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American Society of Clinical Oncology 2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer

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ABSTRACT

Purpose
To update the recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of breast cancer.

Methods
For the 2007 update, an Update Committee composed of members from the full Panel was formed to complete the review and analysis of data published since 1999. Computerized literature searches of MEDLINE and the Cochrane Collaboration Library were performed. The Update Committee's literature review focused attention on available systematic reviews and meta-analyses of published tumor marker studies. In general, significant health outcomes (overall survival, disease-free survival, quality of life, lesser toxicity, and cost-effectiveness) were used for making recommendations.

Recommendations and Conclusions
Thirteen categories of breast tumor markers were considered, six of which were new for the guideline. The following categories showed evidence of clinical utility and were recommended for use in practice: CA 15-3, CA 27.29, carcinoembryonic antigen, estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, urokinase plasminogen activator, plasminogen activator inhibitor 1, and certain multiparameter gene expression assays. Not all applications for these markers were supported, however. The following categories demonstrated insufficient evidence to support routine use in clinical practice: DNA/ploidy by flow cytometry, p53, cathepsin D, cyclin E, proteomics, certain multiparameter assays, detection of bone marrow micrometastases, and circulating tumor cells.

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INTRODUCTION

The American Society of Clinical Oncology (ASCO) first published evidence-based clinical practice guidelines for the use of tumor markers in breast cancer in 1996. ASCO guidelines are updated at intervals by an Update Committee of the original Expert Panel. The last update of the tumor markers guideline was published in 2000. For the 2007 update, the Panel expanded the scope of the guideline to include a broader range of markers in breast cancer. In addition, the impact of genomic technologies was considered in the Update. While molecular subtyping is still in its infancy, and subgroups are not well defined, the use of multiparameter technologies in clinical practice has considerable potential. The updated recommendations are summarized in Table 1.

UPDATE METHODOLOGY

For the 2007 update, an Update Committee composed of members from the full Panel was formed to complete the review and analysis of data published since 1999 (Appendix Table A1). Computerized literature searches of MEDLINE and the Cochrane Collaboration Library were performed. The searches of the English-language literature spanned 1999 to February 2007 (or from 1966 to February 2007 for the new markers). Details of the literature searches are provided in the Appendix.

The Update Committee's literature review focused attention on available systematic reviews and meta-analyses of published tumor marker studies, although primary data were also reviewed. By and large, however, the primary literature is characterized by studies that included small patient numbers, that are retrospective, and that commonly perform

Table 1. Summary of Guideline Recommendations

Recommendations for the Use of Tumor Markers in Breast Cancer	
Specific Marker	2007 Recommendation
CA 15-3 and CA 27.29 as markers for breast cancer as screening, diagnostic, or staging tests	Present data are insufficient to recommend CA 15-3 or CA 27.29 for screening, diagnosis, and staging. ***There is no change from the guideline published in 2000.***
CA 15-3 and CA 27.29 to detect recurrence after primary breast cancer therapy	Present data do not support the use of CA 15-3 and CA 27.29 for monitoring patients for recurrence after primary breast cancer therapy. ***There is no change from the guideline published in 2000.***
CA 15-3 and CA 27.29 to contribute to decisions regarding therapy for metastatic breast cancer	For monitoring patients with metastatic disease during active therapy, CA 27.29 or CA 15-3 can be used in conjunction with diagnostic imaging, history, and physical examination. Present data are insufficient to recommend use of CA 15-3 or CA 27.29 alone for monitoring response to treatment. However, in the absence of readily measurable disease, an increasing CA 15-3 or CA 27.29 may be used to indicate treatment failure. Caution should be used when interpreting a rising CA 27.29 or CA 15-3 level during the first 4-6 weeks of a new therapy, since spurious early rises may occur. ***There is no change from the guideline published in 2000.***
CEA for screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy	CEA is not recommended for screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy. ***There is no change from the guideline published in 2000.***
CEA to contribute to decisions regarding therapy for metastatic breast cancer	For monitoring patients with metastatic disease during active therapy, CEA can be used in conjunction with diagnostic imaging, history, and physical examination. Present data are insufficient to recommend use of CEA alone for monitoring response to treatment. However, in the absence of readily measurable disease, an increasing CEA may be used to indicate treatment failure. Caution should be used when interpreting a rising CEA level during the first 4-6 weeks of a new therapy, since spurious early rises may occur. ***There is no change from the guideline published in 2000.***
ERs and PgRs	ER and PgR should be measured on every primary invasive breast cancer and may be measured on metastatic lesions if the results would influence treatment planning. In both pre-and postmenopausal patients, steroid hormone receptor status should be used to identify patients most likely to benefit from endocrine forms of therapy in both the early breast cancer and metastatic disease settings. In patients with DCIS who are candidates for hormonal therapy, data are insufficient to recommend routine measurement of ER and PgR for therapy recommendations.
DNA flow cytometry–based parameters	Present data are insufficient to recommend use of DNA content, S phase, or other flow cytometry–based markers of proliferation to assign patients to prognostic groups. ***There is no change from the guideline published in 2000.***
Immunohistochemically based markers of proliferation (Note: This topic is new to the guideline)	Present data are insufficient to recommend measurement of Ki67, cyclin D, cyclin E, p27, p21, thymidine kinase, topoisomerase II, or other markers of proliferation to assign patients to prognostic groups.
HER2 evaluation in breast cancer	HER2 expression and/or amplification should be evaluated in every primary invasive breast cancer either at the time of diagnosis or at the time of recurrence, principally to guide selection of trastuzumab in the adjuvant and/or metastatic setting. Other utilities for HER2 evaluation are also discussed separately above.
HER2 to define prognosis for early-stage breast cancer patients in the absence of systemic therapy	HER2 amplification, overexpression, and the presence of HER2 extracellular domain are generally associated with a poorer prognosis. However, the value of this information in clinical practice is questionable and the use of HER2 for determining prognosis is not recommended. ***There is no change from the guideline published in 2000.***
HER2 to select patients for anti-HER2–based therapy	High levels of tissue HER2 expression or *HER2* gene amplification should be used to identify patients for whom trastuzumab may be of benefit for treatment of breast cancer in the adjuvant or metastatic disease settings. ***There is no change from the guideline published in 2000.***
The utility of HER2 for predicting response to specific chemotherapeutic agents	Level II evidence (prospective therapeutic trials in which marker utility is a secondary study objective) suggests that overexpression of HER2 (3+ by protein or $>$ 2.0 FISH ratio by gene amplification) identifies patients who have greater benefit from anthracycline-based adjuvant therapy. If a clinician is considering chemotherapy for a patient with HER2-positive breast cancer, it is recommended that an anthracycline be strongly considered, assuming there are no contraindications to anthracycline therapy. In the context of trastuzumab therapy, there is Level I evidence (single, high-powered, prospective, randomized, controlled trials specifically designed to test the marker or a meta-analyses of well-designed studies) that a nonanthracycline regimen may produce similar outcomes. At present, the Update Committee does not recommend that HER2 be used to guide use of taxane chemotherapy in the adjuvant setting.
HER2 to determine sensitivity to endocrine therapy	HER2 should not be used to withhold endocrine therapy for a patient with hormone receptor–positive breast cancer, nor should it be used to select one specific type of endocrine therapy over another. ***There is no change from the guideline published in 2000.***
Utility of circulating extracellular domain of HER-2	Measuring circulating extracellular domain of HER2 is not currently recommended for any clinical setting. ***There is no change from the guideline published in 2000.***
p53 as a marker for breast cancer	Present data are insufficient to recommend use of *p53* measurements for management of patients with breast cancer. ***There is no change from the guideline published in 2000.***

(continued on following page)

Table 1. Summary of Guideline Recommendations

Recommendations for the Use of Tumor Markers in Breast Cancer	
Specific Marker	2007 Recommendation
uPA and PAI-1 as a marker for breast cancer (Note: This topic is new to the guideline)	uPA/PAI-1 measured by ELISAs on a minimum of 300 mg of fresh or frozen breast cancer tissue may be used for the determination of prognosis in patients with newly diagnosed, node negative breast cancer. IHC for these markers is not accurate, and the prognostic value of ELISA using smaller tissue specimens has not been validated. Low levels of both markers are associated with a sufficiently low risk of recurrence, especially in hormone receptor–positive women who will receive adjuvant endocrine therapy, that chemotherapy will only contribute minimal additional benefit. Furthermore, CMF-based adjuvant chemotherapy provides substantial benefit, compared with observation alone, in patients with high risk of recurrence as determined by high levels of uPA and PAI-1.
Cathepsin D as a marker for breast cancer	Present data are insufficient to recommend use of cathepsin D measurements for management of patients with breast cancer. ***There is no change from the guideline published in 2000***
Cyclin E fragments as markers for breast cancer (Note: This topic is new to the guideline)	Present data are insufficient to recommend use of whole length or fragment measurements of cyclin E for management of patients with breast cancer.
Proteomic analysis for breast cancer (Note: This topic is new to the guideline)	Present data are insufficient to recommend use of proteomic patterns for management of patients with breast cancer.
Multiparameter gene expression analysis for breast cancer (Note: This topic is new to the guideline)	In newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, the Onco*type* DX assay can be used to predict the risk of recurrence in patients treated with tamoxifen. Onco*type* DX may be used to identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy. In addition, patients with high recurrence scores appear to achieve relatively more benefit from adjuvant chemotherapy (specifically (C)MF) than from tamoxifen. There are insufficient data at present to comment on whether these conclusions generalize to hormonal therapies other than tamoxifen, or whether this assay applies to other chemotherapy regimens. The precise clinical utility and appropriate application for other multiparameter assays, such as the MammaPrint assay, the "Rotterdam Signature," and the Breast Cancer Gene Expression Ratio are under investigation.
Bone marrow micrometastases as markers for breast cancer (Note: This topic is new to the guideline)	Present data are insufficient to recommend assessment of bone marrow micrometastases for management of patients with breast cancer.
Circulating tumor cell assays as markers for breast cancer (Note: This topic is new to the guideline)	The measurement of circulating tumor cells (CTCs) should not be used to make the diagnosis of breast cancer or to influence any treatment decisions in patients with breast cancer. Similarly, the use of the recently FDA-cleared test for CTC (CellSearch Assay) in patients with metastatic breast cancer cannot be recommended until further validation confirms the clinical value of this test.

Abbreviations: CEA, carcinoembryonic antigen; ER, estrogen receptor; PgR, progesterone receptor; DCIS, ductal carcinoma in situ; FISH, fluorescent in situ hybridization; uPA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor 1; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; CMF, cyclophosphamide, methotrexate, and fluorouracil; FDA, US Food and Drug Administration.

multiple analyses until one reveals a statistically significant result. Furthermore, many tumor marker studies fail to include descriptions of how patients were treated or analyses of the marker in different treatment subgroups. The Update Committee hopes that adherence to a recently published set of suggested guidelines for reporting of tumor marker results (designated the Reporting Recommendations for Tumor Marker Prognostic Studies [REMARK] criteria) will provide more informative data sets in the future.[1,2]

The Update Committee has attempted to review tumor markers in reference to a Levels of Evidence framework, which defines the quality of the data on a given marker.[3] Most published studies could be designated as Level of Evidence III (evidence from large but retrospective studies), which may generate hypotheses but are insufficient to change clinical practice. The Update Committee attempted, wherever possible, to base the updated recommendations on studies deemed to be Level of Evidence II (prospective therapeutic trials in which marker utility is a secondary study objective), or, ideally, Level of Evidence I (single, high-powered, prospective, randomized controlled trials specifically designed to test the utility of the marker or meta-analyses of well-designed studies).

The Update Committee had two face-to-face meetings to consider the evidence for each of the 2000 recommendations. The guideline was circulated in draft form to the Update Committee. ASCO's Health Services Committee and the ASCO Board of Directors also reviewed the final document.

It is important to emphasize that guidelines and technology assessments cannot always account for individual variation among patients. They are not intended to supplant physician judgment with respect to particular patients or special clinical situations, and cannot be considered inclusive of all proper methods of care or exclusive of other treatments reasonably directed at obtaining the same result.

Accordingly, ASCO considers adherence to this guideline assessment to be voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances. In addition, this guideline describes the use of procedures and therapies in clinical practice; it cannot be assumed to apply to the use of these interventions performed in the context of clinical trials, given that clinical studies are designed to evaluate or validate innovative approaches in a disease for which improved staging and treatment is needed. In that guideline development involves a review and synthesis of the latest literature, a practice guideline also serves to identify important questions and settings for further research.

GUIDELINE RECOMMENDATIONS

CA 15-3 AND CA 27.29 AS MARKERS FOR BREAST CANCER

2007 recommendation for CA 15-3 and CA 27.29 as screening, diagnostic, or staging tests. Present data are insufficient to recommend CA 15-3 or CA 27.29 for screening, diagnosis, and staging. There is no change from the original guideline.

