Value of post-operative reassessment of estrogen receptor α expression following neoadjuvant chemotherapy with or without gefitinib for estrogen receptor negative breast cancer

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Statement

Results of the NICE trial has been published previously (Bernsdorf et al, reference no.18). The results of the current study has not been presented previously

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Abstract

Background

The NICE trial was designed to evaluate the possible benefits of adding epidermal growth factor receptor targeted therapy to neoadjuvant chemotherapy in patients with estrogen receptor α (ER) negative and operable breast cancer. Preclinical data have suggested that signalling through the ErbB receptors or downstream effectors may repress ER expression. Here we investigated whether gefitinib, given neoadjuvant in combination with epirubicin and cyclophosphamide (EC), could restore ER expression.

Materials and Methods

Eligible patients in the NICE trial were women with unilateral, primary operable, ER negative invasive breast cancer ≥ 2 cm. Material from patients randomized and completing treatment (four cycles of neoadjuvant EC plus 12 weeks of either gefitinib or placebo) in the NICE trial having available ER status both at baseline and after neoadjuvant treatment were eligible for this study.

Tumors with indication of changed ER phenotype (based on collected pathology reports) were immunohistochemically reassessed centrally.

Results

115 patients were eligible for this study; 59 patients in the gefitinib group and 56 patients in the placebo group. Five (4.3%) of 115 tumors changed ER phenotype from negative to positive. No difference between the two treatment groups was observed, as changes were seen in 3 patients in the gefitinib (5.1%) and in 2 patients in the placebo (3.6%) group with a difference of 1.51% (95% CI, -6.1 – 9.1; p=1.0). Results of the NICE trial has been reported previously.

Conclusion

Postoperative reassessment of ER expression changed the assessment of ER status in a small but significant fraction of patients and should, whenever possible, be performed following neoadjuvant chemotherapy for ER negative breast cancer. Gefitinib did not affect the reversion rate of ER negative tumors.

Introduction

Expression of the estrogen receptor (ER) possesses a strong predictive value and constitutes a requisite for the efficacy of adjuvant endocrine therapy in breast cancer patients (1). Measurement of ER has for that reason become standard on all newly diagnosed invasive breast cancers (2;3) and accurate and standardized assessment of ER is therefore essential.

A negative association between ER expression and expression of receptors from the ErbB-family, like epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor II (HER2), has been demonstrated in a large number of clinical studies and overexpression of EGFR and HER2 are primarily observed in ER negative tumors (4;5). Both clinical and preclinical data demonstrate that progression of ER dependent breast cancer may occur via a shift from ER to EGFR/HER2 signalling (6-8), although the shift occurs with only modest upregulation of the ErbB receptor mediated signalling (8-10).

Absent ER expression is a complex multi-step and potentially reversible process and it has been suggested that absent ER expression, may be reverted by blocking of the ErbB system. Thus, several cell culture studies have shown that blocking EGFR and/or HER2 receptor signaling resulted in regain of endocrine sensitivity (10-16). Furthermore, in both clinical and preclinical studies, it has been demonstrated that treatment with trastuzumab and/or gefitinib restores ER expression in HER2 positive and ER negative breast cancer (16;17). In Munzone et al (17), three of ten patients changed ER negative phenotype to ER positive following treatment, indicating that re-evaluation of ER expression may be recommended in general.

Consequently, we sought to examine the effect of neoadjuvant treatment on ER expression in patients with early ER negative breast cancer and whether treatment with gefitinib, a specific EGFR tyrosine kinase inhibitor, would increase the possibility of endocrine responsiveness. The present study is an exploratory analysis but according to and defined by the study protocol. The study is based on collection of local pathology reports, followed by central immunohistochemistry (IHC) confirmation of tumors with ER phenotype change following definitive surgery. All patients received preoperative chemotherapy in the NICE trial and were randomized to gefitinib against placebo.

