Original article

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Locally advanced/inflammatory breast cancers treated with intensive epirubicin-based neoadjuvant chemotherapy: are there molecular markers in the primary tumour that predict for 5-year clinical outcome?

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Background: Locally advanced and/or inflammatory breast cancer (LABC) is a heterogeneous disease. Molecular markers may help to understand this heterogeneity. This paper reports the results of a study assessing the potential prognostic or predictive value of HER-2, p53, cyclinD1, MIB1, ER and PgR expression by immunohistochemistry from patients included in an EORTC–NCIC–SAKK trial.

Patients and methods: A total of 448 patients with a cytological or histological diagnosis of LABC were randomised into a trial comparing two anthracycline-based neoadjuvant regimens. Chemotherapy was followed by standard locoregional therapy. Survival was comparable in both arms. We collected and analysed centrally paraffin-embedded tumour specimens from 187 (72.5%) of 258 patients that had a histological diagnosis.

Results: Of the patients included in this molecular marker study 114 relapsed and 91 died. In the multivariate analysis p53 positivity was associated with a shorter progression-free survival [hazard ratio (HR) = 1.96; 95% CI 1.33–2.91; $P = 0.0008$) and a shorter overall survival (HR = 1.98; 95% CI 1.28–3.06; $P = 0.002$). PgR positivity predicted for a longer overall survival (HR = 0.54; 95% CI 0.35–0.83; $P = 0.0045$).

Conclusions: p53 was an independent factor predicting for survival. In order to clarify whether p53 is a pure prognostic and/or a predictive factor, a phase III trial is being conducted (EORTC 10994/BIG 00-01 study) using functional assay in yeast from frozen tumour samples.

Key words: breast cancer, locally advanced/inflammatory breast cancer, neoadjuvant chemotherapy, p53

Introduction

Molecular predictive factors of response or resistance to chemotherapy in breast cancer are lacking in clinical practice. The list of candidates is long and MDR1/gp170, topoisomerase II, S-phase, HER-2, p53 and others have been analysed in many breast cancer trials. Within this list two markers, namely HER-2 and p53, seem very promising. Preclinical data regarding the relationship between HER-2 and response to chemotherapy are contradictory [1]. In the clinic the role of HER-2 as a predictive factor of response to chemotherapy, specifically to anthracyclines, has been suggested by several retrospective analyses conducted in the context of prospective trials. Taken together these data suggest that HER-2 is associated with sensitivity to doxorubicin [2]. *TP53* is a key regulatory gene in the apoptotic pathway and preclinical and clinical studies have shown that anticancer agents achieve their cytotoxic effect through apoptosis. *In vitro* and *in vivo* studies indicate that tumours containing wild-type *p53* respond better to anthracyclines than *p53*-mutant tumours [3, 4]. One clinical study [5], recently updated [6], where *TP53* gene was analysed suggested that specific mutations may confer resistance to anthracyclines.

The results of a large intergroup (EORTC–NCIC–SAKK) phase III trial conducted on 448 patients presenting with locally advanced and/or inflammatory breast cancers were recently reported [7]. Patients were randomised between two different neoadjuvant anthracycline chemotherapy regimens. After six cycles of chemotherapy, locoregional treatment was planned, followed by tamoxifen for 5 years. After a median follow-up of 5.5 years, no significant difference in terms of progression-free survival (PFS) or overall survival (OS) was found between the two chemotherapy arms. Patients with inflammatory breast cancer

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had a shorter median PFS than patients with locally advanced breast cancer (24 months and 44 months, respectively). However, both subgroups were heterogeneous, some patients having progressed very early and others remaining without progression several years after diagnosis. Beyond the clinical presentation (locally advanced versus inflammatory breast cancer) we attempted to understand this heterogeneity with the help of molecular markers. Therefore, we collected tumour specimens from patients included in the EORTC–NCIC–SAKK study and performed a central immunohistochemical analysis of the following markers: estrogen receptor (ER) and progesterone receptor (PgR), MIB-1, HER-2, p53 and cyclin D1. This paper reports the results of a retrospective assessment of the potential prognostic or predictive value of these markers in relation to clinical response, PFS and OS. By definition "prognostic factors are associated with either the metastatic and/or growth rate potential of the primary tumour and predictive factors are associated with relative sensitivity and/or resistance to specific therapeutic agents" [8]. Some factors can be both prognostic and predictive. We will elaborate on this in the discussion.

