5 Minute Journal Club

POST-SABCS Issue 3, 2013

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CME Information

LEARNING OBJECTIVES

- Evaluate the EndoPredict® signature together with a predefined combination of clinicopathologic factors and molecular data as a predictor of late metastases for patients with estrogen receptor-positive and HER2-negative breast cancer.
- Assess the utility of the Onco*type* DX® Recurrence Score® in predicting benefit from the addition of paclitaxel to adjuvant doxorubicin/ cyclophosphamide for patients with lymph node-negative and estrogen receptor-positive breast cancer treated concurrently with endocrine therapy.
- Compare the performance of the Breast Cancer Index℠ biomarker to that of the Onco*type* DX Recurrence Score and IHC4 score as prognostic factors for distant recurrence of hormone receptor-positive, lymph node-negative primary breast cancer.
- Determine the impact of metabolic syndrome on breast cancer recurrence for patients with high-, intermediate- or low-risk disease as defined by the 21-gene Onco*type* DX Recurrence Score assay.
- Appraise the reproducibility of IHC-based Ki-67 biomarker assays and ongoing strategies to increase concordance in analysis and scoring methods.

CME Information (Continued)

CREDIT DESIGNATION STATEMENT

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FACULTY DISCLOSURES

The following faculty (and their spouses/partners) reported real or apparent conflicts of interest, which have been resolved through a conflict of interest resolution process:

CME Information (Continued)

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5 MinuteJournalClub

POST-SABCS Issue 3, 2013

Adjuvant chemotherapy for patients with node-positive luminal A breast cancer

The December San Antonio Breast Cancer Symposium (SABCS) once again featured a bounty of papers focused on tissue predictors of response to systemic agents, and although none will shake up clinical practice like Dr Soon Paik's legendary 2004 SABCS presentation documenting the predictive value of the 21-gene Recurrence Score® (RS) in tumor samples from patients on the NSABP-B-20 trial of tamoxifen alone or with chemotherapy (CT), on a macro level these translational and clinical findings contribute significantly to our knowledge base and help further divide this disease into specific biologic subsets. However, other than being an outlet for new research data, SABCS is also an exceptional educational event where fascinating sessions often provide new perspectives on patient care.

To that end, the spectacular clinical science symposium on the first day of the conference included a thoughtful and thought-provoking overview by Dr Kathy Albain on the critical and controversial issue of adjuvant treatment for patients with the common luminal A phenotype, defined by Dr Charles Perou and others as having high ER and normal HER2 levels and relatively low proliferation.

Dr Albain noted that last year's international meta-analysis demonstrated an overall benefit of CT in ER+ tumors, but the emergence of contemporary assays like the RS has now identified patients with ER+ tumors who are less likely to benefit from CT.

A critical issue in this regard is the patient with a node+ tumor, and to explore this Dr Albain took a unique tack by directly comparing the results of her work (presented in SABCS 2010) evaluating the RS in available tissue from the SWOG-8814 node+ study to the findings from Paik's initial evaluation of the assay in node-negative tumors from B-20. Interestingly, there was a remarkably similar correlation between CT benefit and RS in B-20 and S8814. However, more than 2 years later controversy still surrounds the issue. Dr Albain referred to a 2012 ***JCO* editorial** by Dr Dan Hayes in which he enthusiastically supported enrolling eligible patients on the ongoing RxPONDER node+ trial but vehemently objected to withholding CT (and the potential to improve the chance of remaining disease free) in such individuals outside a study setting. As is often the case with education sessions of this type, Dr Albain did not provide a definitive recommendation about the use of tissue predictors in node+ disease, but at **our CME symposium that evening** a number of the faculty members, including Drs Hal Burstein, Kim Blackwell, George Sledge and Cliff Hudis, noted that they will selectively obtain a RS in patients with node+, ER+, HER2-negative tumors and a low nodal burden.

After hearing Dr Albain's talk, I invited her to participate in an audio interview, during which she was particularly enthused about the next generation of prospective trials, including TAILORx and RxPONDER (RS in node-negative and node+ settings) and MINDACT and I-SPY 1 (70-gene signature in the adjuvant

and neoadjuvant settings), which have the potential to drastically shift how predictive assays are employed in clinical practice. However, until these trials begin to report, oncologists must make these difficult decisions with a less than optimal evidence base and keep abreast of incremental steps forward. In that regard, here's the bottom line on the most recent crop of related SABCS data sets.

1. More on RS in node+ tumors

A prominent SABCS paper focused on another retrospective/prospective analysis of tissue in patients with ER+ tumors in a large Phase III trial (NSABP-B-28, evaluating AC alone or with paclitaxel in patients with node+ disease) and provided additional evidence that RS can predict outcome in this population. Interestingly, the incremental gain from paclitaxel was not correlated with RS, but the analysis was underpowered to make that determination.

2. Molecular profiles to predict risk of delayed recurrence (DR) in patients completing 5 years of adjuvant endocrine treatment

The SABCS presentation of the ATLAS trial of 5 versus 10 years of tamoxifen was yet another data set demonstrating the critical role of DR in ER+ tumors, and 2 early but encouraging papers reported on novel RT-PCR assays to identify patients at particular risk for these events. The first looked at the **"EndoPredict® Score"** in tissue from 2 major Austrian trials, and the second examined the **"Breast Cancer Index"** versus RS and IHC4 in 665 primary tumor samples from the TransATAC tissue bank. Although not definitive, it appears that these or other similar assays may one day be able to provide important input on the critical clinical decision of extending endocrine treatment to 10 years or more while also yielding clues about specific genes correlated with the almost mysterious syndrome of DR, particularly in luminal A tumors.

3. Another dagger in the heart of Ki-67

Show this abstract to your friendly local pathologist the next time he or she offers a home brew that can save you the cost of a RS. After reviewing these scary numbers on lack of Ki-67 reproducibility among pathologists, unless perhaps Dr Mitch Dowsett or Dr Matt Ellis is doing your assay, you may want to rethink this approach.

4. Correlation between metabolic syndrome (MS) and breast cancer recurrence in luminal A tumors

This fascinating effort from Dr Albain's group attempted to determine whether the presence of MS is predictive of breast cancer recurrence in RS subtypes. In addition to documenting an overall eye-popping 27% rate of MS (glucose intolerance/diabetes and 2 other factors, including hypertension, dyslipidemia, central obesity and microalbuminemia) among the 332 patients in the study, of great interest was the correlation of MS and recurrence rate in patients with low RS. This intriguing finding suggests that the work of Dr Rowan Chlebowski and many others demonstrating a link between recurrence and metabolic factors like diet, obesity and exercise may be particularly relevant in luminal A tumors.

Next on this series: SABCS papers on CT, including a surprising potentially practice-changing paper on "pseudoadjuvant" treatment for patients with resected local recurrences rendered Stage IV with no evidence of disease.

Neil Love, MD
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Association between the 21-Gene Recurrence Score (RS) and Benefit from Addition of Adjuvant Paclitaxel in Node-Positive, ER-Positive Breast Cancer Patients: Results from NSABP B-28

Mamounas EP et al.

Proc SABCS 2012;Abstract S1-10.

Background

- The 21-gene Onco*type* DX® Recurrence Score® (RS) assay predicts the 10-year risk of distant recurrence and survival in lymph node (LN)-negative and LN-positive, ER-positive breast cancer (BC) treated with adjuvant endocrine therapy (*Breast Cancer Res Treat* 2011;127:133).
- The RS predicts benefit from chemotherapy in patients with ER-positive BC (*Lancet Oncol* 2010;11:55).
- In addition, the RS predicts the risk of death for patients with ER-positive BC with ≤3 positive nodes that has been treated with adjuvant chemoendocrine therapy (*Breast Cancer Res Treat* 2012;134:683).
- **Study objective:** To evaluate the association between the RS and benefit of adding paclitaxel (P) to doxorubicin/ cyclophosphamide (AC) therapy for patients with LN-positive, ER-positive BC from the NSABP-B-28 trial.

Mamounas EP et al. *Proc SABCS* 2012;Abstract S1-10.

