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CLINICAL TRIAL

Increasing steroid hormone receptors expression defines breast cancer subtypes non responsive to preoperative chemotherapy

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Abstract The predictive role of the degree of endocrine responsiveness to preoperative chemotherapy (PCT) is unclear. We reviewed pretreatment biopsies of 553 patients with locally advanced breast cancer who were treated with PCT. The incidence of pathological complete remission (pCR) and outcome were assessed with respect to the degree of estrogen (ER) and progesterone receptor (PgR) expression (ER and PgR absent, vs. ER or PgR 0–49%, vs. ER and PgR ≥50% of the cells positive). A statistically significant higher pCR rate was observed at the multivariate analysis for patients with ER and PgR absent tumors (17.7%) versus patients with tumors expressing high ER and PgR (0%) (OR 14.4 $P < 0.001$). Despite the higher incidence of pCR, a statistically significant worse disease-free survival (DFS), and overall survival (OS) was observed for patients with ER and PgR absent tumors versus patients with tumors expressing high ER and PgR (HR 6.4, 95% CI 3.5–11.6, for DFS; HR 3.6 95% CI 2.4–5.6 for OS). Response and outcome after PCT are correlated with the degree of expression of steroid hormone receptors. Studies on tailored preoperative therapies are needed.

Keywords Predictive factors · Primary therapy · Breast cancer

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Introduction

The early identification of features associated with response or resistance to primary therapy are important in the development of the most effective multimodal approaches, and identifying cohorts of patients most likely to benefit from preoperative chemotherapy (PCT) [1–3]. Features predictive of response and outcome include steroid hormone receptor expression. It was recently shown that the pathological complete remission (pCR) rate was significantly higher following PCT for patients with tumors

not expressing estrogen (ER) and progesterone receptor (PgR), compared with the receptor positive cohort [4–6]. Regardless of the significantly higher incidence of pCR for patients with ER and PgR absent disease, the 5-year disease-free survival (DFS) was significantly worse for this cohort compared with the low/positive expression cohort in several studies [5–8]. In these studies analyses were performed based on a so-called 'receptor-negative grouping', which combines receptor-absent disease with that expressing low receptor levels, and 'receptor positive grouping' which combines all patients with tumors expressing ER and/or PgR in $\geq$10% of the cells. Although setting thresholds in a biological continuum, as the extent of steroid hormone receptors, may be considered arbitrary, the percentage of neoplastic cells immunoreactive for hormone receptors is currently considered of paramount value in assessing tumor endocrine responsiveness and planning adjuvant targeted treatments [9]. In particular, three categories were described at the recent St. Gallen Consensus Conference as highly endocrine responsive (high expression of both ER and PgR in a majority of tumor cells), incompletely endocrine responsive (lower expression of ER and or PgR), and endocrine non-responsive (complete absence of both ER and PgR).

No data are currently available on the role of the degree of endocrine responsiveness and its correlation with the response to a preoperative treatment.

The aim of the present study is to seek information on the predictive and prognostic value of the degree of steroid hormone receptor expression, according to the REMARK recommendations [10]. We therefore evaluated the course of disease in 553 patients with large operable primary breast cancer who had preoperative diagnosis and surgery performed at the European Institute of Oncology (EIO).

Patients and methods

Patients

For the present study we considered eligible all the patients with a histologic diagnosis of invasive T2-T4a-d, N0-3 M0 breast cancer admitted at the EIO for a PCT. Other eligibility criteria included no previous chemotherapy/hormonotherapy, performance status 0–2 (ECOG), measurable lesions, age $\geq$18 years, white blood cells $\geq$4,000/mm^3; platelets $\geq$100,000/mm^3, AST, ALT, LDH, gamma-GT $\leq$2.5 $\times$ upper normal limit and bilirubin $\leq$3 mg/100 ml.

Chest X-ray, abdomen ultrasound and bone scan were performed to exclude distant metastasis, blood tests were performed to assess bone marrow, renal and hepatic function within 2 weeks from the start of treatment.

Patients were treated with PCT as previously reported [7]. The regimens used included anthracycline or taxane or vinorelbine containing regimens. Patients with partial or complete remission were candidates to receive a maximum of six courses.

Response criteria

Responses were evaluated by both radiological (breast ultrasound or Rx mammography) and clinical evaluation according to standard WHO criteria. Pathological complete remissions were evaluated according to Kuerer et al. [11]. In particular the absence of invasive cancer on both the primary breast tumor and axillary lymph nodes qualified for pCR.

