p53 Expression as a Prognostic Marker in Inflammatory Breast Cancer

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ABSTRACT

Purpose: **Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer. Nuclear expression of p53 protein in breast cancer correlates with more aggressive tumors. We retrospectively analyze the expression of p53 as a prognostic marker to predict pathological complete response and survival in patients with IBC.**

Experimental Design: **Fifty-nine patients with IBC were treated from January 1994 to April 2000. Forty-eight patients were included. Diagnostic core biopsies were taken before treatment was started. Expression of hormone receptors and p53 was determined by immunohistochemistry. All patients received an anthracycline-based regimen preoperatively; 22 patients (46%) also received paclitaxel. Forty-four patients (92%) achieved an objective clinical response and underwent mastectomies.**

Results: **Median age at diagnosis was 48 years. Thirty patients (63%) had hormone receptor-negative tumors. Twenty-eight patients (58%) had p53-positive tumors, and 20 patients (42%) had p53-negative tumors. Nine patients (19%) achieved a pathological complete response. At a median follow-up of 77 months, 28 recurrences (58%) and 26 deaths (54%) had occurred. Patients with p53-positive tumors were younger ($P = 0.02$) and tended to have lower 5-year progression-free survival rates (35% *versus* 55%; $P = 0.3$) and overall survival rates (44% *versus* 54%; $P = 0.4$).**

Conclusions: **This retrospective analysis demonstrates that nuclear p53 protein expression may represent an adverse prognostic marker in IBC and may provide a valuable tool for selecting treatment for this aggressive disease.**

INTRODUCTION

Inflammatory breast cancer (IBC) is the most aggressive form of primary breast cancer. It is rare, with an incidence rate of 1% to 6% in the United States (1). However, data from the Surveillance, Epidemiology and End Results Program comparing trends and patterns for breast cancer revealed that the incidence of IBC has increased from 0.3 to 0.7 case per 100,000 person-years (1). Compared with non-IBC stage II breast cancer, primary IBC is associated with lower overall survival (OS) rates (2, 3).

IBC is a clinical diagnosis characterized by the presence of symptoms and signs such as erythema, tenderness, edema, pain, and ulceration that rapidly extend to the entire breast (4, 5). Pathologically, IBC is frequently diagnosed by the presence of cancer cells penetrating dermal lymphatic channels (Fig. 1), causing the inflammatory signs (6).

The management of IBC has evolved over the past 25 years. A multidisciplinary approach has improved both local control and survival, with about 30% of patients living more than 5 years (7, 8). Primary chemotherapy (PC) is considered to be the main component of the treatment. The presence of pathological residual disease in the breast and lymph nodes after PC is considered an important adverse prognostic factor (7–9).

Missense mutations of the *p53* gene and cytoplasmic sequestration are thought to be the main mechanism behind the inactivation of p53 protein and loss of function in IBC (10, 11). Nuclear expression of mutated p53 in breast cancer has been associated with more aggressive tumors, early metastases, anthracycline resistance, and less favorable long-term outcome (12). The prognostic role of p53 expression and/or mutation in IBC has not been clearly established. Higher levels of nuclear p53 expression have been detected in IBC compared with other locally advanced breast cancers (13–15). Only one report describes an association between poor prognoses and p53 mutation and nuclear overexpression of the p53 protein in a relatively small group of patients with primary IBC (16). We retrospectively analyzed whether the nuclear expression of p53 protein predicts for pathological complete response (pCR) and is a prognostic marker for disease recurrence and survival for patients with IBC.

PATIENTS AND METHODS

Study Population. Fifty-nine patients with IBC identified through the University of Texas M. D. Anderson Cancer Center Breast Cancer Management System Database were treated between January 1994 and April 2000 in clinical trials at The University of Texas M. D. Anderson Cancer Center. Diagnostic biopsies were taken before treatment was started. All investigations were performed after approval by the institutional

