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Prospective Comparison of Clinical and Genomic Multivariate Predictors of Response to Neoadjuvant Chemotherapy in Breast Cancer

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Abstract

Purpose: Several different multivariate prediction models using routine clinical variables or multigene signatures have been proposed to predict pathologic complete response to combination chemotherapy in breast cancer. Our goal was to compare the performance of four conceptually different predictors in an independent cohort of patients.

Experimental Design: Gene expression profiling was done on fine-needle aspirations of 100 stage I to III breast cancers before preoperative paclitaxel, 5-fluorouracil, doxorubicin, and cyclophosphamide combination chemotherapy. Pathologic response was correlated with prediction results from a clinical nomogram, a human cancer–derived genomic predictor (DLDA30), a cell line–based genomic predictor [*in vitro* coexpression extrapolation (COXEN)], and an optimized cell line–derived (*in vivo* COXEN) predictor. None of the 100 test cases were used in the development of these predictors.

Results: The *in vitro* COXEN using a combination of four individual drug sensitivity predictions derived from cell lines was not predictive [area under the receiver operator characteristic curve (AUC), 0.5; 95% confidence interval, (95% CI), 0.41-0.59]. The clinical nomogram (AUC, 0.73; 95% CI, 0.65-0.80) and the DLDA30 (AUC, 0.73; 95% CI, 0.66-0.80) genomic predictor had similar performances. The *in vivo* COXEN that used informative genes from cell lines but was trained on a separate human data set also showed significant predictive value (AUC, 0.67; 95% CI, 0.60-0.74). These three different prediction scores correlated with each other and were significant in univariate but not in multivariate analysis.

Conclusions: Three conceptually different predictors performed similarly in this validation study and tended to identify the same patients as responders. A genomic predictor that relied solely on a composite of individual drug sensitivity predictions from cell lines did not show any predictive value. *Clin Cancer Res; 16(2); 711–8. ©2010 AACR.*

Not all patients with breast cancer respond to chemotherapy, and different drugs induce different responses in different patients. Therefore, molecular predictors that identify who may benefit from what drug with sufficient accuracy could aid treatment selection. Accurate response predictors could also streamline drug development and facilitate the conduct of clinical trials through the identification of patients who do or do not benefit from existing therapies. Several investigators suggested that cell line–derived predictors are useful to predict response to chemotherapy in patients (1, 2). A commonly used strategy is to use gene expression data and *in vitro* drug response information from the NCI-60 or other cell line panels and use these information to develop drug-specific pharmacogenomic response predictors than can be applied to human data (3–5). It is also possible to develop multigene predictors of response to therapy from supervised analysis of gene expression data using human cancers with known response to chemotherapy (6, 7). Many routinely available clinical variables are also associated with chemotherapy sensitivity in breast cancer. Estrogen receptor (ER)–negative, high-grade, and HER2-positive cancers all tend to show greater sensitivity to chemotherapy, and these variables can also be combined into a fairly accurate multivariate prediction model (8).

The goal of the current study was to compare the predictive accuracy of four conceptually different predictors. Each predictor was applied to the same 100 cases of breast cancers that received uniform preoperative chemotherapy

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Translational Relevance

Chemotherapy response predictors can be constructed from clinical variables or from gene expression data of human cancers with known response to chemotherapy, or using data from cell lines with known drug sensitivity. We compared these three methods testing four different *a priori* defined predictors in 100 cancers treated with neoadjuvant chemotherapy. The clinical variable–based and empirically derived genomic predictors worked equally well but the pure, cell line–trained genomic predictor showed poor performance. Many genes that were informative of drug response in cell lines were not informative in human cancers, and even genes that retained informative value needed to be trained on human data, rather than cell lines, to achieve good predictive performance. The empirically derived genomic response predictor developed from all breast cancers relied heavily on identifying known clinical phenotypes that limits its practical value; the next generation of such markers will need to be developed separately for distinct breast cancer subtypes.

and were not included in the predictor development process. Each of these cases received neoadjuvant chemotherapy with paclitaxel followed by 5-fluorouracil (5-FU), doxorubicin, and cyclophophosphamide (T/FAC), and pathologic response was assessed. The four predictors included the following; (*a*) a clinical variable–based nomogram to predict pathologic complete response (pCR; ref. 8), (*b*) a 30-probe set pharmacogenomic predictor that was developed from the supervised analysis of 81 breast cancers that received T/FAC preoperative chemotherapy (7), (*c*) a cell line–derived response marker that combined individual drug-specific predictive signatures from the NCI-60 cell line data (1); and (*d*) a revised cell line–derived predictor that used human gene expression data to train a prediction model that used drug-specific sensitivity features selected from the cell lines. Each of these predictors was finalized before application to the current validation data set.

