

Feasibility of Breast Conservation after Neoadjuvant Chemotherapy in 58 Patients with Locally Advanced Breast Cancer Using p53 and MDR1 Genes as Predictors of Response

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ABSTRACT

Purpose: The aim of this prospective trial was to evaluate the feasibility and outcome of breast conservation therapy for patients with locally advanced breast cancer after neoadjuvant chemotherapy. We studied also the value of p53 and MDR1 as predictors to neoadjuvant chemotherapy.

Patients and methods: Between August 1997 and May 2000, 58 patients with locally advanced breast carcinoma (stages IIIA and IIIB) completed treatment consisting of 5-fluorouracil 600 mg/m², epirubicin 60 mg/m² and cyclophosphamide 600 mg/m² (FEC) administered intravenously, at intervals of 3 weeks. The number of cycles of chemotherapy given depended on the clinical tumor response. Surgery (local excision if sufficiently downstaged, or mastectomy if not, both with axillary dissection) was performed. Surgery was followed by radiation therapy and 4 more cycles of FEC chemotherapy as an adjuvant therapy. P53 and MDR1 were assessed in the initial tissue biopsy by means of reverse transcriptase-mediated polymerase chain reaction (RT-PCR). p53 and MDR1 findings were correlated with treatment response and linkage between p53 function and cellular response was assessed by terminal deoxynucleotidyl transferase-mediated nick end labeling assay.

Results: The overall response (CR+PR) was 87.9%, with a clinical complete response rate of 29.3%. Six patients had a pathological complete response and 10 patients had only minimal residual disease. Median follow-up from the start of chemotherapy was 24 months (range 6 to 40). Twenty two patients (43%) underwent BCS with actuarial 3-year disease-free and overall survival of 58% and 75% respectively. Cosmetic results were good to excellent in 77.2% of the patients. Modified radical mastectomy was done in 29/51 (56.9%) patients with actuarial 3-year disease-free and overall survival of 60% and 78% respectively. In 13 out of 58 patients (22.4%), p53 mutations could be identified, including eight point mutations, three

minor deletions and two complex deletions. Mutations were located in exons 4,6,7,8 and 10 of the p53 gene, including two mutations in the intron region affecting the splice sites. The seven non-responders showed p53 mutations while 6/51 responding patients had p53 mutations. Treatment failure was related to the presence of p53 gene mutations ($p = 0.0029$). Presence of apoptosis was related to a normal p53 status and treatment response ($p < 0.0001$). In patients responding to FEC, the mean percentage of apoptotic cells was seven. Of 7 patients with treatment failure, 5 had 0% and two patients had 1% apoptotic cells. Twelve patients showed the specific band corresponding to the MDR1 mRNA. All patients with no response to neoadjuvant chemotherapy had MDR1 gene expression. MDR1 expression was significantly correlated with resistance to neoadjuvant chemotherapy ($p = 0.0026$). The remaining five patients with MDR1 expression had (PR) to neoadjuvant chemotherapy and also had p53 mutations.

Conclusion: In conclusion, the results of the present study compare favorably with previous studies in patients with locally advanced breast cancer (LABC). Our results suggest that breast conservation was feasible and safe for patients with LABC, with careful selection based on response to chemotherapy. We have demonstrated that p53 plays a distinct drug-specific role in chemoresistance. The response to a combination of FEC was directly related to normal p53 and tumor cell apoptosis in breast cancer patients. These results provide clinical evidence of a p53-dependent cytotoxic effect of these DNA-damaging agents. It seems that resistance to chemotherapy is a multifactorial phenomenon, in which many genes are involved.

Key Words: Locally advanced breast cancer - Neoadjuvant chemotherapy - p53 and MDR1.

INTRODUCTION

Patients with locally advanced breast cancer (LABC) have poor prognosis when treated with

surgery and/or radiotherapy [4,6,10]. These tumors include stage IIIA and IIIB breast cancer according to the American Joint Committee of Cancer (AJCC) [13]. As a consequence of these poor results, neoadjuvant chemotherapy has been introduced. The principal goals of neoadjuvant chemotherapy in breast cancer are to improve local control and to improve survival [19]. Neoadjuvant chemotherapy offers several additional advantages: inoperable tumors or tumors that are too large to be removed by lumpectomy can be effectively downstaged by use of neoadjuvant chemotherapy. A decrease in the extent of surgery leads to improved cosmetic results and the possibility of a reduction in local recurrences [29]. Another advantage of neoadjuvant therapy is the *in vivo* assessment of tumor sensitivity to chemotherapy, which allows optimization of available therapeutic agents [36]. The main disadvantages of neoadjuvant chemotherapy are: preoperative treatment causes loss of information regarding lymph node status prior to systemic treatment and delays surgery which is the main treatment. Although lymph node status is the most important prognostic indicator for patients with early-stage breast cancer, patients with tumors larger than 1 cm are currently offered postoperative systemic therapy regardless of lymph node involvement [25,36]. Furthermore, regional metastatic disease that involves axillary lymph nodes retains prognostic information after neoadjuvant chemotherapy [25,28,44]. Several studies have been performed; using three to four cycles of neoadjuvant chemotherapy prior to surgery and/or radiotherapy, often followed by adjuvant chemotherapy. The reported clinical response rate to neoadjuvant chemotherapy varies between 30% and 90%, with 10%-35% clinical complete remissions. The five-year overall survival (OS) rate is reported to range between 40% and 60% [4,6,12,13,15], while one study showed a five-year survival of 80% in patients with stage IIIA disease [24].

