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Biomarker Analysis of Neoadjuvant Doxorubicin/Cyclophosphamide Followed by Ixabepilone or Paclitaxel in Early-Stage Breast Cancer

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Abstract

Purpose: Predictive biomarkers offer the potential to improve the benefit:risk ratio of a therapeutic agent. Ixabepilone achieves comparable pathologic complete response (pCR) rates to other active drugs in the neoadjuvant setting. This phase II trial was designed to investigate potential biomarkers that differentiate response to this agent.

Experimental Design: Women with untreated, histologically confirmed primary invasive breast adenocarcinoma received neoadjuvant doxorubicin/cyclophosphamide, followed by 1:1 randomization to ixabepilone ($n = 148$) or paclitaxel ($n = 147$). Rates of pCR were compared between treatment arms based on predefined biomarker sets: *TUBB3*, *TACC3*, and *CAPG* gene expression, a 20- and 26-gene expression model, MDR1 protein expression, and other potential markers of sensitivity. βIII-tubulin protein expression is reported separately but is referred to here for completeness. All patients underwent a core needle biopsy of the primary cancer for molecular marker analysis before chemotherapy. Gene expression profiling data were used for molecular subtyping.

Results: There was no significant difference in the rate of pCR in both treatment arms in βIII-tubulin–positive patients. Higher pCR rates were observed among βIII-tubulin–positive patients than in βIII-tubulin–negative patients. Furthermore, no correlation was evident between *TUBB3*, *TACC3*, and *CAPG* gene expression, MDR1 protein expression, multi-gene expression models, and the efficacy of ixabepilone or paclitaxel, even within the estrogen receptor–negative subset.

Conclusion: These results indicate that βIII-tubulin protein and mRNA expression, MDR1 protein expression, *TACC3* and *CAPG* gene expression, and multigene expression models (20- and 26-gene) are not predictive markers for differentiating treatment benefit between ixabepilone and paclitaxel in early-stage breast cancer. *Clin Cancer Res; 19(6); 1587–95. ©2013 AACR.*

Introduction

Breast cancer is a heterogeneous disease that can be classified into subgroups on the basis of hormone receptor status, HER2 expression levels, and gene expression profiling (1–3). Some breast cancer subgroups may have high response rates to specific chemotherapeutic drugs, whereas others may derive a relatively small benefit, but at the same time be exposed to treatment-related toxicity (4–8). This underscores the need for predictive biomarkers that can be used prospectively to select which patients with breast cancer are most likely to respond to a given treatment and which should be offered an alternative regimen with a greater likelihood of benefit. Accordingly, predictive biomarkers offer the potential to improve the benefit:risk ratio of a given therapeutic agent.

The neoadjuvant setting provides an opportunity for identifying biomarkers that are predictive of treatment response in patients with breast cancer (9, 10). Pathologic complete response (pCR) after neoadjuvant therapy is associated with favorable disease-free survival and overall survival (11–13) and is thus a valid endpoint for correlation with biomarker expression. Indeed, various gene expression profiles have been shown to correlate with pCR after neoadjuvant chemotherapy regimens in patients with breast cancer (14–26).

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Translational Relevance

The neoadjuvant setting provides a unique scenario for identifying biomarkers that are predictive of treatment response in patients with breast cancer. Single-agent neoadjuvant ixabepilone has previously shown promising activity in invasive breast cancer, particularly in patients with high βIII-tubulin expression. This randomized, phase II trial evaluated potential biomarkers, including βIII-tubulin, which may differentiate response to neoadjuvant ixabepilone relative to paclitaxel in early-stage breast cancer. No correlation was evident between βIII-tubulin protein and mRNA expression, MDR1 protein expression, *TACC3* and *CAPG* gene expression, and multigene expression models (20- and 26-gene), and the efficacy of ixabepilone or paclitaxel, indicating that these markers are not predictive of differentiating treatment benefit in this patient setting. Higher pathologic complete response rates were observed among βIII-tubulin–positive patients than among βIII-tubulin–negative patients; however, this was true for both the ixabepilone- and paclitaxel-treated cohorts.

Ixabepilone, an epothilone agent that is currently approved for the treatment of chemotherapy-resistant metastatic breast cancer in the United States, achieves a pCR rate (18% in a single-arm phase II study) similar to that seen with other agents commonly used in the neoadjuvant setting [i.e., docetaxel, paclitaxel, and doxorubicin/cyclophosphamide (AC); refs. 27–31]. Ixabepilone has a similar, but distinct, mechanism of action to that of taxanes and appears to be less sensitive to mechanisms that confer taxane resistance (32, 33). Current research is focused on the role of ixabepilone in patients with early relapse after taxane-based adjuvant therapy.

Preclinical evidence and retrospective analyses of clinical studies suggest that expression of βIII-tubulin may be a valid biomarker of differential tumor sensitivity to ixabepilone and the taxanes in breast cancer (34–39). These data indicate that βIII-tubulin confers resistance to paclitaxel, but not to ixabepilone. *In vitro*, βIII-tubulin expression correlates with degree of resistance to paclitaxel in breast cancer cell lines (38). Downregulation of the expression of βIII-tubulin by various manipulations (RNA interference, antisense, hypoxia, etc.), consistently increases sensitivities of cell lines to paclitaxel, whereas upregulation of βIII-tubulin expression decreases sensitivity to paclitaxel (35, 36). In contrast, ixabepilone retains activity in taxane-resistant tumor cells with high βIII-tubulin expression (33).

In the neoadjuvant setting (27), ixabepilone monotherapy shows a higher pCR rate among estrogen receptor (ER)-negative patients than in the overall population (29% vs. 18%). Further analysis of this patient subgroup indicates a markedly higher pCR rate in those with ER negativity and overexpression of βIII-tubulin ($n = 24$) than patients with ER negativity, but no βIII-tubulin overexpression ($n = 38$; 25% vs. 5%; ref. 40). Patients with triple-negative, basal-like, or HER2-positive tumors may have higher βIII-tubulin expression than other breast cancer subtypes, which may contribute to the aggressiveness of these subtypes, and predict for ixabepilone clinical response (40).