Patients and methods

Full details of the NICE study (clinicaltrials.gov identifier NCT00239343) have been described previously (18). In summary, NICE was a Nordic phase II, multicenter, two-armed double-blind randomized trial

involving 12 centers in Denmark, Norway and Sweden, in which 181 patients were assigned to either four cycles of epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2)(EC) plus 12 weeks of daily treatment with gefitinib (250 mg) or EC plus 12 weeks daily treatment with placebo.

Eligible patients were women with unilateral, primary operable, ER negative invasive breast cancer ≥ 2 cm. Patients with inflammatory breast cancer, involvement of skin or muscle, involvement of supraclavicular lymph nodes or evidence of distant metastases were not eligible.

The primary clinical objective of the NICE trial was to compare the difference in pathologic complete response (pCR) rate in the two treatment arms and secondary objectives were complete and overall response. Tumor response was assessed by the response evaluation criteria in solid tumors (RECIST) criteria.

Written informed consent was obtained from all study participants prior to study entry and ethical committees with jurisdiction for the participating institutions approved the study protocol.

Patients who completed treatment in the NICE study and had available ER status, before and after neoadjuvant treatment, were eligible for this study.

Local pathology assessment

Local pathologists determined ER expression before random assignment. Immunohistochemistry was used in all participating institutions and ER was classified as negative for a proportion score of < 10% staining cells and ignoring intensity of staining.

Central pathology assessment

Formalin fixed paraffin-embedded tumor tissue was prospectively collected at baseline and following definite surgery (pre- and postoperative specimens, respectively). In all, complete specimens was obtained from 134 patients (74% of the randomized population). The Central DBCG Pathology Laboratory in Herlev, Denmark reviewed the material for residual tumor tissue and expression of tumor markers.

Assessment of ER and HER2

In tumors where local pathology reports indicated a change in ER phenotype, a central review of ER status was performed using IHC and scored with percentage of staining.

HER2 and *EGFR* copy number Fluorescence In Situ Hybridization (FISH) analysis was performed by use of DakoCytomation (*HER2* pharm Dx™ and *EGFR* pharm Dx™, Dako, Glostrup, Denmark). Tumors were scored as *HER2* or *EGFR* positive/amplified when the gene to centromere ratio ≥ 2 and *EGFR* as deleted when the ratio was < 0.8.

Statistical methods

The percentage of ER staining cells were dichotomised as present (> 0%) or absent (0%), and as positive (≥ 10%) or negative (< 10%). Concordance denoted the proportion of tumors with the same classification by local and central assessment. Fisher's exact test was used to evaluate the difference in ER changes in the two groups.

Results

In all, 158/181 (87%) patients randomized into the NICE study completed treatment, of which 115 patients were eligible for this study; 59 patients in the gefitinib group and 56 patients in the placebo group. Reasons for excluding 43 patients were: a pCR was observed, leaving no tumor material for posttreatment ER evaluation (n=23); tumor was retrospectively identified as baseline ER positive (n=6); or no posttreatment ER evaluation was done locally (n=14). Baseline patient characteristics were well balanced in the two groups (table 1).

5.1% (3/59) of tumors in the gefitinib group and 3.5% (2/56) of tumors in the placebo group, with a total of 4.3% (5/115) and a difference of 1.51% (95% CI, -6.1 – 9.1) changed from ER negative at baseline to ER positive after neoadjuvant treatment. All tumors with change in ER were revised and confirmed centrally. Local and central ER evaluation and staining scores for tumors with ER phenotype change are listed in table 2. It is noteworthy, that in the tumors with ER reversion, between 15 to 90% of the tumor cells were ER positive (fig 1).

Tumors with change in ER phenotype tended to be of ductal histology and *HER2*, *EGFR* and PgR normal/negative as this was randomly present in 4 tumors. Pathologic characteristics at baseline for the five tumors with change in ER are listed in table 3.

Other pathologic characteristics for tumors with a change in ER phenotype, such as *HER2, EGFR* and histology, were not different from the entire cohort (table 1 and 2).

