Impact of age, tumor characteristics, and treatment on local control and disease outcome in early stage breat cancer : an EORTC translational research project

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CHAPTER 4

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

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Abstract

The aim of this retrospective study was to identify markers capable of predicting pathological complete (pCR) and overall clinical tumour response to preoperative anthracycline-based chemotherapy and clinical outcome in women with operable breast cancer. Therefore, we used the pre-treatment core biopsies from 107 patients who were enrolled in the EORTC trial 10902 to analyse tumour characteristics and the oncogenic markers *bcl-2*, *p53*, ER, PgR, *HER2*, and *p21*. Median follow-up was 7 years (95% confidence interval [CI], 6.89-7.45). pCR was seen in seven patients (6.5%) and was associated with improved overall survival (hazards ratio, 0.39; 95% CI, 0.05-2.56; P = 0.30). In multivariate logistic regression analysis, pCR was independently predicted by *p53* overexpression estimated by immunohistochemistery (odds ratio [OR], 16.83; 95% CI, 1.78-159.33; P = 0.01). Fifty-eight patients showed clinical tumour response (>50% decrease in tumour size), however responders experienced no benefit in clinical outcome. Clinical tumour response was independently predicted by *p53* overexpression (OR, 5.57; 95% CI, 1.58-19.65; P = 0.008) and small clinical tumour size (OR, 10.26; 95% CI, 2.01-52.48; P= 0.005). In multivariate Cox regression analysis, negative pathological lymph node status, low tumour grade and use of tamoxifen showed improved overall survival. 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. Tumour downstaging due to preoperative chemotherapy was found to increase breast-conserving therapy rates [1,2].
Response of breast tumours following preoperative chemotherapy can be assessed either clinically or pathologically. Patients with responding tumours 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 tumour tissue biopsies is an excellent study model to analyse the predictive value of different tumour 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 tumour characteristics and pathological and clinical tumour response are still limited.
We used data from a prospective randomized trial comparing pre- versus postoperative chemotherapy to study the correlation between pathological and clinical tumour response and patient and tumour characteristics. Tumour characteristics included oncogenic markers analysed on pre-treatment biopsy specimens and classic tumour characteristics. In addition, we assessed the prognostic significance of these clinical characteristics including pathological and clinical tumour response on overall and distant disease-free survival.

Patients and Methods

Patients

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 tumour 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 the 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 tumour 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 oestrogen receptor and nodal status.

Pathological tumour response

Surgical tumour specimens were examined for the presence of microscopic residual tumour. 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 tumour response

The tumour response classification system used in EORTC 10902 was according to the UICC [11]. Clinical tumour 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 tumour 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 $\geq$ 50% decrease in total tumour size after four cycles of preoperative chemotherapy. An increase of $\geq$ 25% in tumour 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 tumours (cSD and cPD).

Histology and immunohistochemistery
Blocks were collected from core needle biopsies taken before the start of chemotherapy. All immunohistochemical (IHC) 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]. Oestrogen receptor status (ER) was estimated immunohistochemically 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 Methods
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 tumour response classification systems and patient and tumour 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 0.10 in the univariate analysis. The effect of patient and tumour 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 0.10 in the univariate analysis. Survival curves of the tumour 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 tumour characteristics
EORTC 10902 trial randomised 350 patients to preoperative chemotherapy and 321

patients received this allocated treatment. Tumour 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 tumour characteristics are listed in Table 1.
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 tumours. All but one of the patients with pCR were clinically graded as responders. At the time of analysis, the median follow-up period was seven years (95% confidence interval [CI], 6.89-7.45); thirty-one (29%) patients have died and of the patients alive, ten (9.3%) have experienced a distant relapse. Although otherwise stipulated in the treatment protocol, nine (17%) women older than 50 years were not administered to tamoxifen treatment and four (7.4%) women in the younger group did use tamoxifen.

Prognostic value of pathological tumour response
The association of pathological tumour response with overall survival and distant disease-free survival is shown in Figure 1 and 2, respectively. Patients with complete pathological response 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, 0.39-21.14; P = 0.30). Patients with a complete pathological response 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, 0.50-26.33; P = 0.21).

