

Breast Cancer Subtypes and Response to Docetaxel in Node-Positive Breast Cancer: Use of an Immunohistochemical Definition in the BCIRG 001 Trial

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ABSTRACT

Purpose

To investigate the prognostic and predictive significance of subtyping node-positive early breast cancer by immunohistochemistry in a clinical trial of a docetaxel-containing regimen.

Methods

Pathologic data from a central laboratory were available for 1,350 patients (91%) from the BCIRG 001 trial of docetaxel, doxorubicin, and cyclophosphamide (TAC) versus fluorouracil, doxorubicin, and cyclophosphamide (FAC) for operable node-positive breast cancer. Patients were classified by tumor characteristics as (1) triple negative (estrogen receptor [ER]-negative, progesterone receptor [PR]-negative, HER2/*neu* [HER2]-negative), (2) HER2 (HER2-positive, ER-negative, PR-negative), (3) luminal B (ER-positive and/or PR-positive and either HER2-positive and/or Ki67^{high}), and (4) luminal A (ER-positive and/or PR-positive and not HER2-positive or Ki67^{high}), and assessed for prognostic significance and response to adjuvant chemotherapy.

Results

Patients were subdivided into triple negative (14.5%), HER2 (8.5%), luminal B (61.1%), and luminal A (15.9%). Three-year disease-free survival (DFS) rates (*P* values with luminal B as referent) were 67% (*P* < .0001), 68% (*P* = .0008), 82% (referent luminal B), and 91% (*P* = .0027), respectively, with hazard ratios of 2.22, 2.12, and 0.46. Improved 3-year DFS with TAC was found in the luminal B group (*P* = .025) and a combined ER-positive/HER2-negative group treated with tamoxifen (*P* = .041), with a marginal trend in the triple negatives (*P* = .051) and HER2 (*P* = .068) subtypes. No DFS advantage was seen in the luminal A population.

Conclusion

A simple immunopanel can divide breast cancers into biologic subtypes with strong prognostic effects. TAC significantly complements endocrine therapy in patients with luminal B subtype and, in the absence of targeted therapy, is effective in the triple-negative population.

J Clin Oncol 27. © 2009 by American Society of Clinical Oncology

INTRODUCTION

There has been a steady evolution in the adjuvant treatment of early breast cancer. In patients with hormone-sensitive tumors, tamoxifen reduces the risk of recurrence and death by more than 30%.¹ Subsequently, anthracycline-based chemotherapy¹ and, more recently, taxane-containing regimens,² have been shown to be most efficacious in improving disease-free (DFS) and overall survival (OS) in patients with operable node-positive breast cancer.

Since the study of Perou et al³ using gene expression studies to identify discrete genetic subtypes of breast cancer with distinct prognoses, there has been a paradigm shift in adjuvant therapy decisions,

away from risk categories based on stage alone to the determination of tumor responsiveness.⁴ This molecular advance has been difficult to translate into current clinical practice because a number of highly significant but nonoverlapping gene signatures with prognostic effect have been proposed,⁵⁻⁹ complicating their application, and because the large clinical trials that inform current evidenced-based treatment decisions generally do not have available materials suitable for gene expression profile-based molecular subtyping.

Subsequent work has shown that although the genetic definitions may vary across classification schemes, most of these systems will classify tumors into categories with similar clinical implications.^{10,11}

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Submitted May 8, 2008; accepted November 10, 2008; published online ahead of print at www.jco.org on February 9, 2009.

Supported by Grants No. P50-CA58223 and U01 CA114722-01 (to C.M.P.).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/09/2799-1/\$20.00

DOI: 10.1200/JCO.2008.18.1024

The primary molecular subdivision is by estrogen receptor (ER) status,^{3,8,12} with further subdivision of ER-positive tumors into luminal A and a worse-prognosis luminal B category. The ER-negative tumors are subdivided into HER2/*neu* (HER2)-positive and a basal-like group, the latter being approximated by a triple negative (ER-negative, progesterone receptor [PR]-negative, HER2-negative) phenotype.¹³ The most significant discriminator in the subset assignment of ER-positive tumors is proliferation.¹⁴⁻¹⁶ Recent examination of 357 invasive breast cancers subtyped by gene expression analysis has

determined and validated an optimal cut point for a Ki67 proliferation index to distinguish luminal B from luminal A (Cheang et al, manuscript submitted for publication), suggesting that a simple immunopanel of ER, PR, HER2, and Ki67 can serve as a proxy for much of the clinically relevant information generated by genetic subtyping.

In this unplanned, subset analysis of the Breast Cancer International Research Group (BCIRG) 001 trial, immunohistochemically defined subsets of patients were examined to test the prognostic and predictive significance of these subsets in a clinical trial setting.

Table 1. Patient Characteristics for the Total Study Population and Biologic Subtypes With Univariate Analysis of Disease-Free Survival in the Total Population

Characteristic	No.	Total HR	P	Triple Negative		HER2		Luminal B		Luminal A	
				No.	%	No.	%	No.	%	No.	%
Age, years											
< 50	711	1.00	—	111	58	53	47	450	56	84	40
50+	639	0.88	.2449	81	42	60	53	360	44	127	60
Menopausal status											
Pre	531	1.00	—	83	43	50	44	293	36	97	46
Post	819	0.90	.3362	109	57	63	56	517	64	114	54
Tumor size, cm											
≤ 2	576	1.00	—	67	35	45	40	347	43	107	51
> 2	774	1.61	< .0001	125	65	68	60	463	57	104	49
Histologic subtype											
Lobular	286	1.00	—	6	3	3	3	183	23	90	43
Ductal other	1064	1.72	.0003	186	97	110	97	627	77	121	57
Overall histologic grade											
1	245	1.00	—	1	1	1	1	131	16	108	52
2	603	1.96	.0007	26	14	37	33	436	54	96	46
3	489	3.54	< .0001	163	86	75	66	236	29	4	2
Vascular invasion											
None/minimal	938	1.00	—	132	70	71	68	557	72	164	85
Extensive	338	1.60	< .0001	56	30	33	32	213	28	29	15
Positive lymph nodes											
1-3	903	1.00	—	129	68	74	66	550	69	131	64
4-9	327	1.81	< .0001	50	26	30	27	189	24	54	26
10+	103	2.79	< .0001	12	6	8	7	62	8	20	10
Estrogen receptor											
Negative	318	1.00	—	192	100	113	100	3	0	3	1
Positive	1021	0.52	< .0001	0	0	0	0	807	100	208	99
Progesterone receptor											
Negative	498	1.00	—	192	100	113	100	156	19	29	14
Positive	841	0.50	< .0001	0	0	0	0	654	81	182	86
HER2*											
Negative	1034	1.00	—	192	100	0	0	626	78	211	100
Positive	288	1.56	.0002	0	0	113	100	175	22	0	0
Ki67-1 index											
Low	242	1.00	—	5	3	5	4	14	2	211	100
High	1099	1.85	.0002	187	97	108	96	796	98	0	0
p53 staining											
Negative	1014	1.00	—	96	50	56	50	650	81	200	95
Positive	320	1.52	.0003	96	50	56	50	155	19	11	5
Treatment											
FAC	663	1.00	—	90	48	57	50	397	49	107	51
TAC	679	0.73	.0027	99	52	56	50	408	51	104	49
Tamoxifen											
No	442	1.00	—	167	87	94	83	139	17	30	14
Yes	908	0.43	< .0001	25	13	19	17	671	83	181	86

