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PRECLINICAL STUDY

Relationship between oestrogen receptor status and proliferation in predicting response and long-term outcome to neoadjuvant chemotherapy for breast cancer

Robin L. Jones · Janine Salter · Roger A'Hern · Ash Nerurkar · Marina Parton · Jorge S. Reis-Filho · Ian E. Smith · Mitchell Dowsett

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Abstract Oestrogen receptor (ER) negative breast cancers are more likely to achieve a pathological complete response (pCR) to neoadjuvant chemotherapy compared to those with ER positive tumours. ER positive tumours exhibit low proliferation and ER negative cancers high proliferation. The aim of this study was to determine to what extent the better response of ER negative cancers correlates with proliferation rate. A retrospective analysis of a prospectively maintained database identified 175 neoadjuvant chemotherapy patients with tissue available for Ki67 analysis. On univariate analysis, pre-therapy Ki67 ($P = 0.04$), ER status ($P = 0.002$), HER2 status ($P = 0.004$) and grade ($P = 0.0009$) were associated with a pCR. In a multivariate model, HER2 was the only significant predictor of pCR. No significant relationship between pre-therapy Ki67 and relapse-free and overall survival was demonstrated. Ki67 is not an independent predictor of clinical CR or pCR. Aspects of ER status beyond its inverse relationship with proliferation may contribute to its predictive value for pCR.

Keywords Breast cancer · Neoadjuvant chemotherapy · Proliferation · ER · Pathological complete response

R. L. Jones · J. Salter · R. A'Hern · M. Dowsett (✉)
Academic Department of Biochemistry, Royal Marsden Hospital, Fulham Road, London SW3 6JJ, UK
e-mail: mitch.dowsett@icr.ac.uk

R. L. Jones
e-mail: robin.jones@icr.ac.uk

A. Nerurkar · M. Parton · I. E. Smith
Breast Unit, Royal Marsden Hospital, London, UK

J. S. Reis-Filho
Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, London, UK

Introduction

The administration of neoadjuvant chemotherapy is well established in the treatment of large potentially operable and locally advanced breast cancer [1]. Large randomised trials have demonstrated no significant difference in survival between patients treated with adjuvant and neoadjuvant chemotherapy [1]. Albeit, not perfect, pathological complete response (pCR), following neoadjuvant chemotherapy, has been shown to be a good surrogate marker for overall survival. In fact women achieving a pCR following neoadjuvant therapy have significantly better survival compared to those with residual carcinoma [1].

There is evidence to suggest that ER negative tumours are more likely to achieve a pCR following neoadjuvant chemotherapy compared to ER positive cancers [2–4]. An inverse relationship exists between ER expression and proliferation as assessed by various methods including the MIB-1 antibody against Ki67 [2, 3, 5], with ER positive cancers showing low proliferation rates, whereas ER negative breast cancers show high proliferation rates. Most investigators have confirmed that high proliferation is associated with a better short-term response to neoadjuvant chemotherapy [5–7]. However, previous studies of women treated with or without adjuvant chemotherapy have demonstrated that patients with high proliferation have a poor long-term outcome [6]. In fact, despite having a poorer prognosis, patients with triple negative (ER, PgR and HER2 negative) cancers have the highest prevalence of pCR following neoadjuvant chemotherapy [8].

This study aimed to determine to what extent the better response rate of ER negative tumours to neoadjuvant chemotherapy is potentially explained by their high proliferation. This would be clinically significant because the inverse relationship between ER and proliferation is not

absolute: if the determinant of response was proliferation and not ER, proliferation rather than ER should be measured and used for prediction of response to neoadjuvant chemotherapy. The second aim was to determine the influence of pre-chemotherapy proliferation on long-term outcome in a cohort of breast cancer patients treated with neoadjuvant chemotherapy.