The biological functions of the p53 tumor suppressor gene make it a potential predictive marker. p53 has been observed to function as a transcription factor that regulates normal cell growth by controlling genes that promote progression through the cycle and by controlling those that cause arrest in G₁, when the genome is damaged [19,32]. Active p53 can further promote apoptosis in growth-arrested cells and has been related to the efficient execution of pro-

grammed cell death in response to DNA damage in the presence of oncogenic triggers [2,45]. Intact p53 has been shown to induce apoptosis in response to ionizing radiation, whereas loss of p53 function has been reported to enhance cellular resistance to a number of chemotherapeutic agents [17,26,27]. Because p53 has been recognized to influence response to chemotherapy, efforts have been undertaken to study its usefulness as a predictive factor in LABC patients [17,22,30]. The combination therapy of FEC is widely used in the treatment of breast cancer [22,24]. Alkylating substances (cyclophosphamide) as well as anthracyclines (epirubicin) induce DNA damage by cross-linking DNA strands. This results in apoptosis due to p53 activation [9,11,28,43]. Because cancer-related death in breast cancer patients is ultimately caused by distant metastasis in most cases, it seems that P-glycoprotein expression could indicate the presence of multidrug-resistant clones, from which tumor recurrence and dissemination might arise [7,8,40]. However, expression of the gene encoding multidrug resistance associated protein (MDR1) is not the sole factor responsible for multidrug resistance, either in breast cancer or in any other tumor [33,42]. If anything is clear at present about multidrug resistance, it is the fact that its possible causes are manifold and increasing with every new resistance-associated gene studied [32,42]. Therefore, the difficulty instead lies in which of the genes to investigate and for which reasons, because the potential candidates are many. We have centered the present study on P-glycoprotein because in part anthracyclines are the main chemotherapeutic agents in most combinations used when treating breast cancer patients with neoadjuvant therapy and on the other part, because we used epirubicin in the present study. P-glycoprotein (MDR1)-associated resistance is the paradigm of anthracycline-mediated resistance, at least under experimental conditions [26,33]. We have studied the expression of MDR1 before neoadjuvant chemotherapy in the present series of patients. The results have been correlated with the response to treatment. So, the aim of this prospective trial was to evaluate the outcome and the possibility of breast conservation therapy for patients with locally advanced breast cancer after neoadjuvant chemotherapy and to study the value of p53 and MDR1 for predicting response to neoadjuvant chemotherapy.

PATIENTS AND METHODS

Fifty-eight patients with locally advanced breast carcinoma were enrolled in the study between August 1997 and May 2000.

Eligibility criteria:

- 1- Histopathologically proven breast carcinoma.
- 2- Patients with newly diagnosed clinical stage IIIA and IIIB disease according to the AJCC guidelines.
- 3- Normal cardiac function, with a left ejection fraction of at least 60% measured by echocardiography.
- 4- Adequate hematologic, renal and hepatic function.

The initial clinical evaluation consisted of a careful history and physical examination for all patients before enrollment. Baseline studies included complete blood count, routine chemistry, electrocardiogram, lipid profile, chest X-ray, bone scan, liver ultrasound or C.T., bilateral mammography and left ventricular ejection fraction (LVEF) by echocardiography were performed before starting treatment. All cases were diagnosed by means of a core biopsy, at which time hormone receptors, mutant p53 expression and MDR1 were determined. We studied the expression of the genes encoding multi-drug resistance associated protein (MDR1) and P53 in tumor specimens from 58 patients before neoadjuvant chemotherapy. P-glycoprotein expression and P53 were assessed by means of reverse-transcriptase-mediated polymerase chain reaction (RT-PCR).

To monitor for cardiotoxicity, we evaluated LVEF every three cycles and after the end of treatment. Blood counts and chemistry were performed at the beginning of each 21-day cycle of treatment.

Determination of ER and PR:

Estrogen receptors (ER) and progesterone receptor (PR) were estimated by the ligand binding assay technique [5]. A concentration \geq 10 fmol ER/mg protein was considered positive and a value lower than 10 fmol ER/mg protein was considered negative.

Assessment of P53 and MDR1 genes:

300 μ l of RNA extraction solution (RNA-

zole. Cinna Biotic Inc., Houston) were pipeted in polypropylene microcentrifuge tubes placed in ice wet. In case of paraffin-embedded tumor tissue, three or four 10 μ m paraffin sections, corresponding to about 10 mg of tissue, were trimmed of excess paraffin and placed in 1.5 ml Eppendorf tubes with 1 ml of xylene, vortex-mixed and centrifuged for 5 min at room temperature or 5x2x2 mm frozen sections of tumor specimens were added to each tube and squeezed against tube walls. 500 μ l of RNazole and 80 μ l chloroform and isoamyle alcohol mixture (24:1) were added to each tube, mixed for 10 seconds by vortex and placed in wet ice for 5 minutes then centrifuged for 10 min at 14000g. 350 μ l aqueous upper phase were transferred to a new tube containing isopropanol. Mixed by vortex and placed on wet ice for 30 minutes and then centrifuged at 14000g for 20 minutes. The supernatant was discarded and pelleted RNA was washed twice with 70% ethanol, dried and finally dissolved in 50 μ l of diethyl pyrocarbonate-treated water and 1 μ l of RNA guard. The RNA samples were heat denatured at 90°C for 3 minutes, followed by chilling on wet ice for 3 minutes. To prepare cDNA reaction mixture, 25 μ l of a given RNA sample were transferred to a microcentrifuge tube containing 10 μ l of moloney murine leukemia virus reverse transcriptase, 25 μ l of RNA guard, 37.5 μ l of cDNA mix 90 nM Tris-HCl (pH 8.3), 138 mM KCl, 18 mM MgCl₂, 30 mM DTT (dithiothreitol), 3.6 mM of each of dCTP, dATP, dTTP and dITP, 0.9 mM dGTP and 0.152 A₂₆₀ U of pd (N) random primers (approximately 2.5 pmol of primers) to yield a final volume of 75 μ l. The cDNA reaction mixture was incubated at 37°C for 1 hour and the reaction products were heat denatured at 90°C for 3 minutes and stored at -20°C. PCR and determination of p53 mRNA cDNA and MDR1 mRNA cDNA were performed according to Sjogren et al. [38] and Chevillard et al. [7] respectively.

Assessment of cellular response: Apoptosis assay (TUNEL):

The immunohistochemical detection of apoptosis in paraffin-embedded tumor tissue sections was performed by TUNEL staining (Boehringer Mannheim, Mannheim, Germany). Terminal deoxynucleotidyl transferase labeling with fluorescein-dUTP was performed according to the manufacturer's recommendations. As negative control, all samples were incubated

with a nucleotide mixture lacking terminal transferase. The tissue sections were counterstained with DAPI (4',6-diamidino-2'-phenylindole dihydrochloride; Boehringer Mannheim). Cells carrying fluorescent signals were counted and the percentages were calculated.

