

Prognostic Value of p53 for Local Failure in Mastectomy-Treated Breast Cancer Patients

By R.C. Zellars, S.G. Hilsenbeck, G.M. Clark, D.C. Allred, T.S. Herman, G.C. Chamness, and R.M. Elledge

Purpose: The loss of p53 function is a recognized adverse prognostic factor in invasive breast cancer. Several studies have shown a relationship between the nuclear accumulation of p53 protein (a surrogate marker of p53 inactivation) and poor disease-free and overall survival. In general, however, these studies did not report the prognostic value of p53 for local failure, which we have therefore assessed retrospectively here.

Materials and Methods: Accumulation of p53 protein was evaluated by immunohistochemistry in 1,530 mastectomy-treated breast cancer patients (259 radiation therapy [RT]- and 1,271 mastectomy only [No RT]-treated patients). Statistical comparisons were made between p53 protein accumulation, estrogen/progesterone receptors, nodal status, tumor size, and local failure rate (LFR). Local failure was defined as tumor recurrence involving the chest wall and/or the ipsilateral supraclavicular/axillary lymph nodes. The median follow-up period was 62 months.

Results: In the No RT group, the LFR was 9.1% and 16.5% in p53-negative and p53-positive patients, respectively ($P < .001$). Multivariate analysis revealed that p53 protein accumulation was significantly associated with an increased risk of local relapse (relative risk [RR], 1.7; 95% confidence interval [CI], 1.2 to 2.4). Nodal status and tumor size were also significant factors. In the RT group, the LFR was 9.3% and 21.5% in p53-negative and p53-positive patients, respectively ($P = .009$). Multivariate analysis revealed that p53 protein accumulation was significantly associated with an increased risk of local relapse (RR, 2.5; 95% CI, 1.1 to 5.7), as was nodal status.

Conclusion: Nuclear accumulation of p53 protein is independently associated with a significantly increased local failure rate in breast cancer patients treated with mastectomy, with or without radiation.

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TWENTY PERCENT TO 30% of node-positive breast cancer patients will develop a local-regional recurrence after being treated by mastectomy with or without chemotherapy.¹⁻³ Ultimately, 50% of these recurrences will be controlled with local modalities; however, the remaining 50% will die with uncontrolled local-regional disease.^{4,5}

In the hope of preventing such events, early, randomized clinical trials evaluated the role of postmastectomy radiation therapy (RT). Although these trials consistently demonstrated a significant improvement in local control, evidence of survival benefit was lacking.⁶ As a result, many considered RT ineffectual in improving survival, and some suggested that post-mastectomy RT may be detrimental.⁶ The debate over this

topic continues⁷⁻⁹ with the recent publication of two randomized prospective trials in premenopausal node-positive women.^{1,2} In these trials, the two arms of each study differed only with respect to the addition of postmastectomy radiation. The results showed that there was not only an improvement in local control but also a significant improvement in survival in the patients who received adjuvant RT. Although confirmatory trials would provide greater certainty on this issue, it is reasonable to expect that a population exists with sufficient risk of local-regional failure that adjuvant RT will not only improve local-regional control but also possibly improve survival.

However, because the personal, social, and financial cost of postmastectomy RT can be substantial, it would be helpful to identify breast cancer patients at greatest risk of local-regional recurrence and therefore most likely to benefit from postmastectomy RT. Known predictors of an increased risk of local-regional failure are lymph node positivity, tumor size, grade of anaplasia, extent of surgery, and possibly lymph-vascular invasion.^{1,7,10-13} Because some of these prognostic factors parallel those of distant failure, we hypothesized that some other factors prognostic of distant failure may be associated with local-regional recurrence. Some well-recognized prognostic factors of distant failure are estrogen/progesterone receptor status, S-phase fraction, DNA ploidy, and, more recently, p53 accumulation. Although many of these prognostic factors have been studied for some time, the tumor suppressor gene *p53* is relatively new and is currently the focus of much attention

From the Departments of Radiology (Division of Radiation Oncology), Medical Oncology, and Pathology, University of Texas Health Science Center at San Antonio, San Antonio, and Baylor Methodist Breast Center at Baylor College of Medicine, Houston, TX.

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Address reprint requests to Richard C. Zellars, MD, Division of Radiation Oncology, Department of Radiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr, San Antonio, TX 78284-7800.