Study Methods

- The Phase III NSABP-B-28 trial compared 4 cycles of AC to AC → P, and patients younger than 50 years or 50 and older with hormone receptor-positive BC also received tamoxifen for 5 years with concurrent chemotherapy (n = 3,060).
- This subset analysis of NSABP-B-28 included patients with ER-positive BC by central microarray IHC assay and with successful 21-gene RS assay results (n = 1,065).
  - Patients who received AC: 519
  - Patients who received AC → P: 546
- Kaplan-Meier estimates and log-rank tests were used to assess survival outcomes in patient subgroups by RS and treatment.
- Multivariate Cox regression models were adjusted for traditional clinicopathologic factors and used to determine the predictive utility of adding paclitaxel to AC (AC → P).

Mamounas EP et al. *Proc SABCS* 2012;Abstract S1-10.

Analysis of Prognosis by RS Risk Groups (10-Year Follow-Up)

Outcome	Low risk (n = 386)	Interm risk (n = 364)	High risk (n = 315)	*p*-value
DFS	75.8%	57.0%	48.0%	<0.001
DRFI	80.9%	64.9%	55.8%	<0.001
OS	90.0%	74.7%	63.0%	<0.001
BCSS	95.0%	78.9%	68.2%	<0.001

Interm = intermediate; DFS = disease-free survival; DRFI = distant recurrence-free interval; OS = overall survival; BCSS = BC-specific survival

- The distribution of patients by RS was not significantly different according to treatment, surgery type and number of positive nodes.
- There were statistically significant differences in the distribution of the RS according to age and tumor size:
  - Older patients and those with small tumors were more likely to have a low RS.

Mamounas EP et al. Breast Cancer Symposium 2012;Abstract 1; Mamounas EP et al. *Proc SABCS* 2012;Abstract S1-10.

Benefit of Adding P to AC (10-Year Follow-Up)

Outcome	AC (n = 519)	AC → P (n = 546)	HR	*p*-value
DFS	58.9%	63.2%	0.87	0.14
DRFI	66.4%	69.6%	0.89	0.26
OS	74.9%	78.5%	0.87	0.26

HR = hazard ratio

Mamounas EP et al. *Proc SABCS* 2012;Abstract S1-10.

DFS and DRFI: Benefit of Adding P to AC by RS Risk Group (10-Year Follow-Up)

DFS*	AC	AC → P	HR	*p*-value
Low risk (n = 186, 200)	75.5%	76.1%	1.01	0.99
Interm risk (n = 180, 184)	53.4%	60.4%	0.84	0.26
High risk (n = 153, 162)	45.3%	50.5%	0.81	0.21
DRFI†	**AC**	**AC → P**	**HR**	***p*-value**
Low risk (n = 186, 200)	80.8%	80.9%	0.95	0.78
Interm risk (n = 180, 184)	62.5%	67.3%	0.88	0.49
High risk (n = 153, 162)	53.2%	58.2%	0.86	0.40

* Test for common treatment benefit of adding paclitaxel to AC ($p = 0.65$)

† Test for common treatment benefit of adding paclitaxel to AC ($p = 0.93$)

Mamounas EP et al. *Proc SABCS* 2012;Abstract S1-10.

OS Benefit of Adding P to AC by RS Risk Group (10-Year Follow-Up)

OS*	AC	AC → P	HR	*p*-value
Low risk (n = 186, 200)	91.5%	88.5%	1.28	0.40
Interm risk (n = 180, 184)	69.9%	79.3%	0.74	0.12
High risk (n = 153, 162)	60.7%	65.3%	0.86	0.45

* Test for common treatment benefit of adding paclitaxel to AC ($p = 0.30$)

Mamounas EP et al. *Proc SABCS* 2012;Abstract S1-10.

Author Conclusions

- The 21-gene RS significantly predicted risk of recurrence and death for patients with LN-positive and ER-positive BC treated with adjuvant chemoendocrine therapy.
- The RS did not significantly predict benefit from the addition of paclitaxel to AC among 1,065 patients from the NSABP-B-28 trial with ER-positive BC included in this analysis:
  - A possible explanation may be that the overall benefit from paclitaxel was small and statistical power to detect treatment by RS interaction was low.
  - An insignificant trend was observed toward benefit with the addition of paclitaxel to AC for patients with intermediate and high RS results.
- Future studies are planned to identify new genes that may be used to predict taxane benefit.

Mamounas EP et al. *Proc SABCS* 2012;Abstract S1-10.

Investigator Commentary: Association between the 21-Gene RS and Benefit from Adding P to AC in LN-Positive, ER-Positive BC

This is a subset study of prognosis for patients with ER-positive, LN-positive BC who had received tamoxifen with concurrent chemotherapy (CT) on the NSABP-B-28 trial. The RS is known to be prognostic for LN-positive BC, but this study determined whether it could predict benefit from adding CT. It demonstrated that the RS is a good prognostic tool when patients are classified according to RS risk groups. The DFS was 75% for patients in the low-risk group and 48% in the high-risk group. However, analysis of RS as a predictor of benefit from adding P to AC was not statistically significant. There was a hint that in the low-risk group patients didn't gain much with additional CT, but the interaction term wasn't significant and it's not certain that the RS can be used in that way. Nevertheless, I believe it's an excellent prognostic tool. I believe these results do add to our comfort level that this tool is prognostic whether you have administered endocrine therapy or have added chemotherapy, whether the BC is node-negative or node-positive. The "devil is in the details" of figuring out how exactly it helps to make decisions.

Interview with Lisa A Carey, MD, January 17, 2013

The EndoPredict Score Identifies Late Distant Metastases in ER+ / HER2- Breast Cancer Patients

Dubsky P et al.

Proc SABCS 2012;Abstract S4-3.

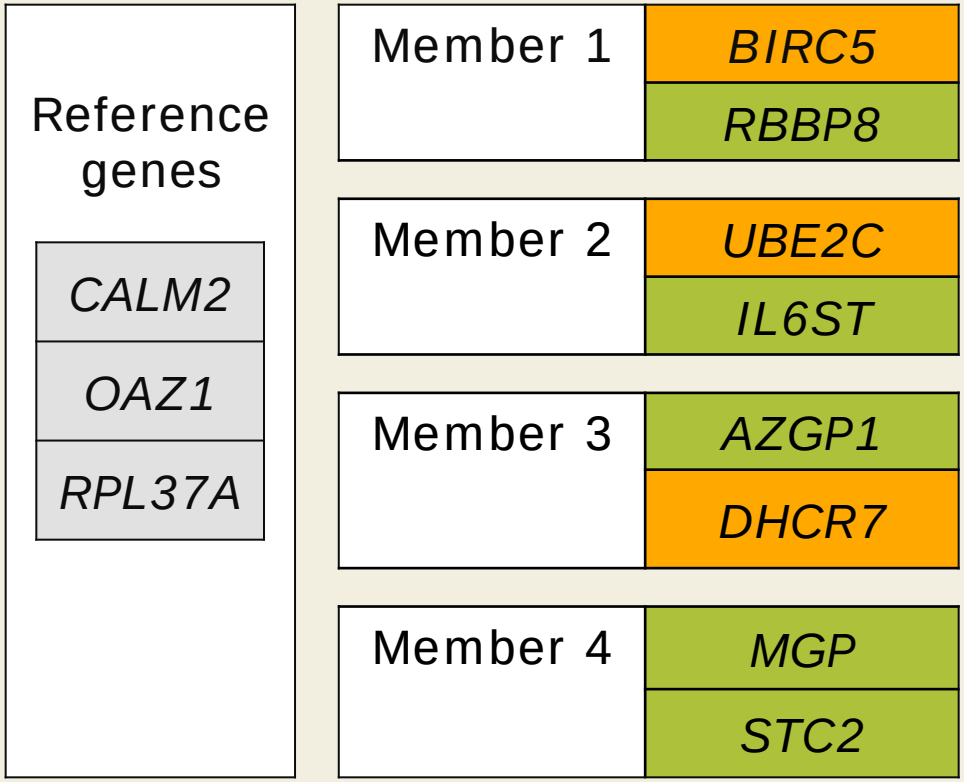
Background

- In contrast to ER-negative breast cancer (BC), annual recurrence rates persist beyond 5 years for ER-positive disease, and the risk of BC-specific mortality is higher after 5 to 10 years of follow-up (*J Clin Endocrin Metab* 2012;97:e2201).
- Several ongoing trials are evaluating the benefit of extended aromatase inhibitor (AI) therapy and have identified factors, such as nodal positivity, tumor size, premenopausal status and ER/PR status, as useful elements for predicting benefit from extended AI therapy.
- Currently available multigene signatures have been trained to predict early recurrences but commonly fail to predict late events (*Breast Cancer Res Treat* 2011;129:607).
- **Current study objective**: To evaluate whether the EndoPredict® (EP) signature can identify late metastases in patients with ER-positive/HER2-negative BC, and to determine if a predefined combination of nodal status and tumor size with molecular data (EPclin) could further improve this prediction.