Of the 115 patients, 99 and 101 had both pre and post treatment *HER2* and *EGFR* status performed, respectively. Changes in *HER2* were observed in 3 tumors (3%, gefitinib, n=1; placebo, n=2) and in *EGFR* in 7 tumors (6.9%; gefitinib, n= 3; placebo, n=4). None of the five tumors with change in ER phenotype had any changes in *HER2* or *EGFR*.

A partial response was observed in 3 tumors and stable disease in 2 tumors with change in ER phenotype (table 3). Because of the low numbers of tumors with change in ER status, no analysis of difference in response for tumors with and without ER phenotype change was done.

Discussion

This is the first report exploring ER-negative phenotype changes after a neoadjuvant gefitinib regime. All patients received EC with or without gefitinib and pathological tumor characteristics were collected at baseline and at surgery.

Since central review revealed 1% of tumors to be a wrongly ER classified locally in the BIG 1-98 study (19), a central review of tumors with change in ER phenotype was conducted. The central review revealed no discrepancy with local ER classification (table2).

The primary goal of this study was to evaluate the effect of neoadjuvant treatment on ER expression and whether gefitinib, given neoadjuvant with EC, would have an increased impact on ER expression. Interestingly, 4.3% (5/115) of ER negative tumors changed ER phenotype, supporting that ER expression, is reversible, as also reported by others (6;7;12;17;20;21). The reversibility of ER expression challenges the clinical approach to patients with an ER resistent tumor, as patients with an initially ER negative tumor, that revert to ER positive, might benefit from endocrine therapy.

Together with our results, the significant shift in ER phenotype, from ER positive to negative, following neoadjuvant therapy observed in the trial by Taucher et al (21) and the variation in ER expression between primary and metastatic breast cancer reported by Arslan et al (22), further emphasizes the importance for renewed ER evaluation after neoadjuvant treatment and in the event of recurrence or metastases.

A higher turnover rate was anticipated in the gefitinib group, due to the inverse relation between expression of ER and EGFR/HER2 and due to the reversal of ER expression by suppression of EGFR/HER2 signaling, but no difference between the two treatment groups was observed. These results indicate that the inhibition of EGFR with gefitinib, may not be the only mechanism which restores ER expression and suggests that

EGFR, may not alone drive cell growth in ER negative breast cancer. This is further supported by a recent review, describing different mechanisms of reversing ER expression and sensitization to endocrine therapy in ER negative breast cancer (15). In four of five tumors, ER reversion occurred in tumors with normal *EGFR* and *HER2*, and no conclusion on a change from ErbB to ER signalling can be made.

It remains unknown, whether the clinical activity of gefitinib can be equated to gefitinibs ability to revert ER expression, if such an ability excist. However, the clinical effect of gefitinib is neither depended on EGFR expression nor inhibition of EGFR phosphorylation (23;24) as well as gefitinib does not affect the expression of EGFR (23). In our study the majority of patients included were *EGFR* normal (table 1) and whether selecting patients based on *EGFR* amplification would have resulted in more tumors converting ER phenotype remains unknown, but no tumor that changed ER phenotype was *EGFR* amplified in the present study.

In the study by Baselga et al (23), the complete inhibition of EGFR phosphorylation, which did not translate into a clinical benefit, was achieved with gefitinib 500 mg/daily as monotherapy for treatment of advanced breast cancer. In the NICE study, a dose of gefitinib 250 mg/daily was chosen since no clinical benefit was observed in a comparison of gefitinib 500 mg/dailly to 250 mg/daily (25;26). Whether a dose of gefitinib 500 mg/daily could have increased the rate of ER reversions remain unknown.

The exact mechanism underlying the change in ER phenotype is unknown. As no discrepancy was observed between local and central review, the changes are likely due to a biological phenomenon, rather than inconsistent measurements. The intratumoral heterogeneity in breast cancer, may result in genetic subclones with different sensitivity to anti-neoplastic treatment (27). This could be a possible explanation for a change in ER phenotype, where, under the pressure of chemotherapy, a possible shift (clonal selection) from an ER negative dominant clone to an ER positive dominant clone could have occurred.