Materials and Methods

Patients and materials. Gene expression data from 100 stage I to III breast cancers were generated at the University of Texas M.D. Anderson Cancer Center. These patients were prospectively and sequentially accrued to a pharmacogenomic marker discovery trial. None of these cases were included in the development process of any of the four predictors. Gene expression profiling was done on fine-needle aspiration specimens of newly diagnosed breast cancers before any therapy. These specimens contain 70% to 90% neoplastic cells with minimal stromal contamination (9). All patients signed informed consent for genomic analysis of their cancer. Patients received 6 mo of preoperative T/FAC chemotherapy followed by surgical resection of the cancer. No patient received preoperative trastuzumab in this study. The clinical characteristics of the patient population are presented in Table 1. Response to chemotherapy was dichotomized as pCR [no residual invasive cancer (RD) in the breast and lymph nodes] or RD. The prognostic importance of pCR has extensively been discussed in the literature (10, 11).

Gene expression profiling. RNA extraction and gene expression profiling were done in multiple batches over time as described previously (7). Normalization and quality control assessment of the hybridization results were done with the SimpleAffy software of the Bioconductor package[8] (Supplementary Materials and Methods). All CEL files are available at the GEO Web site (accession number GSE16716, Microarray Quality Control Consortium II data set).

Statistical analysis. The clinical variable–based nomogram to estimate the probability of pCR was applied as it was described in the original publication (8). It is also available as a free Web-based tool.[9] The overall development and validation schema for the three genomic predictors is illustrated in Supplementary Fig. S1. The DLDA30 predictor uses diagonal linear discriminant analysis (DLDA) to calculate a prediction score based on the expression of 30 probe sets that are differentially expressed between cases with pCR and RD to neoadjuvant T/FAC chemotherapy (7). This predictor was trained and the threshold was defined on a discovery set of 82 cases. For this analysis, the current gene expression data were normalized using the dCHIP V1.3 software and the same code was used to perform prediction estimates as described in the original publication.[10]

The *in vitro* coexpression extrapolation (COXEN) gene expression prediction model (GEM) was derived from the microarray data and *in vitro* drug sensitivity results (1). Informative probe sets that were associated with paclitaxel, 5-FU, doxorubicin, and cyclophosphamide sensitivity, respectively, were identified from the NCI-60 microarray data. The 10% to 20% most and least sensitive cell lines based on GI_{50} values were compared to define the informative probe sets that were further filtered based on the *COXEN coefficient*, which represents the degree of concordance of expression between cell line and human breast cancer gene expression data (12). Genes with the highest COXEN coefficient were included in the drug-specific prediction models that used linear discriminant analysis and were trained on the NCI-60 cell lines (13, 14). A combined score from the four individual drug predictors, assuming their independence, was calculated to generate a combination chemotherapy (T/FAC) predictor

[8] http://www.bioconductor.org
[9] http://www.mdanderson.org/pcr
[10] Code is available at http://bioinformatics.mdanderson.org/pubdata.html.

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Table 1. Patient characteristics

Clinical and pathologic data	Validation set (n = 100)
Median age, y	50 (26-76)
Race	
Caucasian	68
African-American	12
Asian	7
Hispanic	13
Histologic type	
Invasive ductal	85
Mixed ductal/lobular	8
Invasive lobular	7
Prechemotherapy tumor size	
T_0 (with positive axillary node)	2
T_1	8
T_2	62
T_3	13
T_4	15
Clinical N stage	
N_0	27
N_1	47
N_2	13
N_3	13
Nuclear grade	
1	11
2	42
3	47
ER status*	
Positive	60
Negative	40
HER-2 status†	
Positive	7
Negative	93
Neoadjuvant chemotherapy‡	
Weekly T × 12 + FAC × 4	98
Thrice weekly T × 12 + FAC × 4	2
PCR	15
Residual disease	85

*Cases where >10% of tumor cells stained positive for ER with immunohistochemistry were considered positive.
†Cases that showed either more than three immunohistochemistry staining or had a gene copy number of >2.0 were considered HER-2 "positive."
‡T, paclitaxel; FAC, 5-FU, doxorubicin, and cyclophosphamide.

