Improving breast cancer outcome by preoperative systemic therapy and image-guided surgery

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Chapter 3

Tumor response to preoperative anthracycline-based chemotherapy in operable breast cancer: the predictive role of p53 expression

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ABSTRACT

Introduction

The aim of this retrospective study was to identify markers capable of predicting pathological complete (pCR) and overall clinical tumor response to preoperative anthracycline-based chemotherapy and clinical outcome in women with operable breast cancer.

Methods

Therefore, we used the pre-treatment core biopsies from 107 patients who were enrolled in the EORTC trial 10902 to analyze tumor characteristics and the oncogenic markers Bcl-2, p53, ER, PgR, HER2, and p21. Median follow-up was 7 years (95% CI = 6.89 to 7.45).

Results

pCR was seen in seven patients (6.5%) and was associated with improved overall survival (hazard ratio = 0.39, 95% CI = 0.05 to 2.56, *P* = .30). At multivariate logistic regression analysis, pCR was independently predicted by p53 overexpression estimated by immunohistochemistry (odds ratio (OR) = 16.83, 95% CI = 1.78 to 159.33, *P* = .01). Fifty-eight patients showed clinical tumor response (>50% decrease in tumor size), however responders experienced no benefit in clinical outcome. Clinical tumor response was independently predicted by p53 overexpression (OR = 5.57, 95% CI = 1.58 to 19.65, *P* = .008) and small clinical tumor size (OR = 10.26, 95% CI = 2.01 to 52.48, *P* = .005). At multivariate Cox regression analysis, negative pathological lymph node status, low tumor grade and use of tamoxifen showed improved overall survival.

Conclusion

In conclusion, our data suggest p53 expression is of predictive significance in anthracycline-containing chemotherapeutic regimens.

INTRODUCTION

Preoperative chemotherapy for large, but early stage breast cancer, has been subject of interest for over two decades. The efficacy of preoperative chemotherapy has been demonstrated in several prospective randomized trials showing similar survival and locoregional control rates in patients receiving preoperative chemotherapy and postoperative chemotherapy. Tumor down staging due to preoperative chemotherapy was found to increase breast-conserving therapy rates.[1 2]

Response of breast tumors following preoperative chemotherapy can be assessed either clinically or pathologically. Patients with responding tumors showed an improved overall and disease-free survival and particularly pathological complete response (complete disappearance of malignant cells on microscopic examination; pCR) is suggested as a surrogate marker for these clinical endpoints.[2-5]

Translational research using preoperative tumor tissue biopsies is an excellent study model to analyze the predictive value of different tumor characteristics for response to chemotherapy.[6] To date, a large number of oncogenic markers in breast cancer have been studied using classical survival analyses.[7 8] However, published data on the relation between tumor characteristics and pathological and clinical tumor response are still limited.

We have used data from a prospective randomized trial comparing pre- versus postoperative chemotherapy to study the correlation between pathological and clinical tumor response and patient and tumor characteristics. Tumor characteristics included oncogenic markers analyzed on pre-treatment biopsy specimens and classic tumor characteristics. In addition, we assessed the prognostic significance of these clinical characteristics including pathological and clinical tumor response on overall and distant disease-free survival.

MATERIAL AND METHODS

Study participants

All patients participated in a prospectively randomized trial (EORTC 10902) that compared preoperative chemotherapy versus the same chemotherapeutic regimen administered postoperatively in patients with operable breast cancer.[1] This trial accrued 698 women with early stage breast cancer between 1991 and 1999. The eligibility criteria for this trial have been described previously.[1] Efforts were made to obtain diagnostic biopsy material from all patients randomized to preoperative chemotherapy. For the present analysis, we included patients who had received preoperative chemotherapy with known pathological and clinical tumor response and from whom biopsy material were available for pathological evaluation. We used pre-treatment biopsy material for

immunohistochemical analyses in order to avoid interference of the chemotherapeutic regime on the expression levels of oncogenic markers.[9 10]

