JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

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

Melinda L. Telli, Kristin C. Jensen, Shaveta Vinayak, Allison W. Kurian, Jafi A. Lipson, Patrick J. Flaherty, Kirsten Timms, Victor Abkevich, Elizabeth A. Schackmann, Irene L. Wapnir, Robert W. Carlson, Pei-Jen Chang, Joseph A. Sparano, Bobbie Head, Lori J. Goldstein, Barbara Haley, Shaker R. Dakhil, Julia E. Reid, Anne-Renee Hartman, Judith Manola, and James M. Ford

See accompanying article doi: 10.1200/JCO.2014.57.6660

Author affiliations appear at the end of this article.

Published online ahead of print at www.jco.org on April 6, 2015.

Supported by sanofi-aventis, the Breast Cancer Research Foundation, a Stanford University Clinical and Translational Science Award from the National Institutes of Health, the Stanford Cancer Institute, the American Society of Clinical Oncology Conquer Cancer Foundation, the Triple-Negative Breast Cancer Foundation, Susan G. Komen for the Cure, and Myriad Genetics.

Presented at the 49th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, May 31-June 4, 2013; 35th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 4-8, 2012; and 34th Annual San Antonio Breast Cancer Symposium, December 6-10, 2011.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Clinical trial information: NCT00813956.

Corresponding author: Melinda L. Telli, MD, Stanford University School of Medicine, Department of Medicine, Division of Oncology, 875 Blake Wilbur Dr, MC 5826, Stanford, CA 94305-5826; e-mail: mtelli@stanford.edu.

0732-183X/14/3399-1/$20.00

DOI: 10.1200/JCO.2014.57.0085

ABSTRACT

Purpose
This study was designed to assess efficacy, safety, and predictors of response to iniparib in combination with gemcitabine and carboplatin in early-stage triple-negative and *BRCA1/2* mutation–associated breast cancer.

Patients and Methods
This single-arm phase II study enrolled patients with stage I to IIIA (T ≥ 1 cm) estrogen receptor–negative (≤ 5%), progesterone receptor–negative (≤ 5%), and human epidermal growth factor receptor 2–negative or *BRCA1/2* mutation–associated breast cancer. Neoadjuvant gemcitabine (1,000 mg/m^2 intravenously [IV] on days 1 and 8), carboplatin (area under curve of 2 IV on days 1 and 8), and iniparib (5.6 mg/kg IV on days 1, 4, 8, and 11) were administered every 21 days for four cycles, until the protocol was amended to six cycles. The primary end point was pathologic complete response (no invasive carcinoma in breast or axilla). All patients underwent comprehensive *BRCA1/2* genotyping, and homologous recombination deficiency was assessed by loss of heterozygosity (HRD-LOH) in pretreatment core breast biopsies.

Results
Among 80 patients, median age was 48 years; 19 patients (24%) had germline *BRCA1* or *BRCA2* mutations; clinical stage was I (13%), IIA (36%), IIB (36%), and IIIA (15%). Overall pathologic complete response rate in the intent-to-treat population (n = 80) was 36% (90% CI, 27 to 46). Mean HRD-LOH scores were higher in responders compared with nonresponders (P = .02) and remained significant when *BRCA1/2* germline mutations carriers were excluded (P = .021).

Conclusion
Preoperative combination of gemcitabine, carboplatin, and iniparib is active in the treatment of early-stage triple-negative and *BRCA1/2* mutation–associated breast cancer. The HRD-LOH assay was able to identify patients with sporadic triple-negative breast cancer lacking a *BRCA1/2* mutation, but with an elevated HRD-LOH score, who achieved a favorable pathologic response. Confirmatory controlled trials are warranted.

J Clin Oncol 33. © 2015 by American Society of Clinical Oncology

INTRODUCTION

Breast cancers that arise in *BRCA1* and *BRCA2* mutation carriers are characterized by homologous recombination DNA repair deficiency. The *BRCA1* and *BRCA2* gene products are critical for DNA double-strand break repair, and as such, DNA repair–targeted therapeutics have been investigated to exploit the inherent homologous recombination deficiency of these tumors to therapeutic advantage.[1] Preclinical studies have demonstrated that *BRCA1/2*-deficient breast tumors exhibit differential chemosensitivity compared with *BRCA1/2*-proficient cancers, with greater sensitivity to platinum and gemcitabine and less sensitivity to taxanes.[2-5] In clinical studies, data have shown high-level activity

of cisplatin as neoadjuvant therapy in *BRCA1* mutation carriers and poorer response rates and progression-free survival in *BRCA1* mutation carriers (compared with noncarriers) with metastatic hormone receptor–negative breast cancer treated with taxane therapy.[6]

Sporadic triple-negative breast cancer (TNBC) shares many pathologic and molecular features with breast cancers caused by hereditary *BRCA1* germline mutations.[7] On the basis of this and the observations described, the hypothesis emerged that sporadic TNBC may possess similar DNA repair defects and demonstrate similar chemosensitivity profiles as *BRCA1* mutation–associated breast tumors. Preclinically, basal-like breast cancer cell lines, like *BRCA1*-deficient cancer cell lines, demonstrate increased sensitivity to poly (ADP-ribose) polymerase (PARP) inhibition, cisplatin, and gemcitabine and are deficient in base excision repair, leading to enhanced sensitivity to oxidative DNA damage.[8,9]

Therefore, we set out to investigate a neoadjuvant combination chemotherapy regimen targeting DNA repair defects in early-stage TNBC and *BRCA1/2* mutation–associated breast cancers. Specifically, PrECOG 0105 was designed to assess the efficacy and safety of and predictors of response to iniparib (BSI-201; sanofi-aventis, Paris, France) in combination with gemcitabine and carboplatin. Over the course of this study, knowledge regarding the mechanism of action of iniparib evolved. Initially investigated as a PARP1 inhibitor, it was subsequently demonstrated that iniparib does not possess characteristics typical of the PARP inhibitor class.[10-12] Preclinically, the metabolites of iniparib are believed to be involved in the uncoupling of electron transport from oxidative phosphorylation, which in turn produces reactive oxygen species at cytotoxic levels.[13]

