Changes in Recurrence Score by neoadjuvant endocrine therapy of breast cancer and their prognostic implication

Takayuki Ueno,[1] Shigehira Saji,[2] Norikazu Masuda,[3] Hiroji Iwata,[4] Katsumasa Kuroi,[5] Nobuaki Sato,[6] Hiroyuki Takei,[7] Yutaka Yamamoto,[8] Shinji Ohno,[9] Hiroko Yamashita,[10] Kazufumi Hisamatsu,[11] Kenjiro Aogi,[12] Hironobu Sasano,[13] Masakazu Toi[14]

Correspondence to
ProfessorShigehiraSaji; ss-saji@wa2.so-net.ne.jp

ABSTRACT

Background Neoadjuvant endocrine therapy (NET) can improve surgical outcomes in postmenopausal patients with hormone receptor-positive breast cancer. The Ki67 labelling index after NET has a better prognostic power than that at baseline. However, it remains unknown whether a multigene assay with post-treatment samples could predict the prognosis better than that with pretreatment samples.

Methods The prognostic value of the multigene assay Oncotype DX Recurrence Score (RS) was investigated using pretreatment and post-treatment samples from a multicentre NET trial, JFMC34-0601 (UMIN C000000345), where exemestane was given at 25 mg/day for 24 weeks.

Results Both pretreatment and post-treatment RSs were significantly associated with disease-free survival (DFS) (p=0.005 and 0.002, respectively). The combination of pretreatment and post-treatment RSs was also a predictor of DFS (p=0.002) and superior to preoperative endocrine prognostic index (PEPI). Furthermore, combined RS was the only independent prognostic factor in the multivariate analysis among the three RSs (p=0.04). In addition, combined RS could differentiate early recurrence in the high-risk group from mid/late recurrence in the intermediate-risk group, suggesting possible differential treatment strategies based on the risk categories indicated by the combined RS.

Conclusions The combination of pretreatment and post-treatment RSs could provide pivotal information for predicting DFS and differentiating early recurrence in the high-risk group from mid/late recurrence in the intermediate-risk group in patients with hormone receptor-positive breast cancer. A larger study is required to validate the results.

Key questions

What is already known about this subject?
► Ki67 labelling index provides more accurate information after 2 weeks of neoadjuvant endocrine therapy than at baseline for predicting the clinical outcome.
► Oncotype DX Recurrence Score predicts clinical response to neoadjuvant endocrine therapy.

What does this study add?
► The combination of pretreatment and post-treatment Recurrence Scores predicted disease-free survival better than either alone.
► The combined Recurrence Score differentiated early recurrence in the high-risk group from mid/late recurrence in the intermediate-risk group.

How might this impact on clinical practice?
► Possible differential treatment strategies including addition of chemotherapy and extension of endocrine therapy can be applied based on the risk categories indicated by the combined Recurrence Score.

INTRODUCTION

Neoadjuvant endocrine therapy (NET) has been employed to improve surgical outcomes for postmenopausal patients with hormone receptor-positive breast cancer. It has been shown to increase the rate of breast conservation.[1–3] The conversion rate from mastectomy to breast-conserving surgery has been reported to be 44%, 31% and 24% in those who received neoadjuvant anastrozole, tamoxifen and both, respectively.[3]