Patients and methods

Patients and treatment

From May 1993 to April 1996, patients with locally advanced or inflammatory breast cancer were included in a large EORTC–NCIC–SAKK randomised study that compared two neoadjuvant anthracycline-based regimens, with dose-intensity in one regimen being twice that of the other. The eligibility criteria for this trial are described by Therasse et al. [7]. Patients were randomised to receive six cycles of neoadjuvant chemotherapy, either fluorouracil 500 mg/m^2 days 1 and 8 intravenously (i.v.), epirubicin 60 mg/m^2 days 1 and 8 i.v., cyclophosphamide 75 mg/m^2/day from day 1 to day 14 orally, q 4 weeks (FEC 120), or epirubicin 120 mg/m^2 day 1, cyclophosphamide 830 mg/m^2 i.v. and granulocyte colony-stimulating factor (G-CSF) (Filgrastim®) 5 µg/kg/day from day 1 to day 13 subcutaneously (EC regimen), every 2 weeks. Tumour assessment was planned after three and six cycles of chemotherapy in both arms. Locoregional treatment was planned after chemotherapy and was flexible according to each centre's policy. Tamoxifen was started 4 weeks following day 1 of the last cycle of chemotherapy in all patients irrespective of hormone-receptor status and was planned for a total of 5 years. All patients were followed in a standardised fashion after treatment completion to determine the PFS and OS.

Central pathology review

Formalin-fixed or Bouin Hollande-fixed, paraffin-embedded tumour samples from the primary tumour were obtained prior to the initiation of neoadjuvant chemotherapy from participating EORTC institutions. Tumours were provided as unstained 5-µm tissue sections or as tumour blocks, in which case the slides were prepared centrally. The tumours were graded on haematoxylin and eosin (H&E) slides according to the Elston modified scoring scheme of Bloom and Richardson [9] by a reference pathologist (S.D.B.) blinded to the clinical outcome of the patient.

Immunohistochemical analysis

All immunohistochemical analyses were performed in a single reference laboratory, using the unstained 5-µm tissue sections. The general protocol for immunohistochemical staining was performed according to a standardised method previously described by Clahsen et al. [10]. This standardised method has been used in several retrospective studies on tissue sections from patients treated within EORTC trials.

Except for HER-2, an antigen retrieval procedure was applied: tissue sections were pre-incubated in 10 mM sodium citrate solution and incubated three times for 5 min in a 600 W microwave oven. Slides were then pre-incubated for 15 min in phosphate-buffered saline (PBS) with 5% bovine serum albumin (BSA) and incubated for 1 h at room temperature with the following primary antibodies: mouse monoclonal HER-2 Abs CB11 (Biogenex, undiluted), mouse monoclonal p53 Abs DO-7 (Dako, 1/100 dilution), mouse monoclonal Cyclin D1 Abs DCS-6 (Novocastra, 1/40 dilution), mouse monoclonal estrogen receptors Abs 1D5 (Dako, 1/100 dilution), mouse monoclonal progesterone receptors Abs PGR-1A6 (Biogenex, 1/20 dilution), mouse monoclonal Ki-67 antigen Abs MIB-1 (Immunotech, 1/20 dilution). Sections were washed with PBS and reacted with streptavidin–biotin peroxidase reagents (Dako) and diaminobenzidine chromogen. Sections were finally counterstained with haematoxylin (5 min).