A major goal of this study was to identify markers of response to this neoadjuvant therapy among patients with TNBC. Given the clinical potential of DNA repair–targeted therapeutics, many groups have focused on developing methods to characterize changes in the genomic landscape resulting from underlying homologous recombination defects in cancers.[14-17] In addition to *BRCA1* and *BRCA2*, there are many DNA repair genes that may be altered by germline or somatic mutations, rearrangements, DNA methylation, or dysregulated mRNA expression that are hypothesized to result in impairment of the homologous recombination pathway. The homologous recombination deficiency loss of heterozygosity (HRD-LOH) assay allows for the detection of HRD regardless of etiology or mechanism as measured by levels of genomic LOH.[14] During assay development, LOH regions of intermediate size were observed more frequently in ovarian tumors with defective *BRCA1/2*. On the basis of this finding, the HRD-LOH assay was developed and represents a count of the number of LOH regions of intermediate size ($>$ 15 Mb and $<$ whole chromosome) observed in the tumor genome. In this article, we report the first assessment to our knowledge of the HRD-LOH biomarker in TNBC and evaluate its ability to distinguish responders from nonresponders treated with neoadjuvant platinum-based therapy.

PATIENTS AND METHODS

Patients, Study Design, and Treatment Regimen

This single-arm phase II neoadjuvant study enrolled patients with newly diagnosed, treatment-naive stage I to IIIA (T size $\geq$ 1 cm by magnetic resonance imaging [MRI]) TNBC (estrogen receptor [ER] $\leq$ 5%, progesterone receptor [PR] $\leq$ 5%, and human epidermal growth factor receptor 2 [HER2] negative [0 or 1+ by immunohistochemistry or fluorescent in situ hybridization nonamplified]) or *BRCA1/2* mutation–associated breast cancer. A core biopsy of the primary breast tumor was required for research purposes. All patients underwent comprehensive *BRCA1* and *BRCA2* genotyping.

Patients were treated with carboplatin intravenously (IV) at an area under the curve of 2 on days 1 and 8, gemcitabine 1,000 mg/m^2 IV on days 1 and 8, and iniparib 5.6 mg/kg IV on days 1, 4, 8, and 11 every 21 days before definitive surgery. In the original protocol, patients at Stanford University were treated with four cycles of therapy (total of 13 patients) before the protocol was amended to increase treatment duration to six cycles and expand the trial to multiple centers within PrECOG, with the goal of treating 80 patients with the six-cycle regimen. After completion of surgery, adjuvant systemic therapy and radiotherapy were recommended at the discretion of the treating physician.

This protocol was approved by the institutional review board at each participating center. Informed consent was obtained from all patients.

Study End Points and Statistical Considerations

The primary end point was pathologic complete response (pCR) by central assessment, defined as the absence of invasive carcinoma in the breast and axillary lymph nodes. The extent of residual disease was assessed using the residual cancer burden (RCB) index.[18] This index has been validated as an independent prognostic marker of distant relapse-free survival in patients with breast cancer treated with neoadjuvant chemotherapy (RCB 0, complete pathologic response; RCB I, minimal residual disease; RCB II, moderate residual disease; and RCB III, extensive residual disease). Additional secondary end points included safety of the combination, radiographic response by MRI (central review), rate of conversion to breast conservation eligibility, and correlation of baseline tumor gene expression and gene copy number profiles with treatment response.

Per protocol, the primary analysis was to combine patients enrolled to receive four or six cycles of neoadjuvant therapy. At the time the study was expanded, the revised design was based solely on the patients to be treated with six cycles. Therefore, both the 6-cycle cohort alone and the combined cohorts are reported. The primary analysis included all patients registered with the intent to treat (ITT). Efficacy analyses were performed for all eligible patients and safety analyses for all patients who received at least one dose of trial therapy. Assuming that 76 of 80 patients were eligible and treated, the regimen would be deemed of interest if the lower bound of the 90% exact binomial CI for the pCR rate exceeded 25%. This design had an 87.5% power to detect a 15% absolute improvement in pCR over historical data with cisplatin alone,[19] using a binomial test with a one-sided α level of 5%.

RESULTS

Patient and Tumor Characteristics

A total of 93 patients were treated in the study. Thirteen patients were enrolled onto the four-cycle protocol, and 80 patients were enrolled onto the six-cycle protocol. All patients in the four-cycle group completed treatment. Of the 80 patients in the six-cycle group, 11 (13.8%) discontinued treatment prematurely: five (6.3%) because of progressive disease, five because of unacceptable toxicity (four with wild-type *BRCA*, one with mutant *BRCA*), and one because of a protocol violation (patient lost to follow-up with mutant *BRCA*). There were no ineligible patients, so the ITT and safety populations included the same number of patients.

