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Mutation and Accumulation of p53 Related to Results of Adjuvant Therapy of Postmenopausal Breast Cancer Patients

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p53 protein accumulation and gene mutation have been implicated in resistance to cytotoxic treatment. This study was performed to further assess the predictive value of p53 in breast cancer. Postmenopausal patients were randomized to adjuvant chemotherapy with cyclophosphamide, metothrexate, or 5-fluorouracil (CMF) vs. postoperative radiotherapy. The patients were also randomized to adjuvant tamoxifen vs. no endocrine treatment. Immunohistochemistry (IHC) and single-strand conformation polymorphism (SSCP), followed by direct sequencing, was performed. The p53 altered group, regarded as positive for p53 gene mutation and/or p53 protein accumulation, tended to benefit more from CMF than from radiotherapy as compared with others regarding distant recurrences. In the group lacking p53 alteration there was a significantly decreased local recurrence rate in the radiotherapy group as compared with the CMF group (RR = 0.24, 95% CI = 0.083–0.62), whereas no benefit from radiotherapy was found for patients showing p53 alterations. Tamoxifen significantly decreased the rate of distant recurrence for estrogen receptor-positive patients with no apparent difference in relation to p53 alteration. It is suggested that p53 alteration indicates benefit from CMF compared with radiotherapy regarding distant recurrence-free survival and the best local control with radiotherapy is achieved in the absence of p53 alteration. Finally. altered p53 status is probably not a marker of resistance to tamoxifen.

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Several studies have shown that p53 is a prognostic factor of distant recurrence in breast cancer (1). The principal methods used have been immunohistochemistry (IHC) and different gene analyses. The predictive value of p53 has also been investigated but so far the results are not consistent. Several of the clinical studies performed have analyzed advanced or metastatic disease and have either not found any predictive value (2, 3) or resistance to chemotherapy in cases with altered p53 (4–6). One study has reported on p53 mutation and tumor response in advanced cancer (7). Poor response has also been found in early breast cancer (8–10) though substantial benefit from chemotherapy has also been reported (11, 12). In the two latter studies the chemotherapy regime consisted of cyclophosphamide, metothrexate, and 5-fluorouracil (CMF).

In advanced breast cancer p53 mutations have been reported to be associated with poor response to tamoxifen (13). However, results from immunhistochemical studies do not indicate that normal expression of p53 is a necessity for response to tamoxifen (14–17). Altered p53 has been reported to predict good response to postoperative radiotherapy in breast cancer in preventing local recurrence (18) and death from breast cancer (19), although several studies have reported that p53 accumulation is associated with an increased risk of local relapse (20–23).

Inconsistent results from several studies may have many reasons. In most studies either immunohistochemistry or gene analysis has been performed, and relatively few studies have been based on randomized clinical trials.

The importance of p53 status for therapy response may also depend on type of treatment as different cellular damage activates different signaling pathways. This study was performed to address the following three questions. First, is it possible to have a good response to adjuvant

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chemotherapy with CMF despite inactivated p53 shown as p53 protein accumulation or p53 gene mutation? Second, does a normal p53 status improve the result of radiotherapy? Third, is a normal p53 status necessary for a good response to adjuvant tamoxifen?