Patients and methods

Clinical methodology

A retrospective analysis of a prospectively maintained clinical database was performed to identify patients treated with neoadjuvant chemotherapy for operable or locally advanced breast cancer between 1985 and 2005. In order to address the first aim of the study, patients treated with neoadjuvant chemotherapy alone (i.e. those treated with no endocrine therapy for the neoadjuvant period with ER positive tumours) were selected. Patients with ER negative tumours treated with concurrent neoadjuvant tamoxifen were included in this study, as tamoxifen has minimal activity in such cases. Neoadjuvant chemotherapy regimens included: (1) anthracycline-based schedules comprising doxorubicin 60 mg/m^2 or epirubicin 60 mg/m^2 once every 3 weeks often within the context of clinical trials; (2) CMF (cyclophosphamide 100 mg orally days 1–14, methotrexate 30 mg/m^2 days 1 and 8, and 5-fluorouracil 1 g/m^2 days 1 and 8); and occasionally (3) mitoxantrone containing regimens (up to 11 mg/m^2). Treatment was usually given to a total of 6 courses, and occasionally to eight in specific trials. Sixty-two of the patients selected were also included in another study assessing the prognostic value of pre- and post-neoadjuvant chemotherapy Ki67 [9].

The association between pathological and clinical response with the following pre-neoadjuvant chemotherapy factors was assessed; age, menstrual status, clinical T and N stage, initial operability (i.e. operable or locally advanced), Ki67, ER, PgR, HER2, grade, histological type (invasive ductal and lobular), treatment with an anthracycline-based regimen or not and dual ER and HER2 negative phenotype.

The influence of the following factors with relapse-free (RFS) and overall (OS) survival was investigated:

1. Pre-therapy: age, menstrual status, clinical T and N stage, initial operability, Ki67, ER, PgR, HER2, grade, histological type and dual negative phenotype.
2. Others: treatment with an anthracycline-based regimen, clinical response, the attainment of a pCR, type of surgery performed, adjuvant endocrine or chemotherapy.

Clinical response was assessed according to World Health Organisation criteria, following each cycle of chemotherapy by measuring the two largest diameters of the tumour [10]. Those with no residual invasive or in situ disease were classified as having achieved a pCR [10].

Surgical and post-operative management followed standard institutional guidelines as described elsewhere [10]. Prior to 1995, women achieving a clinical CR to neoadjuvant chemotherapy were frequently offered the option of radiotherapy without surgery. This approach was discontinued following a retrospective analysis of these patients, which demonstrated a high local recurrence rate [11].

Tissue acquisition

Tissue was acquired using standard techniques [9]. Haematoxylin and eosin staining was performed to obtain the histological type and where possible the histological grade as part of routine clinical management [12].

Immunohistochemistry

A standard procedure for Ki67 staining was followed [9], 4 μm sections were dewaxed in xylene and then hydrated by means of a series of graded ethanol baths and rinsed in water. Endogenous peroxidase activity was blocked. By microwaving at full power (750 W) in citrate buffer pH 6.0 for 10 min antigen retrieval was performed. MIB-1 primary antibody (Dako, Denmark) was used at a dilution of 1:50, and incubated for an hour at room temperature. All washes and dilutions were performed with phosphate-buffered saline (PBS). Biotinyalated rabbit anti-mouse immunoglobulin was applied and subsequently avidin-biotin complex (ABC; Dako). Diaminobenzene (DAB; Sigma, USA) was applied to develop peroxidase activity and counterstaining performed with haematoxylin. The observer (blinded to patient outcome) examined stained sections using a standard light microscope 40× objective using a 10 × 10 eye-piece graticule. Ki67 score was defined as the percentage of total number of tumour cells (at least 1,000) with nuclear staining over 10 high powered fields (40×).

The same staining procedure as described above, with microwave antigen retrieval, was used for ER. The primary antibody used 6F11 (Novocastra, UK) was incubated at a dilution of 1:40 for 2 hr at room temperature. The Histoscore (H-score) was used to assess ER, incorporating evaluation of intensity of stain (0–3) and number of cells staining (range of score 0–300). Using this method ER positive tumours have a score of >1.