Treatment schedule:

- Phase I: Neoadjuvant chemotherapy:

Neoadjuvant chemotherapy consisted of 5-fluorouracil 600 mg/m², epirubicin 60 mg/m² and cyclophosphamide 600 mg/m² (FEC) administered intravenously, every 3 weeks. Chemotherapy was given until either complete response (CR), with no palpable tumor, was achieved or until the maximum response had been achieved (no change in tumor size for two consecutive treatment cycles). The median number of neoadjuvant chemotherapy courses given was 4 courses (range 3-5). Clinical objective response was assessed and categorized according to WHO criteria [35], after each cycle by physical examination when appropriate and after the third cycle by imaging techniques. Complete response (CR) was defined as complete disappearance of all lesions for at least 4 weeks; partial response (PR) was defined as a reduction of greater than or equal to 50% in the sum of the products of all measurable lesions; stable disease (SD) defined as less than 25% reduction in measurable lesions and with no new lesions. Any increase in measurable lesions greater than 25% and/or appearance of new lesions was defined as progressive disease (PD).

- Phase II: Loco-regional therapy:

After neoadjuvant chemotherapy, three different loco-regional therapeutic approaches were proposed depending on the clinical, mammographic and sonographic response status, as well as on the site of the tumor, multicentricity and on the volume of the breast compared to the residual tumor. Local treatment was planned as follows:

- 1- When the residual tumor size was greater than 3 cm (largest diameter) or it had a retro-areolar localization, or when initial tumor was multifocal. Patey mastectomy and axillary dissection was performed followed by radiation therapy.
- 2- When the residual tumor was less than or equal to 3 cm in its largest diameter, conser-

vative surgery with a wide excision and axillary dissection limited to level I and II lymph nodes was carried out, followed by radiation therapy.

- 3- When the primary tumor and palpable nodes had disappeared, patients underwent open biopsy of the sites of previous disease, including axillary dissection. Histologically confirmed complete responders were treated with radiation therapy. Pathologically partial responders underwent re-excision of the site of primary lesion followed by radiation therapy.

The pathological response in the surgically resected specimen was based on detailed microscopic examination of multiple sections from the breast and axillary lymph nodes. No residual tumor in the mastectomy specimen and axillary lymph nodes was defined as a pathological complete response. When only tumor was present at microscopic examination the response was graded as minimal residual disease. And when tumor was visible at macroscopic evaluation of the mastectomy specimen and axillary lymph nodes, the pathological response was graded as macroscopic disease [41].

Radiotherapy:

Radiation therapy started within 2 weeks after surgery. A dose of 50 Gy in 25 fractions over 5 weeks was delivered to the breast/chest wall with medial and lateral tangential fields, using wedges to correct for inhomogeneity by ⁶⁰Co or 6-MV photons, followed by an electron beam boost of 16 to 20 Gy, varying from 8 MeV to 15 MeV, according to the depth from skin to base of wound cavity on ultrasonography for patients treated with breast preservation. Patients with four or more histologically positive axillary lymph nodes underwent irradiation to the supraclavicular area (45 Gy) and internal mammary nodes (45 Gy). The supraclavicular nodes were treated with an anterior field. The internal mammary nodes were irradiated by a direct anterior photon field.

- Phase III: Adjuvant chemotherapy:

After locoregional treatment, all patients received 4 more courses of FEC chemotherapy at three weeks interval as in neoadjuvant therapy. Tamoxifen, 20 mg daily was given after chemotherapy if estrogen (ER) or progesterone (PR) receptors were positive.

Patient follow-up:

During the first 2 years after treatment, patients were seen every three months. At every visit physical examination, complete blood counts and liver function tests were done. Any abnormalities in liver function tests were followed by ultrasonography of the liver. A chest X-ray was obtained every 3 months. Mammograms of the contralateral breast and in case of breast conservation therapy, mammograms of the treated breast were obtained every 3 months. Bone scan was performed every 6 months. After 2 years, these studies were performed at yearly intervals.

Statistical analysis:

Data were entered, checked and analyzed using EPI-INFO (version 6.1) software package [23].

RESULTS*Patient characteristics:*

Patient characteristics are listed in table (1). The median patient age was 55 years (range, 26 to 60 years). The average tumor diameter by clinical and mammographic evaluation was 61 mm (range 40-130).

Response to neoadjuvant chemotherapy:

Clinical response to neoadjuvant FEC therapy was observed in 51 patients (87.9%): Seventeen patients (29.3%) achieved a clinical complete response and 34 patients (58.6%) had a clinical partial response. Seven patients had a stable disease (Table 2). Usually, best response was observed after three cycles of neoadjuvant chemotherapy. At pathologic examination of surgical specimens, six patients had a pathologic complete remission (10.3%) and 10 patients (17.2%) had only minimal residual disease. In 35 patients macroscopic tumor was still present (Table 3). Twenty-six of 51, or 51% of the patients were found to be node-negative at surgery (Table 4). No statistically significant association was observed between clinical and pathological response ($p = 0.62$). In 13 of 58 patients (22.4%), P53 mutations could be identified, including eight point mutations, three minor deletions and two complex deletions. Mutations were located in exons 4,6,7,8 and 10 of the p53 gene, including two mutations in the intron region affecting the splice sites (Table 5). 7/7 non-responding patients to neoadjuvant

chemotherapy had p53 mutations while 6 out of 51 responding cases (11.8%) had p53 mutations ($p = 0.0029$). Resistance to FEC was correctly predicted by p53 sequencing in 53.9%. Presence of apoptosis was related to a normal p53 status and treatment response ($p < 0.0001$; Table 6). In patients with response, the mean percentage of apoptotic cells was seven. Of 7 patients with treatment failure, 5 had 0% and two patients had only 1% apoptotic cells (Fig. 1). Of 58 patients, 12 showed the specific band corresponding to the MDR1 mRNA (Fig. 2) and were therefore classified as expressors of the gene. All patients with no response to neoadjuvant chemotherapy (7SDs) had MDR1 gene expression. MDR1 expression was significantly correlated with resistance to neoadjuvant chemotherapy ($p = 0.0026$). The remaining five patients with MDR1 expression had (PR) to neoadjuvant chemotherapy and also had P53 mutations.

