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PRECLINICAL STUDY

TP53 genomics predict higher clinical and pathologic tumor response in operable early-stage breast cancer treated with docetaxel-capecitabine ± trastuzumab

Stefan Glück · Jeffrey S. Ross · Melanie Royce · Edward F. McKenna Jr. · Charles M. Perou · Eli Avisar · Lin Wu

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Abstract To determine rates of pathologic complete response (pCR) and near-complete response (npCR) in operable early-stage breast cancer using neoadjuvant capecitabine plus docetaxel, with or without trastuzumab, and investigate biomarkers of pathologic response. Women with operable early-stage breast cancer were enrolled in a multicenter study of neoadjuvant therapy for four 21-day cycles with capecitabine 825 mg/m^2 plus docetaxel 75 mg/m^2 if human epidermal growth factor receptor 2 (HER2)-negative, and additionally, a standard trastuzumab dose if HER2-positive. Primary endpoint was rate of pCR and npCR. Secondary endpoints were potential associations between response and TP53 mutational analysis using the AmpliChip TP53 assay or immunohistochemical (IHC) staining, and genomic subtyping using the PAM50 assay. In patients who completed treatment and surgery, pCR and npCR rates were 15.8% in patients with HER2-negative and 50% in patients with HER2-positive tumors. Stratified by genomic subtype, patients of HER2-enriched subtype had the best response (72.2%), and luminal A (9.1%) and B (4.8%) subtypes, the poorest. Of 147 patients tested for TP53 mutations using the AmpliChip assay, 78 variants were detected; 55 were missense. Response rate among TP53-mutated patients was 30%, significantly higher than TP53 wild-type patients (10%; $P = 0.0032$). Concordance between AmpliChip mutation status versus TP53 IHC staining was 65%, with AmpliChip status predictive of response and IHC status not predictive. Capecitabine plus docetaxel in HER2-negative, and with trastuzumab in HER2-positive patients, provided a good response rate with four cycles of non-anthracycline-containing therapy. TP53 mutational analysis and genomic subtyping were predictive.

Some of the data were previously presented. Ross JS, Perou CM, Aki N, Patten N, Wu L, McKenna EF, Lawrence HJ, Royce M, Avisar E, and Gluck S. p53 mutational status, but not immunohistochemical staining (IHC), is associated with a clinical response of the primary tumor in women receiving neoadjuvant docetaxel-capecitabine chemotherapy for locally advanced breast cancer. Presented at the 31st Annual San Antonio Breast Cancer Symposium; San Antonio, Texas, December 10–14, 2008.

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S. Glück (✉) · E. Avisar
Division of Hematology/Oncology, Department of Medicine, Sylvester Comprehensive Cancer Center, University of Miami, Leonard M. Miller School of Medicine, 1475 NW 12th Ave., Suite 3510, Miami, FL 33136, USA
e-mail: sgluck@med.miami.edu

J. S. Ross
Albany Medical Center, 43 New Scotland Avenue, Albany, NY 12208, USA

M. Royce
University of New Mexico Cancer Research and Treatment Center, 7801 Academy, Albuquerque, NM 87109, USA

E. F. McKenna Jr.
Roche Inc., 340 Kingsland Street, Nutley, NJ 07110, USA

C. M. Perou
Lineberger Comprehensive Cancer Center and University of North Carolina at Chapel Hill, CB 7295, Chapel Hill, NC 27599, USA

L. Wu
Roche Molecular Diagnostics, 4300 Hacienda Drive, Pleasanton, CA 94588, USA

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Introduction

In women with operable non-metastatic breast cancer, the long-term clinical outcome in terms of survival and rate of recurrence is the same with neoadjuvant chemotherapy as with adjuvant chemotherapy [1], or the addition of adjuvant to neoadjuvant chemotherapy [2]. The addition of a taxane to anthracycline-based preoperative chemotherapy significantly improved the clinical response rate and pathologic complete response (pCR) rate but did not result in increased survival [3, 4]. Sequential anthracycline/taxane neoadjuvant therapy demonstrates pCR rates of 14.3–34%, inclusive of residual ductal carcinoma in situ [3, 5, 6]. Lengthening the duration of treatment in earlier responders, using non-cross-resistant therapy early in nonresponders, and intensifying therapy with more than three drugs does not appear to improve pCR rates [7, 8]. Neoadjuvant therapy offers several advantages, including the potential for breast-conserving surgery and the identification of molecular and other prognostic and predictive markers [4, 9–11].