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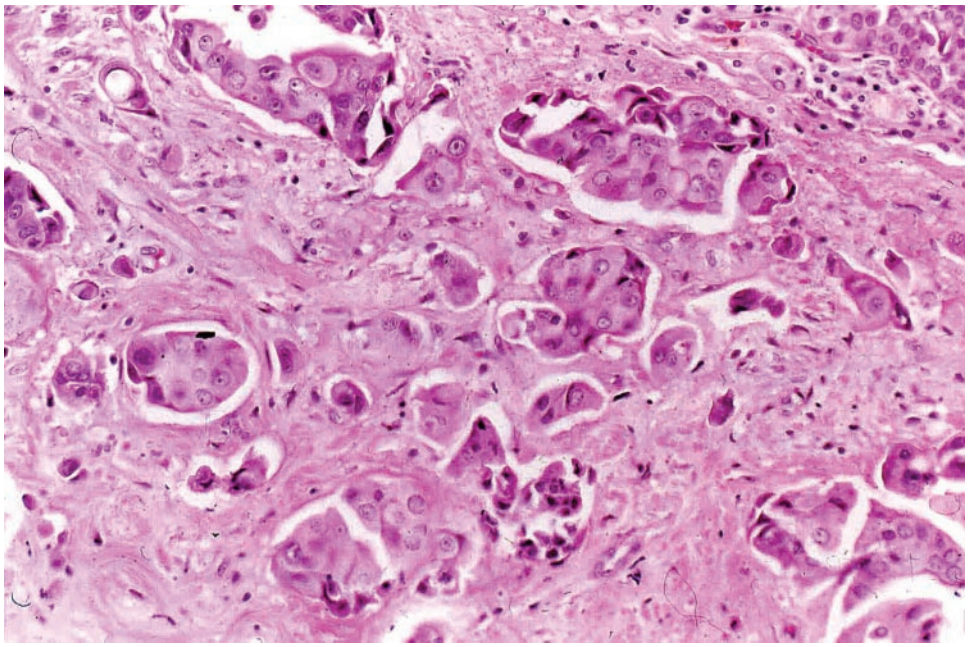

Fig. 1 Tissue section showing IBC with prominent involvement of vascular spaces.

review board. Forty-eight patients for whom biopsy specimens and adequate follow-up data were available were included in this study.

Clinical diagnosis of IBC required the presence of erythema, heat, ridging, and peau d'orange with or without evidence of dermal lymphatic invasion on pathological evaluation. All patients were presented at the Breast Center's multidisciplinary conference to confirm their diagnosis of primary IBC and later to assess their response to treatment. Patients were treated with PC and then underwent mastectomy if they achieved an objective clinical remission (partial or complete response). After surgery, patients received adjuvant radiotherapy and hormonal treatment with tamoxifen if their disease was hormone receptor positive. In all patients, PC included an anthracycline-based therapy with of 5-fluorouracil (500 mg/m^2), doxorubicin (500 mg/m^2), and cyclophosphamide (500 mg/m^2) [FAC] every 21 days. Twenty-two patients (46%) also received paclitaxel (175–250