Patient characteristics are detailed in Table 1. All enrolled patients were women, and the majority were white (72%). Most patients had clinical stage II breast cancer (72%), and most had TNBC, except for three *BRCA1/2* mutation carriers who had ER-positive and/or PR-positive/HER2-negative breast cancer. A total of 19 patients (24%) treated with six cycles had a deleterious germline mutation in *BRCA1*, *BRCA2*, or both genes. Sixty-five patients treated with six cycles of

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	Patients Receiving Six Cycles (n = 80) No.	%	All Patients (N = 93) No.	%
Female sex	80	100.0	93	100.0
Race				
Asian	10	12.5	11	11.8
Black or African American	7	8.8	9	9.7
Other	1	1.3	2	2.2
Unknown	4	5.0	4	4.3
White	58	72.5	67	72.0
Ethnic group				
Hispanic or Latino	7	8.8	10	10.8
Not Hispanic or Latino	72	90.0	82	88.2
Unknown	1	1.3	1	1.1
Age at screening, years				
Median	48.0		48.0	
Range	26-74		26-74	
Clinical stage				
I	10	13	12	13
IIA	29	36	35	38
IIB	29	36	32	34
IIIA	12	15	14	15
Breast cancer subtype				
Triple negative	77	96	90	97
ER and/or PR positive/HER2 negative	3	4	3	3
BRCA1/2 mutation status*				
BRCA1 mutation	14	18	15	16
BRCA2 mutation	4	5	4	4
BRCA1 and *BRCA2* mutations	1	1	1	1

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor 2; PR, progesterone receptor.
*Germline.

therapy had fresh frozen tumor tissue available for gene expression profiling (Affymetrix U133 plus 2.0; Santa Clara, CA), and of these, 78% were basal-like by PAM50 analysis[20] (Data Supplement). Fifty-one of the 65 samples passed the ER filter used by the Vanderbilt TNBC type calculator (http://cbc.mc.vanderbilt.edu/tnbc/), allowing for triple-negative molecular subtype assignment with the following distribution: basal-like 1 (BL1), n = 8 (16%); basal-like 2 (BL2), n = 2 (4%); immunomodulatory (IM), n = 14 (27%); luminal androgen receptor (LAR), n = 4 (8%); mesenchymal, n = 14 (27%); mesenchymal stem-like (MSL), n = 3 (6%); and unstable, n = 6 (12%; Data Supplement).[21]

Response Data

The primary efficacy results for pathologic response in the ITT population are summarized in Table 2. Among all 80 enrolled patients treated with six cycles of therapy, 29 patients (36.3%) achieved a pCR. Among those wild type for *BRCA1/2*, the pCR rate was 33%. Among *BRCA1/2* mutation carriers, the pCR rate was 47%, and in *BRCA1/2* mutation carriers with TNBC, it was 56%. One *BRCA1* mutation carrier had bilateral breast cancer and achieved a pCR in both breasts, although this was counted as one response. Among all 93 patients, 31 (33.3%) achieved a pCR (90% CI, 25.3% to 42.2%). Rates of combined RCB 0/1 are also detailed in these subgroups.

In the six-cycle and combined groups, respectively, 58 (72.5%; 90% CI, 63.1% to 80.6%) and 68 patients (73.1%; 90% CI, 64.5% to 80.6%) achieved an objective response by MRI. Among the 23 and 27 patients not eligible for breast-conservation surgery at baseline in the six-cycle and combined groups, 14 (60.9%; 90% CI, 41.7% to 77.8%) and 15 (55.6%; 90% CI, 38.2% to 72.0%), respectively, became eligible for breast conservation.

Adverse Events

All patients had at least one treatment-emergent adverse event (TEAE). The most common treatment-related TEAEs among all 93 patients and 80 patients treated with six cycles, respectively, were fatigue (84.9% and 85.0%), nausea (81.7% and 81.3%), neutropenia or neutrophil count decreased (49.5% and 53.8), alopecia (46.2% and 51.3%; [grade 2, 4.3% and 5.0%]), anemia (33.3% and 35%), dysgeusia (25.8% and 28.8%), diarrhea (24.7% and 26.3%), and rash (20.4% and 23.8%). All grade 4 TEAEs occurred in patients receiving six cycles of treatment. There were no deaths during the study. Table 3 summarizes all grade 3 to 4 adverse events possibly, probably, or definitely related to the combination treatment regimen. Notably, among 80

Table 2. Pathologic Response in ITT Population

Response	All Patients No.	%	*BRCA1/2* Wild Type No.	%	*BRCA1/2* Mutant No.	%	TN and *BRCA1/2* Mutant No.	%
Patients receiving six cycles	n = 80		n = 61		n = 19		n = 16	
pCR, RCB 0	29	36	20	33	9*	47	9*	56
90% CI, %	27 to 46		23 to 44		27 to 68		33 to 77	
RCB 0 or 1	45	56	31	51	14	74	12	75
90% CI, %	46 to 66		40 to 62		52 to 89		52 to 91	
Patients receiving four and six cycles	n = 93		n = 73		n = 20		n = 17	
pCR, RCB 0	31	33	22	30	9	45	9	53
90% CI, %	25 to 42		21 to 40		26 to 65		31 to 74	
RCB 0 or 1	51	55	36	49	15	75	13	76
90% CI, %	46 to 64		39 to 60		54 to 90		54 to 92	

Abbreviations: ITT, intent to treat; pCR, pathologic complete response; RCB, residual cancer burden; TN, triple negative.
*One *BRCA1* mutation carrier had bilateral TN breast cancer and achieved pCR in both breasts.

Table 3. Grade 3 to 4 Adverse Events by CTCAE (version 3.0)

Adverse Event	Six Cycles (n = 80)				Four and Six Cycles (N = 93)			
	Grade 3		Grade 4		Grade 3		Grade 4	
	No.	%	No.	%	No.	%	No.	%
Neutropenia	33	41	6	8	36	39	6	6
Febrile neutropenia	0	0	0	0				
ALT elevation	12	15	0	0	14	15	0	0
Anemia	8	10	0	0	8	9	0	0
AST elevation	7	9	0	0	7	8	0	0
Thrombocytopenia	4	5	2	3	4	4	2	2
Fatigue	2	3	0	0	3	3	0	0
Subdural hematoma	0	0	1	1	0	0	1	1
Cerebrovascular accident	0	0	1	1	0	0	1	1
Pulmonary embolism	0	0	1	1	0	0	1	1
Headache	1	1	0	0	1	1	0	0
Nausea	1	1	0	0	1	1	0	0
Vomiting	1	1	0	0	1	1	0	0
Flu-like illness	1	1	0	0	1	1	0	0
Urinary tract infection	1	1	0	0	1	1	0	0

NOTE. Adverse events possibly, probably, or definitely related to treatment with gemcitabine, carboplatin, and iniparib.
Abbreviation: CTCAE, Common Terminology Criteria for Adverse Events.