In all series and for each antibody, positive controls (known cases of breast carcinoma) and negative controls (primary antibody omitted) were included.

All slides were scored independently by two investigators (S.D.B. and H.B.) without patient information. The method of counting and scoring the immunostaining and the definition of a cut-off value were established for each antibody according to previous studies before starting the analyses; all details are described below. All discrepancies in scoring between the two investigators were resolved by consensus.

p53 overexpression. Scoring was done using a semiquantitative system according to a method previously described [10]. Mean nuclear staining intensity (MSI) was evaluated and could vary from 0 (none), 1 (weak), 2 (moderate) to 3 (strong). Percentage of positive tumour cell nuclei (PPN) was estimated and values were given: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (>75%). The addition of these two values (MSI and PPN) results in the p53 staining score (range 2–7). Expression of p53 was considered as positive for scores ≥4 [10].

HER-2 overexpression. As previously described, a tumour was considered as positive if a clear circonferential membranous positivity was found in the tumour cells either focally or throughout the tumour, as defined in previous studies [11]. Tumour cells which showed a granular cytoplasmic staining were considered to have normal HER-2 expression. The proportion of HER-2 positive cells was determined semi-quantitatively as the percentage of invasive tumour cells ranging from 0% to 100% from the entire tissue section.

MIB-1 positivity. Only clear nuclear staining in tumour cells was considered positive. The percentage of positively stained cells was calculated from the number of brown nuclei seen in a sample of 200 tumour cells [12]. We selected the threshold of 20% (<20%, negative; ≥20%, positive), as used in a previous EORTC immunohistochemical study [10].

ER and PgR status. Only clear nuclear staining in tumour cells was considered positive. The proportion of ER and PgR-positive cells was determined as the percentage of invasive tumour cells ranging from 0% to 100% from the entire tissue section. The threshold of 10% positivity was chosen as the cut-off value, according to a current consensus recommendation [13]. The staining intensity was scored from 0 to 3, in a similar fashion as for p53 (see above).

Cyclin D1 expression. Only clear nuclear staining in tumour cells was considered positive. Rarely, a weak cytoplasmic staining was observed; this was considered to be non-specific. The percentage of positive cells and the staining intensity were determined as described above. We chose a cut-off value of 10% (<10%, negative tumours; ≥10%, positive tumours), as used in previous studies [14].

Statistical methods

Life tables were constructed to examine PFS and OS, using the Kaplan–Meier method [15] and were compared using the log-rank test [16]. A proportional hazards regression model [17] with stratification for treatment [18] was used in the univariate and multivariate analysis. A step-down (backward) variable selection procedure was used to fit the multivariate model [19]. A two-sided test was used at the 5% level of significance.

In the statistical analysis of prognostic value, we planned to analyse molecular marker expression by immunohistochemistry both as continuous and dichotomous variables. For each marker the definitions of positivity and negativity were selected before we performed the analysis based on previous studies, as described in the previous section. The correlation between the response status and the predictive value of each biological factor has been analysed in a univariate and multivariate logistic regression model [17, 19]. All analyses testing prognostic significance of the different factors identified have been performed with SAS software. The survival data and response status were provided directly from Software for the Management and Analysis of Randomised Trials (SMART). The correlation between the response status and the predictive value of each biological factor has been analysed in a univariate analysis.

Table 1. Comparison of patient characteristics included in the European Organisation for Research and Treatment of Cancer (EORTC) 10921 study: group A, patients included in the prognostic factor study (PF study) and group B, patients not included in the PF study

Characteristics	Group A	Group B
	n (%)	*n* (%)
No. of patients (*n*)	179	258
Age (years)		
Median	49	49
Range	27–75	26–74
WHO performance status		
0	163 (91)	223 (86)
1	16 (9)	35 (14)
Tumour		
Locally advanced cancer (any T, N2,3, M0 or T4a,b,c, any N, M0)	103 (58)	131 (51)
Inflammatory cancer (T4d, any N, M0)	76 (42)	127 (49)
Menopausal status		
Pre	89 (50)	130 (50)
Post	88 (49)	121 (47)
Unknown	2 (1)	7 (3)
Clinical response to chemotherapy		
CR	48 (27)	75 (29)
PR/NC/PD	131 (73)	183 (71)
Treatment[a]		
FEC	88(49)	129(50)
EC + G-CSF	91(51)	129(50)

[a]No survival differences between the two treatment arms (FEC versus EC + G-CSF).