Literature update and discussion. CA 15-3 and CA 27.29 are well-characterized assays that allow the detection of circulating MUC-1 antigen in peripheral blood. Several studies have been published since the last ASCO guideline that support the prognostic relevance of this circulating marker in early-stage breast cancer.[4-8] In one study of 1,046 patients, Ebeling et al[4] reported CA 15-3 to be a predictor of worse outcome in univariate but not multivariate analysis including tumor size, lymph node status, histologic grade, and estrogen receptor (ER) status. Gion et al[5] further reported a highly significant prognostic contribution for CA 15-3 in a Cox regression model that included age, ER status, and tumor stage in a group of 362 node-negative breast cancers. While it is likely that serum tumor markers CA 15-3 and CA 27.29 have prognostic value, their role in the management of early-stage breast cancer is unclear.[9,10] It has yet to be determined that MUC-1–based serum markers are helpful in making treatment decisions in this setting. Therefore, the Update Committee did not recommend their measurement at diagnosis.

2007 recommendation for CA 15-3 and CA 27.29 to detect recurrence after primary breast cancer therapy. Present data do not support the use of CA 15-3 and CA 27.29 for monitoring patients for recurrence after primary breast cancer therapy. There is no change from the guideline published in 2000.

Literature update and discussion. Several well-designed studies have shown that an increase in CA 15-3 or CA 27.29 after primary and/or adjuvant therapy can predict recurrence an average of 5 to 6 months before other symptoms or tests. While additional studies have been published since the last ASCO guideline that address the value of these serum markers at detecting recurrence,[11-16] there are no prospective randomized clinical trials to demonstrate whether detection and treatment of occult or asymptomatic metastases using tumor markers impact on the most significant outcomes (disease-free survival, overall survival, quality of life, toxicity, or cost-effectiveness). Although the assay was approved by the US Food and Drug Administration, the US Food and Drug Administration does not require tests to show clinical benefit if that is not part of the manufacturer's indication. Given the limited evidence, and until clinical benefit is established, present data are insufficient to recommend routine use of CA 15.3 or CA 27.29 for this application. This recommendation is in line with that of the ASCO guideline for follow-up and management of patients with breast cancer.[9]

2007 recommendation for CA 15-3 and CA 27.29 to contribute to decisions regarding therapy for metastatic breast cancer. For monitoring patients with metastatic disease during active therapy, CA 27.29 or CA 15-3 can be used in conjunction with diagnostic imaging, history, and physical examination. Present data are insufficient to recommend use of CA 15-3 or CA 27.29 *alone* for monitoring response to treatment. However, in the absence of readily measurable disease, an increasing CA 15-3 or CA 27.29 may be used to indicate treatment failure. Caution should be used when interpreting a rising CA 27.29 or CA 15-3 level during the first 4 to 6 weeks of a new therapy, given that spurious early rises may occur. There is no change from the guideline published in 2000.

Literature update and discussion. No relevant studies were identified from the review of the literature conducted for this topic.

CARCINOEMBRYONIC ANTIGEN AS A MARKER FOR BREAST CANCER

2007 recommendation for carcinoembryonic antigen for screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy. Carcinoembryonic antigen (CEA) is not recommended for screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy. There is no change from the guideline published in 2000.

Literature update. No relevant studies were identified from the review of the review of literature conducted for this topic.

2007 recommendation for CEA to contribute to decisions regarding therapy for metastatic breast cancer. For monitoring patients with metastatic disease during active therapy, CEA can be used in conjunction with diagnostic imaging, history, and physical examination. Present data are insufficient to recommend use of CEA *alone* for monitoring response to treatment. However, in the absence of readily measurable disease, an increasing CEA may be used to indicate treatment failure. Caution should be used when interpreting a rising CEA level during the first 4 to 6 weeks of a new therapy, given that spurious early rises may occur. There is no change from the guideline published in 2000.

Literature update and discussion. CEA levels are less commonly elevated than are levels of the MUC-1 assays, CA 27.29, or CA 15-3. Only 50% to 60% of patients with metastatic disease will have elevated CEA levels, compared with 75% to 90% who have elevated levels of the MUC-1 antigen.[17-22] CEA levels are minimally complementary with MUC-1 levels. For example, in one study of 53 women with metastatic breast cancer, CA 15-3 and CEA levels were elevated in 94% and 69%, respectively. CEA was elevated in only a single case in which CA 15-3 was not.[23] Nonetheless, in several studies there have been selected cases in which CEA is informative (elevated) and CA 15-3 or CA 27.29 is not.[13,24-31] Older studies suggest that, like the MUC-1 assays, CEA levels appear to track with disease status.[20,32-34] Taken together, these data suggest that it is reasonable to evaluate one of the MUC-1 assays and CEA initially in a patient with metastatic disease. If the MUC-1 assay is elevated, there appears to be no role for monitoring CEA, but if not, then CEA levels may provide supplementary information to the clinician in addition to clinical and radiographic investigations.

ERs AND PROGESTERONE RECEPTORS AS MARKERS FOR BREAST CANCER

2007 recommendation for ERs and progesterone receptors. ER and progesterone receptor (PgR) should be measured on every primary invasive breast cancer and may be measured on metastatic lesions if the results would influence treatment planning. In both pre- and postmenopausal patients, steroid hormone receptor status should be used to identify patients most likely to benefit from endocrine forms of therapy in both the early breast cancer and metastatic disease settings. In patients with ductal carcinoma in situ (DCIS) who are candidates for hormonal therapy, data are insufficient to recommend routine measurement of ER and PgR for therapy recommendations.

Literature update and discussion. ER and probably PgR content are associated with a favorable prognosis, and more importantly, highly predictive of benefit from endocrine treatment in both the

adjuvant and metastatic settings.[35-37] These treatments include tamoxifen, ovarian ablation (surgical or chemical), aromatase inhibitors (anastrazole, letrozole, exemestane), and irreversible ER inhibitors (eg, fulvestrant). Endocrine treatments are used for prevention of new cancers and of recurrent distant metastases as well as for the treatment of metastatic disease.[38] Fortunately, the majority of contemporary clinical trials have incorporated estrogen and progesterone receptor testing with the evaluation of newer antiestrogens and continue to demonstrate the value of these markers for predicting response to hormonal therapy.[39] Nonetheless, the Update Committee acknowledges the deficits in standardization for ER and PgR assays (in particular, immunohistochemistry [IHC]), and further efforts at defining reproducibility and accuracy for particular reagents are an important priority. With those caveats, the previous guideline recommendations regarding the use of ER and PgR for diagnosis and treatment of invasive breast cancer remain unchanged.

A topic that has emerged since the 2000 update is the potential role of hormone receptor determination in the management of DCIS. DCIS is a complex group of diseases that have diverse outcomes and account for approximately 20% to 30% of breast cancer cases.[40-42] Most physicians accept the concept that high nuclear grade and necrosis predict a worse outcome for patients with DCIS.[43-48] Although ER negativity is associated with a worse outcome in patients with DCIS, it is not an independent predictor in the context of high nuclear grade and necrosis.[49] Therefore the Update Committee does not recommend the use of the ER as a predictor of outcome in patients with DCIS.

The current treatment options for DCIS include mastectomy, lumpectomy followed by breast radiation therapy,[50-53] or lumpectomy alone in selected patients.[54-57] The addition of tamoxifen to the lumpectomy followed by breast radiation therapy is supported by the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-24 trial,[51,58] which showed a significant decrease in the recurrence of both in situ and invasive breast cancer in the tamoxifen group, with no impact on overall survival. A single report, available in abstract form only, suggested the benefits of tamoxifen in regard to reduction of local recurrence, and second primary breast cancers might be confined to those patients whose original DCIS expressed ER.[59] Another large randomized trial of adjuvant tamoxifen in DCIS, the United Kingdom Coordinating Committee on Cancer Research trial, failed to show an advantage for the tamoxifen-treated group in either the recurrence of breast cancer or overall survival.[52] Data from the Early Breast Cancer Trialists' Collaborative Overview are mixed regarding whether the hormone responsiveness of a contralateral breast cancer is related to the ER content of the first primary.[35] These data were retrospective in design at best. At present, the Update Committee felt that data were insufficient to support using the ER status of DCIS to elect to treat with or withhold tamoxifen in a patient who undergoes breast preservation.

MARKERS OF PROLIFERATION

2007 recommendation for flow cytometry–based proliferation markers. Present data are insufficient to recommend use of DNA content, S phase, or other flow cytometry–based markers of proliferation to assign patients to prognostic groupings. There is no change from the guideline published in 2000.

Literature update and discussion. DNA flow cytometry determination of S phase is one of several markers of proliferative rate in breast tumor specimens. In general, markers of elevated proliferative rate correlate with a worse prognosis in untreated patients, and may predict benefit from chemotherapy.[60] The implementation of DNA flow cytometry as a marker of proliferative rate is complicated by the variation in methods of tissue preparation and differences in instrumentation and methods for converting information on the histograms to the S-phase estimate. In addition, interpretation of individual studies is complicated by the fact that many are too small to have statistical power, cut-offs have not been prospectively defined, and study populations have not been controlled for adjuvant systemic treatments.

Table 2 summarizes results published from 1999 to 2004 showing the prognostic value of S phase on outcome of node-negative patients. In studies with more than 200 patients, S phase was a consistent univariate predictor of outcome, whereas smaller studies were generally negative. The prognostic value seen in the larger studies was usually maintained after multivariate analysis. In the one large study where multivariate analysis did not confirm its value, the inclusion of another measure of mitotic index eliminated S phase. Of the five larger studies, one that claimed to use prospectively defined methodologies and cut points was strongly positive.[61]

Table 2. Recent Studies of S Phase and Ploidy in Breast Cancer (1999-2007)

Reference	No. of Patients	F/U (months)	Tx	Cut Point	OS*	DFS*	OS†	DFS†
Michels et al[62]	476	48	No	Tertiles	Y	Y (3.0)	NA	Y
Chassevent et al[63]	408	69	20% C	Tertiles	NA	Y	NA	Y (3.7)
Mandard et al[61]	281	82	50%	Tertiles	Y	Y	N	N
Malmström et al[64]	237nn	48	8% C, 4% H	12%	Y	Y	NA	Y (3.8)
Lackowska et al[65]	209	74	NA	NA	NA	Y	NA	Y
Pinto et al[66]	175	40	NA	6.1%	N	N	N	N
Prasad et al[67]	129	144	NA	6%	N	N	N	N
Harbeck et al[68]	125	72	NA	6%	N	Y	N	N
Reed et al[69]	115	> 60	NA	Continuous	N	N	N	N

Abbreviations: F/U, follow-up; Tx, treatment; OS, overall survival; DFS, disease-free survival; NA, not available; C, chemotherapy; H, hormonal therapy; Y, significant improvement in end point; N, no significant improvement in end point.
*Univariate; numeric values represent relative risk.
†Multivariate; numeric values represent relative risk.

Because of the technical variation in flow cytometry determination of S phase, it is not possible to endorse results produced by all methodologies. Nonetheless, if the flow cytometry–determined S phase is determined using a validated method, in a laboratory with experience using the technique, it appears that an elevated S-phase fraction is associated with a worse outcome (Table 2). However, the data are insufficiently consistent to recommend routine use of flow cytometry to make clinical decisions.

2007 recommendation for immunohistochemically based markers of proliferation in breast cancer. Present data are insufficient to recommend measurement of Ki67, cyclin D, cyclin E, p27, p21, thymidine kinase (TK), topoisomerase IIα, or other markers of proliferation to assign patients to prognostic groupings.

Marker definition. Additional markers of proliferation have been measured by IHC to determine their prognostic and predictive value in breast cancer. These include but are not limited to Ki 67, TK, cyclin E, cyclin D, cyclin inhibitors p27 and p21, and topoisomerase IIα. These measures of proliferation are typically enzymes involved in DNA metabolism (eg, TK), cell cycle checkpoint functions (eg, cyclins, p27, p21), and DNA-modifying enzymes (eg, topoisomerase II). Ki67, MIB-1 and PCNA are proliferating cell nuclear antigens of unknown function and are present exclusively in dividing cells.

Literature review and discussion. The prognostic and predictive role of Ki67, cyclin D, cyclin E, p27, p21, TK, and topoisomerase IIα are discussed by Colozza et al[60] in an exceptionally thorough review of 132 articles including 159,516 patients. The authors appropriately point out that all studies concerning these markers are level IV or III at best, and demonstrate the difficulty in interpreting the literature due to lack of standardization of assay reagents, procedures, and scoring. In addition, the majority of marker studies address the prognostic role of the marker, whereas studies of the predictive value for efficacy of treatment are either lacking or performed on small sample sizes without a randomized comparison for a particular marker. These issues led the authors to conclude that Ki67, cyclin D, cyclin E, p27, p21, TK, and topoisomerase II are not recommended for clinical practice. The Update Committee concurs with these conclusions and refers the reader to this elegant review for additional details. In addition, cyclin E is discussed further in this guideline.