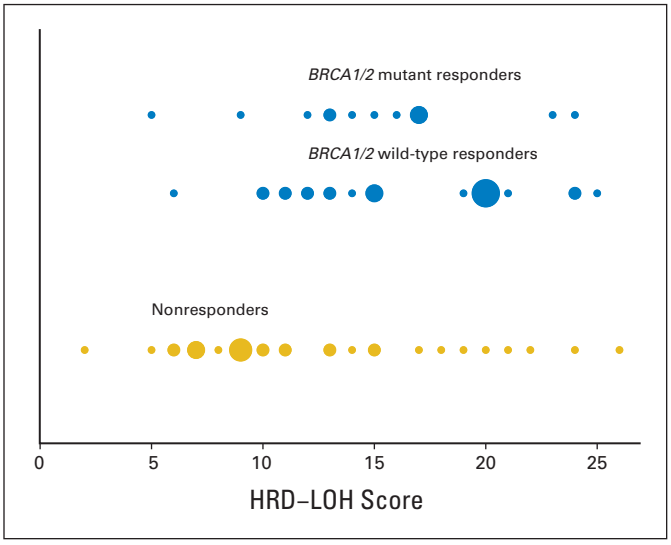

Fig 1. Homologous recombination deficiency–loss of heterozygosity (HRD-LOH) score distribution among responders (residual cancer burden, 0 or 1) and nonresponders (residual cancer burden, 2 or 3). Plot shows distribution of HRD-LOH scores among responders in blue (*BRCA1/2* mutant and wild type) and nonresponders in gold. Size of each dot corresponds to number of patients with that score; larger dot indicates more patients with that HRD-LOH score.

patients treated with six cycles of therapy, the rate of grade 2 (complete) alopecia was only 5%, and the rate of grade ≥ 2 neuropathy was 1%.

Correlative Studies

HRD-LOH. Given the hypothesis of underlying DNA repair defects in sporadic TNBC, we set out to evaluate a novel measure of genomic instability to detect the accumulation of changes in the genomic landscape of a tumor attributable to defective homologous recombination DNA repair. Methods for determination of the HRD-LOH score are detailed in the Appendix (online only).

Among 80 patients treated in the six-cycle protocol, 77 (18 with mutant *BRCA1/2*) had sufficient DNA extracted from their tumor core biopsies to proceed with the HRD assay (Data Supplement). Of these, 66 samples (17 with mutant *BRCA1/2*) passed the quality filter based on the level of discrimination between balanced and unbalanced regions of the tumor genome, and 11 did not (including one with mutant *BRCA1/2*). One *BRCA1* mutation carrier was excluded because she had no pathologic outcome data available, leaving 65 patients with HRD-LOH and response data. In total, four somatic mutations were identified in *BRCA1* or *BRCA2* on tumor sequencing; two of these patients achieved a pCR.

In Figure 1, the distribution of HRD-LOH scores among responders in blue and nonresponders in gold is depicted. Mean HRD-LOH scores were higher in responders compared with nonresponders (15.7 *v* 12.5; $P = .020$; Table 4). Importantly, mean HRD-LOH scores were similar in *BRCA1/2*-mutant versus intact responders. When those with *BRCA1/2* germline mutations were excluded, mean HRD-LOH scores in *BRCA1/2* wild-type responders were higher than in wild-type nonresponders (16.1 *v* 12.3; $P = .021$). When the data were analyzed using a cutoff of ≥ 10 as indicative of homologous recombination deficiency, responders were more likely to exhibit HRD-LOH scores ≥ 10 compared with nonresponders in all patients and *BRCA1/2* wild-type patients ($P = .0026$ and .0024, respectively). As shown in Figure 2, rates of favorable pathologic response, defined as RCB of 0 or 1, were 66% versus 20% for patients with an HRD-LOH score ≥ 10 compared with < 10; 81% versus 47% for patients harboring an underlying germline *BRCA1/2* mutation compared with wild type; and 66% versus 8% for patients with a high HRD-LOH score or *BRCA1/2* mutation compared with patients with a low HRD-LOH score who were also *BRCA1/2* wild type, respectively (Fig 2). When

Table 4. Association of Response (RCB 0 or 1) and HRD-LOH Score (n = 65 of 80 patients receiving six cycles)

Response	HRD-LOH Score Mean	*P**
All patients (n = 65)		.020
Responders (n = 36)	15.7	
Nonresponders (n = 29)	12.5	
BRCA1/2 wild type (n = 49)		.021
Responders (n = 23)	16.1	
Nonresponders (n = 26)	12.3	

	HRD-LOH Score		
	Low (< 10)	High (≥ 10)	*P*†
All patients (n = 65)			.0026
Responders	3	33	
Nonresponders	12	17	
BRCA1/2 wild type (n = 49)			.0024
Responders	1	22	
Nonresponders	11	15	

Abbreviations: HRD-LOH, homologous recombination deficiency–loss of heterozygosity; RCB, residual cancer burden.
*Wilcoxon rank sum test.
†Fisher's exact test.

