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Evaluating the 21-Gene Assay Recurrence Score® as a Predictor of Clinical Response to 24 Weeks of Neoadjuvant Exemestane in Estrogen Receptor-Positive Breast Cancer

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ABSTRACT

Background

The aim of this study was to investigate the association of the Recurrence Score (RS) result with the clinical response to neoadjuvant endocrine therapy in postmenopausal women with breast cancer.

Methods

Core biopsy samples at baseline and post-treatment surgical samples were obtained from 80 and 77 of 116 patients, respectively, enrolled onto the multicenter prospective study of neoadjuvant exemestane therapy, JFMC34-0601. The 21 gene assay was performed after appropriate manual microdissection. Estrogen receptor (ER), progesterone receptor (PgR), HER2 and Ki-67 by immunohistochemistry were centrally evaluated. Clinical response was assessed based on the RECIST criteria.

Results

Sixty-four core biopsy samples and 52 resection samples met the Recurrence Score quality requirements. The clinical response rate in the low RS group (19/32, 59.4%) was significantly higher than that in the high RS group (3/15, 20.0%) ($P = 0.015$) and similar to that in the intermediate RS group (10/17, 58.8%). Breast conserving surgery (BCS) rates were 90.6% (29/32) in the low RS group, 76.5% (13/17) in the intermediate RS group and 46.7% (7/15) in the high RS group. The odds ratio for BCS adjusted for continuous baseline Ki-67 was 0.114 (95% CI, 0.014 to 0.721; P = 0.028) between the high and low RS groups. Recurrence Score

values in pre-treatment samples were highly correlated with those in post-treatment samples (Spearman correlation coefficient 0.745, 95% CI 0.592-0.846).

Conclusion

We demonstrated the predictive value of Recurrence Score results for clinical response to neoadjuvant exemestane therapy in postmenopausal women with ER-positive breast cancer.

Key words

Recurrence Score (RS); Neoadjuvant endocrine therapy; Ki67; clinical response; breast conserving surgery (BCS) rate

Mini-abstract

Recurrence Score (RS) was compared with the clinical response to neoadjuvant exemestane therapy in postmenopausal patients with ER-positive breast cancer. Baseline RS showed predictive value for clinical response.

Introduction:

Neoadjuvant therapy of breast cancer has potential advantages in improving outcomes in women with operable and inoperable early stage disease [1, 2]. Both neoadjuvant chemotherapy and endocrine therapy have been shown to enable less extensive resection and improve rates of breast-conserving surgery (BCS) [3-6]. The ACOSOG Z1031 trial, which compared three aromatase inhibitors in neoadjuvant settings, showed that 51% (81/159) of the patients who were designated candidates for mastectomy experienced down staging to BCS [7]. Neoadjuvant endocrine therapy is now an acceptable option for postmenopausal patients with endocrine responsive disease [8].

Despite the use of standard biomarkers, considerable heterogeneity of response to therapy still represents a challenge for clinical decision making when considering neoadjuvant therapy. The ability to better identify which patients will respond to therapy would represent a major clinical advance. Although Ki-67 labeling index (LI) shows some consistency in predicting response to chemotherapy, its ability to predict response to neoadjuvant endocrine therapy is controversial [9, 10].

We previously reported results from a neoadjuvant exemestane study in postmenopausal women [11]. In the study, the objective response rate was 51% (59/116) and 40 (77%) of 59 patients who would have required mastectomy were converted to BCS. Neither baseline Ki-67 LI nor changes in Ki-67 LI were associated with clinical response in the study.

The Onco*type* DX® assay has been shown to assess recurrence risk in women with hormone receptor positive (HR+), lymph node negative or positive, early stage breast cancer who are treated with adjuvant endocrine therapy [12-15]. It has also been shown to predict the likelihood

of benefit from adjuvant chemotherapy [12, 16]. Accordingly, the assay is included in clinical guidelines for use in patients with HR+ lymph node negative disease; however, its applicability to HR+ postmenopausal women with lymph node positive disease is considered controversial, pending results of the RxPONDER trial [8, 17-19]. Additionally, studies in the neoadjuvant setting have shown that the test can be used to predict the response to chemotherapy [20, 21]. More recently, a study suggested that the Recurrence Score may predict responses to neoadjuvant endocrine therapy with either tamoxifen or anastrozole [22]. The Onco*type* DX assay may improve the ability to discriminate between clinically similar tumors based on the tumor's underlying biology. This study was conducted to investigate the clinical usefulness of the Recurrence Score result for prediction of the response to neoadjuvant endocrine therapy.