Written informed consent was obtained from all patients. The study was notified to the Institutional Review Board.

Pathology and immunohistochemistry

All patients had pathological evaluation performed at the EIO. The original receptor status determinations, performed before the patient was evaluated in the study were used. The histotype was defined on the specimens at final surgery. For those patients achieving a pCR, the histotype was defined on the pretreatment core biopsies. Invasive Lobular Carcinoma (ILC) was defined according to the criteria proposed by Fechner [12].

Immunostaining experiments for the localization of ER and PgR, HER2 protein and Ki-67 antigen were performed on consecutive tissue sections of the pretreatment core biopsies, as previously reported [7]. The following primary antibodies were used: the monoclonal antibody (MAb) to ER (Dako, at 1/100 diluition), the Mab to PgR (Dako, 1/800), the MIB-1 Mab to the Ki-67 antigen (Immunotech, Marseille, France, 1/1200) and the polyclonal antiserum (Dako, 1/3200) to the HER2 protein.

The immunostained slides were evaluated independently by two of the authors. Only nuclear reactivity was taken into account for ER, PgR, and Ki-67 antigen, whereas only an intense and complete membrane staining >10% of the tumor cells was taken as evidence of Her2/neu overexpression (3). The percentage of cells positive for steroid hormone receptors was available for all patients. According to steroid hormone receptor status tumors were classified as highly endocrine responsive (ER and PgR $\geq$50% of the cells positive), incompletely endocrine responsive (ER or PgR 0–49% of the cells positive) and endocrine non responsive (ER and PgR 0% of the cells positive). The cut off used for the selection of endocrine non responsive patients (0% of the cells) was based on previous studies indicating a different response and outcome after PCT and

adjuvant therapy for this patient population if compared with those patients whose tumors had some expression of steroid hormone receptors [7, 13]. The cutoff of 50% of positive cells for ER and PgR used for defining highly endocrine responsiveness was arbitrarily selected according to the results of retrospective analysis of several trials (SWOG Intergroup 0100, IBCSG trials VIII and IX) asking the question of the addition of adjuvant chemotherapy to endocrine therapy. In these studies the benefit from the addition of chemotherapy was observed mostly in patients with tumors showing low-intermediate (e.g., <50%) ER and PgR levels [14–16].

Study design

We collected information on all consecutive breast cancer patients treated with preoperative therapy at the EIO in Milan between 1995 and 2004. The primary endpoint was pCR. Cochran-Armitage exact test for trend was used to test whether there was a trend in response rates across the three categories of endocrine responsiveness.

In addition, DFS and overall survival (OS) were also evaluated. Disease-free survival was defined as the length of time from the date of first treatment to any relapse (including ipsilateral breast recurrence), the appearance of a second primary cancer (including contralateral breast cancer) or death, whichever occurred first. Overall survival was determined as the time from the date of first treatment until the date of death (from any cause).

Statistical methods

The patient population was described according to the degree of endocrine responsiveness. Fisher exact test was used to test for association between categorical variables and degree of endocrine responsiveness. Baseline factors that predicted a pCR were firstly evaluated in univariate analysis, using Fisher exact test. Factors that were significant at a *P*-value of 0.10 or less were entered into a multiple logistic regression model and were tested for an independent effect. Exact logistic regression was performed because some of the variables tested had sparse data between their levels. Exact odds ratios (ORs) along with their 95% exact CI were estimated.

Factors included in the multiple regression analyses were age, histologic type, grade, cT, cN, degree of endocrine responsiveness, Ki-67, Her-2/neu and treatment regimen.

The median time of follow-up was calculated as the median observation time among all patients. Plots of the survival curves were drawn using the Kaplan–Meier method. The log-rank test was used to assess survival differences between groups identified by patient or tumor characteristics.

Log-rank test for trend was used to evaluate whether there was a trend in the overall and disease free survival probabilities across the three categories of endocrine responsiveness.

Multivariate Cox proportional hazard regression models were used to assess factors predicting DFS and OS. Each model included histologic type, age at diagnosis, grade, pT, number of positive lymph nodes, degree of endocrine responsiveness, Her-2/neu, Ki-67 and peritumoral vascular invasion. Results from Cox models were expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). All analyses were performed with the SAS software version 9.1 (Cary, NC). All *P*-values were two-sided.