The Hercep test (Dako) was used to perform HER2 immunohistochemistry. Specimens were classified as positive if immunohistochemical staining was 3+ or if staining was 2+ and FISH (fluorescence in situ hybridisation) positive. PgR was evaluated using the Allred score [13].

Statistical analysis

Associations between two variables were assessed as follows: nominal tabulated data were analysed using the Chi-squared test (for 2 × 2 tables Fisher's exact test was used); if one factor was ordinal the Kruskal–Wallis test was used if more than two groups were being compared, the Mann Whitney test for trend being employed to compare two groups. If two ordinal factors were being assessed Spearman Rank correlation was employed. Multivariate analysis of pCR (a binary variable) was undertaken using logistic regression. Univariate and multivariate analysis of RFS and OS was carried out using Cox regression. RFS was defined as the time from the date of presentation to the date of first local relapse, distant relapse or occurrence of a new primary tumour. OS was defined as the time from presentation to death. Patients without an event were censored at the time of last follow-up.

Multivariate analysis was performed in a forward stepwise fashion, the most significant additional variable (satisfying $P < 0.05$) being added at each stage, cases with missing values for any of the variables in the model were excluded from analysis. About 95% confidence intervals were used to express ranges within which true parameter values were likely to lie.

All P values were two-tailed and 95% confidence intervals were employed for all tests.

For Ki67 analysis, the centred linear component was first calculated by log transforming the variable and the average of all log transformed values was then deducted from each log transformed observation. This had the effect of 'centering' the values, e.g. the average would be zero and values below the average would be below zero, those above the average would be above zero. A 'centred' quadratic component was then calculated by squaring this linear component and similarly a cubic component was calculated by cubing the linear component. The centred quadratic variable would allow a U-shaped relationship between outcome and the factor to be detected, e.g. low values could be high risk relative to values in the middle of the distribution and similarly high values would also be high risk. Fitting the linear, quadratic and cubic components, or any combination of them allows investigation of the pattern between the log hazard of death (or of relapse, or log odds ratio of response) to be investigated.

Results

The clinical characteristics of 175 patients with pre-therapy histopathology blocks that had sufficient material available for immunohistochemical assessment of Ki67 are displayed in Table 1. The median age was 48 years, with a range of 26 to 75 years. At the time of analysis 56 patients had died and 71 had relapsed.

A significant association between pre-therapy Ki67 and histological grade ($P < 0.001$) was observed and an inverse association with ER status ($P = 0.0004$). No association between pre-therapy Ki67 and HER2 was seen ($P = 0.9$).

Table 1 Clinical characteristics of the 175 patients

Characteristic	Total number of patients (%)
Menstrual status	
Pre	95 (54.3%)
Peri	12 (6.9%)
Post	48 (27.4%)
Hysterectomy	20 (11.4%)
T stage	
T1	2 (1.1%)
T2	82 (46.9%)
T3	68 (38.9%)
T4	23 (13.1%)
N stage	
N0	84 (48.0%)
N1	82 (46.9%)
N2	6 (3.4%)
N3	3 (1.7%)
Initial operability	
Operable	154 (88.0%)
Locally advanced	21 (12.0%)
ER	
Positive	97 (55.4%)
Negative	78 (44.6%)
Clinical response	
Complete response	55 (31.4%)
Partial response	69 (39.4%)
Stable disease	35 (20.0%)
Progressive disease	16 (9.1%)
pCR	
Yes	18 (10.3%)
No	131 (74.9%)
Radiotherapy alone	23 (13.1%)
Unknown	3 (1.7%)

Table 2 Univariate and multivariate analysis of factors predictive of clinical and pathological response and multivariate analysis of factors predictive of pCR