CR, complete response; EC, epirubicin, cyclophosphamide and granulocyte colony-stimulating factor (G-CSF) (Filgrastim®); FEC, 5-fluorouracil, epirubicin, cyclophosphamide; PR, partial response; NC, no change; PD, progressive disease.

Results

Patient and tumour characteristics

From May 1993 to April 1996 448 patients were included in the EORTC–NCIC–SAKK trial. Eleven patients were not eligible. One of the inclusion criteria for this protocol was a cytological or histological diagnosis of breast carcinoma. We were able to collect tumour specimens from 187 (72.5%) of 258 patients that had a histological diagnosis. Eight cases were not evaluable for the immunohistochemical analysis (no invasive carcinoma detected in six cases; technically unsuitable specimen in two cases). Consequently tumour specimens from 179 patients were included in the immunohistochemical study, leaving 258 patients who were treated in the clinical trial but not entered in this translational research study (no tumour specimen collected). Characteristics of patients in these two groups were well balanced for age, tumour presentation (locally advanced versus inflammatory), menopausal status, chemotherapy regimen allocated and clinical response to chemotherapy (Table 1). Tumour characteristics (histological grade and molecular markers) of the 179 patients included in this study are listed in Table 2.

Table 2. Tumour characteristics: histological grade and molecular markers

Tumour characteristics	PF study (*n* = 179)
	n (%)
Histological grade	
Grade I and II	82 (58)
Grade III	60 (42)
ER	
Negative <10%	102 (57)
Positive ≥10%	77 (43)
PgR	
Negative <10%	60 (34)
Positive ≥10%	117 (66)
MIB-1	
Negative <20%	42 (25)
Positive ≥20%	128 (75)
HER-2	
Negative	132 (74)
Positive	47 (26)
p53 overexpression	
Negative (score <4)	126 (70)
Positive (score ≥4)	53 (30)
Cyclin D1	
Negative <10%	126 (71)
Positive ≥10%	52 (29)

ER, estrogen receptor; PF, prognostic factor; PgR, progesterone receptor.

Survival

Of the 179 patients included in this molecular markers study 114 relapsed and 91 died.

Univariate analysis for PFS and OS

A first univariate analysis was performed on the following parameters analysed as dichotomous variables: tumour grade (I/II versus III), ER status [percentage of cells stained (%CS) <10 versus ≥10], PgR status (%CS <10 versus ≥10), MIB-1 percentage (%CS <20 versus ≥20), HER-2 overexpression (%CS = 0 versus >0), p53 overexpression (score <4 versus ≥4) and cyclin D1 overexpression (% CS <10 versus ≥10). This analysis demonstrated that two factors were significantly associated with a shorter PFS and OS: ER negativity and p53 positivity (Table 3). PgR negativity was associated with a shorter PFS and OS; this difference was statistically significant for OS ($P = 0.007$), but not for PFS ($P = 0.23$). Progression-free survival and OS curves according to immunohistochemically defined p53 status are shown in Figures 1 and 2.

A second univariate analysis was performed on immunohistochemical parameters which were analysed as continuous variables. Again ER negativity and p53 overexpression were significantly associated with a worse outcome in terms of PFS and OS (Table 4). Progesterone receptor negativity and MIB-1 positivity were associated with a shorter PFS and OS; with both markers this difference was statistically significant for OS ($P = 0.002$ and 0.004, respectively), but not for PFS ($P = 0.22$ and 0.09, respectively).