HER2 AS A MARKER FOR BREAST CANCER

2007 recommendation for HER2 evaluation in breast cancer. HER2 expression and/or amplification should be evaluated in every primary invasive breast cancer either at the time of diagnosis or at the time of recurrence, principally to guide selection of trastuzumab in the adjuvant and/or metastatic setting. Other utilities for HER2 evaluation are also discussed separately below.

Literature update and discussion. HER2 is a member of the epidermal growth factor receptor (EGFR) family.[70] It is amplified and overexpressed in 15% to 30% of newly diagnosed breast cancers and is associated with more aggressive behavior.[71] Several potential clinical applications have been proposed for determination of HER2 status in breast cancer patients, including (1) determination of prognosis in untreated patients; (2) prediction of resistance to endocrine therapy or of selective resistance to tamoxifen but not aromatase inhibitors; (3) prediction of relative resistance to certain chemotherapies, such as cyclophosphamide, methotrexate, and fluorouracil (CMF) –like regimens; (4) prediction of benefit from anthracycline or paclitaxel; and (5) prediction of benefit from anti-HER2 therapies, in particular trastuzumab and lapatinib. Circulating HER2 extracellular domain (ECD) levels have been proposed as a surrogate for tissue measures of HER2, to monitor patients for early relapse or to monitor response to standard therapies or HER2-targeted therapies. These utilities were considered and commented on in the Guideline. HER2 can be measured in tissue by assays for expression, most commonly by IHC, or for gene amplification, most commonly by fluorescent in situ hybridization (FISH). A separate Expert Panel convened jointly by the College of American Pathologists (CAP) and ASCO has recently published a set of guideline recommendations regarding analysis of tissue HER2 status, in which it was strongly recommended that laboratories offering this service be accredited on an annual basis.[72] The Update Committee endorses the ASCO-CAP guideline; hence, this topic was not covered further in the present guideline update.

The ECD of HER2 can be detected in serum or plasma, most commonly by a commercially available enzyme-linked immunosorbent assay (ELISA), and is elevated in approximately 30% of patients with metastatic breast cancer.[73-84]

2007 recommendations for HER2 to define prognosis for early-stage breast cancer patients in the absence of systemic therapy. HER2 amplification, overexpression, and the presence of HER2 extracellular domain are generally associated with a poorer prognosis. However, the value of this information in clinical practice is questionable and the use of HER2 for determining prognosis is not recommended. There is no change from the guideline published in 2000.

Literature update and discussion. The prognostic significance of HER2 overexpression in tumor tissue has been evaluated in several clinical trials with most, but not all, studies suggesting that HER2 positivity is associated with worse prognosis in untreated patients.[71,85,86] Due to the variability in immunohistochemical assays and scoring systems used, there is insufficient evidence to endorse IHC-based testing for HER2 in determining prognosis for breast cancer patients. The results of HER2 amplification as a prognostic factor are more consistent, with HER2 amplification usually associated with worse prognosis, including node-negative populations.[87-89] As discussed below, most studies of serum HER-2 extracelluar domain have found an association with higher tumor stage and increased tumor burden.[74,90,91] As might be expected, elevated levels of HER2/ECD correlate with worse prognosis.[75,92] However, serum HER2 appears to retain its prognostic effect in multivariate models, suggesting a biologic role beyond its association with HER-2 tissue expression.[83,90-94] While the weight of evidence suggests that HER2 amplification/overexpression and/or shedding of ECD are associated with worse outcome, the role of this marker purely to determine prognosis in clinical practice is unclear, given that outcomes are so heavily influenced by subsequent therapy. Hence, the Update Committee does not recommend the measurement of HER2, by any method, for the sole purpose of determination of patient prognosis.

2007 recommendation for use of HER2 to select patients for anti-HER2–based therapy. High levels of tissue HER2 expression or *HER2* gene amplification should be used to identify patients for whom trastuzumab may be of benefit for treatment of breast cancer in the adjuvant or metastatic disease settings. There is no change from the guideline published in 2000.

Literature update and discussion. Trastuzumab is a humanized monoclonal antibody that binds to the extracellular domain of HER2. A prospective randomized clinical trial has demonstrated that trastuzumab improves response rates, time to progression,

and overall survival when combined with chemotherapy compared with chemotherapy alone in the metastatic setting.[95] Phase II monotherapy studies have demonstrated that trastuzumab induces responses in approximately 15% to 25% of selected patients.[96-98] Eligibility for all of these trials was based on HER2 positivity, either by IHC or FISH. It has been assumed that patients without HER2-positive cancers will not benefit from trastuzumab. A single unpublished prospective randomized clinical trial has addressed the value of trastuzumab added to paclitaxel in patients with HER2 low (or "equivocal") metastatic breast cancer, and no statistically significant differences were reported for any outcome.[99]

Five prospective randomized clinical trials have now been reported in the adjuvant setting, as well as a single, small, prospective, randomized neoadjuvant clinical trial. Each has shown a remarkable beneficial effect of trastuzumab on pathologic complete response, disease-free survival, and overall survival.[100-104] As in the metastatic setting, eligibility for these trials depended on some measure of HER-2 positivity (either 3+ staining by IHC or FISH amplification more than 2.0). Therefore, at present, trastuzumab is indicated only for HER2-positive patients, and patients with HER2-negative status (IHC 0-2+ and FISH negative) should not receive trastuzumab. The Update Committee refers the reader to the recently published ASCO-CAP detailed guideline for methodology and accreditation of assays for HER2.[72]

Recently published data from a prospective randomized clinical trial suggest that the addition of the epidermal growth factor family tyrosine kinase inhibitor, lapatinib, to capecitabine resulted in better outcomes than capecitabine alone in patients with HER2-positive metastatic breast cancer. The Update Committee anticipates that HER2 status may also be used to guide lapatinib therapy in the future.[105]

Sensitivity to Chemotherapy

2007 recommendation for the utility of HER2 for predicting response to specific chemotherapeutic agents. Level II evidence (prospective therapeutic trials in which marker utility is a secondary study objective) suggests that overexpression of HER2 (3+ by protein or $>$ 2.0 FISH ratio by gene amplification) identifies patients who have greater benefit from anthracycline-based adjuvant therapy. If a clinician is considering chemotherapy for a patient with HER2-positive breast cancer, it is recommended that an anthracycline be strongly considered, assuming there are no contraindications to anthracycline therapy. In the context of trastuzumab therapy, there is Level I evidence (single, high-powered, prospective, randomized controlled trials specifically designed to test the marker or a meta-analyses of well-designed studies) that a nonanthracycline regimen may produce similar outcomes. At present, the Update Committee does not recommend that HER2 be used to guide use of taxane chemotherapy in the adjuvant setting.

Literature update and discussion. The role of HER2 in both tissue and serum in predicting response to specific agents has been evaluated. Most trials involving CMF-based regimens suggest that patients with HER2-positive tumors benefit less with this therapy than do patients with HER2-negative tumors.[106-109] However, results from randomized phase III trials of CMF versus no chemotherapy and CMF with or without the addition of anthracycline-containing therapy suggest that patients with HER2-positive breast cancers still derive some benefit from CMF, but it appears that the addition of an anthracycline further improves their prognosis.[110,111]

It is not clear if HER2 is specific for benefit from anthracyclines, or whether HER2 is associated with benefit from addition of any therapy that is more effective overall.[112-118] Indeed, it is not clear whether HER2 itself is the target of anthracyclines or if HER2 status serves as a surrogate for a different gene product that may be the target of the anthracycline. In this regard, several groups have evaluated the abnormalities (amplification and/or deletion) of topoisomerase IIα (Topo II), which is located on the same amplicon on chromosome 17 as HER2. Anthracyclines directly bind Topo II and function, at least in part, by inhibiting its activity in DNA replication, therefore making it an attractive marker for anthracycline activity.[119] Topo II may increase sensitivity to anthracyclines and also confer relative resistance to alkylating agents in preclinical studies.[120,121] While several clinical cohorts have been evaluated for Topo II amplification and the results generally support this explanation for altered sensitivity to anthracyclines in HER2-amplified breast tumors, other studies do not confirm these findings.[122-125] Although these studies approach Level of Evidence II quality as defined earlier (prospective therapeutic trials in which marker utility is a secondary study objective), the uncertainty regarding the biologic relationship between Topo II protein expression, copy number, proliferation, and benefit from anthracylines makes assessment of Topo II unreliable at this time. In fact, recent trials suggest that the model of a direct relationship between Topo II amplification, overexpression of Topo II protein, and benefit from anthracyclines is overly simplistic.[126,127] The fact that Topo II protein level corresponds to proliferation rate, but not Topo II copy number, suggests that the coamplification of Topo II may not be associated with increased target for anthracycline-containing therapy as predicted. Furthermore, both deletion and amplification of the Topo II region are associated with benefit from anthracycline-containing therapy in HER2-amplified tumors.[124,126,127] Since topoisomerase IIα protein is essential for chromosome segregation and proliferation, and is more abundant in aneuploid tumors, it seems unlikely that Topo II amplification fully explains benefit from anthracyclines in the setting of HER2 amplification.[128,129]

The previous discussion notwithstanding, most correlative studies have suggested that HER2 amplification and/or overexpression identifies those patients in randomized trials who benefit from anthracycline-based chemotherapy compared with CMF, while in HER2-negative patients there appears to be no difference between the two regimens.[115,130,131] Thus, given the weight of the evidence for HER2, it seems prudent to recommend anthracycline-based adjuvant chemotherapy for a patient with HER2-positive breast cancer, assuming adjuvant chemotherapy is indicated, the patient has no contraindication to an anthracycline, and trastuzumab administration is not planned.

The benefit of taxane-based therapy for HER2-positive tumors is controversial. Some studies suggest improved response to docetaxel or paclitaxel, while others suggest relative resistance.[122,130,132,133] This may relate, in part, to the method for detecting HER2, given that serum HER2 has been used to determine HER2 positivity in some studies and is associated with tumor burden (as discussed below), which confounds the ability to discern the independent predictive value of HER2 in this setting. In a retrospective analysis of a trial comparing three different doses of paclitaxel monotherapy in patients with metastatic breast cancer, tissue HER2 status was not associated

with response rate, disease-free survival, or overall survival.[134] In contrast, another retrospective analysis reported that HER2 amplification was associated with benefit from paclitaxel and doxorubicin compared with cyclophosphamide and doxorubicin, while there was no difference in outcomes for HER2-negative patients with metastatic breast cancer.[135]

A recent study of HER2 by FISH and IHC in Cancer and Leukemia Group B 9344/Intergroup 0148 trial suggests that the benefit from the addition of adjuvant paclitaxel after four cycles of doxorubicin and cyclophosphamide in node-positive breast cancer patients is more pronounced in those with HER2-positive breast cancers.[132] Indeed, there was no detectable benefit from addition of paclitaxel in HER2-negative, ER-positive patients. This observation may explain the variability in studies looking at taxane benefit in HER2-positive tumors because ER status varies by cohort. Again, this study does not distinguish between a benefit from taxane-based therapy versus the addition of more effective chemotherapy in HER2-positive tumors. Until this study is published and corroborated, these results must be viewed as preliminary.

In summary, the data regarding the predictive value of HER2 and response to chemotherapy generally support the concept that the benefit of adjuvant anthracycline therapy is most marked in the HER2-positive subgroup of patients. However, the benefit of taxane-based therapy in HER2-positive patients remains controversial and definitive conclusions have not been reached.

SENSITIVITIES TO ENDOCRINE THERAPY IN GENERAL OR TO SPECIFIC ENDOCRINE THERAPIES

2007 recommendation for use of HER2 to determine sensitivity to endocrine therapy. HER2 should not be used to withhold endocrine therapy for a patient with hormone-receptor positive breast cancer, nor should it be used to select one specific type of endocrine therapy over another. There is no change from the guideline published in 2000.

