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Breast Cancer

High Proliferation Predicts Pathological Complete Response to Neoadjuvant Chemotherapy in Early Breast Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Ki67 • Breast cancer • Neoadjuvant chemotherapy • Chemosensitivity • Predictive factor

Abstract

Background. In the neoadjuvant setting, changes in the proliferation marker Ki67 are associated with primary endocrine treatment efficacy, but its value as a predictor of response to chemotherapy is still controversial.

Patients and Methods. We analyzed 262 patients with centralized basal Ki67 immunohistochemical evaluation derived from 4 GEICAM (Spanish Breast Cancer Group) clinical trials of neoadjuvant chemotherapy for breast cancer. The objective was to identify the optimal threshold for Ki67 using the receiver-operating characteristic curve method to maximize its predictive value for chemotherapy benefit. We also evaluated the predictive role of the defined Ki67 cutoffs for molecular subtypes defined by estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2).

Results. A basal Ki67 cutpoint of 50% predicted pathological complete response (pCR). Patients with Ki67 >50% achieved a pCR rate of 40% (36 of 91) versus a pCR rate of 19% in patients with Ki67 ≤50% (33 of 171) ($p = .0004$). Ki67 predictive value was especially relevant in ER-HER2− and ER-HER2+ patients (pCR rates of 42% and 64%, respectively, in patients with Ki67 >50% versus 15% and 45%, respectively, in patients with Ki67 ≤50%; $p = .0337$ and .3238, respectively). Both multivariate analyses confirmed the independent predictive value of the Ki67 cutpoint of 50%.

Conclusion. Basal Ki67 proliferation index >50% should be considered an independent predictive factor for pCR reached after neoadjuvant chemotherapy, suggesting that cell proliferation is a phenomenon closely related to chemosensitivity. These findings could help to identify a group of patients with a potentially favorable long-term prognosis. ***The Oncologist*** 2016;21:150–155

Implications for Practice: The use of basal Ki67 status as a predictive factor of chemotherapy benefit could facilitate the identification of a patient subpopulation with high probability of achieving pathological complete response when treated with primary chemotherapy, and thus with a potentially favorable long-term prognosis.

Introduction

In breast cancer, Ki67 immunohistochemical (IHC) determination is the most widely used biomarker of cell proliferation. Despite this, Ki67 status is not considered a robust prognostic or predictive factor because of the limited reproducibility of results, the variability in cutpoints used, and the different clinical scenarios in which it has been studied. Thus, it has not been recommended as a predictive factor in common clinical practice [1].

The role of Ki67 as a predictive factor of response to neoadjuvant hormone therapy has been well-established [2, 3]. However, its value is less obvious in the prediction of response to neoadjuvant chemotherapy [4, 5]. Several retrospective studies have associated high levels of Ki67 with higher pathological complete response (pCR) rates [6–11]. However, other studies have failed to confirm these data

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[12, 13]. This inconsistency could be related to the fact that in nearly all studies the choice of cutpoints to define high Ki67 levels has been based on empirical observations without any biological justification or proper statistical approach. A wide range of high Ki67 levels has been communicated, with the cutpoint of 10% to 25% being the most commonly used [14]. In addition, its predictive role regardless estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status has not been ascertained.

The main objective of this study was to analyze the predictive role of Ki67 for neoadjuvant chemotherapy efficacy. Our investigation was based on data from four clinical trials carried out by GEICAM (the Spanish Breast Cancer Group). First, we identified the optimal cutpoint of Ki67 to maximize its predictive value; then, we evaluated the predictive role of Ki67 in relation to ER and HER2 status.

Patients And Methods

Study Population

We analyzed the data from 262 patients with available centralized Ki67 IHC determination and pathological response data, derived from four GEICAM clinical trials of neoadjuvant chemotherapy for breast cancer: GEICAM/2002-01 (34 patients, all subtypes), GEICAM/2003-03 (32 patients, HER2 overexpressed), GEICAM 2006-03 (119 patients, luminal subtype [defined by IHC as ER+ and/or progesterone receptor (PR)+, HER2−] and triple-negative subtype [ER−, PR−, HER2−]), and GEICAM/2006-14 (77 patients, HER2 overexpressed). In all these trials, patients were treated with anthracycline and taxane-based neoadjuvant chemotherapy; anti-HER2 therapy (trastuzumab or lapatinib) was administered for most patients with HER2 overexpression (Table 1). Detailed descriptions of these trials have been published elsewhere [15–19]. The 262 patients involved represented 73% of the 361 patients participating in the four trials combined. These trials were performed in accordance with the Declaration of Helsinki, approved by the ethics committees at all participating institutions (supplemental online Table 3) and the Spanish Health Authority, and registered at http://www.clinicaltrials.gov (NCT00128856, NCT00129896, NCT00432172, NCT00841828). All patients provided written informed consent for trial participation and molecular analyses.