Prognostic value of clinical tumour response
Patients with a clinical tumour response had an overall survival rate after 7 years of 67% (Figure 3). Non-responders had an overall survival rate of 75% (HR, 0.71; 95% CI, 0.34-1.45; P = 0.35). Patients with clinical response had a distant disease-free survival rate after 7 years of 61% compared to 61% for patients with non-responding tumours (HR, 0.94; 95% CI, 0.51-1.74; P = 0.84; Figure 4).

Predictive characteristics for pathological and clinical response
We assessed the predictive value of patient and tumour characteristics and expression of oncogenic markers in pre-treatment core needle biopsies.
Table 2 lists the relationships between dichotomized characteristics and pathological and clinical tumour response. Pathological lymph node status and *p53* status were significantly correlated with pathological tumour response. Including both variables in the multivariate analysis (Table 3) revealed an independent relationship of positive *p53* expression with pCR (odds ratio [OR], 16.83; 95% CI, 1.78-159.33; P = 0.01) and a non-significant association of negative pathological lymph node status. Clinical tumour response was predicted by clinical tumour size, tumour grade, *p53* status, PgR status, and *HER2* status (Table 2). In multivariate analysis, positive *p53* expression (OR, 5.57; 95% CI, 1.58-19.65; P = 0.008) and small clinical tumour size (OR, 10.26; 95% CI, 2.01-52.48; P = 0.005) remained as independent predictive factors of clinical tumour response (Table 3).

Prognostic characteristics for overall survival and distant disease-free survival
Table 4 shows the prognostic value of patient and tumour characteristics in

Table 1. Patient and tumour 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 tumour size†		
T1	18	17
T2	64	60
T3	21	19
T4	4	3
Clinical tumour response‡		
complete	7	7
partial	51	48
stable disease	47	44
progressive disease	2	2
Pathological tumour size‡		
pT0/pCR	7	7
pT1	43	40
pT2	48	45
pT3	7	7
pT4	2	2
Clinical lymph node status†		
negative	65	58
positive	45	42
Pathological lymph node status‡		
negative	45	42
positive	65	58
Grade†		
I	13	12
II	69	64
III	19	18
unknown	6	6
BCL-2 expression†		
negative	25	23
positive	59	55
unknown	23	22
P53 expression†		
negative	73	68
positive	26	24
unknown	8	8
ER status†		
negative	21	20
positive	71	66
unknown	15	14
PgR status†		
negative	50	47
positive	49	46
unknown	8	7
HER2 expression†		
negative	92	86
positive	10	9
unknown	5	5
P21 expression†		
negative	45	42
positive	47	44
unknown	15	14

† Assessed prior to the delivery of chemotherapy; ‡ Assessed after the delivery of chemotherapy;
BCT= breast conservative treatment; pCR= pathological complete response

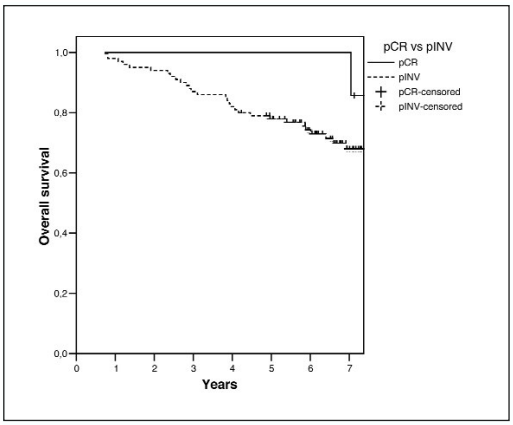

Figure 1. Pathological tumour response and overall survival.
pCR= pathological complete response;
pINV= invasive tumour cells on pathological examination

