ASCO SPECIAL ARTICLE

2000 Update of Recommendations for the Use of Tumor Markers in Breast and Colorectal Cancer: Clinical Practice Guidelines of the American Society of Clinical Oncology*

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Objective: **To update the 1997 clinical practice guidelines for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of breast and colorectal cancers. These guidelines are intended for use in the care of patients outside of clinical trials.**

Options: **Six tumor markers for colorectal cancer and eight for breast cancer were considered. They could be recommended or not for routine use or for special circumstances. In addition to carcinoembryonic antigen (CEA) and CA 15-3, CA 27.29 was also considered among the serum tumor markers for breast cancer.**

Outcomes: **In general, the significant health outcomes identified for use in making clinical practice guidelines (overall survival, disease-free survival, quality of life, lesser toxicity, and cost-effectiveness) were used.**

Evidence: **A computerized literature search from 1994 to March 1999 was performed.**

Values: **The same values for use, utility, and levels of evidence were used by the committee.**

Benefits, Harms, and Costs: **The same benefit, harms, and costs were used.**

Recommendation: **Changes were recommended (see Appendix).**

Validation: **The updated recommendations were validated by external review by the American Society of Clinical Oncology's (ASCO's) Health Services Research Committee and by ASCO's Board of Directors.**

Sponsor: **American Society of Clinical Oncology.**

J Clin Oncol 19:1865-1878.

THE AMERICAN Society of Clinical Oncology (ASCO) published evidence-based clinical practice guidelines for the use tumor markers in breast and colon 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 1997.

For the 2000 update, an update committee composed of members from the full panel was formed to review and to analyze data published since 1994. Computerized literature searches of MEDLINE and CancerLit were performed. The searches of the English-language literature from 1994 to 1999 combined each of the markers with the corresponding disease site.

The update committee had a single face-to-face meeting to consider the evidence for each of the 1997 recommendations. The guideline was circulated in draft form to the update committee and to the full expert panel for review and approval. Each guideline from the 1997 update is listed below, followed by the 2000 update, if applicable, and then by the 2000 recommendation.

It is important to realize that guidelines 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 results. ASCO considers adherence to these guidelines to be voluntary. The ultimate determination regarding their application is to be made by the physician in light of each patient's individual circumstances. In addition, these guidelines describe administration of therapies in clinical practice; they cannot be assumed to apply to interventions performed in the context of clinical trials, given that such clinical studies are designed to test innovative and novel therapies in which better treatment is of paramount importance. In that guideline development involves a review and synthesis of the latest literature, a practice guideline also serves to identify important questions for further research and those settings in which investigational therapy should be considered.

From the American Society of Clinical Oncology, Alexandria, VA.

Accepted January 4, 2001.

**Adopted on November 3, 2000, by the American Society of Clinical Oncology.*

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0732-183X/01/1906-1865

GUIDELINES: COLORECTAL CANCER

Carcinoembryonic Antigen as a Marker for Colorectal Cancer

1a. 1997 Recommendation: Carcinoembryonic antigen (CEA) is not recommended to be used as a screening test for colorectal cancer.

2000 Update: None.

2000 Recommendation: No change.

1b. 1997 Recommendation: CEA may be ordered preoperatively in patients with colorectal carcinoma if it would assist in staging and surgical treatment planning. Although elevated preoperative CEA (> 5 ng/mL) may correlate with poorer prognosis, data are insufficient to support the use of CEA to determine whether to treat a patient with adjuvant therapy.

2000 Update: None.

2000 Recommendation: No change.

1c. 1997 Recommendation: If resection of liver metastases would be clinically indicated, it is recommended that postoperative serum CEA testing may be performed every 2 to 3 months in patients with stage II or III disease for 2 or more years after diagnosis. An elevated CEA, if confirmed by retesting, warrants further evaluation for metastatic disease but does not justify the institution of adjuvant therapy or systemic therapy for presumed metastatic disease.