The long-term outcomes of NET have been studied in association with post-treatment tumour biology. It has been reported that the Ki67 labelling index provides more accurate information after 2 weeks of NET than at baseline for predicting the eventual clinical outcome.[4] A cumulative index or scoring system has been proposed, which comprises post-treatment clinical and biological characteristics, such as tumour size, nodal status, oestrogen receptor (ER) status and Ki67 index. The index is called as preoperative endocrine prognostic index (PEPI); PEPI indicates the long-term clinical outcome of patients better than baseline tumour characteristics.[5 6] However, it remains unclear whether a multigene assay using
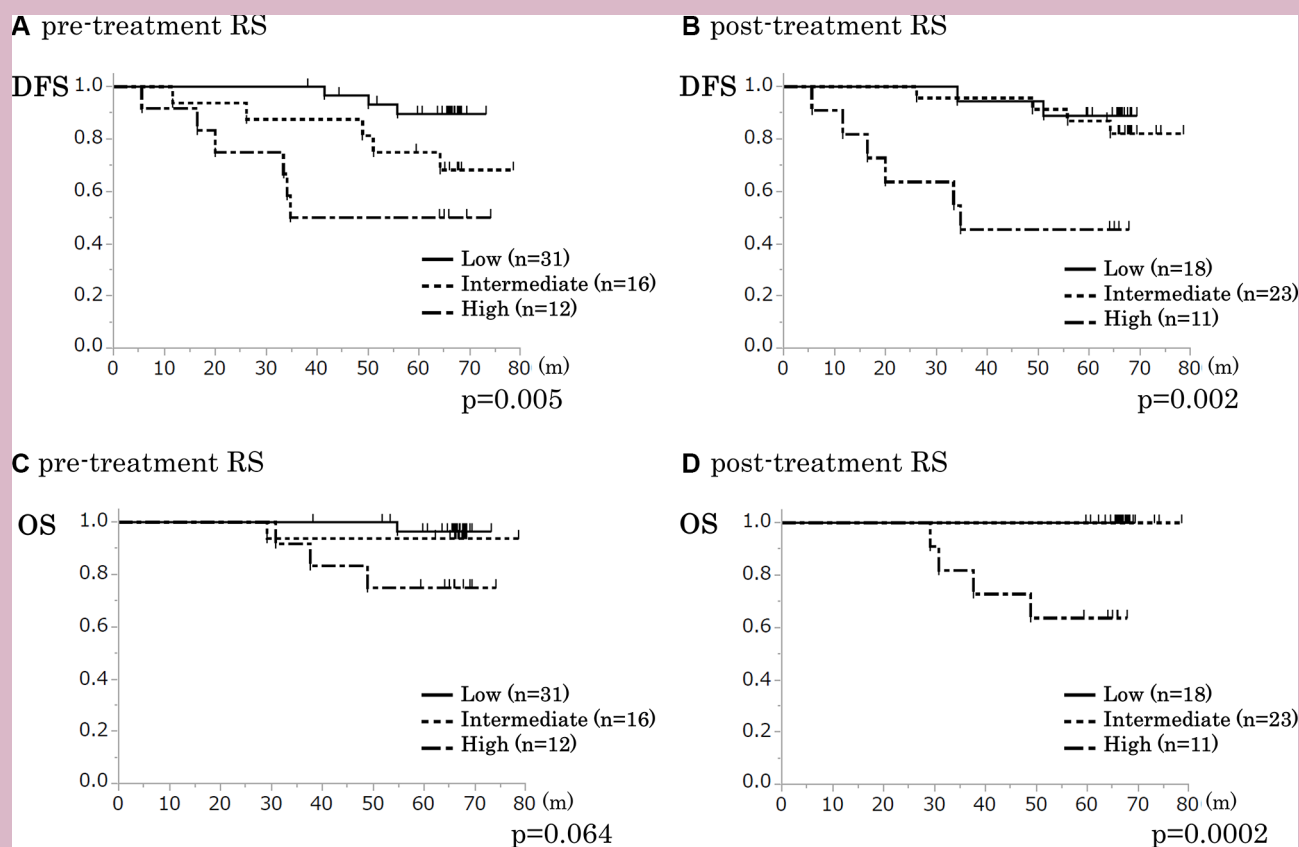
Figure 1 (A) Disease-free survival (DFS) according to pre-treatment Recurrence Score (RS). RS measured with pre-treatment biopsy samples correlated with DFS (p=0.005). (B) DFS according to post-treatment RS. Post-treatment RS correlated with DFS (p=0.002). (C) Overall survival (OS) according to pre-treatment RS. Pre-treatment RS was not significantly associated with OS (p=0.064). (D) OS according to post-treatment RS. Post-treatment RS significantly associated with OS (p=0.0002).

post-treatment samples predicts the long-term outcomes better than that using pretreatment samples.