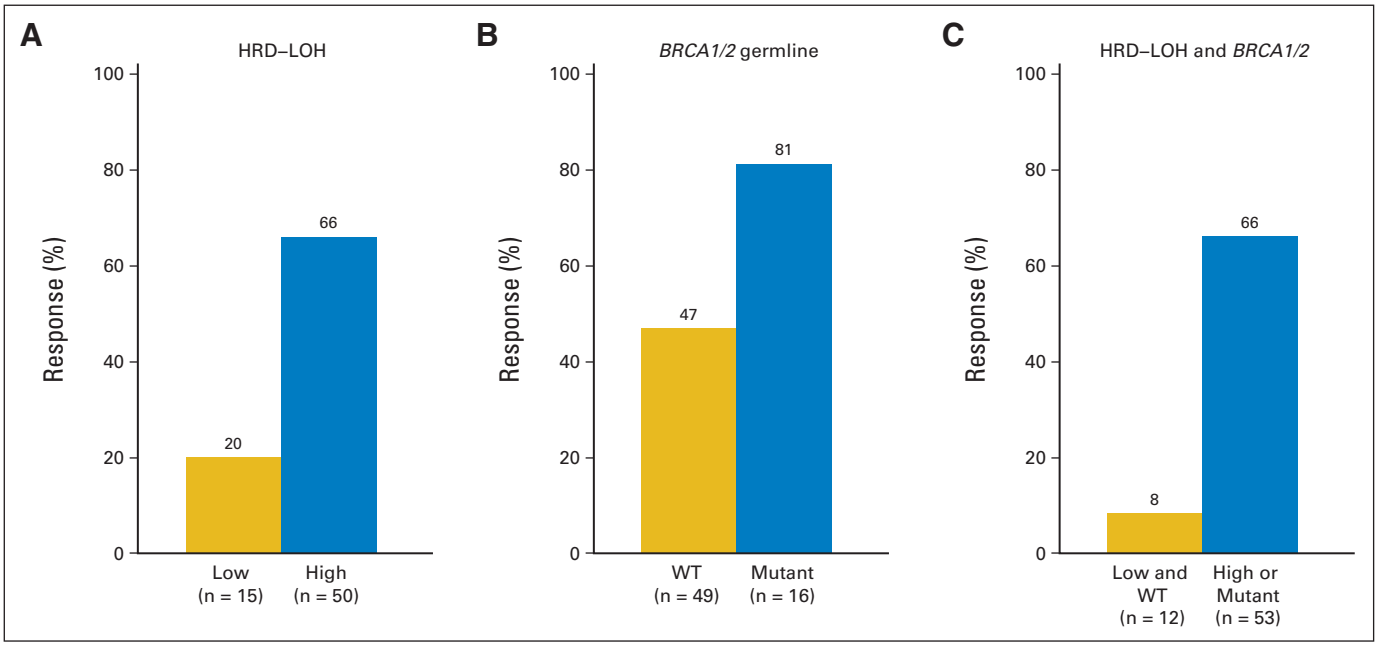

Fig 2. Rate of favorable response (residual cancer burden [RCB], 0 or 1) by homologous recombination deficiency–loss of heterozygosity (HRD-LOH) score, germline *BRCA1/2* status, and their combination (n = 65). Proportion of patients achieving favorable response, defined as RCB index score of 0 or 1 based on (A) HRD-LOH score low (< 10) versus high (≥ 10), (B) germline *BRCA1/2* status wild type (WT) versus mutant, and (C) combination of HRD-LOH low (< 10) and germline *BRCA1/2* WT versus HRD-LOH high (≥ 10) or germline *BRCA1/2* mutant. Total of 65 of 80 patients in six-cycle protocol had HRD-LOH data available.

data were analyzed using RCB 0 (pCR) versus no pCR, mean HRD-LOH scores were 16.0 among those with a pCR and 13.4 among those with no pCR ($P = .058$). When looking at HRD-LOH as a dichotomous variable (< 10 *v* ≥ 10), patients with a pCR were more likely to exhibit HRD-LOH scores ≥ 10 compared with patients who did not achieve a pCR ($P = .0012$).

In an attempt to better understand the underlying mechanism for a high HRD score and/or clinical response in patients with TNBC without germline or tumor *BRCA1/2* mutations, a preliminary analysis of tumor *BRCA1* promoter methylation and subsequent gene expression was performed for 25 of 45 *BRCA1/2* wild-type patients with HRD-LOH data. In total, 15 samples had *BRCA1* promoter methylation, and all had an HRD-LOH score ≥ 10. Nine (60%) of 15 were responders (RCB 0 or 1; Data Supplement).

Gene expression. In an exploratory analysis in the six-cycle group, we assessed response by RCB group (0 or 1 *v* 2 or 3) across the TNBC molecular subtypes. As previously reported,[22] we also observed considerable variation in rates of favorable response across these subtypes, although numbers in some of the groups were small, and results must be interpreted cautiously (Data Supplement). When germline *BRCA1/2* mutation status was overlaid on this, we observed that those with mutant *BRCA1/2* were distributed across the various subtypes, with only one *BRCA*-mutant patient in the BL1 and BL2 groups combined. Mean HRD-LOH scores across the TNBC subtypes were as follows: BL1, 17 (range, 2 to 24; n = 8); BL2, 20 (n = 1); IM, 15.9 (range, 6 to 24; n = 10); LAR, 6.7 (range, 5 to 8; n = 3); mesenchymal, 13.1 (range, 6 to 25; n = 14); MSL, 13 (range, 10 to 16; n = 2); and unstable, 10.6 (range, 6 to 17; n = 6). Appendix Table A1 (online only) lists the molecular characteristics of the five patients with progressive disease.