IHC and/or Fluorescence In Situ Hybridization Determination of Ki67, ER, PR, and HER2

Biomarker analysis was carried out by IHC and/or fluorescence in situ hybridization (FISH) at a central laboratory on the available pretreatment tumor samples from those patients. We performed immunostaining using formalin-fixed, paraffin-embedded tissue sections as previously described [20].

Ki-67 was assessed using the mouse monoclonal antibody (mAb) clone MIB1 (Dako, Glostrup, Denmark, http://www.dako.com). The Ki67 proliferation index was defined as the mean of tumor cells with marker expression, including the hot spots.

For ER and PR expression, sections were incubated with primary mAb to ER: clone SP1 for GEICAM/2002-01 and GEICAM/2006-03 (Dako), clone EP1 for GEICAM/2006-14 (Dako); mAb to PR clone 1A6 (Novocastra, Leica Biosystems, Nussloch, Germany, http://www.leicabiosystems.com) for GEICAM/2002-01, clone PgR636 (Dako) for 2006-14, and clone Y85 (Vitro, Madrid, Spain, http://www.vitro.bio/) for GEICAM/2006-03. IHC determination was performed by using the EnVision FLEX system (Dako). ER and PR were scored with reference to the proportion of stained tumor cells and were classified as positive according to American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines [21].

HER2 expression was determined by HercepTest (Dako) for GEICAM/2002-01, GEICAM/2003-03, and GEICAM/2006-03, and amplification was confirmed by PathVysion FISH probes (Abbott Molecular, Des Plaines, IL, https://www.abbottmolecular.com/us/home.html), following ASCO/CAP guidelines [22].

All assays were evaluated by three expert pathologists (F.R., F.P., J.A.A.) blinded to pathological response. Discrepancies were solved by consensus between them.

Definition of pCR

We defined pCR on the basis of the Miller and Payne criteria [23] as the complete disappearance of the invasive tumor in the mammary gland (grade 5) and the absence of tumor in the axillary lymph nodes examined by axillary clearance after neoadjuvant therapy or negative sentinel node before the start of therapy.

Statistical Analysis

Receiver-operating characteristic (ROC) curve analysis was used to determine the optimal cutpoint for Ki67 by calculating the sensitivity and specificity indices corresponding to Ki67 cutpoints selected for every 10 units (range, 1–100). Cutpoints divide the study population into groups of high and low expression, which we correlated with pCR. We considered the optimal cutpoint as the one having the highest combined sensitivity and specificity values [24]; we prioritized those with higher sensitivity indices under equal circumstances. We then performed univariate and multivariate logistical regression analyses to examine the association between clinical-pathological variables and pCR. Multivariate models included only variables that exhibited a univariate association with the dependent variable, pCR ($p < .25$). We used the area under the curve (AUC) parameter to evaluate the model, including Ki67 and other clinical-pathological variables, and to assess Ki67's ability to discriminate between patients with and without pCR. All analyses were performed by using the Statistical Analysis System (SAS) Enterprise Guide 5.1 software (SAS Institute Inc., Cary, NC, https://www.sas.com/).