Breast-conserving surgery (BCS):

Modified radical mastectomy was performed in 29 patients (56.9%) and breast conservation was feasible in 43.1% (Table 7). Of the Twenty-two patients who underwent breast - conserving surgery, 17 (77.2%) had a cosmetic results judged "excellent" or "good" on the Harris scale [18]. Five patients judged "fair" and none judged "poor". Reasons for mastectomy are given in Table (8).

Patterns of failure:

In terms of first relapse, whatever the follow-up, following conservative surgery ($n = 22$), 7 patients had a relapse (four isolated breast tumor recurrence, one breast tumor relapse with distant metastases and 2 distant metastases). Following modified radical mastectomy ($n = 29$), there were 9 relapses (one isolated local recurrence, 3 local recurrence with distant metastases and 5 distant metastases). The four observed isolated local tumor recurrences following conservative treatment were salvaged by mastectomy (Table 9).

Survival:

The median follow-up period was 24 months (range 6 to 40). Not a single patient progressed during chemotherapy. The disease-free and overall 3-year survival rates were calculated for mastectomy and breast conservation separately (Figs. 3&4). The overall survival at 3-years for

patients with mastectomy and breast conservation were 78% and 75% respectively. The disease-free survival at 3-years for patients with mastectomy was 60% and for patients with

breast conservation was 58%. The differences in overall and disease-free survival between both groups of patients were not statistically significant.

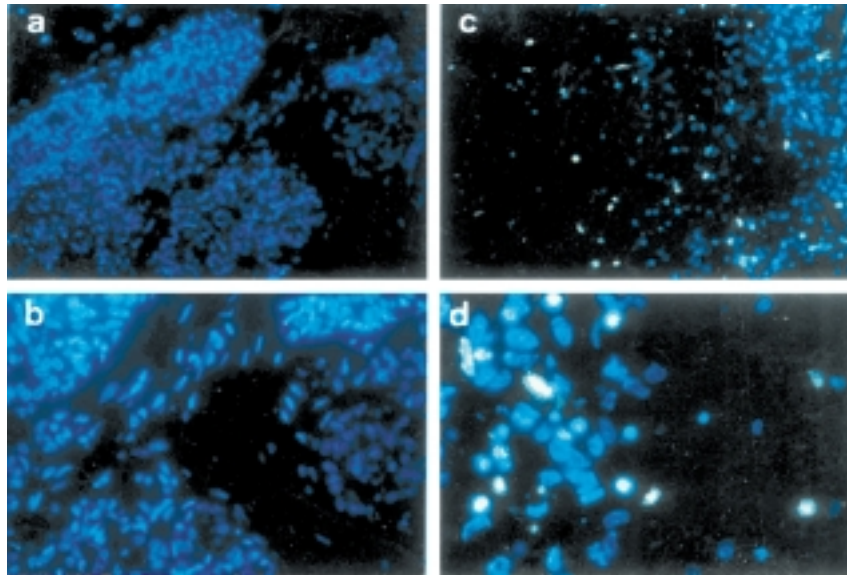


Fig. (1): Detection of apoptosis by TUNEL assay in two breast cancer patients after treatment with FEC. Tumors with clinical response and resistance are compared. Apoptotic cells appear fluorescent green, the counterstain is DAPI. TUNEL assay of the tumor showing 0% apoptotic cells [original magnification, x20 (a) and x40 (b)]. Patient 23 showed no response to chemotherapy and carried a nine-base deletion in the p53 gene. Tumor of patient 9 showing 8% apoptotic cells x20 (c) and x40 (d); patient 9 achieved PR after chemotherapy, the p53 gene of the tumor was normal.

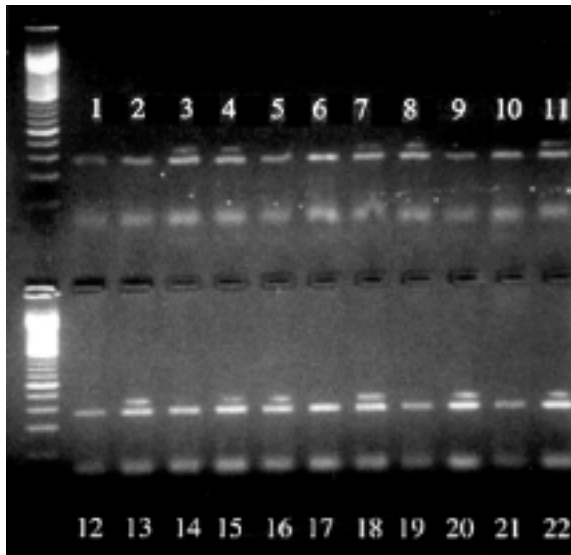


Fig. (2): Ethidium-bromide-stained agarose gel after RT-PCR amplification of MDR1 mRNA. The MDR1-expressing tumors are demonstrated by the presence of a 168 base-pair band, above the β -globin positive control (lanes 3,4,7,8,11,13,15, 16,18,20 and 22).

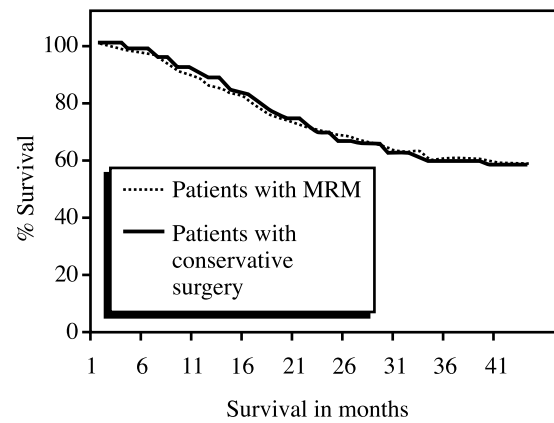


Fig. (3): Disease free survival for patients underwent conservative surgery or modified radical mastectomy.

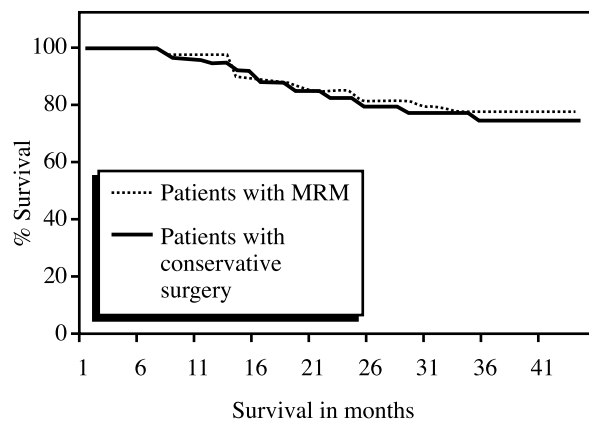


Fig. (4): Overall survival for patients underwent conservative surgery or modified radical mastectomy.