DISCUSSION

The study met its primary end point, with a pCR rate of 36% (90% CI, 27% to 46%) among patients treated with six cycles of therapy and a pCR rate of 33.3% (90% CI, 25.3% to 42.2%) in all patients. Given the nonrandomized nature of this study, the relative contribution of iniparib therapy cannot be assessed. Nevertheless, this platinum-based, non–anthracycline- and non–taxane-based regimen was well tolerated and produced pathologic responses that were on par with those recently reported with third-generation anthracycline/taxane-based regimens (pCR, 26% to 39%).[23,24] In molecularly defined subgroups of our TNBC cohort selected for DNA repair deficiencies using *BRCA1/2* mutation status and/or the HRD-LOH assay, we observed favorable pathologic response rates (RCB, 0 or 1) ≥ 65%, supporting the targeted nature of the therapy. Our results do not have direct impact on clinical practice today, but they strongly suggest that patient selection based on underlying DNA repair deficiency in future randomized trials of standard versus DNA repair defect–targeted therapy in TNBC should be pursued.

A strength of this study is the comprehensive molecular phenotyping that was performed on all patient tumors. Microarray gene expression analysis confirmed that the majority of patients selected for triple-negative status based on immunohistochemical characterization were basal-like (78% by PAM50). Further subtyping using the Vanderbilt gene expression criteria suggest that some patients with TNBC may exhibit particular chemosensitivity (IM and MSL groups), whereas others are more resistant (LAR) and that these differences are not directly related to *BRCA1/2* germline mutation status.

This study also comprehensively evaluated *BRCA1/2* germline mutation status of all enrolled patients as well as pretreatment tumor

biopsy samples for *BRCA1/2* mutations and a measure of genomic instability using a novel diagnostic approach. The pCR rate with this platinum-based regimen was highest in patients with germline *BRCA1/2* mutations, although comparisons in our study were underpowered. Among patients lacking a germline *BRCA1/2* mutation, the HRD-LOH assay was able to identify additional patients with sporadic TNBC and an elevated HRD-LOH score associated with underlying defects in homologous recombination who achieved a favorable pathologic response. Therefore, the HRD-LOH assay seems to be a powerful diagnostic tool for assessing DNA repair capacity of tumors, as reflected by a so-called genomic scar, without knowledge of an underlying genetic cause and without the need for assessing a DNA damage–inducible response to therapy (eg, gamma-H2AX focus formation). Prospective evaluation of the HRD-LOH assay is necessary to confirm whether this biomarker is prognostic or truly predictive of therapeutic benefit to DNA-damaging therapy such as platinum as well as newer-generation PARP inhibitors. The molecular mechanism for HRD in our patients with TNBC without a *BRCA1/2* mutation is not known, but epigenetic downregulation of *BRCA1* expression secondary to promoter methylation may explain this in part.

Whether the carboplatin and gemcitabine backbone of our regimen represents an improved therapy for the HRD-selected patients with TNBC in our trial, or whether these patients simply respond better to any cytotoxic regimen, cannot yet be assessed. To date, the results of three randomized neoadjuvant TNBC platinum studies have been reported, all of which examined an add-on approach of carboplatin to anthracycline- and taxane-based therapy. The GEICAM (Grupo Español de Investigación en Cáncer de Mama) 2006-03 study of epirubicin and cyclophosphamide followed by docetaxel with or without carboplatin showed no improvement in pCR with the addition of carboplatin.[25] In the phase II GeparSixto trial, which assessed a regimen of dose-intense anthracycline- and taxane-based chemotherapy with bevacizumab with or without carboplatin, patients receiving carboplatin achieved a pCR rate of 53.2% compared with 36.9% in patients who did not.[26] In the phase II CALGB 40603 (Cancer and Leukemia Group B) study of standard anthracycline/taxane-based chemotherapy with or without carboplatin and with or without bevacizumab, the addition of carboplatin significantly increased the pCR rate (breast and axilla) in the per-protocol population by 13% (pCR with carboplatin, 54% *v* without, 41%).[23] Toxicity was increased and early discontinuation was more common in carboplatin-treated patients.

Placing these results, along with the results of the GeparSixto and CALGB 40603 studies, in the broader context deserves careful consideration. In the conduct of PrECOG 0105, we found that certain toxicities commonly encountered with anthracycline/taxane-containing adjuvant therapy, specifically alopecia (grade 2, 5%), chemotherapy-induced amenorrhea, and peripheral neuropathy (grade 2, 1%; grade 3 to 4, 0%), were infrequent. In a randomized setting, we would expect that important differences in toxicity would be observed among patients treated with gemcitabine and carboplatin compared with a regimen such as paclitaxel and carboplatin followed by doxorubicin and cyclophosphamide. These toxicity differences are important and may affect endocrine symptom burden, reproductive concerns, and overall quality of life in this group of predominantly young, premenopausal breast cancer survivors.