MATERIAL AND METHODS

Patients and tumors

The patients included in the trial had either histologically verified lymph node metastases or a tumor diameter exceeding 30 mm (24). The patients did not receive any preoperative treatment. Surgery consisted of modified radical mastectomy. For most of the patients randomized to chemotherapy, the treatment consisted of 12 courses of CMF given according to the original Milan protocol (cyclophosphamide 100 mg/m^2 on days 1–14, methotrexate 40 mg/m^2 i.v. on days 1 and 8, and 5-fluorouracil 600 mg/m^2 i.v. on days 1 and 8). However, during the first 18 months of the trial cyclophosphamide was replaced by chlorambucil 10 to 15 mg orally on days 1 to 8, and up to 18 months was allowed for the 12 courses to avoid dose reductions. In a previous report of the trial, it has been described that a CMF dose level of less than 65% of the planned dose was received by 55% of the postmenopausal patients compared with 38% among the premenopausal patients (24). Radiotherapy was given with high-voltage technique. The dose was 46 Gy with 2 Gy per fraction 5 days a week for a total treatment time of about 4.5 weeks. The target volume included the chest wall, axilla, supraclavicular fossa, and the ipsilateral internal mammary nodes. Using a 2×2 factorial study design, the patients were also randomized between tamoxifen for 2 or 5 years, and no adjuvant endocrine therapy. Thus, there were a total of four treatment groups: adjuvant chemotherapy, adjuvant chemotherapy plus tamoxifen, radiotherapy, or radiotherapy plus tamoxifen. Tamoxifen treatment at a dose of 40 mg daily was initiated within 4–6 weeks of surgery. The current study included a subset consisting of 266 patients of those included in the randomized trial. In these patients primary tumor material had been sent to a central laboratory for hormone receptor determinations. The material that was not needed for the receptor assays was stored in liquid nitrogen until further processed. The patients were followed up for a median period of 11.5 years, and distant metastasis or death from breast cancer was registered in 91 cases after 5 years and in 125 cases after the complete follow-up period. Forty-seven local recurrences were registered. Tumor and treatment characteristics from all patients in the trial and from the patients in the present study are presented in Table 1.

Table 1

Tumor and treatment characteristics of the patients in the main and present study

	All patients included in the trial n = 679 (%)	Patients in the present study n = 266 (%)
No. of positive nodes:		
0	12	12
1–3	56	58
> 3	32	30
Tumor size:		
≤ 20 mm	43	43
> 20 mm	57	57
Estrogen receptor: status		
Negative	29	30
Positive	71	70
Adjuvant treatment:		
Radiotherapy	45	43
Chemotherapy (CMF)	55	57
Endocrine treatment:		
None	49	50
Tamoxifen	51	50

Immunohistochemistry

The monoclonal antibody PAb1801 (Oncogene Science, Manhasset, New York, USA) was used for immunohistochemistry on frozen sections. As a negative control we used IgG antibodies (Sigma Chemical Co, St Louis, Missouri, USA). The tumors were collected from fresh surgical resections and stored at -70°C before they were cut. The 5 μm frozen sections were kept in the freezing cabinet during the section procedure and thereafter immediately stored at -70°C until preparation. The sections were fixed in acetone (4°C) for 10 min. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol for 5 min. Normal rabbit serum (10%) was used for 10 min in order to block non-specific immunostaining. The slides were incubated with the primary antibody (1 μg/ml) at room temperature for 1 h. The sections were subsequently incubated with biotinylated rabbit anti mouse immunoglobulin (1:200) and streptavidin horseradish peroxidase (1:300), 30 min for each step, staining with 3.3-diaminobenzidine tetrahydrochloride in phosphate-buffered saline (pH 7.5) with 0.036% (v/v) hydrogen peroxidase for 8 min. After each step, except the incubation with normal rabbit serum, the sections were rinsed in phosphate-buffered saline containing 0.1% bovine serum albumin. Thereafter the sections were counterstained with haematoxylin, dehydrated in a series of ethanols, and mounted. The slides were independently examined by two of us. The sections with clearly stained nuclei in more than 1% of the tumor cells were scored as positive.

Extraction of DNA from fresh tissue

Tumor tissue was first disintegrated with scissors and then digested with 50 µl proteinase-K (10 mg/ml, Boeringer Mannheim), 500 µl TEN buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl pH 8.0), 100 µl SDS (20%) and incubated at 55°C. DNA was separated from proteins by repeated extractions with phenol, phenol/chloroform (1:1) and chloroform. Nucleic acids were precipitated by addition of 2 volumes of ice-cold 95% ethanol and 1/10 volume 3M sodium acetate incubated over night at −20°C. DNA was pelleted by centrifugation at 15 000 × g for 1 h, after which salt residues and RNA were removed by washing with 70% ethanol. Nucleic acids were repelleted as above for 10 min and then vacuum-dried and suspended in sterile Milli-Q water.