Studies of gene expression profiles from ER-negative patients who received neoadjuvant ixabepilone or a taxane-containing regimen [neoadjuvant paclitaxel followed by fluorouracil, and AC (FAC)] have identified 4 other potential biomarkers that differentiate pCR with ixabepilone from the taxane-containing regimen (41). These biomarkers include 2 microtubule-related genes, transforming acidic coiled-coil–containing protein 3 (*TACC3*) and chromosome condensation protein G (*CAPG*), and 20- and 26-gene models. TACC3 localizes to the centriole and has a role in microtubule dynamics (42), and CAPG is a component of a condensin complex that impacts centromere and kinetochore function, although the mechanism is not clearly defined (43).

This randomized, phase II trial was designed to compare pCR rates induced by neoadjuvant AC followed by ixabepilone or paclitaxel in women with early-stage breast cancer, based on the 5 predefined biomarker sets described above: βIII-tubulin protein expression measured by immunohistochemistry (IHC), *TACC3* and *CAPG* gene expression, and expression of 20- and 26-gene lists. This study also compared pCR rates in treatment arms defined by multidrug resistance protein 1 (MDR1) protein expression and assessed the predictive value of mRNA expression of *TUBB3* and other β-tubulin isoform genes; preclinical evidence has suggested that β-tubulin isoforms other than βIII-tubulin may also exhibit resistance to taxanes (44, 45).

Materials and Methods

Study design and patients

This randomized, open-label, multicenter, phase II trial (NCT00455533) enrolled previously untreated women with histologically confirmed primary invasive breast adenocarcinoma (T2–3, N0–3, M0, tumor size $\geq$ 2.0 cm), regardless of hormone receptor or HER2 expression status. The trial was initially restricted to triple-negative breast cancer (TNBC) but was later amended to include all tumor types, independent of any knowledge of accumulated outcome data, based on slow patient accrual.

Patients received sequential neoadjuvant therapy starting with 4 cycles of AC (doxorubicin 60 mg/m^2 intravenously and cyclophosphamide 600 mg/m^2 intravenously) given every 3 weeks, followed by 1:1 randomization to either ixabepilone (40 mg/m^2 3-hour infusion) every 3 weeks for 4 cycles, or paclitaxel (80 mg/m^2 1-hour infusion) weekly for 12 weeks. Patients were stratified by tumor size at baseline, ER status, clinical response to AC and investigator site. To provide 81% power to detect a 14% or greater difference in pCR rates using a one-sided, $\alpha = 0.05$ level Fisher exact test, approximately 300 patients (150 per arm) were randomized to either ixabepilone or paclitaxel.

All patients underwent definitive breast surgery 4 to 6 weeks after the last dose of ixabepilone or paclitaxel, consisting of either a lumpectomy with axillary dissection or modified radical mastectomy. Surgical specimens were evaluated by a staff pathologist at each study site; no central pathology review was conducted. The pCR rate was evaluated as the primary endpoint, with pCR defined by no histologic evidence of residual invasive adenocarcinoma in the breast and axillary lymph nodes, with or without the presence of ductal carcinoma *in situ*. Full details of the study design are reported separately (Saura and colleagues, manuscript in preparation).

The trial was conducted in accordance with the ethical principles originating in the Declaration of Helsinki, and in compliance with Good Clinical Practice and regulatory guidelines. The study was approved by the Institutional Review Board or Independent Ethics Committee at all participating sites. All patients provided written informed consent.

Tissue specimens

Four core needle tumor tissue biopsies (4 passes) were obtained before neoadjuvant therapy with AC. Three biopsy specimens were combined at the study site and immediately placed in RNAlater solution for subsequent gene expression analysis. The remaining biopsy specimen was formalin-fixed and paraffin-embedded (FFPE) at the study site and then underwent IHC analysis for selected protein antigens.

In a small subset of patients with incomplete pathologic information before study entry, an additional (fifth) core needle biopsy was obtained (during the same procedure at the study site) to confirm the diagnosis of invasive carcinoma and assess HER2, ER, and progesterone receptor (PR) status.

Analysis of mRNA expression

The mRNA expression levels of 2 single-gene models, *TACC3* and *CAPG*, as well as the multigene (20- and 26-gene) expression models were measured using an Affymetrix gene expression profiling approach. Affymetrix gene expression data are available from the Gene Expression Omnibus website (GSE41998).

The core tumor biopsy specimens in RNAlater solution were processed for extraction of total RNA. Downstream labeling reactions were conducted on all RNA samples with an RNA integrity number (RIN) $\geq$2.8 and >50 ng total RNA. Biotin-labeled cRNA targets were synthesized using an Affymetrix in vitro transcription (IVT) labeling kit, and all labeled cRNA targets with $\geq$10 μg yield of product were then hybridized to Affymetrix Human Genome (HG) U133A 2.0 GeneChips. Hybridization quality was assessed using several different metrics, including scaling factor, percentage of probesets above the threshold of detection, and ratios of intensity of 3′ and 5′ probes for 2 ubiquitously expressed genes. Probesets representing the 2 single-gene models (*TACC3* and *CAPG*), the 20- and 26-gene models, and the β-tubulin genes are present on the HG U133A 2.0 GeneChips. Intensity levels detected by the corresponding probes were normalized using a Robust Multi-Chip Average method. The resultant values correlated with mRNA expression levels for each of the genes. When genes were represented by multiple probes, the average expression level was calculated.

Breast cancer subtyping

Gene expression profiling data were used for molecular subtyping: genes that constitute the PAM50 (46) were converted into Affymetrix probesets; hierarchical clustering using centroid linkage in Array Studio to define basal-like, luminal-like A, luminal-like B, HER2-enriched, and normal-like subtypes.

In addition, information about HER2, ER, and PR status was collected from the sites that participated in the clinical study. This information was used to classify subjects with triple-negative (HER2-negative, ER-negative, and PR-negative) or non-TNBC.