Anthracyclines have been an important part of polychemotherapy for breast cancer. Major disadvantages of anthracyclines are the potential for cardiotoxicity and a small but detrimental risk of secondary leukemia (0.2–1.7%) [12–14]. Recently, comparable efficacy has been documented for anthracycline-based and non-anthracycline chemotherapy regimens in the adjuvant setting [12].

The addition of capecitabine (Xeloda®, Genentech, Inc., South San Francisco, CA) 1,250 mg/m^2 twice daily to docetaxel (Taxotere®, sanofi-aventis US, LLC, Bridgewater, NJ) improves patient outcomes, including objective response rate (42 vs. 30%; $P = 0.006$), median time to progression (6.1 vs. 4.2 months; $P < 0.0001$), and median overall survival (14.5 vs. 11.5 months; $P = 0.0126$) versus docetaxel alone in patients with anthracycline-resistant metastatic breast cancer [15]. Combining a taxane with capecitabine avoids the cardiotoxicity associated with anthracycline therapy. However, gastrointestinal events (i.e., diarrhea and stomatitis) and hand-foot syndrome are commonly reported. Capecitabine/taxane combinations are comparable in efficacy to anthracycline/taxane combinations in the treatment of metastatic breast cancer [16].

Capecitabine also has demonstrated efficacy in the metastatic setting in combination with agents such as trastuzumab [17] (Herceptin®, Genentech, Inc., South San Francisco, CA) and bevacizumab [18] (Avastin®, Genentech, Inc., South San Francisco, CA) at doses lower than the approved 1,250 mg/m^2 twice-daily dose. In addition, capecitabine has a favorable safety profile without cumulative toxicity. Capecitabine therefore represents a logical partner to further improve outcomes for patients with early breast cancer; a number of key trials are evaluating capecitabine-based combinations in the neoadjuvant setting [15, 19]. The benefits of adding trastuzumab to capecitabine in women with human epidermal growth factor receptor 2 (HER2)-positive breast cancer also have been demonstrated in two phase II trials [17, 20].

The tumor suppressor gene TP53 is involved in the regulation of cellular proliferation, survival, and genomic integrity in breast cancer. The TP53 gene is mutated frequently in breast cancer, and this mutation has been associated with an adverse prognosis [21]. The clinical utility of TP53 mutation analysis as a predictor of response to a specific treatment is controversial. The majority of previous investigations, however, relied up immunohistochemistry (IHC) to detect nuclear accumulation of TP53 protein, which cannot accurately identify the TP53 gene mutation status or differentiate between the several functional defects that arise from mutations at specific sites on the multifunctional TP53 gene [22, 23].

The primary objective of the Xeloda in NeoAdjuvant (XeNA) trial was to determine the rate of pCR and near-complete response (npCR) in women with operable, early-stage breast cancer receiving neoadjuvant capecitabine plus docetaxel, with or without trastuzumab. The trial also evaluated whether potential associations exist between response and TP53 alterations as assessed by the AmpliChip TP53 assay, immunohistochemical (IHC) staining, and genomic subtype assessed by the PAM50 assay [24].

Methods

XeNA was an open-label, multicenter study. Simon's optimal two-stage design was used to independently assess the efficacy of preoperative combination therapy with capecitabine and docetaxel in women with HER2-negative breast cancer as well as the addition of trastuzumab in women with HER2-positive breast cancer [25]. The study was approved by the institutional review boards at participating institutions in accordance with the precepts of the Helsinki Declaration. All patients provided written, informed consent.

Patient population

Adult women (age ≥18 years) with histologically confirmed, infiltrating (invasive) HER2-negative or HER2-positive stage II/III breast cancer (T2, N0, N1, and T3, N0, N1) were eligible. A central laboratory (Albany Medical College Department of Pathology and Laboratory Medicine, Albany, NY) confirmed the presence of infiltrating breast

cancer and estrogen receptor (ER), progesterone receptor (PgR) (Ventana Medical Systems Inc., Tucson, AZ), and HER2 status (FISH, Ventana Inform™, Ventana Medical Systems Inc., Tucson, AZ). Patients were excluded if they had evidence of metastatic disease, received primary breast cancer treatment, tumor size <2 cm, breast cancer that could not be measured clinically, inflammatory carcinoma, clinically significant cardiac disease, inadequate renal function, received treatment with an investigational agent within 4 weeks, or severe uncontrolled systemic disease.