However, in the gefitinib treated group, two of the three tumors displayed a posttreatment ER level of 90%. In one case, this was associated with stable disease, strongly supporting that gefitinib treatment may be responsible for the ER reversion. In the two patients with a partial response, selective survival and growth of a minor undetected population of ER positive cells cannot be excluded, although a posttreatment ER level of 90% indicate that reversion may have occurred.

Regardless of the underlying mechanism of restoring ER expression, in order to translate into a clinical benefit, the re-expressed ER must be functional in order to respond to endocrine therapy. In the study by

Monzone et al (17), two of three patients with advanced disease, who reverted to ER positivity, were treated with endocrine therapy and one of these patients was without progression at three years. Whether or not re-expression of ER after neoadjuvant treatment indicate benefit from endocrine therapy remains unknown and requires further investigations.

Conclusion

Our data show that ER expression is reversible in a minor but significant fraction of ER negative tumors, emphasizing the importance for reliable and correct pathologic evaluation, in order to provide best treatment possible. Eventhough, gefitinib did not have an impact on tumors reverting ER phenotype, a possible role of gefitinib in the change of ER expression cannot be excluded.

The reversion of ER from negative to positive might indicate benefit from endocrine treatment in patients otherwise exempt from this option. Therefore, a reassessment of ER status and whenever possible, also markers associated with functional ER e.g. the progesterone receptor, should be performed following neoadjuvant treatment.

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Table 1. Pretreatment patient and tumor characteristics.

Characteristics		Gefitinib (n=59)		Placebo (n=56)	
		Number (n)	Percent (%)	Number (n)	Percent (%)
Age, years					
	Mean	52.8		53,3	
	SD	10.1		10.5	
	Range	32, 70		32, 69	
Histology					
	Ductal	56	94.9	52	92.9
	Lobular	2	3.4	2	3.6
	Other	1	1.7	2	3.6
Histologic grade					
	1	0	0	1	1.9
	2	26	46.4	22	40.7
	3	30	53.6	31	57.4
	Missing	3		2	
Tumor status					
	< 30 mm	35	59.3	26	46.8

	30 – 50 mm		19	32.2	23	41.1	
	>30 mm		5	8.5	7	12.5	

	HER2	PgR	Grade	Tumor size (mm)	Histology	EGFR	Response (tumor reduction %)
Gefitinib							
Tumor 1	normal	negative	2	21	ductal	normal	SD (29%)
Tumor 2	normal	negative	2	21	ductal	normal	PR (52%)
Tumor 3	normal	negative	2	70	lobular	normal	PR (57%)
Placebo							
Tumor 4	amplified	negative	3	40	ductal	deleted	SD (0%)
Tumor 5	normal	positive	3	26	ductal	normal	PR (42%)

HER2					
	Normal	34	58.6	32	57.1
	Amplified	24	41.4	24	42.9
	Unsuitable/missing	1			
EGFR					
	Deleted	5	9.3	5	9.4
	Normal	46	85.2	45	84.9
	Amplified	3	5.6	3	5.7
	Unsuitable/missing	5		3	

SD: Standard deviation.
HER2: Human epidermal growth factor receptor II.
EGFR: Epidermal growth factor receptor.

Table 2. Results of local and central ER evaluation.

	Local[1]		*Central*[2]	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Gefitinib				
Tumor 1	negative	positive (25%)	0%	90%
Tumor 2	negative	positive (30%)	0%	15%
Tumor 3	negative	positive (NA)	0%	90%
Placebo				
Tumor 4	negative	positive (100%)	0%	20%
Tumor 5	negative	positive (80%)	0%	70%

ER: Estrogen receptor. NA: Not available.
[1]Dichotomized data from local evaluation with 10% cut-off.
[2]Central evaluation with percent of ER staining.

Table 3. Baseline pathologic characteristics and response to NAC for ER changing tumors.

NAC: Neoadjuvant chemotherapy, ER: Estrogen receptor, PgR: Progesterone receptor, HER2: Human epidermal growth factor receptor II, EGFR: Epidermal growth factor receptor, PR: Partial response, SD: Stable diesease.