βIII-Tubulin IHC

βIII-tubulin protein expression was measured by IHC using a prototype pharmacodiagnostic assay developed by Dako North America, Inc. The assay was based on previously reported IHC assays for βIII-tubulin (47, 48). βIII-tubulin cytoplasmic staining was scored on a 0 to 3 scale (negative, weak, moderate, and strong), and the percentage of tumor cells at each intensity level was determined. Endothelial cells present in most tissue specimens, which consistently stained at a 2 to 3 intensity level, were used as an internal positive control. An isotype-matched antibody was used as a negative control to evaluate background staining. A prespecified cutoff for βIII-tubulin–positive staining was defined as staining in $\geq$50% of tumor cells at an intensity of 2 to 3. In addition, the Histo-score of βIII-tubulin staining was determined from the following formula:

$$\text{Histo-score} = 100 \times (\%\text{ cells with intensity } 1) + 200 \times (\%\text{ cells with intensity } 2) + 300 \times (\%\text{ cells with intensity } 3)$$

MDR1 IHC

MDR1 protein levels were measured by IHC using a protocol adapted from previously published methods and included the use of a monoclonal antibody (49). Briefly, 4 μm sections were deparaffinized and epitope recovered by the steam heat–induced epitope recovery method described by Ladner and colleagues (50). Subsequent to a 15-minute incubation with UltraVision block at room temperature, tissue sections were incubated with the JSB-1 antibody (dilution 1:150; Santa Cruz Biotechnology) overnight. Antibody binding was detected with the diaminobenzidine-based UltraVision chromogenic detection system. Slides were counterstained with hematoxylin. The method was optimized to reduce background cytoplasmic staining and enhance membrane staining; however, cytoplasmic staining (presumably cross-reactivity with another protein) was not completely eliminated. Average cytoplasmic and membrane staining intensities were measured on a 0 to 3 scale (negative, weak, moderate, and strong), and

the percentage of tumor cells with any staining was also captured for both cytoplasmic and membrane localizations. Two thresholds were used to define patients with negative and positive MDR1 status with the first threshold stringently defining IHC-positive status as any membrane staining and the second threshold being more inclusive of samples with any membrane staining or cytoplasmic Histo-score of $\geq$200.

Statistics

βIII-tubulin, *TACC3* and *CAPG* gene expression, as well as expression of other β-tubulin genes, were also summarized descriptively. For each, a logistic regression model (full model) was built with biomarker expression, treatment status, ER status, and all 2- and 3-way interactions as covariates, and pCR as a response variable. In addition, a reduced model was constructed with treatment status, ER status, and their interaction as covariates, and another reduced model with biomarker expression, treatment status, and their interaction as covariates. Comparison of the full and reduced models was made using a likelihood ratio test.

To identify the optimal threshold level for each biomarker-defined subpopulation, a logistic model was constructed using pCR status as the response variable and biomarker status, treatment status, and their interaction as covariates. The cutoff for biomarker positivity was identified by the minimal *P* value of the interaction, with the constraint that the prevalence of the biomarker-defined subpopulation was >15% and <85%.

A multigene expression model was built for the 20- and 26-gene biomarker sets using penalized logistic regression for each treatment arm separately. Receiver-operating characteristic (ROC) plots were generated using 5-fold cross-validation within each arm. For each arm, patients were partitioned into 5 equal-sized subsets. Four subsets were used as the training set to fit the multigene model and the fifth subset was used as the testing set to calculate sensitivities and specificities. All of the 5 subsets were rotated as the testing set and the weighted averages of the sensitivities and specificities were used to generate the ROC plots.

The pCR rate and 90% confidence interval (CI) in the ixabepilone and paclitaxel arms in biomarker population defined by βIII-tubulin protein, *TACC3* and *CAPG* gene expression were estimated by the cross-validation method using a 5-fold cross-validation scheme. The secondary efficacy endpoint, pCR/minimal residual cancer burden (RCB-1) rate, was also analyzed by the cross-validation method using a 5-fold cross-validation scheme. RCB was calculated as a continuous index combining pathologic measurements of primary tumor and nodal metastases for prediction of distant relapse-free survival in multivariate Cox regression analyses. Detailed methodology has previously been reported (51).

Results

Patient disposition and baseline characteristics (biomarker evaluable population)

A CONSORT diagram detailing the patients that were enrolled, randomized, and assessed for gene expression analysis, βIII-tubulin IHC, MDR1 IHC, and pCR/RCB-1 data are shown in Fig. 1. The baseline characteristics of patients in the biomarker evaluable population and the entire study cohort are described in Supplementary Table S1.

Of a total of 295 patients randomized in the study, RNAlater specimens for mRNA expression profiling were submitted for 283 patients; however, specimens from 10 patients yielded poor quality RNA (RIN $\leq$2.8), and RNA from an additional 13 patients did not meet labeling standards for hybridization to Affymetrix GeneChips (IVT yield <10 μg). Therefore, gene expression data from 260 randomized patients were available; 15 patients in this subset did not have pCR data, thus a total of 245 patients had both gene expression profiling and pCR data available (Supplementary Table S2).

βIII-tubulin IHC data were available for 247 randomized patients (Saura, and colleagues, manuscript in preparation); 16 patients in this randomized subset did not have pCR data, thus a total of 231 patients had both βIII-tubulin IHC data and pCR data available.

FFPE tumor tissue was available from 290 randomized patients for MDR1 IHC assessment; however, IHC data were available from only 244 patients, as the submitted sample sections for 42 patients had no evidence of tumor, and sections for 4 patients had no tissue present. An additional 16 patients did not have available pCR data, so 228 randomized patients had MDR1 IHC data available.

Predictive value of the single- and multigene expression models

For single-gene models, *TACC3* and *CAPG*, mRNA expression was measured using Affymetrix gene expression chips, and an optimized cutoff of normalized $\log_2$ expression was established. However, neither model predicted benefit for ixabepilone versus paclitaxel: pCR rates did not differ between treatment arms, and logistic regression did not identify any significant correlation between *TACC3* and *CAPG*, mRNA expression and treatment response (Table 1; Supplementary Table S3). A similar finding was evident in the ER-negative subset (data not shown).