2000 Update: A study from the Eastern Cooperative Oncology Group followed patients on INT 0089 after surgical resection for high-risk stage B2 and C colon carcinoma.[1] For the 421 patients who developed recurrent disease, investigators tried to determine which tests were the most effective and cost-effective in detecting metastases. Follow-up testing was done by protocol guidelines. Ninety-six of the 421 patients with recurrent disease underwent surgical resection with curative intent. For the subgroup of resectable patients, the first test to detect recurrence was CEA, chest x-ray, colonoscopy, and other tests.[2] The physician's examination was unsuccessful in finding resectable disease. CEA was the most cost-effective approach to detecting potentially resectable metastases from colon cancer. Another study followed patients with a specified testing strategy after curative colorectal surgery. Here, 64% of recurrences were detected first by CEA, far more than the other tests in the battery.[3]

2000 Recommendation: No change.

1d. 1997 Recommendation: Present data are insufficient to recommend routine use of the serum CEA alone for monitoring response to treatment. If no other simple test is available to indicate a response, CEA should be measured at the start of treatment for metastatic disease and every 2 to 3 months during active treatment. Two values above baseline are adequate to document progressive disease even in the absence of corroborating radiographs. CEA is regarded as the marker of choice for monitoring colorectal cancer.

2000 Update: None.

2000 Recommendation: No change.

Lipid-Associated Sialic Acid as a Marker for Colorectal Cancer

2. 1997 Recommendation: Present data are insufficient to recommend lipid-associated sialic acid (LASA) for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.

2000 Update: None.

2000 Recommendation: No change.

CA 19-9 as a Marker for Colon Cancer

3. 1997 Recommendation: Present data are insufficient to recommend CA 19-9 for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.

2000 Update: None.

2000 Recommendation: No change.

DNA Ploidy or Flow Cytometric Proliferation Analysis as a Marker for Colon Cancer

4. 1997 Recommendation: Present data are insufficient to recommend DNA flow cytometrically derived ploidy (DNA index) for the management of colorectal cancer.

2000 Update: This update encompassed publications in English that appeared from April 1994 to April 1999, determined flow cytometrically derived parameters for at least 50 colorectal cancer patients, and supplied survival data. Ten articles evaluating the role of DNA ploidy or index determined by flow cytometry after surgical therapy met these criteria and were reviewed.[4-13] Two articles that contained previously published data included in the original guideline review were not included.[14,15] There were too few patients and articles to comment on the role of DNA ploidy in the prognosis of liver metastases.[16-18] Studies using other techniques to assess DNA ploidy, such as image analysis, were not evaluated.

Nine of the 10 articles included patients with both colon and rectal cancer; one addressed DNA ploidy only in colon cancer.[4] Three of the studies were of fresh or frozen material[7,9,13]; the other seven derived their material from paraffin blocks. Two studies were of all (consecutive) patients from a defined time period[6,9]; one was of selected patients enrolled onto a randomized trial of adjuvant chemotherapy[4]; the remainder were of selected cases from a given time period.

Of the 10 studies, four found that patients with an aneuploid tumor or an elevated DNA index had a significantly worse survival after surgery than patients with a diploid tumor or a low DNA index.[7,8,10,12] These parameters had no statistically significant effect on prognosis in the other six articles. In three of three positive studies in which it was analyzed, DNA ploidy or index remained prognostic in a multivariate analysis. In these studies, the subset of stage I/II (Duke's B) was evaluated; in all three, DNA ploidy or index did not give additional prognostic information within the stage.

Three articles evaluating DNA flow cytometric proliferation analysis (% S phase) after surgical therapy of colorectal cancer met the criteria for inclusion in the update.[4,9,13] One study was a randomized trial of adjuvant therapy in colon cancer that analyzed paraffin blocks retrospectively; the others were prospective studies using fresh or frozen tissues. None of the three found % S phase to be of prognostic significance for overall survival.

These studies do not support the use of flow cytometrically derived DNA ploidy or proliferation analysis to determine prognosis or therapy of operable colorectal cancer. They should not be ordered routinely other than as part of a research trial.