Factors	Clinical response	pCR	
	Univariate analysis *P* value (odds ratio 95%CI)	Univariate analysis *P* value (odds ratio 95%CI)	Multivariate analysis *P* value (odds ratio 95%CI)
Menopausal status (post: pre)	0.6	0.1 (0.4, 0.1–1.3)	NS
T stage	0.09	0.4	NS
N stage	0.1	0.3	NS
Ki67 (per 2.7 fold increase)	0.5	0.04 (2.9, 1.1–8.1)	NS
ER status (positive: negative)	0.3	0.002 (0.2, 0.05–0.5)	0.05 (0.3, 0.1–1.0)
HER 2 (positive: negative)	0.2	0.004 (10, 2.0–54)	[a]
Grade	0.01 (3, 1.3–6.2)	0.0009 (14, 19–115)	0.04 (8.7, 1.0–72)
Histological type[b]	0.1	0.4	NS

[a] When included in the model HER2 status was a significant independent predictor of pCR ($P = 0.04$, odds ratio = 10, 2.0–54), but ER status and grade lost significant independent predictive value

[b] Histological type: invasive ductal carcinoma of no special type and invasive lobular carcinoma

Pathological response

Patients with higher pre-therapy Ki67 were significantly more likely to achieve a pCR than those with lower pre-therapy Ki67 ($P < 0.04$; Table 2). Lack of ER expression, HER2 positivity and high tumour grade were significantly associated with higher pCR rates ($P = 0.002$, $P = 0.004$ and $P = 0.0009$, respectively). No statistically significant correlation between pCR and other clinicopathological variables was observed (age, menopausal status, clinical T and N stage, initial operability, PgR status, dual ER/ HER2 negative phenotype, histological type and treatment with an anthracycline-based regimen).

Multivariate analysis was performed with and without HER2 included in the model (Table 2). Pre-therapy grade and ER status were found to be independent predictors of pCR, but not Ki67. When included in the model HER2 status was a significant independent predictor of pCR ($P = 0.04$), but ER status and grade lost significant independent predictive value.

Clinical response

On univariate and multivariate analyses, high histological grade was associated with more prevalent clinical response ($P = 0.01$). No significant correlation between clinical response and the other clinicopathological variables was observed (Table 2).

Relapse-free survival

On univariate analysis the following factors were significantly associated with shorter RFS; higher clinical T ($P < 0.001$) and higher N ($P < 0.001$) stage, locally advanced disease status ($P = 0.05$), higher pre-therapy Ki67 ($P < 0.001$), pre-therapy ER negativity ($P = 0.004$), dual ER/ HER2 negative ($P = 0.002$), clinical progressive disease ($P = 0.003$), type of surgery performed ($P = 0.01$) and no adjuvant endocrine therapy ($P < 0.001$; Table 3). A trend for improved RFS was observed in patients with lower pre-therapy grade ($P = 0.06$) and those achieving a pCR ($P = 0.07$).

On multivariate analysis, higher clinical T ($P < 0.001$) and higher N ($P = 0.001$) stage, locally advanced disease status ($P = 0.02$), ER negativity ($P = 0.04$), lack of clinical response to chemotherapy ($P = 0.001$) and no adjuvant endocrine therapy ($P = 0.001$) were significant independent factors for shorter RFS.

Overall survival

On univariate analysis the following factors were associated with shorter OS; higher clinical T ($P = 0.001$) and N stage ($P = 0.01$), pre-therapy Ki67 (linear function, $P = 0.03$ and quadratic function, $P = 0.01$), treatment with non-anthracycline-based neoadjuvant regimens ($P = 0.03$), lack of cCR to first-line chemotherapy ($P = 0.02$), the absence of a pCR to neoadjuvant therapy ($P = 0.05$), mastectomy as opposed to breast conserving surgery ($P < 0.001$) and no adjuvant endocrine therapy ($P = 0.001$; Table 4).

On multivariate analysis, higher clinical T ($P < 0.001$) and higher N ($P = 0.001$) stage, locally advanced disease status ($P = 0.03$), type of surgery performed ($P = 0.03$) and no adjuvant endocrine therapy ($P = 0.005$) were the only independent prognostic factors associated with shorter OS.