Treatments

Patients received four 21-day cycles of capecitabine 825 mg/m^2 orally twice daily on days 1–14 plus docetaxel 75 mg/m^2 intravenously (IV) on day 1. Patients with HER2-positive breast cancer also received trastuzumab 4 mg/kg IV over 90 min on day 1. This was followed by trastuzumab 2 mg/kg IV over 30 min weekly for 11 doses. The safety and initial efficacy of treatment were evaluated by an independent data monitoring board after a predetermined number of patients had been enrolled (30 with HER2-negative and 13 with HER2-positive tumors). Following review of the data, the board allowed the study to continue without change to the original treatment schedule. The board, however, suggested changing the primary efficacy endpoint to pCR plus npCR [26], since both have a similar clinical outcome (residual cancer burden [RCB] 0 and 1).

Primary and secondary endpoints

The primary study endpoint was the rate of pCR (RCB = 0) and npCR (RCB = 1) in the affected breast following four cycles of assigned therapy. This was based on pathologic assessment of the resected tissue at the time of definitive surgery. pCR was defined as the absence of histologic evidence of invasive breast cancer. npCR was defined as the presence of invasive tumor ≤5 mm (T1a). A secondary endpoint was the association between TP53 alterations and response to neoadjuvant chemotherapy.

TP53 mutational analysis was performed on pretreatment tumor tissue using the AmpliChip TP53 assay (in development, Roche Molecular Systems, Pleasanton, CA), which is a DNA microarray-based resequencing assay designed to detect single-base substitutions and single-base deletions in all coding regions of the TP53 gene [27, 28]. IHC staining was conducted using the Bp-53-11 antibody (CONFIRM™, Ventana Medical Systems, Inc., Tucson, AZ) and an automated method (Ventana). Gene expression profiling for breast cancer intrinsic subtypes also was performed using custom-designed full genome 44,000 feature Agilent microarrays, with tumor intrinsic subtype assigned using a new 50-gene centroid-based method called the PAM50 assay. The protocols for microarray hybridizations and the PAM50 predictor were performed as described by Parker et al. [24], with all microarray data deposited into the Gene Expression Omnibus repository at the National Center for Biotechnology Information under the accession number of GSE22358.

Handling of tumor specimens and genomic profiling

See Supplementary material.

Statistical methods

Sample size was calculated independently based on the null hypothesis of ≤10% (uninterested) versus the alternative hypothesis of ≥20% in the pCR and npCR rate for HER2-neu-negative patients, and the null hypothesis of ≤18% (uninterested) versus the alternative hypothesis of ≥40% in the pCR and npCR for HER2-neu-positive patients using Simon's optimal two-stage design with $P = 0.05$ and power of 80%. Sample size was adjusted for a 10% drop-out/nonevaluable rate. A total of 99 HER2-neu-negative patients and 38 HER2-neu-positive patients were required based on these criteria. The treatment would have to be concluded to have sufficient activity that warrants further investigation if the number of pCR and npCR exceeded 12 for HER2-neu-negative patients and 8 for HER2-neu patients. All enrolled patients who met the study entry criteria and completed the four cycles of neoadjuvant treatment were eligible for pathologic response and biomarker analyses.

An exact 95% confidence interval (CI) was calculated for the pathologic response rate and the overall objective response rate using the Clopper–Pearson formula [29]. Chi-square test was used to test for significance of proportional differences of TP53 mutations and overexpression of TP53 protein (IHC staining) among intrinsic breast cancer subtype, and Fisher's exact test was used for pathologic responses in patients with TP53 sequence mutations and without mutations, and pathologic responses in IHC-negative and -positive cases.

Results

Patient population

Women ($N = 157$) with invasive breast cancer (34 with HER2-positive, 123 with HER2-negative disease) were assessed for eligibility, and 156 patients were enrolled. Baseline demographics and patient and tumor characteristics are shown in Table 1. Figure 1 provides details regarding enrolled patient disposition.