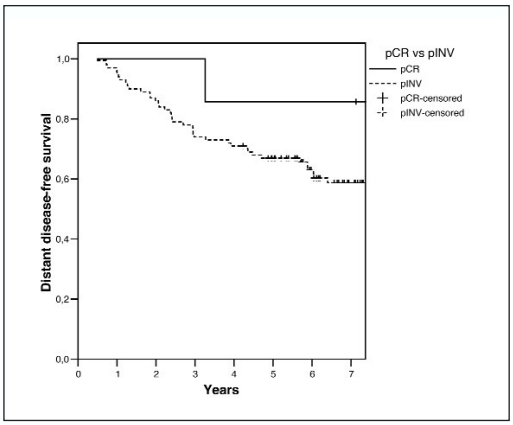

Figure 2. Pathological tumour response and distant disease-free survival.
pCR= pathological complete response;
pINV= invasive tumour cells on pathological examination

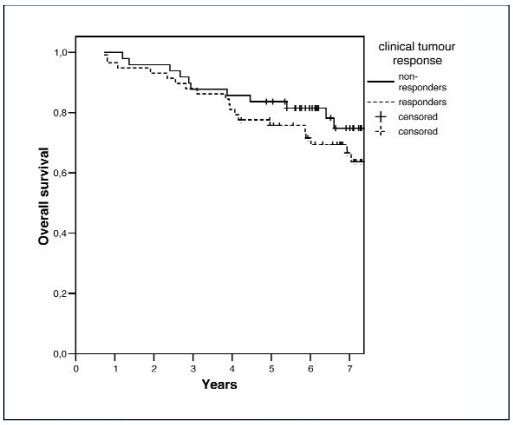

Figure 3. Clinical tumor response and overall survival

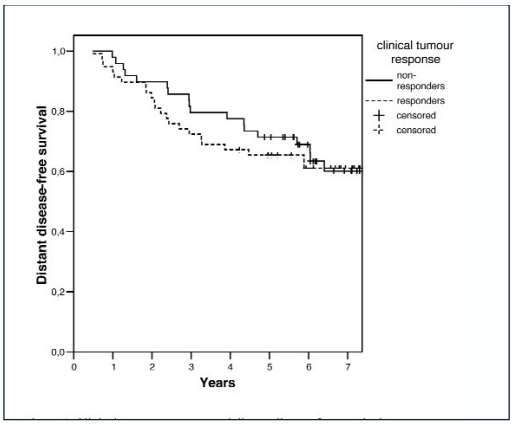

Figure 4. Clinical tumor response and distant disease-free survival

predicting clinical outcome. In this univariate analyses, significant prognostic variables for overall and distant disease-free survival were age, use of tamoxifen, and pathological lymph node status. In addition, histological tumour grade was significantly associated with overall survival. Overexpression of *p53* was non-significantly related with poorer overall (HR, 1.72; 95% CI, 0.82-3.62; P = 0.15) and distant disease-free survival (HR, 1.39; 95% CI, 0.70-2.74; P = 0.35).
The prognostic factors found to be trend significant in the 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 tumour grade III was an independent prognostic factor of poorer overall survival.

Characteristic	Pathological tumour response					Clinical tumour response				
	pCR		pINV			responders		non-responders		
	N	%	N	%	P value	N	%	N	%	P value
Age at diagnosis										
< 40 years	0	0	11	100		8	73	3	27	
≥ 40 years	7	7	89	93	1.00	50	52	46	48	.22
Clinical tumour size†										
≤ 2 cm	0	0	18	100		16	89	2	11	
> 2 cm	7	8	82	92	.60	42	47	47	53	.001
Clinical lymph node status†										
negative	4	7	58	93		36	58	26	42	
positive	3	7	42	93	1.00	22	49	23	51	.43
Pathological lymph node status‡										
negative	6	13	39	87		28	62	17	38	
positive	1	2	61	98	.04	30	48	32	52	.17
Grade†										
I & II	5	6	77	94		40	49	42	51	
III	2	11	17	89	.61	14	74	5	26	.05
BCL-2 expression†										
negative	3	12	22	88		15	60	10	40	
positive	3	5	56	95	.36	27	46	32	54	.23
P53 expression†										
negative	1	1	72	99		32	44	41	56	
positive	5	19	21	81	.004	21	81	5	19	.001
ER status†										
negative	3	14	18	86		14	67	7	33	
positive	3	4	68	96	.13	34	48	37	52	.13
PgR status†										
negative	4	8	46	92		33	66	17	34	
positive	2	4	47	96	.68	19	39	30	61	.007
HER2 expression†										
negative	5	5	87	95		46	50	46	50	
positive	1	10	9	90	.47	8	80	2	20	.09
P21 expression†										
negative	3	7	42	93		25	56	20	44	
positive	3	6	44	94	1.00	23	49	24	51	.53

