

# Clinical and Pathological Response to Primary Chemotherapy in Patients with Locally Advanced Breast Cancer Grouped According to Hormonal Receptors, Her2 Status, Grading and Ki-67 Proliferation Index

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**Abstract.** *Objectives: Biological markers that reliably predict clinical and pathological response to primary systemic therapy may have considerable clinical potential; this study evaluated response compared to expression of ER, PgR and Her2, grading and Ki-67 proliferation index before and after neoadjuvant chemotherapy in patients with locally advanced breast cancer (LABC). Patients and Methods: Fifty-five patients received neoadjuvant chemotherapy for LABC. The incidence of clinical and pathological responses was assessed with respect to basal clinical stage, absent/low vs. high ER and PgR status, low vs. high proliferation index, grading and Her2 overexpression. Results: Overall, 30 patients (54%) underwent downstaging of their primary tumor; pathological complete remission was observed in only one patient with Her2 positive breast tumor. Patients with pre-treatment Ki-67 >20%, Her2 overexpression, T2b/T3 vs. T4 clinical stage achieved higher response rate. Conclusion: The future of neoadjuvant therapy lies in tailoring treatment to individual patients by identifying response predictors; although the number of patients reported is small, this study confirms that clinical stage at diagnosis, Ki-67 reduction and Her2 overexpression are predictive of tumor response to neoadjuvant regimens.*

Neoadjuvant chemotherapy is frequently administered to patients with large or locally advanced invasive breast

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*Key Words:* Neo-adjuvant chemotherapy, locally advanced breast cancer, biological markers.

carcinomas. The aim is to extend the range of patients eligible for breast conserving surgery by surgically downsizing carcinomas; however this benefit is almost exclusively restricted to the subgroup of patients who achieve a complete pathological response (CPR) (1). In addition preoperative treatment of breast cancer is a model for the evaluation of the predictive value of molecular markers, as tumor tissue can be obtained before and after treatment. Predicting the chance of response to treatment is an important goal; patients with a low chance of a clinically useful response to neoadjuvant chemotherapy might be spared unnecessary toxicity. Negative hormone receptor status, Her2 positivity and high proliferation activity are strong predictive markers of chemo-response, although the consensus on the most suitable biological markers in this setting has not been established yet (2). This study evaluated the pre- and post treatment expression levels and changes in Ki-67 proliferation index, ER, PgR and Her2 expression in 55 patients with locally advanced breast cancer for the potential role in predicting treatment response to neoadjuvant combination chemotherapy.

## Patients and Methods

Between 2003 and 2008, samples from 55 patients with locally advanced breast cancer, who received neoadjuvant chemotherapy were retrospectively collected. Histological diagnosis and biological characteristics including hormone receptor status, Ki-67 proliferation index and Her2 status were assessed before the start of chemotherapy, performing a tru-cut biopsy of the primary tumour, and after surgery; grading of invasive carcinoma was obtained from the surgical pathology report. Clinical characteristics including age, menopausal status, number of chemotherapy cycles administered, breast surgery and objective clinical and pathological response in breast and axilla were recorded. Neoadjuvant chemotherapy mostly included anthracycline based regimens using epirubicin 75 mg/m<sup>2</sup>,

epirubicin and taxanes sequentially administered and anthracycline, taxane and trastuzumab in Her2 positive tumours; 5 patients of age  $\geq 70$  years were treated with CMF neoadjuvant chemotherapy. Tumour response was measured clinically in two principal diameters at each cycle of chemotherapy; breast ultrasound and mammography were performed before the treatment start and after four cycles of chemotherapy; MRI was carried out if clinically indicated. Clinical Responses were evaluated according to WHO criteria (3). Pathological complete remission was defined as the total absence of invasive tumour in both breast and axillary nodes.