We previously reported that Oncotype DX Recurrence Score (RS) predicts clinical response to NET and that RS changes after NET, although the change is not statistically significant.[7] In this study, we investigated the prognostic value of the multigene assay RS using both pretreatment and post-treatment tissue samples from a multicenter prospective clinical trial of neoadjuvant exemestane therapy. We found that both pretreatment and post-treatment RSs had prognostic values. However, combined RS, comprising both pretreatment and post-treatment RSs, had a better prognostic value for long-term outcomes in patients who received NET.

PATIENTS AND METHODS

JFMC34-0601 is a multicentre phase II trial to assess the response and safety of neoadjuvant exemestane treatment in postmenopausal patients with ER-positive breast cancer (registration number: UMIN C000000345, figure 1). Postmenopausal female patients with histologically confirmed stage II or IIIa infiltrating ER-positive breast cancer were eligible. ER positivity was defined as ≥10% nuclear staining. Exemestane was given at 25 mg/day for 16 weeks with an 8-week extension unless

progressive disease (PD) was found. Patients underwent surgery at 24 weeks. Patients with PD were excluded and offered appropriate alternative treatment, including surgery. Clinical responses were assessed by investigators according to the Response Evaluation Criteria in Solid Tumours V.1.0, as previously described, by combining the calliper measurements and images, including those of ultrasound, CT and MRI.[8]

The methods for measuring biomarker levels have been described.[7 8] The Oncotype DX RS was calculated using core biopsy and resection samples by Genomic Health (Redwood City, California, USA).[9]

After surgery, patients received standard adjuvant therapy, including endocrine therapy, chemotherapy and radiation. Exemestane was continued after surgery, except for patients with PD. Adjuvant chemotherapy included anthracycline-based regimen, taxane-based regimen, combination of anthracyclin and taxane and cyclophosphamide, methotrexate, 5-fluorouracil.

Informed consent was obtained from all patients who participated in this study. The study was performed in accordance with the Helsinki Declaration.

Statistical analysis

Disease-free survival (DFS) and overall survival (OS) were estimated using the Kaplan-Meier method and

log-rank test. Multivariate analysis was performed using the Cox proportional hazards model. A two-sided p-value of <0.05 was considered significant. All statistical analyses were performed by JMP V.13.2.0 (SAS Institute Japan, Tokyo).

RESULTS

In total, 116 patients were enrolled in the JFMC34-0601 study between March 2006 and December 2007, of whom 102 completed 24 weeks of neoadjuvant exemestane treatment (online supplementary figure 1). Core biopsy and surgical resection samples were retrieved from 80 and 77 patients, respectively. Of the available core biopsy samples, 16 could not be evaluated for RS because of insufficient quality or quantity of RNA. Five patients were excluded from the analysis because three had human epidermal growth factor receptor 2-positive tumour and two had no follow-up data. Therefore, 59 core biopsy samples were used for the analysis in this study. Fifty-two matching resection samples were available for providing RS. The background data of the 59 analysed patients are summarised in table 1.

Prognostic value of pretreatment and post-treatment RSs

The median follow-up period of the study was 67.0 months. RS measured with pretreatment biopsy samples correlated well with DFS in patients who received NET (p=0.005, figure 1A). The 5-year DFS was 90.0%, 75.0% and 50.0% in the low-risk, intermediate-risk and high-risk groups, respectively.

RS was also measured using post-treatment surgical samples. The post-treatment RS also correlated with DFS (p=0.002; figure 1B). The 5-year DFS was 88.9%, 87.0% and 45.5% in the low-risk, intermediate-risk and high-risk groups, respectively.

RSs were also analysed in association with OS. The post-treatment RS significantly associated with OS (p=0.0002), whereas pretreatment RS was not significantly associated with OS (p=0.064, figure 1C,D).

Combined analysis of pretreatment and post-treatment RSs

The combined analysis of both pretreatment and post-treatment RSs was performed. The risk was defined as low when both pretreatment and post-treatment RSs were low and as high when either pretreatment or post-treatment RS was high. All other situations were classified as intermediate risk. The risk classification is depicted in figure 2A.