Multivariate analysis for PFS and OS

Characteristics were analysed as dichotomous variables in the first multivariate analysis and as continuous variables in the second.

All the factors found to be significant at the 0.1 level in the univariate analysis were included in the multivariate Cox regression model for their relation to PFS and OS. Consequently, grade and HER-2 were not analysed in the first multivariate analysis, and HER-2 and cyclin D1 were not analysed in the second multivariate analysis.

In both analyses, p53 positivity (tumour with a score ≥4 or with a high percentage of positive cells) was found to be an independent prognostic factor associated with a statistically significant higher risk of progression and death (Tables 5 and 6).

In both multivariate analyses, PgR negativity was found to be an independent prognostic factor associated with a statistically significant higher risk of death (Tables 5 and 6).

Table 3. Univariate analysis of grade and molecular markers predicting for progression-free survival (PFS) and overall survival (OS) (characteristics analysed as dichotomous variables)

Variable	Progression-free survival					Overall survival				
	O/N	Median (months)	HR	95% CI	*P*	O/N	Median (months)	HR	95% CI	*P*
Grade										
I/II	52/82	33	0.97	0.64–1.48	0.89	38/82	79	1.2	0.74–1.91	0.47
versus III	37/60	38				31/60	58			
ER										
Negative (%CS <10)	70/102	19	0.6	0.41–0.88	0.008	61/102	39	0.45	0.29–0.7	0.0004
Positive (%CS ≥10)	44/77	54				30/77	NR			
PgR										
Negative (%CS <10)	39/60	19	0.79	0.53–1.15	0.23	37/60	37	0.56	0.37–0.85	0.007
Positive (%CS ≥10)	73/117	39				52/117	79			
MiB-1										
Negative (%CS <20)	25/42	46	1.22	0.78–1.91	0.38	17/42	79	1.67	0.98–2.85	0.06
Positive (%CS ≥20)	84/128	26				68/128	56			
HER-2										
Negative (%CS = 0)	83/132	34	1.12	0.74–1.70	0.58	68/132	58	0.91	0.57–1.47	0.71
Positive (%CS >0)	31/47	25				23/47	NR			
p53										
Negative (score <4)	70/126	47	2.16	1.48–3.16	<0.0001	57/126	79	1.95	1.27–2.98	0.002
Positive (score ≥4)	44/53	16				34/53	33			
Cyclin D1										
Negative (%CS <10)	84/126	24	0.70	0.46–1.07	0.1	68/126	50	0.65	0.40–1.04	0.07
Positive (%CS ≥10)	30/52	53				23/52	79			

O/N, observations (events)/number of patients (total); HR, hazard ratio; NR, not reached; CI, confidence interval; %CS, percentage of cells stained.

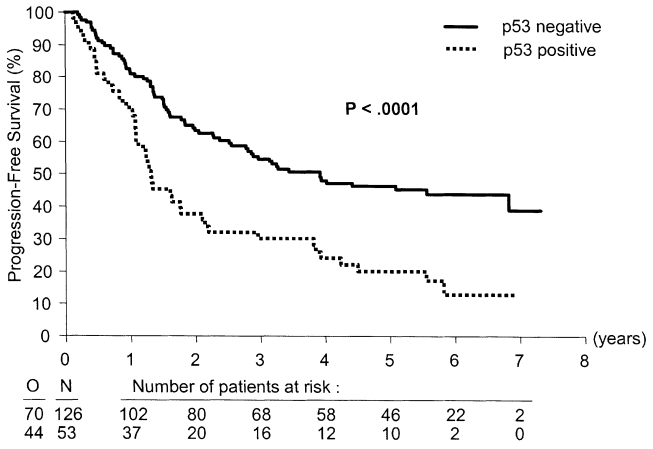

Figure 1. Progression-free survival of patients according to p53 status defined by immunohistochemistry. The numbers below the years on the *x* axis refer to patients at risk.