Polymerase chain reaction (PCR)

The DNA was examined for mutations in exons 5–8 of the p53 gene. Amplification of DNA was carried out by 30–35 cycles of PCR with denaturation and extension steps performed at 94°C for 45 s and 72°C for 40 s respectively. Annealing temperatures were optimized for each set of primers and ranged between 58 and 61°C. The primer sequences were as follows, forward (5′→3′) for exon 5 GCCCTGACTTTCAACTCT, exon 6 GTCCCCAGGCCTCTGATTC, exon 7 TCTTGGGCCTGTGTTATCTC, exon 8 CTGCCTCTTGCTTCTCTTTT and reverse (5′→3′) for exon 5 CAGCCCTGTCGTCTCTCC, exon 6 AACCCCTCCTCCCAGAGAC, exon 7 GGTGGATGGGTAGTAGTATG, exon 8 CTCCTCCACCGCTTCTTGTC.

PCR was performed in a total reaction volume of 22 µl, containing 25 ng of DNA, 2 mM $MgCl_2$, 1 × Taq Polymerase Buffer solution (20 mM $(NH_4)_2SO_4$, 75 mM TRIS-HCl (pH 8.5), 0.1% Tween 20 (v/v), 1 µM of each primer, 0.2 mM of each dNTP and 0.5 u Taq Polymerase (SDS/Promega). An identical reaction mixture, but excluding DNA, was used as a negative control. PCR products and control were confirmed using agarose (2%) gel separation and ethidium bromide staining.

Single strand conformational polymorphism (SSCP) analysis

Amplified products were subjected to radioactive labeling using PCR by incorporation of α-$dATP^{32}$ (Amersham). The labeling was performed by 15 cycles of PCR using the same conditions as for the primary reaction. Five µl labeled PCR product was diluted in 15 µl loading buffer (0.2% SDS, 20 mM EDTA) denatured at 94°C for 5 min and thereafter put on ice before loaded on a non-denaturing MDE gel (FMC BioProducts). Electrophoresis was carried out in a TBE buffer (40 mM TRIS, 45 mM boric acid, 1 mM EDTA pH 8.0) and run overnight at 3W and room temperature. The gel was dried and exposed on X-ray film (Cronex 4, DU PONT) for 6–48 h. The film was viewed independently by at least two investigators. Bands showing mobility shifts were selected for DNA sequencing.

DNA sequencing

DNA excised from SSCP gel was amplified 30 cycles of PCR and purified from primers using Wizard™ PCR Preps DNA Purification System (Promega/SDS). The sequencing was performed with a Thermo sequenase radiolabeled terminator cycle sequencing kit (Amersham), where α-$ddNTP^{33}$ (G, A, T, and C) were incorporated into the amplified DNA fragments. For each exon the forward primer or reverse primer was used for sequencing. The samples were run on a denaturing polyacrylamide (6%) gel containing 8 M urea at 45 W in a buffer solution containing 50 mM TRIS-HCl (pH 8.0), 50 mM boric acid, 1 mM EDTA. The running time was optimized for each exon. Finally, the gel was dried and exposed on X-ray film (Kodak, BioMax MR).

Statistics

Distant recurrence-free survival time and local recurrence-free interval were calculated as the number of years from the date of randomization until the date of distant recurrence or death from breast cancer, respectively, or the date of local recurrence. The relative rates of distant and local recurrence were estimated using Cox's proportional hazards model. Relationships between grouped variables were tested by means of χ^2 tests for contingency tables. The survival curves were made as described by Kaplan–Meier (25) and computed for the total follow-up period. The follow-up period used was the total follow-up period if nothing else is stated in the text. A p-value less than or equal to 0.05 was regarded as statistically significant. Confidence intervals (CI) were within 95%.

RESULTS

Immunohistochemistry and mutational analysis

Fifty-four patients (20%) had clear nuclear staining in at least 1% of the tumor cells and were scored as positive for p53 protein accumulation. The negative controls were all negative.

One hundred and ninety-one patients did not show any mobility shift of any of the 4 exons investigated in this study. Seventy-five patients exhibited mobility shifts in the SSCP analysis for at least one of the exons. Forty-four cases exhibited mutation when sequenced whereas 28 were normal, including four patients with a polymorphism in codon 213, exon 6. Twenty-eight showed point mutations of which 23 were missense mutations and 5 nonsense mutations. Fifteen of the missense mutations were within the L2–L3 region. Twelve tumors had a deletion or an insertion; one of these was in frame, the other led to frame shifts. Four tumors showed mutations at splicing sites.