Discussion

Pathological complete response to neoadjuvant chemotherapy is a clear predictor of survival. We have previously

Table 3 Univariate and multivariate analyses for relapse-free survival

Factors	Univariate analysis		Multivariate analysis	
	P value	HR (95%CI)	*P* value	HR (95%CI)
Age	NS (0.2)	1.2 (0.9–1.5)	NS	
Menstrual status	NS (0.1)		NS	
Pre		1		
Peri		2.2 (1.0–5.0)		
Post		1.3 (0.9–2.1)		
T stage	<0.001		<0.001	2.6 (1.7–3.9)
T1				
T2		1		
T3		2.5 (1.4–4.3)		
T4		3.4 (1.7–6.6)		
N stage	<0.001		0.001	1.9 (1.3–2.8)
N0		1		
N1		1.9 (1.1–3.1)		
N2		1.2 (0.3–5.0)		
N3		22.4 (6.2–80.9)		
Pre-therapy operability	0.05		0.019	0.4 (0.2–0.6)
Operable		1		
Locally advanced		1.9 (1.0–3.5)		
Pre-therapy Ki67	<0.001	0 (0.8–1.4)	NS	
Pre-therapy ER status	0.004		0.04	0.6 (0.4–1.0)
Negative		1		
Positive		0.5 (0.3–0.8)		
Pre-therapy PgR status	NS (0.3)		NS	
Negative		1		
Positive		0.5 (0.1–1.8)		
Pre-therapy HER2 status	NS (0.1)		NS	
Negative		1		
Positive		0.5 (0.2–1.2)		
Pre-therapy ER/ HER2 status	0.002		NS	
Dual negative		3.3 (1.6–7.2)		
Not dual negative		1.0		
Pre-therapy grade	NS (0.06)		NS	
1				
2		1		
3		1.7 (0.9–3.1)		
Pre-therapy histology	NS (0.5)		NS	
IDC		1		
ILC		0.7 (0.3–1.8)		
Anthracycline therapy	NS (0.1)		NS	
No		1		
Yes		0.6 (0.3–1.2)		
Response to neoadjuvant therapy	0.003		0.001	1.5 (1.2–1.9)
CR		1		
PR		1.2 (0.7–2.2)		
SD		0.9 (0.4–2.0)		
PD		4.4 (2.2–8.8)		

Table 3 continued

Factors	Univariate analysis		Multivariate analysis	
	P value	HR (95%CI)	*P* value	HR (95%CI)
pCR to neoadjuvant therapy	NS (0.07)		NS	
No		1		
Yes		0.4 (0.1–1.1)		
Type of surgery performed	0.01		NS	
None		1		
BCS		0.6 (0.3–1.0)		
Masectomy		1.3 (0.7–2.3)		
Adjuvant endocrine therapy	<0.001		0.001	0.4 (0.2–0.7)
No		1		
Yes		0.4 (0.2–0.7)		
Adjuvant chemotherapy	NS (0.1)		NS	
No		1		
Yes		0.4 (0.1–1.3)		

BCS breast conserving surgery, *IDC* invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *pCR* pathological complete response

shown that in patients who do not achieve a pCR, post-neoadjuvant chemotherapy Ki67 to be an independent prognostic factor [9]. The aim of the current study was to assess the prognostic and predictive role of pre-therapy Ki67.

Pre-therapy Ki67, grade, ER and HER2 status were significant predictors of pCR on univariate analysis. However, in a multivariate model, HER2 status was the only significant independent predictor of pCR. These findings corroborate those of previous studies, where grade [2, 14] and HER2 [15, 16] were shown to be significant independent predictors of pCR in multivariate models including Ki67, HER2 and ER status. Furthermore, in analysing ER positive and negative subgroups separately, Ki67 did not have a significant influence on pCR (data not shown). Here we demonstrate that the inverse correlation between ER status and Ki67 does not fully explain the higher pCR rate observed in ER negative tumours. This effect is unlikely to be due to ER status per se, but may in part be explained by HER2 status. These observations regarding the sensitivity of HER2 positive tumours to anthracycline chemotherapy may due to frequent co-amplification of *HER2* and *topoisomerase II* [17]. In contrast, other investigators have found Ki67 to be an independent predictor of pCR in models including histological grade, ER and HER2 status [17–20]. These discordant results may be due to the small patient numbers, the heterogeneous patient populations and chemotherapy regimens analysed and the investigation of varying biomarkers in each individual study as well as differing selection criteria. Another major limitation of most of these studies (including the present) is their retrospective nature.