(see Supplementary Materials and Methods). The higher the COXEN score is, the higher is the predicted probability to achieve response to a given drug or combination.

The *in vivo* COXEN GEMs were developed using the same informative probe sets for each drug as used in the *in vitro* COXEN GEM described above. However, the linear discriminant analysis classification model for the *in vivo* COXEN GEM was trained on a publicly available human data set that was used to develop and validate the original DLDA30 model (n = 133; ref. 7). The main difference between the *in vivo* COXEN GEM strategy and the strategy that lead to the development of the DLDA30 is that the informative features for the *in vivo* COXEN GEM were derived from cell lines and may represent drug-specific sensitivity markers, whereas the features for DLDA30 were derived from the human data. The performance of the *in vitro* and *in vivo* COXEN GEM was evaluated on the 100 new cases blindly. Prediction scores were calculated from the CEL file information provided to the team at University of Virginia without knowledge of the response outcome. The prediction scores were returned to investigators at University of Texas M.D. Anderson Cancer Center to determine correlation with response.

Predictor performances are described using standard metrics including area under the receiver operator characteristic (ROC) curve (AUC), sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values. To calculate the misclassification error rates, we defined the best predictor for each method using the Youden point (YP) on the ROC curve. The Youden index is defined as maximum sensitivity (YP) + specificity (*YP*) − 1, occurring at the optimum threshold, which is the YP (15). The YP corresponds to the point on the ROC curve that is farthest from chance and defines predictor thresholds that maximize both the sensitivity and the specificity, and minimize the misclassification rate in a given data set. The sensitivity, specificity, PPV, and NPV for the COXEN GEM predictors were calculated based on this optimized cutoff that was defined in an external reference set of 133 cases.

Univariate and multivariate logistic regression on pCR/RD status were done to determine which routine clinical variables and prediction scores were associated significantly with pCR. The routine clinical variables used were patient age, pretreatment tumor size, lymph node status, nuclear grade, ER status, and HER2 status. Patient age, nuclear grade, and all prediction scores were treated as continuous variables. Because some of the prediction scores were a combination of the clinical variables (e.g., the clinical nomogram score), multivariate logistic regression using only the prediction scores were also done. Backward elimination was done during the multivariate regression analysis with a significance level of 0.05 for a covariate to stay in model. To make the odds ratios (OR) comparable across the four predictors, we standardize the four score to a similar scale from 0 to 10.

Results

Prediction performance of the clinical nomogram. We calculated for each patient the probability of pCR using the clinical nomogram and compared the predicted pCR rates to the observed rates. Discrimination (i.e., whether the relative ranking of individual predictions is in the correct order) was quantified with the AUC (16). Fifteen of the 100 patients had pCR. This is less than what has been observed in the past in a larger series of patients who received the