Results

Data

A total of 533 consecutive patients with locally advanced breast cancer, treated with preoperative therapy were available for the present analysis. Patients' characteristics are shown in Table 1. One hundred and eighty-one had endocrine non responsive tumors, whereas 241 had incompletely responsive tumors and 111 patients had highly endocrine responsive tumors. A total of 489 patients (91.7%) had IDC and 44 (8.3%) had ILC. A statistically significant difference in the expression of ER and PgR was observed between IDC and ILC (ER and PgR absent 36.4 vs. 6.8%, $P < 0.0001$).

A total of 268 patients (50.3%) underwent breast conserving surgery (BCS) and 265 patients (49.7%) patients had a total mastectomy. No significant difference in the percentage of BCS was observed between highly endocrine responsive tumors and those with lower expression of ER and PgR as well as between ILC and IDC. Radiotherapy was performed in 402 (75.4%) patients with no difference among groups. The majority of the patients (473, 88.7%) were candidate to adjuvant therapy. Patients with highly endocrine responsive tumors and those with incompletely endocrine responsive tumors were less likely to receive adjuvant chemotherapy if compared with those with endocrine non responsive tumors (36.9 vs. 34.4 vs. 69.6%, $P < 0.0001$).

Response

Of the 553 evaluable patients, 40 (7.5%) had a pCR, 313 (58.7%) had objective clinical remission (CR + PR), 167 (31.3%) had stable disease and 13 (2.4%) had progressive disease (Table 2). Objective responses were observed in 79, 63, and 52% in the subgroups of patients with absent, incomplete and high endocrine responsiveness,

Table 1 Patient characteristics according to tumor endocrine responsiveness at presentation

	All patients $n = 533$		Endocrine responsiveness at presentation						P-value[d]
			Non responsive[a] $n = 181$ (34.0%)		Incompletely responsive[b] $n = 241$ (45.2%)		Highly responsive[c] $n = 111$ (20.8%)		
	No.	Col %	No.	Col %	No.	Col %	No.	Col %	
Histotype									
IDC	489	91.7	178	98.3	219	90.9	92	82.9	<0.0001
ILC	44	8.3	3	1.7	22	9.1	19	17.1	
Age (in years)									
< 35	67	12.6	28	15.5	29	12.0	10	9.0	0.35
35–49	252	47.3	81	44.8	110	45.6	61	55.0	
50–59	150	28.1	53	29.3	67	27.8	30	27.0	
60+	64	12.0	19	10.5	35	14.5	10	9.0	
Menopausal status									
Postmenopausal	225	45.3	79	47.3	95	42.2	51	48.6	0.45
Premenopausal	272	54.7	88	52.7	130	57.8	54	51.4	
Unknown	36	–	14	–	16	–	6	–	
Nuclear grade									
1–2	272	62.4	72	46.8	135	68.2	65	77.4	<0.0001
3	164	37.6	82	53.2	63	31.8	19	22.6	
Unknown	97	–	27	–	43	–	27	–	
Clinical T									
T2	307	57.6	89	49.2	141	58.5	77	69.4	0.019
T3	94	17.6	38	21.0	43	17.8	13	11.7	
T4	132	24.8	54	29.8	57	23.7	21	18.9	
Clinical nodal status									
Positive	381	76.5	141	82.0	163	73.1	77	74.8	0.10
Negative	117	23.5	31	18.0	60	26.9	26	25.2	
Unknown	35	–	9	–	18	–	8	–	
HER2									
Overexpressed	78	17.1	44	27.5	30	14.4	4	4.5	<0.0001
Not expressed	379	82.9	116	72.5	178	85.6	85	95.5	
Unknown	76	–	21	–	33	–	22	–	
Ki-67									
<20%	138	26.7	14	8.0	77	32.9	47	43.5	<0.0001
≥220%	378	73.3	160	92.0	157	67.1	61	56.5	
Unknown	17	–	7	–	7	–	3	–	
Regimen									
Antracyclines	284	53.3	117	64.6	114	47.3	53	47.7	<0.0001
Antracyclines and taxanes	29	5.4	16	8.8	9	3.7	4	3.6	
Others	220	41.3	48	26.5	118	49.0	54	48.6	
Concomitant HT									
Yes	108	30.7	n.a.	–	82	34.0	26	23.4	0.046
No	244	69.3			159	66.0	85	76.6	
n.a.	181	12.6			–	–	–	–	

IDC invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *ER* estrogen receptor, *PgR* progesterone receptor, *HT* hormonotherapy, *n.a.* not applicable

[a] (ER and PgR = 0%)

[b] (ER or PgR 0–49%)