Correlations of treatment outcomes with estrogen and progesterone receptor status, Her2, Ki-67 and grading were evaluated. Only nuclear activity was taken into account for Er, PgR and Ki67 antigen, whereas only an intense and complete membrane staining  $>10\%$  of the tumor cells was taken as evidence of HER2 overexpression; the following primary antibodies were used: the mAb to ER (Dako, Glostrup, Denmark, at 1/100 dilution), the mAb to PgR (Dako 1/800), the MIB-1 mAb to the Ki-67 antigen (Immunotech, Marseille, France, 1/1200). Tumors were categorized into two groups in relation to proliferative activity; a Ki-67 proliferation index threshold of 20% was used to distinguish tumours with low ( $<20\%$ ) and high ( $\geq 20\%$ ) proliferative fraction; (the value of 20% for proliferative activity was selected as this value was previously shown to significantly correlate with higher response rate to preoperative chemotherapy) (4). According to steroid hormone receptor status, tumours were arbitrarily divided into two groups: the absent and low hormone responsive group included tumours with ER and PgR nuclear staining up to 20% of cells positivity, and the high endocrine positive group included tumors with ER and PgR nuclear staining from  $> 20\%$  up to 100% of cells; for Her2, all carcinomas were assessed immunohistochemically using the HercepTest test; only Her2 3+ tumors were regarded as positive for overexpression; Her2 2+ tumors were regarded as positive only if Fish assay demonstrated Her2 gene amplification (Pathvision Vysis Abbott, Downers Grove, Ill.) (5). The histologic grade was defined according to Eston – Ellis grading system (6).

*Statistical analysis.* The Chi-square test or Fisher's exact test was used to compare downstaging of primary tumor based on pathological stage vs. clinical stage at diagnosis and Her2 tumor positivity. All *p*-values will be two-tailed.

## Results

All patients had locally advanced breast cancer: TNM stage IIB (n=13), IIIA (n=25) and T4b (n=17). Patient characteristics are shown in Table I. Biological characteristics of the tumours at the pre-treatment biopsy and at surgery were available in 52 patients; 3 patients who, following 4 cycles of neoadjuvant chemotherapy had progressive disease, were treated with definitive radiotherapy. All 55 patients were evaluable for clinical response on completion of chemotherapy; the objective clinical response rate (CR + PR) was 58%. Histopathological response was achieved in 30 patients (54%); higher downstaging of tumor after primary chemotherapy was observed in T2b/T3 tumors compared to T4b ( $p=0.025$ ). However, complete pathological response was observed in only

one patient with Her2 positive breast tumour after combination of taxane, anthracycline and trastuzumab neoadjuvant chemotherapy (Table II).

ER receptors were low or absent in 29% and high in 71% of the tumours, PgR receptors low or absent in 46% and positive in 54%; there was no correlation between ER status, clinical and pathological response: of the patients with high ER receptor positivity, objective clinical remission (CR + PR) was observed in 16 (41%) and stable disease in 23 (59%) patients; 7 patients (44%) achieved objective clinical response and 9 patients (56%) had stable disease in the group of low or absent ER receptor status; no changes in ER positivity score were observed in the 52 patients with both pre and post treatment ER results available. Among 30 patients within the high PgR positive cohort, 40% of objective clinical response and 60% of stable disease was observed compared with 44% of clinical response and 56% of stable disease within the 25 patients with PgR receptor absent or low, respectively. Differences in response rate according to tumour grading were not significant: 33% of patients with grade 3 tumours had objective response compared with 43% of patients with grades 1 and 2 tumours. Differences in remission rate according to number of cycles administered or whether or not taxanes were added to anthracycline were not observed.

Between the 30 patients who achieved histopathological response, 67% of patients showed pre-treatment Ki-67  $>20\%$ .

All the eight patients with Her2 overexpressing tumours received neoadjuvant anthracyclines containing regimens and trastuzumab was added to chemotherapy according to MD Anderson regimen; overall, seven patients (87.5%) achieved an objective clinical response ( $p=0.045$ ) and a pathological complete remission was observed in one patient with Her2 overexpression and hormonal negative receptor tumour after anthracycline, taxane and trastuzumab neoadjuvant regimen.