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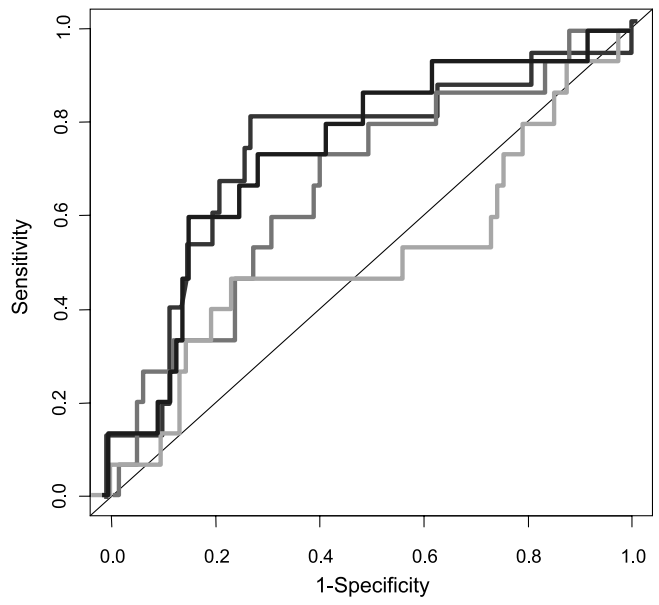
same preoperative chemotherapy (17). The lower pCR rate in this patient cohort is likely due to the relative absence of HER-2-positive patients, only 7% of cases were HER2 positive in this series compared with the typical 20% to 25% seen in the general population and in the previous study. HER2-positive breast cancers have significantly higher rates of pCR compared with HER2-normal cancers (18). Their absence from this more recent patient cohort is due to changing clinical practice; in recent years, many HER-2-positive cases received trastuzumab concomitant with chemotherapy in our center (19). Despite this difference in patient population, the clinical variable–based prediction showed good discrimination. The AUC for the clinical nomogram was 0.72 [95% confidence interval (95% CI), 0.65-0.78], which is similar to the AUC (0.79) observed in the validation cohort reported in the original publication (8). The ROC curves for the nomogram, DLDA30, *in vivo* T/FAC COXEN GEM, and *in vitro* TFAC COXEN GEM are plotted on Fig. 1.

We also assessed calibration that measures agreement between observed outcome frequencies and predicted probabilities of response (Supplementary Fig. S2; ref. 16). Calibration was less good than previously reported; we observed a significant difference between the predicted probabilities and the observed frequencies. The average difference and maximal differences in prediction and observations were 12% and 33%, respectively ($P = 0.006$), and showed that the nomogram tended to err toward overestimating the probability of pCR.

Prediction performance of the DLDA30. The 31-gene pharmacogenomic predictor had an AUC of 0.73 (95% CI, 0.65-0.80; Fig. 1). Using the previously established threshold, the overall accuracy was 72% (95% CI, 62-81); the sensitivity was 60% (95% CI, 32-84); the specificity was 74% (95% CI, 63-83); the PPV was 29% (95% CI, 14-48); and the NPV was 91% (95% CI, 82-97). These values are similar but generally lower than observed in the previous small validation study ($n = 51$, AUC = 0.88;



Clinical nomogram score	0.73	0.65-0.80
DLDA30 score	0.73	0.66-0.80
in vivo-TFAC COXEN	0.67	0.60-0.74
in vitro-TFAC COXEN	0.5	0.41-0.59

pCR = Pathological Complete Response; AUC = Area Under the ROC Curve, ROC = receiver operating characteristic, CI = confidence interval; DLDA30 = Human cancer-derived genomic predictor; in vivo-COXEN GEM = an optimized cell line-derived TFAC predictor, in vitro-COXEN GEM = a cell line-based TFAC genomic predictor.

Fig. 1. ROC curve analysis of the four conceptually different chemotherapy response predictors. ROC curves are shown for the clinical nomogram, the DLDA30 predictor, the *in vitro* combined T/FAC COXEN GEM, and the *in vivo* combined T/FAC COXEN GEM.

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Table 2. Comparison of mean COXEN GEM chemotherapy sensitivity scores for individual drugs and for their combination between patients with pCR and residual cancer

Predictor	Drug	pCR group GEM Scores (mean ± 95% CI)	Residual Cancer GEM Score (mean ± 95% CI)	*P*
In vitro COXEN GEM	**Paclitaxel**	**0.531 ± 0.225**	**0.289 ± 0.079**	**0.045**
	5-FU	0.447 ± 0.229	0.426 ± 0.074	0.848
	Doxorubicin	0.168 ± 0.170	0.235 ± 0.078	0.459
	Cyclophosphamide	0.146 ± 0.176	0.160 ± 0.061	0.879
	TFAC	0.659 ± 0.192	0.601 ± 0.075	0.562
In vivo COXEN GEM	**Paclitaxel**	**0.449 ± 0.172**	**0.221 ± 0.048**	**0.015**
	5-FU	0.262 ± 0.057	0.254 ± 0.023	0.787
	Doxorubicin	**0.365 ± 0.098**	**0.239 ± 0.037**	**0.019**
	Cyclophosphamide	0.366 ± 0.135	0.251 ± 0.049	0.106
	TFAC	**0.755 ± 0.133**	**0.595 ± 0.052**	**0.028**