Table 1 Baseline demographics and characteristics of enrolled patients

	HER2-negative ($n = 122$)	HER2-positive ($n = 34$)
Age, years		
Median	51	52.2
Range	24–80	31–68
Race		
White	81 (66.4%)	24 (70.6%)
Hispanic	21 (17.2%)	2 (5.9%)
Black	16 (13.1%)	7 (20.6%)
Asian	2 (1.6%)	1 (2.9%)
American Indian or Alaska Native	1 (0.8%)	–
Native Hawaiian/other Pacific Islander	1 (0.8%)	–
Menopausal status		
Premenopausal	60 (49.2%)	12 (35.3%)
Postmenopausal	60 (49.2%)	22 (64.7%)
Missing	2 (1.6%)	–
Body surface area, m^2		
Median	1.8	1.8
Range	1.3–2.6	1.5–2.3
Eastern Cooperative Oncology Group Status		
0	111 (91.0%)	29 (85.3%)
1	10 (8.2%)	4 (11.8%)
Not done	1 (0.8%)	1 (2.9%)
Breast cancer subtype/initial diagnosis		
Ductal	95 (77.9%)	32 (94.1%)
Lobular	10 (8.2%)	1 (2.9%)
Mucinous	1 (0.8%)	–
Other	1 (0.8%)	–
Comedo, ductal	3 (2.5%)	–
Ductal, inflammatory	1 (0.8%)	–
Ductal, lobular	7 (5.7%)	–
Ductal, other	1 (0.8%)	–
Lobular, other	2 (1.6%)	–
Ductal, mucinous, other	–	1 (2.9%)
Comedo, ductal, lobular, other	1 (0.8%)	–
Primary tumor		
T2	59 (48.4%)	18 (52.9%)
T3	62 (50.8%)	16 (47.1%)
T4	1 (0.8%)	–
Regional lymph nodes		
NX	1 (0.8%)	1 (2.9%)
N0	57 (46.7%)	15 (44.1%)
N1	64 (52.5%)	18 (52.9%)
Distant metastases		
Distant metastases cannot be assessed	10 (8.2%)	2 (5.9%)
No distant metastases	32 (94.1%)	32 (94.1%)
Stage grouping TNM		
Stage II	82 (67.2%)	24 (70.6%)
Stage III	40 (32.8%)	10 (29.4%)
Tumor size, cm		
Median	5.5	5
Range	2.0–35.0	2.5–12.0