2000 Recommendations: No change.

5. p53 as a Marker for Colorectal Cancer

1997 Recommendation: Present data are insufficient to recommend the use of *p53* expression or mutation for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.

2000 Update: None.

2000 Recommendation: No change.

6. ras as a Marker for Colorectal Cancer

1997 Recommendation: Present data are insufficient to recommend the use of the *ras* oncogene for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.

2000 Update: None.

2000 Recommendation: No change.

Future Directions

Although there are no changes in the recommendations for colon markers as originally proposed, it should be noted that several markers have been shown to be independent prognostic factors in multivariate analyses from more than one single-institution study. These markers would be measured in the serum preoperatively or intratumorally and include serum interleukin-6 levels and intratumoral expression of $p27^{Kip1}$, the deleted in colorectal cancer (*DCC*) gene, and microsatellite instability. The expression of thymidylate synthase within a tumor may be associated with response to fluorouracil. All of these markers are still under development and are not recommended for routine use.

GUIDELINES: BREAST CANCER

1. CA 15-3 as a Marker for Breast Cancer

1997 Recommendation: Present data are insufficient to recommend CA 15-3 or CA 27.29 for screening, diagnosis, staging, or surveillance after primary treatment. Although a rising CA 15-3 or CA 27.29 level can detect recurrence after primary treatment, the clinical benefit is not established; therefore, it cannot be recommended. One well-designed study has shown that an increase in CA 27.29 can predict recurrence an average of 5.3 months before other symptoms or tests.[19] Options for therapy, however, remain unchanged, and there has been no demonstrated impact on the most significant outcomes (improved disease-free or overall survival, better quality of life, lesser toxicity, or improved cost-effectiveness).[20] The data used by the Food and Drug Administration (FDA) to approve CA 27.29 were available to the panel previously; although the assay was approved by the FDA, the FDA does not require tests to show clinical benefit. Given the small body of evidence and until there is evidence of clinical benefit, present data are insufficient to recommend routine use of CA 27.29.

2000 Update: CA 15-3 and CA 27.29 have been evaluated for their ability to determine diagnosis and prognosis, monitor therapy, and predict recurrence of breast cancer after curative surgery and radiotherapy. Multiple studies have shown that the incidence of CA 15-3 elevation increases with an increasing stage of the disease.[21,22] Data compiled from the literature using assays for MUC1 antibodies (CA 15-3, CA549, and MCA) show an incidence of elevation ranging from 5% to 30%, 15% to 50%, 60% to 70%, and 65% to 90% for breast cancer stages I, II, III, and IV, respectively.[23] A recent report comparing CA 27.29 with CA 15-3 suggested that the former was a more sensitive test than the latter, with an incidence of elevation of 29%, 36%, and 59% in stages I, II, and III, respectively, for CA 27.29.[21] In contrast, the incidence of elevation for CA 15-3 measured in the same samples was 15%, 23%, and 54.5% for the patients with stages I, II, or III, respectively. While retrospective, this study included 275 patients (level of evidence III) and found CA 27.29 to be more sensitive in every patient population studied. Despite this reliable correlation with stage, low CA 15-3 levels do not exclude metastases, and a given CA 15-3 level cannot be used to determine the stage of disease.

A number of studies have addressed the question of whether CA 15-3 or CA 27.29 can detect recurrence of breast cancer after primary therapy. The trial reported to the FDA that accomplished approval of CA 27.29 for use as an adjunct in monitoring patients for recurrence was a prospective study at five centers.[19] The trial objective was to determine the ability of CA 27.29 to predict relapse and to determine the lead time provided by the marker elevation. There were 193 patients enrolled; 163 were assessable and 80% of the patients followed had stage II breast cancer. There were 26 recurrences, 15 with CA 27.29 positivity, for a sensitivity of 57.7% and a lead time from the first marker elevation of 5.3 months. Three patients with CA 27.29 elevation had no evidence of recurrence and were considered to have false-positive elevation. The specificity was thus 98% for this study, which had a mean follow-up of 13.6 months. In addition to achieving FDA approval, the data were also construed to show that CA 27.29 was better than CA 15-3 in monitoring for recurrent disease. However, CA 15-3 was not measured simultaneously, so that conclusion must be regarded as preliminary. A phenomenon of "marker migration" (the newer marker is always better) may instead account for the improved results.