Abbreviations: HR, hazard ratio; FAC, fluorouracil, doxorubicin, cyclophosphamide; TAC, docetaxel, doxorubicin, cyclophosphamide.

*HER2 status was determined using fluorescent in situ hybridization (1,264 patients) or CB11 (53 patients for whom fluorescent in situ hybridization results were not available).

METHODS

Patients

BCIRG 001 was a multicenter, prospective, randomized, phase III trial comparing docetaxel, doxorubicin, and cyclophosphamide (TAC) with fluorouracil, doxorubicin, and cyclophosphamide (FAC) as adjuvant chemotherapy in 1,491 women with operable, node-positive breast cancer. A representative tumor sample in the form of formalin-fixed paraffin blocks and/or diagnostic histologic slides was available for 1,350 patients (91%). Other data, including baseline characteristics of the patients and clinical outcomes, were extracted from the BCIRG database used for the second interim analysis of survival (median, 55 months of follow-up), results of which have been previously reported.² All patients provided informed consent. The trial protocol was approved by the research ethics board of the Alberta Cancer Board.

Immunohistochemical and Fluorescent In Situ Hybridization Analysis of Tissue Specimens

Slide review, immunohistochemistry, and fluorescence in situ hybridization (FISH) assays were performed in a central laboratory and read by one pathologist (J.H.). Automated slide processing platforms (Ventana Medical Systems, Tucson, AZ) were used for both immunohistochemical and FISH assays. Representative unstained tumor specimens were stained for ER (clone 6F11, Ventana), PR (clone 636; Dako, Carpinteria, CA), HER2 (clone CB11, Ventana), Ki67 (clone MIB-1, Dako), and p53 (clone 1801; Novocastra Laboratories Ltd, Newcastle upon Tyne, United Kingdom). Immunohistochemical data were recorded as the percentage of positive cells and grade 1 to 3 (greatest) staining intensity. The FISH assay for HER2 was performed according to the manufacturer's instructions (PathVysion; Abbott Molecular Inc, Des Plaines, IL). Tumors were classified as ER- or PR-positive if staining was present in 1% or more of tumor nuclei and p53 positive if more than 10% of nuclei stained. The Ki67 cut point of 13% established by Cheang et al (Cheang et al, manuscript submitted for publication) was used to designate a tumor as high proliferation. For HER2, either intense staining (3+) or a HER2:Cep17 ratio

greater than 2.2 were regarded as positive for immunohistochemical analysis or FISH, respectively.¹⁷

Statistical Analysis

All data generated during central review were recorded in the BCIRG database. A random sample of 500 cases (hematoxylin-eosin and immunohistochemistry samples) were reviewed by Ian Ellis (Nottingham, United Kingdom). For histologic grade and ER status, there was complete agreement in 69% and 85% of cases, with κ values of 0.56 (moderate) and 0.87 (almost perfect), respectively. The level of agreement for histologic grade was consistent with that of published studies.^{18,19} In addition, the concordance rate for the FISH results was 97% (29 of 30) with an external laboratory (Michael Press, Los Angeles, CA) on nonstudy cases.

The occurrence of a DFS event, defined as relapse, second primary malignancy, or death, was the primary outcome and was analyzed for each prognostic variable. Univariate and multivariate analyses were performed using the SAS phreg procedure (SAS Institute, Cary, NC) for the Cox regression model for survival. The SAS lifetest procedure was used to calculate Kaplan-Meier probability estimates for DFS and OS. The SAS logistic procedure was used to obtain significance levels with odds ratios (ORs) and 95% CIs for 3-year DFS rates analysis and histologic tumor type, tumor size, extent of vascular invasion, tumor grade, proliferation index, and p53 by biologic subtype relative to the most common subtype in this study cohort, luminal B.

Univariate Cox regression analysis was performed for each prognostic variable. Subsequently, those variables that were not used in the computation of the biologic subtypes and yielded a univariate result of $P < .10$ were included in the multivariate model. Multivariate Cox regression analysis was performed using the stepwise backward elimination method with model removal set at $P > .10$. The multivariate model included as primary prognostic variables biologic subtype and chemoendocrine treatment (TAC, FAC, and tamoxifen), with interaction terms for tamoxifen treatment and biologic subtype. Tumor grade, primary tumor size, extent of vascular invasion, and number of positive lymph nodes were added as covariables.

The results are presented in accordance with reporting recommendations for tumor marker prognostic studies criteria.²⁰