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in breast cancer research. Research has shown that altered function of this gene is associated with decreased disease-free and overall survival.¹⁴⁻¹⁷ Interestingly, considering the extent to which *p53* has been evaluated for prognostic purposes, there is little in the literature concerning its prognostic value for local-regional failure in mastectomy-treated breast cancer patients. Accordingly, to identify breast cancer patients who have the highest risk of local relapse and would most likely benefit from adjuvant RT, we evaluated the local failure prognostic value of *p53*.

MATERIALS AND METHODS

Patient Data

The following patient information is from the San Antonio breast cancer database. A team of experienced data managers regularly visits each physician's office or clinic to obtain follow-up information. The primary source of information is the patient chart or clinic record. Hospital tumor registries are a second source of information. Once the updated information is entered into the data files, a computer-generated report is produced and sent back to the medical oncologist, surgeon, or tumor registry. This report lists each patient and selected demographic and patient status variables and requests that errors or omissions be corrected and returned.

For the purposes of quality assurance, audits are performed. Patients' charts are reviewed and compared with the database. No significant differences have been found between randomly chosen patient charts and the corresponding database printouts. Information in the database has been independently verified on a sampling basis on multiple occasions over time.

The records of 1,530 mastectomy-treated breast cancer patients from the San Antonio database were reviewed. The patient population was subdivided into two groups: those who received mastectomy followed by adjuvant RT and those who received mastectomy only (No RT). Data concerning the following prognostic factors were collected and analyzed: tumor size, axillary lymph node status, estrogen/progesterone receptor status (estrogen receptor-negative, < 3 fmol/mg; progesterone receptor-negative, < 5 fmol/mg),^{18,19} DNA ploidy and S-phase fraction (determined by flow cytometry),²⁰ type of systemic therapy, local failure rate, and *p53* protein accumulation.

Local failure was defined as a recurrence on the chest wall or in the axillary or supraclavicular nodal regions. Local recurrences as a first site of recurrence were included in the analysis. In all local-regional failure analyses, simultaneous local-regional and distant failure was counted as an event (ie, a local-regional failure). In the competing-risks analyses, distant failure alone as a first failure was a competing risk, preventing further observation of local-regional failure. In the standard survival analysis, distant failure alone as a first failure was treated as a censoring event. Median follow-up for local recurrence was 62 months, with 80% of the patients followed up for more than 2 years (range, 1 to 228 months).

Assessment of p53 Function

p53 protein accumulation, as measured by immunohistochemistry, was used as an indirect assessment of *p53* function. The technical details of this assessment have been published.¹⁴ Briefly, fresh-frozen pellets of breast cancer specimens were thaw-mounted and fixed to glass slides before they were rehydrated and washed. The specimens

were exposed to a monoclonal *p53* antibody cocktail containing both PAb1801 and PAb240 antibodies (Novocastra, Newcastle, United Kingdom). The specimens were then sequentially exposed to a linking antibody, peroxidase-labeled streptavidin, and hydrogen peroxide chromogen substrate and chromogen signal enhancer before being counterstained. The immunostained slides were then scored microscopically, with the observers blind to outcome, on a scale representing the estimated proportion of positive-staining tumor cells ranging from 0 to 5 (none = 0; < 1/100 = 1; 1/100 to 1/10 = 2; 1/10 to 1/3 = 3; 1/3 to 2/3 = 4; > 2/3 = 5). An intensity score was also given, representing the estimated average staining intensity of positive cells (1 = weak; 2 = intermediate; 3 = strong). On the basis of an earlier study,¹⁴ a formal cut point analysis was performed using disease-free survival as the end point. The "best" cut point (ie, lowest score dichotomizing patients into groups with statistically significant distinct outcomes [low risk and high risk]) was greater than zero (ie, any positive staining). This study was based on a subset of the same patients (ie, those treated by mastectomy with or without postoperative radiation). In addition, another formal cut point analysis was performed in the mastectomy-only patients with local failure as the end point. In this analysis, all possible cut points were highly significant ($P = .005$). Thus, we used the same previously validated and published cut point (any positive staining) to define *p53* positivity.