Multigene expression models also did not differentially predict pCR between treatment arms (the genes that constitute these multigene expression models are shown in Supplementary Table S4). ROC curves generated separately for the ixabepilone and paclitaxel arms using the cross-validation method did not indicate that the 20- and 26-gene models differentially predicted pCR between treatment arms (Fig. 2). Further analyses to estimate the optimal cutoff and pCR rates in positive and negative groups were consequently not conducted in these multiple gene models.

Prevalence and predictive value of βIII-tubulin

Expression of βIII-tubulin was assessed at both the protein level, using an IHC assay, and at the mRNA level, using data from Affymetrix gene expression data [IHC data from this study are reported separately (Saura and colleagues, manuscript in preparation), and also included here for completeness].

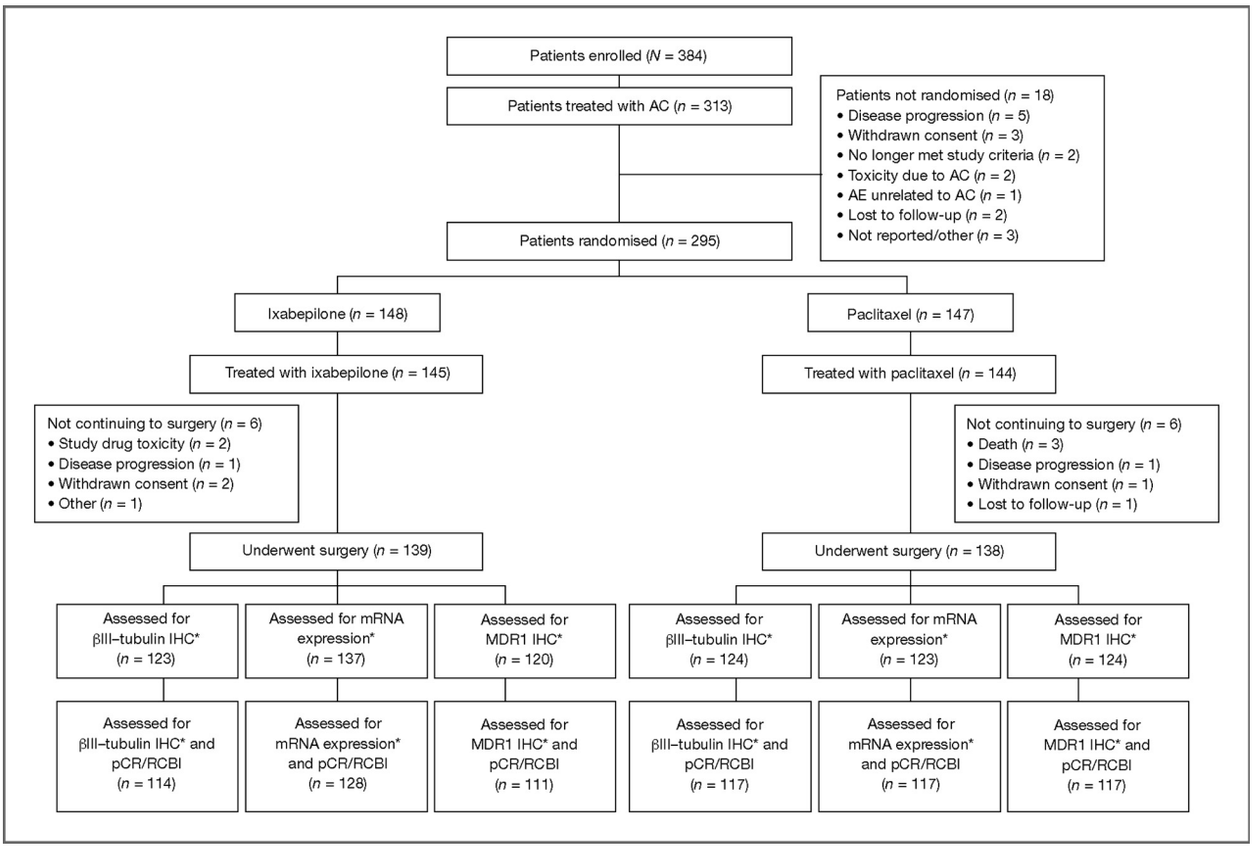

Figure 1. CONSORT diagram. *, assessments conducted on core needle tumor tissue biopsies obtained before neoadjuvant therapy with AC. AE, adverse event.

A correlation between protein and mRNA levels for βIII-tubulin was observed. Relative βIII-tubulin mRNA expression levels were significantly higher in patients classified as βIII-tubulin–positive by IHC than in those classified as βIII-tubulin–negative ($P < 0.0001$; Supplementary Fig. S1). In addition, gene expression correlated with βIII-tubulin IHC Histo-score ($r = 0.49$).

Table 1. Biomarker evaluable populations: efficacy data

	Ixabepilone		Paclitaxel	
Patient group	***n***	**pCR rate,% (90% CI)**	***n***	**pCR rate,% (90% CI)**
All randomized	148	24.3 (18.6–30.8)	147	25.2 (19.4–31.7)
All treated	145	24.8 (19.0–31.4)	144	25.7 (19.8–32.4)
TACC3 mRNA-positive	—	32.0 (18.4–44.7)	—	28.3 (16.7–49.9)
TACC3 mRNA-negative	—	21.1 (10.5–33.4)	—	26.5 (6.8–46.2)
CAPG mRNA-positive	—	32.4 (19.8–49.2)	—	33.1 (21.3–50.4)
CAPG mRNA-negative	—	21.9 (11.3–34.8)	—	19.0 (3.3–34.7)
MDR1 IHC-positive[a]	62	32.3 (22.5–43.3)	58	25.9 (16.7–37.0)
MDR1 IHC-negative[a]	49	14.3 (6.9–25.2)	59	28.8 (19.3–40.0)
MDR1 IHC-positive[b]	19	31.6 (14.7–53.0)	15	20.0 (5.7–44.0)
MDR1 IHC-negative[b]	92	22.8 (15.8–31.2)	102	28.4 (21.1–36.7)

Abbreviations: *CAPG*, chromosome condensation protein G; *TACC3*, transforming acidic coiled-coil-containing protein 3.
[a]Any MDR1 membrane staining or at least 200 cytoplasmic Histo-score.
[b]Any MDR1 membrane staining.