Evaluation of CA 15-3 in assessing response to metastatic cancer therapy would be a much-needed clinical tool. The FDA submission for CA 27.29 included a study of patients with advanced disease.[24] Levels of CA 27.29 were elevated in 81% of patients. Forty-five patients with progressive disease had a median increase of 32%, whereas patients with stable or regressing disease had a median decrease of 19%. Among 43 patients with levels increased 20% or more, progressive disease was confirmed in 33 patients. Further studies are required to determine whether the proposed greater sensitivity of the CA 27.29 assay will allow earlier determination of disease progression or will be achieved at the price of decreased specificity in the metastatic disease setting.

2000 Recommendation: No change.

CEA as a Marker for Breast Cancer

2a. 1997 Recommendation: CEA is not recommended for screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy.

2000 Update: None.

2000 Recommendation: No change.

2b. 1997 Recommendation: Routine use of CEA for monitoring response of metastatic disease to treatment is not recommended. However, in the absence of readily measurable disease, a rising CEA may be used to suggest treatment failure.

2000 Update: Routine use of CEA for monitoring response of metastatic disease to treatment is not recommended. However, in the absence of readily measurable disease, or an elevated MUC-1 marker (CA 15-3 and/or CA 27.29), a rising CEA may be used to suggest treatment failure.

2000 Recommendation: No change.

Estrogen Receptors and Progesterone Receptors as Markers for Breast Cancer

3. 1997 Recommendation: Estrogen and progesterone receptors are recommended to be measured on every primary 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 may be used to identify patients most likely to benefit from endocrine forms of adjuvant therapy and therapy for recurrent or metastatic disease.

2000 Update: Since the publication of the ASCO guidelines, we have witnessed widespread acceptance of the immunocytochemical techniques for estrogen and progesterone receptors (ERICA and PgRICA) over the dextran-coated charcoal (DCC) assay and the sucrose gradient ultracentrifugation (SGU) assay to measure the estrogen and progesterone receptors.[25,26] Although all of the guidelines have been made based on studies that used the DCC and SGU techniques for the estrogen receptor and the progesterone receptor determinations, most oncologists accept the ERICA or PgRICA as equivalent or superior to the older techniques. The concordance of these newer and older techniques is still on the order of 70% to 90%.[27-30] Although most oncologists and pathologists accept that the ERICA and PgRICA are measuring the same biologic phenomenon as the estrogen receptor and the progesterone receptor measured by DCC and SGU assays, this has not been proven.[26] Most oncologists also assume that if the older studies on which the guidelines were based were repeated using the ERICA and PgRICA in place of the DCC and SGU assays, the results would be the same. This has also not been proven.[26] There is strong bias that the ERICA and PgRICA may actually be better techniques than the DCC and SGU techniques, since the immunohistochemical techniques actually measure the estrogen receptor and the progesterone receptor in the cells of interest, ie, the invasive carcinoma and not intraductal carcinoma or normal breast tissue. This also has not been proven.[26] There have been some problems with standardization of these techniques from laboratory to laboratory. The ERICA and PgRICA have become so widely used that they are now considered by most oncologists to be the standard for measurement of

the estrogen receptor and the progesterone receptor. The ASCO guidelines will be interpreted by most oncologists in light of immunohistochemical data, even though the immunohistochemical assays did not generate the data that were used to formulate the guidelines.