Predictors of a pCR to neoadjuvant chemotherapy are also markers of poorer survival. However, recent studies have shown that women with triple negative tumours have a higher pCR rate than those with non-triple negative tumours [8, 21, 22]. Furthermore, those with triple negative disease who do achieve a pCR to neoadjuvant chemotherapy have an excellent prognosis. However, triple negative patients with residual disease following neoadjuvant therapy have worse survival compared to those with non-triple negative tumours [8, 21, 22].

There has been inconsistency regarding the prognostic value of clinical response to neoadjuvant chemotherapy possibly due to inter observer variability, and therefore for the purpose of this study clinical response is not as good an end point as pCR. Our study did not show a significant independent correlation between pre-therapy Ki67 and clinical response.

ER status, clinical T and N stage and the use of adjuvant endocrine therapy were independent prognostic factors for RFS in our study. On multivariate analysis pre-therapy Ki67 was not an independent predictor of RFS or OS. In agreement, most other studies have demonstrated that pre-neoadjuvant chemotherapy Ki67 is not an independent predictor of relapse-free, disease-free and progression-free survival [2, 15, 16, 18, 23–27], or OS [16, 19, 23–27]. A recent meta analysis, of patients treated with and without adjuvant systemic chemotherapy, has found that high Ki67 is associated with worse survival [28]. Further studies assessing the predictive impact of Ki67 in specific chemotherapy regimens are warranted.

In conclusion, pre-therapy ER and Ki67 were predictors of pCR on univariate analysis. However, in a multivariate model (including grade, ER and HER2 status), Ki67 lost significance. This suggests that aspects of ER status beyond its inverse association with proliferation may contribute to its predictive value for pCR. This in part may be explained by the inverse correlation observed between ER and HER2 status. Pre-therapy Ki67 was not an independent predictor of RFS and OS.

Table 4 Univariate and multivariate analyses for overall survival

Factors	Univariate analysis		Multivariate analysis	
	P value	HR (95%CI)	*P* value	HR (95%CI)
Age	NS (0.1)	1.3 (1.0–1.6)	NS	
Menstrual status	NS (0.4)		NS	
Pre		1		
Peri		1.7 (0.7–4.4)		
Post		1.3 (0.8–2.3)		
T stage	0.001		<0.001	2.3 (1.4–3.5)
T1				
T2		1		
T3		2.5 (1.4–4.3)		
T4		3.4 (1.7–6.6)		
N stage	0.01		0.001	2.0 (1.3–3.0)
N0		1		
N1		2.2 (1.2–3.8)		
N2		2.4 (0.7–8.2)		
N3		2.0 (0.3–14.8)		
Pre-therapy operability	NS (0.2)		0.03	0.4 (0.2–0.9)
Operable		1		
Locally advanced		1.6 (0.8–3.2)		
Pre-therapy Ki67			NS	
Linear	0.03	1.6 (1.0–2.3)		
Quadratic	0.01	1.1 (1.0–1.2)		
Pre-therapy ER status	NS (0.1)		NS	
Negative		1		
Positive		0.7 (0.4–1.1)		
Pre-therapy PgR status	NS (0.2)		NS	
Negative		1		
Positive		0.3 (0–2.1)		
Pre-therapy HER2	NS (0.4)		NS	
Negative		1		
Positive		0.6 (0.2–1.7)		
Pre-therapy ER/HER2 status	0.2		NS	
Dual negative		1.7 (0.7–4.0)		
Not dual negative		1.0		
Pre-therapy grade	NS (0.1)		NS	
1				
2		1		
3		1.7 (0.9–3.1)		
Pre-therapy histology	NS (0.5)		NS	
IDC		1		
ILC		0.7 (0.3–2.0)		
Anthracycline therapy	0.03		NS	
No		1		
Yes		0.5 (0.2–0.9)		
Response to neoadjuvant therapy	0.02		NS	
CR		1		
PR		1.3 (0.7–2.5)		
SD		1.3 (0.6–3.0)		
PD		3.2 (1.5–7.1)		