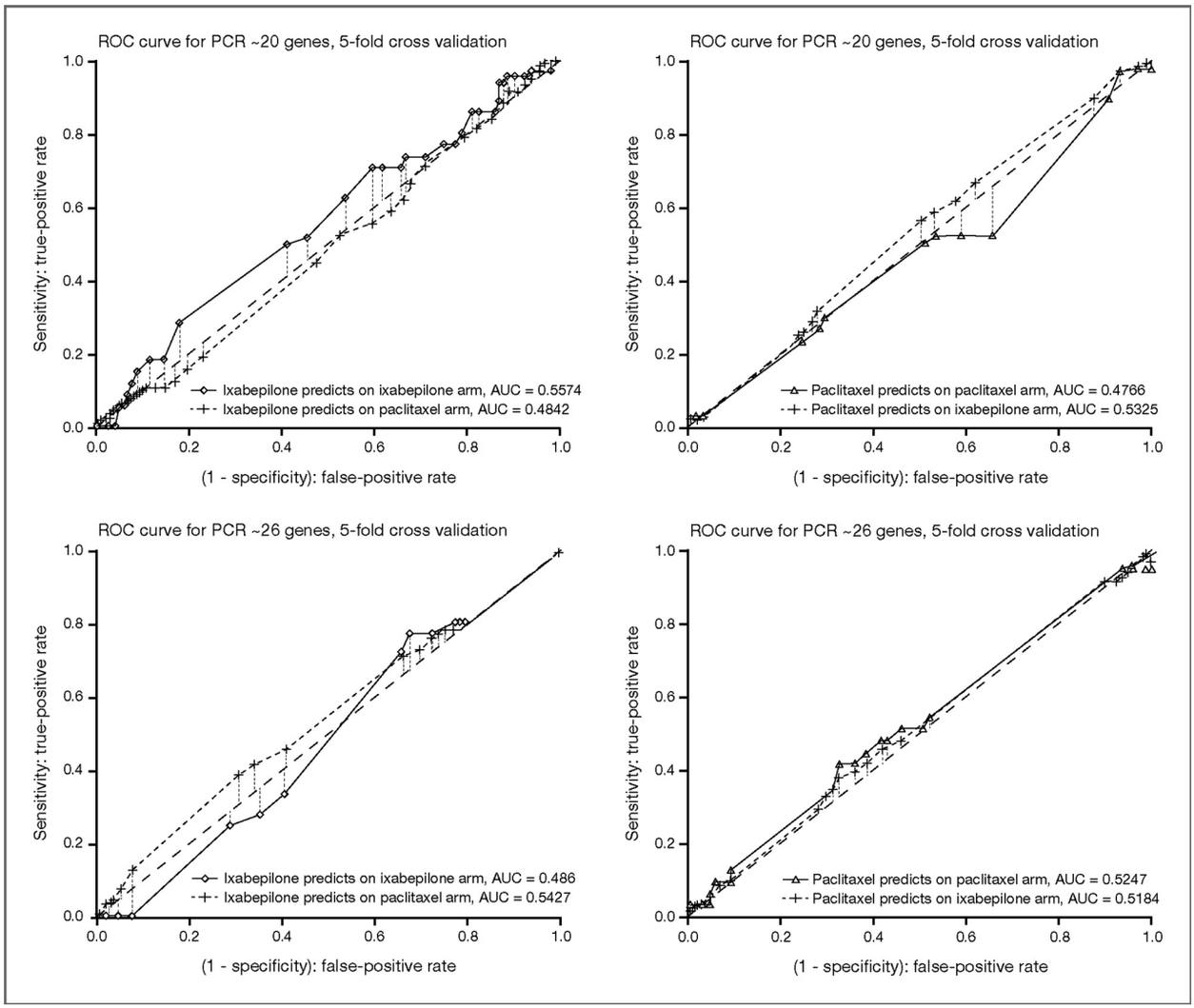

Figure 2. Multigene expression models: ROC curves. AUC, area under the curve.

Eighty-two percent (108 of 132) of triple-negative specimens with gene expression data were classified as basal-like; 47% (106 of 224) of randomized patients with both βIII-tubulin IHC and gene expression data were classified as basal-like by intrinsic gene clustering. Distribution of βIII-tubulin positivity (defined using a prespecified cutoff) was non-random among subtypes (Table 2), with a significantly higher frequency in basal-like and HER2-enriched versus

Table 2. Association between βIII-tubulin status and breast cancer subtype

Tumor classification	βIII-Tubulin expression[a]	
Intrinsic breast cancer subtypes ($N = 224$)[b]	Positive ($n = 86$)	Negative ($n = 138$)
Basal-like (106; 47%)	58 (55)	48 (45)
HER2-enriched (17; 8%)	9 (53)	8 (47)
Luminal-like A (40; 18%)	8 (20)	32 (80)
Luminal-like B (50; 22%)	10 (20)	40 (80)
Normal-like (11; 5%)	1 (9)	10 (91)

[a]Prespecified cutoff (defined as ≥50% 2+ or 3+ cells).
[b]224 patients had both βIII-tubulin IHC data and gene expression profiling data (Saura and colleagues, manuscript in preparation).

other breast cancer subtypes ($P < 1 \times 10^{-5}$, χ^2 test). Fifty-five percent (58 of 106) of basal-like specimens were classified as βIII-tubulin–positive. Conversely, 67% (58 of 86) of βIII-tubulin–positive specimens were classified as basal-like.

The pCR rate for the overall study population was similar between the treatment arms (ixabepilone: 24.3%; paclitaxel: 25.2%) and similar to that reported historically for anthracycline- and taxane-based regimens in this setting. The pCR rates for the subset of patients with tumor specimens for both gene expression analysis are provided in Table 1, along with pCR rates for the entire study cohort.