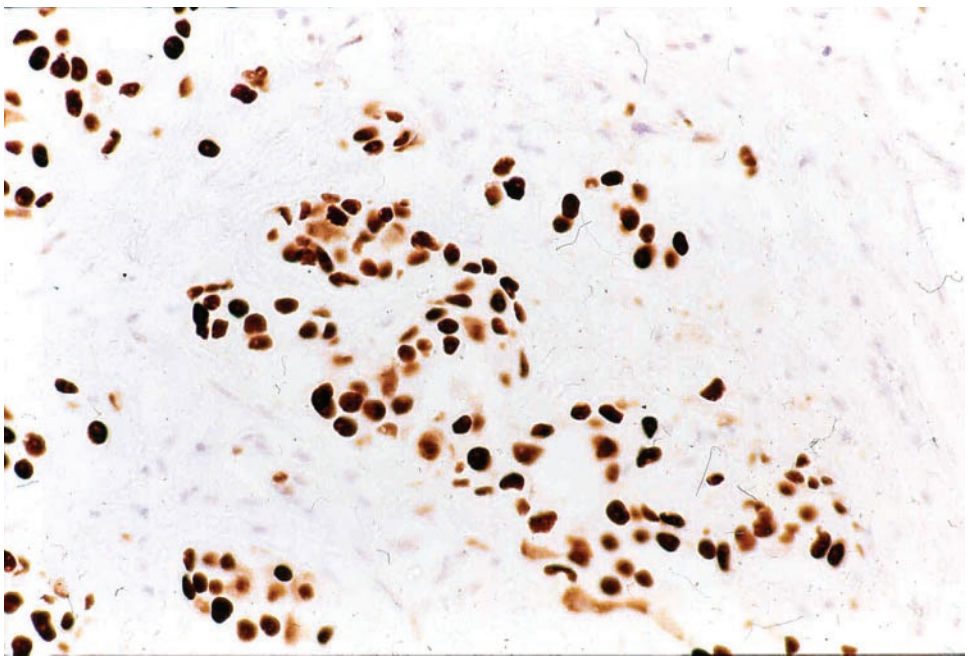

Fig. 2 Immunostain for p53 showing strong positive nuclear staining of the tumor cells.

Table 1 Patient characteristics

	Overall no. of patients (%)	p53 negative	p53 positive	*P*
Total	48	20	28	
Age (y)				
<50	28 (58)	9 (45)	19 (68)	
>50	20 (42)	11 (55)	9 (32)	0.11
Race				
Asian	2 (4)	1 (5)	1 (4)	
Black	3 (6)	1 (5)	2 (7)	
Hispanic	6 (13)	3 (15)	3 (11)	
Caucasian	37 (77)	15 (75)	22 (79)	0.43
Lymphovascular invasion				
No	2 (6)	0 (0)	2 (12)	
Yes	29 (94)	14 (100)	15 (88)	0.49
Nuclear grade				
2	11 (24)	7 (39)	4 (15)	
3	34 (76)	11 (61)	23 (85)	0.09
Histology				
Ductal	41 (89)	16 (84)	25 (93)	
Lobular	4 (9)	3 (16)	1 (4)	
Metaplastic	1 (2)	0 (0)	1 (4)	0.29
ER				
Negative	32 (58)	9 (47)	23 (64)	
Positive	23 (42)	10 (53)	13 (36)	0.003
PR				
Negative	37 (82)	13 (68)	24 (92)	
Positive	8 (18)	6 (32)	2 (8)	0.05
Taxane use				
No	10 (21)	5 (25)	5 (18)	
Yes	38 (79)	15 (75)	23 (82)	0.72
pCR				
Yes	9 (19)	4 (20)	5 (18)	
No	39 (81)	16 (80)	23 (82)	1.00

mg/m^2) every 21 days (17). Forty-four patients (92%) obtained objective clinical responses (partial or complete response) and underwent mastectomies. After mastectomy, 16 patients received adjuvant paclitaxel.

Tumor Response. Extent of primary tumor and regional lymph node involvement was measured at the time of presentation using mammography, clinical examination, and ultrasonography. The same methods were used to monitor response to PC. Clinical response was documented and classified as follows: a complete response was defined as complete disappearance of evident disease for at least 4 weeks based on physical exam, ultrasound, and X-ray. A partial response was a >50% decrease of bidimensionally measurable disease for at least 4 weeks. Stable disease was defined as a change in measurable disease that was too small to be called a partial response or progressive disease, with no new lesions appearing for a period of at least 4 weeks. Progressive disease required an unequivocal 50% or greater increase in the size of any measurable lesion (two diameters), the worsening of existing lesions, the appearance of new lesions, or deterioration of symptoms related to cancer. Clinical assessment was recorded monthly, and complete tumor assessment was repeated before surgery. Surgical specimens were histologically examined to determine pCR. A pCR was defined as the absence of invasive disease in the breast and the axillary lymph nodes at the time of definitive surgery.

Immunohistochemistry Methods. Forty-eight paraffin-embedded tissue samples were available for the study. Histologic sections of 4-μm thickness were cut from paraffin blocks and incubated overnight at 4°C with a 1:50 dilution of the human-specific monoclonal antibody DO7 (Dako, Carpinteria, CA), which recognizes mutant forms of the p53 protein. Sections from breast cancers with known p53 mutation and immunoreactivity and sections from normal breast tissue were included as positive and negative controls to confirm the consistency of the analysis. A specimen was scored as positive (Fig. 2) when nuclear staining was equal to or greater than 10% (18, 19). The estrogen receptor (ER) status and progesterone receptor (PR) status were available by immunohistochemical

