Survival of Patients with Metastatic Breast Carcinoma

Importance of Prognostic Markers of the Primary Tumor

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BACKGROUND. Women with metastatic breast carcinoma have a highly variable clinical course and outcome. Intrinsic genetic heterogeneity of the primary breast tumor may play a role in this variability and may explain it in part. Therefore, the authors tested the hypothesis that the characteristics of primary breast tumors are important determinants of prognosis and survival in patients with metastatic breast carcinoma.

METHODS. The prognostic significance of the biology of the primary tumor for outcome in patients with metastatic breast disease was assessed in 346 patients with lymph node positive breast carcinoma who developed distant, recurrent disease. Traditional prognostic indicators (age, tumor size, number of involved lymph nodes, sites of recurrence, disease free interval [DFI], adjuvant treatments, estrogen receptor [ER] expression, progesterone receptor [PgR] expression, S-phase fraction [SPF], and DNA ploidy), together with three newer biologic markers (c-*erb*B-2, p53, and bcl-2) were assessed. Sites of recurrence were defined as nonvisceral (bone and locoregional lymph nodes) or visceral (lung, liver, brain, and other organs).

RESULTS. The median duration of survival was 17.8 months (95% confidence interval, 15.2–21.5 months). Univariate analysis showed that age $>$ 50 years, visceral disease, and shorter DFI were associated significantly with poor outcome ($P < 0.05$). In addition, the molecular phenotype of the primary breast tumor was significant, with primary tumors that showed ER negativity and PgR negativity, high SPF, aneuploidy, accumulation of p53 protein, and lower bcl-2 expression, together with c-*erb*B-2 overexpression, all associated with a poorer clinical outcome ($P < 0.05$). In a multivariate analysis, older age, visceral disease, shorter DFI, PgR negativity, high SPF, and lower bcl-2 expression were significant predictors of worse survival ($P < 0.05$).

CONCLUSIONS. In addition to traditional risk factors, bcl-2 negativity was associated significantly with a worse clinical outcome. Biologic features of primary tumors were correlated independently with outcome after first recurrence in patients with metastatic breast carcinoma and may be used as indicators of prognosis in the metastatic setting. ***Cancer*** **2003;97:545–53.** © *2003 American Cancer Society.*
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KEYWORDS: breast carcinoma, prognosis, progesterone receptor, S-phase fraction, bcl-2.

Although significant advances in survival have been achieved with adjuvant treatments in patients with early-stage, primary breast carcinoma, patients with recurrent metastatic breast carcinoma usually die of their disease.[1] However, the clinical course of these patients varies greatly; some patients die a short time after they develop recurrent disease, whereas others may live well for many years. Treat-

ments in these patients frequently provide long-term remission with concomitant improvement in the quality of life.

The value of prognostic factors in patients with early-stage breast carcinoma has been confirmed in a large number of studies. However, there have been only a few reports on prognostic factors in patients with metastatic disease. Traditional biologic markers of the primary breast tumor, such as estrogen receptor (ER) expression and lymph node involvement, can predict clinical outcome after patients develop recurrent disease.[2,3] Other factors, such as shorter disease free interval (DFI) and visceral sites of involvement, also indicate a worse clinical outcome after recurrence.[4] However, the contribution of the newer biologic markers has not been systematically analyzed in patients with metastatic breast carcinoma. Better characterization of an individual's prognosis may aid in their clinical management, for example, by providing adequate information to help with planning and identifying patients who are at poorest risk who may choose from the onset to participate in novel investigational therapies.

We hypothesized that the specific biology of the primary tumor can determine the duration of survival after the appearance of macroscopic distant metastases. To test this hypothesis, we examined newer molecular determinants of breast carcinoma in addition to traditional prognostic factors in patients with positive lymph nodes who developed recurrent disease to determine which features of the presenting primary tumor are capable of predicting the clinical outcome of patients with metastatic disease.

