizumab in patients (pts) with metastatic triple-negative breast cancer (TNBC), a phase 2 trial. *J Clin Oncol* 36:15s, 2018 (suppl; abstr 1011).
- Domchek SM, Postel-Vinay S, Im S-A, Hee Park Y, Delord J-P, Italiano A, et al. An open-label, phase II basket study of olaparib and durvalumab (MEDIOLA): updated results in patients with germline BRCA-mutated (gBRCAm) metastatic breast cancer (MBC). [abstract]. In: Proceedings of the 2018 San Antonio Breast Cancer Symposium; 2018 Dec 4–8; San Antonio, TX. Philadelphia (PA): AACR; 2019;79. Abstract nr PD5-04.