Table 2 describes the p53 protein accumulation vs. the mutation pattern. The 4 cases with mutations affecting splicing regions were all negative for protein accumulation as were cases with mutations leading to frame shifts. The case exhibiting protein accumulation in the 'insertions/deletions' group was in frame. There was only one case that exhibited positive immunostaining of the nonsense mutations. The missense mutations were to a large extent positive for immunohistochemistry, 83%. Sixty-one percent of the tumors with p53 accumulation were negative for mutation.

p53 as a predictive factor was best shown as p53 alteration defined as protein accumulation and/or gene mutation. Further on, we have therefore focused on p53 alteration.

p53 alteration in relation to lymph node status, tumor size, and estrogen receptor status

p53 alteration was significantly correlated to a negative estrogen receptor status ($p < 0.0001$) and tumor size larger than 20 mm ($p = 0.004$). The patients were randomized and there was no significant correlation between p53 status and given adjuvant treatment.

p53 alteration in relation to distant and local recurrence

After 5 years follow-up those with p53 alteration exhibited a significantly increased rate of distant recurrence (RR = 1.9, CI = 1.2–2.9) (Fig. 1). The same was true when regarding protein accumulation (RR = 1.6, CI = 1.02–2.6) and there was a tendency in the same direction for gene mutation (RR = 1.3, CI = 0.77–2.2). These associations were weakened or lost after the total follow-up period. A stronger prognostic value of p53 alteration was seen when analyzing the subgroup of patients that had not received chemotherapy. After 5 years' follow-up the relative rate of distant recurrence for this group was 2.7 (CI = 1.4–5.1). p53 alteration was an independent prognostic indicator of distant recurrence, as were the number of positive nodes and tumor size exceeding 20 mm. The multivariate analysis further showed that treatment with tamoxifen significantly decreased the rate of distant recurrence (Table 3).

Table 2

Immunohistochemistry and p53 protein accumulation vs. gene mutation exons 5–8

Gene mutation	Immunohistochemistry		Row total
	p53 − (n)	p53 + (n)	
Normal	189	33	222
Missense	4	19	23
Nonsense	4	1	5
Insertions/deletions	11	1	12
Splicing	4	0	4
Totals	212	54	266

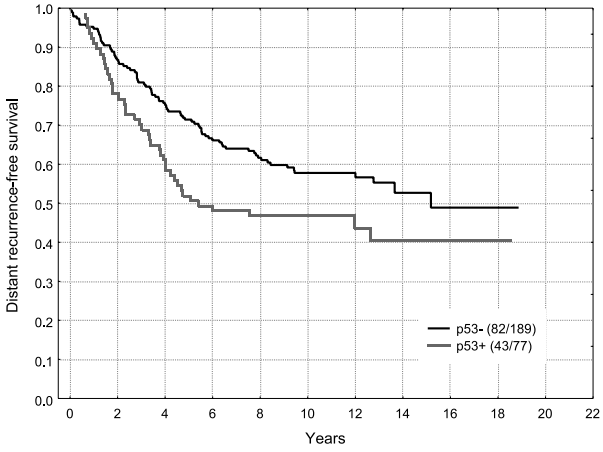

Fig. 1. Distant recurrence-free survival related to p53 alteration. p53 − : lacking both protein accumulation and gene mutation, p53 + : immunoreacitvity for nuclear PAb 1801 and/or mutation within exons 5–8 of the p53 gene. Number of distant recurrences and number of patients within the different groups are presented within parentheses.

The strongest association between p53 status and local recurrence was seen in the group randomized to radiotherapy with p53 alteration showing increased rate of recurrence (RR = 3.4, CI = 1.05–11) (Fig. 2a). This association was not significant when analyzing all patients (RR = 1.3, CI = 0.70–2.4). In multivariate analysis of local recurrence, number of positive nodes and tumor size were independent prognostic factors. Postoperative radiotherapy compared with chemotherapy significantly decreased the rate of local recurrence (Table 4).