MATERIALS AND METHODS

Patients

Patients were identified retrospectively from a large data base of patients who had hormone receptor assays or flow cytometry performed in a central laboratory (University of Texas Health Science Center at San Antonio, San Antonio, TX). Eligible patients were diagnosed initially between 1970 and 1991 and presented with primary breast carcinoma and underwent radical or modified radical mastectomy or underwent lumpectomy and axillary lymph node dissection with postoperative radiation therapy. More than 250 hospitals submitted tumor specimens to this central laboratory for steroid hormone receptor analysis. To obtain demographic information (age and menopausal status), including extent of disease (including the number of axillary lymph nodes), the individual hospitals were contacted, and retrospective chart reviews were requested for each patient. These patients had no evidence of distant metastases at the time of their diagnosis and were followed, as described previously,[5] for recurrent disease (defined as the first clinically recognized evidence of local or distant recurrence) and survival. Patients who presented with lymph node positive disease and had interpretable assays for the various biomarkers were included in this analysis.

Recurrences were defined as nonvisceral (locoregional lymph nodes and bone) or visceral (brain, liver, lung, and other organs). Sites of recurrences were documented by physical examination, X-rays, and/or other imaging modalities. Patients were classified with nonvisceral metastases if these were the only sites of involvement; otherwise, they were categorized with visceral metastases.

Survival was defined as the time from recurrence to the last contact or death. Of 346 patients, there were 299 deaths as of January 2000, with a median of 346 months of follow-up for live patients.

Biologic Markers and Prognostic Variables

Primary tumor size and the number of positive axillary lymph nodes were extracted from the medical records. The DFI, defined as the time from diagnosis to first recurrence, was divided into three groups ($>$ 12 months, 12–60 months, and $>$ 60 months) to create DFI groups. Central evaluation for ER, progesterone receptor (PgR), Bcl-2, S-phrase fraction (SPF), and ploidy were performed in a central laboratory (University of Texas Health Science Center at San Antonio). ER and PgR were measured by ligand-binding assays. Tumors were considered ER positive or PgR positive if they contained $>$ 3 fmol/mg protein or $>$ 5 fmol/mg protein, respectively.[5]

Standard methods for immunohistochemical analysis have been described in detail elsewhere.[6] Briefly, for p53 and bcl-2 immunostaining, the sections were blocked with 10% ovalbumin before incubation with a monoclonal antibody to p53 (cocktail of clones 1801 and 240; Novocastra, Newcastle, United Kingdom) at 1:20 dilution and 1:10 dilution, respectively; bcl-2 monoclonal antibody (clone 124; DAKO, Carpinteria, CA) at 1:25 dilution; Ki-67 (clone MiB1; Immunotech, Westbrook, ME) at 1:100 dilution; and c-*erb*B-2 (clone TAB 250; Triton, Alameda, CA) at 1:500 dilution for 60 minutes. Heat-induced antigen retrieval (citrate buffer, pH 6.0, boiled in a pressure cooker for 5 minutes) was employed when assessing p53, and bcl-2. Secondary rabbit antimouse antibody was then applied. After rinsing, the slides were incubated with streptavidin horseradish peroxidase (1:100) for 30 minutes, rinsed with phosphate-buffered saline (PBS), exposed to diaminobenzidine tetrahydrochloride chromogen for 10 minutes, rinsed with autobuffer

and PBS, counterstained with 1% methyl green, rinsed with deionized water, and then mounted.

Slides that were immunostained for p53, bcl-2, and c-*erb*B-2 were scored by estimating the proportion and average intensity of positive tumor cells (proportion score: 0, none; 1, $<$ 1/100; 2, 1/100–1/10; 3, 1/10–1/3; 4, 1/3–2/3; 5, $>$ 2/3; intensity score: 0, none; 1, weak; 2, intermediate; 3, strong). The proportion and intensity scores were added to obtain a total score (ranging from 0 to 8). For bcl-2, only cytoplasmic staining of tumor cells was evaluated. For p53, only positively stained nuclei were scored. For c-*erb*B-2, only membranous staining was evaluated. Based on prior studies,[7,8] a cut-off value of ≥ 6 ($\geq$ 33% positively stained cells) was chosen prospectively for bcl-2. For p53, the cut-off value of ≥ 2 ($\geq$ 1% positively stained cells) was used.[6] For c-*erb*B-2, a cut-off value of > 2 prospectively was deemed positive, based on previous studies.[9]