In the past 3 years since the publication of the ASCO guidelines, there has been a better understanding of the various components of the estrogen receptor and the progesterone receptor cascades.[31-35] There have also been different ways (polymerase chain reaction, mRNA, and enzyme immunoassay) of measuring these variants of the estrogen receptor and the progesterone receptor.[36-39] These newer methods of measuring the variants of the estrogen receptor and the progesterone receptor may prove to be better than the standard ERICA and PgRICA in predicting the success of hormonal therapy or even the success of cytotoxic chemotherapy or the prognosis for a particular patient.[40,41] However, this needs to be proven. What is more exciting is the development of drugs, for example, the selective estrogen receptor modulators, that may effect these various components of the estrogen receptor and the progesterone receptor pathways.[42] Although the discoveries in the past 3 years since the publication of the ASCO guidelines for estrogen receptor and progesterone receptor have not necessitated a change in the guidelines, newer developments on the horizon may make more specific guideline recommendations a reality over the next few years.

2000 Recommendation: No change.

DNA Flow Cytometrically Derived Parameters as Markers for Breast Cancer

4a. 1997 Recommendation: Present data are insufficient to recommend obtaining DNA flow cytometry–derived estimates of DNA content or S phase in breast tissue.

2000 Update: Present data are insufficient to recommend obtaining flow cytometric or immunohistochemical measures of DNA content and/or S phase fraction (proliferation) in breast tissue to determine prognosis or treatment in the adjuvant or metastatic setting.

2000 Recommendation: No change.

4b. 1997 Recommendation: DNA flow cytometry–derived ploidy are not recommended to be used to assign a patient to prognostic groupings. There is insufficient evidence to recommend the use of S phase determination for assigning patients to prognostic groupings.

2000 Update: Present data are insufficient to recommend obtaining flow cytometric or immunohistochemical measures of DNA content and/or S phase fraction (proliferation) in breast tissue to determine prognosis or treatment for carcinoma-in-situ of the breast.

2000 Recommendation: No change.

c-erb*B*-2 (HER-2/*neu*) *as a Marker for Breast Cancer*

5a. 1997 Recommendation: Present data are insufficient to recommend the use of c-*erb*B-2 (*HER-2*/*neu*) gene amplification or overexpression for management of patients with breast cancer.

2000 Recommendation: c-*erb*B-2 overexpression should be evaluated on every primary breast cancer either at the time of diagnosis or at the time of recurrence. Measures of c-*erb*B-2 amplification may also be of value.

Methods for Measuring c-erb*B-2*

2000 Update: Various methods have been used to measure c-*erb*B-2 and its gene product. These include direct measurement of gene amplification, mRNA level, and protein expression. The most widely studied method is immunohistochemical staining (IHCS). The FDA has approved IHCS for detecting c-*erb*B-2 overexpression and fluorescence in situ hybridization (FISH) for quantifying c-*erb*B-2 gene amplification. At this time, both of these methodologies have been validated as having clinical utility for different clinical purposes. It has not yet been proven that these methods are interchangeable. Non-FDA–approved methodologies may also be of value, but the clinical utility of these methods is less established, and for some, highly suspect. At a minimum, any specific c-*erb*B-2 test used to make clinical decisions should be backed by documentation that the test is accurate and reproducible and has been correlated either with clinical outcomes or with another c-*erb*B-2 test that has been correlated with clinical outcome.

5b. 2000 Recommendation: Because of the uncertain interchangeability, reproducibility, and clinical utility of different c-*erb*B-2 tests, it is important that clinical laboratories report not only an estimate c-*erb*B-2 but also a statement about the test's quality controls, the method, the specific kit or critical reagents, details of the scoring system, a statement regarding reproducibility, sensitivity, and specificity of the assay, and a reference to the clinical validation of the assay or its correlation with a clinically validated c-*erb*B-2 test.

Sensitivity to Trastuzumab

2000 Update: Use of c-*erb*B-2 to select patients for trastuzumab (Herceptin; Genentech, South San Francisco, CA) therapy is supported by preclinical studies that indicate the anti–c-*erb*B-2 antibody has little or no effect on c-*erb*B-2–negative cells.[43] Nearly all patients treated on clinical trials of trastuzumab to date have been c-*erb*B-2–"positive," since this was an entry criterion for these studies.[44-48]

Consequently, it is currently presumed, and highly probable, that overexpression of c-*erb*B-2 by a patient's cancer cells is required for this agent to be of benefit.