Methods:

Study Design

This was a prospectively designed study using archived tumor tissues from the previously conducted JFMC34-0601 study. The primary objective was to assess the association between the Recurrence Score result at baseline and clinical response, by comparing the response rates between the low (<18) and high (≥31) Recurrence Score groups. Secondary objectives included assessment of the associations of continuous baseline Recurrence Score, quantitative estrogen receptor (ER) by RT-PCR and Ki-67 with clinical response and with BCS, as well as associations of changes from baseline to post-treatment values of these markers with clinical response. The study protocol was approved by the Ethics Committee in each institution. Informed consent was obtained from all patients. The study was performed in accordance with the Helsinki Declaration.

Patient Cohort and Tumor Samples

Eligibility criteria for the parent JFMC34-0601 study included: age 55-75 years, ER+ and stage II or IIIa invasive breast cancer (T2-3, N0-2, M0). Patients were confirmed positive for ER or progesterone receptor (PgR) by IHC (≥10% nuclear staining). Study treatment was 25 mg/day exemestane for 16 weeks with an 8-week extension according to the assessment of clinical response. Patients with progressive disease (PD) were withdrawn from the study. At week 24, patients underwent surgery, except those with PD, who had the option of selecting another treatment approach.

Clinical Outcomes Measures

Clinical response was assessed by comparing the longest diameter of the target lesions with the baseline measurement, based on Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.0 by caliper measurement of palpable lesions and ultrasound as previously described [11]. Briefly, complete response (CR) was defined by disappearance of all target lesions; partial response (PR) by at least a 30% decrease in the sum of diameters of target lesions; PD by at least a 20% increase in the sum of diameters of target lesions; stable disease (SD) by neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

Biomarker Assessments

The Onco*type* DX® 21-gene assay was performed on core biopsy and resection samples by Genomic Health [14].

Ki-67, ER and PgR by IHC were performed centrally and assessed by three independent pathologists as described previously [11]. In brief, IHC staining was performed using a Histofine

Kit (Nichirei, Tokyo, Japan). The Ki-67 was stained using the following antibody dilution: 1:100 (Dako, Glostrup, Denmark). Ki-67 LI was obtained by counting 500 to 1000 tumor cells at the sites of hot spots. Ki-67 groups were defined post-hoc as <10, 10-30, and >30%. ER and PgR immuno-reactivity were scored according to Allred's procedure.

Expression of HER2 was determined by the HercepTest (Dako). Positive HER2 status was defined as either 3+ or 2+ with confirmed c-erbB2 gene amplification by the FISH test.

Statistical Analyses

Analyses of baseline markers included all patients with an evaluable RT-PCR result from core biopsies. Analyses of changes from baseline to post-treatment markers included the subset of patients with results from both core biopsies and surgical resections. Changes of continuous markers were defined as "post-treatment value - pre-treatment value". In the primary analysis, the rates of clinical response were compared between the high and low baseline Recurrence Score groups using Fisher's exact test. Logistic regression models were fit to both clinical response and surgery type. Odds ratio (OR) estimates are presented with Wald p-values and 95% confidence intervals (CIs). All p-values are two-sided. In exploratory analyses, the Spearman rank correlation coefficient (and associated 95% CI) was calculated for baseline continuous Recurrence Score and either post-treatment Recurrence Score or baseline continuous Ki-67 by IHC. A paired t-test was applied to compare baseline and post-treatment Recurrence Score values. A two-sample t-test was used to compare the percent reduction in tumor size between the high and low Recurrence Score groups. Fisher's exact test was used to compare the conversion rate from mastectomy to BCS among risk groups.