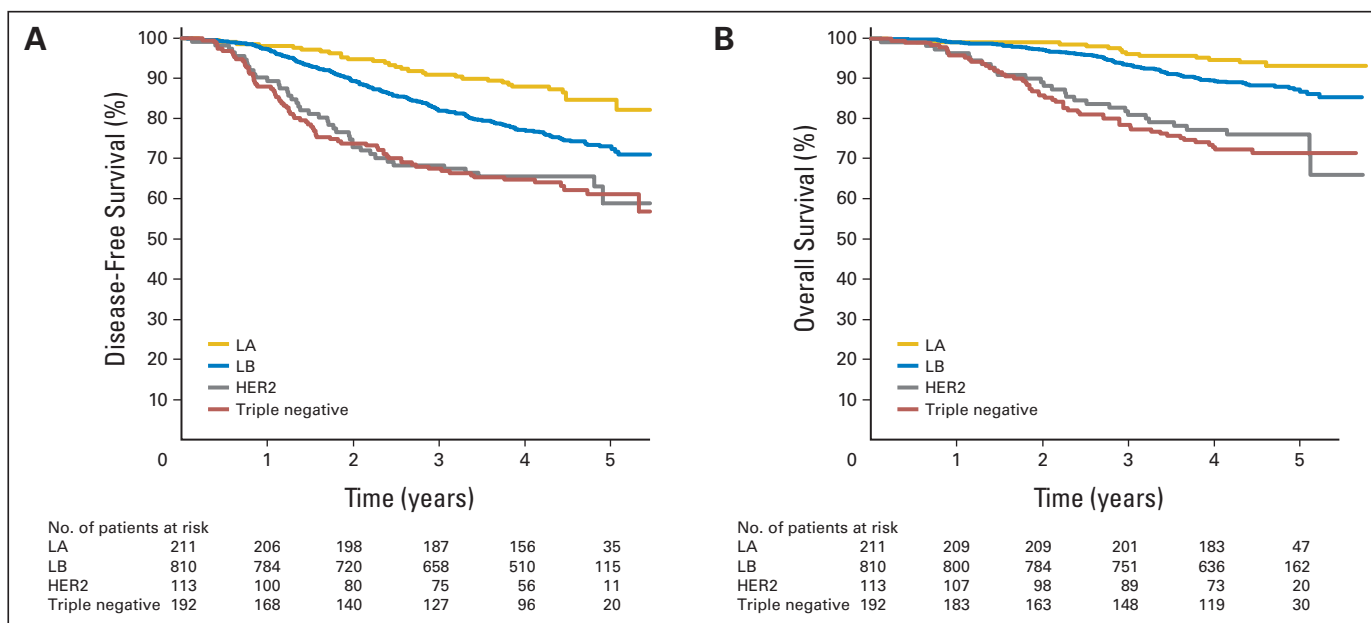


Fig 1. Disease-free and overall survival among patients according to biologic subtype. (A) Disease-free survival and (B) overall survival for patients stratified into triple-negative (estrogen receptor [ER] negative, progesterone receptor [PR] negative, HER2/*neu* [HER2] negative), HER2 (HER2-positive, ER-negative, PR-negative), luminal B (LB; ER-positive or PR-positive and either HER2-positive or Ki67^{high}) and luminal A (LA; ER-positive or PR-positive and not HER2-positive or Ki67^{high}) groups. Pairwise comparisons between subtypes were statistically significant (see text).

Table 2. Stepwise Multivariate Analysis of Disease-Free Survival for All Cases and for Luminal A and Luminal B

Characteristic	Hazard Ratio	95% CI	P
All cases			
TAC treatment	0.73	0.58 to 0.91	.0051
Tamoxifen treatment	0.41	0.31 to 0.56	< .0001
Tamoxifen* triple negative	4.01	2.00 to 8.05	< .0001
Tamoxifen* HER2	2.56	0.95 to 6.93	.0633
Tamoxifen* luminal A	0.56	0.34 to 0.93	.0257
Triple negative	0.62	0.43 to 0.90	.0124
HER2	0.65	0.42 to 1.02	.0602
Tumor size > 2 cm	1.33	1.05 to 1.69	.0178
Histologic subtype	1.39	0.99 to 1.96	.0612
Grade 3	1.39	1.07 to 1.80	.0137
Vascular invasion	1.34	1.06 to 1.69	.0141
4-9 positive nodes	1.87	1.46 to 2.40	< .0001
10+ positive nodes	3.09	2.23 to 4.27	< .0001
Luminal B			
TAC treatment	0.71	0.53 to 0.95	.022
Tamoxifen treatment	0.44	0.32 to 0.61	< .0001
Tumor size > 2 cm	1.49	1.10 to 2.02	.011
Histologic subtype	1.62	1.06 to 2.49	.027
1-3 positive nodes	1.98	1.46 to 2.67	< .0001
4-9 positive nodes	1.61	1.15 to 2.26	.005
10+ positive nodes	4.33	2.93 to 6.41	< .0001
Luminal A			
TAC treatment	0.97	0.46 to 2.06	.943
Tamoxifen treatment	0.15	0.07 to 0.33	< .0001
4-9 positive nodes	4.30	1.81 to 10.20	.001
10+ positive nodes	5.11	1.82 to 14.37	.002

NOTE. Variables involved in the definition of biologic subtypes were not included in this model.
Abbreviation: TAC, docetaxel, doxorubicin, cyclophosphamide.

RESULTS

The frequency of the clinicopathologic variables in the total population are presented in Table 1. On univariate analysis, a significant prognostic effect was detected for tumor size; histologic subtype; tumor grade; extent of vascular invasion; nodal involvement; hormone receptor status; expression of HER2, Ki67, and p53; chemotherapy;

and tamoxifen treatment, with increases in hazard ratios (HRs) for recurrence associated with increases in tumor size and number of positive nodes. No statistically significant prognostic effect was found for age or menopausal status.

We were able to classify the study population into four breast cancer subtypes (Table 1): triple negative (ER-negative, PR-negative, HER2-negative), HER2 (ER-negative, PR-negative, HER2-positive), luminal B (ER-positive and/or PR-positive and either HER2-positive and/or Ki67^{high}), and luminal A (ER-positive and/or PR-positive and not HER2-positive or Ki67^{high}). These subtypes accounted for 14.5%, 8.5%, 61.1%, and 15.9%, respectively. Twenty-four (1.8%) were unassigned, primarily because of missing HER2 values owing to insufficient tumor material or nonformalin fixation.

The biologic subtypes were prognostically significant for DFS and OS (Fig 1). The 3-year DFS rates, HR, and P values for pairwise comparison with luminal B were 67% (HR = 2.22; 95% CI, 1.56 to 3.16; P < .0001) for triple negative, 68% (HR = 2.12; 95% CI, 1.37 to 3.29; P = .0008) for HER2, 82% (referent) for luminal B, and 91% (HR = 0.46; 95% CI, 0.28 to 0.77; P = .0027) for luminal A. The triple-negative and HER2 groups had a similarly poor prognosis (P = .788), as has been described.¹⁴

A multivariate analysis, which excluded variables defining the subtypes (ER, PR, HER2, Ki67), identified independent significance for treatment with TAC/FAC, treatment with tamoxifen, size more than 2 cm, grade 3, extensive vascular invasion, number of involved lymph nodes, treatment, and biologic subtypes (Table 2).