Given the presently available data, we conclude that trastuzumab is currently only indicated for patients with c-*erb*B-2–positive tumors. In this regard, precise breadth ofthe definition of c-*erb*B-2 positivity is uncertain. The only formally published data from the reported clinical trials are from studies using IHCS (+2 or +3) to select patients on the basis of c-*erb*B-2 overexpression, but the role of measures of gene amplification for selecting patients who might benefit from trastuzumab seems nearly certain. At the 2000 Annual Meeting of ASCO, data were presented demonstrating that FISH also identified patients likely to respond to trastuzumab.[49]

6. 2000 Recommendation: High levels of c-*erb*B-2 expression or c-*erb*B-2 amplification can be used to identify patients for whom trastuzumab may be of benefit for the treatment of metastatic, recurrent, and/or treatment-refractory unresectable locally advanced breast cancer.

Response to Cyclophosphamide/Methotrexate/Fluorouracil or Nonanthracycline-Based Adjuvant Chemotherapy

2000 Update: Two early publications suggested that patients with c-*erb*B-2–negative tumors, as determined by IHCS, gained substantially more benefit from cyclophosphamide, methotrexate, and fluorouracil (CMF) than did c-*erb*B-2–positive patients.[50,51] Although these studies were performed using tissues collected from patients enrolled prospectively onto randomized clinical trials, evaluation of c-*erb*B-2 was retrospective in nature and included only a fraction of the entire group of patients who participated in the therapeutic protocols, and in only one of the studies was c-*erb*B-2 overexpression shown to be a statistically independent predictor of less benefit from CMF. Other studies have generally supported the notion that patients with c-*erb*B-2–positive tumors may not benefit as much as patients with c-*erb*B-2–negative tumors from CMF-like adjuvant therapy.[52-54] However, another study of tissue from the original Milan CMF adjuvant trial, presented only as an abstract, did not confirm the adverse prognosis of c-*erb*B-2 positivity. If anything, this study suggested that c-*erb*B-2–positive patients may gain more benefit from CMF than those who are c-*erb*B-2–negative.[55]

7. 2000 Recommendation: The question of whether c-*erb*B-2 overexpression affects the relative benefit of adjuvant CMF chemotherapy remains open, and the Update Committee cannot make a definitive practice recommendation at present.

Response to Anthracycline-Based Adjuvant Chemotherapy

2000 Update: Other data suggest that c-*erb*B-2–positive tumors might be particularly sensitive to doxorubicin-based adjuvant therapies. This possibility was first suggested by the results of a study reported by the Cancer and Leukemia Group B in which c-*erb*B-2 was studied using IHCS in tissues collected from a subset of patients who participated in an adjuvant trial addressing dose of cyclophosphamide, doxorubicin, and fluorouracil (CAF).[56,57] The impact of c-*erb*B-2 overexpression was tested prospectively in a recent study by the National Surgical Adjuvant Bowel and Breast Project.[58] Tissues from more than 90% of patients who participated in B-11 (prednisone, doxorubicin, and fluorouracil [PAF] *v* prednisone and fluorouracil [PF]) were evaluated using IHCS. Overall, the outcomes of c-*erb*B-2–negative patients who received (PF) or (PAF) were similar. However, the outcome of those c-*erb*B-2–positive patients who received PAF were statistically superior to that of those who received PF. These results support the hypothesis that patients with tumors that overexpress c-*erb*B-2 particularly benefit from doxorubicin-based adjuvant chemotherapy.

Very similar results were obtained from a prospective study of specimens from Intergroup 0100, led by the Southwest Oncology Group.[59] In this study, node-positive, postmenopausal, hormone receptor–positive patients were randomly assigned to tamoxifen or tamoxifen plus CAF. In this study, among patients with cancers that overexpressed c-*erb*B-2, chemoendocrine therapy was more effective than endocrine therapy. Results consistent with these have been reported in abstract form from analysis of tissues collected from patients who participated in a randomized trial comparing CMF and fluorouracil, doxorubicin, and cyclophosphamide.[60] Not all adjuvant studies have demonstrated a treatment interaction between anthracyclines and c-*erb*B-2. For example, results in abstract form from an investigation of tissues from a randomized trial comparing CMF and single-agent epirubicin did not find a statistically significant interaction between c-*erb*B-2 expression and the benefit of anthracycline-based therapy, but this study was small ($n = 266$) and had low statistical power for detecting such an interaction.[61]