[c] (ER and PgR ≥50%)

[d] Fisher exact test comparing frequencies among degree of endocrine responsiveness. Unknowns and not applicables were excluded

Table 2 Response to neoadjuvant treatment

	All patients $n = 533$		Endocrine responsiveness at presentation						P-value[d]
			Non responsive[a] $n = 181$ (34.0%)		Incompletely responsive[b] $n = 241$ (45.2%)		Highly responsive [c] $n = 111$ (20.8%)		
	No.	Col %	No.	Col %	No.	Col %	No.	Col %	
Response									
Pathological complete	40	7.5	32	17.7	8	3.3	0	0.0	<0.0001
Partial	313	58.7	111	61.3	144	59.8	58	52.3	
Stable disease	167	31.3	34	18.8	82	34.0	51	45.9	
Progression	13	2.4	4	2.2	7	2.9	2	1.8	
pT									
pT0, is	40	7.5	32	17.7	8	3.3	0	0.0	<0.0001
pT1-pTX	219	41.1	71	39.2	106	44.0	42	37.8	
pT2	195	36.6	55	30.4	90	37.3	50	45.0	
pT3	66	12.4	18	9.9	30	12.4	18	16.2	
pT4									
Positive nodes at surgery									
None	168	31.5	81	44.8	66	27.4	21	18.9	<0.0001
1–3	127	23.8	29	16.0	66	27.4	32	28.8	
4+	238	44.7	71	39.2	109	45.2	58	52.3	

[a] (ER and PgR = 0%)

[b] (ER or PgR 0–49%)

[c] (ER and PgR ≥50%)

[d] Fisher exact test comparing frequencies among degree of endocrine responsiveness

respectively, difference not statistically significant (P value <0.001). However, the increased responses in the receptor absent group did not translate in an higher BCS rate due to a significantly greater proportion of T4 tumors in this group as compared to the intermediate and high endocrine responsive groups (30 vs. 24 vs. 19%, respectively, P for trend = 0.03). Within the endocrine non responsive cohort, 32 (17.7%) achieved a pCR which compares with 3.3 and 0% of those with incompletely and high endocrine responsive disease (P for trend <0.001). When comparing pCR in highly and incomplete endocrine responsive tumors, no statistically significant difference was observed (P-value adjusted for pair-wise multiple comparisons = 0.18). The probability of response was also higher for IDC if compared with ILC (8.2 vs. 0%, $P = 0.065$) (Fig. 1).

Analysis and presentation

Median follow-up was 65 months (range: 4–143 months). DFS and OS were significantly better for patients with highly endocrine responsive disease if compared with those with incomplete endocrine responsive and endocrine non responsive tumors. Five-year DFS for patients with highly, incomplete, and non endocrine responsive tumors was 76 versus 67 versus 43% (log-rank P for trend <0.001), and 5-years OS was 89 vs. 86 vs. 64%, respectively (log-rank P for trend <0.0001). Survival curves for DFS, and OS according to different levels of ER and PgR expression are shown in Fig. 2. When comparing OS and DFS between highly and incomplete endocrine responsive tumors, no statistically significant differences were observed (log-rank P values adjusted for pair-wise multiple comparisons: 0.54 for OS and 0.84 for DFS).

A significant predictor of DFS within the univariate analysis was the histotype. Hazard ratio for patients with IDC versus ILC tumors was 1.94 (95% CI 1.10–3.41). However, after stratifying by degree of endocrine responsiveness, no significant difference in DFS was observed between lobular and ductal highly endocrine responsive tumors (HR adjusted for endocrine responsiveness 1.10, 95% CI 0.48–2.51) (Fig. 3).

Patients achieving a pCR had a better 5 years DFS (73 vs. 59%, log rank $P = 0.13$) and OS (78 vs. 92%, log-rank $P = 0.03$).

Multivariate analysis

The results of multivariate exact logistic regression evaluating baseline factors that predicted pCR are presented in Table 3.