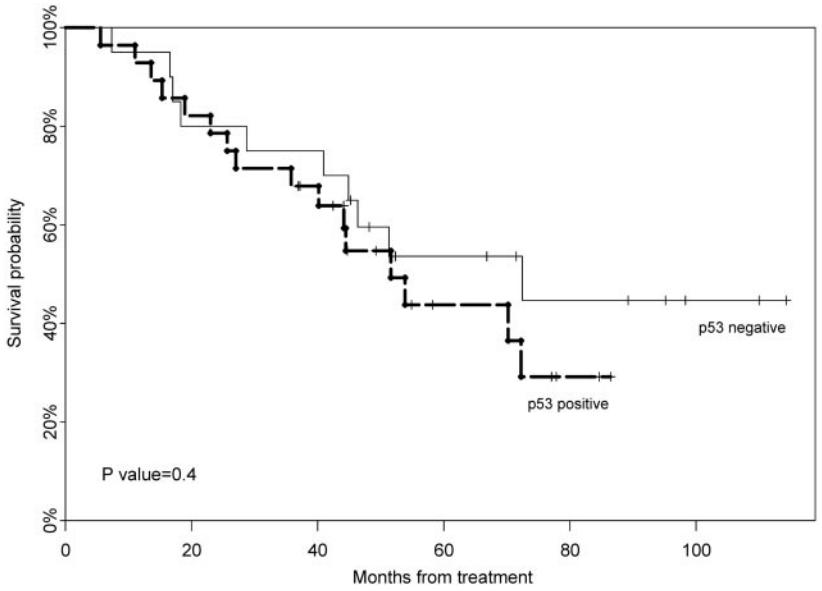

Fig. 3 Kaplan-Meier estimates of the PFS of patients with IBC by p53 status.

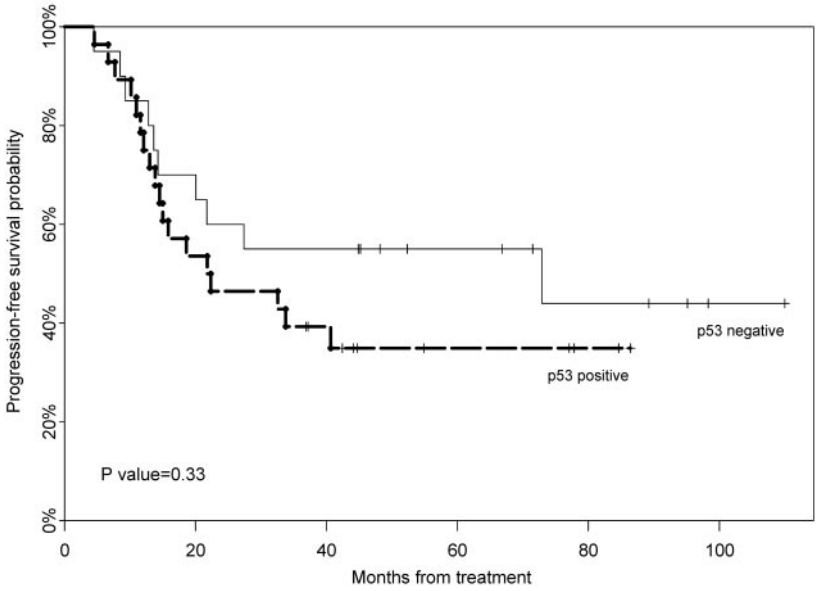

Fig. 4 Kaplan-Meier estimates of the OS of patients with IBC by p53 status.

Table 2 PFS by p53 status, hormone receptor status, nuclear grade, and pCR

	No. of patients	No. of events	5-year PFS Estimate	95% CI	*P*
All patients					
p53 negative	20	10	55%	37–82	
p53 positive	28	18	35%	21–59	0.33
Hormone receptor negative					
p53 negative	9	5	44%	21–92	
p53 positive	21	14	33%	18–61	
Hormone receptor positive					
p53 negative	10	5	60%	36–100	
p53 positive	5	3	40%	14–100	0.61
Nuclear grade 2					
p53 negative	7	4	57%	30–100	
p53 positive	4	1	75%	43–100	
Nuclear grade 3					
p53 negative	11	5	55%	32–94	
p53 positive	23	17	25%	12–52	0.33
No pCR					
p53 negative	16	10	44%	25–76	
p53 positive	23	16	30%	16–56	
pCR					
p53 negative	4	0			
p53 positive	5	2	60%	29–100	0.38

Abbreviation: CI, confidence interval.

stain for 45 patients. Immunohistochemistry was performed following standard procedures on 4-μm sections of paraffin-embedded tissue with monoclonal antibody 6F11 (Novocastra Laboratories Ltd., Burlingame, CA) for ER and monoclonal antibody 1A6 (Novocastra Laboratories Ltd.) for PR. Nuclear staining equal to or greater than 10% was considered positive.