Table (1): Patients characteristics.

Age (years):			
Median	55		
Range	26-60		
Menopausal status:			
Premenopausal	30	51.7%	
Postmenopausal	25	43.1%	
Perimenopausal	3	5.2%	
Hormone receptor status:			
ER-/ PG+	6	10.4%	
ER+/ PG-	18	31%	
ER-/ PG+	8	13.8%	
ER+/ PG+	26	44.8%	
Clinical stage:			
IIIA	40	69%	
IIIB	18	31%	
Clinical nodal status:			
N0	10	17.2%	
N1	20	34.5%	
N2	28	48.3%	

Table (2): Clinical response to neoadjuvant chemotherapy.

	Tumor		Nodes	
	No.	%	No.	%
CR	17	29.3	17	29.3
PR	34	58.6	34	58.6
SD	7	12.1	7	12.1
Total	58	100	58	100

Table (3): Relation between clinical and pathological response of the primary tumors among 51 cases underwent surgery.

Pathological response	Clinical response		Total	
	CR	PR	No.	%
No residual carcinoma	5	1	6	11.8
Microscopic residual	10	-	10	19.6
Macroscopic residual	2	33	35	68.6
Total	17	34	51	100

Table (4): Relation between clinical and pathologic response of axillary nodes among 51 patients underwent surgery.

Pathological response	Clinical response		Total	
	CR	PR	No.	%
Negative	7	19	26	51
1-3 positive	6	9	15	29.4
4-10 positive	3	5	8	15.7
> 10 positive	1	1	2	3.9
Total	17	34	51	100

Table (5): Characterization of TP53 mutations detected in 58 patients with locally advanced breast cancer.

Patient No.	Clinical response	p53 gene mutation	Exon	Codon	Normal sequence	Mutant sequence	AS-change	MDR1
6	SD	T-A transversion	10	338	5' CGC TTC 3'	5' CGC ATC 3'	Phe-Ile	+ve
11	SD	C-A transversion	8	276	5' TGT GCC 3'	5' TGT GAC 3'	Ala-Asp	+ve
12	SD	Deletion	10			Complex	Frameshift	+ve
16	SD	C-T transition	7	248	5' CGG 3'	5' TGG 3'	Arg-Try	+ve
20	SD	C-T transition	8	278	5' GCC TGT CCT 3'	5' GCC TGT CTT 3'	Pro-Leu	+ve
23	SD	9b deletion	7	261-intron	5' TCC AGg tcaggaccactt 3'	5' TCC gccactt 3'	Splicing	+ve
29	SD	2b del	7	260-261	5' GAC TCC AG 3'	5' GAC TCT 3'	Frameshift	+ve
2	PR	1b deletion	4	102	5' ACC TAC 3'	5' ACT ACC...3'	Frameshift	+ve
31	PR	Deletion	10			Complex	Frameshift	-ve
37	PR	g-t transversion	8	307-intron	5' CGA Ggtaac 3'	CGA Gttaac 3'	Splicing	+ve
40	PR	A-G transition	6	214	5' CGA CAT 3'	5' CGA CGT 3'	His-Arg	+ve
45	PR	G-A transition	7	245	5' GGC 3'	5' AGC 3'	Gly-Ser	+ve
53	PR	C-T transition	250	250	5' AGG CCC ATC 3'	5' AGG CTC ATC 3'	Pro-Val	+ve

Table (6): Relation of clinical response to p53 sequencing results and to apoptosis in breast cancer patients with neoadjuvant treatment. Sensitivity and specificity of p53 sequencing, MDR1 expression and apoptosis to detect responders (CR, PR) and nonresponders (SD, PD) is presented and corresponding confidence intervals are added in brackets.

	CR, PR	SD			
<i>p53 sequencing:</i>			<i>p</i> = 0.0029		
Mutant	6	7	Sensitivity	53.9%	(33.8; 69.2)
Normal	45	0	Specificity	100%	(82.6; 100)
<i>Apoptosis:</i>			<i>p</i> < 0.0001		
Positive	50	1	Sensitivity	98%	(89.9; 100)
Negative	1	7	Specificity	87.5%	(70.1; 95)
<i>MDR1 expression:</i>			<i>p</i> = 0.0026		
Expressed	5	0	Sensitivity	10%	(6.2; 26)
Not expressed	46	7	Specificity	86.8%	(60.3; 95.1)

Table (7): Type of surgical procedure.

	No.	%
Breast conservation	22	43
Modified radical mastectomy	29	57
Total	51	100

22 out 58 cases (38%) can keep their breasts.

Table (8): Reasons for mastectomy.

15/29	51.7%	Disease did not regress sufficiently for BCS
6/29	20.7%	Had diffuse disease on presentation
8/29	27.6%	Had gross multicentric disease on presentation

Table (9): Patterns of failure.

	BCS (n = 22)	MRM (n = 29)
local	4	1
Local + distant	1	3
Distant	2	5
Total	7 (31.9%)	9 (31%)

BCS : Breast conservation surgery.
MRM: Modified radical mastectomy.

DISCUSSION

In patients with locally advanced technically operable cancer (IIIA), total or radical mastectomy has been the therapeutic method of choice while with technically inoperable cancer (IIIB), the method of choice was radiotherapy with subsequent mastectomy [28]. This approach was associated with poor 5-year survival rates and it has not been until the advent of primary chemo-

therapy that the outcome has improved [16,28, 29]. There is no doubt that the main problem of locally advanced cancer (in addition to the primary tumor per se) is the presence of the anticipated micrometastases formed months, perhaps years, before presentation [20,21,29]. As a result, systemic chemotherapy combined with locoregional treatment seemed to be the most effective approach [20,21,41]. Neoadjuvant chemotherapy improves operability and allows for breast-sparing operations (lumpectomy, segmentectomy, quadrantectomy), i.e., conservative surgical procedures [20,41]. Another goal of primary chemotherapy is to destroy presumed undetectable micrometastases [20,21], while the ultimate intention, in addition to spare the breast, is to extend the tumor-free interval and overall survival [16,28].