ORs for the association of biologic subtype with prognostic tumor characteristics are shown in Table 3, with the most common subtype, luminal B, as the referent group. Compared with luminal B, the triple-negative and HER2 subtypes were more likely to have a ductal histogenesis (OR = 9.2; 95% CI, 4.00 to 21.04; P < .0001; and OR = 10.6, 95% CI, 3.33 to 33.85; P < .0001, respectively), whereas luminal A cases were more likely to have nonductal disease (eg, lobular or mixed ductal-lobular histogenesis, OR = 0.4; 95% CI, 0.27 to 0.52; P < .0001). Patients with the luminal A subtype were less likely to have tumors larger than 2 cm (OR = 0.7; 95% CI, 0.54 to 0.99; P < .05), extensive vascular invasion (OR = 0.5; 95% CI, 0.30 to 0.71; P = .0004), grade 3 (OR = 0.05; 95% CI, 0.02 to 0.13; P < .0001), or p53 positivity (OR = 0.2; 95% CI, 0.12 to 0.43; P < .0001). p53 positivity was more frequent in the triple-negative (OR = 4.19; 95% CI, 3.01 to 5.85; P < .0001) and HER2 (OR = 4.19; 95% CI, 2.78 to

Table 3. Odds Ratios for Patient and Tumor Characteristics by Biologic Subtype

Variable Category	Triple Negative		HER2		Luminal B (referent)	Luminal A	
	OR	P	OR	P		OR	P
N1 v N2							
Type, ductal v lobular*	9.17	< .0001	10.61	< .0001	1.0	0.38	< .0001
Size, > 2 v ≤ 2 cm	1.40	.0450	1.13	.5436	1.0	0.73	.0408
Grade, 1 v 2	0.13	.0447	0.09	.018	1.0	3.74	< .0001
Grade, 3 v 1 + 2	14.50	< .0001	4.74	< .0001	1.0	0.05	< .0001
Vascular invasion, positive v negative	1.11	.5612	1.22	.3871	1.0	0.46	.0004
MIB-1 index, high v low	1.52	.4270	2.63	.0683	1.0	NA	
p53, positive v negative	4.19	< .0001	4.19	< .0001	1.0	0.23	< .0001
PR, positive v negative	NA		NA		1.0	1.50	.0655

Abbreviations: OR, odds ratio; NA, not applicable; PR, progesterone receptor.
*Includes lobular mixed and pure lobular carcinomas.

6.32; $P < .0001$) groups, compared with luminal B. Patients with triple-negative tumors had the greatest likelihood of having grade 3 tumors (OR = 14.5; 95% CI, 9.39 to 22.40; $P < .0001$).

Compared with patients in the luminal B group, the hazard ratio for a DFS event in patients in the luminal A group was significantly decreased for tumor size more than 2 cm (HR = 0.47; 95% CI, 0.28 to 0.79; $P = .0042$), moderate (HR = 0.46; 95% CI, 0.23 to 0.91; $P = .027$) and high (HR = 0.54; 95% CI, 0.33 to 0.87; $P = .012$) levels of ER expression, as well as in the subgroup with one to three positive nodes (HR = 0.37; 95% CI, 0.19 to 0.70; $P = .002$).

Nineteen percent and 14% of patients in the luminal B and luminal A groups, respectively, were negative for PR. A multivariate analysis controlling for the presence of PR showed a persistent significant difference between luminal A and luminal B (HR = 0.46; 95% CI, 0.29 to 0.74; $P = .0014$), excluding differences in PR as the basis for differences between the subtypes in patients treated with tamoxifen.

Patients in the luminal B group showed a statistically significant improvement in DFS favoring the taxane arm, with 3-year DFS of 85.2% versus 79% (HR = 0.66; 95% CI, 0.46 to 0.95; $P = .025$) for TAC and FAC, respectively (Fig 2). There was marginal significance in the triple-negative and HER2 subtypes, with 3-year DFS of 73.5% and 60.0% (HR = 0.50; 95% CI, 0.29 to 1.00; $P = .051$) and 60.3% and 60.3% (HR = 0.46; 95% CI, 0.20 to 1.06; $P = .068$) for TAC and FAC in the respective subtypes. There was no difference between chemotherapy regimens in patients in the luminal A group: 92.2% and 89.3%, respectively (HR = 0.70; 95% CI, 0.27 to 1.83; $P = .472$).

Although the trial protocol specified adjuvant tamoxifen after chemotherapy for all ER-positive patients, 5% did not receive tamoxifen for a variety of reasons and an additional 11% of patients had a negative agreement between the central laboratory and the originating laboratory, such that 17% of patients in the luminal B and 14% of patients in the luminal A groups were not treated with tamoxifen. This