Statistical Methods. The patient population was described by tabulating categorical variables with p53 status (Table 1). A χ^2 test was used to evaluate the significance of differences between the variables. The Kaplan-Meier product limit method was used to assess differences between both OS and progression-free survival (PFS) rates of the two groups and, after adjustment for ER and PR status, nuclear grade and pCR. The log-rank test and the stratified log-rank test were used to compare survival estimates between the groups. OS was measured in months from the date of diagnosis to the date of death or last follow-up. PFS was measured in months from the date of diagnosis to the date of recurrence or last follow-up. Statistical analyses were carried out using SPSS software version 12.0 (SPSS Inc., Chicago, IL).

Table 3 OS by p53 status, hormone receptor status, nuclear grade, and pCR

	No. of patients	No. of events	5-year OS Estimate	95% CI	*P*
All patients					
p53 negative	20	10	54%	35–82	
p53 positive	28	16	44%	27–70	0.4
Hormone receptor negative					
p53 negative	9	5	56%	31–100	
p53 positive	21	13	38%	21–69	
Hormone receptor positive					
p53 negative	10	5	48%	25–94	
p53 positive	5	2	75%	43–100	0.73
Nuclear grade 2					
p53 negative	7	3	57%	30–100	
p53 positive	4	1	67%	30–100	
Nuclear grade 3					
p53 negative	11	6	53%	30–94	
p53 positive	23	15	37%	21–67	0.51
No pCR					
p53 negative	16		41%	22–76	
p53 positive	23		40%	23–68	
pCR					
p53 negative	4				
p53 positive	5		67%	30–100	0.51

Abbreviation: CI, confidence interval.

RESULTS

The median age of the patients was 48 years (range, 29–71 years), and 77% were Caucasian. Hormone receptor status results were available in 45 cases (94%), and 30 patients (67%) had hormone receptor-negative disease. Twenty-eight patients (58%) had nuclear p53-positive tumors, and 20 patients (42%) had p53-negative tumors. There were no cytoplasmic p53-positive tumors. Patients with p53-positive tumors tended to be younger (median age, 45.2 *versus* 52.2 years; $P = 0.02$) and have tumors with high nuclear grade (85% *versus* 61%; $P = 0.09$). Hormone receptor status was available in 45 patients and inversely correlated with p53 expression ($P = 0.02$): 23 of 26 (88%) p53-positive tumors were ER negative, and 24 of 26 (92%) p53-positive tumors were PR negative. There were no differences in histology, presence of lymphovascular invasion, or race between the two groups.

Nine patients (19%) achieved a pCR. Pathological complete response was not associated with p53 expression: four patients who achieved pCR had p53-negative tumors, and the remaining five patients had p53-positive tumors. The use of neoadjuvant or adjuvant taxanes between the two groups did not differ significantly. At a median follow-up of 77 months, 28 recurrences (58%) and 26 deaths (54%) had occurred.

The estimated 5-year PFS rate was 35% for patients with p53-positive tumors *versus* 55% for patients with p53-negative IBC ($P = 0.33$; Fig. 3). Similarly, the estimated 5-year OS rate was 44% among patients with p53-positive IBC and 54% among patients with p53-negative disease ($P = 0.4$; Fig. 4). Significantly, patients with p53-positive IBC had a 55% higher risk of dying from their disease (hazard ratio, 1.55) than patients with p53-negative disease.

Kaplan-Meier product limit estimates of OS and PFS were also done after adjustment for hormone receptor status and nuclear grade; similar trends were observed (Tables 2 and 3). In patients with hormone receptor-negative tumors, the 5-year estimated OS rate was 38% for the p53-positive group and 56% for the p53-negative group. The 5-year estimated PFS rates were 33% and 44%, respectively. In patients with modified Black's nuclear grade 3 tumors, the 5-year estimated OS rate was 37% for the p53-positive group and 53% for the p53-negative group. The 5-year PFS rates were 25% and 55%, respectively.