Results:

One hundred sixteen patients were enrolled in JFMC34-0601 between March 2006 and December 2007. One hundred two patients completed 24 weeks of neoadjuvant exemestane treatment [11]. Core biopsy and resection samples were obtained for 80 (69%) and 77 (66%) patients, respectively. Of the 157 samples sent for Onco*type* DX testing, 2 were deemed ineligible per blinded Genomic Health pathology review. Insufficient RNA (<375ng) was extracted from 18 samples (15 core biopsy and 3 resection). Standard quality metrics were not met for 8 samples (all resections), leaving 64 core biopsy samples, of which 52 had matching resection samples with evaluable RT-PCR results.

Baseline characteristics and clinical outcomes for the 64 patients are shown in Table 1. Forty-nine (76.6%) patients had BCS while 32 patients (50%) had been candidates for BCS before the treatment. Four patients refused surgery after exemestane therapy and are treated as not BCS.

In the primary analysis, the clinical response rate in the low Recurrence Score group (19/32=59.4%) was significantly higher than that in the high Recurrence Score group (3/15=20.0%) (p=0.015) (Table 2). The clinical response rate in the intermediate risk group (10/17=58.8%) was similar to that in the low risk group. When analyzed by logistic regression, the OR for clinical response between the intermediate and low Recurrence Score groups was 0.977 (95% CI 0.296-3.233, p=0.970) and between the high and low Recurrence Score groups was 0.171 (95% CI 0.040-0.728, p=0.017). In an exploratory analysis, percent reduction in tumor size determined by ultrasound was compared between low and high Recurrence Score groups. Patients in the low Recurrence Score group showed 31.8% reduction in size on average while those in the high Recurrence Score group showed 12.5% reduction, indicating significant

difference between the groups (p=0.045). The average reduction (27.6%) in patients in the intermediate risk group was similar to that in the low risk group.

When treated as a continuous variable, the baseline Recurrence Score was significantly associated with clinical response in a logistic regression analysis (p=0.042, Table 3). There was a trend between continuous baseline ER by RT-PCR and clinical response (p=0.076). Continuous baseline Ki-67 by IHC was not associated with clinical response (p=0.273).

The associations between changes from baseline to post-treatment values of continuous markers and clinical response were examined in logistic regression analyses. Changes in Recurrence Score, ER by RT-PCR, and Ki-67 by IHC were not associated with clinical response (p=0.240, p=0.343, and p=0.629, respectively).

Analysis of Recurrence Score categories and BCS is shown in Table 2. The OR for BCS between the intermediate and low Recurrence Score groups was 0.336 (95% CI 0.066-1.722, p=0.19) and between the high and low Recurrence Score groups was 0.091 (95% CI 0.019-0.432, p=0.003). The logistic regression analyses of continuous baseline Recurrence Score, ER by RT-PCR and Ki-67 by IHC with BCS are shown in Table 3. Continuous baseline Recurrence Score was significantly associated with BCS in both unadjusted (p=0.001) and covariate-adjusted (for tumor size and PgR) (p=0.004) analyses. Continuous baseline ER by RT-PCR was also significantly associated with BCS in both unadjusted (p=0.001) and covariate-adjusted (p=0.023) analyses. Continuous baseline Ki-67 by IHC was significantly associated with BCS in unadjusted analysis (p=0.024) but lost its significance when adjusted for tumor size and PgR (p=0.060). When both continuous Recurrence Score values and continuous Ki-67 were included in the logistic regression model for BCS, the Recurrence Score retained its statistical significance

(p=0.012) whereas Ki-67 did not (p=0.868). The conversion rate from mastectomy planned at baseline to BCS performed after the treatment was 88% (15/17) in the low Recurrence Score group, 70% (7/10) in the intermediate Recurrence Score group and 20% (1/5) in the high Recurrence Score group. The rate was significantly different among groups (p=0.010).

The associations between Recurrence Score and Ki-67, and their respective and joint associations with BCS were examined in exploratory analyses. Figure 1(a) is a scatter plot of baseline Ki-67 by IHC vs. baseline Recurrence Score results. The Spearman correlation coefficient was 0.672 (95% CI 0.506-0.785). All patients with PD had high Recurrence Score values (range: 32 - 73) while three of five PD patients showed intermediate Ki-67 LI {Figure 1(a)}.

No statistically significant difference was observed between baseline and post-treatment Recurrence Score values (p=0.484). A scatter plot is shown in Figure 1(b). The Spearman correlation analysis showed a high correlation (correlation coefficient 0.745, 95% CI 0.592-0.846).