HER2 human epidermal growth factor receptor 2; *TNM* tumor, node, metastasis

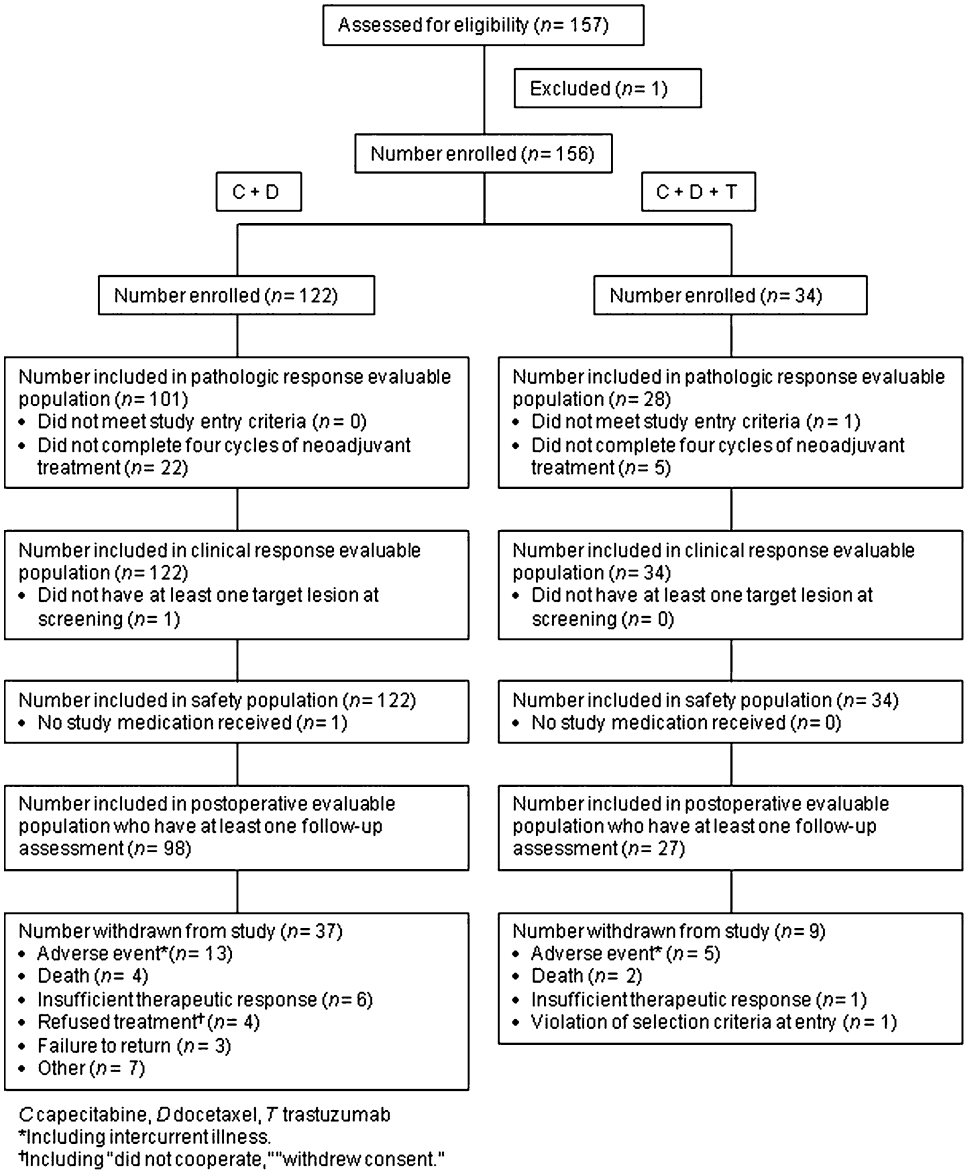

Fig. 1 Disposition of enrolled subjects

Efficacy and safety

The pCR plus npCR rate was 15.8% (16/101; 95% confidence interval [CI], 9.7–25.4) in patients with HER2-negative and 50.0% (14/28; 95% CI, 31.9–71.3) in patients with HER2-positive tumors (Table 2). Ten patients in each group achieved a pCR. When grouped by genomic subtype according to the PAM50 gene expression assay, the pCR plus npCR rate was greatest in the HER2-enriched subtype (72.2%). The pCR plus npCR rate was 31.3% in the basal-like genomic subtype and 9.1% in luminal A and 4.8% in luminal B subtypes.

The clinical response (complete response + partial response) rates by Response Evaluation Criteria in Solid Tumors (RECIST) were 23.8% (29/122; 95% CI, 18.6–35.9) in patients with HER2-negative and 23.5% (8/34; 95% CI, 12.3–45.9) in those with HER2-positive early-stage breast cancer. A complete response was observed in 4.1% (5/122) and 8.8% (3/34), respectively.

Adverse events (AEs) reported as treatment-related and occurring in $\geq$10% of patients in the HER2-negative or HER2-positive groups are shown in Table 3. Fatigue, hand-foot syndrome, nausea, alopecia, and diarrhea were most common. A grade 3/4 AE occurred in 59 (48%) patients with HER2-negative and 19 (56%) with HER2-positive tumors, with neutropenia and hand-foot syndrome observed most frequently. Treatment was discontinued because of treatment-related toxicity in 13 (11%) patients with HER2-negative and 5 (15%) patients with HER2-positive tumors.

Table 2 Pathologic response by HER2 status or genomic subtype

	HER2 status		Genomic subtype				
	HER2-negative ($n = 101$)	HER2-positive ($n = 28$)	Luminal A ($n = 44$)	Luminal B ($n = 21$)	HER2-enriched ($n = 18$)	Normal-like ($n = 12$)	Basal-like ($n = 32$)
pCR + npCR (%)	15.8	50.0	9.1	4.8	72.2	16.7	31.3
pCR (%)	9.9	35.7	6.8	–	50.0	16.7	18.8
npCR (%)	5.9	14.3	2.3	4.8	22.2	–	12.5
Non-responders (%)	80.2	46.4	84.1	95.2	22.2	83.3	65.6
Missing (%)	4.0	3.6	6.8	–	5.6	–	3.1

HER2 human epidermal growth factor receptor 2, *pCR* pathologic complete response, *npCR* near-complete pathologic response

Table 3 Summary of treatment-related adverse events with an incidence ≥10%

	HER2-negative ($n = 122$)		HER2-positive ($n = 34$)	
	All grades	Grade 3/4	All grades	Grade 3/4
Total patients	–	59 (48%)	–	19 (56%)
Fatigue	59 (48%)	–	19 (56%)	–
Hand-foot syndrome	57 (47%)	10 (8%)	21 (62%)	7 (21%)
Nausea	56 (46%)	–	18 (53%)	–
Alopecia	59 (48%)	–	11 (32%)	–
Diarrhea	42 (34%)	–	18 (53%)	–
Neutropenia	28 (23%)	27 (22%)	9 (26%)	10 (29%)
Stomatitis	18 (15%)	–	13 (38%)	–
Rash	26 (21%)	–	4 (12%)	–
Vomiting	21 (17%)	–	4 (12%)	–
Mucosal inflammation	20 (16%)	–	4 (12%)	–
Dyspepsia	18 (15%)	–	5 (15%)	–
Constipation	17 (14%)	–	4 (12%)	–
Dysgeusia	17 (14%)	–	3 (9%)	–
Peripheral neuropathy	16 (13%)	–	2 (6%)	–
Peripheral edema	14 (11%)	–	3 (9%)	–
Anemia	6 (5%)	–	9 (26%)	–
Nail disorder	10 (8%)	–	5 (15%)	–
Insomnia	7 (6%)	–	4 (12%)	–
Dyspnea	6 (5%)	–	4 (12%)	–