† Assessed prior to the delivery of chemotherapy; ‡ Assessed after the delivery of chemotherapy; pCR= pathological complete response; pINV= invasive tumour cells on pathological examination

Table 2. Pathological and clinical tumour response and dichotomized patient and tumour characteristics

Characteristic	Pathological complete response			Clinical response		
	Odds Ratio	95 % CI	P value	Odds Ratio	95 % CI	P value
Negative pathological lymph node status‡	8.47	0.88-81.82	.07			
Positive *p53* expression†	16.83	1.78-159.33	.01	5.57	1.58-19.65	.008
Tumour size ≤ 2 cm†				10.26	2.01-52.48	.005
Grade III†				1.58	0.41-6.13	.51
Negative PgR status†				2.37	0.89-6.31	.08
Positive *HER2* expression†				2.93	0.47-18.14	.25

† Assessed prior to the delivery of chemotherapy; ‡ Assessed after the delivery of chemotherapy; CI= confidence interval

Table 3. Multivariate logistic regression analyses of correlation between dichotomized tumour characteristics and pathological complete tumour response (N=99) and clinical response (N=94)

Discussion

In this analysis, we demonstrated a significant independent association between *p53* overexpression and pathological complete and clinical tumour 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 in this patient population not reach statistical significance although a clear trend was demonstrated (Figures 1 and 2). This finding is in accordance with other randomised controlled trials studying preoperative chemotherapy in primary operable breast cancer while pCR was in these studies a significant prognostic factor [2, 16-18].

In this study, clinical tumour response showed no prognostic benefit (Figures 3 and 4). This result is in discordance 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, 0.91-2.24; P = 0.12). However, the fact that clinical responders in the current group had no favourable prognosis implies that the results concerning the predictive value of characteristics for clinical response must be interpreted with caution. Moreover,