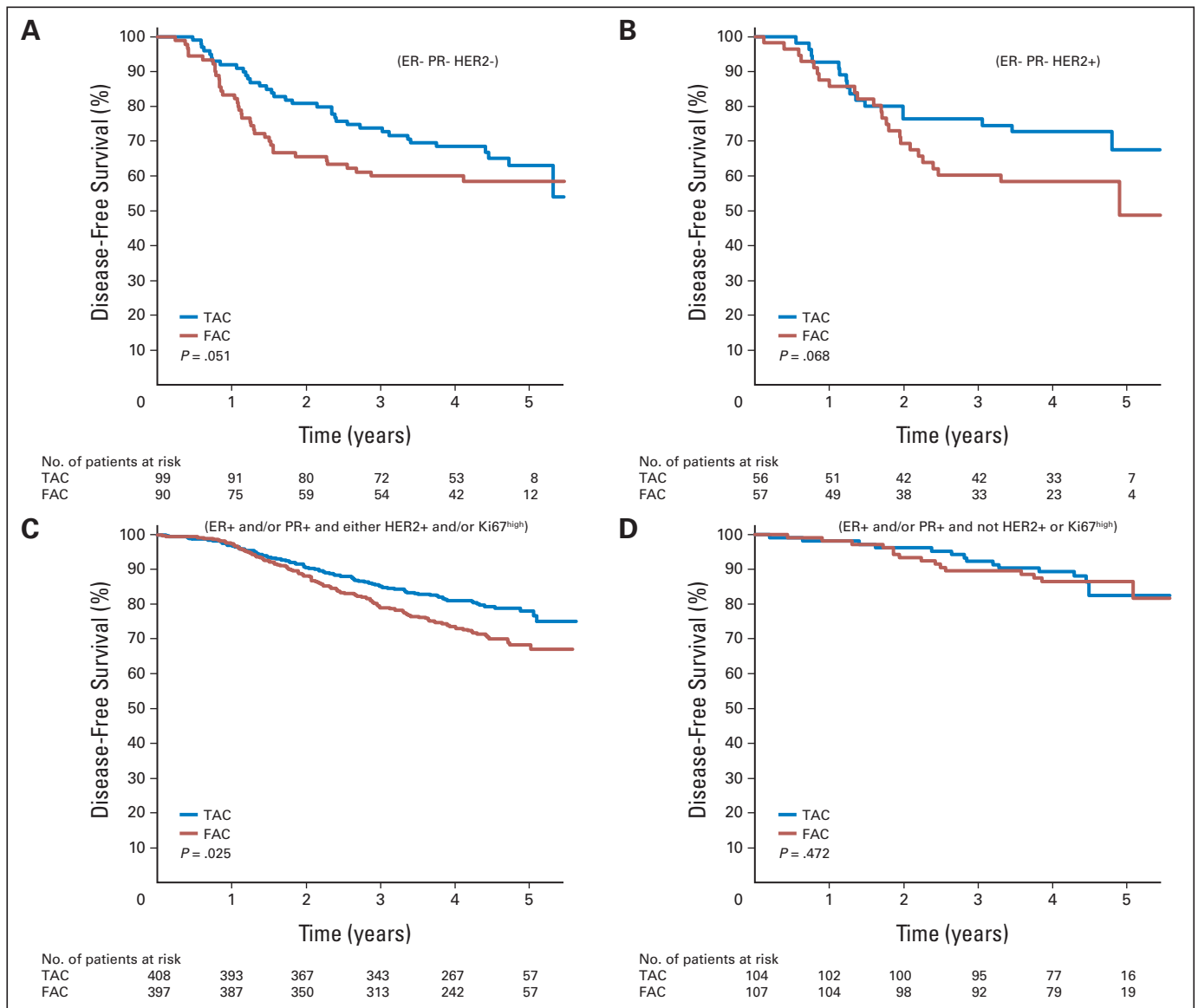


Fig 2. Disease-free survival (DFS) among patients treated with fluorouracil, doxorubicin, and cyclophosphamide (FAC) or docetaxel, doxorubicin, and cyclophosphamide (TAC) according to biologic subtype. Disease-free survival is shown in patients classified as (A) triple negative, (B) HER2, (C) luminal B, or (D) luminal A treated with FAC or TAC. P values in each panel are logistic regression calculations based on 3-year DFS. ER, estrogen receptor; PR, progesterone receptor.

magnitude of negative agreement is similar to that reported in the Eastern Cooperative Oncology 2197 trial²¹ and is a well-described phenomenon in interlaboratory testing.^{22,23} When tamoxifen treatment was entered into the multivariate Cox and DFS model, significant interactions were found, with biologic subtype indicating that subgroup analysis was appropriate. There were few patients treated with tamoxifen in the triple-negative (13%) and HER2 (17%) subtypes, and there was no significant effect of tamoxifen treatment in these groups ($P > .3$), so the interaction in these groups is not considered further. The multivariate analysis for luminal A and luminal B subtypes is presented in Table 2. Both the luminal A and luminal B subtypes showed significant responses to tamoxifen, with HR = 0.44 (95% CI, 0.32 to 0.61; $P < .0001$) for luminal B and HR = 0.15 (95% CI, 0.07 to 0.33; $P < .0001$) for luminal A. As in the unadjusted comparisons, when patients were treated with tamoxifen, TAC was superior to FAC in patients in the luminal B group, with a 3-year DFS of 89.4% and 82%, respectively (HR = 0.71; 95% CI, 0.53 to 0.95; $P = .02$, multivariate model) and not in patients in the luminal A group, with a 3-year DFS of 93% and 96.6% (HR = 0.97; 95% CI, 0.46 to 2.06; $P = .90$, multivariate model; Fig 3).

We performed an exploratory analysis similar to a recently published²⁴ examination of the response of ER-positive/HER2-positive and ER-positive/HER2-negative patients to adjuvant taxane chemotherapy. As in that study, log-rank P values are provided as a measure of discordance and should be viewed as descriptive, not inferential. We found that TAC was associated with improved DFS among the ER-positive/HER2-positive patients (Fig 4A), with similar trends seen in both the tamoxifen-treated and untreated patients (Fig 4B). Contrary to that study, in the ER-positive/HER2-negative patients who had received tamoxifen, there was a benefit to the taxane-containing chem-

otherapy arm (Fig 4D). This advantage was not seen in those patients who did not receive tamoxifen (Fig 4D), nor in the unsegregated population (Fig 4C).

DISCUSSION

It is now accepted that breast cancer is heterogeneous at a molecular level.⁴ There is increasing evidence that these molecular subtypes differ in their response to therapeutic agents.^{14,16,25} Our results build on these findings and indicate that patients with ER-negative tumors (both triple-negative and HER2) show a better response to TAC than to FAC, with the difference in response to the taxane regimen being marginally statistically significant. Despite this response, these subtypes have a worse DFS and OS, with the majority of events occurring early. This is consistent with neoadjuvant reports that document increased chemosensitivity in these subtypes, but poor outcome as a result of higher and faster relapse among those with residual disease.^{16,25} It is expected that if trastuzumab had been included in this early protocol, the 3-year recurrence risk would have been halved in the HER2 population.²⁶

The triple-negative phenotype used in this study only approximates the basal-like subtype,²⁷ although the profile of the DFS^{14,27} and phenotypic characteristics (eg, predominantly ductal [97%], grade 3 [86%], p53 abnormal [50%] with a high proliferation index [97%]) are in keeping with characteristics of the basal subtype.^{28,29} The basal-like subtype itself is heterogeneous and encompasses the majority of *BRCA1*-related carcinomas, medullary carcinomas, and metaplastic carcinomas¹³ and is overrepresented in the aggressive tumors seen in premenopausal African Americans.^{30,31} Although this heterogeneity can theoretically alter chemosensitivity,³¹ we were not able to further define subgroups within this subtype to investigate that possibility.