HER2 human epidermal growth factor receptor 2

TP53 mutation results

The AmpliChip TP53 assay was performed on samples from 147 (94%) patients. No data were obtained for 10 samples because of insufficient DNA yields. Seventy-eight TP53 mutations were detected in 74/147 (50.3%) cases (more than one mutation was observed in four patients). Of the 78 mutations observed, 70.5% were missense, 15.4% nonsense, 11.5% frameshift, and 1.3% splice site. Silent mutations were rare (1.3%). Mutations were distributed widely in exons 2, 4, 5, 6, 7, 8, 9, and 10, with the highest number seen in exons 5, 6, and 8 (see Supplemental Fig. 1). The distribution of the types of TP53 mutations observed and the exons involved were consistent with previously reported studies (see Supplemental Fig. 2).

Concordance between mutation testing and IHC staining

IHC staining for overexpression of TP53 protein was performed on samples from 134 (86%) patients; 127 samples were analyzed by both the AmpliChip assay and IHC staining (Table 4). Concordance of mutation status with IHC staining was only 65% (82/127 samples; Fig. 2). Of the 67 TP53 mutation-positive cases based on the AmpliChip assay with available IHC data, 19 (28%) samples did not stain positive for TP53 by IHC, the large majority of which (16/19; 84%) had non-missense mutations. Of the 60 TP53 mutation-negative cases with available IHC data, there were 26 (43%) cases that stained positive with IHC. While most of these cases showed relatively weak (2+)

Table 4 Concordance of AmpliChip testing and immunohistochemistry for TP53 status

TP53 gene testing	HER2-negative ($n = 122$)			HER2-positive ($n = 34$)		
	n	Positive	Negative	*n*	Positive	Negative
Mutated	50	34 (68.0%)	16 (32.0%)	17	14 (82.4%)	3 (17.6%)
Wild type	49	21 (42.9%)	28 (57.1%)	11	5 (45.5%)	6 (54.5%)

HER2 human epidermal growth factor receptor 2

staining by IHC, approximately one-third of such cases showed 3+/4 staining. These data strongly suggest that IHC staining for TP53 protein fails to identify a significant proportion of cases that harbor TP53 mutations, and that positive IHC staining is an imperfect surrogate for the presence of a mutation.

Correlation between TP53 mutation status and intrinsic breast cancer subtype

TP53 mutation status also was correlated with the intrinsic subtypes, as determined by the PAM50 gene expression profile. The prevalence of TP53 mutations varied significantly between subtypes and was highest in basal-like (73%) and HER2-enriched (68%) subtypes and lowest in luminal A (28%) and luminal B (20%) subtypes ($P < 0.001$). By contrast, overexpression of TP53 protein as determined by IHC staining varied little among the subtypes (49–56%; $P = 0.98$).

Correlation between TP53 alterations and pathologic responses

The frequency of pCR and npCR was significantly higher in patients with TP53 sequence mutations (22/74 or 30%) than in patients without mutations (7/73 or 10%; $P = 0.0032$; two-tailed Fisher's exact test), and this trend was apparent in both HER2-positive and HER2-negative tumors. Furthermore, the frequency of pathologic responses did not differ between cases with missense mutations versus those cases with other types of mutations (nonsense, frameshift, and splice site mutations).