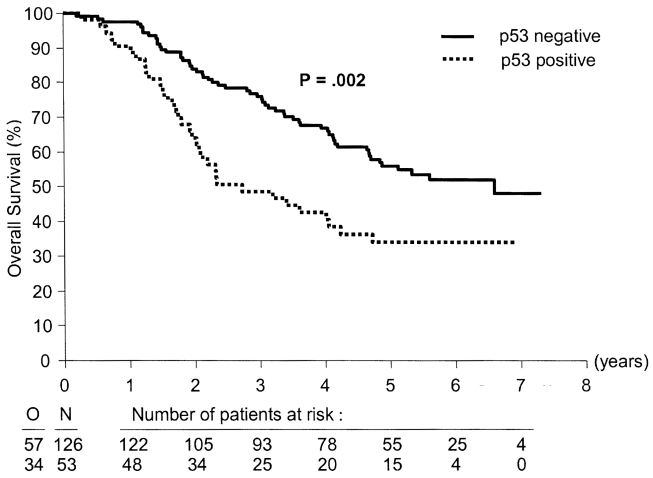

Figure 2. Overall survival of patients according to p53 status defined by immunohistochemistry.

Predictive factors of a clinical complete response to chemotherapy

A complete clinical response to neoadjuvant chemotherapy was observed in 48 of 179 patients (27%). In the univariate analysis, cyclin D1-negative tumours were associated with a lower rate of complete clinical response ($P = 0.051$) (Table 7). No statistically significant correlation between grade or immunohistochemical status of ER, PgR, MIB-1, HER-2 or p53 and a clinical complete response to chemotherapy was found (Table 7).

Discussion

In the literature few studies report on biological factors in locally-advanced/inflammatory breast cancer [5, 20, 21]. This is the largest biological markers study conducted so far in this setting.

In early breast cancer ER and PgR status are weak prognostic factors [22] and well established predictive factors of response to tamoxifen and other hormonal manipulations [23]. In locally advanced and/or inflammatory breast cancers the prognostic or predictive value of hormone receptors remains controversial [21, 24]. In our study, PgR status was found to be an independent factor predicting for OS (Tables 5 and 6). In the multivariate analysis for OS, ER status lost its significance (Tables 5 and 6). This is most probably because of its close correlation with PgR (in the multivariate analysis for OS, ER was removed from the model in favour of PgR). The fact that PgR is an independent factor for OS, but not for PFS seems paradoxical. We hypothesised that we missed its prognostic information for PFS due to the relatively small number of patients analysed.

In our series HER-2 did not predict for either PFS or OS in the univariate analysis. The potential pure prognostic value of HER-2 remains controversial, as shown in a recent meta-analysis [25]. Regarding its predictive value, data in the literature suggest that HER-2 overexpression is associated with anthracycline sensitivity and that this sensitivity increases with dose escalation [2]. In our study patients received chemotherapy regimens with a high dose of anthracyclines in both treatment arms. Therefore, our study design is not optimal to evaluate the hypothesis of an association between HER-2 overexpression and anthracycline sensitivity. Similarly, there are data in the literature that suggest

Table 4. Univariate analysis of molecular markers predicting for progression-free survival and overall survival (characteristics analysed as continuous variables)

Variable	Progression-free survival			Overall survival		
	HR[a]	95% CI	*P*	HR[a]	95% CI	*P*
ER (%CS)	0.83	0.72–0.97	0.015	0.74	0.62–0.88	<0.001
PgR (%CS)	0.92	0.81–1.05	0.22	0.79	0.68–0.92	0.002
MIB-1 (%CS)	1.23	0.97–1.56	0.09	1.45	1.13–1.88	0.004
HER-2 (%CS)	1.03	0.92–1.16	0.61	0.99	0.86–1.12	0.82
p53 (%CS)	1.30	1.17–1.46	<0.0001	1.3	1.15–1.48	<0.0001
Cyclin D1 (%CS)	0.92	0.7–1.19	0.51	0.94	0.7–1.26	0.68

[a]Hazard ratio (HR) represents the increase of the relative risk when the percentage of cells stained (%CS) rises from *x*% to *x*+25%.