The use of a simple proliferation index results in a highly effective separation of ER-positive patients into two intrinsically different populations, luminal A and luminal B (Cheang et al, manuscript submitted for publication).^{11,14-16,32} These subtypes have a different outcome when compared across tumor size, nodal status, and level of estrogen or progesterone receptor positivity. This underscores the importance of proliferation and supports suggestions that incorporation of a proliferation score into therapy decisions may complement¹¹ or even supplant^{16,33} histologic grade. Assignment of biologic subtype without consideration of histologic type resulted in significant separation, with the triple-negative and HER2 groups being almost exclusively of ductal origin. Approximately one half of the luminal A subtype consisted of lobular and mixed ductal-lobular carcinomas, consistent with evidence supporting a close genetic relationship between low-grade ductal carcinoma and lobular carcinomas.^{34,35}

In patients treated with tamoxifen, patients in the luminal B but not luminal A group show a significant benefit of TAC over FAC chemotherapy. The benefit of taxanes in hormone receptor-positive tumors is controversial.³⁶ Analysis of first-generation taxane trials in adjuvant therapy including the Grupo Espanol de Investigacion del Cancer de Mama 9906³⁷ and a pooled analysis of this present trial and PACS01³⁸ found no statistically significant interaction between hormone receptor status and taxane response, with benefit in both the ER-positive and ER-negative patients. However, subset analyses of two other taxane trials, the E2197³⁹ and the Cancer and Leukemia Group B (CALGB),^{24,40} as well as neoadjuvant data,^{16,41} suggest that

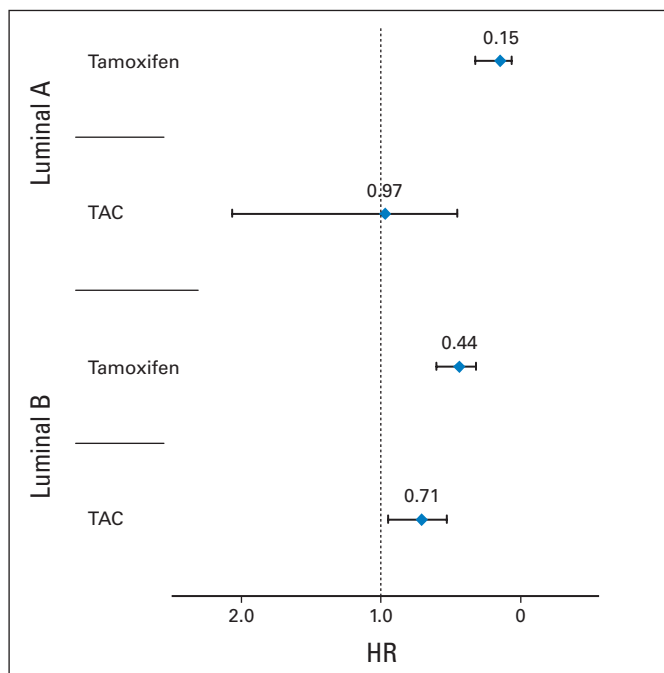


Fig 3. Forest plot of covariate adjusted hazard ratios (HR) for disease-free survival in patients in the luminal A and luminal B groups by treatment (hazard ratios and 95% CIs using fluorouracil, doxorubicin, and cyclophosphamide and no tamoxifen as baseline treatments). Values less than 1 show an advantage to docetaxel, doxorubicin, and cyclophosphamide (TAC) or tamoxifen.

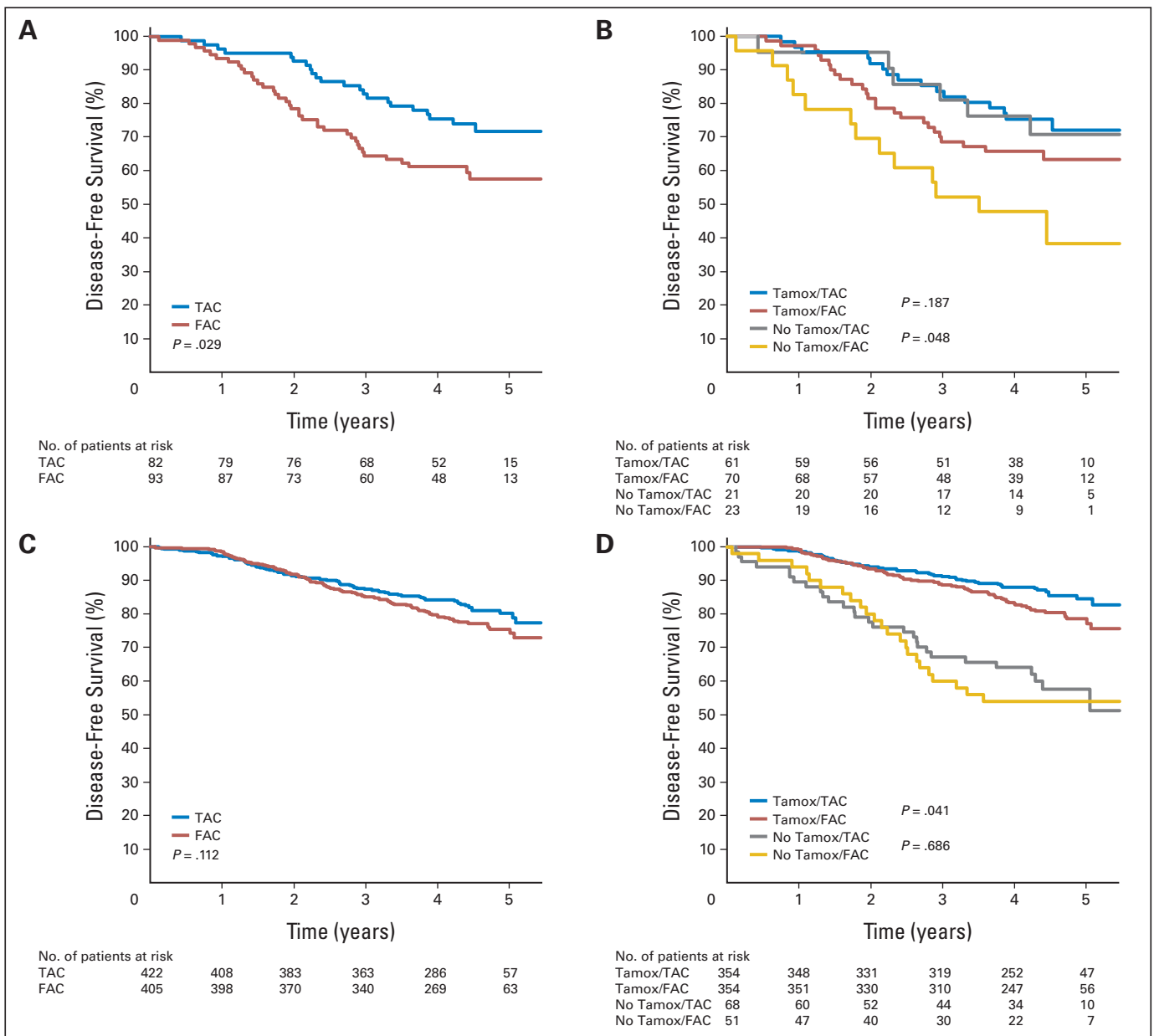


Fig 4. Disease-free survival among estrogen receptor positive patients treated with fluorouracil, doxorubicin, and cyclophosphamide (FAC) or docetaxel, doxorubicin, and cyclophosphamide (TAC) according to HER2 status and tamoxifen (Tamox) treatment. Disease-free survival for estrogen receptor (central lab)-positive patients, (A) positive or (C) negative for HER2, by treatment arm. (B, D) Subdivision of these patients by tamoxifen treatment. Log-rank P values are descriptive only.