Sensitivity analyses were conducted using pCR/RCB-1 as the endpoint; and evaluating pCR in the subset of ER-negative patients; and βIII-tubulin data per mRNA expression (Table 1). The results were consistent, with no correlation observed between biomarker and treatment outcome.

Predictive value of MDR1

There was no significant difference in pCR or pCR/RCB-1 between ixabepilone and paclitaxel for MDR1 protein levels (Table 1). pCR rates were also assessed within the ER-negative subset, and again no association between treatment and MDR1 IHC status was observed (data not shown).

Discussion

Neoadjuvant chemotherapy is an effective treatment option for patients with operable breast cancer. This type of therapy, in which tumor tissue is collected before and after chemotherapy, allows for biomarker analyses to guide patient selection (predictive biomarker), assess the biologic effect of treatment (pharmacodynamic biomarker), and risk stratification (prognostic biomarker). Identifying a biomarker that differentially predicts benefit within a class of chemotherapeutic agents, such as microtubule-stabilizing agents, could help patients to achieve the maximal clinical benefit from therapy.

Ixabepilone has well-established clinical activity in taxane-resistant breast cancer (52–54). Several candidate biomarkers have been proposed that may predict a differential benefit of ixabepilone versus paclitaxel in breast cancer, based on prior published literature and/or preclinical/clinical evidence (35, 36, 38, 41). These include βIII-tubulin protein and mRNA expression, *TACC3* and *CAPG* gene expression, and the multi 20- and 26-gene models. Although this randomized, phase II study was adequately designed to explore the potential for these candidate biomarkers, no correlation was seen between any biomarker and differential treatment response (pCR) to ixabepilone and paclitaxel (both with prior AC).

Clinically, overexpression of βIII-tubulin has been associated with resistance to paclitaxel in many tumor types, including breast, ovarian, and non–small cell lung cancer (37, 38, 47, 48). Given the activity of ixabepilone seen in taxane-resistant tumor cells with high βIII-tubulin expression (33), it was expected that ixabepilone would show greater efficacy than paclitaxel among βIII-tubulin–positive patients. However, the results of our study did not show βIII-tubulin protein and mRNA expression to be predictive markers for differentiating treatment benefit between ixabepilone and paclitaxel. While higher pCR rates were seen among βIII-tubulin–positive patients than among βIII-tubulin–negative patients, this was true for both the ixabepilone- and paclitaxel-treated cohorts. This suggests that overexpression of βIII-tubulin may be associated with a general increase in sensitivity to chemotherapy.

Our data (plus that reported by Saura and colleagues, manuscript in preparation) show that βIII-tubulin overexpression correlates with TNBC, a subtype of breast cancer with increased chemosensitivity relative to ER-positive breast cancer (7). Fifty-three percent of triple-negative specimens were classified as βIII-tubulin–positive, compared with only 22% (21 of 97) of ER-positive patients and 28% (7 of 25) or HER2-positive patients. Ixabepilone has shown efficacy in TNBCs (55). However, long-term follow-up data are required to determine the prognostic value of βIII-tubulin overexpression; the current trial was not designed to collect long-term data.

Previous data indicate that ER negativity is associated with increased pCR in patients receiving ixabepilone neoadjuvant therapy (27). When comparisons were made between ER-negative patients receiving neoadjuvant ixabepilone and those receiving a paclitaxel-containing regimen (paclitaxel followed by FAC) in another trial, *TACC3* and *CAPG* gene expression, and the 20- and 26-gene expression models were found to differentiate ixabepilone-induced pCR (41). However, the present study showed no apparent correlation between *TACC3* and *CAPG* gene expression, MDR1 protein expression, multigene expression models, and the efficacy of ixabepilone or paclitaxel, even within the ER-negative subset (Supplementary Table S3).

AC may have had a confounding effect in the present study. Although there is a lack of evidence suggesting a relationship between AC sensitivity and βIII-tubulin status, an interaction between biomarker expression and AC clinical activity cannot be ruled out. Treatment with AC within the current study may have muted any potential correlation between gene expression models and treatment with a microtubule-stabilizing agent. Furthermore, this study may have had additional power if it was carried out within triple-negative or ER/HER2-negative populations only.

The dosing schedule of ixabepilone has been assessed in the first-line treatment of metastatic breast cancer. In a randomized, phase II study, ixabepilone (40 mg/m^2) every 3 weeks was more active than weekly ixabepilone (16 mg/m^2), in combination with bevacizumab (56). A more recent Cancer and Leukemia Group B (CALGB) phase III study showed that weekly ixabepilone in combination with bevacizumab was not superior to weekly paclitaxel in combination with bevacizumab (57). These results support selection of every-3-week ixabepilone dosing in the present study, in which pCR rates were similar in both the ixabepilone and paclitaxel arms.

No examples of a validated predictive biomarker for individual chemotherapeutic regimens have yet been described, although numerous gene signatures and candidates (*ERCC1*, *RRM1*, etc.) have been proposed. The reason for this may be attributed to the complexity and redundancy of pathways related to resistance to cytotoxic agents. Alternatively, the prevalence of biomarkers/signatures that may discriminate between 2 cytotoxic agents may be so infrequent that they cannot be properly tested in phase II studies. The current phase II study highlights the challenges in development of predictive biomarkers and the need to conduct properly designed, prospective, randomized studies in validating predictive biomarker before launching a phase III study.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.-M. Tseng, S. Chan, D. Liu

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.E. Horak, L. Pusztai, G. Xing, O.C. Trifan, S. Chan, D. Liu

Writing, review, and/or revision of the manuscript: C.E. Horak, L. Pusztai, O.C. Trifan, C. Saura, C.-M. Tseng, S. Chan, D. Liu