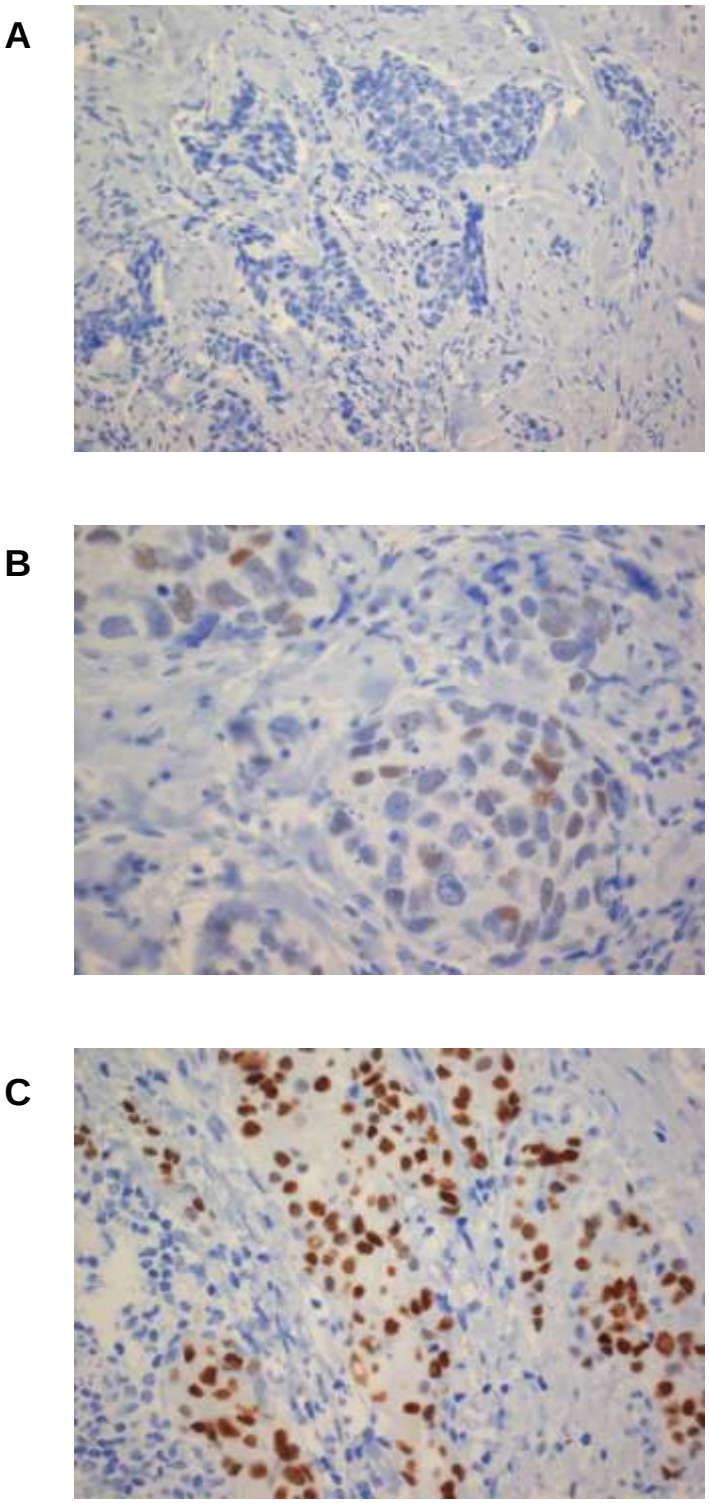

Fig 1 Immunohistochemical estrogen receptor staining in pre- and posttreatment tumor samples. (**a**) Baseline tumor sample from tumor 2 with 0% ER staining. (**b**) 40% ER staining in posttreatment sample from tumor 4 recieving EC + placebo treatment. Average ER staining throughout the sample was 20%. (**c**) 90% ER staining in