Journal of Mammary Gland Biology and Neoplasia, Vol. 6, No. 4, October 2001 (© 2002)

Prognostic Factors in Breast Cancer: Current and New Predictors of Metastasis

Daniel F. Hayes,[1,3] Claudine Isaacs,[2] and Vered Stearns[1]

Decisions regarding the use of adjuvant therapy for breast cancer are strongly influenced by the risk of disease recurrence and death. These risks are now determined by examining the currently recognized breast cancer prognostic factors, including clinical stage, axillary nodal status, tumor size and grade, hormone receptor status, and presence of lymphovascular involvement. Newer factors are being evaluated in an attempt to more precisely define disease-related prognosis. This paper provides an overview of issues that need to be considered when analyzing studies of prognostic factors as well as a review of the currently recognized and the newer candidate prognostic factors.

KEY WORDS: breast cancer; prognostic factors; adjuvant systemic therapy; tumor marker utility grading system.

INTRODUCTION

Approximately 180,000 American women will be told they have breast cancer this year and nearly 40,000 will die from it. Almost all of them will receive some sort of treatment, including surgery, radiation, chemotherapy, endocrine therapy, or even one of the more recently introduced biological therapies. Early application of these therapies has now been shown, in well-designed, prospective randomized clinical trials, to reduce the odds of recurrence and death from breast cancer. Overall the risk of mortality due to breast cancer has been declining in the Western world over the last decade (1–6). However, these therapies are frequently, if not always, associated with substantial short- and long-term morbidity and, occasionally, mortality. Therefore, the challenging tasks are to both identify breast cancers early and to distinguish those breast cancers that are likely to cause morbidity and mortality from those that will not. Accurate performance of the latter task will permit selection of those therapies most likely to be effective for a given patient.

[1] Breast Oncology Program, University of Michigan Comprehensive Cancer Center, Ann Arbor, Michigan.

[2] Breast Cancer Program, Lombardi Cancer Center, Georgetown University, Washington, District of Columbia.

[3] To whom correspondence should be addressed at University of Michigan Comprehensive Cancer Center, 6312 CCGC, 1500 E. Medical Center Dr., Ann Arbor, Michigan 48109-0942. e-mail: hayesdf@umich.edu

Abbreviations used: ASCO, American Society of Clinical Oncology; TMUGS, Tumor Marker Utility Grading System; LOE, levels of evidence; CALGB, Cancer and Leukemia Group B; AC, adriamycin (doxorubicin)/cyclophosphamide; RR, relative risk; TNM, tumor/nodes/metastases; SEER, Surveilance, Epidemiology, and End Results; OS, overall survival; SBR, Scharff–Bloom–Richardson; LVI, lymphatic vessel invasion; BVI, blood vessel invasion; NSABP, National Surgical Adjuvant Bowel and Breast Project; ER, estrogen receptor; PgR, progesterone receptor; DFS, disease free survival; SPF, S-phase fraction; TLI, thymidine labeling index; BrDu, bromodeoxyuridine; IHC, immunohistochemistry; FISH, fluorescent in situ hybridization; PCR, polymerase chain reaction; H&E, hematoxylin and eosin; BMM, bone marrow micrometastases; ELISA, enzyme-linked immunosorbent assay; MVD, microvessel density; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; UPA(R), urinary plasminogen activator (receptor); PAI, plasminogen activator inhibitor; CMF, cyclophosphamide/methotrexate/5-fluorouracil); TIMP, tissue inhibitors of metalloproteinases; TF, tissue factor.

1083-3021/01/1000-0375/0

EVALUATION OF PROGNOSTIC FACTORS: GENERAL CONSIDERATIONS

Substantial efforts have been made to subdivide patient populations into groups that behave differently, so that therapy can be applied more efficiently. Indeed, the diagnosis of breast cancer itself divides the general population into those with it and those without it. Separation of these two groups is crude, and estimation of prediction of outcomes based solely on the diagnosis of "breast cancer" is very poor. Therefore, as early as the end of the nineteenth century, doctors began to observe that outcomes were related to clinical cancer size and the presence or absence of pathologically involved lymph nodes (7). These early efforts led to what is now commonly designated as "staging," which has now become highly codified within an internationally coordinated effort (8).

These efforts only partially separate patients into subgroups with different biological behaviors. In the context of the explosion of molecular biology over the last four decades laboratory and clinical scientists have studied a series of "molecular prognostic factors." Hundreds of these putative markers have been reported, yet very few have actually gained widespread clinical use. In part, this frustrating lack of progress is a function of the incredible biological diversity of the disease. However, much of the confusion and controversy in the field stems from poorly designed and analyzed clinical studies. Although observations from these studies may generate important hypotheses, they are much less likely to provide reliable estimates of the true clinical utility of a marker than prospective studies that specifically address predefined hypotheses (9–11).

Prognosis Versus Prediction

The distinction between prognostic and predictive factors can critically affect the results of a clinical/laboratory investigation (12–14). Markers of prognosis are associated with patient outcome independent of the treatment they receive (14–16). In this regard, prognostic factors reflect the ability of the tumor to metastasize, invade, and proliferate. It is critical to distinguish prognostic factors from those that are predictive of response to therapy. These may do so either because they are a direct target of the agent or because their expression is an epiphenomenon of the mechanisms of action (or resistance) of the therapy. Many markers may be both prognostic and predictive, further confusing interpretation of tumor marker results.

The distinction between prognostic and predictive factors is illustrated by the schematic diagrams presented in Fig. 1(A)–(C). Figure 1(A) represents two hypothetical pure prognostic factors. Factor 1 is a strong prognostic factor, while Factor 2 is a weak one (see below). For each factor, patients who are "positive" have a worse prognosis than patients who are "negative." Since these markers do not predict benefit from therapy, the benefits of therapy are the same for both factor negative and factor positive patients. Figure 1(B) illustrates two pure predictive factors. In this case, these are predictors for response to therapy. In the absence of therapy, patients who are "positive" and "negative" have the same outcomes. However, patients who are "positive" for each of the markers have better outcomes than those who are "negative" for the respective marker when the specific therapy is applied. In Fig. 1(C), the factors have mixed prognostic and predictive utilities. In this case, the factors are favorable prognostic factors ("positive" patients do better than "negative" patients in the absence of therapy), and they are also favorable predictive factors ("positive" patients are more likely to benefit than "negative" patients). A thorough understanding of these critical distinctions is essential in the evaluation of clinical utility of a tumor marker, and yet, very often, they are lost in study design.

Technical and Methodologic Problems With Evaluation of Prognostic Factors

In addition to the often unappreciated issue of prognosis and prediction, many other issues further complicate the interpretation of prognostic factor studies. Most studies are usually retrospective, include only small numbers of patients, and do not control for other confounding prognostic variables. Other problems that contribute to study differences include differing lengths of follow-up and different endpoints (response, disease-free survival, overall survival, and relative risk of relapse or death). In addition, studies examining the same prognostic factor may use different methods of analysis and different cutoff points. The ideal study should be prospectively designed for a defined population of patients to address the clinical utility of the prognostic factor, using predetermined methods and cutoffs. In an effort to avoid the confounding effect of treatment, only patients who do not receive systemic therapy should

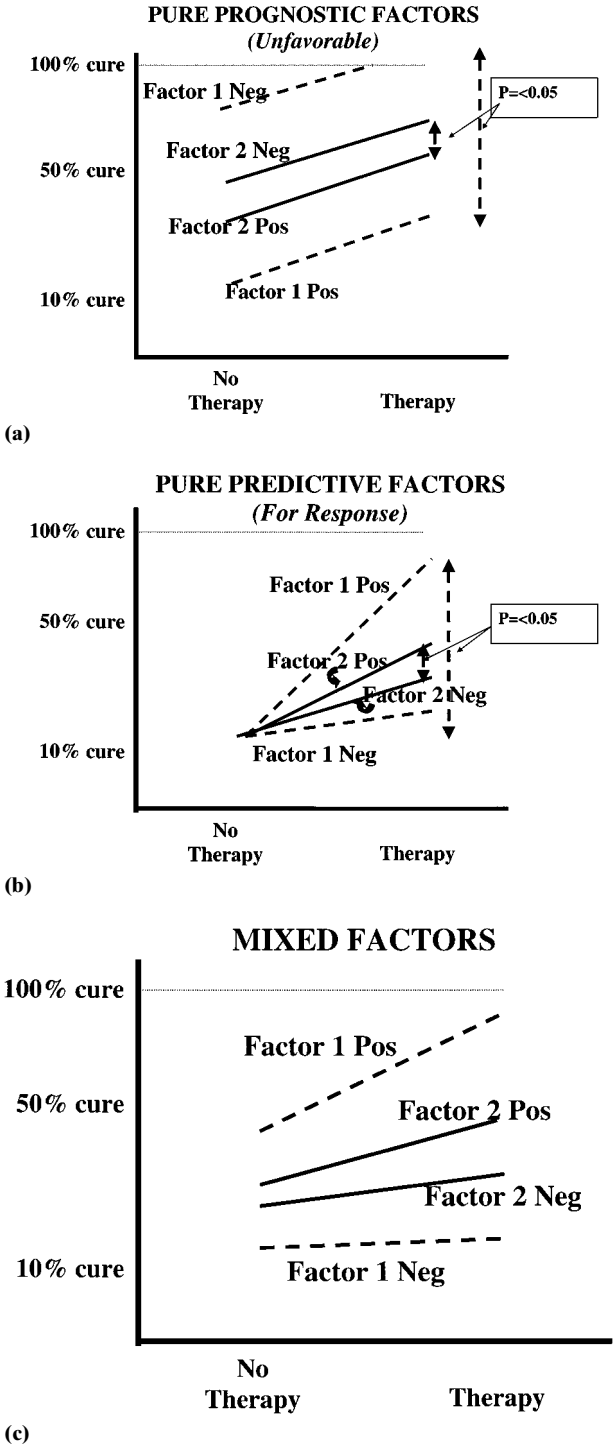


Fig. 1. Schematic examples of prognostic (A), predictive (B), and mixed prognostic/predictive (C) factors. In each schema, Factor 1 (dashed line) is a strong factor, and Factor 2 (solid line) is a weak factor. The length of the vertical arrows illustrate the relative strengths of the factors. The *p*-values represent the likelihood that the separation of the two groups (positive vs. negative) is not due to chance alone. From Ref. 14 with permission.

be studied. In addition, studies should report the relative risk of recurrence and/or mortality associated with a specific factor. This approach would allow the reader to compare the strength of different prognostic factors.