This combined RS system was well associated with DFS (p=0.002, figure 2B). Early recurrence occurred mostly in the high-risk group and mid/late recurrence occurred mainly in the intermediate-risk group. No recurrence was observed in the low-risk group in this study population.

Combined RS was also significantly associated with OS (p=0.002, figure 2C).

Table 1 Patient's background characteristics

Factor	N
Total	59
Age	
55–59	9
60–69	33
70–79	17
T	
2	57
3	2
N	
0	43
1	16
Clinical stage	
IIA	43
IIB	14
IIIA	2
ER	
+	59
–	0
PgR	
+	55
–	4
HER2	
+	0
–	59
Adjuvant chemotherapy	
+	18
–	41

ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor.

Prognostic value of RS in comparison with PEPI

We have shown that PEPI is associated with DFS in the JFMC34-0601 study.[10] Therefore, we compared all the three types of RSs with PEPI. Because pretreatment and post-treatment and combined RSs were correlated with each other, each RS was separately compared with PEPI using the Cox proportional hazards model (table 2). Among the three models, combined RS showed independent prognostic power (p=0.0096), and the post-treatment RS was marginally significant (p=0.047), whereas pretreatment RS did not show statistical significance (p=0.058, table 2). PEPI was not an independent prognostic factor for DFS in any of the three models (table 2).

Subset analyses

Because nodal involvement (No) and adjuvant chemotherapy are considered to be associated with prognosis, it is important to assess the prognosis in patients
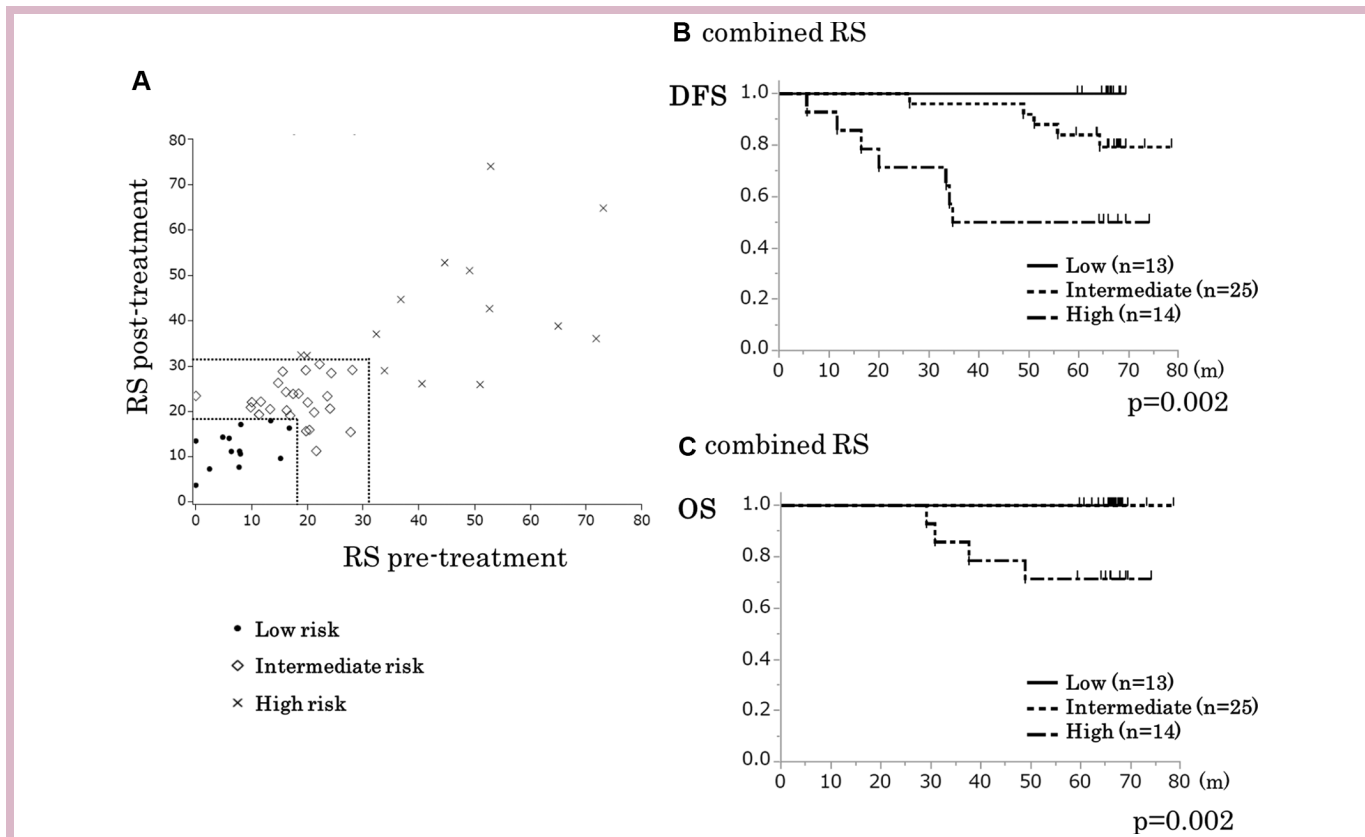
Figure 2 (A) Risk classification based on the combination of pretreatment and post-treatment Recurrence Scores (RSs). Low risk: both pretreatment and post-treatment RSs were low. High risk: either pretreatment or post-treatment RS was high. Intermediate risk: all other situations. (B) Disease-free survival (DFS) according to combined RS. Combined RS was well associated with DFS (p=0.002). (C) Overall survival (OS) according to combined RS. Combined RS was significantly associated with OS (p=0.002).