## Discussion

Existing studies show that patients who benefit the most from neoadjuvant chemotherapy are those who achieve a pathological complete response (CPR) with no residual microscopic tumour; the measurement of clinical responses is to an extent subjective and the assessment of pathological response is more robust and reproducible; however achievement of pathological complete response is uncommon occurring in only 3-16% of patients (7), while clinical response are reported in up to 50-70% of patients (4, 8). In this study 54% of patients underwent downstaging of their primary tumor; however only one patient with Her2 positive and hormonal receptor negative tumor obtained complete pathological response.

Table I. Patient's characteristics.

	No. %
Evaluable patients	55
Median age (range)	55 (30-75)
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	No. %
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Menopausal status	
Pre	21 (38%)
Post	34 (62%)
Histological type	
Ductal	48 (87%)
Lobular	7 (13%)
ER status	
0-20%	16 (29%)
21-100%	39 (71%)
PgR status	
0-20%	25 (45%)
21-100%	30 (55%)
Ki-67(a)	
≥20%	32 (71%)
<20%	13 (29%)
HER2 overexpression	
3+	8 (15%)
Tumor grade (b)	
G1-G2	36 (70%)
G3	15 (30%)
Neoadjuvant chemotherapy	
Antracycline-based	30 (54%)
Taxane+Antracycline	12 (22%)
Antracycline+Taxane+herceptin	8 (15%)
CMF	5 (9%)
Number of chemotherapy cycles	
≤4	30 (54%)
>4	25 (46%)
Surgery	
Mastectomy	32 (58%)
Lumpectomy/Quadrantectomy	20 (36%)
Definitive Radiotherapy	3 (6%)

(a) information missing in 10 cases; (b) information missing in 4 patients: 1 patient was not evaluable for grading at surgery because she achieved a PCR and 3 patients because underwent definitive radiotherapy.

Patients with base-line advanced local stage had lower pathological complete response and higher number of positive lymph nodes after primary chemotherapy (9). Jeruss *et al.* (10) recently elaborated a prognostic scoring system including pre-treatment clinical stage, final pathologic stage and biological markers in patients treated with neoadjuvant chemotherapy: patients who presented with clinical stage IIB or IIIA disease had favourable projected outcomes with respect to patients presenting with stage IIIB or IIIC disease. In the present study 76% of patients had T3 - T4b baseline clinical stage. Considering that tumor diameter has been indicated as a predictor of preoperative treatment response, one of the plausible reasons for the lower rate of response observed in this study might be the large tumor size on baseline in this series: in fact results showed higher

Table II. Downstaging of primary tumor and nodal status according to baseline clinical stage.

Clinical TNM stage at diagnosis (n=55)	Downstaging of primary tumor (n=30*)				
	pT0	pTis	pT1	pT2	pT3
T2b (n=13)	1	1	8	0	0
T3 (n=25)	0	0	8	7	0
T4b (n=17)	0	0	0	3	2
<hr/>		Clinical nodal status (n=55)		Pathological nodal status (n=52)	
		N0 (n=13)		N0 (n=14)	
		N1 (n=42)		N1 (n=38)	

\*Overall histopathological response with downstaging of primary tumor was achieved in 30 patients (54%); surgery was not performed in 3 patients (6%) who had progressive clinical disease following neoadjuvant chemotherapy; in 22 patients (40%) downstaging of primary tumor was not achieved. n=number of patients.

downstaging of tumor after primary chemotherapy in T2b / T3 tumor compared to T4b.

Apart from the achievement of CPR in the breast, one of the most important prognostic factors after neoadjuvant therapy is the post-treatment lymph node status; previously investigators have shown the importance of CPR in positive lymph nodes in response to neoadjuvant chemotherapy, independent of the response of the primary tumor. Patients who achieved a CPR in previously documented positive lymph nodes had considerably better outcomes than patients who did not, regardless of the response in the primary tumor (11, 12). In the present study, 76% of patients had clinical pre-treatment positive lymph nodes and 73% remained with lymph nodes positive at pathological staging.