CI, confidence interval; ER, estrogen receptor; HR, hazard ratio; PgR, progesterone receptor.

Table 5. Multivariate analysis of molecular markers predicting for progression-free survival and overall survival (characteristics analysed as dichotomous variables)

Variable	Progression-free survival			Overall survival		
	HR	95% CI	*P*	HR	95% CI	*P*
ER			NS (0.07)			NS (0.16)
PgR			–[a]	0.54	0.35–0.83	0.0045
MIB-1			–[a]			NS (0.1)
p53	1.96	1.33–2.91	0.0008	1.98	1.28–3.06	0.002
Cyclin D1			NS (0.6)			NS (0.48)

[a]In the univariate analysis for progression-free survival, PgR and MIB-1 were not found to be significant at the 0.1 level, and therefore were not included in the multivariate analysis.
CI, confidence interval; ER, estrogen receptor; HR, hazard ratio; NS, not significant; PgR, progesterone receptor.

Table 6. Multivariate analysis of molecular markers predicting for progression-free survival and overall survival (characteristics analysed as continuous variables)

Variable	Progression-free survival			Overall survival		
	HR[a]	95% CI	*P*	HR[a]	95% CI	*P*
ER (%CS)			NS (0.22)			NS (0.09)
PgR (%CS)			–[b]	0.79	0.68–0.93	0.004
MIB-1 (%CS)			NS (0.57)			NS (0.32)
p53 (%CS)	1.27	1.13–1.43	<0.0001	1.29	1.13–1.47	0.0001

[a]Hazard ratio (HR) represent the increase of the relative risk when the percentage of cells stained (%CS) rises from *x*% to *x*+25%.
[b]In the univariate analysis for progression-free survival, PgR was not found to be significant at the 0.1 level, and therefore was not included in the multivariate analysis for PFS.
CI, confidence interval; ER, estrogen receptor; NS, not significant; PgR, progesterone receptor.

that HER-2-positive tumours may be less sensitive to tamoxifen than HER-2-negative tumours [2]. In our study all patients received tamoxifen and the results do not suggest an inverse association between HER-2 overexpression and tamoxifen sensitivity.

In this study p53 was a strong independent factor predicting for PFS and OS. We first performed a multivariate analysis with molecular markers as dichotomous variables using a predefined score detailed in the methods section. In this multivariate analysis p53 was the only factor associated with an increased risk of progression (HR = 1.96) and the strongest factor associated with an increased risk of death (HR = 1.98) (Table 5). We then performed a second multivariate analysis, the factors being analysed as continuous variables. Again p53 emerged as the only factor predicting for a shorter PFS and the strongest factor predicting for a shorter OS (Table 6).

The risk of false positive and false negative results is higher when p53 is assessed by the immunohistochemical method as compared with molecular biology methods (e.g. denaturating gradient gel electrophoresis, genomic p53 sequencing method) [6, 26]. With immunohistochemistry there is a risk of false negative cases related to the type of *p53* mutation. With many *p53* gene mutations, p53 protein half-life is increased and immunohistochemistry detects the protein in the nucleus, but when *p53* gene mutations encode unstable proteins (nonsense mutations, splicing mutations) immunohistochemistry remains negative ('null mutations'). These mutations have been found to account for 25–47% of all *p53* mutations in series of patients with early breast cancers [27, 28]. These specific mutations were probably uncommon in our series and this may explain our observation: p53 by immunohistochemistry was a strong independent prognostic factor in this subset of high risk tumours. We could hypothesise that the use of a more sensitive method (e.g. denaturating gradient gel electrophoresis, genomic p53 sequencing method) could only have increased the independent prognostic value of p53 assessed by immunohistochemistry.