Treatment

Chemotherapy consisted of four cycles of preoperative fluorouracil 600 mg/m^2, epirubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2 (FEC) administered intravenously, at intervals of every 3 weeks. Surgical therapy followed within 4 weeks of the fourth course of chemotherapy. Surgery consisted of either a modified radical mastectomy or breast-conserving surgery (wide local excision of the tumor or quadrantectomy plus axillary dissection and adjuvant radiotherapy). Recommended guidelines for radiotherapy have been described previously.[1] If radiotherapy was indicated, it was administered after surgery. Patients older than 50 years also received tamoxifen 20 mg daily for at least 2 years, regardless of their estrogen receptor and nodal status.

Pathological tumor response

Surgical tumor specimens were examined for the presence of microscopic residual tumor. If no signs of residual malignant cells at the primary site were seen with histological examination, this was scored as a pathological complete response (pCR). The specimens still containing invasive malignant cells were graded as pINV.

Clinical tumor response

The tumor response classification system used in EORTC 10902 was according to the UICC.[11] Clinical tumor size was scored by the local investigators before the start of chemotherapy as well as at the time of surgery by both clinical examination and mammography. The product of the two greatest perpendicular diameters was used to compare tumor size before and after chemotherapy.

Clinical complete response (cCR) was defined as complete disappearance of all clinically detectable malignant disease by palpation and mammography. Clinical partial response (cPR) was defined as 50% decrease in total tumor size after four cycles of preoperative chemotherapy. An increase of 25% in tumor size after a minimum of two courses of preoperative chemotherapy was considered to be progressive disease (cPD). If patients did not meet one of the above-mentioned criteria after four cycles of chemotherapy, they were classified as having stable disease (cSD). For the purpose of this analysis, we distinguished between patients with overall clinical response (cCR and cPR) and patients with non-responding tumors (cSD and cPD).

Histology and immunohistochemistry

Blocks were collected from core needle biopsies taken before the start of chemotherapy. All immunohistochemical analyses were performed in one reference laboratory by two pathologists who were unaware of the clinical outcome of the patients. Invasive carcinomas were histologically graded according to the method of Bloom and Richardson, adapted by Elston and Ellis.[12] Bcl-2 was assessed using Clone 124 (Boehringer Mannheim, Germany) and scored according to van Slooten and colleagues (staining 3 indicates positive status).[13] p53 accumulation was detected using Do-7 monoclonal antibody (NovaCastra, Newcastle on Tyne, United Kingdom) and a semi-quantitative system based on the sum of the mean staining intensity (0 to 3; none to strong) and an estimation of the percentage of positive cell nuclei (0 to 4; 0% to >75%); this allowed a sum score of 0 to 7, with staining 4 being considered positive.[14] Estrogen receptor status (ER) was estimated using the monoclonal antibody DAKO-ER 1D5 (Dako, Glostrup, Denmark; staining indicates positive status).[14] Progesterone receptor status (PgR) was measured using mPRI monoclonal antibody (Transbio, Paris, France; staining indicates positive status).[14] HER2 expression was assessed using the monoclonal antibody 3B5 (staining score 0, 1 and 2 indicates a negative result and 3 resembles a positive result).[15] p21 was measured using the monoclonal antibody EA10 (Calbiochem, Cambridge, MA, USA; 3 indicates a positive result).[13 14]

Statistical analysis

Overall survival time was defined as the time between randomization and death from any cause. Distant disease-free survival was defined as the time between the date of randomization and the date of distant disease relapse or death from any cause whichever came first. Correlations between the two tumor response classification systems and patient and tumor characteristics were tested using the Pearson's Chi-square test or the Fisher's Exact test. A multivariate logistic regression model was fitted that was based on all characteristics that had a *P*-value up to .10 in the univariate analysis. The effect of patient and tumor characteristics on the survival endpoints was assessed using the Cox proportional hazards regression model to estimate hazard ratios and their 95% confidence intervals. A multivariate Cox regression model was fitted that was based on all characteristics that had a *P*-value up to .10 in the univariate analysis. Survival curves of the tumor response groups were estimated using the Kaplan-Meier technique. The statistical analyses were performed using SPSS software (SPSS Inc., Chicago, II, USA). A two-sided significance level of 0.05 was used.