without N0 and in those who have not received chemotherapy. When analyses were restricted to N0 patients, similar results on DFS were observed for pretreatment and post-treatment and combined RSs (online supplementary figure 2A-C). All N0 patients had T2 tumour. In this population, combined RS indicated early recurrence in the high-risk group and mid/late recurrence in the intermediate-risk group, which is consistent with the result from the whole study population (online supplementary figure 2C).

Subsequently, DFS was analysed according to chemotherapy use. Patients who received adjuvant chemotherapy had higher T and N stages and exhibited a poorer DFS than those who did not (online supplementary table 1 and online supplementary figure 3A). Then, DFS was analysed in a subset of patients who did not receive adjuvant chemotherapy (online supplementary figure 3B-D). Combined RS indicated early recurrence in the high-risk group and mid/late recurrence in the intermediate-risk group (p=0.032), while neither

Table 2 RS and PEPI

| | | Univariate analysis | Multivariate analysis | | |
			1	2	3
PEPI	0 versus ≧1	**0.022**	0.53	0.35	0.49
RS	Low versus intermediate versus high				
Pretreatment		**0.005**	0.058		
Post-treatment		**0.002**		**0.047**	
Combined		**0.002**			**0.0096**

Bold values mean statistical significance.
PEPI, preoperative endocrine prognostic index; RS, Recurrence Score.

Table 3 Univariate and multivariate analysis for DFS

		Univariate analysis	Multivariate analysis		
			1	2	3
T	T2 versus T3	**0.005**	0.45	0.39	0.5
N	Negative versus positive	0.100	0.34	0.59	0.43
Ki67	<11 versus ≧11	**0.050**	0.87	0.92	0.47
Clinical response	PR versus SD versus progressive disease	**<0.0001**	0.15	0.55	0.28
Adjuvant chemotherapy	Negative versus positive	**0.015**	0.93	0.48	0.77
RS	Low versus intermediate versus high				
Pretreatment		**0.005**	0.47		
Post-treatment		**0.002**		0.42	
Combined		**0.002**			**0.04**

Bold values mean statistical significance.
DFS, disease-free survival; PR, partial response; RS, Recurrence Score; SD, stable disease.

pretreatment nor post-treatment RS significantly correlated with the outcomes.