By contrast, IHC status did not correlate significantly with pathologic response (20% pCR and npCR in

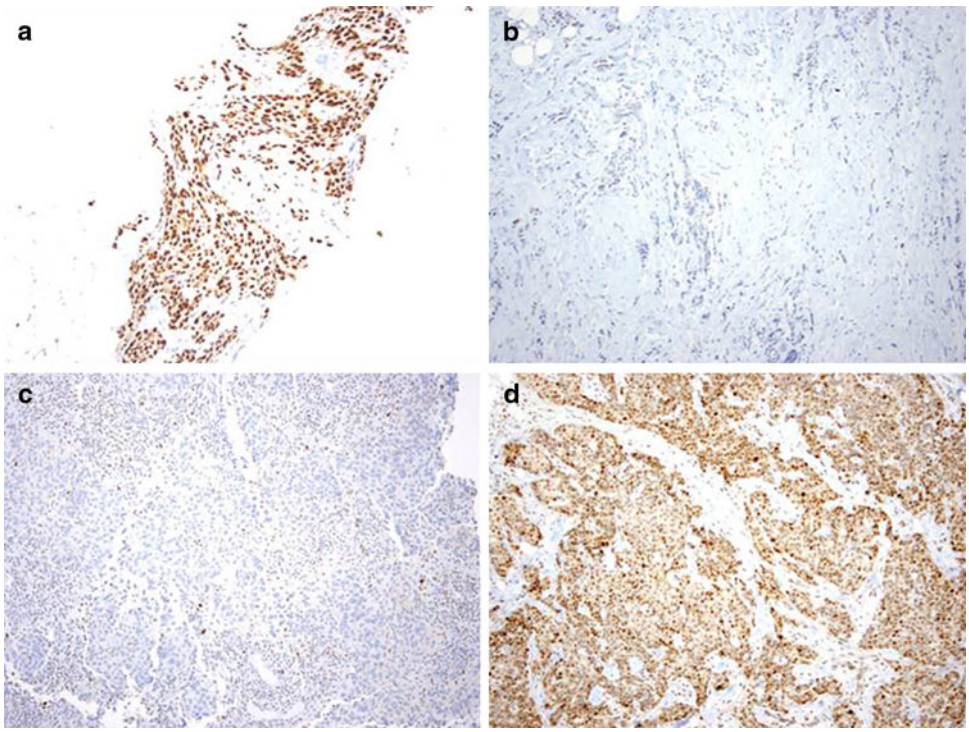

Fig. 2 Images of cases. **a** Case A: 66-year-old woman with a 50-mm, estrogen receptor (ER)-negative, progesterone receptor (PgR)-negative, human epidermal growth factor receptor 2 (HER2)-positive tumor. TP53 immunohistochemistry (IHC) is strong positive; TP53 mutation also is positive by AmpliChip. The patient achieved a pathologic complete response (pCR) after neoadjuvant treatment. **b** Case B: 51-year-old woman with an ER-positive, PgR-negative, HER2-negative tumor. TP53 IHC is negative; TP53 mutation is positive by AmpliChip. The patient achieved a partial response after neoadjuvant therapy. **c** Case C: 55-year-old woman with a 45-mm, triple-negative (ER, PgR, and HER2) tumor. TP53 IHC is negative; TP53 mutation is wild type by AmpliChip. The patient achieved a pCR after neoadjuvant treatment. **d** Case D: 54-year-old woman with a 50-mm, ER-positive, PgR-positive, HER2-negative tumor. TP53 IHC is strong positive; TP53 is wild type by AmpliChip. No response was achieved with neoadjuvant treatment

IHC-negative cases vs. 28% in IHC-positive cases; $P = 0.38$, two-sided Fisher's exact test). For patient samples with concordant AmpliChip and IHC data, the pathologic response rate among patients whose tumors were TP53 mutant and IHC-positive was 43.6%, whereas in patients whose tumors were TP53 wild type and IHC-negative, the response rate was only 11.5%. By comparison, among the cases with discordant AmpliChip and IHC data, the response rate in cases that were TP53 mutant but IHC-negative was 29.4%, while the response rate in cases that were TP53 wild type but IHC-positive was only 8.7%. Thus, TP53 mutational status as determined by the AmpliChip assay was a better predictor of clinical response than conventional IHC staining for TP53.

Discussion

The combination of lower-than-standard dose capecitabine plus docetaxel for four cycles in patients with HER2-negative breast cancer, and with trastuzumab in patients with HER2-positive breast cancer, is a clinically active, non-anthracycline treatment in women with operable, early-stage breast cancer. pCR rates appear comparable to those achieved with three to four cycles of anthracycline/taxane doublet [5, 30, 31]. pCR rates also appear comparable to those achieved with four cycles of anthracycline-based treatment [3, 4]. These results also compare favorably with those of other capecitabine-based, neoadjuvant studies [15, 19, 20]. Importantly, pathologic response varied by genomic subtype, being higher in the HER2-enriched and basal-like groups and lower in the luminal subtypes. This pattern of response is consistent with the results observed by others for anthracycline/taxane-containing, neoadjuvant treatment [24, 32].