RESULTS

Patient and tumor characteristics

EORTC 10902 trial randomized 350 patients to preoperative chemotherapy and 321 patients received this allocated treatment. Tumor response was assessable in 301 patients. For 194 of these patients no data was available on histological and immunohistochemical analyses. Thus, we were able to include 107 patients in this study. Patient and tumor characteristics are listed in Table 1.

Table 1. Patient and tumor characteristics

Characteristic	N	%
Age at diagnosis		
< 40 years	11	10
> 40 years	96	90
Type of surgery		
Mastectomy	57	53
BCT	50	47
Tamoxifen		
No	59	55
Yes	48	45
Radiotherapy		
No	20	19
Yes	87	81
Clinical tumor size[a]		
T1	18	17
T2	64	60
T3	21	19
T4	4	3
Clinical tumor response[b]		
Complete	7	7
Partial	51	48
Stable disease	47	44
Progressive disease	2	2
Pathological tumor size[b]		
pCR	7	7
pT1	43	40
pT2	48	45
pT3	7	7
pT4	2	2
Clinical nodal status[a]		
Negative	65	58
Positive	45	42
Pathological nodal status[b]		
Negative	45	42
Positive	65	58
Grade[a]		
I	13	12
II	69	64
III	19	18
Unknown	6	6
BCL-2 status[a]		
Negative	25	23
Positive	59	55
Unknown	23	22
P53 status[a]		
negative	73	68
positive	26	24
unknown	8	8
ER status[a]		
Negative	21	20
Positive	71	66
Unknown	15	14
PgR status[a]		
Negative	50	47
Positive	49	46
Unknown	8	7
HER2 status[a]		
Negative	92	86
Positive	10	9
Unknown	5	5
P21 status[a]		
Negative	45	42
Positive	47	44
Unknown	15	14

a Assessed prior to the delivery of chemotherapy.
b Assessed after the delivery of chemotherapy.
BCT, breast conservative treatment;
pCR, pathological complete response.

The median age at diagnosis was 49.8 years. Seven (6.5%) pathological complete responses following preoperative chemotherapy were seen and 58 (54%) patients had clinically responding tumors. All but one of the patients with pCR was clinically graded as responders. At the time of analysis, the median follow-up period was seven years (95% CI = 6.89 to 7.45); 31 (29%) patients have died and of the patients alive, 10 (9.3%) have experienced a distant relapse. Although otherwise stipulated in the treatment protocol, 9 (17%) women older than 50 years did not use tamoxifen and four (7.4%) women in the younger group did use tamoxifen.

Prognostic value of pathological tumor response

The association of pCR with overall survival and distant disease-free survival is shown in Figure 1. Patients with a pCR had an overall survival rate after 7 years of 86% compared with 68% for patients with residual disease (pINV) on pathological examination (hazards ratio (HR) = 2.87, 95% CI = 0.39 to 21.14, $P = .30$). Patients with a pCR had a distant disease-free survival rate at 7 years follow-up of 86%, compared to 59% for patients with pINV (HR = 3.62, 95% CI = 0.50 to 26.33, $P = .21$).