The details of DNA flow cytometry have been described elsewhere.[5] In brief, approximately 100 mg of frozen pulverized tumor were homogenized manually, filtered, and centrifuged at 759 $\times$ *g* for 45 minutes. Chicken red cells in PBS were added as an internal standard. Cells were lysed and stained for DNA by incubation in a modified Krishan hypotonic sodium citrate staining buffer containing propidium iodide as the DNA fluorochrome. DNA-stained nuclei were prepared and run on an Epics V flow cytometer (Coulter Electronics, Hialeah, FL). Fifty thousand tumor events were acquired on a single-parameter, 256-channel fluorescence histogram, and the cell cycle distributions (G0 G1, presynthetic phase; S, synthetic phase; G_2M, postsynthetic and mitotic phase) were analyzed by a cell-cycle software program (Modfit; Verity Software House, Inc.). DNA content was regarded as diploid if G0 G1 peaks were superimposed and were considered aneuploid only if separate peaks were seen. SPF measurements were divided in to low ($<$ 6%), intermediate (6–10%), and high ($>$ 10%), based on earlier studies.[5]

Statistical Methods

Univariate analyses of survival after first recurrence were performed by calculating Kaplan–Meier survival curves and comparing subsets of patients using log-rank tests. Results of these analyses are expressed either as median survival with 95% confidence intervals obtained from Proc LIFETEST in SAS software (version 8.2 for Windows; SAS, Inc., Cary, NC) or as hazard ratios with 95% confidence intervals obtained from Proc PHREG. Multivariate analyses were performed by creating a Cox proportional hazards model using Proc PHREG. Significant prognostic factors initially were identified using a stepwise variable-selection technique, and the process was then repeated using backward elimination of nonsignificant factors. Both procedures produced the same final model. A prognostic index (PI) was then created for each patient by summing the products of each significant factor in the Cox model and its β coefficient. The distribution of PI scores was then divided into quartiles, and Kaplan–Meier survival curves were calculated for each quartile, from which median survival and 95% confidence intervals were obtained.

RESULTS

In this study, we analyzed 346 patients with interpretable assays for all biomarkers from a total of 1256 patients with recurrent disease who initially had lymph node positive disease. Comparison between the patients analyzed and all patients with recurrent disease showed no selection bias or statistically significant difference for age at diagnosis, tumor size, DFI, site of recurrence, ER status, PgR status, frequency of chemotherapy, p53 expression, or HER-2 expression (results not shown).

Univariate Analysis

The characteristics of 346 patients with metastatic disease at the time of first recurrence are summarized in Table 1. The median age at initial diagnosis of the primary tumor was 56.4 years (range, 26.3–90.4 years). The majority of patients (221 of 346 women; 64%) were age $\geq$ 50 years. The primary tumors were mainly ER positive (75%), PgR positive (51%), aneuploid (62%), had a higher SPF (53%), and had greater p53 accumulation (57%), were without c-*erb*B-2 overexpression (73%), and had low bcl-2 expression (65%). The median number of lymph nodes dissected and examined in these patients was 17 lymph nodes (range, 1–47 lymph nodes) in patients with 1–3 positive lymph nodes, 19 lymph nodes (range 5–61) in patients with 4–9 positive lymph nodes, and 24 lymph nodes (range, 11–56) in patients with $>$ 10 positive lymph nodes. The majority of these patients with positive lymph nodes had received adjuvant chemotherapy (60%), and adjuvant hormonal therapy was administered to 48% of these patients. The sites of initial recurrence were mainly nonvisceral (66%), and the median overall survival after recurrence was 17.8 months (95% confidence interval, 15.2–21.5 months). The median follow-up of live patients was 346 months.