NOTE: TFAC, the sum of individual paclitaxel, 5-FU, doxorubicin, and cyclophosphamide scores. Significant results are highlighted in bold.

ref. 7). The difference in predictive performance is likely due to the relative absence of HER2-positive cases in this study. The calibration of the DLDA30 was excellent (Supplementary Fig. S2).

Prediction performance of the cell line–derived COXEN GEM predictors. We compared the *in vitro* COXEN GEM chemosensitivity scores between cases with pCR and residual cancer for each drug separately and also in

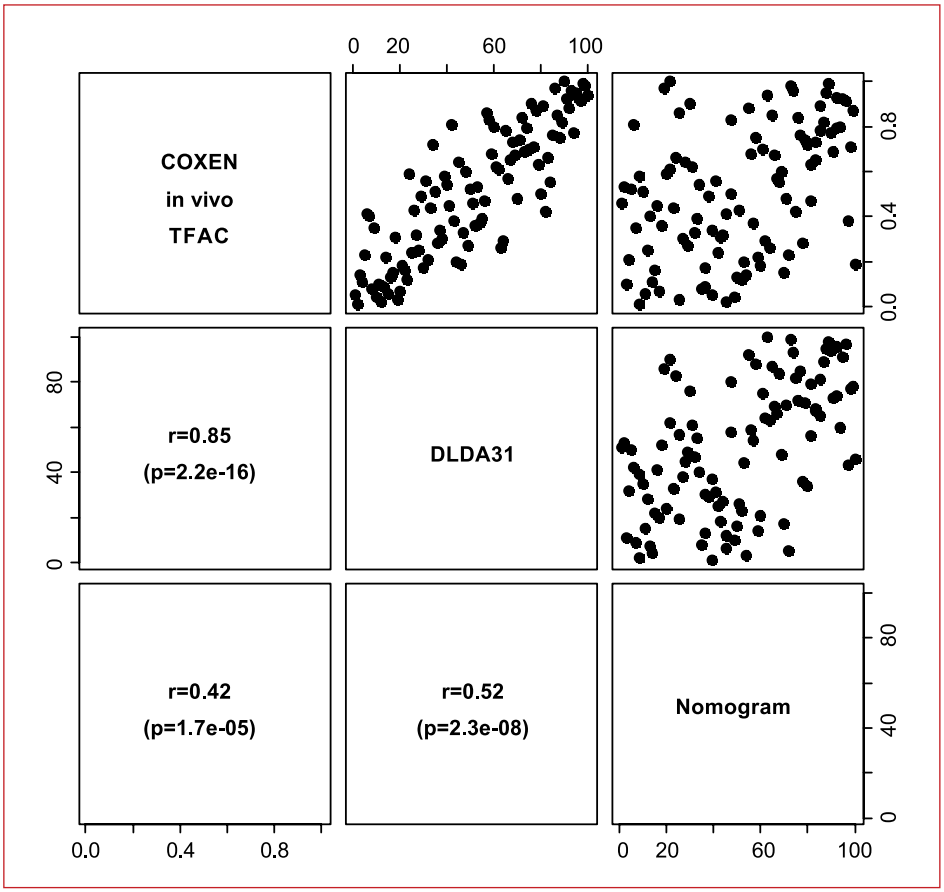

Fig. 2. Correlation between ranks of prediction scores. Ranks of predicted scores of the *in vivo* GEM, the DLDA30 predictor, and the clinical nomogram were plotted against each other. Rank-based Spearman correlation and *P* value were calculated for each pair of comparisons.