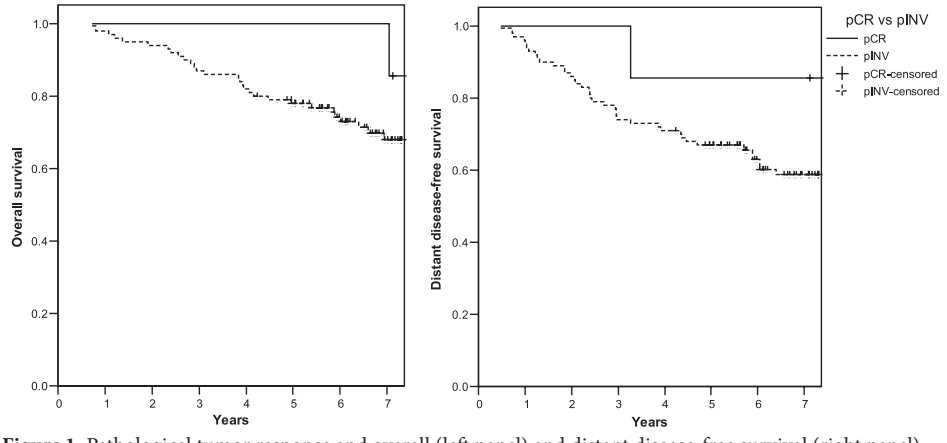

Figure 1. Pathological tumor response and overall (left panel) and distant disease-free survival (right panel). pCR = pathological complete response; pINV = invasive tumor cells on pathological examination.

Prognostic value of clinical tumor response

Patients with a clinical tumor response had an overall survival rate after 7 years of 67% (Figure 2). Non-responders had an overall survival rate of 75% (HR = 0.71, 95% CI = 0.34 to 1.45, $P = .35$). Patients with clinical response had a distant disease-free survival rate after 7 years of 61% compared to 61% for patients with non-responding tumors (HR = 0.94, 95% CI = 0.51 to 1.74, $P = .84$; Figure 2).

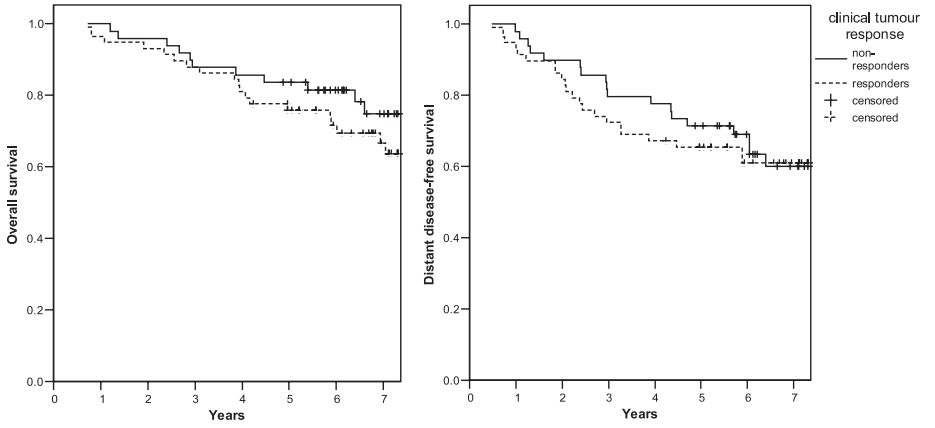

Figure 2. Clinical tumor response and overall (left panel) and distant disease-free survival (right panel).

Predictive characteristics for pathological and clinical response

We assessed the predictive value of patient and tumor characteristics and expression of oncogenic markers in pre-treatment core needle biopsies. Table 2 lists the relationships between dichotomized characteristics and pathological and clinical tumor response. Pathological lymph node status and p53 status were significantly correlated with pathological tumor response. Including both variables in the multivariate analysis (Table 3) revealed an independent relationship of p53 overexpression with pCR (odds ratio (OR) = 16.83, 95% CI = 1.78 to 159.33, *P* = .01) and a non-significant association of negative pathological lymph node status. Clinical tumor response was predicted by clinical tumor size, tumor grade, p53 status, PgR status, and HER2 status (Table 2). At multivariate analysis, p53 overexpression (OR = 5.57, 95% CI = 1.58 to 19.65, *P* = .008) and small clinical tumor size (OR = 10.26, 95% CI = 2.01 to 52.48, P = .005) remained as independent predictive factors of clinical tumor response (Table 3).