In summary, our data from PrECOG 0105 support the notion that understanding the biology of TNBC and *BRCA1*- and *BRCA2*-mutant breast cancer allows for improved therapeutic strategies that target the DNA repair defects of these tumors. Future trials with treatment selection based on tumor DNA repair capacity in TNBC are currently in development and may lead to improved long-term outcomes.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Melinda L. Telli, Allison W. Kurian, Robert W. Carlson, Anne-Renee Hartman, Judith Manola, James M. Ford
Financial support: Melinda L. Telli, James M. Ford
Administrative support: Melinda L. Telli, Pei-Jen Chang, Joseph A. Sparano, James M. Ford
Provision of study materials or patients: Melinda L. Telli, Allison W. Kurian, Irene L. Wapnir, Robert W. Carlson, Joseph A. Sparano, Bobbie Head, Lori J. Goldstein, Barbara Haley, Shaker R. Dakhil, James M. Ford
Collection and assembly of data: Melinda L. Telli, Kristin C. Jensen, Allison W. Kurian, Jafi A. Lipson, Kirsten Timms, Elizabeth A. Schackmann, Irene L. Wapnir, Pei-Jen Chang, Bobbie Head, Barbara Haley, Shaker R. Dakhil, Judith Manola, James M. Ford
Data analysis and interpretation: Melinda L. Telli, Kristin C. Jensen, Shaveta Vinayak, Patrick J. Flaherty, Kirsten Timms, Victor Abkevich, Joseph A. Sparano, Lori J. Goldstein, Julia E. Reid, Anne-Renee Hartman, Judith Manola, James M. Ford
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Ford JM, Kastan MB: DNA damage response pathways and cancer, in Niederhuber JE, Armitage JO, Doroshow J, et al (eds): Abeloff's Clinical Oncology (ed 5). Philadelphia, PA, Churchill Livingstone, 2014, pp 142-153
2. Alli E, Sharma VB, Hartman AR, et al: Enhanced sensitivity to cisplatin and gemcitabine in Brca1-deficient murine mammary epithelial cells. BMC Pharmacol 11:7, 2011
3. Bhattacharyya A, Ear US, Koller BH, et al: The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. J Biol Chem 275: 23899-23903, 2000
4. Farmer H, McCabe N, Lord CJ, et al: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434:917-921, 2005
5. Kennedy RD, Quinn JE, Mullan PB, et al: The role of BRCA1 in the cellular response to chemotherapy. J Natl Cancer Inst 96:1659-1668, 2004
6. Kriege M, Jager A, Hooning MJ, et al: The efficacy of taxane chemotherapy for metastatic breast cancer in BRCA1 and BRCA2 mutation carriers. Cancer 118:899-907, 2012
7. Telli ML, Ford JM: Novel treatment approaches for triple-negative breast cancer. Clin Breast Cancer 10:E16-E22, 2010 (Suppl 1)
8. Hastak K, Alli E, Ford JM: Synergistic chemosensitivity of triple-negative breast cancer cell lines to poly(ADP-Ribose) polymerase inhibition, gemcitabine, and cisplatin. Cancer Res 70:7970-7980, 2010
9. Alli E, Sharma VB, Sunderesakumar P, et al: Defective repair of oxidative dna damage in triple-negative breast cancer confers sensitivity to inhibition of poly(ADP-ribose) polymerase. Cancer Res 69:3589-3596, 2009
10. Liu X, Shi Y, Maag DX, et al: Iniparib nonselectively modifies cysteine-containing proteins in tumor cells and is not a bona fide PARP inhibitor. Clin Cancer Res 18:510-523, 2012
11. Patel AG, De Lorenzo SB, Flatten KS, et al: Failure of iniparib to inhibit poly(ADP-Ribose) polymerase in vitro. Clin Cancer Res 18:1655-1662, 2012

12. Pierce A, McGowan PM, Cotter M, et al: Comparative antiproliferative effects of iniparib and olaparib on a panel of triple-negative and non-triple-negative breast cancer cell lines. Cancer Biol Ther 14:537-545, 2013

13. Licht S, Cao H, Li Z, et al: Mechanism of action of iniparib: Stimulation of reactive oxygen species (ROS) production in an iniparib-sensitive breast cancer cell line. Mol Cancer Ther 10, 2011 (abstr A226)

14. Abkevich V, Timms KM, Hennessy BT, et al: Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. Br J Cancer 107:1776-1782, 2012

15. Birkbak NJ, Wang ZC, Kim JY, et al: Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. Cancer Discov 2:366-375, 2012

16. Popova T, Manié E, Rieunier G, et al: Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. Cancer Res 72:5454-5462, 2012

17. Vollebergh MA, Lips EH, Nederlof PM, et al: An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. Ann Oncol 22:1561-1570, 2011

18. Symmans WF, Peintinger F, Hatzis C, et al: Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 25:4414-4422, 2007

19. Silver DP, Richardson AL, Eklund AC, et al: Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. J Clin Oncol 28:1145-1153, 2010

20. Parker JS, Mullins M, Cheang MC, et al: Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27:1160-1167, 2009

21. Lehmann BD, Bauer JA, Chen X, et al: Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 121:2750-2767, 2011

22. Masuda H, Baggerly KA, Wang Y, et al: Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. Clin Cancer Res 19:5533-5540, 2013

23. Sikov WM, Berry DA, Perou CM, et al: Impact of the addition of carboplatin (Cb) and/or bevacizumab (B) to neoadjuvant weekly paclitaxel (P) followed by dose-dense AC on pathologic complete response (pCR) rates in triple-negative breast cancer (TNBC): CALGB 40603 (Alliance). Presented at the San Antonio Breast Cancer Symposium, San Antonio, TX, December 10-14, 2013 (abstr S5-01)

24. Rugo HS, Olopade OI, Demichele A, et al: Veliparib/carboplatin plus standard neoadjuvant therapy for high-risk breast cancer: First efficacy results from the I-SPY 2 trial. Presented at the San Antonio Breast Cancer Symposium, San Antonio, TX, December 10-14, 2013 (abstr S5-02)

25. Alba E, Chacon JI, Lluch A, et al: A randomized phase II trial of platinum salts in basal-like breast cancer patients in the neoadjuvant setting: Results from the GEICAM/2006-03, multicenter study. Breast Cancer Res Treat 136:487-493, 2012

26. von Minckwitz G, Schneeweiss A, Loibl S, et al: Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): A randomised phase 2 trial. Lancet Oncol 15:747-756, 2014

Affiliations

Melinda L. Telli, Kristin C. Jensen, Shaveta Vinayak, Allison W. Kurian, Jafi A. Lipson, Patrick J. Flaherty, Elizabeth A. Schackmann, Irene L. Wapnir, Robert W. Carlson, Pei-Jen Chang, and James M. Ford, Stanford University School of Medicine, Stanford; Bobbie Head, Marin Specialty Care, Greenbrae, CA; Kirsten Timms, Victor Abkevich, Julia E. Reid, and Anne-Renee Hartman, Myriad Genetics, Salt Lake City, UT; Joseph A. Sparano, Albert Einstein College of Medicine, New York, NY; Lori J. Goldstein, Fox Chase Cancer Center, Philadelphia, PA; Barbara Haley, University of Texas Southwestern Medical Center, Dallas, TX; Shaker R. Dakhil, Cancer Center of Kansas, Wichita, KS; and Judith Manola, Dana-Farber Cancer Institute, Boston, MA.