Statistical Analysis

Relationships between prognostic factors and administration of adjuvant RT were evaluated using χ^2 tests. Distant failure before local failure is a competing risk and a potential source of bias if ignored.²¹ Estimates of local failure rates were therefore computed in two ways, using the usual Kaplan-Meier method with groups being compared by the log rank test and using cause-specific cumulative incidence²² with groups being compared by the method of Pepe and Mori.²³ Cumulative incidence analyses were performed with SAS macros (SAS Institute, Cary, NC) kindly provided by the National Surgical Adjuvant Breast and Bowel Project. All computations were performed with SAS software (version 6.11).

RESULTS

Patient and Tumor Characteristics

A total of 1,530 patients were included in these analyses, 259 (17%) of whom received adjuvant RT and 1,271 (83%) of whom did not (Table 1). Because the patient population was not randomized, there were a number of differences between the study groups. Patients who received RT had more positive axillary lymph nodes ($P < .001$), larger tumors ($P < .001$), and higher S-phase fractions ($P = .04$) and were more often estrogen receptor-negative ($P < .001$) and progesterone receptor-negative ($P < .001$). These patients more frequently received adjuvant chemotherapy ($P < .001$) and adjuvant endocrine therapy ($P = .04$). However, there were no significant differences in age at diagnosis, DNA ploidy status, or *p53* status between patients who did or did not receive adjuvant RT.

All local failure rates were determined by the cumulative incidence method (unless otherwise stated) to account for competing risks. However, for purposes of comparison, the

Table 1. Demographic and Tumor Characteristics

Factor	No RT (n = 1,271)		RT (n = 259)		P
	No. of Patients	%	No. of Patients	%	
Involved nodes					< .001
0	561	44	50	19	
1-3	368	29	73	28	
4+	342	27	136	53	
Tumor size					< .001
≤ 2 cm	387	30	53	20	
> 2 cm	884	70	206	80	
Age at diagnosis					.51
≤ 35 years	47	4	10	4	
35-65 years	804	63	173	67	
> 65 years	420	33	76	29	
ER					< .001
ER+	993	78	176	68	
ER-	278	22	83	32	
PgR					.027
PgR+	704	57	123	49	
PgR-	530	43	126	51	
Unknown	37		10		
DNA ploidy					.24
Diploid	339	34	62	29	
Aneuploid	672	66	149	71	
Unknown	257		48		
S-phase fraction					.036
Low (≤ 6%)	271	30	43	23	
Intermediate (6%-10%)	220	24	34	19	
High (≥ 10%)	426	46	102	59	
Unknown	354		80		
Adjuvant endocrine therapy					.035
Yes	440	35	107	41	
No	828	65	150	59	
Unknown	3		2		
Adjuvant chemotherapy					< .001
Yes	444	35	127	49	
No	823	65	131	51	
Unknown	4		1		
p53 status					.24
p53-	620	49	116	44	
p53+	651	51	143	56	

local failure rate, as determined by the Kaplan-Meier method, is also presented in Tables 2 and 3. The 5-year actuarial local failure rates were 15.9% and 13.0%, respectively, for patients who did or did not receive adjuvant RT after surgery. Five-year actuarial local failure rates are shown in Tables 2 and 3 for each of the factors included in these analyses. The higher local failure rate for patients who received radiation was probably due to biologically more aggressive tumors in this group, as manifested by a greater preponderance of poor prognostic factors, because this was not a randomized study. Table 4 further illustrates this point. It shows the distribution of patients and their local control status by tumor stage and the number of involved nodes.

For the 1,271 patients who did not receive adjuvant RT, the following factors were associated with higher local failure rates in univariate analyses: positive axillary lymph nodes ($P < .001$), large (> 2 cm) tumors ($P < .001$), ER negativity ($P = .04$), high S-phase fractions ($P < .001$), administration of adjuvant chemotherapy ($P = .003$), and positive p53 status ($P < .001$) (Table 2). The apparent negative effect of adjuvant chemotherapy on local control was due to the greater likelihood of poor-risk patients (eg, those with more involved nodes) being given adjuvant treatment, so that any benefit of this treatment was more than offset by the initially higher risk. No significant relationships were observed with regard to age at diagnosis, DNA ploidy, progesterone receptor status, or adjuvant endocrine therapy (Table 2). Risk of local relapse in the mastectomy-only patients was nearly twice as high for p53-positive patients compared with p53-negative patients (16.5% v 9.1%, respectively; $P < .001$) (Fig 1A).