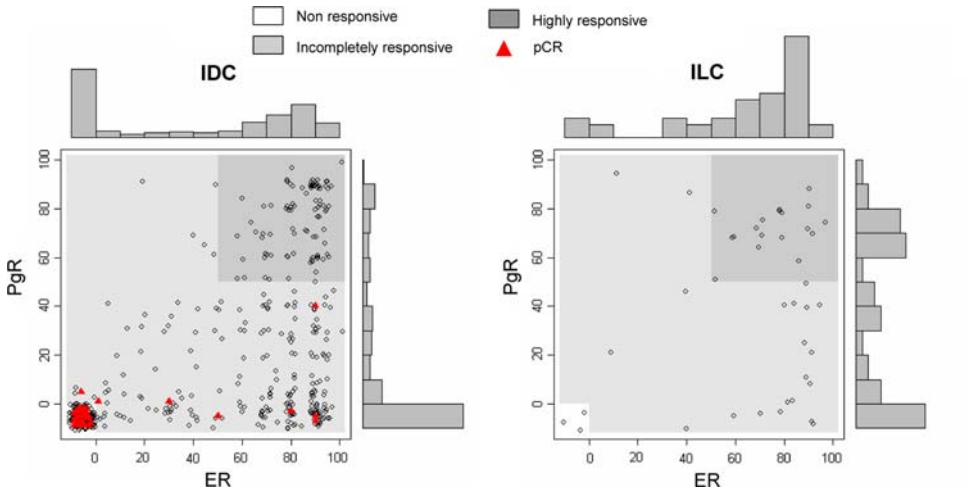

Fig. 1 Correlation between the degree of endocrine responsiveness and pCR, according to histotype. Non responsive: (ER and PgR = 0%). Incompletely responsive: (ER or PgR 0–49%). Highly responsive: (ER and PgR ≥50%)

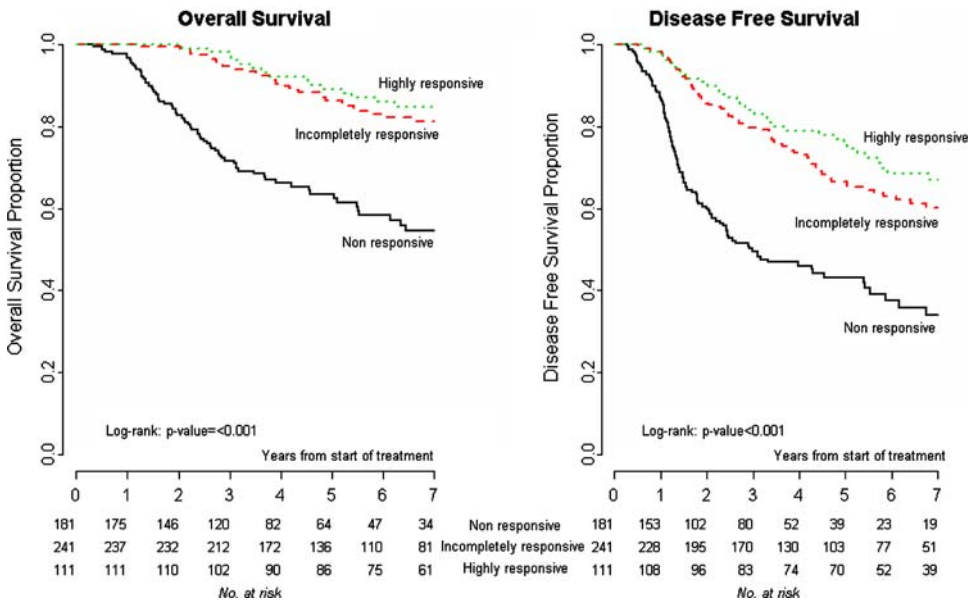

Fig. 2 OS and DFS by degree of endocrine responsiveness. Non responsive: (ER and PgR = 0%). Incompletely responsive: (ER or PgR 0–49%). Highly responsive: (ER and PgR ≥50%)

A statistically significant difference in the probability of achieving a pCR was observed for patients with endocrine non responsive tumors versus patients with highly endocrine responsive tumors (Odd ratio: 14.44 95% CI 2.86–Infinity. The indefinite upper confidence limit reflects the zero observed pCRs in the reference category).

ER and PgR immunoreactivity evaluated as a continuous variable (Odds Ratio for 10% decrease in the number of positive cells both for ER and PgR) was also associated to an higher probability of achieving a pCR (OR 2.14, 95% CI 1.25–3.66, $P = 0.0053$).

Results of multivariate Cox regression models evaluating factors associated to the risk of relapse/death are presented in Table 3. Despite the significantly higher proportion of pCR achieved by PCT for patients with endocrine non responsive tumors a statistically significant worse DFS and OS were observed in multivariate analysis for these patients versus patients with highly endocrine responsive disease (HR 6.41, 95% CI 3.54–11.59, $P < 0.0001$ for OS; HR 3.65 95% CI 2.38–5.6, $P < 0.001$ for DFS) (Fig. 2).