Multivariate analysis for DFS

To further investigate the prognostic value of the three RSs, multivariate analyses including recognised prognostic factors, such as T stage, nodal status, Ki67 index, adjuvant chemotherapy use and clinical response to NET were performed. Among the three models, only combined RS was independently associated with DFS (p=0.04), unlike pretreatment or post-treatment RS (table 3).

DISCUSSION

To the best of our knowledge, this is the first report to show that post-treatment RS after NET is a prognostic factor for DFS and that combined RS is the most potent prognostic factor for DFS among the three RSs in patients with ER-positive breast cancer. In addition, our results suggested that combined RS is useful to differentiate between early recurrence in the high-risk group and mid/late recurrence in the intermediate-risk group.

It is clinically critical to differentially predict early and late recurrence to determine which patients require chemotherapy or endocrine therapy of >5 years. There are several promising multigene assays to predict late recurrence. PAM50 risk of recurrence and EndoPredict are promising assays to predict late recurrence, but they cannot differentiate between the risks of early and late recurrence.[11–13] RS and Breast Cancer Index have also been reported to be useful to predict early and late recurrence; however, they cannot distinguish between early and late recurrence in the high risk group.[14 15] Thus, if the combined analysis of pretreatment and post-treatment RSs is useful in differentially predicting early and mid/late recurrence, it is clinically useful to consider different treatment strategies for adjuvant therapy in different risk

groups. A larger prospective study is required to validate the results.

Although the study included both node-positive and node-negative patients, consistent results were obtained even when analyses were restricted to N0 patients. In addition, after making adjustment by nodal status in the multivariate analysis (table 2), combined RS remained significant, suggesting that it is useful in predicting recurrence regardless of nodal status. However, this must be validated by larger prospective studies, preferably in two independent cohorts with and without nodal involvement.

There were several limitations in this study. First, the sample size was small because of the restricted number of samples for RS. In particular, the combined analysis required matching samples between pretreatment and post-treatment samples, which further reduced the sample size. Second, the population in this study included both patients who had received adjuvant chemotherapy and those who had not. To address this, analyses in the subpopulation of patients who did not receive adjuvant chemotherapy were performed, and the prognostic value of combined RS was confirmed (online supplementary figure 3D). In addition, multivariate analysis was performed to adjust for chemotherapy use, and the results showed that combined RS was independently associated with DFS (table 2). However, the results should be interpreted with caution and keeping this limitation in mind. Third, the study could not determine whether RSs have a predictive value for chemotherapy benefits. It is important to determine whether combined RS predicts chemotherapy benefit better than that by pretreatment or post-treatment RS. This is crucial when considering the clinical use of combined RS; therefore, the results should be verified using samples from larger clinical trials, such as the NEOS (New Primary Endocrine-therapy Origination Study) trial.[16]

In conclusion, both pretreatment and post-treatment RSs were associated with DFS, and combined RS was the most potent prognostic factor for DFS among the three RSs. In addition, combined RS could differentiate early recurrence in the high-risk group from mid/late recurrence in the intermediate-risk group. Larger prospective studies are required to validate these results and investigate the predictive value of combined RS for chemotherapy benefits.

Author affiliations
[1]Breast Surgical Oncology, Cancer Institute Hospital, Tokyo, Japan
[2]Medical Oncology, Fukushima Medical University, Fukushima, Japan
[3]Department of Surgery, Breast Oncology, National Hospital Organization Osaka National Hospital, Osaka, Japan
[4]Department of Breast Oncology, Aichi Cancer Center, Nagoya, Japan
[5]Surgery, Tokyo Metropolitan Health and Medical Treatment Cooperation Ebara Hospital, Tokyo, Japan
[6]Department of Breast Oncology, Niigata Cancer Center Hospital, Niigata, Japan
[7]Department of Breast Surgery and Oncology, Nippon Medical School, Tokyo, Japan
[8]Department of Breast and Endocrine Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan
[9]Breast Oncology Center, Cancer Institute Hospital, Tokyo, Japan
[10]Department of Breast Surgery, Hokkaido University Hospital, Sapporo, Japan
[11]Oikawa Hospital, Fukuoka, Japan
[12]Department of Breast Surgery, National Hospital Organization Shikoku Cancer Center, Ehime, Japan
[13]Department of Pathology, Tohoku University School of Medicine, Sendai, Japan
[14]Breast Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Patient consent for publication Not required.