Results

Patient Characteristics

Patients' characteristics are described in Table 1. The median age was 49 years, and 57% of patients were premenopausal. Fifty-nine percent had T2 stage disease, and 62% were clinically node-positive. Most patients (84%) had an invasive ductal carcinoma, whereas 6% had lobular and 10% other type. The histological grade was mainly 2 (36%) or 3 (44%), ER positivity was seen in 50% of tumors, and PR and HER2 negativity was seen in 60% and 57% of cases, respectively. The distribution by ER and HER2 status was as follows: ER+/HER2− tumors in 69 (26%) patients, ER+/HER2+ in 62 (24%) patients, ER−/HER2+ in 51 (20%) patients, and ER−/HER2− in 80 (30%) patients. The median proportion of cells stained for Ki67 was 30% (quartile 1, 15%; quartile 3, 65%). Supplemental online

Table 1. Patient characteristics by pathological complete response in breast and axilla

Characteristic	pCR = yes (*n* = 69)	pCR = no (*n* = 193)	Total (*n* = 262)
Study, *n* (%)			
GEICAM 2002-01	3 (8.8)	31 (91.2)	34 (13.0)
GEICAM 2003-03	9 (28.1)	23 (71.9)	32 (12.2)
GEICAM 2006-03	27 (22.7)	92 (77.3)	119 (45.4)
GEICAM 2006-14	30 (39.0)	47 (61.0)	77 (29.4)
Treatment, *n* (%)			
EC-D	13 (16.7)	65 (83.3)	78 (29.8)
EC-DCb	14 (34.1)	27 (65.9)	41 (15.6)
EC-DL	11 (28.9)	27 (71.1)	38 (14.5)
EC-DH	19 (48.7)	20 (51.3)	39 (14.9)
GAT	3 (8.8)	31 (91.2)	34 (13.0)
Median age (range), yr	51 (30–77)	48.0 (24–75)	49.0 (24–77)
Menopausal status, *n* (%)			
Premenopausal	35 (23.6)	113 (76.4)	148 (56.5)
Postmenopausal	34 (29.8)	80 (70.2)	114 (43.5)
Tumor size, *n* (%)[a]			
T1	8 (40.0)	12 (60.0)	20 (7.6)
T2	43 (27.9)	111 (72.1)	154 (58.8)
T3	13 (19.7)	53 (80.3)	66 (25.2)
T4	5 (22.7)	17 (77.3)	22 (8.4)
Median (range), cm	3 (1.0–15.8)	4.1 (0.8–13.0)	4 (0.8–15.8)
Nodal status, *n* (%)[a]			
N0	22 (22.0)	78 (78.0)	100 (38.1)
N1	41 (29.7)	97 (70.3)	138 (52.7)
N2	6 (26.1)	17 (73.9)	23 (8.8)
N3	0 (0.0)	1 (100.0)	1 (0.4)
Histologic grade, *n* (%)			
G1	2 (9.1)	20 (90.9)	22 (8.4)
G2	18 (19.1)	76 (80.9)	94 (35.9)
G3,	41 (35.3)	75 (64.7)	116 (44.3)
GX	8 (26.7)	22 (73.311.4)	30 (11.4)
Histopathologic type, *n* (%)			
Invasive ductal carcinoma	60 (27.2)	161 (72.8)	221 (84.4)
Invasive lobular carcinoma	3 (18.8)	13 (81.2)	16 (6.1)
Other	6 (24)	19 (76)	25 (9.5)
Estrogen receptor, *n* (%)			
Positive	16 (12.2)	115 (87.8)	131 (50.0)
Negative	53 (40.5)	78 (59.5)	131 (50.0)
Progesterone receptor, *n* (%)			
Positive	11 (10.3)	96 (89.7)	107 (40.8)
Negative	58 (37.4)	97 (62.6)	155 (59.2)
HER2, *n* (%)			
Positive	40 (35.4)	73 (64.6)	113 (43.1)
Negative	29 (19.5)	120 (80.5)	149 (56.9)
Subtypes (ER/HER2 status), *n* (%)			
ER+/HER2−	1 (1.4)	68 (98.6)	69 (26.3%)
ER+/HER2+	15 (24.2)	47 (75.8)	62 (23.7%)
ER−/HER2+	25 (49.0)	26 (51.0)	51 (19.5%)
ER−/HER2−	28 (35.0)	52 (65.0)	80 (30.5%)
Median Ki67 (range) (%)	60 (5–100)	30 (1–95)	30 (1–100)

[a]Tumor, node, metastasis staging according to the *AJCC Cancer Staging Manual* (5th edition).