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

Study supervision: C.E. Horak, C. Saura, S. Chan, D. Liu

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References

1. Cianfrocca M, Gradishar W. New molecular classifications of breast cancer. CA Cancer J Clin 2009;59:303–13.
2. Schnitt SJ. Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy. Mod Pathol 2010;23 Suppl 2:S60–4.
3. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74.
4. Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 2005;11:5678–85.
5. Isakoff SJ. Triple-negative breast cancer: role of specific chemotherapy agents. Cancer J 2010;16:53–61.
6. Koshy N, Quispe D, Shi R, Mansour R, Burton GV. Cisplatin-gemcitabine therapy in metastatic breast cancer: improved outcome in triple negative breast cancer patients compared to non-triple negative patients. Breast 2010;19:246–8.
7. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007;13:2329–34.
8. Liedtke C, Mazouni C, Hess KR, André F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008;26:1275–81.
9. Wolff AC, Berry D, Carey LA, Colleoni M, Dowsett M, Ellis M, et al. Research issues affecting preoperative systemic therapy for operable breast cancer. J Clin Oncol 2008;26:806–13.
10. Snoj N, Bedard PL, de Azambuja E, Cardoso F, Piccart M. Are we HERting for innovation in neoadjuvant breast cancer trial design? Breast Cancer Res 2009;11:201.
11. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998;16:2672–85.
12. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A, et al. Preoperative chemotherapy: updaters of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol 2008;26:778–85.
13. Montagna E, Bagnardi V, Rotmensz N, Viale G, Pruneri G, Veronesi P, et al. Pathological complete response after preoperative systemic therapy and outcome: relevance of clinical and biologic baseline features. Breast Cancer Res Treat 2010;124:689–99.
14. Chang JC, Wooten EC, Tsimelzon A, Hilsenbeck SG, Gutierrez MC, Tham YL, et al. Patterns of resistance and incomplete response to docetaxel by gene expression profiling in breast cancer patients. J Clin Oncol 2005;23:1169–77.
15. Nagasaki K, Miki Y. Molecular prediction of the therapeutic response to neoadjuvant chemotherapy in breast cancer. Breast Cancer 2008;15:117–20.
16. Ayers M, Symmans WF, Stec J, Damokosh AI, Clark E, Hess K, et al. Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. J Clin Oncol 2004;22:2284–93.
17. Park S, Shimizu C, Shimoyama T, Takeda M, Ando M, Kohno T, et al. Gene expression profiling of ATP-binding cassette (ABC) transporters as a predictor of the pathologic response to neoadjuvant chemotherapy in breast cancer patients. Breast Cancer Res Treat 2006;99:9–17.
18. Kadra G, Finetti P, Toiron Y, Viens P, Birnbaum D, Borg JP, et al. Gene expression profiling of breast tumor cell lines to predict for therapeutic response to microtubule-stabilizing agents. Breast Cancer Res Treat 2012;132:1035–47.
19. Yasojima H, Shimomura A, Naoi Y, Kishi K, Baba Y, Shimazu K, et al. Association between c-myc amplification and pathological complete response to neoadjuvant chemotherapy in breast cancer. Eur J Cancer 2011;47:1779–88.
20. Naoi Y, Kishi K, Tanei T, Tsunashima R, Tominaga N, Baba Y, et al. Prediction of pathologic complete response to sequential paclitaxel and 5-fluorouracil/epirubicin/cyclophosphamide therapy using a 70-gene classifier for breast cancers. Cancer 2011;117:3682–90.
21. Fan C, Prat A, Parker JS, Liu Y, Carey LA, Troester MA, et al. Building prognostic models for breast cancer patients using clinical variables and hundreds of gene expression signatures. MC Med Genomics 2011;4:3.
22. Lin Y, Lin S, Watson M, Trinkaus KM, Kuo S, Naughton MJ, et al. A gene expression signature that predicts the therapeutic response of the basal-like breast cancer to neoadjuvant chemotherapy. Breast Cancer Res Treat 2010;123:691–9.
23. Straver ME, Glas AM, Hannemann J, Wesseling J, van de Vijver MJ, Rutgers EJ, et al. The 70-gene signature as a response predictor for neoadjuvant chemotherapy in breast cancer. Breast Cancer Res Treat 2010;119:551–8.
24. Rody A, Karn T, Gätje R, Ahr A, Solbach C, Kourtis K, et al. Gene expression profiling of breast cancer patients treated with docetaxel,