Characteristic	Overall Survival					Distant Disease-Free Survival				
	N/O	7-years percent	Hazards ratio	95% CI	P value	N/O	7-years percent	Hazards ratio	95% CI	P value
Age at diagnosis										
< 40 years	11/7	45	1.00			11/7	36	1.00		
≥ 40 years	96/24	73	0.34	0.14-0.78	.01	96/34	64	0.40	0.18-0.92	.03
Type of surgery										
mastectomy	57/17	66	1.00			57/24	58	1.00		
BCT	50/14	74	0.83	0.41-1.69	.62	50/17	64	0.72	0.36-1.33	.29
Tamoxifen										
no	59/24	60	1.00			59/30	48	1.00		
yes	48/7	84	0.34	0.15-0.79	.01	48/11	77	0.39	0.19-0.77	.01
Radiotherapy										
no	20/8	56	1.00			20/9	51	1.00		
yes	87/23	74	0.52	0.23-1.16	.11	87/32	63	0.69	0.33-1.44	.32
Clinical tumour size†										
≤ 2 cm	18/4	72	1.00			18/5	67	1.00		
> 2 cm	89/27	70	1.30	0.45-3.72	.63	89/36	59	1.57	0.61-4.00	.35
Clinical tumour response‡										
responders	58/19	67	1.00			58/22	61	1.00		
non-responders	49/12	75	0.71	0.34-1.45	.35	49/19	61	0.94	0.51-1.74	.84
Pathological tumour size‡										
≤ 2 cm	50/13	75	1.00			50/17	64	1.00		
> 2 cm	57/18	66	1.41	0.69-2.88	.35	57/24	58	1.43	0.77-2.67	.26
Pathological tumour response‡										
pCR	7/1	86	1.00			7/1	86	1.00		
pINV	100/30	68	2.87	0.39-21.14	.30	100/40	59	3.62	0.47-26.33	.21
Clinical lymph node status†										
negative	62/17	73	1.00			62/22	64	1.00		
positive	45/14	67	1.27	0.62-2.57	.51	45/19	56	1.33	0.72-2.55	.37
Pathological lymph node status‡										
negative	45/8	84	1.00			45/8	81	1.00		
positive	62/23	61	2.82	1.23-6.44	.01	62/33	46	4.15	1.90-9.06	.00
Grade†										
I & II	82/20	74	1.00			82/29	64	1.00		
III	19/9	55	2.23	1.01-4.91	.05	19/9	50	1.58	0.75-3.33	.23
BCL-2 expression†										
negative	25/8	70	1.00			25/11	54	1.00		
positive	59/12	79	0.62	0.26-1.53	.30	59/16	73	0.55	0.25-1.18	.12
P53 expression†										
negative	73/19	73	1.00			73/27	62	1.00		
positive	26/11	58	1.72	0.82-3.62	.15	26/12	52	1.39	0.70-2.74	.35
ER status†										
negative	21/9	60	1.00			21/9	56	1.00		
positive	71/19	71	0.57	0.26-1.26	.16	71/27	61	0.81	0.38-1.74	.59
PgR status†										
negative	50/19	62	1.00			50/23	52	1.00		
positive	49/12	75	0.58	0.28-1.19	.14	49/16	68	0.64	0.34-1.20	.16
HER2 expression†										
negative	92/27	70	1.00			92/37	59	1.00		
positive	10/3	69	1.11	0.34-3.66	.87	10/3	70	0.82	0.25-2.66	.74
P21 expression†										
negative	45/12	72	1.00			45/16	65	1.00		
positive	47/17	64	1.56	0.74-3.28	.24	47/12	53	1.44	0.75-2.76	.28

† Assessed prior to the delivery of chemotherapy; ‡ Assessed after the delivery of chemotherapy; N/O= number of patients/ observed number of events; CI= confidence interval; BCT= breast

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

Characteristic	Overall survival			Distant disease-free survival		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
Positive pathological lymph node status[‡]	4.30	1.71-10.82	.002	5.19	2.35-11.46	.000
Use of tamoxifen	0.41	0.17-1.00	.05	0.34	0.17-0.69	.003
Age younger than 40 years	2.13	0.81-5.65	.13	2.28	0.98-5.32	.06
Grade III[†]	3.02	1.28-7.12	.01			

N= number of patients; CI= confidence interval; [‡] Assessed after the delivery of chemotherapy; [†] Assessed prior to the delivery of chemotherapy

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

determining clinical tumour response after preoperative chemotherapy is difficult and can be either under- or overestimated due to fibrosis, weakening of the tumour margins and resolution of oedema, suggesting prognostic superiority of pathologically evaluated tumour response [19-22].

Although pCR in our study was associated with *p53* overexpression and higher survival rate, positive *p53* status 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 of *p53* positive tumours may be overcast by rapid regrowth of micro-metastases after initial remission of the primary tumour, reflecting their aggressive biology. Though, analysis of this 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 tumour suppressor. Breast cancer patients with *p53* mutations or protein accumulation measured by IHC in their tumours have worse survival [23-26]. Meanwhile, the literature of the predictive value of *p53* status on tumour response to preoperative anthracycline-based chemotherapy is conflicting.(7) Most studies find no association between *p53* expression and tumour response to anthracyclines [27-32]. Others have associated *p53* overexpression with both resistance [14, 33-35] and sensitivity [10,36] to preoperative anthracycline containing chemotherapy. Interpretation of the above literature is complicated since the definition of response various across studies, the correlation between *p53* protein accumulation and the presence of mutations is not absolute and numerous non-standardized IHC 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 is confirmed 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. 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 micro-array 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 RCT's [42-45].

In conclusion, our data derived from a prospective randomized trial suggest that *p53* overexpression estimated by immunohistochemistery is an independent predictive factor of tumour 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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