Abbreviations: EC-D, epirubicin plus cyclophosphamide followed by docetaxel; EC-DCb, EC-D plus carboplatin; EC-DL, EC-D plus lapatinib; EC-DH, EC-D plus trastuzumab; ER, estrogen receptor; GAT, gemcitabine, Adriamycin (doxorubicin; Hikma Pharmaceuticals PLC, London, UK, http://www.hikma.com/en), and paclitaxel; GEICAM, Spanish Breast Cancer Group; HER2, human epidermal growth factor receptor 2; MDH, Myocet (doxorubicin hydrochloride; Teva Pharmaceutical Industries Ltd., Petah Tikva, Israel, http://www.tevapharm.com), docetaxel, and trastuzumab; pCR: pathological complete response (breast and axilla).

Figure 1 shows the distribution of the proportion of staining for Ki67 in 10-expression intervals.

pCR Rates and ROC Curves

Of the 262 patients, 69 (26%) achieved pCR, similar to the rate observed in the whole population (85 of the 355 [24%] patients with available pathological CR data included in the 4 trials). The pCR rate was higher in small tumors, those with histological grade 3, ER- or PR-negative tumors, and those with high Ki67 indices (Table 1).

Results based on ROC curve method showed that the Ki67 cutpoints with the highest combined sensitivity and specificity values were 60 and 50, with 50% showing a higher sensitivity index (data not shown). On the basis of this cutpoint (Ki67 >50%), high Ki67 was seen in 35% of patients (91 of 262) included in this analysis. Fifty-three percent of the pCRs (36 of 69) were achieved in patients with Ki67 >50%, and 72% of patients without pCR (138 of 193) had tumors with Ki67 ≤50%. Patients with Ki67 >50% achieved a pCR rate of 40% (36 of 91 cases) and patients with Ki67 ≤50% had a pCR rate of 19% (33 of 171 cases) ($p = .0004$) (Table 1).

Table 2 describes pCR rates according to ER and HER2 status and Ki67 index. Only 1 of the 69 patients with ER+/HER2− tumors (1.5%) achieved pCR. In contrast, 15 of the 62 ER+/HER2+ patients (24%), 28 of the 80 ER−/HER2− patients (35%), and 25 of the 51 ER−/HER2+ patients (49%) achieved pCR. The 50% cutpoint of Ki67 predicted better pCRs, specifically in patients with ER−/HER2− (42% high versus 15% low; $p = .0337$) and ER−/HER2+ (64% high versus 45% low; $p = .3238$) tumors.

Univariate and Multivariate Analysis of pCR Predictive Factors

The univariate analyses (supplemental online Table 1) showed a statistically significant association between pCR and small tumor size (considered as a continuous variable) ($p = .0368$), high histological grade ($p = .0185$), ER− status ($p \leq .0001$), PR− status ($p < .0001$), and HER2+ status ($p = .0041$). A high proliferation level determined by Ki67 quantitative measurement (odds ratio [OR]: 1.02; 95% confidence interval [CI]: 1.01–1.03; $p < .0001$) or by considering a Ki67 >50% (OR: 2.74; 95% CI: 1.55–4.82; $p = .0005$) exhibited a significant association with pCR.

In the multivariate analyses (Table 3), factors showing an independent and statistically significant association with pCR included a Ki67 index >50%, ER− and HER2+ status, and smaller tumor size (AUC = 0.7846).

Multivariate analyses including only the most chemosensitive patient subpopulation (that with ER−/HER2− and ER−/HER2+ tumors) (supplemental online Table 2) showed that Ki67 >50% and HER2+ status were independent predictive factors for pCR (AUC: 0.6178).

Table 2. Rate of pathological complete response for breast and axilla by estrogen receptor status, human epidermal growth factor receptor 2 status, and Ki67 score

	pCR		
Variable	**Ki67 ≤50%**	**Ki67 >50%**	**Total**
ER+/HER2−	0/60 (0.0)	1/9 (11.1)	1/69 (1.5)
ER+/HER2+	12/51 (23.5)	3/11 (27.3)	15/62 (24.2)
ER−/HER2+	18/40 (45.0)	7/11 (63.6)	25/51 (49.0)
ER−/HER2−	3/20 (15.0)	25/60 (41.7)	28/80 (35.0)
Total	33/171 (19.3)	36/91 (39.6)	69/262 (26.3)

Values are expressed as *n/n* (%).
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor; pCR, pathological complete response.