The prognostic interaction between pCR and p53 status was also analyzed (Figs. 5 and 6); although p53 status did not influence pCR rate, pCR remains the strongest predictor of long-term outcome. The estimated 5-year PFS and OS rates were, respectively, 78% and 86% for patients who achieved a pCR compared with 36% and 40% for patients who did not. Overexpression of p53 was associated with a higher rate of recurrence and death even in patients who obtained a pCR. Interestingly, there were two recurrences (40%) and one death (20%) among the patients with p53-positive tumors. There were no recurrences or deaths in the p53-negative group ($P = 0.3$ and 0.5, respectively).

DISCUSSION

Our retrospective data suggested that patients with p53-positive IBC have a less favorable prognosis, as demonstrated by the shorter OS and PFS rates, than patients with p53-negative IBC. The differences were not statistically significant, most likely because of the small number of cases studied, a common limitation in IBC studies. The adjustments for hormone receptor status and nuclear grade were also limited by the number of cases.

Several studies have investigated the incidence of p53 overexpression in breast cancer, including locally advanced disease (20–22). Faille *et al.* (14) performed an initial analysis of alterations in p53 in 39 patients with locally advanced breast cancer; the majority of these patients had inflammatory changes. The authors found that the presence of a p53 mutation was significantly associated with larger tumor diameter ($P = 0.0062$) and evidence of metastatic disease ($P = 0.0015$). Also, a nonsignificant association existed between the mutation and negative ER status and lower response rates to therapy (14).

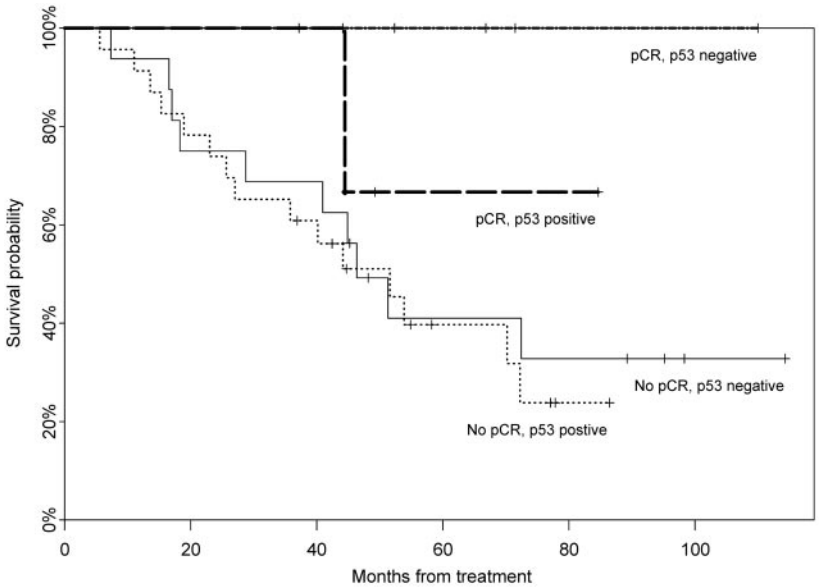

Fig. 5 Kaplan-Meier estimates of the PFS of patients with IBC by p53 status and pathological response (pCR *versus* no pCR).