Literature update and discussion. Complex interactions exist between the HER2 and ER pathways. HER2 expression in human breast cancer cells is downregulated by estrogens.[136] Conversely, overexpression of HER2 promotes estrogen-independent growth and is associated with resistance to tamoxifen in vitro and in animal models, possibly by promoting ligand-independent growth. These observations are consistent with the inverse association of estrogen and progesterone receptors with HER2 overexpression and also provide a rationale for the lower response of HER2-overexpressing tumors to endocrine therapy shown in several clinical studies.[107,137-141] However, most of these studies were retrospective and nonrandomized. To date, randomized trials have not led to consensus on this association.[142-145] The interaction of HER2 with endocrine therapy may vary depending on the type of hormonal agent in question. Ellis et al[146] have shown that HER2- and/or EGFR-positive tumors were more likely to respond to neoadjuvant letrozole than tamoxifen in a randomized trial of 324 primary breast cancer patients. In contrast, an analysis (presented in abstract form only) of the Anastrozole versus Tamoxifen versus a Combination of the two (ATAC) trial, failed to show that HER2-overexpressing tumors benefit more from the aromatase inhibitor.[147,148]

In summary, there are insufficient data to support the use of HER2 in tissue (or serum, as discussed below) as a predictor of response to endocrine therapy, although the evidence does suggest that in patients with ER-positive tumors, the relative benefit from antiestrogens for those with HER2-positive cancers is likely to be lower than for those with HER2-negative cancers. It is not at all clear that the benefit of aromatase inhibitors in this group is any greater than in the HER2-negative, ER-positive group.

Utility of Measures of Circulating ECD of HER2

2007 recommendation for the utility of circulating extracellular domain of HER2. Measuring circulating extracellular domain of HER2 is not currently recommended for any clinical setting. There is no change from the original guideline.

Literature update and discussion. The HER2 extracellular domain was initially isolated in culture media from an HER2-amplified cell line,[149] and in the serum of nude mice bearing xenografts from HER2-amplified cells.[150] It was subsequently isolated from pleural effusions and serum of advanced breast cancer patients.[78] Several studies have shown it to be present in roughly 25% of unselected patients. On comparison with tissue expression, it appears that the majority of patients who shed ECD are positive for HER2 at the level of the primary tumor.[112] The functional significance of ECD shedding has not been determined, but in vitro data suggest that deletion of the extracellular carboxy terminus of the molecule enhances the signaling activity and transforming ability of the NH-2 terminally truncated receptor, p95 HER2.[151,152]

Therefore, the ECD of HER2 might serve as a surrogate marker for tissue HER2 status for any or all of the utilities discussed above, especially prediction of benefit from trastuzumab or anthracyclines. Furthermore, serial HER2 ECD levels might be useful for monitoring, either to detect recurrence in asymptomatic patients who are believed to be free of detectable disease, or to determine disease status in patients with metastatic breast cancer.

As with tissue HER2 status, serum HER2 might be useful to determine prognosis. Studies of serum HER2 more uniformly suggest worse outcome. However, in early-stage disease, as with circulating MUC-1 or CEA, levels of circulating HER2 ECD are directly related to tumor burden in patients with HER2-positive breast cancer, and there are no studies that suggest knowledge of HER2 ECD is of value in this setting.[153] Likewise, in patients with metastases, elevated levels of circulating HER2 are associated with worse outcomes, but not to the extent that a patient might be treated differently based simply on "prognosis."[154,155]

Pretreatment circulating HER2 might be used as a predictive factor for selection of specific therapy, especially in the metastatic setting. Many patients with new or serially progressive metastatic disease may not have had HER2 measured in their primary cancers (although the Update Committee anticipates that this situation will become increasingly less common). Furthermore, several studies have suggested that a small fraction of metastatic HER2 evaluations are discordant from the primary measurements.[156] If HER2 status is important to direct therapy, measurement of the HER2 status may be worthwhile in patients with metastases. A circulating tumor marker that accurately reflects tissue HER2 status has certain advantages over rebiopsy of a metastatic lesion, with less morbidity and ability to monitor changes serially in disease biology. Several publications have attempted to address this utility in the context of both endocrine and trastuzumab-based therapy.[81,157-159]

As noted, one possible indication for HER2 would be to direct endocrine therapy. Several studies have suggested that pretreatment circulating HER2 ECD levels in metastatic patients are associated with

lower response, shorter time to progression, and worse survival in ER-positive patients about to begin a new endocrine treatment. However, most (if not all) of these studies were confounded by the known association of serum HER2 with greater disease burden.[77,84] In a study of patients with advanced breast cancer randomly assigned to receive tamoxifen or letrozole, the presence of elevated ECD correlated with a lower response to both regimens, with no advantage of letrozole over tamoxifen.[81] However, there was a statistically significant improvement in time to progression in patients with shed ECD treated with letrozole versus tamoxifen, suggesting that the aromatase inhibitors may exhibit some advantage in the HER2-positive population.[81] Patients in this trial were randomly assigned to either therapy, but the correlative analysis of ECD and response to therapy was conducted retrospectively. Perhaps the most promising of use of HER2 ECD would be to predict response to trastuzumab (or other HER2-directed therapies, such as lapatinib) and to monitor disease response and progression once treatment has begun.

Given the association of HER2/ECD with HER2 overexpression, it seems likely that this marker could also predict response to trastuzumab. On the other hand, HER2/ECD is associated with a higher tumor burden, which may lower response rates and decrease the half-life of the antibody, due to the abundance of binding sites. Another concern resides in the formation of immune complexes between HER2/ECD and trastuzumab, with the potential for accelerated clearance and reduction in the efficacy of this therapy. Of note, concerns that circulating trastuzumab might interfere with the measurement of HER2/ECD levels have been refuted by results of in vitro experiments.[159] While high levels of HER2/ECD (500 ng/mL) were shown to decrease the half-life of trastuzumab, high levels of serum HER2/ECD do not preclude response in trastuzumab-treated patients and may, in fact, predict a more favorable response.[155,157,160] Most studies show a more precipitous decline in serum HER2 to be associated with favorable response, suggesting that this marker may be useful for monitoring disease course during trastuzumab-containing therapy.[160] However, the definition of a favorable response by HER2/ECD has not been uniformly defined in published studies.

Serum HER2 has been studied to monitor disease for recurrence response and progression in several trials.[158] Although rising ECD has been associated with recurrence in early-stage disease, serum HER2 tracks with response and progression in some patients being treated for metastatic disease, it is frequently discordant with disease course during either chemotherapy or hormonal therapy.[153,160]

In summary, although appealing, use of circulating HER2/ECD is hampered by a lack of high-quality studies and a lack of consistent findings. These are required to understand fully the precise utility of this marker in evaluation or monitoring of patients with breast cancer.

p53 AS A MARKER FOR BREAST CANCER

2007 recommendation for p53. Present data are insufficient to recommend use of *p53* measurements for management of patients with breast cancer. There is no change from the original guideline.

Literature update and discussion. The results from recently reported studies are insufficient to change the recommendation from the 1999 version of the guideline. A number of studies suggest that high tissue p53 protein levels measured by IHC or mutations or deletions in the *p53* gene measured by single-strand conformational gel electrophoresis, manual sequencing, or allele-specific polymerase chain reaction (PCR) appear to be a univariate predictor of poor outcome (Table 3). A meta-analysis performed in 1999[174] suggests that p53 mutations confer an independent relative risk of 1.7 (95% CI, 1.2 to 2.4) for both disease-free survival and overall survival. However, it seems unlikely that IHC for p53 will provide sufficiently accurate results to be clinically useful, given that it detects both mutated p53 and stabilized wild-type p53, and conversely will miss p53 deletions. Methods to define more precisely and conveniently genetic abnormalities in p53 might permit a more accurate analysis of association of p53 and clinical outcomes, either as a pure prognostic factor or as a predictor of benefit from systemic therapies. However, at present, methodologies to do so are cumbersome, expensive, and not widely available as routine clinical assays, limiting the utility of this marker in clinical practice. Furthermore, there are no prospective

Table 3. Recent Studies of p53 in Node-Negative Patients With Early Breast Cancer (1999-2007)

Reference	No. of Patients	F/U (months)	Method	Tx	Cut Point	OS*	DFS*	OS†	DFS†
Joensuu et al[162]	852	≈100	IHC DO7	5%	20%	—	Y (2)	N	N
Reed et al[163]	613	307	IHC CM1	Some	—	N	N	N	N
Gion et al[164]	599	60	IHC	No	—	—	—	—	N
Liu et al[165]	331	190	IHC	> 10%	—	N	Y	N	N
Ferrero et al[166]	297	132	IHC	Some	—	Y	Y	N	N
Mandard et al[61]	280	82	IHC	50%*	—	No	N	N	N
Rudolph et al[167]	261	96	IHC DO1	None	—	Y	Y	N	N
Kato et al[168]	260	240	IHC CM1	—		3.9	3.7	N	Y (3.7)
Bull et al[169]	543	85	SSCP	≈50%	—	Y (1.97)	Y (1.69)	N	N
Goffin et al[170]	141	96	IHC DO7	> 50%	10%	Y (3.0)	—	N	N
Linderholm et al[171]	485	56	Cytosol	5%	—	Y (2.1)	N	Y (2.5)	N
Overgaard et al[172]	160	< 60	Mutations	Some	—	Y	Y	Y (4.5)	Y
Cuny et al[173]	363	66	Mutations	Some	—	Y	Y	Y (2.7)	Y (5.3)
Olivier et al[161]	1,794	120	Mutations	Some	—	Y	NR	Y (2.5)	NR

Abbreviations: F/U, follow-up; Tx, treatment; OS, overall survival; DFS, disease-free survival; IHC, immunohistochemistry; Y, significant improvement in end point; N, no significant improvement in end point; NR, not reached.
*Univariate; numeric values represent relative risk.
†Multivariate; numeric values represent relative risk.

or retrospective studies to confirm the clinical utility of these methods, even if they were logistically feasible.

Of note, a recently reported study from Norway of nearly 2,000 women with newly diagnosed breast cancer again suggests that *p53* gene abnormalities, as defined by sequencing, are associated with worse prognosis.[161] Importantly, subset analysis suggested that p53 mutations/deletions were particularly prognostic in node-negative, ER-positive patients, although treatment was not described. If confirmed, p53 status might be used to determine which patients benefit from the addition of chemotherapy to endocrine therapy.

The Update Committee again had difficulty discerning the potential bias introduced into most studies of p53 by the confounding effects of therapy. As with many of the other markers addressed in this guideline update, it is likely p53 abnormalities are associated with either resistance or sensitivity to different therapeutic agents. Most studies analyzing p53 have not taken therapy into consideration, and the results may be strongly biased in one direction or the other, depending on the agents in question.

UROKINASE PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR 1 AS MARKERS FOR BREAST CANCER (Note. This topic is new to the guideline)

2007 recommendation for urokinase plasminogen activator and plasminogen activator inhibitor 1. Urokinase plasminogen activator (uPA)/plasminogen activator inhibitor (PAI-1) measured by ELISAs on a minimum of 300 mg of fresh or frozen breast cancer tissue may be used for the determination of prognosis in patients with newly diagnosed, node-negative breast cancer. IHC for these markers is not accurate, and the prognostic value of ELISA using smaller tissue specimens has not been validated. Low levels of both markers are associated with a sufficiently low risk of recurrence (especially in hormone receptor–positive women who will receive adjuvant endocrine therapy) that chemotherapy will only contribute minimal additional benefit. Furthermore, CMF-based adjuvant chemotherapy provides substantial benefit, compared with observation alone, in patients with high risk of recurrence as determined by high levels of uPA and PAI-1.

uPA and PAI-1: Marker definition. uPA and PAI-1 are part of the plasminogen activating system, which includes the receptor for uPA and other inhibitors (PAI-2 and PAI-3). This system has been shown experimentally to be associated with invasion, angiogenesis, and metastasis.[175]

uPA and PAI-1: Methodology. Several assay formats for these two markers have been evaluated, including IHC, quantitative real-time reverse transcriptase (RT) -PCR, and enzyme-linked immunosorbent assays (ELISA).[176-178] ELISA, performed on fresh or frozen tissue or cytosolic fractions remaining after biochemical hormone-receptor measurement, is the only method that has been determined to be prognostic.[179] Importantly, all the data from a pooled analysis study[179] and from a prospective randomized clinical trial[180] in which uPA and PAI-1 were used to stratify patients were obtained based on analysis of large tissue sections from tumors that had not been previously biopsied. Although ELISA using tissue from core needle biopsies would be clinically useful, the prognostic value of such a strategy remains to be confirmed.[181] The effects of a prior core biopsy on uPA and PAI-1 levels, which could conceivably alter expression of these tissue-remodeling enzymes, are unknown.

uPA and PAI-1: Literature Review and Analysis

Risk, screening, and monitoring. Currently available data address the impact of uPA and PAI-1 on prognosis for patients with early-stage breast cancer. A retrospective study suggests that ductal fluid uPA/PAI-1 levels might be of use for screening or risk recategorization of high-risk women, but these data require verification.[182] There are few if any data regarding monitoring patients with serial uPA/PAI-1 levels.[183-185]

Prognosis in Early-Stage Breast Cancer

Several studies have suggested that overexpression of uPA and/or PAI-1 have been consistently related to poor prognosis in early-stage breast cancer. These studies suggest that these two factors, combined, are associated with 2- to 8-fold higher risk of recurrence and death.[176,177,186-190] Importantly, studies of node-negative patients who did not receive adjuvant systemic therapy suggest that these two markers are very strong prognostic factors, independent of size, grade, and hormone receptor status.[179,190,191]

A pooled analysis of uPA/PAI-1 data collected from 8,377 breast cancer patients was performed by members of the Receptor and Biomarker Group of the European Organisation for Research and Treatment of Cancer.[179] These results demonstrate the reproducibility of the assay among several sites, and they confirm the strong association of overexpression of uPA and PAI-1 with recurrence and survival during a median follow-up of 79 months. A subset analysis of node-negative, untreated patients also confirmed the potential utility of these markers for identifying a low-risk cohort in this group.