Dubsky P et al. *Proc SABCS* 2012;Abstract S4-3.

The EP and EPclin Signatures

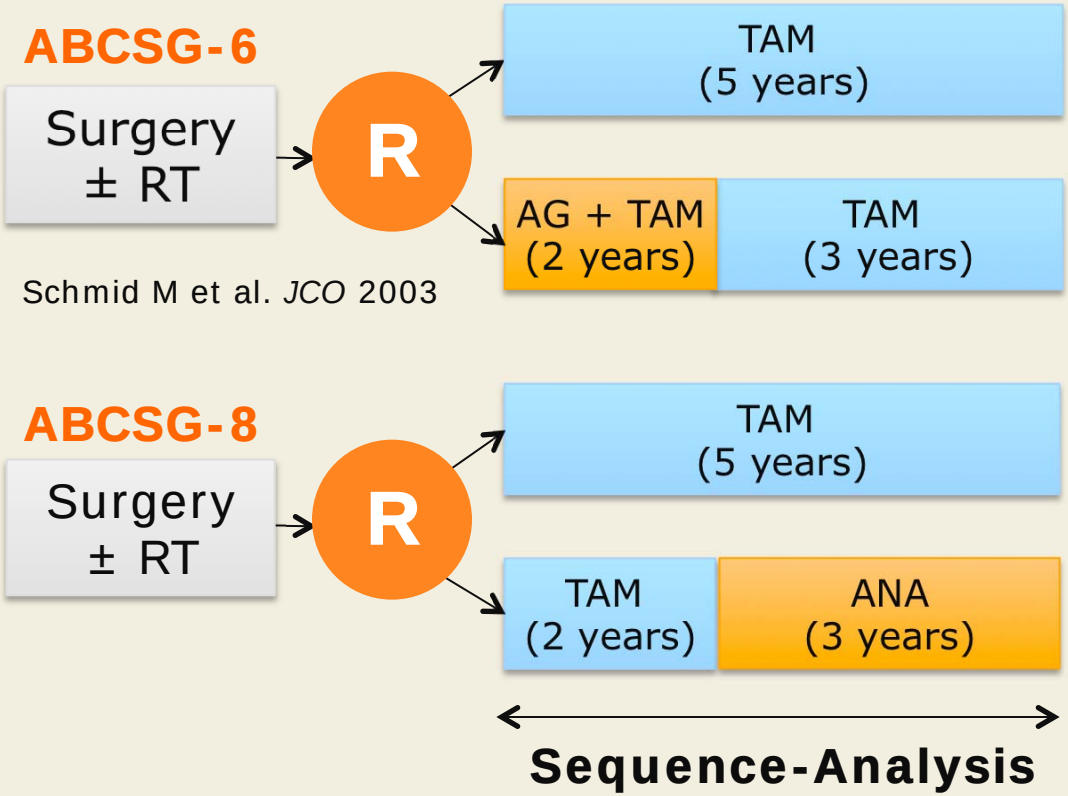
- The EP signature contains 8 cancer-related genes and 3 reference genes (*Clin Cancer Res* 2011;17:6012).



- EP was determined using qRT-PCR and formalin-fixed, paraffin-embedded tissue samples.
- EP signature was trained on samples from tamoxifen (TAM)-treated women (n = 964).
- EP signature was validated using samples from patients on the Phase III ABCSG-6 (n = 378) and 8 (n = 1,324) trials.
- EPclin is a predefined score incorporating EP, tumor size and nodal status.

Dubsky P et al. *Proc SABCS* 2012;Abstract S4-3.

Patients and Methods

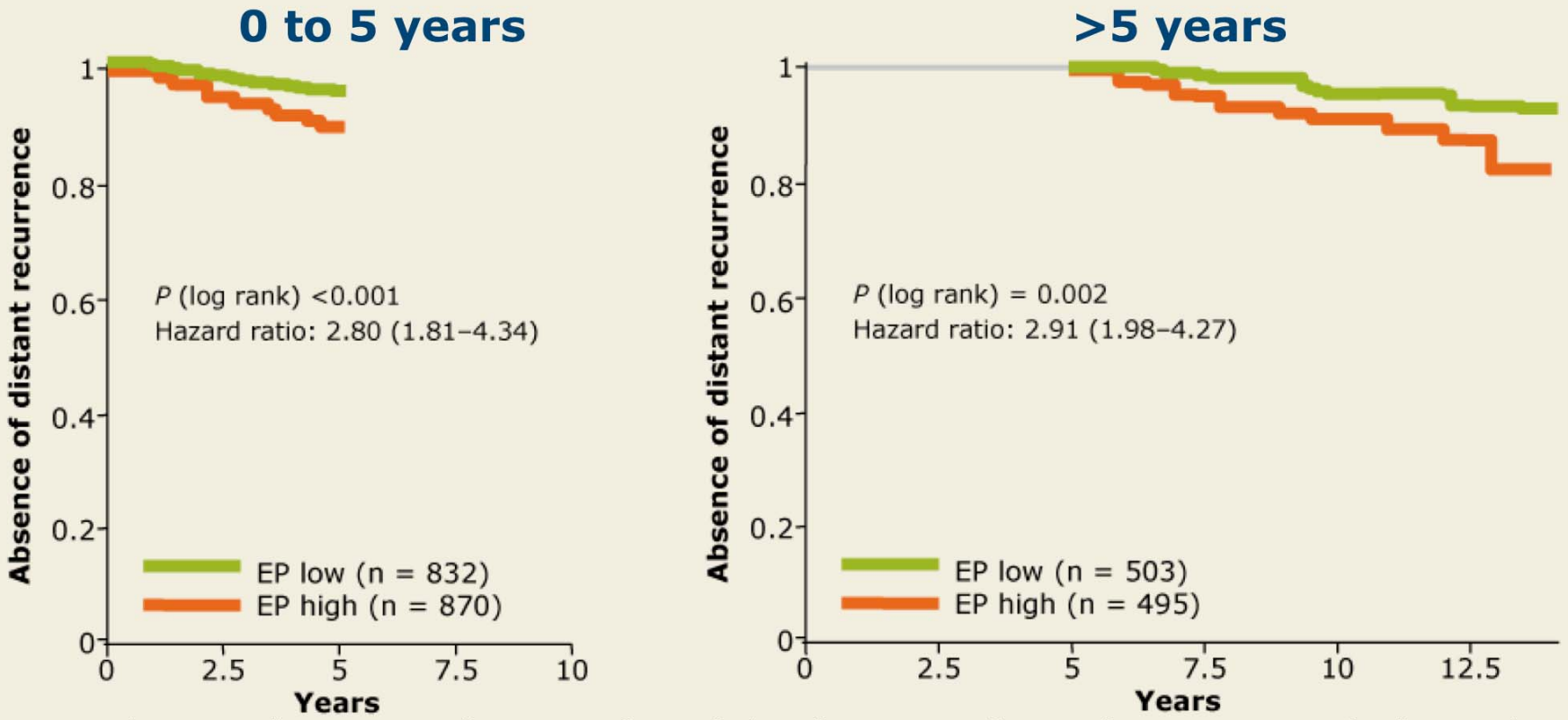


- 1,702 postmenopausal patients with ER-positive/ HER2-negative BC
- Two thirds of patients with tumor size less than 2 cm and N0
- No adjuvant chemotherapy
- 998 patients at risk after 5 y (median follow-up = 7.12 y)
- Primary endpoint was distant metastasis-free survival

TAM = tamoxifen; AG = aminoglutethimide; ANA = anastrozole

Dubsky P et al. *Proc SABCS* 2012;Abstract S4-3.