Discussion:

In this study, we demonstrated the predictive value of Recurrence Score results for response to neoadjuvant endocrine therapy. Patients with low scores showed a better response to neoadjuvant endocrine therapy than those with high scores. Since patients with high Recurrence Score results have been shown to benefit from chemotherapy, the 21-gene assay may provide additional information in selecting neoadjuvant treatment with endocrine therapy for low Recurrence Score and chemotherapy for high Recurrence Score cancers.

ER Allred scores have been reported to correlate with response rates to neoadjuvant letrozole or tamoxifen. The P024 trial of neoadjuvant letrozole or tamoxifen showed that tumors with low ER Allred scores still responded to letrozole [23]. Conversely, some tumors with higher ER levels did not respond to endocrine therapy [23, 24]. Gene expression-based profiles categorize HR+, HER2- breast cancers into two subtypes: luminal-A and luminal-B [25]. However, the classification, as determined by PAM50, is reported not to relate to clinical response or the likelihood of BCS after neoadjuvant AI treatment [7].

In our study, Recurrence Score was the only predictive factor for clinical responses to neoadjuvant endocrine therapy and the most potent predictive factor for BCS in the covariate-adjusted analysis. These results are consistent with other studies that suggest that low Recurrence Score can predict benefit from endocrine therapy [22, 24]. The study by Kim et al. compared the outcomes of the tamoxifen and placebo arms of the NSABP B14 trial and demonstrated that higher levels of quantitative ER expression by RT-PCR correlated with greater benefit from adjuvant tamoxifen as measured by distant recurrence [24].

Our results indicate that Recurrence Score values before and after endocrine therapy were highly correlated. Since a number of studies have suggested that post-treatment biomarkers such as Ki-67 LI and ER have better prognostic values than pre-treatment biomarkers, post-treatment biomarkers are receiving increasing interest for patient stratification in clinical trials [26-28]. Dowsett et al. reported the results of an unplanned, exploratory investigation of the relationship between post-treatment Ki-67 (2 weeks) and recurrence free survival (RFS) using archived tumors from the IMPACT study [26]. Their results indicated that post-treatment Ki-67, larger baseline tumor size, and post-treatment ER level were significantly correlated with DFS. Ellis et al. analyzed the ability of post-treatment Ki-67 and other factors (tumor size, grade, nodal status,

and post-treatment ER expression) to predict RFS and breast cancer specific survival using archived tumors from the P024 study [27]. An interesting study (ACOSOG Z1031, Cohort B) has been conducted to determine whether patients with a high Ki-67 value after two weeks of neoadjuvant AI treatment show a higher than expected pCR rate to neoadjuvant chemotherapy than would be typically observed for those patients with unselected ER-rich tumors and will tell us whether assessment of Ki-67 two weeks after neoadjuvant AI treatment is useful for the identification of a chemotherapy sensitive subgroup of ER+ tumors. However, even if this is the case, intervention of two weeks AI treatment and re-biopsy are necessary. Although further investigations are needed, the comparative stability of Recurrence Score would improve the decision making of the whole treatment before the initiation of treatment.

This study's primary limitation was small sample size. Availability of tumor samples from the parent study was limited and recovery of mRNA was not uniformly adequate. Further investigation in larger prospective studies would better define candidates for neoadjuvant endocrine therapy. Another limitation was no assessment of lymph node response. Although nodal response is clinically relevant, considering one of the major purposes of neoadjuvant endocrine therapy is improvement in surgical outcome, the clinical response in the primary site and the BCS rate are also of clinical importance for the assessment of the effect of neoadjuvant endocrine therapy.

In conclusion, this study showed a predictive value of Recurrence Score results for clinical response to neoadjuvant exemestane therapy. The 21-gene assay shows promise for providing useful information to guide neoadjuvant treatment selection for systemic therapy, with neoadjuvant endocrine treatment for patients with low Recurrence Score disease and neoadjuvant chemotherapy treatment for patients with high Recurrence Score disease.