The positive results we achieved in terms of downstaging (87.9% of cases), was comparable with the results reported by other authors [20,21,24]. Despite these promising results, a conservative surgical procedure was possible in only 38% of cases. We obtained a response rate similar to previous studies on neoadjuvant chemotherapy in LABC, where response rates of 60%-80% have been reported, with 10%-30% clinical complete remissions [20,21,24,41]. Pathologic complete response varied from 7% to 20% with the use of conventional neoadjuvant chemotherapy [20,21,24]. In our study six patients (10.3%) had a pathologically complete response and 10 patients (17.2%) had only minimal residual microscopic disease, which makes a total of 27.5% of patients with major tumor reduction.

DNA damage is a well-characterized stimulus of p53-dependent induction of apoptosis

[34,37,46]. We demonstrated a strong relation between p53 status and response to neoadjuvant treatment with a combination of FEC in breast cancer patients. Alkylating substances such as cyclophosphamide, as well as anthracyclines like epirubicin, intercalate with DNA and induce DNA cross-links and strand breaks considered to produce a p53-dependent induction of apoptosis [34]. The third component of the combination therapy, fluorouracil, has been shown to inhibit processing and function of RNA [1,11]. Recently, the depletion of ribonucleotide triphosphate, necessary for RNA synthesis, by fluorouracil has been detected to be a stimulus for p53 induction [3,39]. Hence, the three substances seem to synergistically trigger p53-dependent apoptosis [16,31]. Our results clinically support the assumption that the response to FEC combination therapy is p53 related. In our study, the TP53 mutation rate was 22.4%, which is in accordance with the published frequencies in breast cancer [31,43]. All patients with no response to neoadjuvant chemotherapy (7SDs) had a TP53 mutation. Treatment failure was related to the presence of P53 gene mutations ($p = 0.0029$), also resistance to FEC was correctly predicted by p53 sequencing in 53.9%. In our study the presence of apoptosis was related to a normal p53 status and treatment response ($p < 0.0001$). The role of resistance-associated genes in the development of resistance to chemotherapy in humans is still controversial [42]. Breast cancer is no exception and the MDR1 gene in particular has been extensively studied in connection with it, although no definitive conclusion has been reached [8,25, 42]. Chung et al. [8] addressed the possible significance of the MDR1 gene in breast cancer through a large meta-analysis and cautiously stated in their conclusion that "we found no evidence to support the assumption that MDR1/gp170 expression has no role in breast cancer and while the precise role of MDR1/gp170 in breast cancer remains to be established definitively, it seems likely that, in tumors where expression is detectable, this expression contributes to the multidrug-resistant phenotype". Our present findings seem only to corroborate this statement as all patients with no response to neoadjuvant chemotherapy showed MDR1 gene expression. Finally, our findings cannot be based on P53 and MDR1 alone because the complex mechanism of apoptosis involves transcription of many genes and their products. A

breast conserving treatment was feasible in 38% of patients. A previous study has looked at the feasibility of breast conservation surgery after induction chemotherapy for locally advanced breast cancer. Schwartz et al., retrospectively reviewed patients at their institution who had received neoadjuvant chemotherapy followed by mastectomy [36]. Of the 143 reviewed patients, only 33 (23%) were retrospectively considered potential breast conservation candidates. This 23% compares to 38% of our patients who actually had lumpectomy. The better response to neoadjuvant chemotherapy in our series may be due to less advanced disease in our patients. In addition to downstaging of the primary tumor, nodal downstaging doubtless also occurs. However, because the initial pathologic nodal stage is not known it is impossible to quantify. However, we can estimate the number of patients with pathologically positive nodes at the time of diagnosis. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 trial [14], 75% of clinically node-positive and 40% of clinically node-negative patients were actually pathologically node-positive. Using this algorithm for our patients allows us to estimate that 75% x (48 patients) + 40% x (10 patients) or about 40 patients would have been node-positive had they undergone immediate surgery. Pathological examination of axillary lymph nodes after axillary dissection showed that only 25 patients were node-positive after neoadjuvant chemotherapy suggesting that at least 15/58 (25.9%) were downstaged to pN0. We know of only one published report on cosmesis for locally advanced disease treated with lumpectomy and radiation, that of the first report by Jacquillat et al. [21]. In this study a good to excellent cosmetic result was seen in 73% of patients and fair in 27%, which is consistent with our data although surgical extirpation was not performed in their patients. A compilation of cosmetic outcomes in early breast cancer by Fowble et al. [15] suggests a "good" to "excellent" cosmetic result rate of 69%-90%, which is also similar to our findings. The optimum number and duration of induction chemotherapy remains unknown. Several authors used more than three or four cycles [6,13,20] a combination with five or six drugs [10,11,29] or an increasing drug dose [22,41] to achieve better tumor response rate. In our study the median follow-up is relatively short, but it appears that actuarial 3-year DSF

and OS for patients underwent mastectomy and those with breast conservation were compared favorably to results reported by other authors [20,21,29,35,41]. The 3-year OS for patients with mastectomy and breast conservation were 78% and 75% respectively and DFS was 60% and 58% respectively. The differences in overall and disease-free survival between both groups of patients were not statistically significant.

In conclusion, the results of the present study compared favorably with previous studies in patients with locally advanced breast cancer (LABC). Our results suggest that breast conservation has been shown to be feasible and its results are comparable to mastectomy for patients with LABC, presuming careful selection based on response to chemotherapy. We have demonstrated that p53 plays a distinct drug-specific role in chemoresistance. The response to a combination of FEC was directly related to normal p53 and tumor cell apoptosis in breast cancer patients. These results provide clinical evidence of a p53-dependent cytotoxic effect of these DNA-damaging agents. From our results, finally, it seems evident once more that resistance to chemotherapy is a multifactorial phenomenon, where not just one but many genes are involved.