doxorubicin, and cyclophosphamide within the GEPARTRIO trial: HER-2, but not topoisomerase II alpha and microtubule-associated protein tau, is highly predictive of tumor response. Breast 2007;16: 86–93.
25. Thuerigen O, Schneeweiss A, Toedt G, Warnat P, Hahn M, Kramer H, et al. Gene expression signature predicting pathologic complete response with gemcitabine, epirubicin, and docetaxel in primary breast cancer. J Clin Oncol 2006;24:1839–45.
26. Gianni L, Zambetti M, Clark K, Baker J, Cronin M, Wu J, et al. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. J Clin Oncol 2005;23:7265–77.
27. Baselga J, Zambetti M, Llombart-Cussac A, Manikhas G, Kubista E, Steger GG, et al. Phase II genomics study of ixabepilone as neoadjuvant treatment of breast cancer. J Clin Oncol 2009;27:526–34.
28. Gradishar WJ, Wedam SB, Jahanzeb M, Erban J, Limentani SA, Tsai KT, et al. Neoadjuvant docetaxel followed by adjuvant doxorubicin and cyclophosphamide in patients with stage III breast cancer. Ann Oncol 2005;16:1297–304.
29. Buzdar AU, Singletary SE, Theriault RL, Booser DJ, Valero V, Ibrahim N, et al. Prospective evaluation of paclitaxel versus combination chemotherapy with fluorouracil, doxorubicin, and cyclophosphamide as neoadjuvant therapy in patients with operable breast cancer. J Clin Oncol 1999;17:3412–7.
30. Demaria S, Volm MD, Shapiro RL, Yee HT, Oratz R, Formenti SC, et al. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin Cancer Res 2001;7: 3025–30.
31. Bear HD, Anderson S, Brown A, Smith R, Mamounas EP, Fisher B, et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2003;21:4165–74.
32. Lee FY, Smykla R, Johnston K, Menard K, McGlinchey K, Peterson RW, et al. Preclinical efficacy spectrum and pharmacokinetics of ixabepilone. Cancer Chemother Pharmacol 2009;63:201–12.
33. Dumontet C, Jordan MA, Lee FF. Ixabepilone: targeting βIII-tubulin expression in taxane-resistant malignancies. Mol Cancer Ther 2009; 8:17–25.
34. Ranganathan S, Benetatos CA, Colarusso PJ, Dexter DW, Hudes GR. Altered β-tubulin isotype expression in paclitaxel-resistant human prostate carcinoma cells. Br J Cancer 1998;77:562–6.
35. Kavallaris M, Burkhart CA, Horwitz SB. Antisense oligonucleotides to class III β-tubulin sensitize drug-resistant cells to Taxol. Br J Cancer 1999;80:1020–5.
36. Hari M, Yang H, Zeng C, Canizales M, Cabral F. Expression of class III β-tubulin reduces microtubule assembly and confers resistance to paclitaxel. Cell Motil Cytoskeleton 2003;56:45–56.
37. Paradiso A, Mangia A, Chiriatti A, Tommasi S, Zito A, Latorre A, et al. Biomarkers predictive for clinical efficacy of taxol-based chemotherapy in advanced breast cancer. Ann Oncol 2005;16 Suppl 4:14–9.
38. Tommasi S, Mangia A, Lacalamita R, Bellizzi A, Fedele V, Chiriatti A, et al. Cytoskeleton and paclitaxel-sensitivity in breast cancer: the role of β-tubulins. Int J Cancer 2007;120:2078–85.
39. Sève P, Dumontet C. Is class III β-tubulin a predictive factor in patients receiving tubulin-binding agents? Lancet Oncol 2008;9:168–75.
40. Horak CE, Lee FY, Xu L, Galbraith S, Baselga J. High β-III tubulin expression in triple-negative (TN) breast cancer (BC) subtype and correlation to ixabepilone response: a retrospective analysis. J Clin Oncol 27:15s, 2009 (suppl; abstr 3587).
41. Wu S, Chasalow S, Lee H, Xu L, Paul B, Mokliatchouk O, et al. Identification of predictive markers to differentiate ixabepilone from paclitaxel activity in ER-negative breast cancer patients. J Clin Oncol 25:18s, 2007 (suppl; abstr 2525).
42. Booth DG, Hood FE, Prior IA, Royle SJ. A TACC3/ch-TOG/clathrin complex stabilises kinetochore fibres by inter-microtubule bridging. EMBO J 2011;30:906–19.
43. Hubert T, Van Impe K, Vandekerckhove J, Gettemans J. The actin-capping protein CapG localizes to microtubule-dependent organelles during the cell cycle. Biochem Biophys Res Commun 2009;380: 166–70.
44. Haber M, Burkhart CA, Regl DL, Madafiglio J, Norris MD, Horwitz SB. Altered expression of M beta 2, the class II beta-tubulin isotype, in a murine J774.2 cell line with a high level of taxol resistance. J Biol Chem 1995;270:31269–75.
45. Bhattacharya R, Cabral F. A ubiquitous beta-tubulin disrupts microtubule assembly and inhibits cell proliferation. Mol Biol Cell 2004; 15:3123–31.
46. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160–7.
47. Sève P, Isaac S, Trédan O, Souquet PJ, Pachéco Y, Pérol M, et al. Expression of class III β-tubulin is predictive of patient outcome in patients with non-small cell lung cancer receiving vinorelbine-based chemotherapy. Clin Cancer Res 2005;11:5481–6.
48. Sève P, Mackey J, Isaac S, Trédan O, Souquet PJ, Pérol M, et al. Class III beta-tubulin expression in tumor cells predicts response and outcome in patients with non-small cell lung cancer receiving paclitaxel. Mol Cancer Ther 2005;4:2001–7.
49. Rudas M, Filipits M, Taucher S, Stranzl T, Steger GG, Jakesz R, et al. Expression of MRP1, LRP and Pgp in breast carcinoma patients treated with preoperative chemotherapy. Breast Cancer Res Treat 2003;81:149–57.
50. Ladner RD, Lynch FJ, Groshen S, Xiong YP, Sherrod A, Caradonna SJ, et al. dUTP nucleotidohydrolase isoform expression in normal and neoplastic tissues: association with survival and response to 5-fluorouracil in colorectal cancer. Cancer Res 2000;60:3493–503.
51. 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.
52. Thomas E, Tabernero J, Fornier M, Conté P, Fumoleau P, Lluch A, et al. Phase II clinical trial of ixabepilone (BMS-247550), an epothilone B analog, in patients with taxane-resistant metastatic breast cancer. J Clin Oncol 2007;25:3399–406.
53. Thomas ES, Gomez HL, Li RK, Chung HC, Fein LE, Chan VF, et al. Ixabepilone plus capecitabine for metastatic breast cancer progressing after anthracycline and taxane treatment. J Clin Oncol 2007; 25:5210–7.
54. Sparano JA, Vrdoljak E, Rixe O, Xu B, Manikhas A, Medina C, et al. Randomized phase III trial of ixabepilone plus capecitabine versus capecitabine in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol 2010;28: 3256–63.
55. Perez EA, Patel T, Moreno-Aspitia A. Efficacy of ixabepilone in ER/PR/HER2-negative (triple-negative) breast cancer. Breast Cancer Res Treat 2010;121:261–71.
56. Rugo HS, Campone M, Amadori D, Wardley AM, Aldrighetti D, Conte PF, et al. Randomized phase II study of weekly versus every 3 week ixabepilone plus bevacizumab (ixa/bev) versus paclitaxel plus bev (pac/bev) as first-line therapy for metastatic breast cancer (MBC): Final results. J Clin Oncol 28:15s, 2010 (suppl; abstr 1040).
57. Rugo HS, Barry WT, Moreno-Aspitia A, Lyss AP, Cirrincione C, Mayer EL, et al. CALGB 40502/NCCTG N063H: Randomized phase III trial of weekly paclitaxel (P) compared to weekly nanoparticle albumin bound nab-paclitaxel (NP) or ixabepilone (Ix) with or without bevacizumab (B) as first-line therapy for locally recurrent or metastatic breast cancer (MBC). J Clin Oncol 30, 2012 (suppl; abstr CRA1002).