Table 4 continued

Factors	Univariate analysis		Multivariate analysis	
	P value	HR (95%CI)	*P* value	HR (95%CI)
pCR to neoadjuvant therapy	0.05		NS	
No		1.0		
Yes		0.1 (0.02–1.0)		
Type of surgery performed	<0.001		0.03	2.2 (1.1–4.4)
None		1		
BCS		0.5 (0.2–1.0)		
Mastectomy		1.8 (0.9–3.5)		
Adjuvant endocrine therapy	0.001		0.005	0.4 (0.2–0.8)
No		1		
Yes		0.4 (0.2–0.6)		
Adjuvant chemotherapy	NS (0.9)		NS	
No		1		
Yes		0.9 (0.3–2.6)		

BCS breast conserving surgery, *IDC* invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *pCR* pathological complete response

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References

1. Jones RL, Smith IE (2006) Neoadjuvant treatment for early-stage breast cancer: opportunities to assess tumour response. Lancet Oncol 7:869–874. doi:10.1016/S1470-2045(06)70906-8
2. Colleoni M, Viale G, Zahrieh D et al (2004) Chemotherapy is more effective in patients with breast cancer not expressing steroid hormone receptors: a study of preoperative treatment. Clin Cancer Res 10:6622–6628. doi:10.1158/1078-0432.CCR-04-0380
3. Pohl G, Rudas M, Taucher S et al (2003) Expression of cell cycle regulatory proteins in breast carcinomas before and after preoperative chemotherapy. Breast Cancer Res Treat 78:97–103. doi:10.1023/A:1022165715043
4. Ring AE, Smith IE, Ashley S, Fulford LG, Lakhani SR (2004) Oestrogen receptor status, pathological complete response and prognosis in patients receiving neoadjuvant chemotherapy for early breast cancer. Br J Cancer 91:2012–2017. doi:10.1038/sj.bjc.6602235
5. Parton M, Krajewski S, Smith I et al (2002) Coordinate expression of apoptosis-associated proteins in human breast cancer before and during chemotherapy. Clin Cancer Res 8:2100–2108
6. Ravaioli A, Bagli L, Zucchini A, Monti F (1998) Prognosis and prediction of response in breast cancer: the current role of the main biological markers. Cell Prolif 31:113–126. doi:10.1111/j.1365-2184.1998.tb01190.x
7. Archer CD, Parton M, Smith IE et al (2003) Early changes in apoptosis and proliferation following primary chemotherapy for breast cancer. Br J Cancer 89:1035–1041. doi:10.1038/sj.bjc.6601173
8. Carey LA, Dees EC, Sawyer L et al (2007) The triple negative paradox: primary tumour chemosensitivity of breast cancer subtypes. Clin Cancer Res 13:2329–2334. doi:10.1158/1078-0432.CCR-06-1109
9. Jones RL, Salter J, A'hern R et al (2008) The prognostic significance of Ki67 before and after neoadjuvant chemotherapy in breast cancer. Breast Cancer Res Treat. doi:10.1007/s10549-008-0081-7
10. Jones RL, Lakhani SR, Ring AE, Ashley S, Walsh G, Smith IE (2006) Pathological complete response and residual DCIS following neoadjuvant chemotherapy for breast carcinoma. Br J Cancer 94:358–362
11. Ellis P, Smith I, Ashley S et al (1998) Clinical prognostic and predictive factors for primary chemotherapy in operable breast cancer. J Clin Oncol 16:107–114
12. Elston CW, Ellis IO (1991) Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19:403–410. doi:10.1111/j.1365-2559.1991.tb00229.x
13. Harvey JM, Clark GM, Osborne CK, Allred DC (1999) Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 17:1474–1481
14. Petit T, Wilt M, Velten M et al (2004) Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. Eur J Cancer 40:205–211. doi:10.1016/S0959-8049(03)00675-0
15. Penault-Llorca F, Cayre A, Bouchet Mishellany F et al (2003) Induction chemotherapy for breast carcinoma: predictive markers and relation with outcome. Int J Oncol 22:1319–1325
16. Tinari N, Lattanzio R, Natoli C et al (2006) Changes of topoisomerase II alpha expression in breast tumours after neoadjuvant chemotherapy predicts relapse-free survival. Clin Cancer Res 12:1501–1506. doi:10.1158/1078-0432.CCR-05-0978
17. Arriola E, Rodriguez-Pinilla SM, Lambros MB et al (2007) Topoisomerase II alpha amplification may predict benefit from adjuvant anthracycline in HER2 positive early breast cancer. Breast Cancer Res Treat 106:181–189. doi:10.1007/s10549-006-9492-5
18. Bottini A, Berruti A, Brizzi MP et al (2005) Cytotoxic and antiproliferative activity of the single agent epirubicin versus epirubicin plus tamoxifen as primary chemotherapy in human breast cancer: a single-institution phase III trial. Endocr Relat Cancer 12:383–392. doi:10.1677/erc.1.00945
19. Faneyte IF, Schrama JG, Peterse JL, Remijnse PL, Rodenhuis S, van de Vijver MJ (2003) Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. Br J Cancer 88:406–412. doi:10.1038/sj.bjc.6600749
20. Prisack HB, Karreman C, Modlich O et al (2005) Predictive biological markers for response of invasive breast cancer to