Ethics approval The study protocol was approved by the Ethics Committees of participating institutions.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES
1. Cataliotti L, Buzdar AU, Noguchi S, et al. Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. *Cancer* 2006;106:2095–103.
2. Eiermann W, Paepke S, Appfelstaedt J, et al. Preoperative treatment of postmenopausal breast cancer patients with letrozole: a randomized double-blind multicenter study. *Ann Oncol* 2001;12:1527–32.
3. Smith IE, Dowsett M, Ebbs SR, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the immediate preoperative anastrozole, tamoxifen, or combined with tamoxifen (IMPACT) multicenter double-blind randomized trial. *JCO* 2005;23:5108–16.
4. Dowsett M, Smith IE, Ebbs SR, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. *J Natl Cancer Inst* 2007;99:167–70.
5. Ellis MJ, Suman VJ, Hoog J, et al. Ki67 proliferation index as a tool for chemotherapy decisions during and after neoadjuvant aromatase inhibitor treatment of breast cancer: results from the American College of surgeons Oncology Group Z1031 trial (Alliance). *JCO* 2017;35:1061–9.
6. Ellis MJ, Tao Y, Luo J, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst* 2008;100:1380–8.
7. Ueno T, Masuda N, Yamanaka T, et al. 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. *Int J Clin Oncol* 2014;19:607–13.
8. Toi M, Saji S, Masuda N, et al. Ki67 index changes, pathological response and clinical benefits in primary breast cancer patients treated with 24 weeks of aromatase inhibition. *Cancer Sci* 2011;102:858–65.
9. 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* 2004;351:2817–26.

10. Ueno T, Saji S, Masuda N, *et al*. Impact of clinical response to neoadjuvant endocrine therapy on patient outcomes: a follow-up study of JFMC34-0601 multicentre prospective neoadjuvant endocrine trial. *ESMO Open* 2018;3:e000314.

11. Dubsky P, Brase JC, Jakesz R, *et al*. The EndoPredict score provides prognostic information on late distant metastases in ER+/HER2- breast cancer patients. *Br J Cancer* 2013;109:2959–64.

12. Sestak I, Cuzick J, Dowsett M, *et al*. Prediction of late distant recurrence after 5 years of endocrine treatment: a combined analysis of patients from the Austrian breast and colorectal Cancer Study group 8 and arimidex, Tamoxifen alone or in combination randomized trials using the PAM50 risk of recurrence score. *JCO* 2015;33:916–22.

13. Sestak I, Dowsett M, Zabaglo L, *et al*. Factors predicting late recurrence for estrogen receptor-positive breast cancer. *J Natl Cancer Inst* 2013;105:1504–11.

14. Sgroi DC, Sestak I, Cuzick J, *et al*. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. *Lancet Oncol* 2013;14:1067–76.

15. Wolmark N, Mamounas EP, Baehner FL, *et al*. Prognostic Impact of the Combination of Recurrence Score and Quantitative Estrogen Receptor Expression (*ESR1* on Predicting Late Distant Recurrence Risk in Estrogen Receptor–Positive Breast Cancer After 5 Years of Tamoxifen: Results From NRG Oncology/National Surgical Adjuvant Breast and Bowel Project B-28 and B-14. *JCO* 2016;34:2350–8.

16. Iwata H, Masuda N, Fujisawa T, *et al*. NEOS: a randomized, open label, phase 3 trial of adjuvant chemotherapy for postmenopausal breast cancer patients who responded to neoadjuvant letrozole: first report of long-term outcome and prognostic value of response to neoadjuvant endocrine therapy. *2017 San Antonio Breast Caner Symposium*, San Antonio, 2017:P3-13–03.