Table 3. Multivariate analyses for factors associated to pathological complete response in breast and axilla

Variable	OR (95% CI)	*p* value
Ki67 >50% (versus Ki67 ≤50%)	2.970 (1.348–6.547)	.0069
ER/HER2 status (versus ER+/HER2−)		<.0001
ER+/HER2+	0.049 (0.006–0.385)	.0009
ER−/HER2+	3.532 (1.528–8.166)	<.0001
ER−/HER2−	0.914 (0.375–2.230)	.3148
Tumor size (continuous)	0.849 (0.735–0.980)	.0255

Area under the curve = 0.7846.
Abbreviations: CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor; OR, odds ratio.

Discussion

According to robust retrospective analyses of clinical trial data [2, 3], the assessment of cellular proliferation based on Ki67 determination may be used as a predictive factor for the efficacy of breast cancer neoadjuvant hormone therapy. However, its role as an independent predictive factor for efficacy of neoadjuvant chemotherapy is not that well-established [4]. Thus, routine Ki67 assessment has not been recommended when patients receive primary chemotherapy [25]. The reason is that despite data indicating the correlation between high ki67 and pCR, most of these data are derived from retrospective studies other than clinical trials [6–11]. Furthermore, Ki67 cutpoints used in these studies were selected empirically (with thresholds associated with the mean of observed values in the study population) or were arbitrarily established at 10%–25% [14].

Our method—ROC curve analysis based on sensitivity and specificity indices to discriminate patients achieving pCR in a specific range of biomarker values—was aimed at identifying an optimal Ki67 cutpoint. Our results showed that 50% was the optimal cutpoint and identified two subpopulations: patients with a high Ki67 level (>50%) achieving a pCR rate of 40% (36 of 91 cases) and patients with a low Ki67 level (≤50%) with a pCR rate of 19% (33 of 171 cases). In the multivariate analyses, this effect was confirmed to be independent of other pCR-related factors, such as ER or HER2 status.

Ki67's predictive value in breast cancer is of special relevance in the population potentially most responsive to neoadjuvant chemotherapy. Including the ER−/HER2− and ER−/HER2+ patients, we observed a pCR rate of 45% (32 of 71 cases) for those with Ki67 >50% and a pCR rate of 35% (21 of 60) in patients with Ki67 ≤50%. In this chemosensitive subgroup, basal Ki67 indices >50% were also an independent predictor for pCR. The long-term prognostic value of pCR

among patients treated with neoadjuvant chemotherapy has been well-established [26, 27]. Recent findings have shed light on this prognostic relationship, which seems to apply especially to patients with HER2+, triple-negative (ER−/PR−/HER2−), or high-risk ER+ tumors [28, 29]. In fact, regarding drug approval, the Food and Drug Administration has adopted pCR in these patient subgroups as a surrogate marker of long-term treatment efficacy [30].

Thus, a Ki67 index >50% may be predictive of high pCR rates of postneoadjuvant chemotherapy (especially in the ER−/HER2− and ER−/HER2+ population). This cutpoint may potentially identify a subpopulation of breast cancer patients with a favorable long-term prognosis after achieving pCR with primary chemotherapy. Similar findings have been described for management of neuroendocrine tumors. Nadler et al. [31] established that Ki-67 is a reliable pathological grading marker in determining tumor grade, according to the European Neuroendocrine Tumor Society guidelines and the 2010 World Health Organization classification. Moreover, in patients with advanced gastrointestinal neuroendocrine carcinoma, Sorbye et al. [32] suggested that the Ki67 threshold of 55% was the best cutoff predicting rate of response to chemotherapy.