The first interim report of a prospective trial using uPA and PAI-1 levels to stratify node-negative patients has been published.[180] Five hundred fifty-six node-negative patients were accrued. Those patients whose tumors expressed low levels of both markers were followed in a prospective registry and were not treated with adjuvant chemotherapy. Patients whose tumors showed elevated uPA and/or PAI-1 levels were randomly assigned to adjuvant chemotherapy (CMF) or no adjuvant chemotherapy. In this report the estimated 3-year recurrence rate for 241 patients with low levels of both uPA/PAI-1 was 6.7%, with a median follow-up of 32 months. The recurrence rate for patients with elevated uPA and/or PAI-1 levels who did not receive chemotherapy was roughly double that, and the hazard rate for recurrence in the group for patients treated with adjuvant chemotherapy was 0.56 of that for patients who were not treated.

Other reports suggest uPA and/or PAI-1 may serve as predictive factors for hormone therapy and/or specific types of chemotherapy, but these are uncontrolled studies.[182,192]

The data support the requirement for both uPA and PAI-1 levels to be performed using ELISAs on whole sections (minimum 300 mg) of fresh or frozen cancer tissue. IHC results do not reliably predict outcomes, and the prognostic value of ELISA using smaller tissue specimens, such as tissue collected by core biopsy, has not been validated.[181] Furthermore, in the modern era of frequent pre-excision, diagnostic core needle biopsies, one must interpret uPA and PAI-1 ELISA results with caution.

Future Studies

Studies are underway in Europe to address further the utility of uPA/PAI-1 measurements. In an ongoing prospective

clinical trial, patients are randomly assigned to two groups: in one group, they will have clinical decisions regarding adjuvant chemotherapy using uPA/PAI-levels; in the other group, these decisions will be made according to existing guidelines. Carefully designed studies addressing the predictive role of uPA/PAI-1 for specific chemotherapy and endocrine therapy are recommended. Finally, components of the urokinase plasminogen activating system appear to be promising targets for future therapeutic studies.

CATHEPSIN D AS A MARKER FOR BREAST CANCER

2007 recommendation for cathepsin D. Present data are insufficient to recommend use of cathepsin D measurements for management of patients with breast cancer. There is no change from the guideline published in 2000.

Literature update and discussion. The role of cathepsin D in breast cancer pathogenesis and outcome has been studied extensively. A Dutch study of 2,810 patients between 1978 and 1992 provides the largest data set used to evaluate the relevance of this marker in breast cancer.[193] In this study 1,412 patients were node negative and did not receive systemic adjuvant therapy. Median follow-up was 88 months. Cathepsin D levels were determined in breast tumor cytosols using a radiometric immunoassay (ELSA-CATH-D; CIS Bio International, Gif-sur-Yvette, France). The use of a cut point of 45.2 pmol/mg of protein cathepsin D was modestly predictive (hazard ratio, 1.39) in both node-negative and node-positive populations by multivariate analysis, which included tumor size, number of nodes, and ER status but not tumor grade.

In a subsequent study[194] of 1,851 patients (1,182 node-negative patients) with 59 months of follow-up, high levels of cathepsin D expression were associated with a 1.7-fold higher hazard of relapse both in univariate and multivariate analyses using a cut point of 10 pmol/mg of protein; this cut point was defined retrospectively to optimize the results. Although these results show cathepsin D determinations to be predictive of outcome, the magnitude of this effect would be expected to be relatively small (if a relative risk of 1.4 was used), splitting a population with a 20% risk into populations with a low 17% risk and a high 23% risk. In general, the Committee has found that studies of cathepsin D measured by IHC are variable, with no assay standardization and inconsistent associations with outcome, and, again, with little regard to the confounding effects of systemic therapy.

CYCLIN E AS A MARKER FOR BREAST CANCER (Note. This topic is new to the guideline)

2007 recommendation for cyclin E. Present data are insufficient to recommend use of whole length or fragment measurements of cyclin E for management of patients with breast cancer.

Cyclin E: Marker definition. Cyclin E is a 50-kd protein expressed in the late G_1 phase of the cell cycle. Association of cyclin E with CDK2 stimulates kinase activity and promotes transition of cells to the S phase, ensuring subsequent cell division by phosphorylating the Rb protein that then releases bound E2F transcription factors and promotes DNA synthesis. Activity of the cyclin E-CDK2 enzyme complex is inhibited by the p21 and p27 proteins. Elevated levels of cyclin E have been observed in a number of different cancers.[195]

In breast cancers, cyclin E is cleaved to lower molecular weight (LMW) fragments (33 to 45 kd) by elastase[196] and by calpain 2.[197] These LMW fragments have greater affinity for CDK2 and resist inhibition by p21 and p27.[198] In addition, the LMW fragments confer resistance to tamoxifen and increase genomic instability.[199] Consequently, there is a biologic rationale for evaluation of cyclin E protein, and particularly its LMW fragments, as a marker of poor prognosis in breast cancer.

Cyclin E: Methodology. Intact cyclin E protein has been measured by IHC in formalin-fixed paraffin-embedded (FFPE) tissue, and mRNA for cyclin E has been quantitated by RT-PCR in fresh frozen specimens.[200] LMW forms of cyclin E have been measured by Western blot analysis of proteins in fresh frozen tissue.[201] Discordance in the prognostic value of cyclin E between IHC and Western blot analysis may be related to the antibodies used for each assay, given that the reagents that detect intact cyclin E may not react with the LMW fragments. Even when antibodies recognize the intact protein and its fragments, however, discordance between IHC and Western blots analysis has been observed in 37% of cases.[201] In a single study, dramatic results regarding use of cyclin E and outcome were reported only when the LMW fragments were considered and the assay for these was performed by Western blotting.[201] However, Western blotting is relatively impractical for routine clinical use, and the antibody used in this study cannot be applied successfully to FFPE issue. Monoclonal antibodies are needed to advance studies of this marker in archived tissue and to make its use in routine clinical practice possible.

Cyclin E: Literature review and analysis. Conclusions regarding the prognostic value of cyclin E in the published literature are mixed, perhaps in part due to methodologic differences in the assays (IHC *v* Western blotting) and due to lack of high-level studies. In addition, cyclin E is closely linked to proliferation and its independent prognostic significance is less clear. Nonetheless, elevated levels of cyclin E protein have been fairly consistently associated with a poor prognosis in breast cancer. In a recent meta-analysis of cyclin E overexpression of 2,534 patients in 12 published studies, overexpression of cyclin E was associated with a 2.32-fold (95% CI, 1.25- to 4.30-fold) increased risk of recurrence in univariate analysis and a 1.72-fold (95% CI, 0.95- to 3.10-fold) risk of recurrence in multivariate analysis.[202] In addition, the combined hazard ratio estimate for overall survival and breast cancer–specific survival was 2.98 (95% CI, 1.85 to 4.78) and 2.86 (95% CI, 1.85 to 4.41) in univariate and multivariate analysis, respectively. In a recently published paper in which all patients received one of two regimens of adjuvant doxorubicin and cyclophosphamide in a prospective Southwest Oncology Group randomized clinical trial (SWOG 9313), cyclin E overexpression, as determined by IHC for the full-length protein, was not associated with a worse outcome.[203] However, the negative results of this study must be considered carefully because all of these patients received chemotherapy and the assay was not specific for cyclin E fragments.

Substantially higher prognostic value has been reported when both the LMW fragments of cyclin E and the intact molecule are considered together.[201] In a single-institution, retrospective study using archived frozen specimens analyzed by Western blot assay, the hazard ratio for death from breast cancer for patients with high total cyclin E levels, as compared with those with low total cyclin E levels on Western blot analysis, was 13.3—about eight times as high as the hazard ratios associated with other independent clinical and pathological risk factors. Although these data are promising, they are from a

retrospective study, and additional properly designed studies are required to ascertain whether this marker has clinical utility, especially in the setting of no adjuvant chemotherapy.

PROTEOMIC ANALYSIS FOR BREAST CANCER (Note. This topic is new to the guideline)

2007 recommendation for proteomic analysis. Present data are insufficient to recommend use of proteomic patterns for management of patients with breast cancer.

Proteomic analysis: Marker definition. The emerging field of proteomics is complex. In theory, different clinical states, including cancer, might be represented by distinct protein patterns, or signatures. These signatures might consist of completely different proteins, of various mixtures of truncated peptide fragments, or of modifications of proteins or peptides, such as glycosylation, cysteinylation, lipidation, and glutathionylation, each of which might be cancer specific. Therefore, one might be able to exploit these differences, either in tissue, in the circulation, or in secreted fluids, for diagnostic purposes. For proteomic pattern analysis, computer-based algorithms have been developed to distinguish breast cancer from benign disease, or to identify individuals at high risk of recurrence based on the pattern of peptide peaks. An alternative method uses proteomic methods to identify a limited number of proteins that can be measured by immunohistochemical or serum-based immunoassays. Markers can then be validated individually or in combination as a profile or signature.

Proteomic pattern analysis: Methodology. There are several different approaches to analyzing multiple proteins or peptide fragments simultaneously, and each has its positive and negative features.[204] These methods include multiplex ELISA, phage display, and aptamer arrays.[205-207] However, the most widely studied methods involve identification of proteomic profiles as peaks on mass spectometric analysis with precise charge-to-mass ratios. In some cases, proteins have been designated by their apparent molecular weight and isoelectric point within two-dimensional (2D) gel analysis. Specific peptides can be identified further based on their amino acid sequence identity or homology to known proteins or their fragments. Peptides have been identified in serum from breast cancer patients[208]; drug-resistant breast cancer cell lines[209]; cancer cell line membranes[210]; nipple aspirate fluid (NAF)[211]; and normal, benign, premalignant, and malignant tumor tissue.[212,213] For analysis of breast cancers, some studies have used whole tumor specimens that include both epithelial cells and stroma, whereas others have used microdissected epithelial cells. If isolation of epithelial cells is not required, fine-needle aspirate has obtained adequate material.[214] Before mass spectoscopic analysis, preliminary separation of proteins can be performed with 2D gel analysis[211,215] or by binding of proteins to surfaces or matrices using surface-enhanced laser desorption and ionization (SELDI)[207,208,214] and matrix-associated laser desorption and ionization (MALDI),[215] respectively. After desorption and ionization, the pattern of charged peptides generally has been analyzed by time-of-flight (TOF) mass spectoscopy. Other methodologies to examine multiple proteins at once have used multiplex ELISAs that can detect several different proteins simultaneously.[216] Similar assays using phage displays or aptamers to detect multiple peptides have also been reported.[205,206]

Proteomic pattern analysis: Literature review and analysis. During the period 1996 to December 2007, more than 200 articles have been published addressing proteomics and breast cancer. However, many of these are primarily methods articles, and those that do address clinical utility are retrospective in design at best.

SELDI-TOF has been used to profile proteins in serum or plasma from breast cancer patients. Several studies have addressed the potential of SELDI to provide serum biomarkers that differentiate breast cancer from benign disease and/or healthy individuals.[208,217-219] Enrolling between 133 and 169 patients, these studies have identified diagnostic protein profiles with sensitivities and specificities of 76% to 93% and 90% to 93%, respectively. Protein peaks that distinguished healthy women from those with cancer were found at *m/z* 4,300 and 8,900 in two studies, respectively. However, no protein identification was provided. It is apparent from studies that perform protein identification that the majority of serum proteins identified that differentiate patient and normal samples are host-specific proteins in high abundance.[220,221] New methods that allow isolation of low abundance serum proteins more likely to represent tumor markers are in development.[222,223]

Given that a more concentrated source of protein from breast cancer ducts may be better able to identify tumor-specific markers, attention has been paid to the proteomic analysis of NAFs or ductal lavage fluid. When 2D gel electrophoretic separation and MALDI-TOF analysis of NAF were used, gross cystic disease fluid protein-15 levels were lower ($P < .001$) and alpha-1-acid glycoprotein levels were higher ($P < .001$) in 52 breast cancer fluids than in 53 nipple aspirates from benign lesions.[214] When subset analysis was performed, significant differences in levels for the two markers were observed in premenopausal but not in postmenopausal women. However SELDI-TOF analysis failed to detect differences in NAF from the breast with unlilateral early-stage (I-II) cancer and NAF from the contralateral breast.[224] When fluid from the cancer-bearing breast was compared with NAF from healthy volunteers, 17 peaks were overexpressed in fluid from breast cancer patients ($P < .0005$). Isotope-coded affinity tag (ICAT) tandem mass spectrometry (MS) permits both qualitative and quantitative analysis of paired protein samples.