In a univariate analysis, women had significantly worse survival from the time of first recurrence if their primary tumors were characterized by ER negativity, PgR negativity, aneuploidy, high SPF, p53 accumulation, low bcl-2 expression, and c-*erb*B-2 overexpression ($P < 0.05$). In addition to these molecular char-

TABLE 1
Demographic, Clinical, and Tumor Characteristics of 346 Patients who Developed Recurrent Disease with Macroscopic Distant Metastases

Variable	No. of patients	%
All patients	346	100
Age at diagnosis (yrs)		
Median	56.4	—
Range	526.3–90.4	—
Adjuvant chemotherapy		
Any	206	60
None	140	40
Adjuvant endocrine therapy		
Any	166	48
None	180	52
Year of diagnosis		
1970–1985	213	62
1986–1997	133	38
Site of first recurrence		
Nonvisceral	227	65
Visceral	119	35
Age at diagnosis (yrs)		
< 50	125	36
≥ 50	221	64
No. of positive lymph nodes		
1–3	122	35
4–9	105	30
≥ 10	119	35
Size of primary tumor (cm)		
< 2	76	22
2–5	160	46
> 5	110	32
Estrogen receptor		
Positive	261	75
Negative	85	25
Progesterone receptor		
Positive	177	51
Negative	169	49
Ploidy		
Diploid	130	38
Aneuploid	216	62
S-phase fraction		
Low	57	16
Intermediate	104	30
High	185	54
p53		
Negative	148	43
Positive	198	57
c-*erb*B-2		
Not overexpressed	251	73
Overexpressed	95	27
bcl-2		
Low	225	65
High	121	35
Overall survival (months)		
Median	17.8	—
95%CI	15.2–21.5	—

95%CI: 95% confidence interval.

acteristics, older age (> 50 years), shorter DFI (< 12 months), and visceral metastases were associated with a worse outcome at recurrence ($P < 0.05$). Neither prior adjuvant chemotherapy nor hormonal therapy was associated with a worse postrecurrence outcome; thus, adjuvant therapy did not appear to select for more aggressive disease at the time of recurrence. Similarly, the year of diagnosis (before 1985 or after 1985), the initial tumor size, and the number of involved lymph nodes did not affect outcome (Table 2).

Multivariate Analysis

Cox regression analysis was used to examine the independent prognostic value of each variable in all 346 patients (Table 3). In the multivariate analysis, older age (> 50 years), shorter DFI (< 12 months), and visceral metastases were associated with an increased risk of death ($P < 0.05$). In addition, a number of biologic features of the primary tumor were associated independently with a worse outcome after first recurrence, including PgR negativity ($P < 0.05$), high SPF ($P < 0.05$), and low bcl-2 expression ($P = 0.07$). Figure 1 illustrates survival after first recurrence for these independent variables. The results also show that c-*erb*B-2 was not an independent predictor of death after recurrence.

PI Score by Cox Analysis

The Cox model was used to produce a PI score that combined the significant variables that were identified in the multivariate analysis. The PI score weighted these significant variables according to the presence or absence of each variable and the strength of their prognostic contribution. PgR expression (PgR positive, + 1; PgR negative, 0) was weighted and multiplied by − 0.4, SPF (low, 0; intermediate, 1; high, 2) was weighted and multiplied by + 0.2, bcl-2 expression (low, 0; high, 1) was weighted and multiplied by − 0.3, site of recurrence (visceral, 1; nonvisceral, 0) was weighted and multiplied by + 0.5, age (< 50 years, 1; > 50 years, 2) was weighted and multiplied by + 0.5, and DFI (> 12 months, 1; 12–60 months, 2; > 60 months, 3) was weighted and multiplied by − 0.3. Therefore, for example, women who had the best prognosis with primary tumors that showed PgR expression (1 × − 0.4), low SPF (0 × + 0.2), high bcl-2 expression (1 × − 0.3) and who had nonvisceral disease (0 × + 0.5), age < 50 years (1 × + 0.5), and DFI > 60 months (3 × − 0.3) had a PI score of − 1.1. Conversely, in women who had the worst outcomes with primary tumors that were PgR negative (0 × − 0.4), high SPF (2 × + 0.2), low bcl-2 (0 × − 0.3) and had visceral disease (1 × + 0.5), age > 50 years (2 × +