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Table 3. Univariate and multivariate logistic regression results including clinical and genomic score variables

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, y	1.01 (0.95-1.06)	0.82	0.98 (0.90-1.06)	0.61
Prechemotherapy T size (2-4 vs 0-1)	0.68 (0.13-3.54)	0.64	0.85 (0.06-12.14)	0.91
Clinical N stage (N_{1-3} vs N_0)	6.17 (0.77-49.41)	0.087	2.99 (0.28-31.40)	0.36
Nuclear grade	5.06 (1.44-17.80)	0.011	1.97 (0.34-11.49)	0.45
ER status (positive vs negative)	0.07 (0.02-0.34)	0.00091	0.20 (0.02-1.87)	0.16
HER-2 status (positive vs negative)	9.94 (1.96-50.42)	0.0056	4.45 (0.52-38.14)	0.17
Clinical nomogram score	1.49 (1.16-1.90)	0.0016	0.98 (0.64-1.49)	0.91
DLDA30 score	1.52 (1.13-2.04)	0.0055	1.29 (0.69-2.39)	0.42
In vivo T/FAC COXEN GEM score	1.40 (1.03-1.90)	0.034	1.05 (0.50-2.20)	0.90
In vitro T/FAC COXEN GEM score	0.95 (0.81-1.13)	0.59	0.83 (0.63-1.90)	0.18

NOTE: OR, ORs for having pCR versus RD. ER status was determined by immunohistochemistry and HER2 status was determined by immunohistochemistry or fluorescence *in situ* hybridization. Predictor scores, age, and nuclear grade were used as continuous variables.

combination. The genes selected as informative for response to individual drugs from the NCI-60 cell lines showed no overlap with the genes included in the DLDA30 predictor (Supplementary Table S1). Mean COXEN GEM scores for each drug and the combined score are shown in Table 2. AUC for the combined score is plotted on Fig. 1. The results indicate that for three of the four drugs, the purely cell line–derived predictor, could not discriminate response in human patients. However, the AUC for the single-agent paclitaxel predictor was significantly better than random (AUC, 0.64; 95 CI%, 0.56-0.72). It showed a sensitivity of 60% (95% CI, 35-85), a specificity of 65% (95% CI, 55-75), a PPV of 23% (95% CI, 10-36), and a NPV of 90% (95% CI, 83-98) at the cutoff value, which was predetermined on a previous patient set treated with similar chemotherapy by maximizing the Youden index.

Next, we tested the *in vivo* COXEN GEM predictors that were trained on a separate T/FAC-treated patient cohort of 133 cases. The single-drug predictors for both paclitaxel ($P = 0.015$) and doxorubicin ($P = 0.019$) had significantly higher mean scores in the pCR cohort but the scores for 5-FU and cyclophosphamide were not significantly different between the two response groups (Table 2). The combined four-drug *in vitro* T/FAC COXEN GEM score also remained significant ($P = 0.028$). The AUCs for the paclitaxel and doxorubicin predictors were 0.64 (95% CI, 0.56-0.71) and 0.71 (95% CI, 0.64-0.78), respectively. The combined T/FAC *in vivo* COXEN GEM had an AUC of 0.67 (95% CI, 0.60-0.74; $P < 0.04$; Fig. 1). However, the calibration of the T/FAC *in vivo* COXEN GEM was poor, indicating that this predictor overestimated the probability of pCR (Supplementary Fig. S1).

The misclassification error rates, at the YP of the ROC, were 0.27 (95% CI, 0.232-0.339) for both the clinical nomogram and the DLDA30 predictor. The misclassification error rates were 0.38 (95% CI, 0.334-0.45) and 0.35 (95% CI, 0.285-0.412) for the *in vivo* and *in vitro* COXEN, respectively. In summary, these results indicate that genes identified in cell lines as predictors of response to certain drugs can carry information about response in human cancers. The results also show that this is not true for all drugs and that training of the model, assigning predictive weights to the informative genes, is best done on human cancers with known response rather than on the cell lines from which the informative features were defined.

Correlation between prediction scores. Because all predictors were applied to the same 100 cases, we could examine the correlation between prediction scores. In this analysis, we only examined the three predictors that showed significant discriminating value in the human validation data. Because the clinical nomogram, the DLDA30, and the *in vivo* T/FAC COXEN GEM scores each represent different scales, their correlation was plotted based on rank. There was a good correlation ($r = 0.85$) between how the two genomic scores ranked individual patients by predicted sensitivity (Fig. 2). The rank correlation was somewhat less but still significant for the nomogram and the genomic predictors. These observations suggest that the various predictors place individuals in the same position in a relative sensitivity scale even if they measure different variables.