the response to taxanes is less in ER-positive compared with ER-negative tumors. Our results show that the benefit of the addition of taxanes to tamoxifen is restricted to the luminal B population, and because this group comprises the majority of ER-positive patients in this study, this benefit is probably responsible for the positive effect of TAC in the tamoxifen-treated ER-positive/HER2-negative (Fig 4D) and in the ungrouped ER-positive patients.³⁸ The patients in the luminal A group show no benefit to docetaxel when added to tamoxifen (Fig 3), a finding that is not surprising given that taxanes' stabilization of microtubules and mitotic arrest⁴² would be expected to preferentially target rapidly dividing cells. Of note, subset analysis of the PACS 01 trial, presented in abstract form, also found that a "luminal" subtype, defined as ER-positive and

basal-like subtype parameters-negative, showed no difference in response to the addition of docetaxel.⁴³

These findings parallel the experience in node-negative patients. Currently, large cooperative trials in North America (Trial Assigning Individualized Options for Treatment)⁴⁴ and Europe (Microarray in Node-Negative Disease May Avoid Chemotherapy⁴⁵ and Node Negative Breast Cancer⁴⁶) are studying the integration of genomic or biochemical profiles into decisions concerning the necessity of adding chemotherapy to hormonal therapy in selected groups of patients with hormone receptor-positive disease.

In the ER-positive/HER2-negative patients treated with tamoxifen, there was a significant benefit to the taxane regimen (Fig 4D). This differs from the analysis of a similar subset in the CALGB 9344/

INTO148 trial of patients with node-positive breast cancer treated with doxorubicin plus cyclophosphamide, followed by paclitaxel or observation.²⁴ In that report, there was no benefit to the addition of paclitaxel in a similar group of patients. Cross-trial comparisons are difficult, but possible reasons for this difference include superior performance of docetaxel to the paclitaxel dosing protocol used in that trial,^{47,48} or alternatively, there may have been a greater admixture of patients with low-proliferative luminal A disease in the CALGB subset, thus diluting the effect of the taxane in the unsegregated ER-positive population.

The high risk of recurrence in ER-positive tumors not treated with tamoxifen is similar to that reported in the subset analysis by Berry et al⁴⁰ and is in keeping with the meta-analysis results¹ showing that the major benefit in adjuvant therapy in the ER-positive tumors is due to tamoxifen. This suggests that the prognostic effect seen with molecular subdivision is due in large part to its ability to predict response to treatments optimized to each subtype, rather than differing metastatic potential between the subtypes. In this regard, our results show that TAC significantly complements endocrine therapy in tamoxifen-treated patients in the luminal B group, analogous to the improved outcomes in HER2-positive patients treated with doxorubicin and cyclophosphamide followed by docetaxel with trastuzumab. In the absence of specific targeted therapy, TAC is also effective in the triple-negative population. Because this is a retrospective, unplanned subset analysis without adjustment for multiple comparisons, these findings, although consistent with existing literature, should be regarded as hypothesis generating.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those

relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Emmanuelle Magherini, Sanofi-aventis (C) **Consultant or Advisory Role:** Judith Hugh, Sanofi-Aventis (U); John Mackey, Sanofi-Aventis (C); Miguel Martin, Sanofi-Aventis (C) **Stock Ownership:** Charles M. Perou, University Genomics; Emmanuelle Magherini, Sanofi-Aventis **Honoraria:** John Mackey, Sanofi-Aventis; Miguel Martin, Sanofi-Aventis; Charles Vogel, Sanofi-Aventis **Research Funding:** Judith Hugh, Aventis; Torsten O. Nielsen, Sanofi-Aventis; Charles Dumontet, Aventis; John Reed, Aventis; Charles Vogel, Sanofi-Aventis **Expert Testimony:** None **Other Remuneration:** None

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REFERENCES

- Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. *Lancet* 365:1687-1717, 2005
- Martin M, Pienkowski T, Mackey J, et al: Adjuvant docetaxel for node-positive breast cancer. *N Engl J Med* 352:2302-2313, 2005
- Perou CM, Sorlie T, Eisen MB, et al: Molecular portraits of human breast tumours. *Nature* 406:747-752, 2000
- Dinh P, Sotiriou C, Piccart MJ: The evolution of treatment strategies: Aiming at the target. *Breast* 16:S10-S16, 2007 (suppl 2)
- Chang HY, Nuyten DS, Sneddon JB, et al: Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. *Proc Natl Acad Sci U S A* 102:3738-3743, 2005
- Paik S, Shak S, Tang G, et al: A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 351:2817-2826, 2004
- Sorlie T, Tibshirani R, Parker J, et al: Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A* 100:8418-8423, 2003
- Sotiriou C, Neo SY, McShane LM, et al: Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci U S A* 100:10393-10398, 2003
- van 't Veer LJ, Dai H, van de Vijver MJ, et al: Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 415:530-536, 2002
- Fan C, Oh DS, Wessels L, et al: Concordance among gene-expression-based predictors for breast cancer. *N Engl J Med* 355:560-569, 2006
- Sotiriou C, Wirapati P, Loi S, et al: Gene expression profiling in breast cancer: Understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 98:262-272, 2006
- Gruvberger S, Ringner M, Chen Y, et al: Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. *Cancer Res* 61:5979-5984, 2001
- Kreike B, van Kouwenhove M, Horlings H, et al: Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. *Breast Cancer Res* 9:R65, 2007
- Desmedt C, Haihe-Kains B, Wirapati P, et al: Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. *Clin Cancer Res* 14:5158-5165, 2008
- Hu Z, Fan C, Oh DS, et al: The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics* 7:96, 2006
- Rouzier R, Perou CM, Symmans WF, et al: Breast cancer molecular subtypes respond differently to preoperative chemotherapy. *Clin Cancer Res* 11:5678-5685, 2005
- Wolff AC, Hammond ME, Schwartz JN, et al: American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *Arch Pathol Lab Med* 131:18, 2007
- Dalton LW, Page DL, Dupont WD: Histologic grading of breast carcinoma: A reproducibility study. *Cancer* 73:2765-2770, 1994
- Frierson HF Jr, Wolber RA, Berean KW, et al: Interobserver reproducibility of the Nottingham modification of the Bloom and Richardson histologic grading scheme for infiltrating ductal carcinoma. *Am J Clin Pathol* 103:195-198, 1995
- McShane LM, Altman DG, Sauerbrei W, et al: Reporting recommendations for tumor marker prognostic studies. *J Clin Oncol* 23:9067-9072, 2005
- Badve SS, Baehner FL, Gray RP, et al: Estrogen- and progesterone-receptor status in ECOG 2197: comparison of immunohistochemistry by local and central

laboratories and quantitative reverse transcription polymerase chain reaction by central laboratory. *J Clin Oncol* 26:2473-2481, 2008