TABLE 2
Univariate Survival Analysis of Prognostic Markers of the Primary Tumor and Survival from First Recurrence in Patients with Metastatic Breast Carcinoma

Variable	Median survival (months)	95%CI (months)	*P* value (log-rank test)
No. of positive lymph nodes			
1–3	18.6	14.3–25.9	0.25
4–9	21.0	15.2–25.2	—
≥ 10	15.9	11.9–20.0	—
Size of primary tumor (cm)			
< 2	19.9	15.3–24.9	0.10
2–5	18.0	14.0–24.1	—
> 5	17.4	12.0–21.5	—
Estrogen receptor			
Positive	21.0	17.0–24.9	0.0007
Negative	12.0	9.0–15.2	—
Progesterone receptor			
Positive	24.7	20.0–30.1	< 0.0001
Negative	13.0	9.8–16.4	—
Ploidy			
Diploid	22.9	17.0–26.9	0.04
Aneuploid	15.2	12.8–19.2	—
S-phase fraction			
Low	31.0	25.9–41.0	0.008
Intermediate	22.9	15.8–26.0	—
High	14.3	12.6–17.6	—
p53			
Negative	24.8	20.5–27.7	0.02
Positive	14.0	10.9–16.9	—
c-*erb*B-2			
Not overexpressed	20.0	16.0–24.0	0.007
Overexpressed	14.3	9.9–18.0	—
bcl-2			
Low	15.9	13.1–19.4	0.004
High	23.3	17.7–31.0	—
Site of recurrence			
Nonvisceral	23.2	19.2–26.6	< 0.0001
Visceral	10.9	8.1–15.2	—
Year of diagnosis			
≤ 1985	17.5	12.6–21.9	0.35
> 1985	18.9	15.2–23.3	—
Adjuvant chemotherapy			
Any	17.6	14.3–22.3	0.09
None	18.0	12.0–23.3	—
Adjuvant endocrine therapy			
Any	21.5	17.7–25.9	0.9
None	14.6	12.6–19.2	—
Age at diagnosis (yrs)			
< 50	22.1	17.0–26.9	0.003
≥ 50	15.3	12.0–19.9	—
Disease free interval (months)			
< 12	14.3	9.0–17.6	0.001
12–60	18.6	14.3–22.4	—
> 60	36.0	24.1–47.7	—

95%CI: 95% confidence interval.

0.5), and DFI < 12 months (1 × − 0.3) had a PI score of 1.6.

The median survival varied significantly ($P < 0.0001$) in groups of patients with differing PI scores (Table 4, Fig. 2). The first quartile represented patients with the best prognosis and the lowest PI scores. Among this group, which included women with primary tumors that showed PgR expression, low SPF, high bcl-2 expression and had nonvisceral disease, age < 50 years, and DFI > 60 months, the median survival was 38 months. Conversely, in patients with the highest PI scores and the worst outcome, including older

TABLE 3
Multivariate Analyses of Prognostic Markers of the Primary Tumor and Survival from First Recurrence in Patients with Metastatic Breast Carcinoma

Variable	Hazard ratio (95%CI)	*P* value
Older age at diagnosis	1.64 (1.28–2.12)	0.0001
Visceral metastases	1.63 (1.27–2.10)	0.0001
Shorter DFI	1.59 (1.14–1.67)	0.0008
PgR negativity	1.52 (1.18–1.94)	0.001
Increased SPF	1.27 (1.07–1.49)	0.005
bcl-2 negativity	1.23 (1.00–1.67)	0.06

95%CI: 95% confidence interval; DFI: disease free survival; PgR: progesterone receptor; SPF: S-phase fraction.

patients with primary tumors that were negative for PgR with high SPF and low bcl-2 expression and who had visceral disease and shorter DFI, the median survival was only 8 months, one-fifth of the best prognosis quartile (Table 4). Therefore, by determining the PI score based on the clinical features and biologic factors of primary tumors, the median survival of patients with metastatic breast carcinoma can be estimated. The median survival of patients with metastatic breast carcinoma in the first quartile (PI score, from −1.2 to −0.2) was 38 months; for patients in the second quartile (PI score, −0.2 to +0.3), it was 23 months; for patients in the third quartile (PI score, +0.3 to +0.7), it was 13 months; and, for patients in the last quartile (PI score +0.7 to +1.6), it was 8 months (Table 4, Fig. 2).