The result of univariate logistic regression showed that nuclear grade, ER and HER-2 status, clinical nomogram, DLDA30, and *in vivo* T/FAC COXEN GEM scores were each significantly associated with pCR status (Table 3). In the multivariate analysis using both clinical variables and prediction scores, none of these remained significant (Table 3), indicating that the various scores identify similar populations. After backward elimination of nonsignificant covariates, only ER status remained significantly associated with pCR status. In the multivariate analysis using just the three significant prediction scores, clinical nomogram, DLDA30, and *in vivo* T/FAC COXEN GEM

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scores, only the clinical nomogram score remained significant after backward elimination.

Discussion

Different subsets of breast cancers have different degrees of chemotherapy sensitivity, and it is increasingly apparent that there are several methods that can estimate someone's probability of response to therapy. ER-negative, high-grade, and HER2-positive cancers have significantly higher rates of response to chemotherapy than other breast cancers; therefore, these clinical variables can be combined to yield a multivariate prediction model. Identification of gene expression differences between responding and nonresponding cases can also yield multigene predictors to commonly used combination chemotherapy regimens. However, these models are not treatment or regimen specific. A theoretically appealing approach is to develop gene expression–based predictors from cell line models in the laboratory. This strategy offers a promise for individual drug-specific predictors that could be combined into multidrug predictors that could be used to select the optimal combination chemotherapy for an individual in the clinic. This approach has also yielded promising results but it is not without controversy (20–22). The current study represents a prospective evaluation of four conceptually different previously established predictors of response to neoadjuvant chemotherapy. The results show that both a clinical variable–based model and a genomic predictor developed through supervised analysis of human gene expression data could discriminate between cases with higher and lower probability to achieve pCR to T/FAC chemotherapy. However, both predictors had lesser performance than seen in the original reports that is partly due to a shift in patient population used for this validation study. This illustrates an inherent challenge to clinical biomarker development; as clinical practice evolves over time, patient populations participating in biomarker (or therapeutic) studies also change. In this instance, both the clinical pCR predictor nomogram and the DLDA30 genomic assay were developed for a general breast cancer population including HER2-positve cases. However, contemporary validation of these predictors is limited to HER2-normal cases due to the widespread use of trastuzumab in HER2-positive breast cancer. This shift in patient population likely contributes to the decreased performance of these two predictors.

A theoretical advantage of cell line–derived genomic predictors is that they may be less sensitive to shifts in patient population because, in their original form, these are not trained on a historical patient cohort but rather on clean and easily manipulated *in vitro* cell line models. However, the purely cell line–derived drug-specific predictors did not perform well; only the paclitaxel predictor showed modest but significant discriminating power in the human cases. To optimally train a predictor that relies on informative genes from cell lines, it was necessary to use information from a separate human data set with known response outcome.

These results raise an important question: why did some of the cell line–derived predictors worked for some drugs but not for others in human cases? For some drugs, it may be easier to find response markers in the RNA space than for others. The mechanisms of drug resistance are substantially more complex *in vivo* than *in vitro*. Important patient-to-patient differences in drug metabolism and tumor microenvironment (immune response, interstitial pressure, and vascular leakiness) cannot be captured by cell line gene expression data. Considering these complexities, it is remarkable that a cell line–derived paclitaxel (and to lesser degree, a doxorubicin) response predictor retained predictive value in patients at all. To move this field forward, it will be important to systematically examine which cell line–derived response markers retain predictive value in human tumors and why.