anthracycline/cyclophosphamide-based primary (radio-)chemotherapy. Anticancer Res 25:4615–4621
21. Liedtke C, Mazouni C, Hess KR et al (2008) Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 26(8):1275–1281. doi:10.1200/JCO.2007.14.4147
22. Rouzier R, Perou CM, Symmans WF et al (2005) Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 11(16):5678–5685. doi:10.1158/1078-0432.CCR-04-2421
23. Takada M, Kataoka A, Toi M et al (2004) A close association between alteration in growth kinetics by neoadjuvant chemotherapy and survival outcome in primary breast cancer. Int J Oncol 25:397–405
24. Lee J, Im YH, Lee SH et al (2008) Evaluation of ER and Ki-67 proliferation index as prognostic factors for survival following neoadjuvant chemotherapy with doxorubicin/docetaxel for locally advanced breast cancer. Cancer Chemother Pharmacol 61:569–577. doi:10.1007/s00280-007-0506-8
25. Vincent-Salomon A, Rousseau A, Jouve M et al (2004) Proliferation markers predictive of the pathological response and disease outcome of patients with breast carcinomas treated by anthracycline-based preoperative chemotherapy. Eur J Cancer 40:1502–1508. doi:10.1016/j.ejca.2004.03.014
26. Honkoop AH, van Diest PJ, de Jong JS et al (1998) Prognostic role of clinical, pathological and biological characteristics in patients with locally advanced breast cancer. Br J Cancer 77:621–626
27. Bonnefoi H, Diebold-Berger S, Therasse P et al (2003) Locally advanced/inflammatory breast cancers treated with intensive epirubicin-based neoadjuvant chemotherapy: are there molecular markers in the primary tumour that predict for 5-year clinical outcome? Ann Oncol 14:406–413. doi:10.1093/annonc/mdg108
28. de Azambuja E, Cardoso F, de Castro G Jr et al (2007) Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12, 155 patients. Br J Cancer 96(10):1504–1513. doi:10.1038/sj.bjc.6603756