GLOSSARY TERMS

BRCA1: a tumor suppressor gene known to play a role in repairing DNA breaks. Mutations in this gene are associated with increased risks of developing breast or ovarian cancer.

BRCA2: a tumor suppressor gene whose protein product is involved in repairing chromosomal damage. Although structurally different from BRCA1, BRCA2 has cellular functions similar to BRCA1. BRCA2 binds to RAD51 to fix DNA breaks caused by irradiation and other environmental agents. Also known as the breast cancer 2 early onset gene.

homologous recombination: genetic recombination whereby nucleotide sequences are exchanged between two similar or identical strands of DNA to facilitate accurate repair of DNA double-strand breaks.

neoadjuvant therapy: the administration of chemotherapy prior to surgery. Induction chemotherapy is generally designed to decrease the size of the tumor prior to resection and to increase the rate of complete (R0) resections.

triple-negative breast cancer (TNBC): Breast tumors that are negative for estrogen and progesterone receptor expression, and that also underexpress HER-neu.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. 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 jco.ascopubs.org/site/ifc.

Melinda L. Telli
Consulting or Advisory Role: Oncoplex DX, Vertex
Research Funding: sanofi-aventis (Inst), Novartis (Inst), Abbott Laboratories (Inst), Calithera Biosciences (Inst), PharmaMar (Inst), Myriad Genetics (Inst), Biomarin (Inst)

Kristin C. Jensen
No relationship to disclose

Shaveta Vinayak
Travel, Accommodations, Expenses: Incyte

Allison W. Kurian
Research Funding: Myriad Genetics (Inst), Invitae (Inst)

Jafi A. Lipson
Employment: Genentech (I)
Research Funding: Hologic (Inst)

Patrick J. Flaherty
No relationship to disclose

Kirsten Timms
Employment: Myriad Genetics
Stock or Other Ownership: Myriad Genetics
Research Funding: Myriad Genetics
Patents, Royalties, Other Intellectual Property: Myriad Genetics (Inst)

Victor Abkevich
Employment: Myriad Genetics
Stock or Other Ownership: Myriad Genetics

Elizabeth A. Schackmann
No relationship to disclose

Irene L. Wapnir
No relationship to disclose

Robert W. Carlson
Research Funding: sanofi-aventis (Inst)

Pei-Jen Chang
No relationship to disclose

Joseph A. Sparano
No relationship to disclose

Bobbie Head
No relationship to disclose

Lori J. Goldstein
No relationship to disclose

Barbara Haley
Research Funding: Novartis (Inst), Pfizer (Inst), Roche (Inst)

Shaker R. Dakhil
No relationship to disclose

Julia E. Reid
Employment: Myriad Genetics
Stock or Other Ownership: Myriad Genetics

Anne-Renee Hartman
Employment: Myriad Genetics
Stock or Other Ownership: Myriad Genetics

Judith Manola
Research Funding: sanofi-aventis (Inst)

James M. Ford
Research Funding: sanofi-aventis (Inst), Myriad Genetics (Inst), Invitae (Inst), Varian (Inst)

Acknowledgment

We thank Ignacio Garcia-Ribas, MD, PhD (sanofi-aventis), for his oversight of the PrECOG 0105 protocol. We thank Barry Sherman, MD, and Charles Bradley, PhD (BiPar Sciences), for their assistance during the early development of this trial.

Appendix

Homologous Recombination Deficiency–Loss of Heterozygosity Assay

To generate a homologous recombination deficiency–loss of heterozygosity (HRD-LOH) assay score, DNA copy number was determined using genome-wide single-nucleotide polymorphism (SNP) data generated from Affymetrix (Santa Clara, CA) MIP arrays (n = 2) or a custom Agilent (Santa Clara, CA) SureSelect XT capture followed by sequencing on an Illumina (San Diego, CA) HiSeq2500 (n = 33) or both (n = 42). When data from both assays were available, the highest quality score was used for analysis. 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 nontumor DNA.

The HRD-LOH score was calculated by counting the number of LOH regions that were > 15 Mb in length but shorter than the length of a complete chromosome. The correlation coefficient for the 30 samples with passing assays on both platforms was 0.93. 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. Read coverage across each exon was used to detect large rearrangements. *BRCA1* promoter methylation was assessed by bisulfite conversion and polymerase chain reaction amplification of the proximal promoter region followed by next-generation sequencing.

In this analysis, we defined an HRD-LOH score < 10 as homologous recombination proficient and an HRD-LOH score ≥ 10 as homologous recombination deficient. This cutoff of 10 corresponds to the 10th percentile of HRD score distribution in a set of 260 ovarian and breast cancer tumors with deleterious mutations in the *BRCA1* or *BRCA2* gene or promoter methylation of the *BRCA1* or *RAD51C* gene.

Table A1. Characteristics of Patients With Progressive Disease

Patient	ER (%)	PR (%)	*BRCA1/2* Germline Mutation Status	HRD-LOH Score	TNBC Subtype
1	0	0	Negative	9	Mesenchymal
2	0	0	Negative	24	Basal-like 1
3	0	0	Negative	9	Sample did not pass ER filter (high degree of contamination)
4	0	0	Negative	8	LAR
5	1	1	Negative	11	No gene expression data available

Abbreviations: ER, estrogen receptor; HRD-LOH, homologous recombination deficiency–loss of heterozygosity; LAR, luminal androgen receptor; PR, progesterone receptor; TNBC, triple-negative breast cancer.