For the 259 patients who did receive adjuvant RT, high S-phase fraction ($P < .001$) and positive p53 status ($P = .009$) were associated with higher local failure rates (Table 3). No significant relationships were observed with positive axillary lymph nodes, tumor size, age at diagnosis, estrogen or progesterone receptor status, or adjuvant endocrine therapy or chemotherapy (Table 3). However, the numbers of patients and local failures were relatively small in some subsets, resulting in decreased statistical power to detect significant differences. Nevertheless, the risk of local failure was substantially higher for p53-positive patients than for p53-negative patients (21.5% v 9.3%, respectively; $P = .04$) (Fig 1B).

Although accounting for the competing risk of distant failure results in lower estimates of local failure rates compared with the Kaplan-Meier estimate, the relative differences among groups were virtually identical, regardless of the method of estimation. That is, accounting for the competing risk of distant failure does not change the direction or relative magnitude of the effect in the univariate setting. Therefore, we used Cox proportional hazards regression, which censors competing risks, to determine which factors provide independent prognostic information for predicting local failure. Both forward stepwise variable selection and backward elimination were used to construct the most parsimonious models.

Separate multivariate analyses in patients who did or did not receive adjuvant RT confirmed that p53 status was a strong independent predictor of local failure (Table 5). The factors that were also significant independent predictors of local failure in the No RT group were involved nodes ($P < .001$), tumor size ($P = .005$), lack of adjuvant endocrine therapy ($P = .003$), and high S-phase fraction ($P = .02$).

Table 2. Univariate 5-Year Local Recurrence Rates in the No RT Patients (n = 1,271)

Factor	No. of Patients	KM Actuarial Estimate* (%)	Log-Rank P†	Cumulative Incidence‡ (%)	Pepe P§
All patients	1,271	14.7		13.0	
Involved nodes			< .001		< .001
0	561	8.4		7.8	
1-3	368	14.3		12.7	
4+	342	27.2		21.9	
Tumor size			< .001		< .001
≤ 2 cm	387	9.8		9.0	
> 2 cm	884	17.0		14.7	
Age at diagnosis			.28		.62
≤ 35 years	47	23.2		19.3	
35-65 years	804	14.9		13.3	
> 65 years	420	13.3		11.7	
ER			.08		.04
ER+	993	13.6		12.0	
ER-	278	18.4		16.4	
PgR			.17		.16
PgR+	704	14.2		12.7	
PgR-	530	15.2		13.4	
DNA ploidy			.29		.29
Diploid	339	13.3		11.9	
Aneuploid	672	17.1		14.8	
S-phase fraction			< .001		< .001
Low (≤ 6%)	271	12.0		10.6	
Intermediate (6%-10%)	220	10.1		9.1	
High (≥ 10%)	426	22.3		19.1	
Adjuvant endocrine therapy			.70		.40
Yes	440	13.6		11.9	
No	828	15.3		13.6	
Adjuvant chemotherapy			< .001		.003
Yes	444	20.4		17.4	
No	823	11.9		10.6	
p53 status			< .001		< .001
Negative	620	10.1		9.1	
Positive	651	19.2		16.5	

*Local recurrence rate estimated as $1 - S(t)$, where $S(t)$ is the Kaplan-Meier estimate of survival at time t , with other events censored.

†Log-rank test comparing time to local recurrence, with other events censored.

‡Local recurrence rate estimated as the cause-specific cumulative incidence of local recurrence (Gaynor et al²²).

§Weighted marginal probability test of Pepe and Mori.²³ This is a two-sample test. In cases of more than two groups, pairwise tests were performed and the minimum Sidak adjusted P value is reported.

The sample size in this analysis was decreased to the 889 patients with complete data for all factors. Nevertheless, the multivariate relative risk of local failure in this subgroup of p53-positive tumors was 1.7 ($P = .006$).

The increased local failure risk of larger tumors and involved nodes was expected. Adjuvant endocrine therapy was shown to be beneficial in this analysis, whereas it did not seem to be beneficial in the univariate analysis. This finding is probably because the benefit of endocrine therapy was counterbalanced by the greater likelihood of its being used in poor-risk patients.