Distant Metastasis-Free Survival (DMFS)

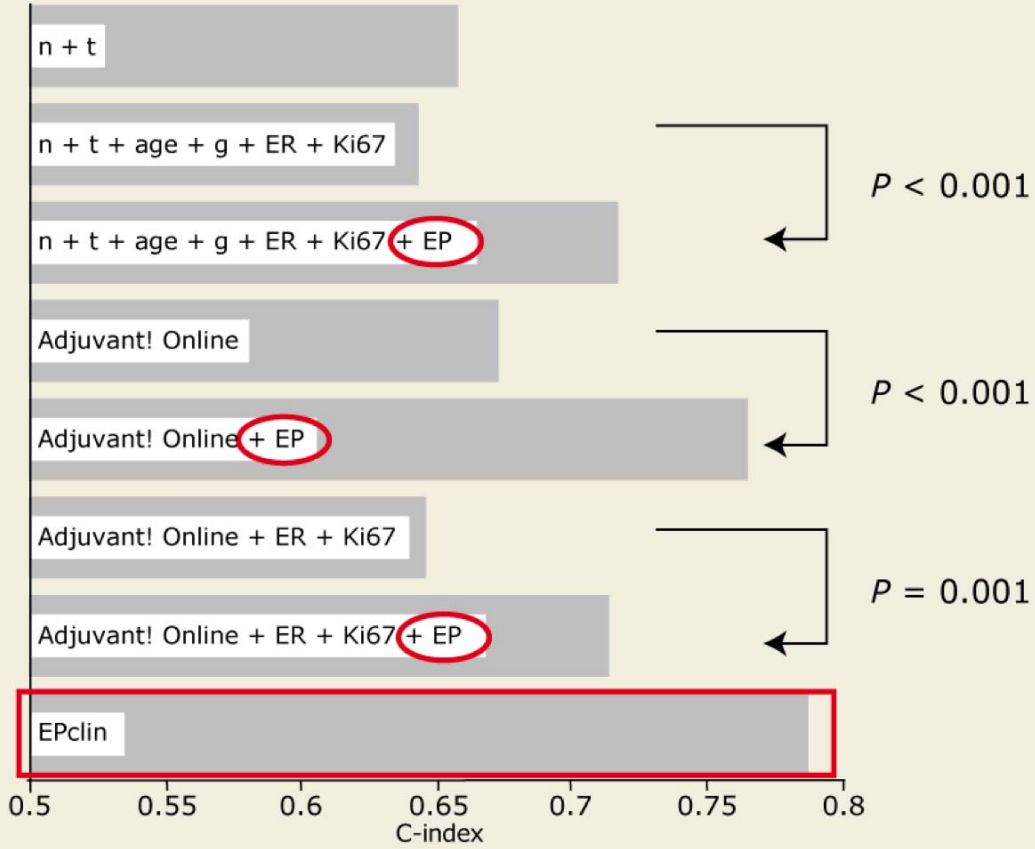


- EP low-risk group (49% of pts) had a significantly improved clinical outcome before and after 5 y of follow-up:
  - 96.3% were distant metastasis free between 5 and 10 y

With permission from Dubsky P et al. *Proc SABCS* 2012;Abstract S4-3.

Prognostic Performance After 5 Years

C-index* (>5 years)



- The EndoPredict Score improves the prognostic performance of all common clinical parameters.
- The EPclin — a predefined score of EndoPredict and the clinical parameters nodal status and tumor size — showed the best performance to predict late metastases.

* C-index = measure of prognostic performance

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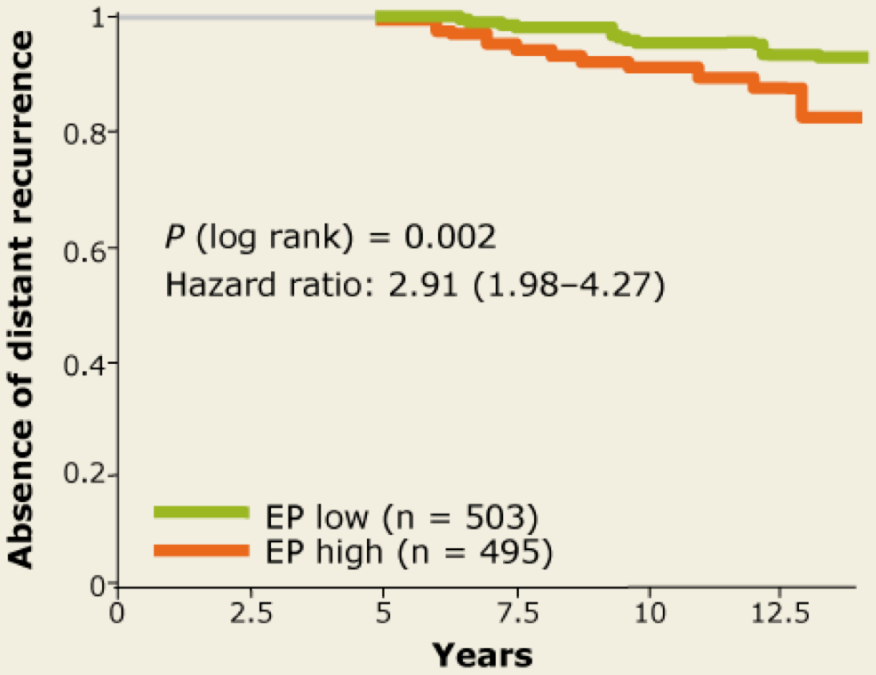
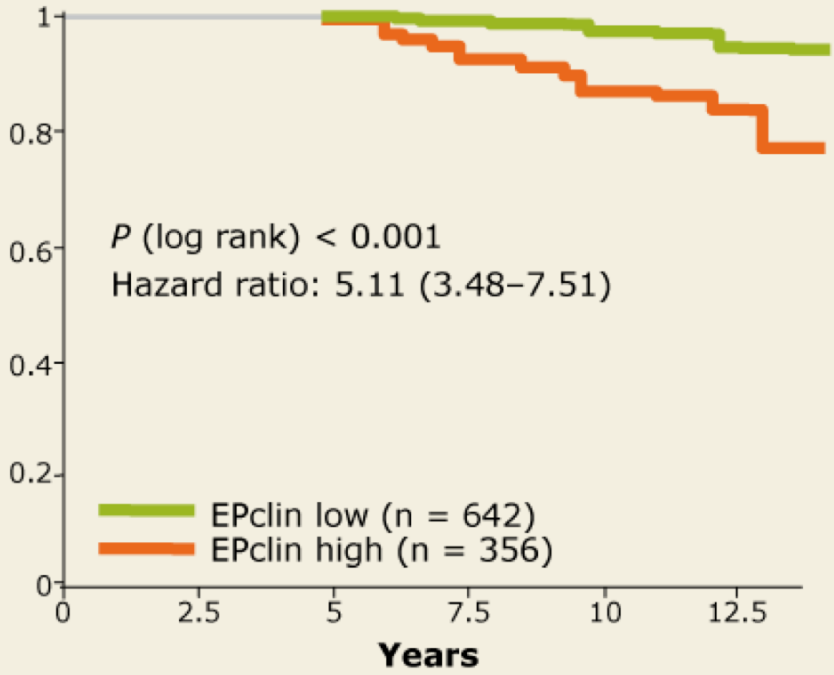
EP Score versus EPclin Score

EP low risk (49% of pts) >5 y



96.3% pts were distant metastasis free at 5-10 y

EPclin low risk (64% of pts) >5 y



>98% pts were distant metastasis free at >5 y

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Prognostic Contribution of ER Signaling and Cell Proliferation Genes

Variable	0-5 years Unit HR (95% CI)	*p*-value	>5 years Unit HR (95% CI)	*p*-value
Proliferation	1.60 (1.33-1.92)	<0.001	1.19 (0.85-1.67)	0.297
ER signaling	0.89 (0.75-1.06)	0.204	0.61 (0.46-0.81)	<0.001
Age	1.03 (1.00-1.06)	0.039	0.98 (0.93-1.02)	0.355
Nodal status	2.20 (1.71-2.83)	<0.001	2.50 (1.60-3.90)	<0.001
Tumor size	1.26 (0.94-1.70)	0.123	1.15 (0.69-1.93)	0.585
Ki67	1.00 (0.98-1.03)	0.727	1.01 (0.97-1.06)	0.502
Grade	1.23 (0.78-1.93)	0.364	0.69 (0.35-1.36)	0.285
Treatment arm	0.92 (0.59-1.43)	0.712	0.89 (0.39-2.05)	0.783

recurrence.

- Genes associated with ER signaling add independent prognostic information for late recurrence.

Dubsky P et al. *Proc SABCS* 2012;Abstract S4-3.