Table 3. Multivariate logistic regression analysis of tumor characteristics and pathological complete tumor response (N = 99) and clinical response (N = 94)

Characteristic	Pathological response			Clinical response		
	OR	95% CI	*P*	OR	95% CI	*P*
Lymph node negative[a]	8.47	0.88–81.82	0.07			
Positive p53 status[b]	16.83	1.78-159.33	0.01	5.57	1.58–19.65	0.008
Tumor size < 2 cm[b]				10.26	2.01–52.48	0.005
Grade IIIa[b]				1.58	0.41–6.13	0.51
Negative PgR status[b]				2.37	0.89–6.31	0.08
Positive HER2 expression[b]				2.93	0.47–18.14	0.25

a Assessed after to the delivery of chemotherapy. b Assessed prior the delivery of chemotherapy.

Table 2. Association of tumor response and patient and tumor characteristics

Characteristic	Pathological response					Clinical response				
	pCR		pINV		*P*	Responders		Non-responders		*P*
	N	%	N	%		N	%	N	%	
Age at diagnosis					1.00					0.22
< 40 years	0	0	11	100		8	73	3	27	
> 40 years	7	7	89	93		50	52	46	48	
Clinical tumor size					0.60					0.001
< 2 cm	0	0	18	100		16	89	2	11	
> 2 cm	7	8	82	92		42	47	47	53	
Clinical nodal status					1.00					0.43
Negative	4	7	58	93		36	58	26	42	
Positive	3	7	42	93		22	49	23	51	
Pathological nodal status					0.04					0.17
Negative	6	13	39	87		28	62	17	38	
Positive	1	2	61	98		30	48	32	52	
Histological grade					0.61					0.05
I & II	5	6	77	94		40	49	42	51	
III	2	11	17	89		14	74	5	26	
BCL-2 status					0.36					0.23
Negative	3	12	22	88		15	60	10	40	
Positive	3	5	56	95		27	46	32	54	
P53 status					0.004					0.001
Negative	1	1	72	99		32	44	41	56	
Positive	5	19	21	81		21	81	5	19	
ER status					0.13					0.13
Negative	3	14	18	86		14	67	7	33	
Positive	3	4	68	96		34	48	37	52	
PgR status					0.68					0.007
Negative	4	8	46	92		33	66	17	34	
Positive	2	4	47	96		19	39	30	61	
HER2 status					0.47					0.09
Negative	5	5	87	95		46	50	46	50	
Positive	1	10	9	90		8	80	2	20	
P21 status					1.00					0.53
Negative	3	7	42	93		25	56	20	44	
Positive	3	6	44	94		23	49	24	51	

pCR, pathological complete response; pINV, invasive tumor cells on pathological examination.

Prognostic characteristics for overall survival and distant disease-free survival

Table 4 shows the prognostic value of patient and tumor characteristics in predicting clinical outcome. Significant prognostic variables for overall and distant disease-free survival were age, use of tamoxifen, and pathological lymph node status. In addition, histological tumor grade was significantly associated with overall survival. Overexpression of p53 was non-significantly related with poorer overall (HR = 1.72, 95% CI = 0.82 to 3.62, $P = .15$) and distant disease-free survival (HR = 1.39, 95% CI = 0.70 to 2.74, $P = .35$).

Table 4. Univariate Cox regression analyses for overall and distant disease-free survival