In a third study, NAF from tumor bearing and contralateral disease-free breasts of patients with unilateral early-stage breast cancer were analyzed using ICAT labeling, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, liquid chromatography, and MS.[225] Alpha2HS-glycoprotein was underexpressed in NAF from tumor-bearing breasts, whereas lipophilin B, beta globin, hemopexin, and vitamin D–binding protein precursor were overexpressed. Western blot analysis of pooled samples of NAF from healthy volunteers versus NAF from women with breast cancer confirmed the overexpression of vitamin D–binding protein in tumor-bearing breasts. Finally, analysis of NAF obtained preoperatively from 114 women and analyzed by SELDI-TOF indicated that three proteins (5,200-H4, $P = .04$; 11,880-H4, $P = .07$; and 13,880 Da-SAX, $P = .03$) were differentially expressed in women with versus those without breast cancer.[226] Although of interest, these studies are all very preliminary. They are hampered by their retrospective design, and the frequent use of incongruent controls. Currently, none would lead to a clinical change in patient management.

At a tissue level, differences in protein profiles have been found between DCIS and normal ductules.[227] Similarly, protein profiling in small numbers of samples with 2D gel electrophoretic separation and MALDI-TOF demonstrated differential expression of several proteins between a fraction of infiltrating ductal carcinomas and normal breast tissue, including gelsolin, vinculin,

lumican, α_1-antitrypsin, heat shock protein-60, cytokeratin-18, transferrin, enolase-1,and γ-actin.[228] Of this group, only heat shock protein-70 (more abundant) and peroxiredoxin-2 (less abundant) displayed the same trend in all of the infiltrating ductal carcinomas examined.

Few published studies have addressed the prognostic significance of protein profiles from breast cancer tissue. Jacquemier et al[229] used IHC on tissue microarrays to profile the expression of 26 selected proteins in more than 1,600 cancer samples from 552 consecutive patients with early breast cancer. Supervised cluster analysis identified a set of 21 proteins whose combined expression significantly correlated with metastasis-free survival (MFS) in a learning set of 368 patients ($P < .0001$) and in a validation set of 184 patients ($P < .0001$). Among the 552 patients, the 5-year MFS was 90% for patients classified in the "good-prognosis class" and 61% for those classified in the "poor-prognosis class" ($P < .0001$). This difference remained significant when the molecular grouping was applied according to lymph node or ER status, as well as the type of adjuvant systemic therapy. In multivariate analysis, the 21-protein set was the strongest independent predictor of clinical outcome. Other studies using analysis of multiple protein biomarkers on tissue microarray have identified subclasses of breast cancer with clinical implications.[230-232] However, these studies are confounded by differences in populations, reagents and analysis methods, and systemic treatments, and therefore the Update Committee is unable to draw conclusions regarding clinical utility of any of these assays. Nevertheless, these studies illuminate the heterogeneity of breast cancer and bring us closer to understanding the relevant subclasses. In summary, these promising results, for the most part, are derived from retrospective studies and require additional confirmation in larger and well-designed prospective studies. At present, none of the proteomic profiling techniques has been validated sufficiently to be used for patient care.

MULTIPARAMETER GENE EXPRESSION ANALYSIS FOR BREAST CANCER (Note. This topic is new to the guideline)

2007 recommendation for multiparameter gene expression analysis. In newly diagnosed patients with node-negative, estrogen receptor–positive breast cancer, the Onco*type* DX assay (Genomic Health Inc, Redwood City, CA) can be used to predict the risk of recurrence in patients treated with tamoxifen. Onco*type* DX may be used to identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy. In addition, patients with high recurrence scores (RSs) appear to achieve relatively more benefit from adjuvant chemotherapy (specifically [C]MF) than from tamoxifen. There are insufficient data at present to comment on whether these conclusions generalize to hormonal therapies other than tamoxifen, or whether this assay applies to other chemotherapy regimens. The precise clinical utility and appropriate application for other multiparameter assays, such as the MammaPrint assay (Agendia BV, Amsterdam, the Netherlands), the so-called Rotterdam Signature, and the Breast Cancer Gene Expression Ratio are under investigation.

Gene expression array analysis: Definition. Gene expression profiling recently has been introduced into the clinical literature during the last decade as research suggests that assessing the expression of multiple genes in a tumor sample may provide useful information about tumor behavior.[233,234] These molecular signatures hold the promise for improving diagnosis, for the prediction of recurrence, and in aiding selection of therapies for individual patients. Molecular classification has identified subtypes of breast cancer that are known to be present based on clinical experience. Among the categories are ER-positive and/or PR-positive tumors and *HER2* gene-amplified tumors, both of which exhibit characteristic transcriptional profiles. In addition, a category of breast cancer termed "basal-like" due to the expression of basal keratins (CK5, CK14, CK15, and CK17) has emerged from these studies.[233-238] These tumors characteristically lack ER, PR, and HER2, although some controversy exists about the HER2 element. Furthermore, basal-like tumors often exhibit p53 mutation and low expression of *BRCA1* (breast cancer associated 1) tumor suppressor genes, and this phenotype is common among *BRCA1* carriers and sporadic triple-negative tumors.[239] The literature surrounding gene expression profiling continues to debate the existence of such molecular subtypes and, if they do exist, the exact definitions of these subtypes. Nevertheless, many clinical trials are now designed to subdivide patients by ER/PR and HER2 status to validate claims that different groups of tumors may be more homogeneous and therapeutic approaches should address these groups rather than the population of breast cancer patients as a whole. At this time, the following profiling platforms have made their way to clinical practice and will be discussed further.

Gene expression array analysis: Methodology. Several technologies have been developed to generate molecular signatures, including cDNA and oligonucleotide arrays and multiplex PCR technologies. An early series of publications specifically described molecular signatures in breast cancer, primarily focused on associations between particular sets of genes with altered expression and survival.[233,234,237,240] A number of studies have attempted to focus those initial observations on clinical outcomes, most notably prognosis in early breast cancer patients.

While the Update Committee recognizes that many such platforms are under development, few have been subjected to rigorous assay quality control and clinical validation. The following four assays have come closest to achieving these goals: the Onco*type* DX, the MammaPrint test, the so-called Rotterdam Signature, and the Breast Cancer Gene Expression Ratio. Only the Onco*type* DX and the MammaPrint assays are available commercially, and the laboratory that performs the Onco*type* DX has been certified by the Clinical Laboratory Improvement Amendments to perform the test for clinical use. The MammaPrint assay has recently received clearance by the US Food and Drug Administration as a class 2, 510(k) product, which ensures independent review of data and labeling, conformance of the device sponsor to good manufacturing practices (the so-called quality system regulations), and postmarketing surveillance and reporting to US Food and Drug Administration. The US Food and Drug Administration does not evaluate treatment outcomes as a result of use of this prognostic device. While quality control is expected for the Update Committee to endorse a particular assay, a clear definition of assay utility is essential for acceptance into clinical practice. Given that these two assays are closest to implementation in clinical practice, they will be discussed in greater detail and commented on specifically in the sections that follow.

Onco*type* DX

Oncotype DX: Definition. Onco*type* DX is an RT-PCR assay that measures the expression of 21 genes—sixteen cancer-related genes

and five reference genes—in RNA extracted from FFPE samples of tissue from primary breast cancer. The levels of expression of the 21 genes are manipulated by an empirically derived, prospectively defined mathematical algorithm to calculate an RS, which is then used to assign a patient to one of three groups by estimated risk of distant recurrence: low, intermediate, and high.[241]

The assay is intended to estimate risk of recurrence of patients with hormone receptor–positive breast cancer with stage I or II breast cancer and negative axillary lymph nodes. It has been suggested that tamoxifen-treated patients with an excellent estimated prognosis may be spared adjuvant chemotherapy. In addition, patients with a high RS appear to achieve a higher proportional benefit from adjuvant (C)MF chemotherapy than those with low or intermediate RSs. In a retrospective and preliminary analysis of tissues collected from 651 patients who participated in NSABP B-20, the test for interaction between chemotherapy treatment and RS was statistically significant ($P = .038$).[242] Patients with high-RS (≥ 31) tumors (ie, high risk of recurrence) had a large benefit from chemotherapy (relative risk, 0.26; 95% CI, 0.13 to 0.53; absolute decrease in 10-year distant recurrence rate: mean, 27.6%; SE, 8.0%). Patients with low-RS (< 18) tumors appeared to receive little if any benefit from chemotherapy treatment (relative risk, 1.31; 95% CI, 0.46 to 3.78; absolute decrease in distant recurrence rate at 10 years: mean, −1.1%; SE, 2.2%). Patients with intermediate-RS tumors did not appear to have a large benefit, but the uncertainty in the estimate cannot exclude a clinically important benefit. Although intriguing, the confidence limits of these estimates are large, and it is not clear whether this effect is limited specifically to CMF or to any chemotherapy. Regardless, even if this apparent differential sensitivity to chemotherapy is not confirmed, there is no a priori reason to suspect that tumors with low RS would be more likely to respond to chemotherapy or that those with high RS profiles would be more resistant. Therefore, the Update Committee believes it is reasonable to use Onco*type* DX to identify those patients with a node-negative, ER-positive cancer and low RS who might avoid chemotherapy because of the very small potential benefit. Conversely, the potential absolute benefit for those with a higher RS is likely to outweigh the risk from treatment.

Development and validation of the assay. The 21 genes in Onco*type* DX were selected from a much larger set of genes following the analysis of retrospective test sets of clinical material from several sources, including specimens from a cooperative group trial in which patients with ER-positive, node-negative breast cancer received tamoxifen versus tamoxifen plus chemotherapy (NSABP B-20). After the prognostic algorithm was developed in these test data sets, Onco*type* DX was validated by the analysis of specimens and data from a second set of patients with node-negative, ER-positive breast cancer treated only with tamoxifen, who were enrolled in the NSABP clinical trial B-14.[243] Adequate RT-PCR profiles were obtained from 668 of 675 tumor blocks. The Kaplan-Meier estimates of the rates of distant recurrence at 10 years in the patients allocated to the low-risk group (comprising 51% of the total) was 6.8% (95% CI, 4.0 to 9.6); in the patients allocated to the intermediate-risk group (22% of the total), it was 14.3% (95% CI, 8.3 to 20.3); and in the patients allocated to the high-risk group (27% of the total), it was 30.5% (95% CI, 23.6 to 37.4). This yielded a statistically significant comparison of the low-risk versus high-risk categories ($P < .001$). By multivariate Cox-model analysis, the test was a significant predictor of recurrence independent of age and tumor size ($P < .001$), and a significant predictor of overall survival ($P < .001$).

A large retrospective set of specimens from Kaiser Permanente cases with long follow-up was also evaluated to determine whether the RSs for these patients correlated with disease outcome. The results were consistent with those from the randomized trials and similar proportions of the patients fell within the low-, intermediate-, and high-risk groups.[244]

A cost-utility analysis applying a Markov decision analytic model was used to forecast overall survival, costs, and cost-effectiveness of using the test in practice.[245] Fifty-three patients (8% of the total population studied) who had been enrolled onto NSABP B-14 were classified as having a low risk of distant recurrence by National Comprehensive Cancer Network (NCCN) clinical guidelines.[246] The application of Onco*type* DX reclassified 15 of these patients (28%) to an intermediate- or high-risk group. The remaining 615 patients (92% of the total population studied) were classified as high risk by NCCN guidelines. The test reclassified 300 of these patients (49%) to a low-risk group. These data and estimates of benefits of therapy (tamoxifen and chemotherapy) from published overview analyses were used to examine the potential impact of using Onco*type* DX to make treatment decisions, instead of NCCN criteria, for 100 theoretical US patients. The authors calculated that using Onco*type* DX would result in an average increase in quality-adjusted survival of 8.6 years and a reduction in overall costs of $202,828.