In patients who received adjuvant RT, involved axillary lymph nodes ($P = .02$), p53 accumulation ($P = .02$), and lack

of adjuvant chemotherapy ($P = .01$) were independent predictors of local failure. In the RT group, the multivariate relative risk for local failure in p53-positive tumors was 2.5 ($P = .02$). Lack of chemotherapy was associated with an increased risk of local failure in the multivariate analysis (relative risk, 2.5), although no similar relationship was identified in the univariate analysis. As with endocrine therapy in the No RT group, the patients most likely to receive chemotherapy were also those at highest risk. Therefore, the potential benefit of chemotherapy was counterbalanced by more biologically aggressive tumors with intrinsically higher relapse rates. Once confounding variables were adjusted for, the benefit of chemotherapy on local control became apparent.

Table 3. Univariate 5-Year Local Recurrence Rates in the RT Patients (n = 259)

Factor	No. of Patients	KM Actuarial Estimate* (%)	Log-Rank P†	Cumulative Incidence‡ (%)	Pepe P§
All patients	259	18.8		15.9	
Involved nodes			.11		.34
0	50	11.6		10.7	
1-3	73	14.1		12.4	
4+	136	24.3		19.5	
Tumor size			.79		.39
≤ 2 cm	53	17.8		14.7	
> 2 cm	206	19.0		16.2	
Age at diagnosis			.41		.78
≤ 35 years	10	10.0		10.0	
35-65 years	173	18.1		15.5	
> 65 years	76	21.8		19.8	
ER			.45		.20
ER+	176	17.2		14.2	
ER-	83	22.2		19.4	
PgR			.53		.86
PgR+	123	19.3		15.9	
PgR-	126	16.9		14.7	
DNA ploidy			.1		.05
Diploid	62	13.0		10.7	
Aneuploid	149	21.0		17.4	
S-phase fraction			.02		< .001
Low (≤ 6%)	43	17.8		15.6	
Intermediate (6%-10%)	34	4.8		3.5	
High (≥ 10%)	102	25.6		20.7	
Adjuvant endocrine therapy			.6		.91
Yes	107	16.7		14.9	
No	150	20.9		17.0	
Adjuvant chemotherapy			.20		.18
Yes	127	17.4		14.1	
No	131	20.6		17.9	
p53 status			.01		.009
Negative	116	10.4		9.3	
Positive	143	26.8		21.5	

*Local recurrence rate estimated as $1 - S(t)$, where $S(t)$ is the Kaplan-Meier estimate of survival at time t , with other events censored.

†Log-rank test comparing time to local recurrence, with other events censored.

‡Local recurrence rate estimated as the cause-specific cumulative incidence of local recurrence (Gaynor et al²²).

§Weighted marginal probability test of Pepe and Mori.²³ This is a two-sample test. In cases of more than two groups, pairwise tests were performed and the minimum Sidak adjusted P value is reported.

DISCUSSION

Given the percentage of women who will have a local-regional recurrence after mastectomy and the local control and survival benefit of adjuvant RT in select patients, it is important to identify novel molecular markers predictive of local failure. These new predictors will aid in the identification of women most likely to benefit from additional local therapy. In this study, we assessed traditional indicators of distant disease-free survival for local failure prognostic value. We found that a promising new predictor of distant disease-free survival also has local failure prognostic value. That putative predictor is the tumor suppressor gene *p53*.

The *p53* tumor suppressor gene is an important negative regulator of cellular proliferation. The *p53* gene product is

induced in response to DNA damage. Evidence shows that the expression of *p53* protein leads to cell cycle arrest in G_1 and in some cases to programmed cell death, or apoptosis.²⁴⁻²⁶ Arrest of the cell cycle, presumably to allow for DNA repair, as well as apoptosis, prevents the replication of damaged DNA and the subsequent propagation of genetic defects. This DNA replication control mechanism maintains fidelity in chromosomal transmission and has earned *p53* the title "guardian of the genome."²⁶

Alterations in *p53* lead to loss of its cell growth-regulatory function, resulting in accelerated cell growth and increased DNA mutation frequency. The unchecked propagation of these mutations is thought to contribute to the development of human cancers. The hypothesis that altered

Table 4. Patient Distribution and Local Control Status by Tumor Stage and Number of Involved Nodes