Some limitations of this study need to be acknowledged. Our grouping of the validation cases into pCR and RD are clinically justified because cases with pCR have excellent long-term survival most probably due to their chemotherapy sensitivity, whereas those with RD have a variable outcome. However, most cases with RD have some degree of tumor response, and therefore, these cases are not strictly resistant to therapy even if their long-term benefit from chemotherapy remains uncertain. To address the effect of this dichotomization, we also correlated prediction scores with residual cancer burden treated as a continuous response variable, and results have not changed. In addition, the cell line–derived predictors were developed for single agents, and their combination, assuming independence between drug sensitivities, was used to define a multidrug sensitivity score. Our combination score approach may be too naïve to capture the complexity of potential multidrug interactions that can occur during treatment. Unfortunately, genomic data from patients treated with single drugs was not available for validation. The lack of data from patients with different single-agent therapies also limits the ability to truly evaluate the regimen specificity of the cell line–derived signatures. From the current results, it is impossible to infer if the paclitaxel signature represents a general chemotherapy sensitivity marker or if it truly predicts for paclitaxel sensitivity. This is an important question that will need to be studied in future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Lee JK, Havaleshko DM, Cho H, et al. A strategy for predicting the chemosensitivity of human cancers and its application to drug discovery. Proc Natl Acad Sci U S A 2007;104:13086–91.
2. Potti A, Dressman HK, Bild A, et al. Genomic signatures to guide the use of chemotherapeutics. Nat Med 2006;12:1294–300.
3. Bonnefoi H, Potti A, Delorenzi M, et al. Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial. Lancet Oncol 2007;8:1071–8.
4. Coombes KR, Wang J, Baggerly KA. Microarrays: retracing steps. Nat Med 2007;13:1276–7; author reply 7–8.
5. Hsu DS, Balakumaran BS, Acharya CR, et al. Pharmacogenomic strategies provide a rational approach to the treatment of cisplatin-resistant patients with advanced cancer. J Clin Oncol 2007;25:4350–7.
6. Chang JC, Wooten EC, Tsimelzon A, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. Lancet 2003;362:362–9.
7. Hess KR, Anderson K, Symmans WF, et al. Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. J Clin Oncol 2006;24:4236–44.
8. Rouzier R, Pusztai L, Delaloge S, et al. Nomograms to predict pathologic complete response and metastasis-free survival after preoperative chemotherapy for breast cancer. J Clin Oncol 2005;23:8331–9.
9. Symmans WF, Ayers M, Clark EA, et al. Total RNA yield and microarray gene expression profiles from fine-needle aspiration biopsy and core-needle biopsy samples of breast carcinoma. Cancer 2003;97:2960–71.
10. Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008;26:1275–81.
11. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007;25:4414–22.
12. Miller LD, Smeds J, George J, et al. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. Proc Natl Acad Sci U S A 2005;102:13550–5.
13. Dressman HK, Berchuck A, Chan G, et al. An integrated genomic-based approach to individualized treatment of patients with advanced-stage ovarian cancer. J Clin Oncol 2007;25:517–25.
14. Takata R, Katagiri T, Kanehira M, et al. Validation study of the prediction system for clinical response of M-VAC neoadjuvant chemotherapy. Cancer Sci 2007;98:113–7.
15. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32–5.
16. Coutant C, Olivier C, Lambaudie E, et al. Comparison of models to predict nonsentinel lymph node status in breast cancer patients with metastatic sentinel lymph nodes: a prospective multicenter study. J Clin Oncol 2009;27:2800–8.
17. Green MC, Buzdar AU, Smith T, et al. Weekly paclitaxel improves pathologic complete remission in operable breast cancer when compared with paclitaxel once every 3 weeks. J Clin Oncol 2005;23:5983–92.
18. Andre F, Mazouni C, Liedtke C, et al. HER2 expression and efficacy of preoperative paclitaxel/FAC chemotherapy in breast cancer. Breast Cancer Res Treat 2008;108:183–90.
19. Buzdar AU, Valero V, Ibrahim NK, et al. Neoadjuvant therapy with paclitaxel followed by 5-FU, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. Clin Cancer Res 2007;13:228–33.
20. Ein-Dor L, Kela I, Getz G, Givol D, Domany E. Outcome signature genes in breast cancer: Is there a unique set? Bioinformatics 2005;21:171–8.
21. Michiels S, Koscielny S, Hill C. Prediction of cancer outcome with microarrays: a multiple random validation strategy. Lancet 2005;365:488–92.
22. Ransohoff DF. Bias as a threat to the validity of cancer molecular-marker research. Nat Rev Cancer 2005;5:142–9.

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