DISCUSSION

Breast carcinoma is a clinically diverse and heterogeneous disease, and patients with metastatic disease have survival ranging from a few weeks to more than a decade. It has been shown that traditional prognostic markers, like ER status of the primary tumor, lymph node involvement, and site of recurrence, are capable of predicting survival in patients with metastatic breast carcinoma.[2,3] Older age was associated significantly with a worse outcome, an observation that may be due to the presence of more comorbid conditions in older women. There have been no comprehensive evaluations in large numbers of patients of the prognostic significance of newer biologic markers of breast carcinoma. The objective of this study was to better refine prognosis in patients with metastatic breast disease based on markers and characteristics of the primary tumor, so that management in these women can be planned.

The patients in this study represent women who had lymph node positive breast carcinoma and who subsequently developed recurrent disease in distant, metastatic sites. Hence, adjuvant chemotherapy or endocrine therapy was administered to the majority of the women. Patients who presented with lymph node positive disease were selected, because all of the desired biologic assays were available in these patients, and there was a higher percentage of events in these women compared with patients who had lymph node negative disease.

This analysis of a large number of patients with lymph node positive breast disease demonstrates that biologic factors of the initial primary tumor indeed are associated with postrecurrence outcome, and the prognosis for these women is determined strongly by intrinsic molecular characteristics of the primary tumor. Although metastases of tumors may continue along a path of further molecular evolution and change, early events in the primary tumor remain crucial determinants of later biologic life. It is interesting to note that the year of diagnosis, which is an indirect indication of differences in treatment for metastatic disease with time, did not have a significant impact on clinical outcome. In addition to established prognostic markers, like ER and site of recurrence, newer biologic markers (like SPF, ploidy, p53, c-*erb*B-2, and bcl-2) predicted for clinical outcome after recurrence on univariate analysis. In the multivariate analysis, independent prognostic variables that predicted a worse outcome after recurrence included visceral site of recurrence, shorter DFI, older age, PgR negativity, high SPF, and low bcl-2 expression. These findings are consistent with the hypothesis that the same intrinsic tumor biology of the primary tumor that predicts for the likelihood of recurrence in patients with early-stage breast carcinoma also plays a critical role in determining outcome after macroscopic metastatic recurrences develop in patients with advanced disease.

One of the strongest traditional prognostic factors in patients with metastatic breast carcinoma is the site of recurrence. Unlike patients with visceral metastases, women with bony metastases have a median life expectancy of many years.[10] Previous investigators also have shown that ER is important in determining prognosis in patients with metastatic breast disease.[2] It has been reported that ER positive breast carcinoma tends to recur more frequently in bone, whereas ER negative tumors tend to recur in visceral sites.[2] Tumor size and axillary lymph node status at the time of diagnosis also have been investigated as prognostic factors at the time of recurrence. Howell et al. found that axillary lymph node status and tumor size were predictors of recurrence,[3] whereas Pater et al. examined disease stage, tumor size, lymph node involve-