Author Conclusions

- The EP score identifies early and late recurrences and offers independent prognostic information (data not shown) beyond what can be achieved with all common clinical parameters.
- Proliferation genes add prognostic information for identifying early recurrences, whereas genes associated with ER signaling are important for late events.
- The EPclin score identified a low-risk subgroup containing 64% of patients at risk after 5 years:
  - 98.2% of these women remain free of distant metastases 10 years after diagnosis.
- The risks and side effects of extended therapy should be weighed against this projected outcome.

Dubsky P et al. *Proc SABCS* 2012;Abstract S4-3.

Investigator Commentary: EndoPredict Score and Identification of Late Distant Metastases in ER-Positive/HER2-Negative BC

EndoPredict (EP) is an 8-gene signature that was developed comprising proliferation and estrogen receptor signaling-related genes. A variation of the EP score, known as EPclin, takes into account the clinical variables of tumor size and nodal status. This group demonstrated that the EP score predicted both early and late relapses and performed better at predicting late relapses. They also performed an ontogenic-like exploration study of what drove the prognostic ability of the score, and the proliferation genes predicted early relapse, whereas the genes involved in estrogen receptor signaling predicted late relapse. To me, these results with the EP score were compelling. This group has examined a late relapse endpoint effectively.

There are now several of these prognostic genomic tests, such as the Onco*type* DX®, MammaPrint®, Breast Cancer Index^{SM} and PAM50, coming into clinical use, and we don't always know which test is better to use. I believe what we would like to have is a big, international, multiorganization, mature data set to analyze in order to perform an ubercomparison.

Interview with Lisa A Carey, MD, January 17, 2013

Comparative Performance of Breast Cancer Index (BCI) vs Onco*type* DX and IHC4 in the Prediction of Late Recurrence in HR-Positive, LN-Negative Breast Cancer Patients: A TransATAC Study

Sgroi DC et al.

Proc SABCS 2012;Abstract S1-9.

Background

- More than 50% of late recurrences for patients with estrogen receptor (ER)-positive breast cancer (BC) occur after 5 years from diagnosis, making residual risk of recurrence a substantial concern (*Lancet Oncol* 2010;11:1135).
- Current multigene signatures have significant prognostic performance in predicting early recurrence 0 to 5 years post-diagnosis (*JNCI* 2006;98:1183; *Lancet Oncol* 2010;11:55).
- However, these signatures have limited performance in predicting the risk of late recurrence (>5 years).
- **Study objective:** To determine whether the BCI biomarker adds prognostic information to clinical variables in predicting distant recurrence in patients with ER-positive, lymph node (LN)-negative BC enrolled on the TransATAC trial.

Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

Breast Cancer Index (BCI)

- The BCI is a PCR-based assay that stratifies patients into 3 risk groups and has been shown to predict distant recurrence beyond clinical and pathophysiological parameters.
- It consists of 2 independently developed biomarkers.
  - HOXB13:IL17BR (H/I) gene expression ratio: Prognostic and predictive for extended adjuvant hormonal therapy benefit
  - Molecular Grade Index: A set of cell cycle-related genes that predicts for distant recurrence beyond tumor grade
- The BCI Linear Model was trained on the untreated arm of the Stockholm Trial and was the BCI model used in the current analysis.

Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

Design for Sample Analyses

Eligibility (n = 1,102)

Centrally confirmed hormone receptor-positive, LN-negative primary tumor
Prior tamoxifen or anastrozole alone
No adjuvant chemotherapy
Sufficient residual RNA for BCI
Available Onco*type* DX® Recurrence Score® (RS) and IHC4 scores
Database of 10-year follow-up

↓

Final study cohort
665 primary tumor samples,
LN-negative and matched for BCI, IHC4 and RS

- **Primary endpoint:** Distant recurrence
- Measurements included the analysis of overall distant recurrence (0-10 years), early distant recurrence (0-5 years) and late distant recurrence (5-10 years)

Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

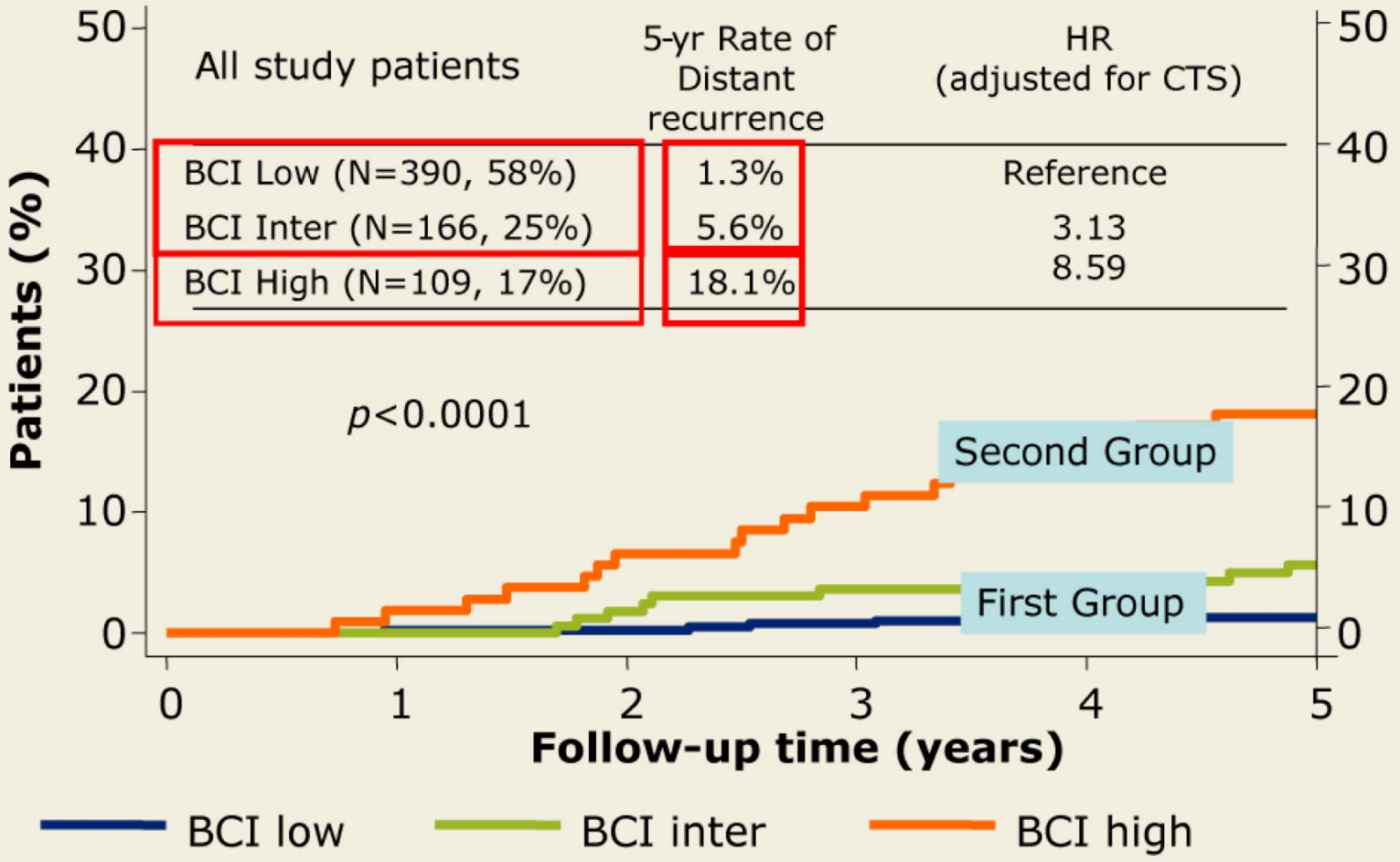
BCI Identifies 3 Risk Groups* (All Patients, 10-Year Follow-Up)

Risk group (n, %)	10-y rate of distant recurrence	Hazard ratio†
BCI-low (n = 390, 58%)	4.2%	Reference
BCI-intermediate (n = 166, 25%)	18.3%	2.89
BCI-high (n = 109, 17%)	30.0%	4.86

* $p < 0.0001$; † Adjusted for Clinical Treatment Score (CTS), an algorithm consisting of nodal status, tumor size and grade, age and treatment

Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

BCI Identifies 2 Early Recurrence Risk Groups



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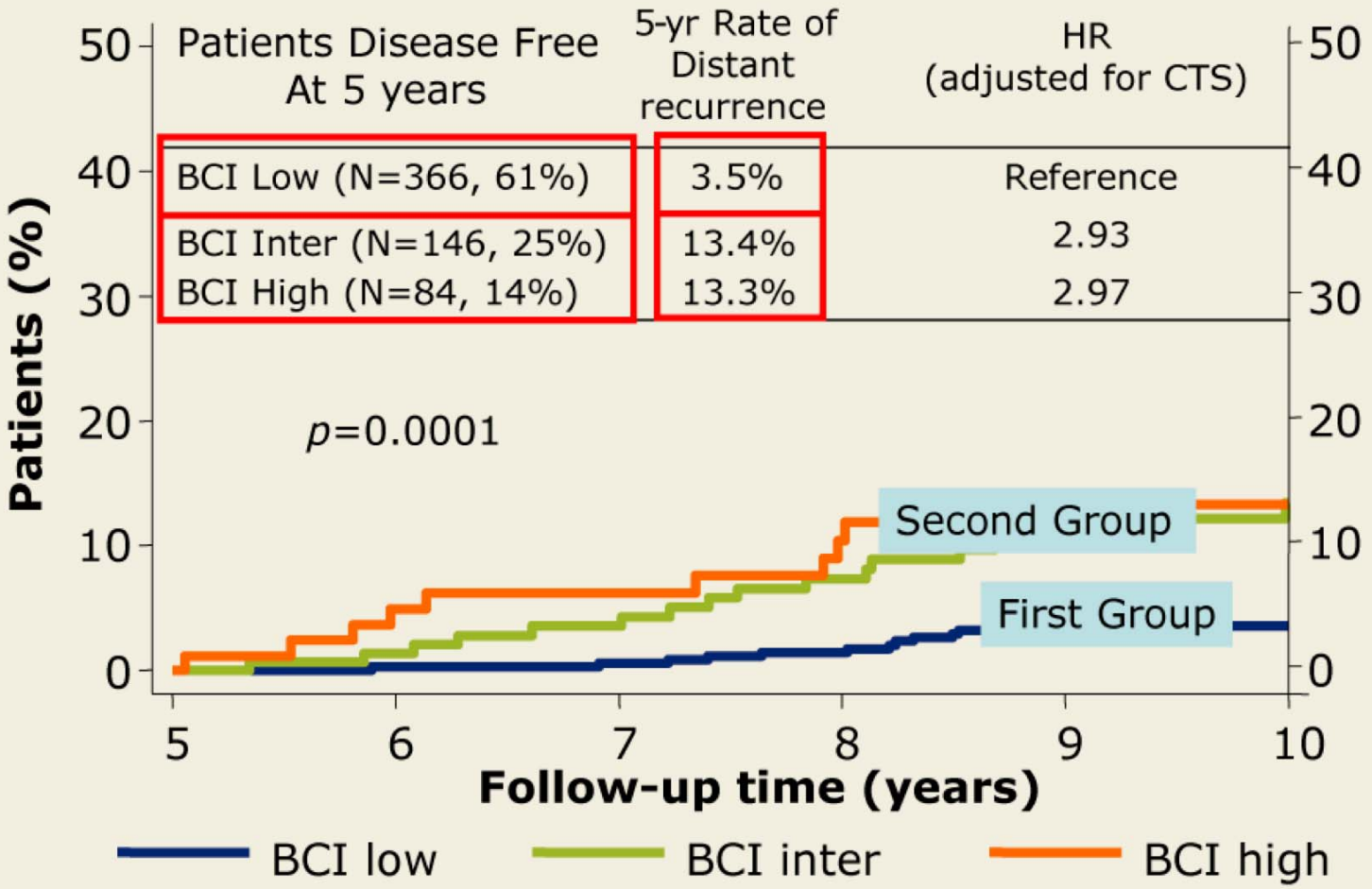
BCI Identifies 2 Late Recurrence Risk Groups



With permission from Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

Comparative Prognostic Performance for 0 to 10 Years

Assay type	LR χ^2 statistical analysis*		
	Univariate	Multivariate†	*p*-value
BCI	48.9	22.7	$p < 0.0001$
IHC4	39.2	22.9	$p < 0.0001$
Onco*type* DX RS	25.2	13.8	$p = 0.0002$

* The likelihood ratio (LR) test was used to measure the amount of additional information provided by the BCI biomarker beyond CTS, and it allowed for a head-to-head comparison with the IHC4 and Onco*type* DX RS assays.

† Multivariate analysis of LR χ^2 test was always adjusted for CTS.

Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

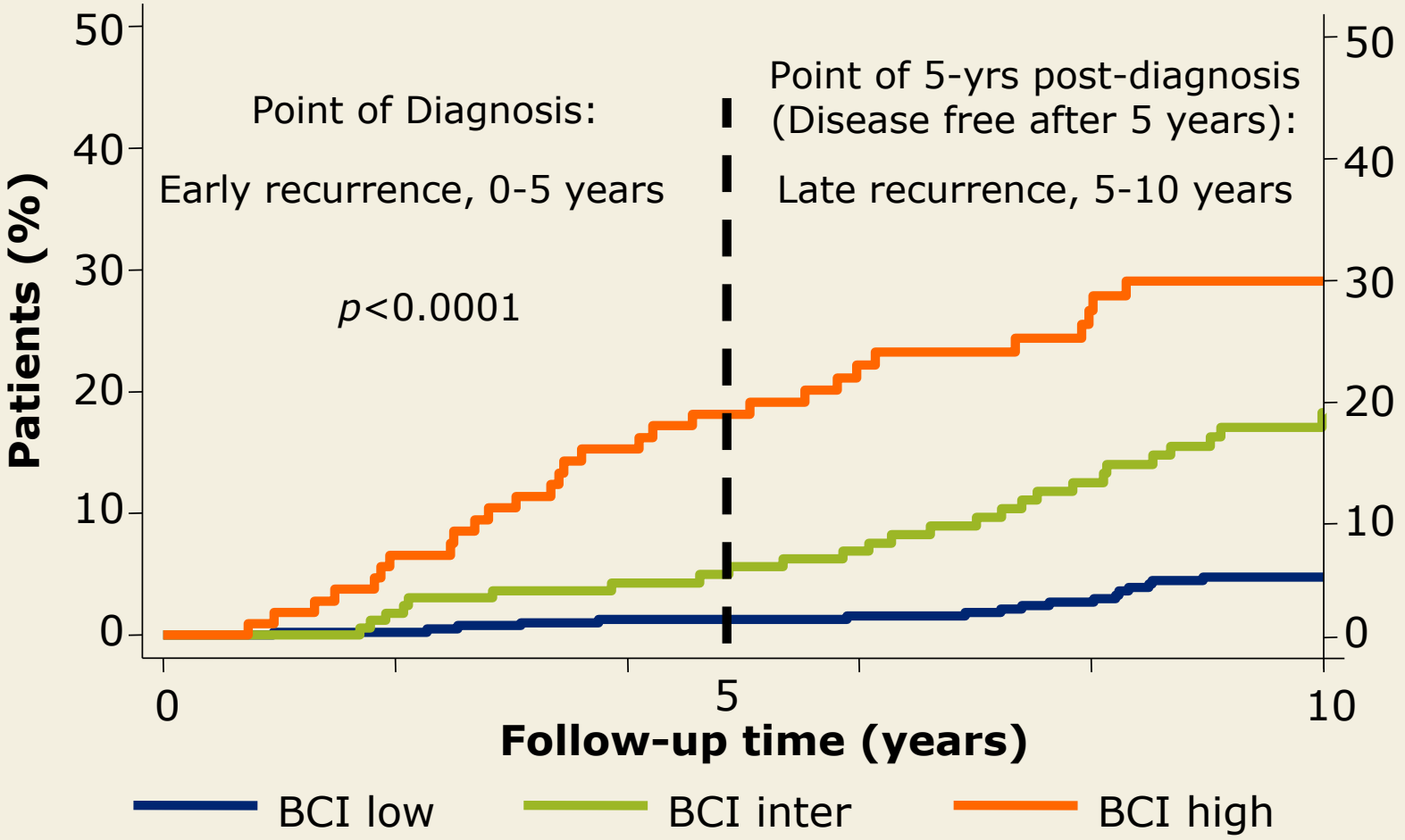
Comparative Prognostic Performance for Early and Late Distant Recurrence

Early recurrence (0-5 years)	LR χ^2 statistical analysis		
	Univariate	**Multivariate**	***p*-value**
BCI	34.7	15.4	$p < 0.0001$
IHC4	43.6	28.8	$p < 0.0001$
Onco*type* DX RS	28.9	18.2	$p < 0.0001$
Late recurrence (5-10 years)	**Univariate**	**Multivariate**	***p*-value**
BCI*	16.6	8	$p = 0.0005$
IHC4	4.8	1.6	$p = 0.2$
Onco*type* DX RS	2.2	0.5	$p = 0.5$

* Only the BCI assay demonstrated sustained significant prognostic performance.

Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

Ten-Year BCI Analysis of Early and Late Recurrence



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Author Conclusions

- BCI significantly predicts a 10-year distant recurrence rate beyond CTS for patients with ER-positive, LN-negative BC.
- BCI is a significant prognostic factor beyond CTS for predicting late distant recurrence of 5 to 10 years.
- Onco*type* DX RS and IHC4 scores are not significant prognostic factors for late distant recurrence of 5 to 10 years.
- The performance of BCI for patients at good risk with LN-negative, ER-positive primary tumors identified the following 2 groups at the point of diagnosis:
  - Those at low risk of early recurrence, who are adequately treated with endocrine therapy (ET)
  - Those at high risk of early recurrence, who do not benefit adequately from simple ET and should be considered for additional therapy (chemotherapy or other)

Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

Author Conclusions (Continued)

- At the point of a follow-up of 5 years disease free, BCI identified 2 groups of patients:
  - Those at low risk of late recurrence, who do not need subsequent therapy
  - Those at significant risk of late recurrence, who should be considered for additional or alternative systemic adjuvant therapy

Sgroi DC et al. *Proc SABCS* 2012;Abstract S1-9.

Investigator Commentary: Comparative Performance of BCI vs RS and IHC4 in Predicting Late Recurrence for Hormone Receptor-Positive, LN-Negative BC

This is a subset study of the BCI in tumors from 665 patients on the large TransATAC trial with HR-positive, LN-negative BC who had received prior tamoxifen or anastrozole only. One must be cautious when interpreting data about the efficacy of a biomarker when including subsets on which the biomarker (IHC4) was trained. It is now being recognized that ER-positive BC carries a constant ongoing risk of relapse beyond the first 5 years. Because the biology that differentiates risk of early versus late relapse is not fully understood, many therapeutic studies are being conducted in this field.

After 10 years of follow-up, this study showed that BCI was prognostic. If the BCI is classified into low-, intermediate- and high-risk groups, patients in the low-risk group had <5% risk of relapse. The intermediate- and high-risk groups had 18% and 30% risk of relapse, respectively. In the first 5 years, it seemed that the low- and intermediate-risk groups had an especially low risk of relapse when grouped together. After year 5, patients with low risk of relapse had a low BCI and the intermediate- and high-risk BCI groups had a 13% risk of relapse. However, for years 5 to 10, the RS score didn't perform well from a prognostic standpoint. These data are interesting, and I believe it's highly important that we get a grip on identifying patients who need to receive extended adjuvant therapy.

Interview with Lisa A Carey, MD, January 17, 2013

An International Ki67 Reproducibility Study

Nielsen TO et al.

Proc SABCS 2012;Abstract S4-6.

Background

- Immunohistochemical (IHC) analysis of the cell proliferation marker Ki67 has the potential for utility in breast cancer management for prognostic determinations and in the prediction of treatment response.
- However, the lack of consistent results across laboratories from Ki67 analysis of samples has limited its utility and value as a reliable biomarker.
- In 2010, a working group was assembled to develop and harmonize methodology for Ki67 analysis and scoring and to identify procedures to improve concordance (*JNCI* 2011;103:1656).
- **Study objective:** To conduct an international Ki67 reproducibility study to devise an approach to harmonize Ki67 analytical and scoring methods with the aim of improving concordance.

Nielsen TO et al. *Proc SABCS* 2012;Abstract S4-6.

Systematic Study Design

“Phase 1” of the Study

Tissue microarray (TMA) slides stained/scored using local methods

↓

“Phase 2” of the Study

Create a web-based calibration method ➔

Staining of TMA slides after calibration + standardized scoring

↓

Analysis of Core Biopsies and Whole Sections

- **Analytical validity:** To determine the extent of reproducibility to which pathologists can reliably quantify Ki67 staining.
- **Endpoints:** Dissemination of analytically valid and meaningful methods for assessing Ki67 and provision of a gold-standard set of web-based calibration cases.

Nielsen TO et al. *Proc SABCS* 2012;Abstract S4-6.

Phase 1 Portion of the Study

- Phase 1 determined whether experienced laboratories can deliver consistent Ki67 percentage scores on the same cases using local visual scoring methods.
  - 1-mm TMA cores from 100 breast cancer cases were scored visually by labs using their own scoring methods
- Experiments:
  - Intraobserver (repeat scoring of same TMA slide)
  - Interobserver using a central staining method
  - Interlaboratory observer using local staining methods
- The study included labs from Canada, France, Italy, UK and USA.
  - Study sites included universities, major cancer centers and a national reference lab

Nielsen TO et al. *Proc SABCS* 2012;Abstract S4-6.

Phase 1: Results and Lessons Learned

- Intraobserver consistency was good.
  - Six laboratories scored the same 50 cases 3 times.
  - Reproducibility was high, with an intraclass correlation coefficient (ICC) of 0.94 (95% CI: 0.93-0.97).
  - Labs using formal counting methods produced more consistent results than those using visual estimation.
- Interobserver variability using centrally stained TMA slides was problematic:
  - Median Ki67 values across 8 labs ranged from 10% to 28%.
  - Overall ICC was 0.71 (95% CI: 0.47-0.78).
  - At a hypothetical 13.5% cutoff, 32% of cases would be scored as high Ki67 by one lab but low Ki67 by another lab.
- Although intraobserver consistency was good, interobserver variability was problematic.

Nielsen TO et al. *Proc SABCS* 2012;Abstract S4-6.

Phase 1: Results and Lessons Learned (Continued)

- Cutoff points are not freely transferable, and local recalibrations against clinical endpoints or reference images are needed.
- Although the staining method added some variability to the results, the major source of Ki67 differences besides patient biology was the scoring method.
  - Estimation versus counting
  - Choice of areas to count
  - Invasive cancer versus other cells
  - Threshold of “brown” considered as “positive” Ki67 staining

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Phase 2 Portion of the Study

- Phase 2 evaluated whether:
  - Ki67 scorers can be trained in a common visual scoring method that might be transferrable for clinic use.
  - A common reference tool for clinical trial studies can be developed.
- A web-based scoring calibration interface was created from digital images of TMA scores from 9 “training” and 9 “test” cases.
  - TMA slides were centrally stained, representing a range of Ki67 scores.
- A standardized practical scoring method with good internal consistency was chosen.
- Simple instructions with visual examples were developed and provided to 11 laboratories around the world.
- These efforts are continuing with additional labs.

Nielsen TO et al. *Proc SABCS* 2012;Abstract S4-6.

Phase 2: Calibration Criteria

- Nine web-based standard images, using a click-tracking application, allowed for the assessment of differences from reference scorers.
- Laboratories with the highest inter- and intralab reproducibility were chosen as reference labs.
  - Their average log2 transformed Ki67 scores = gold standard
- Calibration criteria for study success:
  - Root mean squared differences (RMSE) between volunteer and reference labs among the 9 images: <0.6
  - Maximum absolute difference (MAXDEV) between volunteer and reference lab scores among the 9 images: <1.0

Nielsen TO et al. *Proc SABCS* 2012;Abstract S4-6.

Performance Statistics: Training Phase (First Attempt) versus Testing Phase

Score statistic	RMSE (Pass: <0.6)		MAXDEV (Pass: <1.0)	
	Training	Testing	Training	Testing
Mean	0.68	0.41	1.66	0.93
Standard deviation	0.44	0.19	1.26	0.48
Minimum	0.24	0.14	0.35	0.28
Maximum	1.47	0.59	3.82	1.41
Median	0.56	0.49	1.18	1.12

- Differences between training and test cases did not reach statistical significance, possibly due to sample size.
  - For RMSE, $p = 0.21$
  - For MAXDEV, $p = 0.22$

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Phase 2: Study Outcomes and Lessons Learned from Calibration

- Results of calibration testing from 9 participant laboratories and 2 reference labs:
  - 5 of 9 passed testing
- Labs were “trainable,” with improved performance, although improvement was not statistically significant, probably due to sample size.
- Labs differed on the threshold of “brown” staining considered to be positive.
- The following would be added to web-based instructions:
  - Examples of images showing the level of staining that should be considered positive
  - Reminders to consult hematoxylin and eosin (H&E) staining guidelines and not to score ductal carcinoma in situ

Nielsen TO et al. *Proc SABCS* 2012;Abstract S4-6.