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Figure legends

Figure 1

(a) Scatterplot of Baseline RS and Baseline Ki-67, with Spearman Correlation Coefficient

The Spearman correlation coefficient between baseline RS and baseline Ki67 was 0.672 (95% CI 0.506-0.785). None of five patients with tumor progression was in the low or intermediate RS groups.

(b) Scatterplot of Baseline RS and Post-treatment RS, with Spearman Correlation Coefficient

Baseline RS was highly correlated with RS in post-treatment samples (Spearman correlation coefficient 0.745, 95% CI 0.592-0.846).

Table 1: Baseline Patient Characteristics and Clinical Outcomes (n=64)

Feature	n (%)		Feature	n (%)
Age (years)			HER2 by IHC/FISH	
55-64	34 (53.1%)		Negative	50 (78.1%)
65-74	25 (39.1%)		Positive	2 (3.1%)
75-77	5 (7.8%)		Unknown	12 (18.8%)
Tumor Stage at baseline			RS Risk Group	
T2	62 (96.9%)		Low (< 18)	32 (50.0%)
T3	2 (3.1%)		Intermediate (18 - 30)	17 (26.6%)
			High (≥ 31)	15 (23.4%)
Stage				
IIA	47 (73.4%)		Ki-67 by IHC (%)	
IIB	15 (23.4%)		<10	28 (43.8%)
IIIA	2 (3.1%)		10-30	23 (35.9%)
			>30	13 (20.3%)
ER by IHC (Allred Score)				
4	1 (1.6%)		Clinical Response	
5	3 (4.7%)		CR	0
6	5 (7.8%)		PR	32 (50.0%)
7	14 (21.9%)		SD	24 (37.5%)
8	41 (64.1%)		PD	5 (7.8%)
			NE	3 (4.7%)
ER Status by RT-PCR				
ER- (≤6.5C_T)	1 (1.5%)		Surgery Type	
ER+ (>6.5C_T)	63 (98.4%)		Breast-Conserving	49 (76.6%)
			Mastectomy	11 (17.2%)
PgR by IHC (Allred Score)			No surgery	4 (6.3%)
0	4 (6.25%)			
4	7 (10.94%)			
5	4 (6.25%)			
6	8 (12.5%)			
7	19 (29.69%)			
8	12 (18.75%)			
NE	10 (15.63%)			
PgR Status by RT-PCR				
PgR- (≤ 5.5 C_T)	14 (21.9%)			
PgR+ (> 5.5 C_T)	50 (78.1%)			