Patterns of p53 status and response to adjuvant treatment of radiotherapy, chemotherapy and tamoxifen

Considering distant recurrence during the first 5 years of follow-up, patients with p53 alteration tended to benefit from CMF compared with radiotherapy (RR = 0.72, CI = 0.32–1.6), whereas those without p53 alteration showed a trend towards a decreased benefit (RR = 1.4, CI = 0.78–2.4) (Fig. 2b). The test for interaction between p53 alteration and response to treatment was not significant ($p = 0.49$). There was a significant benefit from radiotherapy in the group with normal p53 status concerning local recurrence (RR = 0.24, CI = 0.083–0.62). This benefit was not seen in the group with altered p53. A test for interaction showed a borderline significance for p53 alteration, $p = 0.051$ (Table 5, Fig. 2a).

The treatment effect of tamoxifen was evaluated in the subgroup of patients with positive estrogen receptor status. Tamoxifen significantly reduced the rate of distant recurrence and there was a non-significant rate reduction of local recurrence (RR = 0.61, CI = 0.39–0.95 and RR = 0.67, 0.32–1.4). Both in the groups having altered p53 and in the group lacking any p53 alteration there was a non-significant benefit from tamoxifen treatment with reduced

Table 3

p53 alteration, nodal status, tumor size, estrogen receptor status, and adjuvant therapy in relation to distant recurrence-free survival—multivariate analysis according to the Cox model

	n	Multivariate analysis		
		Rate ratio	95% CI	p-value
p53 alteration				
Negative	189	1.0		
Positive	77	1.5	1.03–2.3	0.037
Number of positive nodes[1]				
0	31	1.0		
1–3	149	2.1	1.5–2.8	
>3	75	4.4	3.2–5.9	<0.0001*
Tumor size[2]				
≤20 mm	112	1.0		
>20 mm	151	1.6	1.07–2.3	0.021
Estrogen receptor status[3]				
Negative	78	1.0		
Positive	185	0.88	0.58–1.3	0.54
Adjuvant treatment				
Radiotherapy	114	1.0		
Chemotherapy (CMF)	152	0.99	0.68–1.4	0.95
Endocrine therapy				
None	134	1.0		
Tamoxifen	132	0.63	0.43–0.93	0.020

Missing data [1] 11 cases, [2] 3 cases and [3] 3 cases. * Test for trend. Categories were coded 0, 1, and 2.

rate of distant recurrence (RR = 0.51, CI = 0.21–1.3 and RR = 0.65, CI = 0.39–1.08) (Table 6, Fig. 2c). The same was true when analyzing p53 status in relation to local recurrence (RR = 0.69, CI = 0.30–1.6 and RR = 0.41, CI = 0.079–2.1). Similar rate reductions with tamoxifen were seen when protein accumulation and gene mutation were analyzed separately.

DISCUSSION

This study describes p53 alterations in postmenopausal patients diagnosed with early breast cancer. The prognostic and the predictive value of p53 were evaluated in relation to local and distant recurrence. The patients were randomized to adjuvant chemotherapy with CMF versus postoperative radiotherapy. All patients were also randomized to treatment with tamoxifen versus no adjuvant endocrine treatment. The study confirms previous studies that have reported altered p53 status as a poor prognostic factor as reviewed by Elledge and Allred (15).

It could be argued that we have chosen to report on p53 alteration defined as p53 protein accumulation and/or p53 gene mutation in order to strengthen the results. However, we believe that analysis at both the protein and the gene level gives further information and might better reveal impaired p53 function than the two methods separately. Our results show that the type of mutation can largely explain the discrepancy between the presence of gene mutations and negative immunohistochemistry, where mutations leading to shift in the reading frame more often would lead to lack of protein. The relatively large group with positive immunohistochemistry but negative gene mutation could partly be explained by mutations outside exons 5–8. However Sjögren et al. (26) showed that the majority of the mutations outside exons 5–8 consisted of deletions, insertions, or stop codons, which were mainly negative for immunohistochemistry. There could also be false negatives in the SSCP analysis. However, we do not believe this to be the primary explanation since the SSCP was run according to a carefully monitored protocol and all the shifts, including those that could be suspected, were sequenced. Binding to other proteins, such as the MDM2 protein, might be a possible explanation (27, 28). Increased production of wild type p53 has been seen, as a response to different DNA damaging agents (29–31), but this does not explain the increase in preoperatively untreated patients. However, it cannot be excluded that other stress factors could induce accumulation of the wild type protein. A group of patients with p53 protein accumulation but no detectable mutation within exons 5–8 has also been reported by Gretarsdottir et al. (32). Others authors (33, 34) have reported a significant prognostic value of mutations in exons 5–8 while Chevillard et al. (7) did not find any prognostic significance of p53 mutations. There could be different explanations as to why there is not such a strong correlation in the present study. The patients have