Future Directions

- If this study is successful:
  - The same scoring system will be applied to core biopsies.
  - Whole sections (with hot-spot issues) will follow later.
  - Clinical utility of analytically valid methods will be confirmed.
  - The levels of expected residual variability in best-practice scoring methods will be defined.
- If this study is unsuccessful:
  - Whether automated platforms and algorithms can deliver consistent results will be tested.
  - Failure would strongly suggest that the Ki67 index by IHC should be used only after internal validation for a given clinical context or as a research tool.

Nielsen TO et al. *Proc SABCS* 2012;Abstract S4-6.

Discussant Comments: An International Study of Ki67 Reproducibility

This is an elegant study with a strong statistical approach. It points out that pathologists are somewhat “creatures of habit.” The overall concordance rate was 71%, which is not particularly strong. With individual research tools, each pathologist becomes somewhat deviant, which is not acceptable in estimating the proliferation rate on a continuous scale. With centrally stained TMA slides, median Ki67 values across labs ranged from 10% to 28%. If we’re considering cutoffs in the 15% to 25% range, this will be problematic. Importantly, however, pathologists are trainable.

Improvements were noted from the use of the web-based tool, even though they weren't statistically significant. Bias in choosing tissue sites to count was controlled by the use of TMA slides, but this strategy will introduce some intratumor heterogeneity to the analysis. This will in turn add to the lack of concordance when applied to core biopsies or tissue sections. Also, differences were apparent in identifying the threshold for “brown” staining. Clearly a potential exists for improvement with computer assistance in this field. IHC for Ki67 is currently not reliable for precise measurement of the proliferating fraction in breast cancer. It could be improved with a standardized interpretation method.

W Fraser Symmans, MD, ASCO 2012

Investigator Commentary: An International Study of Ki67 Reproducibility

This study illuminates the issues associated with obtaining objective results with Ki67 analysis in terms of the way cells are stained and counted. In order to solve these problems, we're working with a diagnostics company to rapidly develop a rigorous Ki67 test involving the use of a scanner, which will not be so dependent on counting cells with a microscope. With any IHC test variations occur in the quality of the staining, so the staining must be properly controlled for. This can be achieved with the use of an assay kit. If "home brews" by individual labs are used, variation is introduced into the experiments, especially with different incubation periods and the preparation of different antibodies. Thus, different results are generated. Another issue is the heterogeneity of staining as this can be patchy. The same is true for HER2 and ER testing, and the issues mentioned above exist with all IHC tests, not Ki67 only. We believe that the scanner is a good way of solving this problem because it allows for the counting of thousands of cells, which the human eye can't do, and is more objective. If that works out, I believe we will have solved some of these problems. We should be able to push forward with the large validation study that is part of the planned neoadjuvant/adjuvant Phase III ALTERNATE trial, and I am hopeful that in the end Ki67 will enter clinical practice in a more robust and confident manner.

Interview with Matthew J Ellis, MB, BChir, PhD, February 7, 2013

Metabolic Syndrome and Recurrence within the 21-Gene Recurrence Score Assay Risk Categories in Lymph Node Negative Breast Cancer

Lakhani A et al.

Proc SABCS 2012;Abstract PD10-02.

Background

- Metabolic syndrome (MS) refers to a constellation of abnormalities similar to those of diabetes, a disease linked to breast cancer (BC).
- Evidence shows that the incidence of MS is increasing, but its interaction with BC incidence, tumor biology and outcomes are not fully understood (*Am J Clin Nutr* 2007;86:s823).
- The Onco*type* DX® Recurrence Score® (RS) assay quantifies the probability of disease recurrence in early-stage estrogen receptor (ER)-positive BC and has prognostic and predictive significance (*Lancet Oncol* 2010;11:55).
- **Study objective:** To determine whether the presence of MS is predictive of BC recurrence to a variable degree across the different BC types as defined by the risk categories of the 21-gene Onco*type* DX RS assay.

Lakhani A et al. *Proc SABCS* 2012;Abstract PD10-02.

Study Methods

- The study included patients with newly diagnosed ER-positive, lymph node (LN)-negative BC treated between 2006 and 2011 who underwent a 21-gene RS assay (n = 332).
- All patients received standard systemic/local therapy.
- The electronic medical record was searched for key diagnoses including MS, and patients were classified into MS groups using the World Health Organization (WHO) definition.
- Tumor characteristics including grade, size, Ki67 and HER2 status were recorded.
- Patient characteristics including age, race, menopausal status and body mass index were recorded.
- The association of MS, tumor and patient characteristics within the RS risk groups was analyzed.

Lakhani A et al. *Proc SABCS* 2012;Abstract PD10-02.

Overview of Patient Characteristics and Study Outcomes

Characteristic	n = 332
Median age	62 years
Patients with MS	27%

- The WHO classification defines MS as diabetes mellitus or glucose intolerance in addition to at least 2 of the following: hypertension, dyslipidemia, central obesity and microalbuminemia.
- Out of 21 patients with recurrent BC, 13 (61.9%) had MS.
- A significant association was evident between BC recurrence and MS, independent of other factors (p = 0.0002).
- A significant association was evident between MS and race (p = 0.004).
- No significant association was apparent between MS and any of the other patient or tumor characteristics studied, including the 21-gene RS.

Lakhani A et al. *Proc SABCS* 2012;Abstract PD10-02.

Effect of MS on BC Recurrence

Oncotype DX risk category	Odds ratio (presence vs absence of MS)	95% CI
Low risk (RS 0-17)	23.649	2.818-198.435
Intermediate risk (RS 18-30)	3.950	0.984-15.852
High risk (RS 31-100)	0.813	0.063-10.478

- A significant association was evident between BC recurrence and MS in patients with BC categorized as low risk by the Oncotype DX RS score.

Lakhani A et al. *Proc SABCS* 2012;Abstract PD10-02.

Analysis of MS According to Race

Race	MS		*p*-value*
	Presence	Absence	
Caucasian (n = 284)	24%	76%	0.0081
African American (n = 21)	52%	48%	
Hispanic (n = 11)	36%	64%	
Asian (n = 9)	44%	56%	
Other (non-Hispanic) (n = 1)	100%	0%	

* Chi-squared *p*-value

Lakhani A et al. *Proc SABCS* 2012;Abstract PD10-02.

Author Conclusions

- MS is an independent risk factor for BC recurrence among women with LN-negative, ER-positive, low-risk BC treated with standard adjuvant therapy.
- MS has an impact on recurrence for patients with a tumor biology defined by the 21-gene Onco*type* DX RS assay as low risk or, to a lesser extent, intermediate risk.
- However, there is no difference in recurrence risk for patients at high risk using the Onco*type* DX RS assay.
- Interventions directed at modifying MS in patients with newly diagnosed early BC have the potential to favorably affect survival for those with specific tumor characteristics.
- Prospective studies should be conducted to further evaluate the short- and long-term effects of MS on BC outcomes.

Lakhani A et al. *Proc SABCS* 2012;Abstract PD10-02.

Investigator Commentary: Impact of Metabolic Syndrome on BC Recurrence Using the 21-Gene Onco*type* DX Recurrence Score

The study started with 332 patients with ER-positive, LN-negative BC, of which 89 had MS according to the WHO criteria. A patient with MS must have diabetes or glucose intolerance in addition to 2 or more of hypertension, dyslipidemia, central obesity or microalbuminemia, several of which have been independently associated with poorer outcome. One finding was that MS was associated with race, with 52% of the African American study population having MS compared to 24% of Caucasians. With this study design it will be difficult to determine the condition driving poorer outcome. Although a multivariate analysis was performed, it was limited by the inclusion of only 89 patients. If race is the driving factor, it is important to note that it is associated with negative prognostic implications. Although use of the Onco*type* DX RS to study the relationship between MS and BC recurrence didn't add to our understanding in terms of disease outcome, this is an interesting and hypothesis-generating study.

Interview with Lisa A Carey, MD, January 17, 2013