In our series we did not find a correlation between p53 status and clinical response to chemotherapy (Table 7). Amongst other possibilities two reasons may explain this observation. First the number of events (number of clinical complete responses) was small in our series and we may have missed a correlation by a lack of statistical power. Secondly, as discussed previously, immunohistochemistry is not the best method to assess p53. In five trials conducted in the neoadjuvant setting where p53 status was assessed by immunohistochemistry, no correlation was found between p53 status and clinical response to chemotherapy [6, 29–32]. While in one clinical study [5], recently updated [6], where p53 was assessed by temporal temperature gradient gel

Table 7. Univariate analysis of factors predicting for a clinical complete response (CR) (characteristics analysed as dichotomous variables)

Variable	CR/N	Odds ratio	95% CI	*P*
Grade				
I/II	19/82	1.42	0.67–3.02	0.36
III	18/60			
ER				
Negative (%CS <10)	29/102	0.83	0.42–1.62	0.57
Positive (%CS ≥10)	19/77			
PgR				
Negative (%CS <10)	18/60	0.80	0.40–1.6	0.54
Positive (%CS ≥10)	30/117			
MIB-1				
Negative (%CS <20)	8/42	1.93	0.82–4.5	0.13
Positive (%CS ≥20)	40/128			
HER-2				
Negative (%CS = 0)	32/132	1.61	0.78–3.32	0.19
Positive (%CS >0)	16/47			
p53				
Negative (score <4)	36/126	0.73	0.34–1.55	0.41
Positive (score ≥4)	12/53			
Cyclin D1				
Negative (%CS <10)	28/126	2.02	1–4.07	0.051
Positive (%CS ≥10)	19/52			

CR/N, number of patients with a clinical complete response/number of patients (total).
CI, confidence interval; %CS, percentage of cells stained; ER, estrogen receptor; PgR, progesterone receptor.

electrophoresis (TTGE) and genomic sequencing, specific *p53* mutations were associated with resistance to anthracyclines. Pathologically assessed complete response may be a better surrogate for chemotherapy efficacy than clinical response. Therefore biological markers predicting for a pathological complete response (pCR) would be of great value. However, in view of the small number of patients who achieved a pCR, we decided not to evaluate the possible correlation between p53 status (or other markers) and pathological response.

Our results do not allow us to conclude whether p53 is a pure prognostic, or a mixed prognostic and predictive factor. Preclinical data suggest that p53-mutated tumours are less sensitive to anthracyclines [3, 4], but remain sensitive to taxanes [33–35]. Moreover, the correlation between p53 status and pCR, that we were unable to address, needs to be evaluated. In order to test this hypothesis a large intergroup phase III clinical trial is being conducted (EORTC, SAKK, Swedish and Angloceltic groups) under the auspices of the Breast International Group (EORTC 10994/BIG 00-01 study). Patients with large operable or locally advanced breast cancer are randomised to receive six cycles of neoadjuvant chemotherapy, either an anthracycline-based regimen or a docetaxel-based regimen, followed by locoregional treatment and antihormonal treatment when indicated. In this study, p53 status will be determined by using a functional assay in yeast that detects functionally important *p53* mutations [36]. This test gives direct information regarding p53 function (detects biologically important mutations) and is theoretically more sensitive than sequencing because it is insensitive to contamination of samples with normal tissue. We will measure the correlation between p53 assessment by immunohistochemistry method and functional test as part of a side study. The functional test will be performed from frozen tumour samples taken by double trucut biopsy or by single incisional biopsy, as we have shown that the material from both biopsies gives identical results [37]. These frozen samples will also be assessed by cDNA microarray technology, with the hope of identifying a gene expression profile that predicts 'exquisite' sensitivity to a taxane-based regimen.

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