In 1995, the American Society of Clinical Oncology (ASCO) convened an Expert Panel to assess the clinical utility of tumor markers in breast and colorectal cancers (17). A summary of these evidence-based guidelines, which have been updated on two occasions, is provided in Table I (18,19). A feature of the Expert Panel deliberations was the recognition of a lack of organized policies and "rules" for investigating and evaluating tumor markers. This recognition led to a second publication in which a proposal, designated the Tumor Marker Utility Grading System (TMUGS), was offered (9,14). TMUGS was developed as a means to objectively evaluate the clinical utility of individual prognostic factors. In this system, specific tumor markers or factors are evaluated and assigned a semiquantitative score ranging from 0 to 3+ (Table II). A TMUGS score of 2+ or 3+ implies that there are sufficient data to support the use of the specific marker to make decisions regarding a particular outcome. TMUGS scores are assigned based on whether the marker truly distinguishes one group from another with regard to specific outcomes and whether knowledge of that distinction is important in making clinical decisions. For example, preclinical studies might suggest that a marker is appealing for use as a prognostic factor, but clinical studies fail to validate the hypothesis. In this case, the marker would be assigned a score of "0." For a second marker, clinical studies may show that it clearly distinguishes patients who have a favorable outcome from those who are expected to do poorly. However, if effective therapy does not exist for these patients, the clinical utility of this information, although reliable, is of uncertain value. This marker would be assigned a "1+" for this use. More details are provided in Table II and in other publications (9).

A critical part of this assessment is the evaluation of "levels of evidence" (LOE) from which the data have been extracted for evaluation (9). This classification system includes five levels, for which the first three are most relevant (Table III). LOE I provides the strongest support, whereas LOE V is the weakest. LOE I data are derived either from a single high-powered prospective study designed to test a particular marker or from a meta-analysis or overview of lower level LOE studies. LOE II data come from companion studies to large clinical trials

Table I. American Society of Clinical Oncology Clinical Practice Guidelines for Use of Tumor Markers in Breast Cancer (Tissue Factors Only)[a]

Factor	Use	Guideline
Estrogen and progesterone receptors	Predictive factors for endocrine therapy	Measure on every primary breast cancer and on metastatic lesions if results influence treatment planning
DNA flow cytometrically derived parameters	Prognosis or prediction	Data are insufficient to recommend obtaining results
c-erbB-2 (HER-2/neu)	Prognosis	Data are insufficient to recommend obtaining results for this use
	Prediction for trastuzumab, CMF-like regimens, doxorubicin, taxanes, and endocrine Rx	c-erbB-2 should be evaluated on every primary breast cancer at time of diagnosis or at time of recurrence for use as predictive factor for trastuzumab; committee could not make definitive recommendations regarding CMF-like regimens. c-erbB-2 may identify patients who particularly benefit from anthracyline-based therapy but should not be used to exclude anthracycline treatment. c-erbB-2 should not be used to prescribe taxane-based therapy or endocrine therapy
P53	Prognosis or prediction	Data are insufficient to recommend use of p53
Cathepsin-D	Prognosis	Data are insufficient to recommend use of cathepsin-D

[a]Modified from Ref. 19.

in which tumor or blood specimens are collected prospectively. These studies have preestablished endpoints not only for the therapeutic intervention but also for the marker in question. For LOE III data, specimens are usually analyzed retrospectively. The data obtained from LOE III studies are most useful for generating hypotheses, but these require confirmation in either LOE I or II studies. Unfortunately most of the available evidence regarding prognostic markers is based on LOE III or lower studies.

Clinical Utility of Prognostic Factors: Classification by Relative Strengths

Another consideration in evaluating the clinical utility of a marker is its relative strength in separating

Table II. Tumor Marker Utility Grading System[a]

Utility grade	Explanation of scale
0	Marker has been adequately evaluated for a specific use and the data definitively demonstrate it has *no utility*. The marker should not be ordered for that clinical use.
NA	Data are not available for the marker for that use because marker has not been studied for that clinical use.
±	Data are suggestive that the marker may correlate with biological process and/or endpoint, and preliminary data suggest that use of the marker *may* contribute to favorable clinical outcome, but more definitive studies are required. Thus, the marker is still considered highly investigational and should not be used for standard clinical practice.
+	Sufficient data are available to demonstrate that the marker correlates with the biological process and/or endpoint related to the use, and that the marker results might affect favorable clinical outcome for that use. However, the marker is still considered investigational and should not be used for standard clinical practice, for one of three reasons: 1) The marker correlates with another marker or test that has been established to have clinical utility, but the new marker has not been shown to clearly provide any advantage. 2) The marker may contribute independent information, but it is unclear whether that information provides clinical utility because treatment options have not been shown to change outcome. 3) Preliminary data for the marker are quite encouraging, but the level of evidence (see below) is lacking to document clinical utility.
++	Marker supplies information not otherwise available from other measures that is helpful to the clinician in decision making for that use, but the marker cannot be used as sole criterion for decision-making. Thus, marker has clinical utility for that use, and it should be considered standard practice in *selected* situations.
+++	Marker can be used as an independent criterion for clinical decision making in that use. Thus, marker has clinical utility for that use, and it should be considered standard practice.

[a]Modified from Ref. 9 with permission.

Table III. Classification of Level of Evidence (LOE) for Tumor Marker Studies[a]

LOE	Type of evidence
I	Evidence derived from prospective high-powered trial specifically addressing tumor marker utility OR overview or meta-analysis of lower LOE studies
II	Evidence from companion study to large clinical trial. Specimens collected prospectively and tumor marker utility determined as secondary aim of study
III	Evidence from retrospective trials, where data about patient characteristics and treatment often incomplete
IV	Evidence from small retrospective studies which do not have prospectively dictated therapy
V	Evidence from small pilot studies designed to determine or estimate distribution of marker levels in sample population

[a]Modified from Ref. 9 with permission.

two populations ("positive" from "negative") and whether that separation is sufficient to treat these populations differently. Such a consideration requires an understanding of the perspectives in decision-making held by the caregiver and the patient. In this review, we will focus on prognostic factors for early breast cancer; that is, factors that might be used to assign patients with newly diagnosed breast cancer to one of several different outcome categories that might be treated differently, based on their prognosis, as illustrated in Fig. 2 (14). For example, most clinicians and patients feel that women with a less than a 10% chance of recurrence during the 10 years after diagnosis (in the absence of adjuvant systemic therapy) have a very "good" prognosis. The definition of a "moderate" prognosis includes patients with a chance of recurrence of approximately 10–50%, and "poor" prognosis is considered to encompass greater than a 50% chance of recurrence (again, when considered in the absence of adjuvant systemic therapy). Most women in the latter two categories will probably receive adjuvant systemic therapy, while those in the first group might not, since so many patients (90%) will do well without it. This decision depends on the relative value systems of the patient, her caregiver, and her society. Some patients might be willing to accept enormous toxicities for very small benefits, while others would not. Furthermore, limited resources in some societies might prevent application of expensive therapies for patients who have a very small chance of benefiting, either because they have an excellent prognosis or because the therapy is only minimally effective. Limited studies have been performed in which patients (who had previously undergone chemotherapy) have been queried regarding what absolute benefit they would require to accept chemotherapy (21–23). Remarkably, nearly 40% of patients responded that they would be treated if their chances of mortality were decreased by as little as 1%. Of course, these results also mean that 60% would require higher chances of benefit to accept treatment, highlighting the heterogeneity of patient decision-making. Although hard data are lacking, it appears that many oncologists in the United States generally feel that the benefit/risk ratio is sufficiently high to recommend chemotherapy if the absolute percent of patients who are likely to benefit surpasses 3–5% (24). For example, if a given therapy results in a proportional reduction of 30% of whatever the original recurrence or death rate was, then patients in the "very good" prognosis category would not be treated, since a 30% reduction of a 10% or less chance of recurrence is less than the arbitrary threshold of 3% absolute benefit. In contrast, the same therapy would be acceptable to those in the moderate or poor risk categories, assuming that the therapy is equally effective (in other words, that the predictive factor profile is the same in all three groups), since a 30% reduction of the higher mortality risks results in at least 3% or more patients who would be expected to benefit. Recently, computerized programs have been developed to help

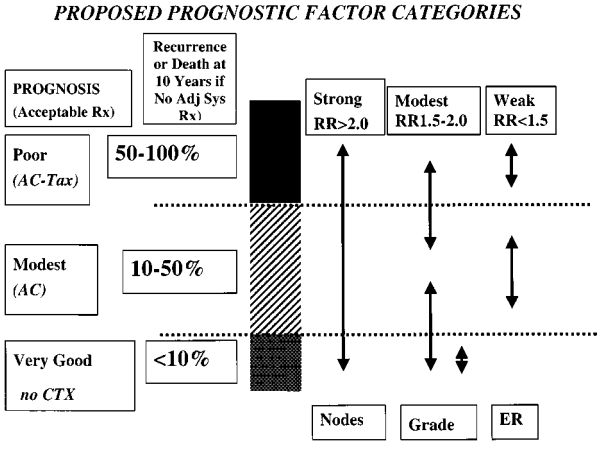


Fig. 2. Schematic illustration of prognostic factor categories. See text for description. From Ref. 20 with permission.

women make these decisions, supplying estimates of outcomes specific to their situation (25). Prospective validation of these programs is underway.

In addition to distinguishing groups of women for whom adjuvant chemotherapy is or is not absolutely indicated, the intensity or amount of the therapy may differ based on these arbitrary prognostic groupings. For example, results from a recent Cancer and Leukemia Group B (CALGB) study indicate that after four cycles of conventional adjuvant chemotherapy (doxorubicin and cyclophosphamide, "AC"), an additional four cycles of paclitaxel provides a further reduction in annual odds of recurrence of approximately 20% (above the 20–30% already expected from AC versus no therapy) (26). For patients who originally fell in the "moderate" risk category illustrated in Fig. 2, the initial reduction in odds of recurrence from the AC alone may be sufficient to place them into the "good" category, and therefore an additional proportional reduction of 20% is not sufficient to justify more chemotherapy. In contrast, for those who initially fell into the poor prognosis category (for example, node positive patients), the benefit from the initial AC will reduce their odds only to the point where they continue to have a "residual" moderate prognosis (Fig. 2). For these patients, a further 20% reduction in annual odds of recurrence is likely to benefit 3% or more of the population, and they might accept the toxicities and risks of the added 3 months of paclitaxel.