REFERENCES

- 1- Aas T., Borresen A.L., Geisler S., Smith-Sorensen B., Johnsen H., Varhaug J.E., Akslen L.A. and Lønning P.: Specific p53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nat. Med.*, 2: 811-814, 1996.
- 2- Alsner J., Yilmaz M., Guldborg P., Hansen L.L. and Overgaard J.: Heterogeneity in the clinical phenotype of TP53 mutations in breast cancer. *Clin. Cancer Res.*, 6: 3923-3931, 2000.
- 3- Angele S., Treilleux I., Taniere P., Martel-Planche G., Guillaume M., Bailly C., Bremond A., Montesano R. and Hall J.: Abnormal expression of the ATM and TP53 genes in sporadic breast cancer. *Clin. Cancer Res.*, 6: 3536-3544, 2000.
- 4- Baltali E., Gunel N., Onat D.A., Atahan I.L., Akcali Z., Buyukunal E., Firat D.: Neoadjuvant chemotherapy in locally advanced breast cancer: a preliminary report. *Turkish Oncology Study Group. Tumori*, 85 (6): 483-487, 1999.
- 5- Barbareschi M., Caffo O., Doglioni C., Fina P., Marchetti A., Buttitta F., Leek R., Morelli L., Leonardi E., Bevilacqua G., Dalla P.P. and Harris A.L.: p21WAF1 immunohistochemical expression in breast carcinoma: correlations with clinicopathological data, oestrogen receptor status, MIB1 expression, p53 gene and protein alterations and relapse-free survival. *Br. J. Cancer*, 74: 208-215, 1996.
- 6- Blajman C., Balbiani L., Block J., et al.: A prospective randomized phase III trial comparing combination chemotherapy with cyclophosphamide, Doxorubicin and 5-Fluorouracil with veinorelbine plus doxorubicin in the treatment of advanced breast carcinoma. *Cancer*, 85: 1091-1097, 1999.
- 7- Chevillard S., Lebeau J., Pouillart P., de Toma C., Beldjord C., Asselain B., Klijanienko J., Fourquet A., Magdelenat H. and Vielh P.: Biological and clinical significance of concurrent p53 gene alterations, MDR1 gene expression and S-phase fraction analyses in breast cancer patients treated with primary chemotherapy or radiotherapy. *Clin. Cancer Res.*, 3: 2471-2478, 1997.
- 8- Chung H.C., Rha S.Y., Kim J.H., Roh J.K., Min J.S., Lee K.S., Kim B.S. and Lee K.B.: P-glycoprotein: the intermediate end point of drug response to induction chemotherapy in locally advanced breast cancer. *Breast Cancer Res. Treat.*, 42: 65-72, 1997.
- 9- Colleoni M., Orvieto E., Nole F., Orlando L., Minichella I., Viale G., Peruzzotti G., Robertson C., Noberasco C., Galimberti V., Sacchini V., Veronesi P., Zurrada S., Orecchia R. and Goldhirsch A.: Prediction of response to primary chemotherapy for operable breast cancer. *Eur. J. Cancer*, 35: 574-579, 1999.
- 10- Crown J.: Evolution in the treatment of advanced breast cancer. *Semin Oncol.*, 25 (Supl. 12): 12-17, 1998.
- 11- Dean A.G., Dean F.A. and Coulmbier D.: EPI-INFO version 6.1: Aword processing, data base and statistics program for epidemiology on microcomputers. Center for disease control, Atlanta, Georgia, USA, 1994.
- 12- El-Didi M.H., Moneer M.M., Khaled H.M. and Makarem S.: Pathological assessment of the response of locally advanced breast cancer to neoadjuvant chemotherapy and its implications for surgical management. *Surg. Today*, 30 (3): 249-254, 2000.
- 13- Eltahir A., Heys S.D., Hutcheon A.W., Sarkar T.K., Smith I., Walker L.G., Ah-See A.K. and Eremin O.: Treatment of large and locally advanced breast cancers using neoadjuvant chemotherapy. *Am. J. Surg.*, 175 (2): 127-132, 1998.
- 14- Fisher B., Brown A. and Mamounas E.: Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. *J. Clin. Oncol.*, 15: 2483-2493, 1997.
- 15- Fowble B., Goodman R., Glick J. and Rosato E.: In: *Breast cancer treatment: a comprehensive guide to treatment*. St. Louis, MO: Mosby-Year Book, 139, 1991.
- 16- Geisler S., Lonning P.E., As T., Johnsen H., Fluge O., Haugen D.F., Lillehaug J.R., Akslen L.A. and Borresen-Dale A.L.: Influence of TP53 gene alterations and c-erbB-2 expression on the response to treatment with doxorubicin in locally advanced breast cancer. *Cancer Res.*, 61: 2505-2512, 2001.