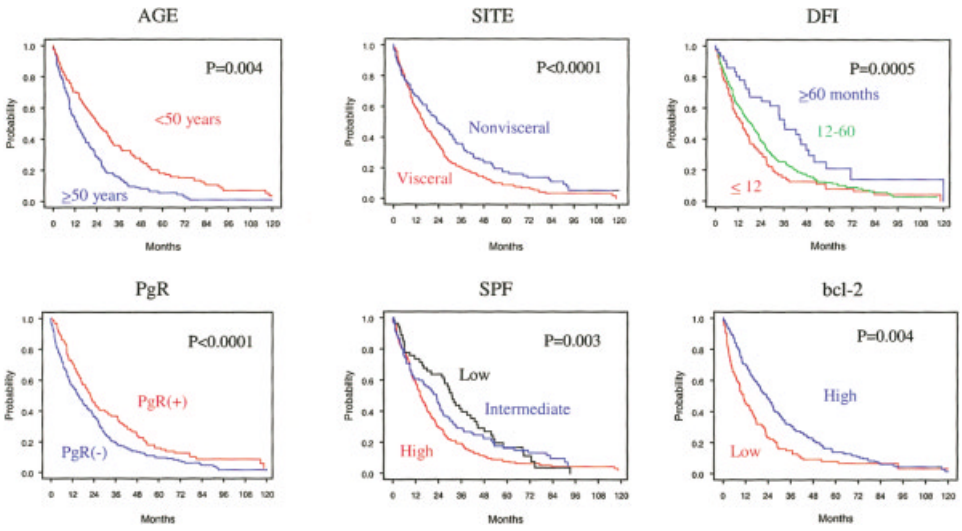

FIGURE 1. Survival after first recurrence in patients with metastatic breast carcinoma. DFI: disease free interval; PgR: progesterone receptor; SPF: S-phase fraction.

TABLE 4
Prognostic Index Score by Cox Analysis[a]

PI score in quartiles	No. of patients		Median survival in months	
	Total	Dead	(95%CI)	Alive
−1.2 to −0.2	88	67	21	38 (30–48)
−0.2 to +0.3	90	77	13	23 (18–27)
+0.3 to +0.7	80	73	7	13 (10–17)
+0.7 to +1.6	88	82	6	8 (6–11)

PI: prognostic index;; 95%CI: 95% confidence interval.

[a] The Cox model was used to produce a PI score that combined the significant variables identified in the multivariate analysis. The PI score weighted these significant variables according to the presence or absence of each variable and the strength of their prognostic contribution. Progesterone receptor (PgR) expression (PgR positive, +1; PgR negative, 0) was weighted and multiplied by −0.4; S-phase fraction (low, 0; intermediate, 1; high, 2) was weighted and multiplied by +0.2; bcl-2 expression (low, 0; high, 1) was weighted and multiplied by −0.3, site of recurrence (visceral, 1; nonvisceral, 0) was weighted and multiplied by +0.5; age (<50 years, 1; ≥50 years, 2) was weighted and multiplied by + 0.5; and disease free survival (<12 months, 1; 12–60 months, 2; >60 months, 3) was weighted and multiplied by −0.3. The median survival of patients in each quartile with differing PI scores varied almost 5-fold from 8 months to 38 months.

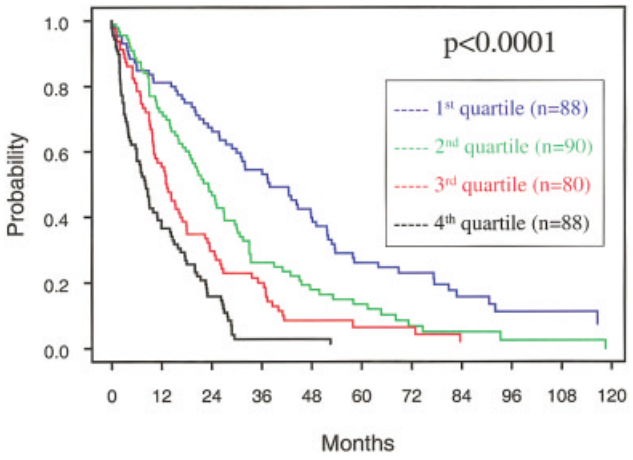

FIGURE 2. Survival after first recurrence in patients with metastatic breast carcinoma by prognostic index.

ment, and DFI and found that disease stage was an independent predictor of survival.[11] Another large study in 433 women confirmed these earlier reports that patients with metastatic disease who had ER negative tumors, visceral metastases, and shorter DFI had significantly worse survival.[12] However, in these earlier series of patients, the newer prognostic markers were not assessed.