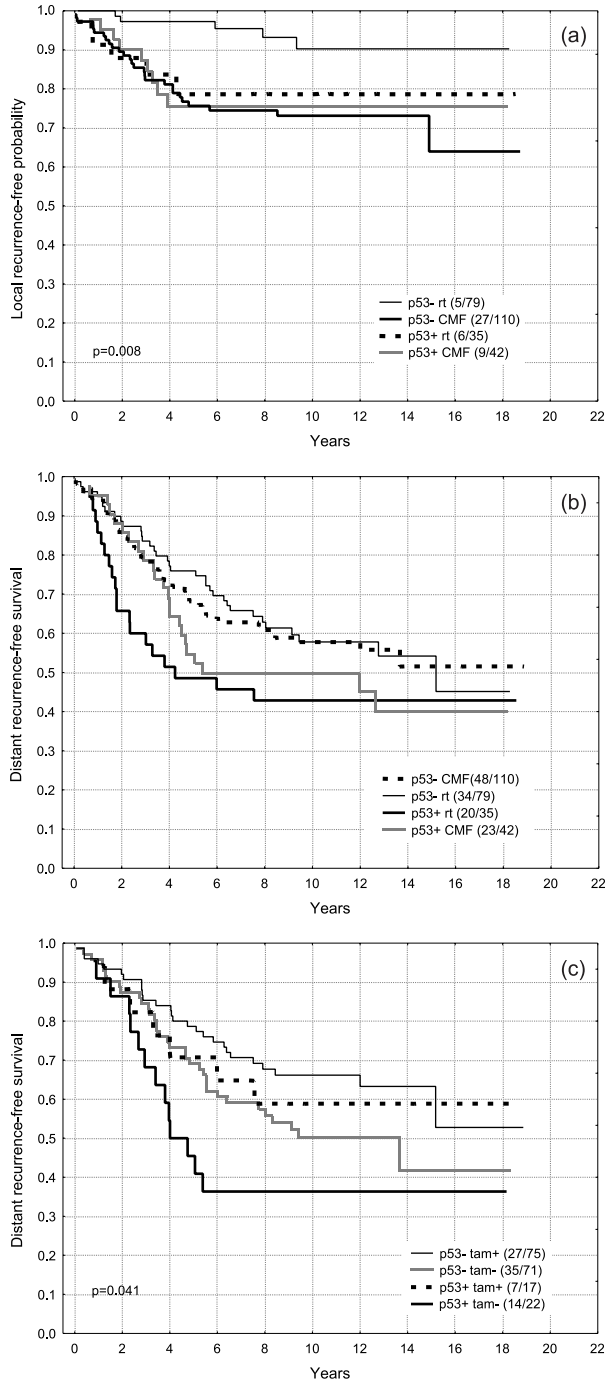

Fig. 2. (A) p53 status in relation to local recurrence-free probability with chemotherapy (CMF) and radiotherapy (RT). (B) p53 status in relation to distant recurrence-free survival with CMF and RT. (C) p53 status in relation to distant recurrence-free survival with (tam+) and without (tam−) tamoxifen. p53−, lack of p53 alteration and p53+, presence of p53 alteration. The number of distant recurrences and number of patients within the different groups are presented within parentheses.

undergone adjuvant treatment where cytotoxic and hormonal treatment interferes with the natural cause of the disease. The impact of p53 was increased when the analysis was restricted to the subgroup that received radiotherapy. p53 accumulation has been reported to be a significant prognostic factor for local recurrence in breast cancer (20, 22, 23). The result in the present study showed an increased rate of local recurrence for patients who had a tumor with an altered p53 status in the group that received radiotherapy.