Characteristic	Overall survival					Distant disease-free survival				
	N/O	7-year SR	HR	95% CI	*P*	N/O	7-year SR	HR	95% CI	*P*
Age at diagnosis					0.01					0.03
< 40 years	11/7	45	1.00			11/7	36	1.00		
> 40 years	96/24	73	0.34	0.14–0.78		96/34	64	0.40	0.18–0.92	
Surgery					0.62					0.29
Mastectomy	57/17	66	1.00			57/24	58	1.00		
Breast conserving	50/14	74	0.83	0.41–1.69		50/17	64	0.72	0.36–1.33	
Tamoxifen					0.01					0.01
No	59/24	60	1.00			59/30	48	1.00		
Yes	48/7	84	0.34	0.15–0.79		48/11	77	0.39	0.19–0.77	
Radiotherapy					0.11					0.32
No	20/8	56	1.00			20/9	51	1.00		
Yes	87/23	74	0.52	0.23–1.16		87/32	63	0.69	0.33–1.44	
Clinical tumor size					0.63					0.35
< 2 cm	18/4	72	1.00			18/5	67	1.00		
> 2 cm	89/27	70	1.30	0.45–3.72		89/36	59	1.57	0.61–4.00	
Clinical response					0.35					0.84
Responders	58/19	67	1.00			58/22	61	1.00		
Non-responders	49/12	75	0.71	0.34–1.45		49/19	61	0.94	0.51–1.74	
Pathological tumor size					0.35					0.26
< 2 cm	50/13	75	1.00			50/17	64	1.00		
> 2 cm	57/18	66	1.41	0.69–2.88		57/24	58	1.43	0.77–2.67	
Pathological response					0.30					0.21
pCR	7/1	86	1.00			7/1	86	1.00		
pINV	100/30	68	2.87	0.39–21.14		100/40	59	3.62	0.47–26.33	
Clinical nodal status					0.51					0.37
Negative	62/17	73	1.00			62/22	64	1.00		
Positive	45/14	67	1.27	0.62–2.57		45/19	56	1.33	0.72–2.55	
Pathological nodal status					0.01					0.00
Negative	45/8	84	1.00			45/8	81	1.00		
Positive	62/23	61	2.82	1.23–6.44		62/33	46	4.15	1.90–9.06	
Histological grade					0.05					0.23
I & II	82/20	74	1.00			82/29	64	1.00		
III	19/9	55	2.23	1.01–4.91		19/9	50	1.58	0.75–3.33	
BCL-2 status					0.30					0.12
Negative	25/8	70	1.00			25/11	54	1.00		
Positive	59/12	79	0.62	0.26–1.53		59/16	73	0.55	0.25–1.18	
P53 status					0.15					0.35
Negative	73/19	73	1.00			73/27	62	1.00		
Positive	26/11	58	1.72	0.82–3.62		26/12	52	1.39	0.70–2.74	
ER status					0.16					0.59
Negative	21/9	60	1.00			21/9	56	1.00		
Positive	71/19	71	0.57	0.26–1.26		71/27	61	0.81	0.38–1.74	
PgR status					0.14					0.16
Negative	50/19	62	1.00			50/23	52	1.00		
Positive	49/12	75	0.58	0.28–1.19		49/16	68	0.64	0.34–1.20	
HER2 status					0.87					0.74
Negative	92/27	70	1.00			92/37	59	1.00		
Positive	10/3	69	1.11	0.34–3.66		10/3	70	0.82	0.25–2.66	
P21 status					0.24					0.28
Negative	45/12	72	1.00			45/16	65	1.00		
Positive	47/17	64	1.56	0.74–3.28		47/12	53	1.44	0.75–2.76	

N/O, number of patients/ observed number of events; SR, survival rate; HR, hazard rate; pCR, pathological complete response; pINV, invasive tumor cells on pathological examination.

The prognostic factors found to be trend significant at univariate analyses were included in multivariate analyses to identify independent prognostic factors of overall and distant disease-free survival (Table 5). Negative pathological lymph node status and use of tamoxifen were both independently associated with improved overall and distant disease-free survival. In addition, histological tumor grade III was an independent prognostic factor of poorer overall survival.

DISCUSSION

In this analysis, we demonstrated a significant independent association between p53 overexpression and pathological complete and clinical tumor response to 4 cycles of preoperative FEC. However, pCR as a prognostic factor for overall survival, as well as for distant disease-free survival, did not reach statistical significance in this patient population, although a clear trend was demonstrated (Figure 1). This finding is in accordance with other randomized controlled trials studying preoperative chemotherapy in primary operable breast cancer, while pCR in these studies was a significant prognostic factor.[2 16-18]