Although data suggest that Onco*type* DX was predictive of 10-year disease-free survival in patients randomly assigned to receive placebo in NSABP B-14, it was not predictive of the likelihood of recurrence in a smaller study of 149 node-negative breast cancer patients with a median follow-up of 18 years who did not receive systemic therapy.[247] In NSABP B-20, in which ER-positive, node-negative patients were randomly assigned to receive tamoxifen ± chemotherapy (either CMF or MF), the RS predicted benefit from the addition of chemotherapy in the high-risk group.[242] In addition, this test was applied to core biopsies from 89 patients with locally advanced breast cancer who received neoadjuvant paclitaxel plus doxorubicin.[248] The RS was positively associated with the likelihood of pathologic complete response ($P = .005$), suggesting that the patients who are deemed by this assay to be at greatest risk of recurrence are more likely to have (at least) short-term benefit from chemotherapy. It is also worth noting that the assay is functional in FFPE tissues, making its use practical in standard practice in most pathology departments in the United States. Careful selection of the appropriate tumor block for Onco*type* DX testing by the pathologist is essential, given that results should reflect the invasive component of the tumor.

In summary, the algorithm used to calculate an RS with the Onco*type* DX was developed using data from prospective therapeutic trials in which marker utility is a secondary study objective (Level of Evidence II) or from large but retrospective studies (Level of Evidence III). Although performed retrospectively, the validation of this assay using a prospectively collected clinical trial data set, but retrospectively collected tissues from the data set, might be considered as Level of Evidence I for use of this assay. It appears that the prediction that a patient with a low RS who takes tamoxifen will have a less than 10% chance of experiencing disease recurrence during 10 years is likely to be accurate. Such a patient appears to be less likely to benefit from adjuvant chemotherapy, based on the recently published update of NSABP B-20.[242] This

analysis further suggests that patients with a high-risk score obtain benefit from the addition of (C)MF chemotherapy and emphasizes the predictive value of this assay.

MammaPrint

MammaPrint: Definition. MammaPrint is a gene expression profiling platform marketed by Agendia. The test requires a fresh sample of tissue that is composed of a minimum of 30% malignant cells and must be received by the company in their kit within 5 days of obtaining the material.

MammaPrint: Development and validation of the assay. The MammaPrint assay was developed based on research initially conducted at the Netherlands Cancer Institute (Amsterdam) and collaborating institutions. Primary tumors from 117 patients with axillary lymph node–negative primary breast cancer were analyzed on oligonucleotide microarrays. The data were subjected to supervised classification to establish a 70-gene RNA expression profile that correlated with a relatively short interval to distant metastases.[234] The signature—largely consisting of genes regulating proliferation plus those involved in invasion, metastasis, stromal integrity, and angiogenesis—was then tested in 295 consecutive stage I or II primary breast cancer patients younger than age 53 years.[249] This second set included 61 patients with lymph node–negative disease used in the prior study that established the test. In this validation trial, the estimated disease-free and overall survival rates at 10 years were 50.6% and 54.6%, respectively, in the 180 patients with the poor-prognosis signature, and 85.2% and 94.5% in the 115 others. The estimated hazard ratio for distant metastases by signature was 5.1 ($P < .001$), and remained significant when adjusted for lymph node status. Furthermore, the profile was independent of other possible prognostic factors (age, node status, tumor diameter, grade, vascular invasion, ER status, type of primary surgery, use of adjuvant chemotherapy, and/or hormone therapy) by multivariable Cox regression analysis. Additional work with this test has demonstrated that in a group of patients whose tumors exhibited high ER expression, the occurrence of metastases is associated with the expression of cell cycle genes.[250] The metastasis-free survival at 10 years was estimated to be 24% for the poor-prognosis group compared with 85% for others. However, the gene expression profile was poorly correlated with outcome in other patient subpopulations.

In an effort to overcome possible biases or inaccuracies in gene selection, error estimation, gene signature stability, or model overfitting in these initial studies, the TRANSBIG research network performed a prospective validation trial in 302 lymph node–negative patients from five European cancer centers.[251-255] At a median follow-up of 13.6 years, this study found that the 70-gene signature added independent prognostic information to conventional clinical and histologic risk factors, although the hazard rate for recurrence in this study was less than that reported in the original studies from Amsterdam. Additional validation has been provided by a study of 96 patients with stage I or II primary breast cancer in which quantitative RT-PCR (rather than microarray analysis) was applied to frozen samples.[256] This study reported that at a minimum follow-up of 5 years, multivariate analysis found that only lymph node status and gene expression profile were significantly correlated to overall survival.

In summary, MammaPrint profiling does appear to identify groups of patients with very good or very poor prognosis. However, due to the nature of the study design, it is difficult to tell if these data pertain to an inherently favorable outcome in untreated patients, to patients whose prognosis is favorable because of the therapy, or to those with poor outcomes in the absence of treatment or despite treatment. Furthermore, the tissue handling requirements for MammaPrint make this assay challenging in current clinical practice: tumor specimens were snap-frozen in liquid nitrogen within 1 hour after surgery; in addition, at present, all data have been generated with whole sections, not with core biopsies. Specimens for analysis had to contain at least 30% malignant cells on hematoxylin and eosin staining, and 30-μm sections were used for isolation of RNA. Despite recent US Food and Drug Administration clearance, the Update Committee judged that more definitive recommendations for use of this assay in clinical practice will require data from more clearly directed retrospective studies or the recently opened Microarray In Node-Negative Disease may Avoid Chemotherapy (MINDACT) study (see below).

Rotterdam Signature

Rotterdam Signature: Definition. A gene expression test based on research initially conducted at the Erasmus MC/Daniel den Hoed Cancer Center, Rotterdam, the Netherlands, has generated the so-called Rotterdam Signature, which consists of a 76-gene microarray assay that does not overlap with either the Onco*type* DX or MammaPrint assays.[257,258]

Rotterdam Signature: Methodology. The Rotterdam Signature was specifically studied in all lymph-node-negative breast cancer patients, regardless of age, tumor size and grade, or ER/progesterone receptor status," and it thus may be distinguished from Onco*type* DX (for hormone receptor–positive female cases) and MammaPrint (for young female cases). This assay is not available commercially at this time.

Rotterdam Signature: Literature review and discussion. In one study, whole sections of frozen tissue from 286 patients with lymph node–negative disease who had not received adjuvant systemic therapy were profiled with this signature.[258] Frozen tumor samples from a 115-case training set were subjected to RNA expression microarray analysis. A 76-gene signature was identified with 60 genes for samples "positive" for ER protein and 16 genes for cases classified as ER negative. The supervision criterion was the development of metastatic disease within 5 years. Validation was performed on 171 different lymph node–negative cases, revealing a hazard ratio of 5.67 ($P < .0001$) uncorrected for conventional prognostic factors (univariate analysis) and 5.55 ($P < .0001$) corrected for these factors (multivariate analysis). The hazard ratios for distant metastasis-free survival in premenopausal (9.60) cases, postmenopausal (4.04) cases, and subsets of lesions between 1.0 and 2.0 cm (14.1) were all statistically significant. Validation was more recently performed in a set of 235 cases (55 treated with tamoxifen) from four medical institutions.[257] As with MammaPrint, tissue collection and preparation requirements may be problematic, given that this assay also requires whole sections of frozen tissue and, at present, is not applicable to FFPE tissue. Neither the results of this assay, nor those of MammaPrint, have been validated in core biopsy specimens, nor have results been validated in whole sections that have been collected after a prior diagnostic core biopsy procedure.

Breast Cancer Gene Expression Ratio

Dr Hayes recused himself from deliberations and Update Committee votes concerning recommendations for this marker due to potential conflicts of interest.

Breast Cancer Gene Expression Ratio: Definition. The Breast Cancer Gene Expression Ratio test (AvariaDx Inc, Carlsbad, CA) is a quantitative RT-PCR–based assay that measures the ratio of the *HOXB6* and *IL17BR* genes, and is marketed as a marker of recurrence risk in untreated ER-positive/node-negative patients.

Breast Cancer Gene Expression Ratio: Methodology. This assay was developed based on the ratio of *HOXB6*:*IL17BR* genes first reported by Ma et al[259,260] as predicting poor outcome in ER-positive patients treated with tamoxifen. The genes were discovered using an oligonucleotide array based on frozen material (Agilent Technologies, Santa Clara, CA) and subsequently validated by quantitative RT-PCR in archived material from the same tumor specimens.

Breast Cancer Gene Expression Ratio: Literature review and discussion. The Breast Cancer Gene Expression ratio is significantly and independently associated with poorer disease-free survival in two studies of lymph node–negative, ER-positive, tamoxifen-treated patients with breast cancer. In these two studies, patients who were low risk by the two-gene expression ratio had average 10-year recurrence rates of approximately 17% to 25%.[261,262] No receiver operating characteristic or reclassification analyses show whether the Breast Cancer Gene Expression Ratio better classifies conventionally classified high-risk patients according to recurrence outcomes. No published studies retrospectively evaluated the ability of the Breast Cancer Gene Expression Ratio to predict chemotherapy benefit in comparison with conventional criteria.

Future Directions

The Blue Cross Blue Shield Association (BCBSA) is currently evaluating gene expression profiling to select women for adjuvant chemotherapy. The BCBSA technology assessment is forthcoming.

Two large, prospective, randomized clinical trials are now underway to confirm the clinical utility of two of these assays: the Trial Assigning Individualized Options for Treatment (TAILORx trial), being conducted by the Breast Cancer Intergroup (TBCI, North American consortium) to test Onco*type* DX; and the MINDACT trial, being conducted by the TRANSBIG (global consortium) to test the MammaPrint assay. The designs of these trials are different but there is overlap in the goals of the two trials. Both are addressing whether the signatures can be used to help patients with node-negative, hormone receptor–positive disease and their physicians determine the most appropriate therapy.

The TBCI TAILORx trial will test whether adjuvant hormonal therapy is not inferior to adjuvant chemohormonal in women whose tumors meet established clinical guidelines for adjuvant chemotherapy and have an RS (measured by Onco*type* DX) between 11 and 25. The primary study end point is disease-free survival, with other coprimary end points to include distant-recurrence–free interval, recurrence-free interval, and overall survival. The TRANSBIG trial, MINDACT, will compare the utility of the Amsterdam signature (MammaPrint) in assigning patients to chemotherapy versus clinicopathologic criteria.[258] The hypothesis is that fewer women in the gene signature arm will receive chemotherapy, but that outcomes in the two arms will be equivalent.[251] Both trials will evaluate prospectively the added value of the prognostic gene signature over clinical and histopathologic prognostic factors currently in use. At this time, neither of these assays addresses prognosis or benefit of specific therapies in two important groups of breast cancer patients: those with ER-negative disease and those with positive axillary lymph nodes, although the MINDACT trial now includes these populations. Data from the Rotterdam group suggest that this signature is highly prognostic in node-negative, ER-negative patients, but these data require validation.[257]

None of the studies addresses early detection or screening so there is no recommendation for use of these technologies for screening. There are also no studies to support recommendations for use in monitoring the response to therapy. Markers of proliferation and genomic instability have also been measured using microarray profiling analyses.[128,263] While these studies are in early stages and require validation, the current data suggest that these measures may capture more meaningful information than single gene markers of proliferation and aneuploidy.

BONE MARROW MICROMETASTASES AS MARKERS FOR BREAST CANCER (Note. This topic is new to the guideline)

2007 recommendation for bone marrow micrometastases as markers. Present data are insufficient to recommend assessment of bone marrow micrometastases for management of patients with breast cancer.

Bone marrow micrometastases: Marker definition. Detection of micrometastases in axillary lymph nodes of a patient with newly diagnosed breast cancer is one of the main features of the TNM system, and axillary lymph node status is widely used in the management of such patients to determine appropriate local and systemic therapy.[264] Therefore, many investigators have hypothesized that detection of micrometastases in the distant organs, such as the bone marrow compartment, of patients with early-stage breast cancer might likewise have prognostic implications, either complementing or perhaps replacing axillary nodal status.[265] Bone marrow micrometastases in breast cancer patients are defined as epithelial cells found within a bone marrow aspirate that may or may not be breast-derived, malignant, or viable. In other words, most studies have relied on the observation that epithelial cells are rarely found in the adult bone marrow and that any appreciable number of such cells detected in excess of the level found in normal volunteers is likely to arise from tumor in a patient with a known breast cancer.