Table 2: Clinical Response and BCS according to Categorical Baseline RS

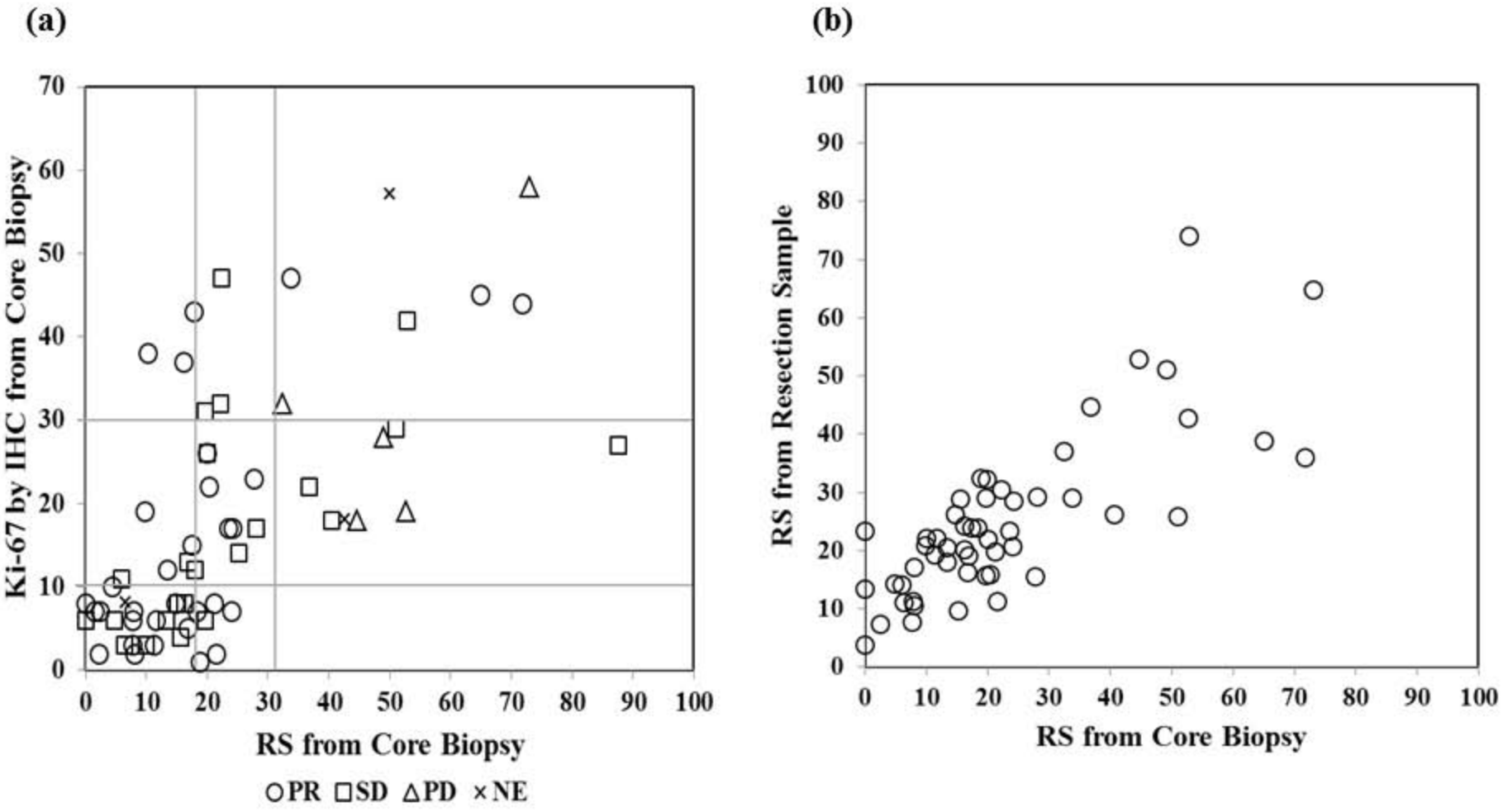
RS Risk Group	Clinical Response		
	Proportion(Response Rate)*	Odds Ratio(95% CI)	p-value
Low (RS <18)	19/32(59.4%)	1	n/a
Int. (RS 18 - 30)	10/17(58.8%)	0.977(0.296, 3.233)	0.970
High (RS ≥31)	3/15(20.0%)	0.171(0.040, 0.728)	0.017
RS Risk Group	Breast-Conserving Surgery		
	Proportion(BCS Rate)	Odds Ratio(95% CI)	p-value
Low (RS <18)	29/32(90.6%)	1	n/a
Int. (RS 18 - 30)	13/17(76.5%)	0.336(0.066, 1.722)	0.19
High (RS ≥31)	7/15(46.7%)	0.091(0.019, 0.432)	0.003

*Primary analysis: p=0.015 by Fisher's exact test for comparison of clinical response rates between the Low and High RS Groups.

Table 3: Continuous Baseline RS, ER by RT-PCR and Ki-67 by IHC and Clinical Response and Breast-Conserving Surgery (BCS)

Endpoint/ Analysis	Continuous Marker					
	Recurrence Score (50 units)		ER by RT-PCR (log2 increase)		Ki-67 by IHC (%)	
	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value
Clinical Response / Unadjusted	0.205 (0.044, 0.946)	0.042	1.436 (0.963, 2.141)	0.076	0.981 (0.948, 1.015)	0.273
BCS / Unadjusted	0.055 (0.009, 0.323)	0.001	1.786 (1.150, 2.774)	0.001	0.957 (0.921, 0.994)	0.024
BCS / Covariate-adjusted*	0.016 (<0.001, 0.259)	0.004	1.881 (1.090, 3.245)	0.023	0.953 (0.907, 1.002)	0.060

* Adjusted for tumor size and PgR Allred score, which were significantly associated with breast-conserving surgery in univariable analyses.


(a)
Ki-67 by IHC from Core Biopsy
RS from Core Biopsy
PR SD PD NE
(b)
RS from Resection Sample
RS from Core Biopsy