In this study, clinical tumor response showed no prognostic benefit (Figure 2). This result is in disagreement with other reports[2 16 17] and most probably resembles a selection bias as the data derived from our larger study population suggest an association of non-response with poorer overall survival (HR = 1.43, 95% CI = 0.91 to 2.24, P = .12). However, the fact that clinical responders in the current group had no favorable prognosis implies that the results concerning the predictive value of characteristics for clinical response must be interpreted with caution. Moreover, determining clinical tumor response after preoperative chemotherapy is difficult and can be either under- or overestimated due to fibrosis, weakening of the tumor margins and resolution of edema, suggesting prognostic superiority of pathologically evaluated tumor response.[19-22]

Although pCR in our study was associated with p53 overexpression and higher survival rate, p53 overexpression was not translated in improved clinical outcome. In contrast, p53 overexpression was non-significantly related with poorer overall and distant disease-free survival. Hypothetically, the short-lived benefits of better response

Table 5. Multivariate Cox regression analyses of characteristics predicting for overall (N = 101) and distant disease-free survival (N = 107)

Characteristic	Overall survival			Distant disease-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
Lymph node negative[a]	4.30	1.71–10.82	0.002	5.19	2.35–11.46	0.000
Use of tamoxifen	0.41	0.17–1.00	0.05	0.34	0.17–0.69	0.003
Age < 40 years	2.13	0.81–5.65	0.13	2.28	0.98–5.32	0.06
Grade IIIa[b]	3.02	1.28–7.12	0.01			

a Assessed after to the delivery of chemotherapy. b Assessed prior the delivery of chemotherapy.
HR, hazard ratio.

of p53 positive tumors may be overcast by rapid re-growth of micro-metastases after initial remission of the primary tumor, reflecting their aggressive biology. However, analysis of the hypothesis that survival in the pCR subgroup is dependent on p53 status was not possible due to the limited power of the current study.

p53, a nuclear protein, plays an essential role in the regulation of cell cycle and functions as a tumor suppressor. Breast cancer patients with p53 mutations or protein accumulation measured by immunohistochemistry in their tumors have worse survival.[23-26] Meanwhile, the literature of the predictive value of p53 status on tumor response to preoperative anthracycline-based chemotherapy is conflicting.[7] Most studies find no association between p53 expression and tumor response to anthracyclines.[27-32] Others have associated p53 overexpression with both resistance[14 33-35] and sensitivity[10 36] to preoperative anthracycline-based chemotherapy. Interpretation of the above literature is complicated since the definition of response varies across studies, the correlation between p53 protein accumulation and the presence of mutations is not absolute and numerous non-standardized immunohistochemistry techniques have been used, limiting the possibility to draw valid conclusions.[37]

The pathological lymph node status after preoperative chemotherapy is in our data an independent prognostic factor for both overall and distant disease-free survival. This finding has also been noted by others.[3 38-40] However, the pre-treatment clinical lymph node status was poorly correlated with clinical outcome. At the time this trial was conducted, the pre-treatment nodal status was determined by palpation only. Nowadays, imaging techniques such as ultrasound are more feasible in establishing nodal status.[41] Future trials should include this technique to provide more reliable information of the actual response of lymph node metastases to preoperative chemotherapy and to determine the subsequent prognostic significance of such a response.

At this time, it is not possible to select patient who will benefit from chemotherapy. However, data have begun to emerge from microarray studies which may lead to the introduction of tailored treatment strategies based upon custom-made risk profiles rather than the classic guidelines derived from traditional randomized clinical trials.[42-45]

In conclusion, our data derived from a prospective randomized trial suggest that p53 overexpression estimated by immunohistochemistry is an independent predictive factor of tumor response after preoperative anthracycline-based chemotherapy in operable breast cancer patients. However, this conclusion must be limited to the regime used in this trial (FE60C), which is probably suboptimal today.[46] Moreover, the relatively small sample size requires conformation in larger studies and the use of p53 measurements should be restricted to clinical trial settings. Prospectively derived data on the predictive and prognostic value of p53 is on the way from the neoadjuvant EORTC trial 10994.[47 48]

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