The preceding discussion highlights the importance of using prognostic factors to separate groups of patients into these prognostic categories. Doing so requires an estimate of the relative strength of the factor and an evaluation of the statistical reliability of that estimate (14). Too often, investigators assume that "statistically significant" ($p \leq 0.05$) means "clinically useful." Rather, clinical utility is based on the strength of the factor in separating two populations, while the statistical analysis simply provides the likelihood that the two populations are separate, regardless of how far apart they may be. In addition to illustrating the differences between prognostic and predictive factors, Fig. 1 also illustrates the difference between strong and weak factors. One factor may reliably separate two groups, but only by a small amount, as illustrated in Fig. 1. For example, for hypothetical Factor 2, the risk of relapse at 10 years may be 45% for positive patients and 40% for negative patients (see Fig. 1(A)). Assuming the study is sufficiently powered, one would be able to distinguish these two groups reliably with a p-value <0.05. However, both have modestly poor prognoses, and one would probably treat them in the same fashion. In contrast, hypothetical Factor 1 might separate positive and negative patients into those with a 90% and those with a 10% risk of relapse. In this case, one would treat these patients substantially differently. The statistical analysis provides assurance that this observation is likely to be real.

Table IV. Proposed Utility of Prognostic Factors Based on Relative Strength

Category	Relative risk for event (relapse, death) for marker positive vs. marker negative
Strong	>2.0
Moderate	1.5–2.0
Weak	<1.5

In a prior publication, we have proposed that one might arbitrarily assign prognostic factors to one of three different categories based on the strength of the factor; that is, the magnitude inherent in that factor's ability to separate groups of patients (Fig. 2 and Table IV) (20). A strong prognostic factor might be defined as one that has at least a twofold increased relative risk (RR) of relapse. Such a factor is likely to move a patient's prognosis across two of the arbitrary prognostic categories illustrated in Fig. 2. A good example of such a factor is lymph node status. A moderate prognostic factor would be associated with a RR of relapse of 1.5–2.0 and could move patients across one category. A weak prognostic factor, RR of <1.5, could move a patient within a category, but probably not across categories (Fig. 1 and 2).

EVALUATION OF SPECIFIC PROGNOSTIC FACTORS

Accepted Prognostic Factors

Clinical and pathological tumor/nodes/metastases (TNM) stage, including axillary node status and tumor size, have long been recognized as the most powerful predictors of breast cancer recurrence (8). In addition, tumor type, grade, presence or absence of lymphatic or vascular invasion, and hormone receptor status, though not included in the American Joint Commission on Cancer staging system, also appear to provide helpful information, although with less strength (Table V).

Table V. Breast Cancer Prognostic Factors

Factor	Strength category	TMUGS score
TNM stage	Strong	3+
Pathologic axillary nodal status	Strong	2 to 3+
Pathologic tumor size	Strong	2 to 3+
Tumor grade	Moderate	1 to 2+
Lymphatic/vascular invasion	Moderate	1 to 2+
Estrogen receptor	Weak	1+[a]
Progesterone receptor	Weak–moderate	1+[a]
Markers of proliferation	Moderate–strong	1–2+
SPF		
TLI		
Ki67 IHC		
Markers of cell death/turnover	?	+/−
p53		
bcl2, bcl-x, bax		
Cyclin D, E, cdk's, cdk inhibitors	?	+/−
Detection of micrometastases		
Axillary lymph nodes	Weak	1+
Sentinel lymph node	?	+/−
Bone marrow	Moderate–Strong	1+
HER-2	Weak–Moderate	1+[a]
Markers of neovascularization, invasion, and metastases		
Neovascularization (IHC fVIII, CD31)	?	+/− to 1+
Angiogenic factors (bFGF, VEGF, etc)	?	+/−
Cathepsin D	Weak–moderate	+/− to 1+
UPA/PAI-1	Moderate–strong	1 to 2+

[a] Very strong predictive factor.

TNM Stage

The 5-year survival rate of newly diagnosed breast cancer patients is highly influenced by the stage of their disease. Results from the National Cancer Database for the year 1989 demonstrated a 5-year survival rate of 87% for Stage I patients, 78% for Stage IIA, 68% for Stage IIB, 51% for Stage IIIA, and 42% for Stage IIIB (8). In this regard, TNM staging is a very strong prognostic factor (Fig. 3).

Axillary Nodal Status

The status of the axillary lymph nodes is also a strong predictor of breast cancer recurrence. Although most older studies have not specified whether patients received adjuvant systemic therapy, the presence or absence of lymph node metastases, as determined by routine histopathology, is strongly prognostic in nearly every study ever performed. In the U.S. Surveilance, Epidemiology, and End Results (SEER) database, the 5-year overall survival (OS) was 92% for node-negative patients, 81% for those with one to three positive axillary lymph nodes, and 57% for those with more than four involved nodes (27). As proof of principle for our proposal of different prognostic categories, an analysis of results from the San Antonio Database of patients who received no adjuvant systemic treatment indicated that the hazard ratio for recurrence and death for node positive as compared to node negative patients was 2.4 and 2.8 respectively (G. M. Clark, personal communication). In summary, axillary nodal status is an extremely important predictor of recurrence.

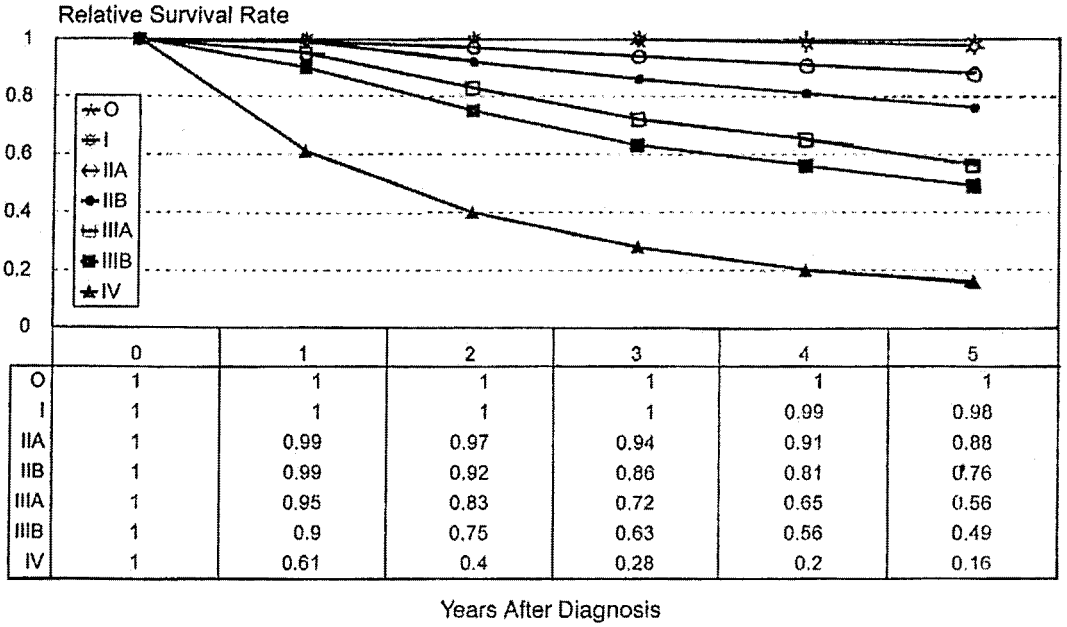
Tumor Size

Tumor size also provides very important prognostic information, which is independent of nodal status. In the SEER database of over 13,000 node-negative women, patients with tumors less than 1 cm had a 5-year relative OS of close to 99%, compared to 89% for those with tumors 1–3 cm, and 86% for those with tumors 3–5 cm (27). Other authors have found similar results, even with followup exceeding 20 years (28,29).

In summary, the results of many large studies indicate that tumor size is a strong marker of breast cancer prognosis and is therefore critical in treatment decision making. Overall, we assign "TMUGS grades" of 2+ or even 3+ to the TNM system (Tables II and V). In other words, TNM is sufficiently strong, and the reliability of the data to support that statement is sufficiently rigorous, that it can be used either in the context of other available information to guide clinical decision making (2+), or as a "stand-alone" factor to make treatment recommendations (3+). The data to support such an assignment for TNM come from mostly accumulated LOE III studies. Although few if any formal LOE I or II studies are available, nearly every study of these factors provides consistently positive results suggesting that the relative risk for "positive" versus "negative" patients is at least 2.0.

Tumor Grade and Type

Pathologic features of invasive breast tumors have been shown to be of prognostic significance, but they appear to be less strong than clinical stage, lymph node status, or size. Certain unusual histopathologic



	0	1	2	3	4	5
O	1	1	1	1	1	1
I	1	1	1	1	0.99	0.98
IIA	1	0.99	0.97	0.94	0.91	0.88
IIB	1	0.99	0.92	0.86	0.81	0.76
IIIA	1	0.95	0.83	0.72	0.65	0.56
IIIB	1	0.9	0.75	0.63	0.56	0.49
IV	1	0.61	0.4	0.28	0.2	0.16

Fig. 3. Prognosis according to TNM staging. From Ref. 8 with permission.

subtypes including pure tubular, papillary, or medullary cancers are associated with a particularly good prognosis with long-term recurrence rates of less than 10% (30,31).

For the more common subtypes, such as invasive ductal or lobular carcinoma, the use of histologic grade has been hampered by interobserver variability. Several investigators have proposed objective grading systems. Perhaps the most widely accepted is the Scarff–Bloom–Richardson (SBR) Classification, subsequently modified by Elston and Ellis, which takes into account mitotic index, differentiation, and pleomorphism, and divides tumors into good, moderate, and poor grades (32,33). Using this and other grading systems, most authors have confirmed that patients with a poor tumor grade have a higher risk of relapse that those with good tumor grade (34,35).

However, the results of these studies are variable, and there is considerable controversy regarding the prognosis of the largest of the three grades (moderate). Given the current lack of standardization of grading systems, at present tumor grade appears to be a moderate strength prognostic factor, with a relative risk of 1.5–2.0. Because of the uncertainty surrounding the prognostic status of the "moderate" grade category, coupled with concerns over interobserver variability, we assign a TMUGS grade of 1+ to tumor grade, although other authors, including the College of American Pathologists, have recommended adoption into routine practice (36,37). It is anticipated that with increasing use of standardized grading systems, the noted interobserver variability in grading will diminish (38–40). Better standardization will permit a more reliable estimate of the strength, and therefore of the clinical utility, of tumor grade as a prognostic factor.