Randomised trials comparing preoperative *vs.* postoperative adjuvant chemotherapy in early breast cancer show similar rates of disease-free and overall survival (13); only mastectomy rate is lower with the preoperative approach (14); preoperative systemic treatment could induce downstaging of the primary tumor but lymph node metastases are less responsive to neoadjuvant chemotherapy and patients with lymph node metastases are at high risk for systemic metastases.

Conversely to the method used to choose an adjuvant systemic therapy regimen, the selection of preoperative therapy does not commonly take into account biological characteristics of the tumor. Recently Kaufmann *et al.* (15) reported recommendations from an international expert panel on the use of neoadjuvant systemic treatment of operable breast cancer: patients whose tumors express markers of good response to chemotherapy are identified as those with

low or absent hormone receptor status, high grade, non-lobular invasive histology and high Ki-67 proliferative index. Recently the largest experience on hormone receptor expression predicted response in the preoperative setting showed that estrogen receptor negative tumors had higher pathological response rates than did estrogen receptor positive tumors (16); however the relative chemoresistance of estrogen receptor positive tumor does not automatically translate into a worse outcome (7). In this study, contrary to the results from previous studies, no significant correlation was detected between response to chemotherapy and estrogen and progesterone receptor status. Several studies have shown that high grade tumors are more likely to decrease in size in response to chemotherapy (1); in this study no significant correlation was detected between response and nuclear grade.

Ki-67 is an antigen present in all phases of the cell cycle except G0; it is a measure of tumor proliferation that has been shown to correlate with outcome in several studies. It has been also suggested that baseline high Ki67 values may be predictive of responsiveness to neoadjuvant chemotherapy (17). Burcombe *et al.* (18) evaluated changes in Ki-67 labeling index in 39 patients before and after neoadjuvant anthracycline chemotherapy: although a reduction in Ki-67 index from pre-treatment values was observed in 69% of patients at surgery, neither pre-treatment nor post-chemotherapy median Ki-67 index differed significantly between clinical or pathological responders and non-responders. By contrast, the results of the present study indicate in the group of patients with baseline higher staining for Ki-67 a subset of patients who appear to benefit from neoadjuvant chemotherapy.

Finally, recent studies have shown higher rates of CPR and survival in Her2 positive patients who receive trastuzumab based neoadjuvant chemotherapy (19). Goldstein *et al.* (20) using a molecular taxonomic classification system, identified the subset of carcinoma patients most likely to achieve a CPR from neoadjuvant chemotherapy: 83% of CPR was achieved in patients classified as having Her2 classic carcinoma. In accordance with the results of Goldstein, in this study higher clinical response rates were achieved in patients with Her2 positive and hormonal receptor negative phenotype, observing here the only CPR achieved by neoadjuvant therapy.

Over the past 20 years there has been a large effort to identify predictive markers associated with CPR in patients submitted to neoadjuvant chemotherapy: ER and PgR status, Her2, Ki-67 proliferative index, and tumor grade and size have all been linked to CPR. Contrary from most published series, the number of patients reported in this study is small; however this study confirms that clinical stage at diagnosis and baseline Ki-67 values are predictive of response to neoadjuvant chemotherapy, irrespective of patient age, menopausal status, tumor grade and hormonal receptor

status. As expected Her2 overexpression is highly predictive of tumor response to neoadjuvant regimens including trastuzumab. Given the lack of a preferred treatment regimen in the neoadjuvant setting, identifying the patients who are likely to respond to specific agents could inform treatment decisions, improve treatment outcomes and aid in avoiding unnecessary exposure to potential toxicities.

## Acknowledgements

We thank Laura Veroni for precious secretarial assistance.

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Received December 1, 2008

Revised January 28, 2009

Accepted February 3, 2009