Overexpression of the protooncogene c-*erb*B-2 is associated with worse survival and prognosis in patients with early-stage breast carcinoma.[13–15] Likewise, mutated or inactivated p53, as shown by nuclear accumulation of protein, is associated with a higher risk of recurrence and shorter survival in women with early-stage breast carcinoma.[16,17] The value of these prognostic markers in women after they develop their first recurrence is uncertain. In one study that involved 127 patients with metastatic disease who were treated with chemotherapy, histologic grade, c-*erb*B-2, p53, and cathepsin D were not significant prognostic markers of outcome or survival,[18] in agreement with our own results showing that c-*erb*B-2 and p53 are not independent predictors of outcome at the time of first disease recurrence. In addition, future studies should address the utility of newer techniques, in particular, gene amplification of c-*erb*B-2 by fluorescent in situ hybridization, as prognostic factors in the metastatic setting.

The results of this study show that the newer biologic markers assessed in the primary tumor can refine the prognosis and outcome of patients with metastatic breast carcinoma. In particular, PgR, SPF, and bcl-2 appeared to be important variables in multivariate analysis. PgR synthesis is increased in estrogen-responsive tissues, including breast carcinoma.[19] Earlier studies on the value of PgR in patients with metastatic disease were small and retrospective. In a large, prospective trial that was designed to investigate the prognostic significance of ER and PgR expression in 342 women with advanced breast carcinoma who were treated with tamoxifen, it was found that PgR was an independent predictor of longer overall survival from the time of recurrence in a multivariate analysis.[20] Our results are consistent with that observation.

bcl-2 is a protein that blocks apoptosis in vitro and would be expected to be associated with a more aggressive phenotype and resistance to some forms of therapy. However, in patients with early-stage breast carcinoma, unexpectedly, high bcl-2 expression is associated with a better outcome and favorable prognostic features, including ER expression, well-differentiated tumors, and intact p53.[6,21–23] In a large prospective trial that was conducted by the Southwest Oncology Group (SWOG 8228) in ER positive patients, high bcl-2 expression was associated with a better response to endocrine therapy and better survival in women with metastatic breast carcinoma.[6] The current study supports the understanding that bcl-2 expression is associated with a more indolent disease and longer survival and appears to be an independent prognostic factor in patients with metastatic disease. In patients with early-stage breast carcinoma, low SPF is associated with better survival.[2,24] However, to the best of our knowledge, there has only been 1 small study in patients with metastatic breast carcinoma that involved 82 women in whom it was found that SPF was an independent prognostic factor of patient outcome.[25]

This is the first systematic study of a large number of metastatic patients in whom the newer biologic factors of primary breast tumors were analyzed as predictors of postrecurrence outcome and survival. Using the PI score created by the contribution of these biomarkers and clinical prognostic features, an estimate of the median duration of survival can be made. The median survival duration for patients with metastatic disease may differ by almost 5-fold, from $>$ 3 years to $<$ 9 months, depending on the PI score. In patients who have favorable prognostic factors, especially women with tumors that express steroid hormone receptors with low proliferation and high bcl-2 expression, less toxic or burdensome therapy may be most appropriate. Conversely, patients with a poor prognostic profile may consider initiation of investigational therapy or treatments that are more complex and potentially more toxic.

The evaluation of these newer biologic markers in primary breast tumors may help in the assessment of outcome and prognosis, and the treatment options for these patients with metastatic breast carcinoma can be based on biologic factors. Although metastatic breast carcinoma usually is ultimately fatal, knowledge of prognosis and life expectancy is of paramount importance to both the patient and their families and is one of the most frequently asked questions in daily practice. After patients develop recurrent disease, the individual biologic characteristics of the primary tumors provide the clinician information to make more specific assessments, allowing patients to plan better plan, to order priorities for the remainder of their lives, and to make adequate preparation for death, a need that often is overlooked by the medical community. In addition to established traditional clinical characteristics, the phenotypic features of the primary tumors are correlated independently with outcome after first recurrence and may be used to estimate the median survival in patients with metastatic disease. This study supports the hypothesis that early events in the primary tumor determine the intrinsic aggressiveness of the disease and are capable of predicting outcome at the time patients with metastatic breast carcinoma develop recurrent disease.

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