22. Francis GD, Dimech M, Giles L, et al: Frequency and reliability of oestrogen receptor, progesterone receptor and HER2 in breast carcinoma determined by immunohistochemistry in Australasia: Results of the RCPA Quality Assurance Program. *J Clin Pathol* 60:1277-1283, 2007

23. Rhodes A, Jasani B, Barnes DM, et al: Reliability of immunohistochemical demonstration of oestrogen receptors in routine practice: Interlaboratory variance in the sensitivity of detection and evaluation of scoring systems. *J Clin Pathol* 53:125-130, 2000

24. Hayes DF, Thor AD, Dressler LG, et al: HER2 and response to paclitaxel in node-positive breast cancer. *N Engl J Med* 357:1496-1506, 2007

25. Carey LA, Dees EC, Sawyer L, et al: The triple negative paradox: Primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res* 13:2329-2334, 2007

26. Baselga J, Perez EA, Pienkowski T, et al: Adjuvant trastuzumab: A milestone in the treatment of HER-2-positive early breast cancer. *Oncologist* 11:4-12, 2006 (suppl 1)

27. Cheang MC, Voduc D, Bajdik C, et al: Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 14:1368-1376, 2008

28. Jones C, Ford E, Gillett C, et al: Molecular cytogenetic identification of subgroups of grade III invasive ductal breast carcinomas with different clinical outcomes. *Clin Cancer Res* 10:5988-5997, 2004

29. Livasy CA, Karaca G, Nanda R, et al: Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol* 19:264-271, 2006

30. Carey LA, Perou CM, Livasy CA, et al: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 295:2492-2502, 2006

31. Di Leo A, Claudino WM, Pestrin M, et al: Using specific cytotoxics with a targeted mind. *Breast* 16:S120-S126, 2007 (suppl 2)

32. Haibe-Kains B, Desmedt C, Sotiriou C, et al: A comparative study of survival models for breast cancer prognostication based on microarray data: Does a single gene beat them all? *Bioinformatics* 24:2200-2208, 2008

33. Meyer JS, Alvarez C, Milikowski C, et al: Breast carcinoma malignancy grading by Bloom-Richardson system vs proliferation index: Reproducibility of grade and advantages of proliferation index. *Mod Pathol* 18:1067-1078, 2005

34. Simpson PT, Reis-Filho JS, Gale T, et al: Molecular evolution of breast cancer. *J Pathol* 205:248-254, 2005

35. Buerger H, Simon R, Schafer KL, et al: Genetic relation of lobular carcinoma in situ, ductal carcinoma in situ, and associated invasive carcinoma of the breast. *Mol Pathol* 53:118-121, 2000

36. Martin M, Mackey J, Vogel C: Benefit from adjuvant taxanes and endocrine responsiveness in breast cancer. *Breast* 16:S127-S131, 2007 (suppl 2)

37. Martín M, Rodríguez-Lescure A, Ruiz A, et al: Randomized phase 3 trial of fluorouracil, epirubicin, and cyclophosphamide alone or followed by paclitaxel for early breast cancer. *J Natl Cancer Inst* 100:805-814, 2008

38. Andre F, Broglio K, Roche H, et al: Estrogen receptor expression and efficacy of docetaxel-containing adjuvant chemotherapy in patients with node-positive breast cancer: Results from a pooled analysis. *J Clin Oncol* 26:2636-2643, 2008

39. Goldstein LJ, O'Neill A, Sparano JA, et al: Concurrent doxorubicin plus docetaxel is not more effective than concurrent doxorubicin plus cyclophosphamide in operable breast cancer with 0 to 3 positive axillary nodes: North American Breast Cancer Intergroup Trial E 2197. *J Clin Oncol* 26:4092-4099, 2008

40. Berry DA, Cirincione C, Henderson IC, et al: Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. *JAMA* 295:1658-1667, 2006

41. von Minckwitz G, Sinn HP, Raab G, et al: Clinical response after two cycles compared to HER2, Ki-67, p53, and bcl-2 in independently predicting a pathological complete response after preoperative chemotherapy in patients with operable carcinoma of the breast. *Breast Cancer Res* 10:R30, 2008

42. Miller ML, Ojima I: Chemistry and chemical biology of taxane anticancer agents. *Chem Rec* 1:195-211, 2001

43. Jacquemier J, Penault-Llorca F, Mnif H, et al: Identification of a basal-like subtype and comparative effect of epirubicin-based chemotherapy and sequential epirubicin followed by docetaxel chemotherapy in the PACS 01 breast cancer trial: 33 markers studied on tissue-microarrays (TMA). *J Clin Oncol* 24:5s, 2006 (suppl; abstr 509)

44. Sparano JA, Paik S: Development of the 21-gene assay and its application in clinical practice and clinical trials. *J Clin Oncol* 26:721-728, 2008

45. Cardoso F, Van't Veer L, Rutgers E, et al: Clinical application of the 70-gene profile: The MINDACT trial. *J Clin Oncol* 26:729-735, 2008

46. Thomssen C, Vetter M, Guerts A, et al: Is tumor-biological risk-assessment realistic in node-negative breast cancer? A status report of the ongoing clinical multicenter trial NNBC 3-Europe: SABCS 2006. *Breast Cancer Res Treat* 100:S114, 2006 (abstr 2100; suppl 1)

47. Jones SE, Erban J, Overmoyer B, et al: Randomized phase III study of docetaxel compared with paclitaxel in metastatic breast cancer. *J Clin Oncol* 23:5542-5551, 2005

48. Hudis C, McArthur H, Dang C: Current status of the taxanes as adjuvant therapy for breast cancer. *Breast* 16:S132-S135, 2007 (suppl 2)

Acknowledgment

We thank the patients and pathologists who shared their material and made this study possible; and Todd Chaba, MD, and Blake Gilks, MD, for their support and guidance.