ER and PgR immunoreactivity evaluated as a continuous variable was also associated with better DFS (HR for 10% decrease in the number of positive cells both for ER and PgR 0.91, 95% CI 0.88–0.95, $P < 0.0001$), and with OS (HR for 10% decrease in the number of positive cells both for ER and PgR 0.89, 95% CI 0.84–0.93, $P = <0.0001$).

In the multivariate analysis grade 3 was significantly associated with poorer DFS (HR 1.69). A significantly poorer DFS and OS were observed for p T3/T4 disease (HRs 6.88 and 4.44, for OS and DFS, respectively), ≥4 positive axillary nodes (HRs 2.02 and 2.21, for OS and DFS, respectively), and vascular invasion (HRs 1.92 and 1.66, for OS and DFS, respectively).

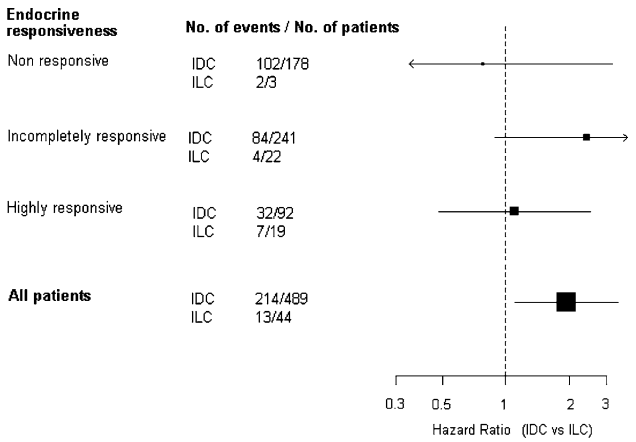

Fig. 3 Analysis of DFS according to degree of endocrine responsiveness. The hazard ratios are for the patients with IDC as compared with those with ILC, and were obtained from the unadjusted Cox model. The dashed vertical line indicates a hazard ratio of 1.00, which is the null-hypothesis value. The size of the squares is proportional to the number of events in the subgroup, the whiskers represent the 95% CI. Non responsive: (ER and PgR = 0%). Incompletely responsive: (ER or PgR 0–49%). Highly responsive: (ER and PgR ≥50%)

Discussion

Recommendations for the selection of therapies in early breast cancer include the identification of different subtypes of breast cancer and, based on genetic profile and immunohistochemistry, the demonstration of selected targets [17, 18]. Treatment strategy should focus mainly on targeted therapies wherever possible, though acknowledging that supplementation with less target-specific chemotherapy is often required [9].

The degree of endocrine responsiveness evaluated quantitatively contribute to a decision about whether endocrine therapy alone may be sufficient. In particular tumors that express high levels of both steroid hormone receptors in a majority of cells (identified with proper immunohistological methods) are defined as highly endocrine responsive. In these patients, particularly in the absence of other adverse factors, only endocrine adjuvant therapy might be prescribed [9].

Conversely, the selection of preoperative therapy did not commonly take into account biological characteristics of the tumor**.** PCT has been given almost universally to patients with large tumors with few exceptions of small series of elderly women for whom an endocrine preoperative therapy seemed the only treatment which could be proposed [19].

Although a change in the algorithm was recently proposed [20] with the expression of steroid hormone receptors considered to be pivotal factors in selecting a program of neoadjuvant therapy, the degree of expression of steroid hormone receptors continues to receive little attention.

The results of the present study provide substantial additional evidence to support the hypothesis that the degree of steroid hormone receptor status of the primary tumor defines distinct biological entities that require a differentiated approach to treatment. According to the present results separate analyses according to the level of steroid hormone receptors must be prospectively planned for future clinical trials and conducted for current and past studies whether or not these were prospectively included in the original protocol.

In particular, in this study the level of expression of ER and PgR was significantly correlated with the probability of response and with the clinical outcome. No pCR was observed within the cohort of patients defined as highly endocrine responsive which compares with 3.3% of those with incompletely responsive and 17.7% of those with endocrine non responsive tumors ($P < 0.0001$). Moreover, the outcome of the patients in terms of 5-years DFS and OS was significantly better for the former cohort if compared with patients with endocrine non responsive tumors.