Lymphatic and Vascular Invasion

Peritumoral lymphatic vessel (LVI) and blood vessel invasion (BVI) have been demonstrated by several groups to predict both for local and distant recurrence (31,41). For example, in one study with 20 years follow-up, the recurrence rate for 461 women with Stage I disease was 38% and 22% for LVI-positive and LVI-negative patients respectively ($p = 0.05$) (31). However, as with tumor grade, reproducibility of LVI among pathologists has been controversial, and the prognostic role of LVI in very small tumors (<1 cm) has not been well-studied. Therefore, although these studies indicate that LVI is a moderate strength prognostic factor, we assign it a 1+, although arguably it could be given a 2+.

Hormone Receptor Status

The impact of hormone receptor (estrogen receptor, ER, and progesterone receptor, PgR) status on prognosis is complex, and many older studies are confounded by the known or unknown presence of adjuvant endocrine therapy (42). Perhaps the best study of the prognostic strength of ER comes from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-06 study, in which women with early stage breast cancer received no adjuvant systemic therapy (34). Patients with ER positive tumors had a 5-year disease free survival (DFS) of 74% and a 5-year OS of 92%. For those with ER negative tumors, the 5-year DFS and OS were 66 and 82%, respectively.

Other studies with longer follow-up indicate that the improved prognosis associated with ER positive tumors does not persist with time (43). These findings suggest that ER positive tumors may not have a different metastatic potential than ER negative tumors. Instead, they may have a slower growth rate. Taken together, these data suggest that ER status is a weak prognostic factor with a relative risk of recurrence of 1.0–1.5 that predicts for more indolent behavior during the first few years after diagnosis more than for overall recurrence. As previously stated, ER status appears to be a much stronger predictor of treatment response than it is a prognostic factor. It should be used primarily in making recommendations regarding the use of endocrine treatment in the adjuvant setting (17–19). Therefore, it is difficult to assign a TMUGS grade to ER. Standing alone as a pure prognostic factor, it would be given a 1+. However, because it provides very powerful information regarding response to endocrine therapy, one must consider assigning it a 3+, as illustrated in the example below.

For instance, consider the prognosis of a woman with a 1 cm (Ti) node negative (No), tumor that is poorly differentiated, is ER negative, and has LVI. Her prognosis is substantially worse than if she had a well-differentiated, ER positive, LVI negative tumor. In the former case, she would be at least in the moderate risk category, where the potential survival benefits of adjuvant systemic chemotherapy are such that she should strongly consider treatment. In the latter case, the patient falls into the very good prognostic category, in which she would at most consider treatment with tamoxifen (Figs. 1 and 2). However, consider a patient with all of the features of the latter case, except that her hormone receptors are positive. In the absence of any therapy, her prognosis is now slightly better than the first patient, by virtue of having positive hormone receptors, but she still probably falls into the moderate risk category. However, adjuvant endocrine therapy substantially reduces her risk of recurrence and death (as much as 40%) moving her into the very good risk category. Therefore, if she were ER negative or endocrine therapy were not available, one might recommend chemotherapy regardless of her ER status, but since endocrine therapy improves her prognosis so much, she might forego chemotherapy. In that case, she would give up a small (1–2%) additional benefit to avoid the toxicities.

Candidate Prognostic Markers

Markers of Proliferation (Flow Cytometry, Thymidine Labeling Index, Bromodeoxyuridine Incorporation)

Logically, faster growing tumors should recur more rapidly than slower growing ones. Indeed, visual estimation of mitotic figures is a key component in the Elston–Ellis SBR tumor grading system. Several different methods exist to more objectively assess the rate of proliferation including DNA flow cytometry (ploidy and S-phase fraction, SPF), thymidine labeling index (TLI), mitotic index, and bromodeoxyuridine (BrDU) incorporation. In addition, immunohistochemical analyses using antibodies directed at antigens present during cell proliferation, such as Ki-67 and PCNA, can be performed on paraffin-embedded tissue and provide a readily available and reproducible alternative means of analysis.

Over 200 articles have examined the impact of SPF on breast cancer prognosis (44). Unfortunately, the field provides a classic illustration of the problems of tumor marker analysis. These studies have used different assays, means of analyses, and cutoff points. In addition they frequently did not include information about systemic therapy, nor have they often controlled for other prognostic variables. All of these factors make many of these studies difficult to interpret. Nonetheless, several large well-designed studies have recently been carried out and indicate that tumors with a high SPF are associated with an inferior outcome when compared to those with a low SPF (44). In a prospective observational study performed by the U.S. Intergroup, SPF was used to identify patient risk and determine treatment (45). Low risk tumors were defined as either having tumors so small that

ER could not be performed, or as <2 cm, ER and PgR positive, and low SPF. Five-year risk of relapse and of death of patients in this category, who were observed without adjuvant systemic therapy, were only 12 and 3%, respectfully. These results suggest that in patients with small ER/PR positive tumors, a low SPF may identify an excellent prognostic group in which only a few patients are likely to benefit from adjuvant systemic chemotherapy. Other studies have indicated that Ki-67 and other measures of proliferation including TLI, mitotic index, and BrDu-incorporation also predict for risk of recurrence (46–48).

Nontheless, the ASCO Tumor Marker Expert Panel has thus far concluded that the data were insufficient to recommend routine use of SPF or related measures for prognostic information or for treatment selection (17–19). This recommendation was due at least in part to a lack of standardization in measurement techniques, reproducibility of test results, and cutoff points. Regardless, the results from new studies suggest that SPF should be reconsidered, particularly for patients with otherwise favorable prognostic features such as small tumor size and positive receptor status. However, certain methodological issues need to be resolved. Flow cytometry and IHC methods need to be standardized, and further data from prospective trials are required for measures of proliferation to be broadly useful. Presently, we assign a TMUGS score of 1+ to those factors, although others have incorporated their use into standard practice.

Markers Regulating Cell Cycle and Cell Death

In addition to gross measures of cellular turnover, one can also assay for abnormalities in protooncogenes and tumor suppressor genes that regulate the process of cell cycle and cell death. Therefore, markers of cell death might provide prognostic information. These include either direct evaluation of apoptosis, or indirect evaluation of the genes and their products that mediate this process.

p53. p53 is a tumor suppressor gene with pleotropic functions located on chromosome 17. The p53-encoded nuclear protein has an important role in regulating transcription of many other genes. In response to cell damage or crisis, wild type p53 can induce cell G1 cycle arrest. If repair mechanisms are not sufficient, p53 also serves as a critical regulator of cellular entry into the apoptotic pathway. The *p53* gene is mutated or deleted in up to 50% of breast carcinomas, and therefore it is an appealing marker for study. However, as with so many other markers, the data regarding *p53* and prognosis are conflicting. Different investigators have used a variety of methods to detect mutated or abnormal p53, including immunohistochemistry (IHC) (with different antibodies), fluorescent in situ hybridization (FISH), and techniques to determine if the gene is intact, such as complete sequencing of the *p53* gene, polymerase chain reaction-single-strand conformation polymorphism, and DNA oligonucleotide arrays. Wildtype p53 protein has a very short half-life, and therefore, in theory, should not be detected by IHC, whereas mutated p53 protein is stabilized and should be detectable. Nonetheless, although IHC does detect mutated p53, it may also identify stabilized wild type (49). In addition, point mutations that are detected in sequencing techniques may not always correlate with protein accumulation on IHC and, of course, deletion of all or the part of the gene encoding the epitopic domain also results in a "negative" IHC result (50). Therefore, when these concerns are added to the usual concerns of interaction with therapy, tissue preparation heterogeneity, and investigator and laboratory variability, it is not surprising that the results of p53 assessment by IHC and outcomes have been mixed. The ASCO Panel reviewed over 40 studies that evaluated the prognostic role of p53 in breast cancer and concluded that there were insufficient data to support the clinical use of p53 (17). Subsequent to the ASCO analysis, a meta-analysis of 11 published studies of somatic mutations of *p53* has been reported (51). Overall, the relative hazards for recurrence and death were 1.5 (95% CI = 1.2–1.9) and 2.0 (95% CI 1.7–2.5) respectively, and for node negative patients, the authors calculated a relative hazard of 2.6 for death (95% CI = 1.7–3.9). In spite of this study, until the more reliable method(s) of analyzing p53 abnormalities are available and until clinical trial designs are used to control for the effects of therapy, it is difficult to recommend incorporation of this marker into clinical practice. Currently, we assign p53 analysis a 1+ TMUGS grade, at best, as a pure prognostic factor in breast cancer.

bcl2 Family. Several members of the *bcl2* family have a critical role in regulation of apoptosis. In general, overexpression of bcl2 is associated with resistance of cells to programmed cell death (52). Enigmatically, bcl2 is regulated in breast cancer by the ER and high levels of the protein are associated with markers of good prognosis and improved outcomes (53). Other members of the *bcl2* family include $bclx_s$, $bclx_L$, and

bax, an inhibitor of apoptosis. In theory, prognosis should be reflected by a balance of bax and bcl2 family members. Only limited data are available to correlate these markers with prognosis in breast cancer. For example, one preliminary study has suggested that low levels of bax are associated with worse outcome in women who received combination chemotherapy for metastatic breast cancer (54). Another study suggested that overexpression of $bclx_L$ is associated with worse outcomes (55). Given the complexity of this family of genes and their protein products, it is likely that alterations in the ratios, rather than the absolute amounts, of bcl2 and other members will play important prognostic and/or predictive roles in breast cancer. In the meantime, evaluation of these genes must be considered highly investigational, with a TMUGS score of +/−.

Other Molecular Measures of Proliferation and Cell Death. The cyclins, their associated kinases, and kinase inhibitors are intimately involved in cell cycle control and cell survival. A number of studies have investigated abnormalities in these genes and their products in relation to breast cancer prognosis (56–58). Although these studies are mostly positive, they are all preliminary, and represent LOE III or less evidence. This category of markers must be assigned a "+/−" TMUGS score.

HER-2

The *HER-2/neu* oncogene, also known as *c-erbB-2*, encodes a 185 kd transmembrane glycoprotein that belongs to the family of epithelial growth factor receptors (59). Amplification of the gene and/or overexpression of the protein occurs in approximately 30% of breast cancers (60). Amplification or overexpression of *HER-2* can be measured in several ways, including IHC and enzyme-linked immunosorbent assay (ELISA) to determine protein expression, and southern blotting or FISH to determine gene amplification.