The results of this study should be interpreted in the context of its weaknesses. First, the sample size is somewhat limited. Second, the centralized Ki67 determination may invite questions about its reproducibility in daily practice. This is especially relevant given that its interobserver variability is often mentioned as one of the limitations of Ki67 being defined by IHC as a robust proliferation biomarker [4]. However, it should be said that the interpretation of different genetic signatures is also subject to variability. Nevertheless, independently of the set of genes used, all of them captured the same subpopulation with a poor prognosis [33, 34], with mainly high-proliferation tumors, especially among ER+ cases [35, 36]. In addition, in other studies IHC-detected Ki67 levels were associated with quantitatively assessed proliferation in first-generation genetic signatures [37–39]. Finally, different studies have reported a high interlaboratory variability in Ki67 scoring on breast tumors among some of the world's most experienced pathologists [40, 41]. Clinical decision-making regarding treatment options in breast cancer often relies on the application of a Ki67 cutoff to classify patients into "Ki67 high" or "Ki67 low" risk groups. Reported data suggest that even if a consensus Ki67 cutoff is agreed upon, discordant Ki67 measurements between observers for low proliferation/luminal A breast tumors have been estimated in 50% of studied cases; the discrepancies in high rates of Ki67 have been lower [41]. Thus, a definition of a Ki67 threshold >50% might improve the reproducibility of scoring results between laboratories and observers and would help to identify tumors with a high proliferation rate and worse outcome.

Conclusion

A basal Ki67 index >50% could be considered an independent predictive factor for pCR reached after neoadjuvant chemotherapy. From a biological perspective, these results suggest that cell proliferation is a phenomenon closely related to chemosensitivity. From a clinical perspective, these findings could facilitate the identification of a patient subpopulation with high probability of achieving pCR when treated with primary chemotherapy, and thus with a potentially favorable long-term prognosis.

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Disclosures

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References

1. Harris L, Fritsche H, Mennel R et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287–5312.

2. Dowsett M, Smith IE, Ebbs SR et al. Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. Clin Cancer Res 2005;11:951s–958s.

3. 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; 100:1380–1388.

4. Dowsett M, Nielsen TO, A'Hern R et al. Assessment of Ki67 in breast cancer: Recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011;103:1656–1664.

5. Andre F, Arnedos M, Goubar A et al. Ki67—no evidence for its use in node-positive breast cancer. Nat Rev Clin Oncol 2015;12:296–301.

6. Colleoni M, Viale G, Zahrieh D et al. Expression of ER, PgR, HER1, HER2, and response: A study of preoperative chemotherapy. Ann Oncol 2008;19:465–472.

7. Denkert C, Loibl S, Müller BM et al. Ki67 levels as predictive and prognostic parameters in pretherapeutic breast cancer core biopsies: A translational investigation in the neoadjuvant GeparTrio trial. Ann Oncol 2013;24:2786–2793.

8. Esserman LJ, Berry DA, DeMichele A et al. Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: Results from the I-SPY 1 TRIAL–CALGB 150007/150012, ACRIN 6657. J Clin Oncol 2012;30:3242–3249.

9. Fasching PA, Heusinger K, Haeberle L et al. Ki67, chemotherapy response, and prognosis in breast

cancer patients receiving neoadjuvant treatment. BMC Cancer 2011;11:486.

10. Jones RL, Salter J, A'Hern R et al. Relationship between oestrogen receptor status and proliferation in predicting response and long-term outcome to neoadjuvant chemotherapy for breast cancer. Breast Cancer Res Treat 2010;119:315–323.

11. Keam B, Im SA, Lee KH et al. Ki-67 can be used for further classification of triple negative breast cancer into two subtypes with different response and prognosis. Breast Cancer Res 2011;13:R22.

12. Guarneri V, Piacentini F, Ficarra G et al. A prognostic model based on nodal status and Ki-67 predicts the risk of recurrence and death in breast cancer patients with residual disease after preoperative chemotherapy. Ann Oncol 2009;20:1193–1198.

13. 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 2008;10:R30.

14. Luporsi E, André F, Spyratos F et al. Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: Analytical and critical review. Breast Cancer Res Treat 2012;132:895–915.

15. Alba E, Albanell J, de la Haba J et al. Trastuzumab or lapatinib with standard chemotherapy for HER2-positive breast cancer: results from the GEICAM/2006-14 trial. Br J Cancer 2014;110:1139–1147.

16. Alba E, Calvo L, Albanell J et al. Chemotherapy (CT) and hormonotherapy (HT) as neoadjuvant treatment in luminal breast cancer patients: Results from the GEICAM/2006-03, a multicenter, randomized, phase-II study. Ann Oncol 2012;23:3069–3074.

17. Alba E, Chacon JI, Lluch A et al. A randomized phase II trial of platinum salts in basal-like breast cancer patients in the neoadjuvant setting. Results from the GEICAM/2006-03, multicenter study. Breast Cancer Res Treat 2012;136:487–493.