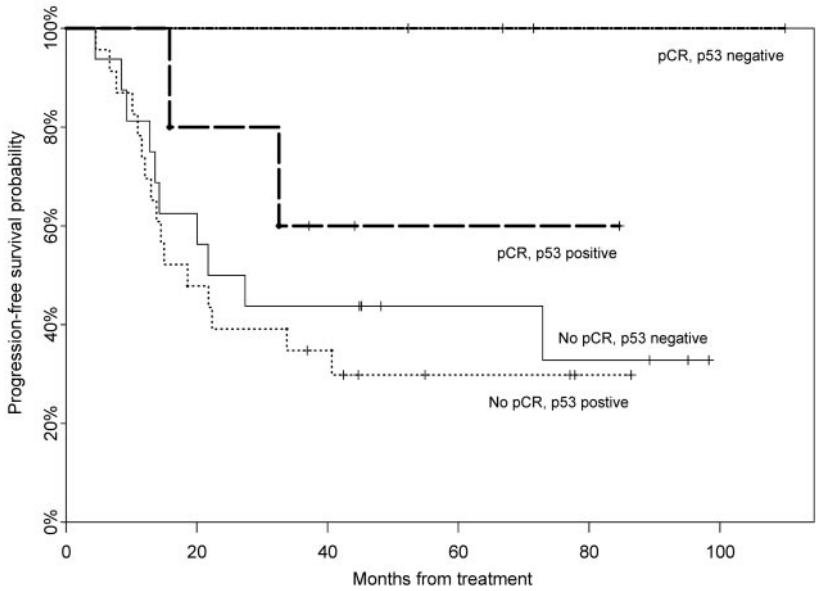

Fig. 6 Kaplan-Meier estimates of the OS of patients with IBC by p53 status and pathological response (pCR *versus* no pCR).

Aziz *et al.* (13) compared the level of p53 expression in 40 IBC patients with that in non-IBC controls: p53 was expressed in 70% of patients with IBC and in 48% of non-IBC controls ($P = 0.0238$). The authors hypothesized that the clinical aggressiveness of IBC may be due to overexpression of p53 (13). More recently, McCarthy *et al.* (15) again showed overexpression of p53 nuclear protein in 24 (53%) of IBC tumors compared with 8 (36%) of 22 non-IBC tumors; however, the difference was not statistically significant ($P = 0.19$). Nuclear exclusion and cytoplasmic sequestration of p53 have been described as the potential mechanisms of protein function inactivation; this phenomenon has been found to occur in 37% of IBCs (13, 15). These observations have not been confirmed by subsequent studies. In the small cohort reported by Riou *et al.*, (26) tissue for 24 patients with IBC was tested for *p53* gene mutation and nuclear overexpression of p53 protein. Overexpression of p53 was not found to be associated with any of the main pathobiologic characteristics, including age, tumor size, histologic grade, inflammation, and hormone receptor and c-erbB2 status. Multivariate analysis indicated that patients with tumors overexpressing p53 had an 8.6-fold higher risk of death ($P = 0.02$). This prognostic value was observed even in the ER-negative group (16).

Our study confirmed that the most important prognostic factor for survival in IBC is pCR after PC (7, 8, 23–25). Interestingly, when PFS and OS were analyzed among patients who achieved a pCR, all of the recurrences and deaths occurred in the group of patients with p53-positive tumors. This important observation suggested that patients with p53-overexpressing IBC have a particularly aggressive form of IBC and that their risk of death from the disease is only marginally reduced by an excellent response to PC. In contrast, patients with IBC who do not have dysfunctional p53 protein expression have a better prognosis when treated with optimal systemic and locoregional treatments and may have a higher chance of cure if they achieve a pCR.

Larger prospective studies need to be performed to confirm these observations and to contribute to a better understanding of the peculiar biology of this particularly aggressive form of breast cancer. Only through coordinated multidisciplinary and multicenter efforts will it be possible to develop more specific and targeted treatments able to significantly affect the prognosis of these patients.

REFERENCES

1. Levine PH, Steinhorn SC, Ries LG, Aron JL. Inflammatory breast cancer: the experience of the Surveillance, Epidemiology, and End Results (SEER) Program. J Natl Cancer Inst (Bethesda) 1985;74: 291–7.

2. Chang S, Parker SL, Pham T, Buzdar AU, Hursting SD. Inflammatory breast carcinoma incidence and survival: the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute, 1975–1992. Cancer (Phila) 1998;82:2366–72.

3. Low JA, Berman AW, Steinberg SM, Lippman ME, Swain SM. Long-term follow-up for inflammatory (IBC) and non-inflammatory (NIBC) stage III breast cancer patients treated with combination chemotherapy. Proc Am Soc Clin Oncol 2002;21:63a.

4. Jaiyesimi IA, Buzdar AU, Hortobagyi GN. Inflammatory breast cancer: a review. J Clin Oncol 1992;10:1014–24.

5. Tabbane F, Bahi J, Rahal K, et al. Inflammatory symptoms in breast cancer. Correlations with growth rate, clinicopathologic variables, and evolution. Cancer (Phila) 1989;64:2081–9.