In a study of 100 node-positive women, Slamon and colleagues first reported that tumors with amplification of *HER-2* were associated with lower DFS and OS (60). Since that time, a large number of studies have examined the prognostic significance of HER-2. Overall, these studies suggest that HER-2 overexpression is of prognostic significance for node positive patients, but variable results have been reported for those with node negative disease (61).

Predictably, the interpretation of many of these studies has been hampered by small sample size, variable methods of HER-2 analyses and cutoffs, short length of follow-up, and perhaps most important the confounding effects of treatment. Recent studies have suggested that HER-2 status is predictive of response to specific types of adjuvant chemotherapy and possibly hormonal therapy (62). Thus, as with other markers, in order to avoid the confounding effect of treatment, studies examining the prognostic significance of HER-2 status are best performed in untreated patients. The preliminary results of a meta-analysis of published and reported results suggest that the hazard rate for recurrence and death for node negative patients appears higher for HER-2 positive patients than for HER-2 negative patients, but the relative difference is weak, at best (RR = 1.12 [95% CI, 1.04–1.22] for recurrence and 1.15 [95% CI, 1.07–1.23] for death, respectively, for patients with HER-2 positive tumors as compared to those with HER-2 negative tumors) (61). Some authors have argued that specific assays, such as FISH, are more reliable than other assays (63). Although intriguing, these LOE III data represent a single study, and confirmation from other data sets is required before one can assume that FISH analysis for HER-2 has a relative prognostic strength equal to clinical stage or nodal status (RR > 2).

In summary, the available data suggest that HER-2 may truly be a prognostic factor in breast cancer but overall appears to be a weak one. It is likely a much stronger predictor of responsiveness to specific therapies (62). At present, we assign a TMUGS score of 1+ to HER-2 as a prognostic factor, although it may be indicated as a predictive factor, especially in selecting anthracycline-based chemotherapy and possibly trastuzumab in the adjuvant setting (Tables I and V). Importantly, methodology used to determine HER-2 status must be standardized, and further LOE I or II studies need to be carried out prior to the integration of HER-2 as a marker of prognosis.

Markers of Metastasis or Metastatic Process

As noted, after clinical stage, the presence or absence of axillary nodal metastases as determined by light microscopy of hematoxylin and eosin (H&E) stained slides, is the most powerful prognostic factor in breast cancer. Therefore detection of occult micrometastases, either in lymph nodes or in other tissues, might identify the 25–30% of "lymph node negative" patients who are destined to relapse without

adjuvant systemic therapy. Although intriguing, the results from studies addressing the presence of occult metastases have not conclusively demonstrated clinical utility of this approach.

Lymph Node Micrometastases. The presence of micrometastatic disease in lymph nodes can be assessed by a number of different means, including serial sectioning and evaluation by conventional H&E staining and light microscopy, IHC staining, or polymerase chain reaction (PCR) for epithelial or tumor-associated antigens (64).

Studies with long patient follow-up have suggested that the presence of lymph node micrometastases, detected either by serial sectioning or by special studies such as IHC or PCR, confers a worse prognosis (64). Overall, these studies suggest that the presence of micrometastatic lymph node involvement predicts for a slightly worse outcome than for patients who are truly node negative. However, most of these studies suggest that occult lymph node involvement is a weak or at most a moderate strength prognostic factor. Moreover, the use of serial sectioning to detect occult nodal involvement is impractical for routine clinical care. We assign a 1+ TMUGS grade to identification of micrometastases in axillary lymph nodes using any special studies (serial sectioning, IHC, PCR, etc.).

Such a cautionary note is further warranted in the case of lymph nodes that have been identified by sentinel node mapping (65,66). Although IHC seems to have been widely adopted by many pathologists as a method of evaluation, only a single study has been reported (and none has been published) that addresses the prognostic strength of positive findings. In this study, the finding of IHC positivity in H&E negative sentinel lymph nodes did not change prognosis (67). In the absence of any meaningful data, this technique is assigned a ± TMUGS grade, at best, and it seems imprudent to incorporate this technique into routine care.

Bone Marrow Micrometastases. Like detection of micrometastases in lymph nodes, occult cancer deposits in bone marrow (bone marrow micrometastases, BMM) might also provide prognostic information. Most studies have utilized immunocytochemical staining of a variety of epithelial antigens, including cytokeratin, membrane bound mucins, or tumor-associated antigens. Current techniques allow the detection of about one tumor cell in 2×10^6 mononuclear cells. The interpretation of the findings from these studies is hampered by the variability in the sensitivity and specificity of the antibodies used, as well as the differences in staining and analysis techniques. A recently published meta-analysis of 20 studies, including many in breast cancer patients, has suggested that this technique may not provide important prognostic information (68). This report noted that the presence of BMM in patients with breast cancer was associated with a worse outcome, but only modestly so (RR of relapse = 1.3; 95% confidence interval [CI], 1.27–1.42) (68). However, overview/meta-analyses may dilute the findings of individual well-designed trials. In this regard, two prospective, large trials from Europe suggest that detection of BMM is strongly prognostic, but recently reported results from a third study, with very long-term follow-up, suggest that BMM was not prognostic in untreated node negative patients (69–71). Although some centers have begun to routinely test for BMM, we feel that more follow-up, especially in those patients with an otherwise particularly good prognosis, is required before widespread adoption of this procedure. If the initially exciting results from these specific studies are substantiated with longer follow-up and other studies, it appears that BMM will be a "strong" factor. However, the inconsistencies and relative sparcity of LOE I or II data lead us to assign this technique a 1+ TMUGS grade at this time.

Markers of Angiogenesis

Tumor growth and metastasis are critically dependent on tumor-induced angiogenesis (72). The increasing appreciation of the importance of angiogenesis prompted speculation that measures of neovascularization, either directly, by counting new blood vessels or indirectly, by monitoring putative angiogenic factors and their receptors, might serve as prognostic factors.

Weidner and colleagues first reported that microvessel density (MVD) count (after IHC staining for Factor VIII-related antigen) is a statistically significant independent predictor of DFS and OS in both node-negative and node-positive women (73). Subsequently reported retrospective studies have both confirmed and refuted these finding (74). As with many of the other tumor marker studies, evaluation of angiogenesis is complicated by technical variation, reader inconsistency, and potential interaction with therapy (75). The data are so confounded that we assign a +/− or 1+ to measures of neovascularization as a prognostic factor, and we do not recommend its use as a basis for making clinical decisions.

In addition to counting MVD, investigators have examined the association between angiogenic factors, produced either by the tumor or recruited from the extracellular matrix, and patient outcome. The most commonly studied angiogenic factors include basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), either in the breast cancer tissue itself or in the circulation (76,77). Although promising, the available LOE III data regarding these factors remains very preliminary. Again, we assign a TMUGS grade of +/− to these factors, and no definite recommendations regarding their use can be made.

In summary, the hypothesis that angiogenic activity in individual specimens might distinguish between tumors that are more indolent or aggressive in nature is appealing. However, the data are mostly derived from small retrospective analyses (LOE III). Large LOE II studies are ongoing, and could help to further delineate the prognostic role of tumor-induced angiogenesis. These studies have at least provided an increasingly sophisticated understanding of the role of various angiogenic factors in the process of malignant transformation and behavior, leading to a number of studies directed toward therapeutic intervention.

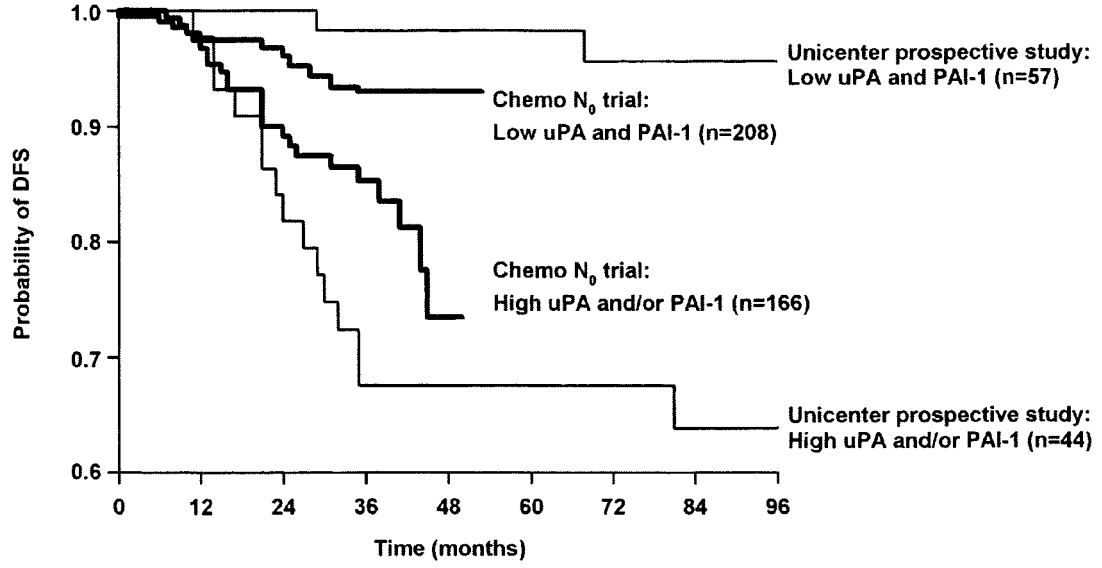
Markers of Invasion

Angiogenesis is just one feature of the complex process of invasion and metastasis. In theory, tumor cells adhere to the extracellular matrix and recruit stromal cells. Subsequently, matrix-degrading proteases mediate tumor cell passage through basement membrane and extracellular matrix leading to tumor progression. Identification of markers that are related to the process of adhesion, invasion, and metastasis might therefore be very valuable, especially in women whose primary breast cancer has not spread to regional nodes. Although a number of promising studies have been reported regarding several of these markers, at present none of those included in this category can be applied for routine clinical use.