Based on cell line experiments and the knowledge of cytotoxic agents' ability to induce apoptosis, it has been proposed that loss of p53 indicate resistance to treatment (35, 36). However, other experiments showed the existence of a p53 independent way to apoptosis (37) and at the same time we reported on results that suggested a benefit of chemotherapy with CMF among premenopausal breast cancer patients with accumulated p53, randomized to adjuvant chemotherapy or postoperative radiotherapy (11). This study was small but, nevertheless, we thought these results were promising and motivated further studies. Although not significant, the benefit from CMF that was seen in the present study is in line with our previous study. The weaker correlation could be due to the fact that the postmenopausal woman has an overall lesser benefit from cytotoxic treatment than the premenopausal. Dublin et al. (12) reported a significant benefit from CMF chemotherapy among breast cancer patients in both the p53 positive and negative group. The controversy in the literature about which role p53 may play as a predictive factor has to a large extent been a question of methodology, and less a question of the action of different drugs. Encouragingly enough, there have been studies showing that the outcome of cytotoxic treatment in relation to p53 seems more to be due to the mechanisms of the drug and its specific cell cycle interactions. Kandioler-Eckersberger et al. (38) have reported a benefit of neoadjuvant paclitaxel compared with FEC that was related to both p53 gene mutation and positive immunohistochemistry. As discussed by Brown and Wouters (39), one problem has also been that conclusions from cell experiments have been extrapolated to the clinical situation. The use of short-term assays to measure cell killing can underestimate overall cell killing among cells lacking wild type p53, which can also falsely indicate lack of an apoptotic response as poor response to cytotoxic treatment.

Almasan et al. (40) described that irradiated cells underwent cell cycle arrest in G1 when both the p53 and retinoblastoma (RB) proteins were intact, but underwent apoptosis in the presence of normal p53 and lack of RB protein. This can give one clue as to why p53-altered tumors may be either sensitive or resistant to cytotoxic agents. More recently, the p53-related protein p73 was shown to play a role in apoptosis induced by certain agents. In the absence of p53, apoptosis can be induced by p73 after activation by E2F1 and the non-receptor tyrosine kinase c-abl (41, 42). Because E2F1 is released when the cell enters

Table 4

p53 alteration, nodal status, tumor size, estrogen receptor status, and adjuvant therapy in relation to local recurrence-free survival—multivariate analysis according to the Cox model

	n	Multivariate analysis		
		Rate ratio	95% CI	p-value
p53 alteration:				
Negative	189	1.0		
Positive	77	1.1	0.57–2.1	0.78
Number of positive nodes:				
0	31	1.0		
1–3	149	2.1	1.3–3.4	
>3	75	4.4	2.7–7.2	0.001*
Tumor size:				
≤20 mm	112	1.0		
>20 mm	151	2.1	1.04–4.1	0.037
Estrogen receptor status:				
Negative	78	1.0		
Positive	185	0.80	0.42–1.5	0.50
Adjuvant treatment:				
Radiotherapy	114	1.0		
Chemotherapy (CMF)	152	2.4	1.2–5.0	0.014
Endocrine therapy:				
None	134	1.0		
Tamoxifen	132	0.70	0.38–1.3	0.26

* Test for trend. Categories were coded 0, 1, and 2.

the S phase, it is possible that the relative importance of the p73 pathway is greatest for agents that exert their effect during this phase.

Some immunohistochemical studies have indicated that p53 accumulation does not predict a survival benefit with chemotherapy (1, 9, 10). In the study by Elledge et al. (1), the patients were randomly selected to adjuvant chemotherapy or no systemic treatment. The results did not show any predictive value of p53 accumulation for adjuvant chemotherapy with CMF + prednisone, nor did p53 status have any prognostic value. In one of the studies the patients were not randomized and thus it was not really conclusive (10). In the study by Clahsen et al. (9), which was randomized, either no chemotherapy or one course of FAC was given perioperatively. Bottini et al. (6) reported that patients with accumulated p53 had a better response to neoadjuvant CMF +/− tamoxifen compared with epirubicine, though patients lacking p53 accumulation had the best response to neoadjuvant CMF +/− tamoxifen.