It was recently shown that the response to primary chemotherapy is lower in terms of pCR in ILC compared with IDC, with a greater need for mastectomy for the former [21, 22]. Conversely, the outcome of ILC appeared to be more favorable than for IDC [20]. Since primary chemotherapy might not achieve main objectives of this strategy such as breast conserving surgery and pCR, its use in ILC was questioned [22]. The results of the present study support the hypothesis that the degree of endocrine responsiveness rather than the histotype should be considered in the selection of patients candidate to preoperative therapy. As shown in Fig. 1, ILC is characterized by significantly higher expression of steroid hormone receptors if compared with IDC which might contribute to the lower response to PCT. Moreover, in the present study we observed that the probability of response and outcome is of similar magnitude in IDC and ILC, if highly endocrine responsive. Finally, no significant difference in terms of DFS and OS was estimated in the multivariate survival model between IDC and ILC. In particular, in the subgroup of patients with highly endocrine responsive similar 5 years DFS (75 and 78%) was observed for IDC and ILC. Cristofanilli et al. [21] found that that ILC responds less frequently to primary chemotherapy if compared with IDC and that the difference in pCR rate between ILC and IDC persisted even after adjusting for hormone-receptor status and type of treatment. In this trial, however, analyses were performed based on a so-called 'receptor-negative grouping,' which combines ER and or PgRabsent disease with that expressing low receptor levels, and receptor positive grouping which include ER and/or PgR ≥10% of the cells.

The present results are of great clinical value since the population of patients with highly endocrine responsive

Table 3 Predictors of response, overall survival and disease free survival

		Response		Overall survival		Disease free survival	
		Observed pCRs (%)	Odds ratio[a] (95% CI)	Observed deaths (%)	Hazard ratio[b] (95% CI)	Observed events (%)	Hazard ratio[b] (95% CI)
Histologic type	ILC	0.0	Reference	24.5	Reference	43.8	Reference
	IDC	8.2	1.12 (0.16–Infinity[c])	18.2	0.72 (0.31–1.64)	29.5	1.02 (0.54–1.93)
Age (in years)	<35	10.4	[d]	28.4	Reference	41.8	Reference
	35–49	8.3		24.2	1.03 (0.60–1.77)	41.7	1.08 (0.70–1.67)
	50+	5.6		22.4	0.68 (0.32–1.45)	43.9	0.86 (0.48–1.54)
Nuclear grade	1–2	4.8	Reference	22.6	Reference	37.1	Reference
	3	15.8	2.06 (0.91–4.87)	28.7	1.46 (0.96–2.23)	52.4	**1.69 (1.22–2.34)**
Clinical T	2	8.8	[d]	[e]		[e]	
	3–4	5.7					
Pathological T	0, is	[e]		7.5	Reference	27.5	Reference
	1/X			17.3	2.29 (0.52–10.04)	35.2	2.48 (0.86–7.11)
	2			27.2	3.84 (0.86–17.07)	48.2	**3.55 (1.22–10.36)**
	3–4			43.0	**6.88 (1.46–32.35)**	57.0	**4.44 (1.44–13.63)**
Clinical nodal status	Negative	10.3	Reference	[e]		[e]	
	Positive	6.8	0.38 (0.16–0.95)				
Pathological nodal status	0	[e]		16.1	Reference	30.4	Reference
	1–3			17.3	1.14 (0.62–2.1)	35.4	1.37 (0.87–2.17)
	4+			33.2	**2.02 (1.17–3.49)**	55.0	2.21 (1.46–3.35)
Endocrine responsiveness	Highly responsive[f]	0.0	Reference	14.4	Reference	35.1	Reference
	Incompletely responsive [g]	3.3	3.54 (0.53-Infinity[c])	17.0	1.53 (0.85–2.75)	34.8	1.19 (0.8–1.77)
	Non responsive[h]	17.7	**14.44 (2.37-Infinity[c])**	39.2	**6.41 (3.54–11.59)**	57.5	3.65 (2.38–5.6)
HER2	Not expressed	9.0	[d]	23.5	Reference	39.8	Reference
	Overexpressed	6.4		26.9	0.77 (0.46–1.3)	53.8	1.35 (0.92–1.96)
Ki–67	<20%	1.5	Reference	16.7	Reference	32.0	Reference
	≥20%	10.0	1.89 (0.39–18.38)	26.7	1.53 (0.9–2.6)	46.6	1.43 (0.96–2.13)
Regimen	Others	4.6	Reference	[e]		[e]	
	AC	9.9	2.02 (0.82–5.42)				
	AC + Taxane	6.9	1.03 (0.09–6.31)				
Perivascular invasion	Absent	[e]		17.5	Reference	33.2	Reference
	Present/focal/diffuse			34.1	**1.92 (1.25–2.94)**	55.8	**1.66 (1.21–2.27)**

pCR pathological complete response, *IDC* invasive ductal carcinoma, *ILC* invasive lobular carcinoma