Bone marrow micrometastases: Methodology. Immunohistochemical staining of bone marrow epithelial cells from aspirates is the most frequently used method to detect micrometastases; however, newer methods have also been explored. Flow cytometry, PCR, and RT-PCR DNA arrays are used to increase the accuracy of finding malignant epithelial cells in the bone marrow.[266-270] A number of studies have pointed out false-positive results that occur with all of these techniques.[271,272] The false positives are usually caused by the staining of normal hematopoietic cells or detection of illegitimate transcription of epithelial genes in hematopoietic cells. In addition, not all studies required identification of the malignant cell by morphology criteria, and hence the presence of normal epithelial cells cannot be excluded. The picture is complicated further by the fact that 1% to 2% of normal volunteers will demonstrate epithelial cells in the marrow by all of these techniques.[273,274] This observation raises the question of whether individuals without cancer have epithelial cells

that may be transiting the marrow at various times as a function of normal physiology.[275]

It is possible that epithelial cells detected in marrow that exhibit morphologic characteristics of cancer do not have long-term malignant potential, either because they lack self-renewal capacity or perhaps because they have been rendered "dormant" by either intrinsic or stromal influences. Thus, not every patient with bone marrow micrometastases will develop clinically apparent metastatic breast cancer. Only approximately 30% to 50% of patients whose marrow contains micrometastases from breast cancer will develop clinically apparent breast cancer metastases during a 5- to 10-year period of follow-up. This same phenomenon has been well demonstrated regarding axillary lymph node metastasis. Even in the absence of adjuvant systemic therapy, up to 25% of patients with axillary metastases, as demonstrated by classic hematoxylin and eosin staining, will not develop detectable systemic recurrence during the ensuing 20 or more years.[276] Given that 50% to 70% of the women with marrow micrometastases do not develop clinically metastatic breast cancer, it is clear that not all detectable breast cancer cells in the bone marrow will have clinical relevance for a particular patient. Numerous investigators have attempted to address this issue by investigating whether these breast cancer cells in the marrow express factors that will predict which breast cancer cells will become truly metastatic. Some of these studies have evaluated the bone marrow micrometastases for expression of cathepsin D, HER2/*neu*, uPA, and so on, as indicators that the visualized cells will become clinical metastases.[277-279]

Bone marrow micrometastases: Literature review and analysis. The fate of breast cancer micrometastases in the bone marrow and their clinical significance for a particular patient are controversial. There is general agreement that bone marrow micrometastases predict a higher risk of relapse and worse survival.[273,280-294] This independent predictor for a poorer outcome usually has been demonstrated with univariate analysis. Approximately half of these studies have not shown bone marrow micrometastasis to be an independent prognostic indicator for disease-free survival or overall survival when multivariate analysis is used. The studies that have evaluated the prognostic importance of bone marrow micrometastases from breast cancer generally have been prospective and usually have contained 200 to 800 patients. However, the subsets are much smaller and make the independent importance of these micrometastases difficult to prove in specific clinical situations such as node-negative breast cancer. Many studies show that the importance of bone marrow micrometastases is linked to tumor size, tumor grade, or possibly nodal status. Therefore, in most cases the patients with bone marrow micrometastasis already have characteristics that will cause their oncologists to treat them with adjuvant therapy, without considering the presence or absence of bone marrow micrometastasis. The subsets of the existing studies are too small to provide adequate data that would allow an oncologist to make a decision about adjuvant therapy for a particular patient based only on the presence of bone marrow micrometastasis.

Recently, an analysis of pooled data from several prospective studies has provided enormous power to evaluate such subsets.[295] In every case, the presence of bone marrow micrometastases was associated with a statistically significantly higher risk of recurrence and death. However, the magnitude of separation of the outcomes for positive versus negative patients was greatest for those patients who received adjuvant systemic therapy. Indeed, although bone marrow positivity did predict for a statistically significantly higher risk of relapse for patients who did not receive adjuvant systemic therapy, the difference in distant-disease–free survival between those patients who had micrometastases versus those who did not was very small (for years 1 to 5, incidence rate ratio = 2.0; 95% CI, 1.2 to 3.35; $P < .007$; and for years 6 to 10, incidence rate ratio = 0.92; 95% CI, 0.3 to 2.78; $P = .88$). These data suggest that the presence of micrometastases may often reflect other prognostic factors already discerned from the primary tumor and the axillary nodal status. The data suggest further that for patients with an apparent very good prognosis (ie, those whose physicians chose not to recommend adjuvant systemic therapy based on primary tumor and the axillary nodal status), bone marrow micrometastases add little prognostic information, given that both marrow-negative and -positive patients appear to have an extremely good prognosis. In summary, these data do not suggest that a patient with bone marrow micrometastases in the presence of a small, low-grade, node-negative breast cancer has a sufficiently worse prognosis such that one can justify making differential recommendations for adjuvant therapy. The data from studying women with micrometastases to the marrow from breast cancer are intriguing and should continue to be evaluated in more directed studies to establish the clinical significance of bone marrow micrometastases in those women in whom they are most likely to be informative.

CIRCULATING TUMOR CELLS AS MARKERS FOR BREAST CANCER (Note. This topic is new to the guideline)

Dr Hayes recused himself from deliberations and Update Committee votes concerning recommendations for this marker due to potential conflicts of interest.

2007 recommendation for circulating tumor cell assays. The measurement of circulating tumor cells (CTCs) should not be used to make the diagnosis of breast cancer or to influence any treatment decisions in patients with breast cancer. Similarly, the use of the recently US Food and Drug Administration–cleared test for CTCs (CellSearch Assay; Veridex, Warren, NJ) in patients with metastatic breast cancer cannot be recommended until additional validation confirms the clinical value of this test.

CTCs: Marker definition. CTCs are those cells present in the blood that possess antigenic or genetic characteristics of a specific tumor type. The source of CTCs is unknown and the clinical significance of CTCs is not yet established. The presence of CTCs in a breast cancer patient may predict for the presence of a micrometastasis or of an aggressive primary tumor.

CTCs: Methodology. CTCs can be detected by several approaches. Most frequently, the CTCs are "captured" by immunomagnetic beads that are coated with an antibody specific for a cell surface, epithelial, or cancer-related antigen. After this positive cell selection, the isolated cells can then be characterized by immunocytochemistry or by gene expression analysis for the presence of cytokeratins and tumor antigens. An alternative method for CTC detection is first to remove leukocytes from the blood sample by positive selection of those cells, and then to interrogate the remaining cells by immunocytochemistry or gene expression analysis using RT-PCR methodology. In addition, RT-PCR methods can be applied directly to whole blood to assess gene expression characteristics of CTCs. In all of these approaches, the use of combinations of cell surface antigens have been proposed to enhance capture efficiency and improve sensitivity,

whereas the use of a panel of tumor antigens or mRNAs for cancer-related genes has been suggested to improve the identification of CTCs and increase the specificity of the test.

One cell detection assay, the CellSearch Assay, has recently received US Food and Drug Administration clearance for application to the metastatic breast cancer patient. In this technique, epithelial cell adhesion molecule antibody–coated magnetic beads are used to capture the CTCs. After the sample is washed to remove the remaining cells, the captured cells are stained with cytokeratin antibody specific for cytokeratins 8, 18, 19, and with antibody to CD45 (a cluster differentiation antigen for leukocytes). Staining with 4′6-diamidino-2-phenylindole-2 (DAPI) confirms the presence of a cell nucleus. A CTC must stain for cytokeratin and DAPI, but not for CP45. The number of cells that have these characteristics is then counted.

CTCs: Literature review and analysis. During the period, January 1996 to December 2006, approximately 400 publications that reported on the detection of CTCs in breast cancer were identified. Most of these publications addressed the development and validation of test methodologies and applications for the assessment of tumor cells in bone marrow. Of the studies that addressed the use of CTCs in the peripheral circulation of breast cancer patients, the majority focused on the use of RT-PCR as the detection method. Many of these studies used single genes to define the presence of CTCs such as cytokeratins 8, 18, 19, or 20[296-302]; CEA,[303] mammaglobin,[304] maspin,[305] and MUC-1.[306] Others used multiple genes[307-313] for characterization of CTCs in blood. In these reports, CTC cell enrichment was accomplished by density gradient centrifugation,[296,298,303,307,312] Ficoll enrichment,[299-302,305,308,311,313] or immunomagnetic separation.[304,306,310] The CellSearch assay was used by three investigators for CTC detection.[314-316] Another commercially available reagent system (AdnaTest BreastCancerSelect and AdnaTest BreastCancerDetect; AdnaGen, Hannover, Germany) uses immunomagetic selection with a panel of membrane antigens and cell identification with a three-gene panel.[317]

Only a few articles addressed the clinical utility of CTCs. Gaforio et al[299] isolated CTCs from 92 patients using double density gradient fractionation followed by immunomagnetic cell separation and immunocytochemical staining for cytokeratin. Cells were detected in 57 of 92 patients and in none of the 16 healthy controls. The presence of cytokeratin-positive cells before chemotherapy correlated with progression-free survival and overall survival.

Weigelt et al[313] performed quantitative RT-PCR of four marker genes (*CK19*, *P1B*, *PS2*, and *EGP2*) to establish a discriminant function. The discriminant function (CTC present) was positive in 24 of 94 patients with metastatic disease. The CTC-positive patients had poorer progression-free survival and overall survival at 2 years than did the CTC-negative patients (17% *v* 36%). These two reports of poorer survival in patients with CTC are consistent with the recently published report by Cristofanilli et al,[315,318] who used the CellSearch test to quantitate CTCs. The CellSearch Assay appears to provide both a prognostic utility and a predictive use in metastatic breast cancer. The presence of more than five CTCs in a patient before any treatment is administered predicts for a poorer outcome than for those patients who have no tumor cells detected. Similarly, the presence of more than five CTCs after the first course of hormone therapy or chemotherapy predicts for no treatment response.[315,318] A subsequent report from Hayes et al[319] showed that the detection of more than five CTCs at any time during therapy was indicative of treatment failure. Recently, Budd et al[320] reported that CTC measurements provided an earlier indication of disease status than did bone imaging. In that report, patients with radiologic evidence of progression who had more than five CTCs demonstrated a significantly shorter survival than those with fewer than five CTC. However, there are no data yet generated to prove that the use of this CTC test leads to a longer survival time or improved quality of life for the patient with metastatic breast cancer. In this regard, the SWOG and the Breast Cancer Intergroup of North America recently have initiated a prospective trial in which patients with metastatic breast cancer who have an elevated CTC after one cycle of first-line chemotherapy will be randomly assigned to either remaining on that therapy until clinical and/or radiographic evidence signals progression, or switching therapy at that time point to a different chemotherapeutic agent (SWOG protocol S0500). Studies of CTCs from patients with early-stage breast cancer suggest their potential utility, although the lower frequency of events makes this area more challenging. Additional studies are necessary to determine the utility of CTCs in early breast cancer.[321]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Appendix

For the 2007 update, a methodology similar to that applied in the original ASCO practice guidelines for use of tumor markers was used. Pertinent information published from 1999 through February 2006 was reviewed for markers that were included in the last update of the guideline; information from 1966 to February 2006 was reviewed for the new markers. The MEDLINE database (National Library of Medicine, Bethesda, MD) was searched to identify relevant information from the published literature for this update. A series of searches was conducted using the medical subject headings or text words for each of the markers with the medical subject heading "breast neoplasms" and related text words. Search results were limited to human studies and English-language articles; editorials, letters, and commentaries were excluded from consideration. The Cochrane Library was searched for available systematic reviews and meta-analyses with the phrases "tumor markers" and "biomarkers." Directed searches based on the bibliographies of primary articles were also performed. Finally, Update Committee members contributed articles from their personal collections. Update Committee members reviewed the resulting abstracts and titles that corresponded to their assigned sections. Inclusion criteria were broad. Update Committee members focused attention on systematic reviews and meta-analyses, and on studies that considered markers in relation to ASCO clinical outcomes for guideline and technology assessment (overall survival, disease-free survival, quality of life, toxicity, and cost-effectiveness).

Table A1. Tumor Markers Panel Members

Panel Members	Institution
Robert C. Bast Jr, MD, *Co-Chair*	M.D. Anderson Cancer Center
Daniel F. Hayes, MD, *Co-Chair*	University of Michigan Medical Center
Dean F. Bajorin, MD	Memorial Sloan-Kettering Cancer Center
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Stanley Hamilton, MD	M.D. Anderson Cancer Center
Jules Harris, MD	Rush-Presbyterian St Luke's Medical Center
Lyndsay Harris, MD	Yale Cancer Center
John M. Jessup, MD	Georgetown University Medical Center
Philip W. Kantoff, MD	Dana-Farber Cancer Institute
Nancy E. Kemeny, MD	Memorial Sloan-Kettering Cancer Center
Ann Kolker	Patient Representative
Susan Leigh, BSN, RN	National Coalition for Cancer Survivorship, Patient Representative
Gershon Y. Locker, MD	Evanston Northwestern Healthcare
Juanita Lyle	George Washington University, Patient Representative
John S. Macdonald, MD	St Vincent's Comprehensive Cancer Center
Pam McAllister, PhD	Science Advocate with the Colorectal Cancer Coalition, Patient Representative
Robert G. Mennel, MD	Texas Oncology PA
Larry Norton, MD	Memorial Sloan-Kettering Cancer Center
Peter Ravdin, MD	M.D. Anderson Cancer Center
Sheila Taube, PhD	National Cancer Institute