18. Antón A, Ruiz A, Plazaola A et al. Phase II clinical trial of liposomal-encapsulated doxorubicin citrate and docetaxel, associated with trastuzumab, as neoadjuvant treatment in stages II and IIIA HER2-overexpressing breast cancer patients. GEICAM 2003-03 study. Ann Oncol 2011;22:74–79.

19. Sánchez-Rovira P, Antón A, Barnadas A et al. Classical markers like ER and ki-67, but also survivin and pERK, could be involved in the pathological response to gemcitabine, adriamycin and paclitaxel (GAT) in locally advanced breast cancer patients: results from the GEICAM/2002-01 phase II study. Clin Transl Oncol 2012;14:430–436.

20. Rojo F, García-Parra J, Zazo S et al. Nuclear PARP-1 protein overexpression is associated with poor overall survival in early breast cancer. Ann Oncol 2012;23:1156–1164.

21. Hammond ME, Hayes DF, Dowsett M et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med 2010;134:907–922.

22. 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. J Clin Oncol 2007;25:118–145.

23. Ogston KN, Miller ID, Payne S et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: Prognostic significance and survival. Breast 2003;12:320–327.

24. Metz CE. Basic principles of ROC analysis. Semin Nucl Med 1978;8:283–298.

25. Berruti A, Generali D, Kaufmann M et al. International expert consensus on primary systemic therapy in the management of early breast cancer: Highlights of the fourth symposium on primary systemic therapy in the management of operable breast cancer, Cremona, Italy (2010). J Natl Cancer Inst Monogr 2011;2011:147–151.

26. Chollet P, Amat S, Cure H et al. Prognostic significance of a complete pathological response after induction chemotherapy in operable breast cancer. Br J Cancer 2002;86:1041–1046.

27. Rastogi P, Anderson SJ, Bear HD et al. Preoperative chemotherapy: Updates of national surgical adjuvant breast and bowel project protocols B-18 and B-27. J Clin Oncol 2008;26:778–785.

28. Cortazar P, Zhang L, Untch M et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014;384:164–172.

29. von Minckwitz G, Untch M, Blohmer JU et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30:1796–1804.

30. Prowell TM, Pazdur R. Pathological complete response and accelerated drug approval in early breast cancer. N Engl J Med 2012;366:2438–2441.

31. Nadler A, Cukier M, Rowsell C et al. Ki-67 is a reliable pathological grading marker for neuroendocrine tumors. Virchows Arch 2013;462:501–505.

32. Sorbye H, Welin S, Langer SW et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): The NORDIC NEC study. Ann Oncol 2013;24:152–160.

33. Fan C, Oh DS, Wessels L et al. Concordance among gene-expression-based predictors for breast cancer. N Engl J Med 2006;355:560–569.

34. Prat A, Parker JS, Fan C et al. PAM50 assay and the three-gene model for identifying the major and clinically relevant molecular subtypes of breast cancer. Breast Cancer Res Treat 2012;135:301–306.

35. Desmedt C, Haibe-Kains B, Wirapati P et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. Clin Cancer Res 2008;14:5158–5165.

36. Wirapati P, Sotiriou C, Kunkel S et al. Meta-analysis of gene expression profiles in breast cancer: Toward a unified understanding of breast cancer subtyping and prognosis signatures. Breast Cancer Res 2008;10:R65.

37. Cuzick J, Dowsett M, Pineda S et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol 2011;29:4273–4278.

38. Niikura N, Iwamoto T, Masuda S et al. Immunohistochemical Ki67 labeling index has similar proliferation predictive power to various gene signatures in breast cancer. Cancer Sci 2012;103:1508–1512.

39. Williams DJ, Cohen C, Darrow M et al. Proliferation (Ki-67 and phosphohistone H3) and oncotype DX recurrence score in estrogen receptor-positive breast cancer. Appl Immunohistochem Mol Morphol 2011;19:431–436.

40. Polley MY, Leung SC, Gao D et al. An international study to increase concordance in Ki67 scoring. Mod Pathol 2015;28:778–786.

41. Polley MY, Leung SC, McShane LM et al. An international Ki67 reproducibility study. J Natl Cancer Inst 2013;105:1897–1906.

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