Tumor Stage	Involved Lymph Nodes	Radiation Therapy											
		No						Yes					
		p53-			p53+			p53-			p53+		
		All	Local Status		All	Local Status		All	Local Status		All	Local Status	
	NED	LRF		NED	LRF		NED	LRF		NED	LRF		
T1	0	108	104	4	106	101	5	4	4	—	5	5	—
	1-3	65	61	4	44	38	6	8	7	1	13	11	2
	4-9	22	20	2	21	14	7	7	6	1	6	3	3
	> 9	8	6	2	13	9	4	6	6	—	4	2	2
T2	0	126	115	11	158	136	22	15	14	1	17	13	4
	1-3	108	95	13	104	86	18	16	14	2	25	22	3
	4-9	61	58	3	70	54	16	16	13	3	14	14	—
	> 9	36	23	13	34	28	6	18	15	3	21	16	5
T3	0	34	32	2	29	22	7	5	5	—	4	4	—
	1-3	16	16	—	31	23	8	6	5	1	5	3	2
	4-9	16	13	3	18	16	2	7	7	—	10	8	2
	> 9	20	12	8	23	10	13	8	6	2	19	13	6

Abbreviations: NED, no evidence of disease; LRF, local-regional failure.

p53 is intimately involved in the genesis of tumors is supported by the fact that it is the most commonly found genetic alteration in human cancers. With regard to breast cancer, altered p53 has been identified in 15% of in situ and 50% of invasive disease. Because loss of p53 function leads to higher proliferative and lower apoptosis rates, altered p53 should therefore be associated with a worse clinical outcome. Indeed, many studies in breast cancer have shown a correlation between p53 protein accumulation and a poor clinical outcome.¹⁴⁻¹⁷ In general, however, these studies did not report the prognostic value of p53 for local failure, which we have retrospectively assessed here.

This is the largest study of its kind in the literature, and it demonstrates that the nuclear accumulation of p53 is a significant adverse prognostic factor for local failure in mastectomy-treated invasive breast cancer. This was also true when the study population was divided according to the presence or absence of adjuvant RT. We present the results of two statistical methodologies for analyzing local failure.

The first, the cause-specific cumulative incidence method, was used because it accounts for competing risks in this population. The second, the more common but less accurate Kaplan-Meier method, was used to make comparisons of our results with other published literature easier. Multivariate analysis revealed that p53 remains a significant independent prognostic factor. The sample size of this study strengthens the reliability of these findings.

We analyzed our patients who received adjuvant RT separately for two reasons. First, the RT group was significantly different from the No RT group in several characteristics. Second, there is substantial controversy in the literature about the effect of p53 on radiation sensitivity.²⁷⁻²⁹ For instance, in a transplanted fibrosarcoma model using tumors that differed only with respect to functional p53 status, mutant p53 tumors were more radioresistant than their wild-type counterparts.³⁰ Conversely, other laboratory studies have found no correlation between p53 status and radiation sensitivity.³¹ However, in a recent clinical publi-

Fig 1. p53-positive tumors had a significantly higher local failure probability in (A) the No RT group and (B) the RT group (dotted line, Kaplan-Meier method; solid line, cumulative incidence method).

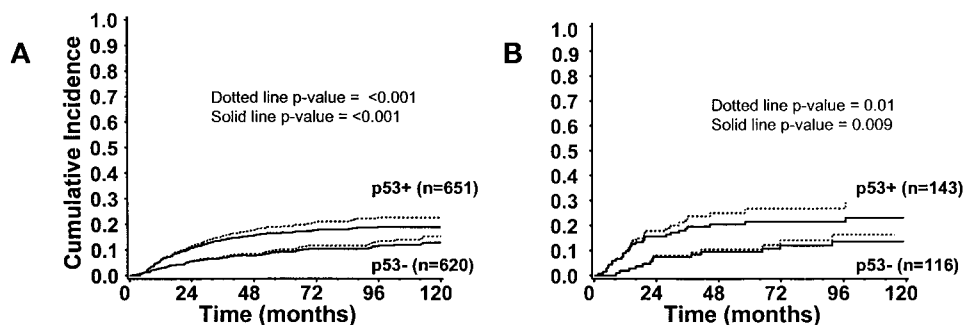


Table 5. Multivariate Cox Model for Patients Who Did and Did Not Receive Adjuvant Radiation Therapy