In this study, those with HER2-positive breast cancer achieved a very good response with capecitabine + docetaxel + trastuzumab, demonstrating a pCR or npCR of 50%. This is particularly noteworthy, considering that only four cycles of chemotherapy were delivered, while many other trials have utilized 6 to 12 cycles of anthracycline/taxane-based combinations [7, 33]. Similarly high pCR rates were achieved in two neoadjuvant studies in patients with HER2-positive disease following six cycles of capecitabine, epirubicin, and docetaxel (40.5%) or six cycles of capecitabine plus trastuzumab and docetaxel (48%) [19, 20].

The study regimen was well tolerated and feasible. A low incidence of grade 3/4 AEs was observed in the present trial, presumably because of the lower capecitabine dose used. Only neutropenia (23.7%) and hand-foot syndrome (10.9%) occurred in >5% of patients. Other studies assessing the neoadjuvant use of capecitabine have reported similar findings, with capecitabine-related AEs easily managed through treatment interruption or dose modification [15, 19, 20].

In this study, three preplanned biomarkers were assessed: (1) TP53 mutation status as assessed by AmpliChip; (2) over-expression of TP53 protein as measured by IHC; and (3) genomic subtype as assessed by microarray and the PAM50 centroid predictor. We identified numerous correlations between response, TP53 sequence-based mutation status determined by AmpliChip assay, and genomic subtype. For example, TP53 mutations were found in 50% of the tumors and were most frequent in basal-like and HER2-enriched subtypes and less common in other subtypes. In addition, both TP53 sequence-based mutation status and genomic subtype were correlated highly with pCR, and pCR plus npCR.

The positive correlation observed between the presence of TP53 mutations and clinical response is, at first glance, surprising, since a number of prior studies in breast cancer have suggested that the presence of TP53 mutations was correlated with a lack of response to a variety of chemotherapy agents, including anthracyclines [34, 35] and combination cyclophosphamide, methotrexate, and fluorouracil [36]. However, two additional studies in breast cancer [37, 38] have suggested that the presence of TP53 mutations was predictive for sensitivity to docetaxel. This hypothesis is supported by in vitro data showing that TP53 mutant tumor cell lines are more sensitive to mitotic poisons like taxanes than are wild-type TP53 cell lines [39], presumably because the mutant cells lack the TP53-dependent capacity to induce G1 and G2 phase arrest and cannot defend themselves from taxane-induced mitotic block and apoptosis. It is notable, perhaps, that a number of other clinical studies failed to show a positive correlation between taxane responsiveness and TP53 alterations when assessed by IHC staining, as demonstrated in this study [40–43].

The use of IHC as a clinical method to identify tumors with TP53 alterations is based on the observation that most missense mutations of the TP53 gene result in a mutant protein that is more stable than the wild-type protein, thus resulting in a higher steady state level of TP53 mutant protein in the cell, and hence readily detectable by IHC staining. However, the poor correlation we have observed between the TP53 mutation test result and IHC staining strongly indicates that IHC staining lacks both sensitivity and specificity for the presence of TP53 mutations. Not surprisingly, IHC staining usually is negative in the 30% of TP53 mutant cases with non-missense mutations, and our study demonstrated that patients with non-missense mutations were as likely to respond as those with missense mutations. Furthermore, we identified a number of IHC-positive cases that lacked any detectable mutation in TP53,

and these cases demonstrated a very low response rate to therapy. It is not clear why TP53 protein is detectable in these wild-type cases, but perhaps this is a reflection of a normal TP53 response to oncogenic stresses.

More importantly, we were the first to demonstrate, in vivo, that TP53 mutational analysis was a statistically significant predictor of treatment response, while TP53 protein expression determined by IHC was not. This study also provides evidence that non-missense mutations of TP53 are clinically important, as five such cases in this trial experienced pCR or npCR despite negative IHC staining.

Conclusion

The combination of lower-than-standard dose capecitabine plus docetaxel in patients with HER2-negative tumors, and with trastuzumab in patients with HER2-positive tumors, provided good pCR and npCR rates with four 21-day cycles. Both TP53 mutational analysis using the AmpliChip TP53 assay, and genomic subtyping using the PAM50 assay, were reliable predictive tests for response to preoperative therapy with capecitabine plus docetaxel with/without trastuzumab. Capecitabine plus docetaxel represents a promising non-anthracycline option in women with early operable HER2-negative breast cancer, and with trastuzumab in women with HER2-positive tumors, with potential enrichment of responders through the use of molecular profiling.

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