6. Taylor GM. A. Inflammatory carcinoma of the breast. Am J Cancer 1938;33:33–4.

7. Rouesse J, Friedman S, Sarrazin D, et al. Primary chemotherapy in the treatment of inflammatory breast carcinoma: a study of 230 cases from the Institut Gustave-Roussy. J Clin Oncol 1986;4:1765–71.

8. Buzdar AU, Singletary SE, Booser DJ, et al. Combined modality treatment of stage III and inflammatory breast cancer. M.D. Anderson Cancer Center experience. Surg Oncol Clin N Am 1995;4: 715–34.

9. Rouzier R, Extra JM, Klijanienko J, et al. Incidence and prognostic significance of complete axillary downstaging after primary chemotherapy in breast cancer patients with T1 to T3 tumors and cytologically proven axillary metastatic lymph nodes. J Clin Oncol 2002;20:1304–10.

10. Moll UM, LaQuaglia M, Benard J, Riou G. Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. Proc Natl Acad Sci USA 1995;92: 4407–11.

11. Soussi T. The p53 tumor suppressor gene: from molecular biology to clinical investigation. Ann N Y Acad Sci 2000;910:121–37.

12. Moll UM, Riou G, Levine AJ. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. Proc Natl Acad Sci USA 1992;89:7262–6.

13. Aziz SA, Pervez S, Khan S, et al. Case control study of prognostic markers and disease outcome in inflammatory carcinoma breast: a unique clinical experience. Breast J 2001;7:398–404.

14. Faille A, De Cremoux P, Extra JM, et al. p53 mutations and overexpression in locally advanced breast cancers. Br J Cancer 1994;69:1145–50.

15. McCarthy NJ, Yang X, Linnoila IR, et al. Microvessel density, expression of estrogen receptor alpha, MIB-1, p53, and c-erbB-2 in inflammatory breast cancer. Clin Cancer Res 2002;8:3857–62.

16. Kandioler-Eckersberger D, Ludwig C, Rudas M, et al. TP53 mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients. Clin Cancer Res 2000;6:50–6.

17. Cristofanilli M, Buzdar AU, Sneige N, et al. Paclitaxel in the multimodality treatment for inflammatory breast carcinoma. Cancer (Phila) 2001;92:1775–82.

18. Stal O, Stenmark Askmalm M, Wingren S, et al. p53 expression and the result of adjuvant therapy of breast cancer. Acta Oncol 1995;34: 767–70.

19. Sjögren S, Inganäs M, Norberg T, et al. The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry. J Natl Cancer Inst (Bethesda) 1996;88: 173–82.

20. Thor AD, Moore DH, Edgerton SM, et al. Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis in breast cancers. J Natl Cancer Inst (Bethesda) 1992;84:845–55.

21. Isola J, Visakorpi T, Holli K, Kallioniemi OP. Association of overexpression of tumor suppressor protein p53 with rapid cell proliferation and poor prognosis in node-negative breast cancer patients. J Natl Cancer Inst (Bethesda) 1992;84:1109–14.

22. Molina R, Segui MA, Climent MA, et al. p53 oncoprotein as a prognostic indicator in patients with breast cancer. Anticancer Res 1998;18:507–12.

23. Harris EE, Schultz D, Bertsch H, et al. Ten-year outcome after combined modality therapy for inflammatory breast cancer. Int J Radiat Oncol Biol Phys 2000;55:1200–8.

24. Arun B, Slack R, Gehan E, Spitzer T, Meehan KR. Survival after autologous hematopoietic stem cell transplantation for patients with inflammatory breast carcinoma. Cancer (Phila) 1999;85:93–9.

25. Ueno NT, Buzdar AU, Singletary SE, et al. Combined-modality treatment of inflammatory breast carcinoma: twenty years of experience at M. D. Anderson Cancer Center. Cancer Chemother Pharmacol 1997; 40:321–9.

26. Riou G, Le MG, Travagli JP, Levine AJ, Moll UM: Poor prognosis of p53 gene mutation and nuclear overexpression of p53 protein in inflammatory breast cancer. JNCI 1993;85:1765–7.

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