In this study missense mutations within the L2–L3 region showed a poorer prognosis (data not shown). Geisler et al. (43) have also reported that mutations involving the L2–L3

Table 5

p53 status as a predictive factor of postoperative radiotherapy vs. adjuvant CMF chemotherapy in relation to local recurrence

	Local recurrence				
	RT	CMF	RR[1]	95%CI	p-value
p53 alteration					
Negative	79(5)	110(27)	0.24	0.083–0.62	0.0030
Positive	35(6)	42(9)	0.95	0.34–2.70	0.92
p53 accumulation					
Negative	87(7)	125(31)	0.33	0.13–0.67	0.0040
Positive	27(4)	27(5)	0.96	0.13–7.14	0.96
p53 mutation exons 5–8					
Normal	96(8)	126(32)	0.31	0.14–0.68	0.0034
Mutated	18(3)	26(4)	1.25	0.28–5.56	0.77

Number of local recurrences is shown within parentheses.
[1] Relative recurrence rate comparing the two treatment groups.

Table 6

p53 status as a predictive factor of adjuvant treatment with tamoxifen vs. without tamoxifen in relation to distant recurrence

	Distant recurrence, ER-positive patients				
	Tamoxifen	Non-tamoxifen	RR[1]	95%CI	p-value
p53 alteration:					
Negative	75(27)	71(35)	0.65	0.39–1.08	0.094
Positive	17(7)	22(14)	0.51	0.21–1.28	0.15
p53 accumulation:					
Negative	82(29)	78(39)	0.62	0.38–1.01	0.054
Positive	10(5)	15(10)	0.61	0.21–1.80	0.37
p53 mutation exons 5–8:					
Normal	81(30)	81(42)	0.63	0.39–1.01	0.054
Mutated	11(4)	12(7)	0.43	0.12–1.47	0.18

Number of distant recurrences is shown within parentheses.
[1] Relative recurrence rate comparing the two treatment groups.

were associated with lack of response to doxorubicin. It would have been interesting to investigate different p53 alteration patterns such as positivity for immunohistochemistry alone and gene mutation alone in addition to different types and locations of gene mutation, which may influence and play a role in the prediction of treatment. However, such analyses were not possible due to very small subgroups.

The systemic effect of radiotherapy with decreased risk of distant recurrence has been reported by Arriagada et al. (44) and Overgaard et al. (45). This effect may be different in p53 positive and negative groups. As we lack one arm without any adjuvant treatment, we can only relate the chemotherapy effect to that of radiotherapy. Jansson et al. (19) reported a benefit from radiotherapy of tumors with mutated p53 when evaluating survival data. However, the material was not fully randomized. Similarly Silvestrini et al. (18) have reported a significantly decreased risk of local recurrence after adjuvant radiotherapy of tumors positive for protein accumulation, which is in contrast with our results where both lack of protein accumulation and absence of gene mutation indicated fewer local recurrences among patients receiving radiotherapy. We suggest that an unaltered p53 status is an important factor for the response to irradiation induced DNA damage and that the mechanisms of response to radiotherapy and CMF, respectively, are differently dependent on p53 status. A normal p53 status and a good response to radiotherapy is also in line with the findings by Adell et al. (46) from a study of rectal cancer. Degeorges et al. (20) also reported an increased rate of local recurrence in the group with p53 accumulation where the patients had received local radiotherapy.

Studies on p53 as a predictor of tamoxifen treatment have shown the response to be the same in groups with or without p53 accumulation (13, 16, 17, 47). However, it has also been concluded that p53 mutation or accumulation predicts poor response to tamoxifen (14, 48). The results in the present study demonstrated that the patients with both normal and altered p53 status appear to have a good response to tamoxifen. As far as we know, the present study is the only one that has analyzed p53 mutations in relation to tamoxifen in randomized material. However, when restricting the analyses to ER positive cases the statistical power becomes relatively low.

We conclude from this study that p53 alteration is a prognostic factor in early postmenopausal breast cancer being associated with an increased risk of distant recurrence. We also conclude that p53 alteration is associated with an increased risk of local recurrence following radiotherapy. We suggest that p53 alteration indicates benefit from CMF compared with radiotherapy regarding distant recurrence-free survival and that the best local control with radiotherapy is achieved in the absence of p53 alteration. Finally, altered p53 status is probably not a marker of resistance to tamoxifen.

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