Factor	P	Relative Risk	95% Confidence Interval
No adjuvant RT			
Involved nodes	< .001	2.0	1.6-2.5
Tumor size > 2 cm	.005	1.9	1.2-3.1
Adjuvant endocrine therapy	.003	0.6	0.4-0.8
p53-positive	.006	1.7	1.2-2.4
High S-phase fraction*	.02	1.6	1.1-2.2
ER/PgR positive	NS	—	—
DNA aneuploidy	NS	—	—
Adjuvant chemotherapy	NS	—	—
Adjuvant RT			
Involved nodes	.02	1.8	1.1-3.1
Tumor size > 2 cm	NS	—	—
Adjuvant endocrine therapy	NS	—	—
p53-positive	.02	2.5	1.1-5.7
High S-phase fraction*	.06	2.2	1.0-5.0
ER/PgR positive	NS	—	—
DNA aneuploidy	NS	—	—
Adjuvant chemotherapy	.01	0.4	0.2-0.8

NOTE. The "No adjuvant RT" group included 889 patients with complete data representing 136 local recurrences. The "Adjuvant RT" group included 173 patients representing 34 local recurrences.

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

*Low and intermediate S-phase fractions combined.

cation, p53 protein accumulation was associated with increased resistance to RT in head and neck cancer patients.³² This finding is consistent with other clinical studies that have shown an increased resistance to chemotherapeutic agents associated with altered p53 function.³³

It is not possible with the present data to definitively assess the influence of p53 on radiation sensitivity. However, the association of p53 protein accumulation with a

similar magnitude of change in local failure (approximately double) in both the No RT and RT groups does not support the hypothesis of differential radiation sensitivity. Interestingly, a comparable yet relatively small study of conservatively treated breast cancer patients by Silvestrini et al³⁴ supports the hypothesis of differential radiation sensitivity. In that study, p53 had local failure-predictive value in patients treated with lumpectomy alone but did not have local failure-predictive value in patients treated with lumpectomy and radiation.

A limitation to this analysis may be the retrospective design of this study. Because therapy was not standardized, treatment preferences could have biased the results. In particular, details of the systemic adjuvant therapies selected were not available. Systemic therapy has been demonstrated to have an effect on local control in some studies.³⁵ In the multivariate analysis, however, p53 status remained an independent prognostic factor after the effect of systemic adjuvant therapy had been considered.

In summary, there is a significant direct correlation between local failure and altered p53 function in mastectomy-treated breast cancer patients. p53 has prognostic significance independent of traditional prognostic factors. Perhaps as more prognostic indicators become available, this information will help to identify those patients who are at greater risk of local failure and therefore may warrant more aggressive local management.

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REFERENCES

- Overgaard M, Hansen P, Overgaard J, et al: Postoperative radiotherapy in high-risk premenopausal women with breast cancer who receive adjuvant chemotherapy. *N Engl J Med* 337:949-955, 1998
- Ragaz J, Stewart JM, Nhu L, et al: Adjuvant radiotherapy and chemotherapy in node-positive premenopausal women with breast cancer. *N Engl J Med* 337:956-962, 1998
- Early Breast Cancer Trialists' Collaborative Group: Effects of radiotherapy and surgery in early breast cancer: An overview of the randomized trials. *N Engl J Med* 333:1444-1455, 1995
- Schwaibold F, Fowble B, Lawrence J, et al: The results of radiation therapy for isolated local regional recurrence after mastectomy. *Int J Radiat Oncol Biol Phys* 21:299-310, 1991
- Aberizk W, Silver B, Henderson C, et al: The use of radiotherapy for treatment of isolated locoregional recurrence of breast carcinoma after mastectomy. *Cancer* 58:1214-1218, 1986
- Cuzick J, Stewart H, Peto R, et al: Overview of randomized trials of postoperative adjuvant radiotherapy in breast cancer. *Cancer Treat Rep* 71:15-25, 1987
- Haagensen CD, Bodian C: A personal experience with Halsted's radical mastectomy. *Ann Surg* 199:143-150, 1984
- Pierce LJ, Lichter AS: Defining the role of postmastectomy radiotherapy: The new evidence. *Oncology* 10:991-1001, 1996
- Pierce L, Glatstein E: Postmastectomy radiotherapy in the management of operable breast cancer. *Cancer* 74:477-485, 1994 (suppl)
- Valagussa P, Bonadonna G, Veronesi U, et al: Patterns of relapse and survival following radical mastectomy. *Cancer* 41:1170-1178, 1978
- Donegan WL, Perz-Mesa CM, Watson F: Biostatistical study of locally recurrent breast carcinoma. *Surg Gynecol Obstet* 122:529-540, 1996