- 17- Gentile M., Ahnstrom M., Schon F. and Wingren S.: Candidate tumour suppressor genes at 11q23-q24 in breast cancer: evidence of alterations in PIG8, a gene involved in p53-induced apoptosis. *Oncogene*, 20: 7753-7760, 2001.
- 18- Harris L. and Swain S.M.: The role of primary chemotherapy in early breast cancer. *Semin Oncol.*, 23 (Suppl. 4): 31-42, 1996.
- 19- Ho G.H., Calvano J.E., Bisogna M., Abouezzi Z., Borgen P.I., Cordon-Cardo C. and Van Zee K.J.: Genetic alterations of the p14ARF-hdm2-p53 regulatory pathway in breast carcinoma. *Breast Cancer Res. Treat.*, 65: 225-232, 2001.
- 20- Hortobagyi G.N., Buzdar A.U. and Strom E.: Primary chemotherapy for early and advanced breast cancer. *Cancer Lett.*, 23: 103-109, 1995.
- 21- Jacquillat C., Weill M. and Baillet F.: Results of neoadjuvant chemotherapy and radiation therapy in the breast conserving treatment of 250 patients with all stages of infiltrative breast cancer. *Cancer*, 66: 119-129, 1990.
- 22- Järvinen T.A., Holli K., Kuukasjärvi T. and Isola J.J.: Predictive value of topoisomerase II α and other prognostic factors for epirubicin chemotherapy in advanced breast cancer. *Br. J. Cancer*, 77: 2267-2273, 1998.
- 23- Kandioler-Eckersberger D., Ludwig C., Rudas M., Kappel S., Janschek E., Wenzel C., Schlagbauer-Wadl H., Mittlbock M., Gnant M., Steger G. and Jakesz R.: TP53 mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients. *Clin. Cancer Res.*, 6: 50-56, 2000.
- 24- Karlsson Y.A., Malmstrom P.O. and Hatschek T.: Multimodality treatment of 128 patients with locally advanced breast carcinoma in the era of mammography screening using standard polychemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide: prognostic and therapeutic implications. *Cancer*, 83: 936-947, 1998.
- 25- Kuerer H.M., Sahin A.A., Hunt K.K., Newman L.A., Breslin T.M., Ames F.C., Ross M.I., Buzdar A.U., Hortobagyi G.N. and Singletary S.E.: Incidence and impact of documented eradication of breast cancer axillary lymph node metastases before surgery in patients treated with neoadjuvant chemotherapy. *Ann. Surg.*, 230 (1): 72-8, 1999.
- 26- Linn S.C., Pinedo H.M., van Ark-Otte J., van der Valk P., Hoekman K., Honkoop A.H., Vermorken J.B. and Giaccone G.: Expression of drug resistance proteins in breast cancer, in relation to chemotherapy. *Int. J. Cancer*, 71: 787-795, 1997.
- 27- MacGrogan G., Mauriac L., Durand M., Bonichon F., Trojani M., de Mascarel I. and Coindre J.M.: Primary chemotherapy in breast invasive carcinoma: predictive value of the immunohistochemical detection of hormonal receptors, p53, c-erbB-2, MIB1, pS2 and GST. *Br. J. Cancer*, 74: 1458-1465, 1996.
- 28- Markris A., Powles T.J., Dowsett M., Osborne C.K., Trott P.A., Fernando I.N., Ashley S.E., Ormerod M.G., Titley J.C., Gregory R.K. and Allred D.C.: Prediction of response to neoadjuvant chemoendocrine therapy in primary breast carcinomas. *Clin. Cancer Res.*, 3: 593-600, 1997.
- 29- McMasters K.M. and Hunt K.K.: Neoadjuvant chemotherapy, locally advanced breast cancer and quality of life. *J. Clin. Oncol.*, 17 (2): 441-444, 1999.
- 30- Molina R., Segui M.A., Climent M.A., Bellmunt J., Albandell J., Fernandez M., Filella X., Jo J., Gimenez N., Iglesias E., Miralles M., Alonso C., Peiro G., Perez-Picanol E. and Ballesta A.M.: p53 oncoprotein as a prognostic indicator in patients with breast cancer. *Anticancer Res.*, 18: 507-512, 1998.
- 31- Norberg T., Klaar S., Karf G., Nordgren H., Holmberg L. and Bergh J.: Increased p53 mutation frequency during tumor progression-results from a breast cancer cohort. *Cancer Res.*, 61: 8317-8321, 2001.
- 32- Pharaoh P.D., Day N.E. and Caldas C.: Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. *Br. J. Cancer*, 80: 1968-1973, 1999.
- 33- Schneider J. and Romero H.: Correlation of P-glycoprotein overexpression and cellular prognostic factors in formalin-fixed, paraffin-embedded tumor samples from breast cancer patients. *Anticancer Res.*, 15: 1117-1121, 1995.
- 34- Samuels-Lev Y., O'Connor D.J., Bergamaschi D., Tripiante G., Hsieh J.K., Zhong S., Campargue I., Namovski L., Crook T. and Lu X.: ASPP proteins specifically stimulate the apoptotic function of p53. *Mol. Cell*, 8: 781-794, 2001.
- 35- Scholl S.M., Fourquet A. and Asselain B.: Neoadjuvant versus adjuvant chemotherapy in premenopausal patients with tumours considered too large for breast conserving surgery: preliminary results of a randomised trial: S6. *Eur. J. Cancer*, 5: 645-652, 1994.
- 36- Schwartz G.F., Birchansky C.A. and Komarnicky L.T.: Induction chemotherapy followed by breast conservation for locally advanced carcinoma of the breast. *Cancer*, 73: 362-369, 1994.
- 37- Shao Z.M., Wu J., Shen Z.Z. and Nguyen M.: p53 mutation in plasma DNA and its prognostic value in breast cancer patients. *Clin. Cancer Res.*, 7: 2222-2227, 2001.
- 38- Sjogren S., Ingandas M., Norberg T., Lindgren A., Nordgren H., Holmberg L. and Bergh J.: The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry. *J. Natl. Cancer Inst.*, 88: 173-182, 1996.
- 39- Smith P.D., Crossland S., Parker G., Osin P., Brooks L., Waller J., Philp E., Crompton M.R., Gusterson B.A., Allday M.J. and Crook T.: Novel p53 mutants selected in BRCA-associated tumours which dissociate transformation suppression from other wild-type p53 functions. *Oncogene*, 18: 2451-2459, 1999.
- 40- Trock B.J., Leonessa F. and Clarke R.: Multidrug-resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance. *J. Natl. Cancer Inst.*, 89: 917-931, 1997.

- 41- Van Praagh I., Charrier S. and Leduc B.: Breast conservation following VEM (Vincorelbine, Epirubicin and Methotrexate) induction chemotherapy in 89 operable breast cancers. *Eur. J. Gynaecol. Oncol.*, 18: 294a-299a, 1997.
- 42- Volm M.: Multidrug resistance and its reversal. *Anti-cancer Res.*, 18: 2905-2917, 1998.
- 43- Wu S.G. and El-Deiry W.S.: p53 and chemosensitivity. *Nat. Med.*, 2: 255-256, 1996.
- 44- Zambetti M., Oriana S., Quattrone P., Verderio P., Terenziani M., Zucali R., Valagussa P. and Bonadonna G.: Combined sequential approach in locally advanced breast cancer. *Ann. Oncol.*, 10: 305-310, 1999.
- 45- Zhang H., Somasundaram K., Peng Y., Tian H., Zhang H., Bi D., Weber B.L. and El-Deiry W.S.: BRCA1 physically associates with p53 and stimulates its transcriptional activity. *Oncogene*, 16: 1713-1721, 1998.
- 46- Zou Z., Gao C., Nagaich A.K., Connell T., Saito S., Moul J.W., Seth P., Appella E. and Srivastava S.: p53 regulates the expression of the tumor suppressor gene maspin. *J. Biol. Chem.*, 275: 6051-6054, 2000.