Cathepsin D. One of the most intensively studied markers in this category is cathepsin D. Cathepsin D is a lysosomal proteolytic enzyme with a critical role in protein catabolism and tissue remodeling (78). Early studies of cathepsin D in breast cancer suggested that high levels of the marker are associated with worse outcomes in some groups of women with breast cancer but subsequent results have been mixed (78,79). After careful review, the ASCO Tumor Marker Expert Panel did not recommend routine clinical use of this marker (17). Nonetheless, considerable interest in this family of enzymes continues. A meta-analysis of 11 studies of cathepsin D in node-negative women suggested that the relative risk of relapse for low versus high levels of cathepsin ranged from 0.53 to 0.63 for follow-up of 12–84 months, which would place it in the "moderate strength" category (80). Regardless, the heterogeneity of methods for analyses of cathepsin D and the broad range of results from the various studies lead us to assign a +/− or 1+ grade, and it must be considered investigational.

Urokinase Plasminogen Activator System. Urokinase plasminogen activator (uPA) is a serine protease with an important role in cancer invasion and metastases (81). When bound to its receptor (uPAR), uPA converts plasminogen into plasmin and mediates extracellular degradation with subsequent tumor cell invasion and spread. In addition, inhibitors of uPA have been identified, including type 1 and 2 plasminogen activator inhibitors (PAI-1 and 2 respectively). PAI-1 level is generally high in tumor tissue and plasma, and it is inactivated when bound to uPA. PAI-2 is usually present in low levels except for some conditions such as pregnancy or myeloid leukemia (81). Several studies have suggested that high levels of uPA, uPAR, and PAI-1 are associated with worse outcomes, while, interestingly, high levels of PAI-2 may be associated with improved outcomes (81,82).

These encouraging LOE III studies led several European investigators to initiate a prospective study in which breast cancer cytosolic uPA and/or PAI-1 levels from node positive breast cancer patients were determined (83). Patients with elevated levels of one or both markers were randomly assigned to six cycles of adjuvant chemotherapy (cyclophosphamide, methotrexate, 5-Fluorouracil (CMF)) versus observation, while patients with low levels of both markers were observed. As expected, those with low levels of both markers had a remarkably favorable prognosis, approaching or surpassing that which most clinicians and patients feel is required to justify adjuvant chemotherapy (<10%) (Fig. 4). In contrast, the prognosis of those with one or both markers elevated who did not receive adjuvant chemotherapy was substantially worse. In the randomized trial, administration of adjuvant chemotherapy to the other 50% of women with elevated uPA and/or PAI-1 levels was associated with a 44% proportional reduction in probability of relapse (at interim analysis with a 32-month median follow-up $p = 0.17$).



Patients at risk: Chemo N_0 trial

Low uPA/PAI-1	173/5	119/7	57/10	p=0.006
High uPA/PAI-1	141/5	106/15	51/19	

Patients at risk: Unicenter prospective study

Low uPA/PAI-1	57/0	57/0	56/1	56/1	46/1	29/2	19/2	p<0.001
High uPA/PAI-1	43/1	35/8	28/14	27/14	24/14	21/14	16/15	

Fig. 4. Disease free survival according to UPA/PAI results from prospective randomized multicenter clinical trial (thick lines) and from prospective unicenter clinical trial (thin lines). From Ref. 83 with permission.

Taken together with the previously published studies, this study, although small and with a short follow-up, very nearly fulfills the requirements put forth to achieve "LOE I" status. Of note, a larger validation trial is now ongoing in Europe. However, at least in the United States, the assay technology is problematic. Nearly all of the data generated for prognosis come from ELISAs performed on relatively large, frozen tissue sections removed at the time of surgery. This situation poses two problems. With widespread advent of screening, primary breast cancers are increasingly smaller, so that the mean size in many centers is now less than 2 cm. Many pathologists are reluctant to exclude a relatively large portion of such a small tumor mass for a molecular assay, precluding its availability for routine light microscopic evaluation. Although promising pilot studies have been performed with core needle biopsy material and microassays for uPA and PAI-1, these results require substantial validation. A second problem involves the requirement for tissue freezing. With the widespread adoption of IHC techniques by most departments of pathology to determine ER and PgR, routine tissue freezing of breast cancer specimens has been discontinued, obviating the need for expensive equipment and supplies for freezing and storage (such as liquid nitrogen). Returning to such a system would require a major paradigm shift in this country. IHC staining for uPA and PAI-1 has been reported, but there are few if any studies correlating these results with poor outcome (84). Therefore, at least in the United States, routine assessment of these markers continues to be considered investigational. Thus, overall, we assign a 1+ TMUGS grade to the UPA/PAI-1 system pending the results of the definitive European validation study and resolution of the technical issues surrounding a practical assay.

Other Markers of Invasion and Metastasis. Many other markers of the metastatic process have been proposed and/or studied. An incomplete list of some of these, for which interesting preclinical rationales and very early LOE III type data have been

reported, include abnormalities in nm23, E-cadherin, the catenins, tissue inhibitors of metalloproteinases (TIMPs), prostate specific antigen, tissue factor (TF), and osteopontin (85) (86–92). Moreover, allelic loss, microsatellite instability, or methylation silencing of tumor suppressor genes may also be prognostic (93–96). However, all of these very interesting concepts require further evaluation and validation, and each is assigned a TMUGS score of +/−.

SUMMARY

Current prognostic factors including TNM stage, axillary node status, tumor size and grade, the presence or absence of lymphatic or blood vessel invasion, and hormone receptor status provide critical information necessary for adjuvant systemic treatment recommendations. Unfortunately, the promise of molecular and biologic markers such as HER-2, p53, cathepsin D, angiogenic factors, and members of the urokinase plasminogen activator system, has yet to be fulfilled. Future research should incorporate rigorous analytic methods for these newer candidate prognostic markers and should involve prospective incorporation into clinical trials. Such an approach will allow clinicians to more precisely identify an individual patient's recurrence risk. This information in turn will lead to a more judicious use of adjuvant therapy for early stage breast cancer.

ACKNOWLEDGMENT

Supported by Grant CI-3 of the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation and a grant from The Fashion Footwear Association of New York (FFANY) "Shoes-On-Sale"/QVC Presents®.

REFERENCES

1. S. Nass, I. C. Henderson, and J. C. Lashoff (2001). *Mammography and Beyond: Developing Technologies for the Early Detection of Breast Cancer*, National Academy Press, Washington, DC.
2. Early Breast Cancer Trialist's Collaborative Group (1998). Polychemotherapy for early breast cancer: An overview of the randomized trials. *Lancet* **352:**930–942.
3. Early Breast Cancer Trialist's Collaborative Group (1998). Tamoxifen for early breast cancer: An overview of the randomised trials. *Lancet* **351:**1451–1467.
4. Early Breast Cancer Trialists Collaborative Group (1996). Ovarian ablation in early breast cancer: Overview of the randomised trials. *Lancet* **348:**1189–1196.
5. Early Breast Cancer Trialists' Collaborative Group (2000). Favourable and unfavourable effects on long-term survival of radiotherapy for early breast cancer: An overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet* **355:**1757–1770.
6. R. Peto, J. Boreham, M. Clarke, C. Davies, and V. Beral (2000). UK and USA breast cancer deaths down 25% in year 2000 at ages 20–69 years. *Lancet* **355:**1822.
7. W. S. Halsted (1894–95). The results of operations for the cure of cancer of the breast performed at the Johns Hopkins Hospital from June, 1889, to January, 1894. *Johns Hopkins Hosp. Rep.* **iv:**297–350.
8. American Joint Committee on Cancer (1997). In I. Fleming, J. Cooper, D. Henson, R. Hutter, B. J. Kennedy, G. Murphy, B. O'Sullivan, L. Sobin, and J. Yarbro (eds.), *AJCC Cancer Staging Handbook, Breast*, 5th edn., Vol. 1, Lippincott-Raven, Philadelphia, pp. 171–180.
9. D. F. Hayes, R. Bast, C. E. Desch, H. Fritsche, N. E. Kemeny, J. Jessup, G. Y. Locker, J. Macdonald, R. G. Mennel, L. Norton, P. Ravdin, S. Taube, and R. Winn (1996). A tumor marker utility grading system (TMUGS): A framework to evaluate clinical utility of tumor markers. *J. Natl. Cancer Inst.* **88:**1456–1466.
10. R. Simon and D. G. Altman (1994). Statistical aspects of prognostic factor studies in oncology. *Br. J. Cancer* **69:**979–985.
11. D. G. Altman and G. H. Lyman (1998). Methodological challenges in the evaluation of prognostic factors in breast cancer. *Breast Cancer Res. Treat.* **52:**289–303.
12. W. L. McGuire (1991). Breast cancer prognostic factors: Evaluation guidelines. *J. Natl. Cancer Inst.* **83:**154–155.
13. G. Gasparini, F. Pozza, and A. L. Harris (1993). Evaluating the potential usefulness of new prognostic and predictive indicators in node-negative breast cancer patients. *J. Natl. Cancer Inst.* **85:**1206–1219.
14. D. F. Hayes, B. Trock, and A. Harris (1998). Assessing the clinical impact of prognostic factors: When is "statistically significant" clinically useful? *Breast Cancer Res. Treat.* **52:**305–319.
15. W. L. McGuire and G. M. Clark (1992). Prognostic factors and treatment decisions in axillary-node-negative breast cancer. *N. Engl. J. Med.* **326:**1756–1761.
16. G. Gasparini (1998). Prognostic variables in node-negative and node-positive breast cancer [editorial]. *Breast Cancer Res. Treat.* **52:**321–331.
17. ASCO Expert Panel (1996). Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer: Report of the american society of clinical oncology expert panel. *J. Clin. Oncol.* **14:**2843–2877.
18. ASCO Expert Panel (1998). 1997 update of recommendations for the use of tumor markers in breast and colorectal cancer. *J. Clin. Oncol.* **16:**793–795.
19. R. C. Bast Jr., P. Ravdin, D. F. Hayes, S. Bates, H. Fritsche Jr., J. M. Jessup, N. Kemeny, G. Y. Locker, R. G. Mennel, and M. R. Somerfield (2001). 2000 Update of recommendations for the use of tumor markers in breast and colorectal cancer: Clinical practice guidelines of the american society of clinical oncology. *J. Clin. Oncol.* **19:**1865–1878.
20. C. Isaacs, V. Stearns, and D. F. Hayes (2001). New prognostic factors for breast cancer recurrence. *Semin. Oncol.* **28:**53–67.
21. P. Ravdin, I. Siminoff, and J. Harvey (1998). Survey of breast cancer patients concerning their knowledge and expectations of adjuvant therapy. *J. Clin. Oncol.* **16:**515–521.