[a] Odds ratios are obtained from a multivariate exact logistic regression model

[b] Hazard ratios are obtained from a multivariate proportional hazard regression model

[c] Infinity reflects zero observed pCRs in the reference category

[d] Factor not considered in the regression model since the associated *P*-value at univariate analysis was >0.10

[e] Not evaluable

[f] (ER and PgR = 0%)

[g] (ER or PgR 0–49%)

[h] (ER and PgR ≥50%)

Bold characters indicate *P*-value < 0.05

tumors represents 20.8% of all tumors which compares with only 8.3% of lobular invasive carcinoma. We recognized that we proposed arbitrary thresholds in a biological continuum, but for the purpose of a pragmatic decision on the benefit from targeted treatments these results might be helpful in the therapeutic algorithm. The number of positive cells has been demonstrated to be relevant in the detection of prognostic and predictive factors in early breast cancer [23]. Previous studies showed a different pattern of response to chemotherapy according to the degree of steroid hormone receptors. In particular, no or limited benefit from the addition of chemotherapy to endocrine therapy was observed in pre-and postmenopausal women with tumors with high expression of ER [14, 15].

Also the higher expression of PgR was correlated in previous studies with a lower degree of response to adjuvant chemotherapy and higher response to endocrine treatment [15, 16].

Genomic expression profiling studies have identified distinct subtypes of breast carcinomas that are associated with different responses to chemotherapy and to different clinical outcomes in the preoperative setting [24]. Luminal types A, B, C, normal breast, HER2-positive, and basal-like phenotypes have been reproducibly separated [25–27]. The luminal subtype is usually divided into at least two subgroups, with different levels of expression of the hormone receptors genes [28]. The luminal A has higher expression of hormone receptors and related genes and low expression of proliferative genes, while the luminal B has lower expression of the ER and higher expression of proliferative genes. Previous studies in breast cancer cell lines have in fact implicated HER1/HER2 signaling in lowering the expression of PgR and/or ER [29–31]. In particular, in the subgroup of patients with ER positive and PgR absent disease, hyperactive cross-talk between ER and growth factor signaling pathways, leading to a more aggressive course of the disease, was recently reported [32, 33]. Previous studies [34, 35] conducted also in the preoperative setting [36], found a correlation between the degree of expression of ER and PgR and HER1/HER2 expression, clearly indicating the higher prevalence of endocrine non responsive disease in the group of patients selected by HER1 and HER2 expression. It is therefore possible that the high expression of ER and PgR identifies a Luminal A phenotype leading to a less aggressive course of the disease as well as poor response to PCT. The Luminal A phenotype has been associated to an improved outcome, albeit the lack of pCR after PCT [37].

Our analysis is limited by the fact that relatively few relapses have occurred within the first 5-years in the population with highly endocrine responsive tumors. Patients with an endocrine responsive disease have the opportunity to respond to proper endocrine therapies and consequently to present an event several years after surgery. In fact, Guarneri et al. [6] showed that in the preoperative setting patients with hormone receptor (HR) positive tumors tended to have better PFS compared with patients with HR-negative breast cancer until just after 100 months after their response assessment. The curves then crossed, and patients with HR-positive tumors tended to have worse PFS compared with patients with HR-negative tumors. Similarly, in a larger series focusing on the behavior of triple negative breast cancer after preoperative therapy as compared to other subsets of tumors, the expression of both ER and PgR was confirmed to be associated with a lower pCR rate and to an improved PFS and OS as compared to HER2 positive or hormone receptor negative tumors [38]. Importantly, patients with ER positive residual tumors fared dramatically better than patients with ER negative and/or HER2 positive tumors not achieving a pCR and this large group may account for the improved outcome observed in ER positive population albeit the lower rate of pCR [38]. In conclusion, the present study indicates the limited, if any, impact of PCT for patients with highly endocrine responsive carcinoma as well as the good outcome in terms of 5-years DFS and OS for this patient population. Further studies using database analyses or prospective trials are required to confirm the limitations of primary chemotherapy in highly endocrine responsive tumors. If confirmed, future selection of preoperative treatment should be based on the degree of tumor ER and PgR expression, and the current practice to treat these patients with chemotherapy should change to a more targeted treatment focusing on endocrine therapies.

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