12. Mentzer SJ, Osteen RT, Wilson RE: Local recurrence and the deep resection margin in carcinoma of the breast. *Surg Gynecol Obstet* 163:513-517, 1986
13. Houghton J, Baum M, Haybittle JL: Role of radiotherapy following total mastectomy in patients with early breast cancer: The Closed Trials Working Party of the CRC Breast Cancer Trials Group. *World J Surg* 18:117-122, 1994
14. Allred DC, Clark GM, Elledge R, et al: Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *J Natl Cancer Inst* 85:200-206, 1993
15. Elledge RM, Fuqua SAW, Clark GM, et al: The role and prognostic significance of p53 gene alterations in breast cancer. *Breast Cancer Res Treat* 27:95-102, 1993
16. Friedrichs K, Gluba S, Eidtmann H, et al: Over-expression of p53 and prognosis in breast cancer. *Cancer* 72:3641-3647, 1993
17. Kovach JS, Hartmann A, Blaszyk H, et al: Mutation detection by highly sensitive methods indicates that p53 genetic mutations in breast cancer can have important prognostic value. *Proc Natl Acad Sci U S A* 93:1093-1096, 1996
18. Powell B, Garola RE, Chamness GC, et al: Measurement of progesterone receptor in human breast cancer. *Cancer Res* 39:1678-1682, 1979
19. McGuire WL, De La Garza M, Chamness GC: Evaluation of estrogen receptor assays in human breast cancer tissue. *Cancer Res* 37:637-639, 1977
20. Dressler LG, Seamer LC, Owens MA, et al: DNA flow cytometry and prognostic factors in 1331 frozen breast cancer specimens. *Cancer* 61:420-427, 1988
21. Gelman R, Gelber R, Henderson IC, et al: Improved methodology for analyzing local and distant recurrence. *J Clin Oncol* 8:548-555, 1990
22. Gaynor JJ, Feuer EJ, Tan CC, et al: On the use of cause-specific failure and conditional failure probabilities: Example from clinical oncology data. *JASA* 88:400-409, 1993
23. Pepe MS, Mori M: Marginal or conditional probability curves in summarizing competing risks failure time data? *Stat Med* 12:737-751, 1993
24. Ruley HE: p53 and response to chemotherapy and radiotherapy. *Principles Pract Oncol Update* 11:7-19, 1997
25. Kastan MB: The p53 tumor suppressor gene: A multifaceted cancer threat. *Adv Oncol* 12:3-7, 1996
26. Lane DP: p53, guardian of the genome. *Nature* 358:15-16, 1992
27. McIlwrath AJ, Vasey PA, Ross GM, et al: Cell cycle arrests and radiosensitivity of human tumor cell lines: Dependence on wild-type p53 for radiosensitivity. *Cancer Res* 54:3718-3722, 1994
28. Kubiczek EK, Yin J, Lin K, et al: p53 mutational status and survival of human breast cancer MCF-7 cell variants after exposure to x-rays of fission neutrons. *Radiat Res* 142:256-262, 1995
29. Lee JM, Bernstein A: p53 mutations increase resistance to ionizing radiation. *Proc Natl Acad Sci U S A* 90:5742-5746, 1993
30. Lowe SW, Bodis S, McClatchey A, et al: p53 status and the efficacy of cancer therapy in vivo. *Science* 266:807-810, 1994
31. Brachman DG, Beckett M, Graves D, et al: p53 mutation does not correlate with radiosensitivity in 24 head and neck cancer cell lines. *Cancer Res* 53:3667-3669, 1993
32. Raybaud-Diogene H, Fortin A, Morency R, et al: Markers of radioresistance in squamous cell carcinomas of the head and neck: A clinicopathologic and immunohistochemical study. *J Clin Oncol* 15:1030-1038, 1997
33. Lowe SW, Ruley HE, Jacks T, et al: p53 dependent apoptosis modulates the cytotoxicity of anti-cancer agents. *Cell* 74:957-967, 1993
34. Silvestrini R, Veronisi S, Benini E, et al: Expression of p53, glutathione S-transferase-pi, and Bcl-2 proteins and benefit from adjuvant radiotherapy in breast cancer. *J Natl Cancer Inst* 89:639-645, 1997
35. Fisher B, Redmond C, Poisson R, et al: Eight-year results of a randomized clinical trial comparing total mastectomy and lumpectomy with or without irradiation in the treatment of breast cancer. *N Engl J Med* 320:822-828, 1989