22. A. S. Coates and R. J. Simes (1992). *Patient Assessment of Adjuvant Treatment in Operable Breast Cancer*, Wiley, New York.
23. C. Lindley, S. Vasa, T. Sawyer, and E. Winer (1998). Quality of life and preferences for treatment following systemic adjuvant therapy for early stage breast cancer. *J. Clin. Oncol.* **16:**1380–1387.
24. S. Rajagopal, P. J. Goodman, and I. F. Tannock (1994). Adjuvant chemotherapy for breast cancer: Discordance between physicians' perception of benefit and the results of clinical trials. *J. Clin. Oncol.* **12**:1296–1304.
25. P. M. Ravdin, L. A. Siminoff, G. J. Davis, M. B. Mercer, J. Hewlett, N. Gerson, and H. L. Parker (2001). Computer program to assist in making decisions about adjuvant therapy for women with early breast cancer. *J. Clin. Oncol.* **19:**980–991.
26. I. C. Henderson, D. Berry, G. Demetri, C. Cirrincione, L. Goldstein, S. Martino, J. Ingle, M. Cooper, G. Canellos, E. Borden, G. Fleming, J. Holland, S. Graziano, J. Carpenter, H. Muss, and L. Norton (1998). Improved disease-free and overall survival from the addition of sequential paclitaxel but not from the escalation of doxorubicin dose level in the adjuvant chemotherapy of patients with node-positive primary breast cancer. *Proc. Am. Soc. Clin. Oncol.* **17:**101a.
27. C. L. Carter, C. Allen, and D. E. Henson (1989). Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. *Cancer* **63:**181–187.
28. P. P. Rosen, S. Groshen, D. W. Kinne, and L. Norton (1993). Factors influencing prognosis in node-negative breast carcinoma: Analysis of 767 T1N0M0/T2N0M0 patients with long-term follow-up. *J. Clin. Oncol.* **11:**2090–2100.
29. C. A. Quiet, D. J. Ferguson, R. R. Weichselbaum, and S. Hellman (1995). Natural history of node-negative breast cancer: A study of 826 patients with long-term follow-up. *J. Clin. Oncol.* **13:**1144–1151.
30. J. F. Simpson and D. L. Page (1994). Status of breast cancer prognostication based on histopathologic data. *Am. J. Clin. Pathol.* **102:**S3–S8.
31. P. P. Rosen, S. Groshen, P. E. Saigo, D. W. Kinne, and S. Hellman (1989). Pathological prognostic factors in Stage I (T1N0M0) and Stage II (T1N1M0) breast carcinoma: A study of 644 patients with median follow-up of 18 years. *J. Clin. Oncol.* **7:**1239–1251.
32. H. J. Bloom and W. W. Richardson (1957). Histological grading and prognosis in breast cancer. *Br. J. Cancer* **11:**359–377.
33. C. W. Elston and I. O. Ellis (1991). Pathological prognostic factors in breast cancer. 1. The value of histological gade in breast cancer: Experience from a large study with long-term follow-up. *Histopathology* **19:**403–409.
34. B. Fisher, C. Redmond, E. R. Fisher, and R. Caplan (1988). Relative worth of estrogen or progesterone receptor and pathologic characteristics of differentiation as indicators of prognosis in node negative breast cancer patients: Findings from National Surgical Adjuvant Breast and Bowel Project Protocol B-06. *J. Clin. Oncol.* **6:**1076–1087.
35. G. Contesso, H. Mouriesse, S. Friedman, J. Genin, D. Sarrazin, and J. Rouesse (1987). The importance of histologic grade in long-term prognosis of breast cancer: A study of 1,010 patients, uniformly treated at the Institut Gustave-Roussy. *J. Clin. Oncol.* **5:**1378–1386.
36. J. W. Yarbro, D. L. Page, L. P. Fielding, E. E. Partridge, and G. P. Murphy (1999). American Joint Committee on Cancer prognostic factors consensus conference. *Cancer* **86:**2436–2446.
37. P. L. Fitzgibbons, D. L. Page, D. Weaver, A. D. Thor, D. C. Allred, G. M. Clark, S. G. Ruby, F. O'Malley, J. F. Simpson, J. L. Connolly, D. F. Hayes, S. B. Edge, A. Lichter, and S. J. Schnitt (2000). Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. *Arch. Pathol. Lab. Med.* **124:**966–978.
38. K. Gilchrest, L. Kalish, V. Gould, H. S. J. Imbriglia, W. Levy, A. Patchefsky, D. Penner, J. Pickren, J. Roth, R. Schinella, I. Schwartz, and J. Wheeler (1985). Interobserver reproducibility of histopathological features in stage II breast cancer. *Breast Cancer Res. Treat.* **5:**3–10.
39. L. W. Dalton, S. E. Pinder, C. E. Elston, I. O. Ellis, D. L. Page, W. D. Dupont, and R. W. Blamey (2000). Histologic grading of breast cancer: Linkage of patient outcome with level of pathologist agreement. *Mod. Pathol.* **13:**730–735.
40. D. L. Page, W. D. Dupont, R. A. Jensen, and J. F. Simpson (1998). When and to what end do pathologists agree? *J. Natl. Cancer Inst.* **90:**88–89.
41. A. M. Neville, R. Bettelheim, R. D. Gelber, J. Save-Soderbergh, B. W. Davis, R. Reed, J. Torhorst, R. Golouh, H. F. Peterson, K. N. Price, *et al*. (1992). Factors predicting treatment responsiveness and prognosis in node-negative breast cancer. The International (Ludwig) Breast Cancer Study Group. *J. Clin. Oncol.* **10:**696–705.
42. W. L. McGuire, A. K. Tandon, D. C. Allred, G. C. Chamness, and G. M. Clark (1990). How to use prognostic factors in axillary node-negative breast cancer patients. *J. Natl. Cancer Inst.* **82:**1006–1015.
43. S. G. Hilsenbeck, P. M. Ravdin, C. A. de Moor, G. C. Chamness, C. K. Osborne, and G. M. Clark (1998). Time-dependence of hazard ratios for prognostic factors in primary breast cancer. *Breast Cancer Res. Treat.* **52:**227–237.
44. C. R. Wenger and G. M. Clark (1998). S-phase fraction and breast cancer—a decade of experience. *Breast Cancer Res. Treat.* **51:**255–265.
45. L. Hutchins, S. Green, P. Ravdin, D. Lew, S. Martino, M. Abeloff, A. Lyss, I. C. Henderson, C. Allred, S. Dakhil, L. Pierce, W. Goodwin, J. Caton, S. Rivkin, R. Chapman, and K. Osborne (1998). CMF vs. CAF with and without tamoxifen in high-risk node-negataive breast cancer patients and a natural history follow-up study in low-risk node-negative patients: First results of intergroup trial INT0102. *Proc. Am. Soc. Clin. Oncol.* **17:**1a.
46. R. W. Brown, C. D. Allred, G. M. Clark, C. K. Osborne, and S. G. Hilsenbeck (1996). Prognostic value of Ki-67 compared to S-phase fraction in axillary node-negative breast cancer. *Clin. Cancer Res.* **2:**585–592.
47. A. D. Thor, S. Liu, D. H. Moore II, and S. M. Edgerton (1999). Comparison of mitotic index, in vitro bromodeoxyuridine labeling, and MIB-1 assays to quantitate proliferation in breast cancer. *J. Clin. Oncol.* **17:**470–477.
48. D. Amadori and R. Silvestrini (1998). Prognostic and predictive value of thymidine labelling index in breast cancer. *Breast Cancer Res. Treat.* **51:**267–281.
49. L. Dressler, K. Conway, J. Geradts, S. Edminston, D. Cowan, M. Burroughs, L. Cui, M. He, J. Pang, K. Mistry, S. Drouin, Y. Li, J. Tse, R. Millikan, and B. Newman (1999). Immunohistochemistry can accurately detect missense mutations in the p53 gene: A comparison with gene sequencing in 196 breast cancers. *Proc. Am. Assoc. Cancer Res.* **40:**690a.
50. C. MacGeoch, G. Turner, L. Bobrow, D. M. Barnes, D. Bishop, and N. Spurr (1995). Heterogeneity in Li-Fraumeni families:

p53 mutation analysis and immunohistochemical staining. *J. Med. Genet.* **32:**186–190.
51. P. D. Pharoah, N. E. Day, and C. Caldas (1999). Somatic mutations in the p53 gene and prognosis in breast cancer: A meta-analysis. *Br. J. Cancer* **80:**1968–1973.
52. J. Reed (1994). Bcl-2 and the regulaton of programmed cell death. *J. Cell Biol.* **124:**1–6.
53. S. Krajewski, A. Thor, S. Edgerton, D. Moore, M. Krajewska, and J. Reed (1997). Analysis of bax and bcl-2 in p53-immunopositive breast cancers. *Clin. Cancer Res.***3:**199–208.
54. S. Krajewski, C. Blomqvist, K. Franssila, M. Krajewska, V. M. Wasenius, E. Niskanen, S. Nordling, and J. C. Reed (1995). Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. *Cancer Res.* **55:**4471–4478.
55. O. I. Olopade, M. O. Adeyanju, A. R. Safa, F. Hagos, R. Mick, C. B. Thompson, and W. M. Recant (1997). Overexpression of BCL-x protein in primary breast cancer is associated with high tumor grade and nodal metastases. *Cancer J. Sci. Am.* **3:**230–237.
56. C. Gillett, P. Smith, W. Gregory, M. Richards, R. Millis, G. Peters, and D. Barnes (1996). Cyclin D1 and prognosis in human breast cancer. *Int. J. Cancer* **69:**92–99.
57. D. M. Barnes and C. E. Gillett (1998). Cyclin D1 in breast cancer. *Breast Cancer Res. Treat.* **52:**1–15.
58. R. Seshadri, C. S. Lee, R. Hui, K. McCaul, D. J. Horsfall, and R. L. Sutherland (1996). Cyclin DI amplification is not associated with reduced overall survival in primary breast cancer but may predict early relapse in patients with features of good prognosis. *Clin. Cancer Res.* **2:**1177–1184.
59. A. L. Schechter, D. F. Stern, L. Vaidyanathan, S. J. Decker, J. A. Drebin, M. I. Greene, and R. A. Weinberg (1984). The neu oncogene: An erb-B-related gene encoding a 185,000-Mr tumour antigen. *Nature* **312:**513–516.
60. D. J. Slamon, G. M. Clark, S. G. Wong, W. J. Levin, A. Ullrich, and W. L. McGuire (1987). Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* **235:**177–182.
61. B. J. Trock, H. Yamauchi, M. Brotzman, V. Stearns, and D. F. Hayes (2000). c-erbB-2 as a prognostic factor in breast cancer: A meta-analysis. *Proc. Am. Soc. Clin. Oncol.* **19:**97a.
62. H. Yamauchi, V. Stearns, and D. F. Hayes (2001). When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. *J. Clin. Oncol.* **19:**2334–2356.
63. M. F. Press, L. Bernstein, P. A. Thomas, L. F. Meisner, J. Y. Zhou, Y. Ma, G. Hung, R. A. Robinson, C. Harris, A. El-Naggar, D. J. Slamon, R. N. Phillips, J. S. Ross, S. R. Wolman, and K. J. Flom (1997). HER-2/neu gene amplification characterized by fluorescence in situ hybridization: Poor prognosis in node-negative breast carcinomas. *J. Clin. Oncol.* **15:**2894–2904.
64. K. Siziopikou, S. Schnitt, J. Connolly, and D. F. Hayes (1999). Detection and significance of occult axillary metastatic disease in breast cancer patients. *Breast J.* **5:**221–229.
65. D. Krag, D. Weaver, T. Ashikaga, F. Moffat, V. S. Klimberg, C. Shriver, S. Feldman, R. Kusminsky, M. Gadd, J. Kuhn, S. Harlow, and P. Beitsch (1998). The sentinel node in breast cancer—a multicenter validation study. *N. Engl. J. Med.* **339:**941–946.
66. A. E. Giuliano, R. C. Jones, M. Brennan, and R. Statman (1997). Sentinel lymphadenectomy in breast cancer. *J. Clin. Oncol.* **15:**2345–2350.
67. N. Hansen, B. J. Grube, W. Te, M. L. Brennan, R. Turner, and A. E. Giuliano (2001). Clinical significance of axillary micrometastases in breast cancer: How small is too small. *Proc. Am. Soc. Clin. Oncol.* **20:**223a.
68. I. Funke and W. Schraut (1998). Meta-analyses of studies on bone marrow micrometastases: An independent prognostic impact remains to be substantiated. *J. Clin. Oncol.* **16:**557–566.
69. I. J. Diel, M. Kaufmann, S. D. Costa, R. Holle, G. von Minckwitz, E. F. Solomayer, S. Kaul, and G. Bastert (1996). Micrometastatic breast cancer cells in bone marrow at primary surgery: Prognostic value in comparison with nodal status. *J. Natl. Cancer Inst.* **88:**1652–1658.
70. S. Braun, K. Pantel, P. Muller, W. Janni, F. Hepp, C. R. Kentenich, S. Gastroph, A. Wischnik, T. Dimpfl, G. Kindermann, G. Riethmuller, and G. Schlimok (2000). Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. *N. Engl. J. Med.* **342:**525–533.
71. G. Gebauer, T. Fehm, E. Merkle, E. P. Beck, N. Lang, and W. Jager (2001). Epithelial cells in bone marrow of breast cancer patients at time of primary surgery: Clinical outcome during long-term follow-up. *J. Clin. Oncol.* **19:**3669–3674.
72. J. Folkman (1990). What is the evidence that tumors are angiogenesis dependent? *J. Natl. Cancer Inst.* **82:**4–6.
73. N. Weidner, J. Folkman, F. Pozza, P. Bevilacqua, E. Allred, S. Meli, and G. Gasparini (1992). Tumor angiogenesis: A new significant and independent prognostic indicator in early stage breast carcinoma. *J. Natl. Cancer Inst.* **84:**1875–1887.
74. G. Gasparini (2001). Clinical significance of determination of surrogate markers of angiogenesis in breast cancer. *Crit. Rev. Oncol. Hematol.* **37:**97–114.
75. A. J. Guidi, D. Berry, G. Broadwater, B. Helmchen, I. Bleiweiss, D. Budman, I. C. Henderson, L. Norton, and D. F. Hayes (submitted). Association ofangiogenesis and disease outcome in node-positive breast cancer patients treated with adjuvant CAF chemotherapy: A CALGB correlative science study from protocols 8541/8869. *J. Clin. Oncol.* **20:**732–742.
76. G. Gasparini, M. Toi, M. Gion, P. Verderio, R. Dittadi, M. Hanatani, I. Matsubara, O. Vinante, E. Bonoldi, P. Boracchi, C. Gatti, H. Suzuki, and T. Tominaga (1997). Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. *J. Natl. Cancer Inst.* **89:**139–147.
77. U. Eppenberger, W. Kueng, J. M. Schlaeppi, J. L. Roesel, C. Benz, H. Mueller, A. Matter, M. Zuber, K. Luescher, M. Litschgi, M. Schmitt, J. A. Foekens, and S. Eppenberger-Castori (1998). Markers of tumor angiogenesis and proteolysis independently define high- and low-risk subsets of node-negative breast cancer patients. *J. Clin. Oncol.* **16:**3129–3136.
78. B. R. Westley and F. E. May (1996). Cathepsin D and breast cancer. *Eur. J. Cancer* **32A:**15–24.
79. P. M. Ravdin, C. A. de Moor, S. G. Hilsenbeck, M. K. Samoszuk, P. M. Vendely, and G. M. Clark (1997). Lack of prognostic value of cathepsin D levels for predicting short term outcomes of breast cancer patients. *Cancer Lett.* **116:**177–183.
80. G. Ferrandina, G. Scambia, F. Bardelli, P. Benedetti Panici, S. Mancuso, and A. Messori (1997). Relationship between cathepsin-D content and disease-free survival in node-negative breast cancer patients: A meta-analysis. *Br. J. Cancer* **76:**661–666.

81. R. W. Stephens, N. Brunner, F. Janicke, and M. Schmitt (1998). The urokinase plasminogen activator system as a target for prognostic studies in breast cancer. *Breast Cancer Res. Treat.* **52:**99–111.
82. J. A. Foekens, H. A. Peters, M. P. Look, H. Portengen, M. Schmitt, M. D. Kramer, N. Brunner, F. Janicke, M. E. Meijer-van Gelder, S. C. Henzen-Logmans, W. L. van Putten, and J. G. Klijn (2000). The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. *Cancer Res.* **60:**636–643.
83. F. Janicke, A. Prechtl, C. Thomssen, N. Harbeck, C. Meisner, M. Untch, C. G. Sweep, H. K. Selbmann, H. Graeff, and M. Schmitt (2001). Randomized adjuvant chemotherapy trial in high-risk, lymph node-negative breast cancer patients identified by urokinase-type plasminogen activator and plasminogen activator inhibitor type 1. *J. Natl. Cancer Inst.* **93:**913–920.
84. D. W. Visscher, F. Sarkar, P. LoRusso, W. Sakr, S. Ottosen, S. Wykes, and J. D. Crissman (1993). Immunohistologic evaluation of invasion-associated proteases in breast carcinoma. *Mod. Pathol.* **6:**302–306.
85. A. B. Tuck, F. P. O'Malley, H. Singhal, J. F. Harris, K. S. Tonkin, N. Kerkvliet, Z. Saad, G. S. Doig, and A. F. Chambers (1998). Osteopontin expression in a group of lymph node negative breast cancer patients. *Int. J. Cancer* **79:**502–508.
86. R. Heimann and S. Hellman (2000). Individual characterisation of the metastatic capacity of human breast carcinoma. *Eur. J. Cancer* **36:**1631–1639.
87. R. Heimann, F. Lan, R. McBride, and S. Hellman (2000). Separating favorable from unfavorable prognostic markers in breast cancer: The role of E-cadherin. *Cancer Res.* **60:**298–304.
88. R. Heimann, D. J. Ferguson, and S. Hellman (1998). The relationship between nm23, angiogenesis, and the metastatic proclivity of node-negative breast cancer. *Cancer Res.* **58:**2766–2771.
89. H. Yu, M. A. Levesque, G. M. Clark, and E. P. Diamandis (1999). Enhanced prediction of breast cancer prognosis by evaluating expression of p53 and prostate-specific antigen in combination. *Br. J. Cancer* **81:**490–495.
90. A. Remacle, K. McCarthy, A. Noel, T. Maguire, E. McDermott, N. O'Higgins, J. M. Foidart, and M. J. Duffy (2000). High levels of TIMP-2 correlate with adverse prognosis in breast cancer. *Int. J. Cancer* **89:**118–121.
91. T. Ueno, M. Toi, M. Koike, S. Nakamura, and T. Tominaga (2000). Tissue factor expression in breast cancer tissues: Its correlation with prognosis and plasma concentration. *Br. J. Cancer* **83:**164–170.
92. R. Yoshida, N. Kimura, Y. Harada, and N. Ohuchi (2001). The loss of E-cadherin, alpha- and beta-catenin expression is associated with metastasis and poor prognosis in invasive breast cancer. *Int. J. Oncol.* **18:**513–520.
93. M. Emi, M. Yoshimoto, T. Sato, S. Matsumoto, Y. Utada, I. Ito, K. Minobe, T. Iwase, T. Katagiri, K. Bando, F. Akiyama, Y. Harada, K. Fukino, G. Sakamoto, M. Matsushima, A. Iida, T. Tada, H. Saito, Y. Miki, F. Kasumi, and Y. Nakamura (1999). Allelic loss at 1p34, 13q12, 17p13.3, and 17q21.1 correlates with poor postoperative prognosis in breast cancer. *Genes Chromosomes Cancer* **26:**134–141.
94. Y. Utada, M. Emi, M. Yoshimoto, F. Kasumi, F. Akiyama, G. Sakamoto, S. Haga, T. Kajiwara, and Y. Nakamura (2000). Allelic loss at 1p34–36 predicts poor prognosis in node-negative breast cancer. *Clin. Cancer Res.* **6:**3193–3198.
95. R. Hui, R. D. Macmillan, F. S. Kenny, E. A. Musgrove, R. W. Blamey, R. I. Nicholson, J. F. Robertson, and R. L. Sutherland (2000). INK4a gene expression and methylation in primary breast cancer: Overexpression of p16INK4a messenger RNA is a marker of poor prognosis. *Clin. Cancer Res.* **6:**2777–2787.
96. X. Yang, L. Yan, and N. E. Davidson (2001). DNA methylation in breast cancer. *Endocr. Relat. Cancer* **8:**115–127.