Overall, there has emerged a fairly consistent picture that anthracycline-based adjuvant therapy is particularly beneficial for treatment of cancers that overexpress c-*erb*B-2. One cannot, however, conclude that such patients receive no benefit from nondoxorubicin-based therapy or that anthracycline-based therapy is ineffective in patients with tumors that do not express c-*erb*B-2.

None of these studies achieve a level of evidence I investigation, and only a few can be considered level of evidence II. It is not clear that the apparent superiority of the anthracycline-based therapy arms in these studies is mechanistically tied in some way to the particular sensitivity to anthracyclines. In studies with metastatic disease, c-*erb*B-2 overexpression has correlated with relative resistance to anthracycline-based therapy[62,63] or was without value for predicting response to therapy.[64]

8. 2000 Recommendation: High levels of c-*erb*B-2 expression, as determined by immunohistochemistry, may identify patients who particularly benefit from anthracycline-based adjuvant therapy, but levels of c-*erb*B-2 expression should not be used to exclude patients from anthracycline treatment.

Sensitivity to Endocrine Therapy

2000 Update: Preclinical models have demonstrated that c-*erb*B-2 overexpression is associated with increased resistance to endocrine therapy or to increased expression of proteins that are associated with such resistance.[65]

Although it is often quoted that c-*erb*B-2 overexpression is associated with resistance to tamoxifen for patients with metastatic disease, a close reading of the literature shows that this resistance is not absolute. For example, although the large Guy's Hospital study is often reported as supporting resistance to tamoxifen, it in fact shows a *relative* resistance.[66] In this study of primary tumors from 241 patients who were treated at first relapse with endocrine therapy, overexpression of c-*erb*B-2 was expressed by IHCS. Although the overall response to treatment and time to progression were significantly lower in patients with c-*erb*B-2–positive tumors compared with those with c-*erb*B-2–negative tumors (38% *v* 56%, $P = .02$; and 4.1 months *v* 8.7 months, $P = .001$, respectively), endocrine therapy was beneficial in approximately 40% of the patients.

The other large study of c-*erb*B-2 and tamoxifen resistance concluded that overexpression of c-*erb*B-2 was not associated with decreased response. In the analysis of c-*erb*B-2 in specimens from 205 patients in the Southwest Oncology Group phase II study of tamoxifen as first-line therapy for metastatic breast cancer, the response rate, time to treatment failure, and survival were slightly inferior in c-*erb*B-2–positive patients,[67] but the overall difference was small and not of statistical or clinical significance.

Several smaller studies also have suggested that c-*erb*B-2 overexpression in breast tumors is associated with a lower rate of responsiveness to tamoxifen.[68] However, not all studies found c-*erb*B-2 to be predictive.[69] In summary, the weight of evidence does not show that c-*erb*B-2 is useful for selecting patients who would not benefit from endocrine therapy.

The use of c-*erb*B-2 to select patients who are unlikely to benefit from endocrine adjuvant therapy also remains controversial. The results from one large study suggests that c-*erb*B-2 overexpression is associated with a lack of benefit for adjuvant tamoxifen,[70] although the interpretation of this study is complicated by the inclusion of estrogen receptor–negative patients. Another study showed no apparent correlation between c-*erb*B-2 expression and the apparent benefit of adjuvant tamoxifen.[71] This question is being addressed in ancillary studies in tissue blocks from National Surgical Adjuvant Bowel and Breast Project B-14 and other randomized studies assessing the utility of tamoxifen in the adjuvant setting, but these results are not yet available. In summary, there is insufficient evidence to assess the value of c-*erb*B-2 for selecting estrogen receptor–positive patients who should not receive adjuvant tamoxifen, and this seems to be an inappropriate use of c-*erb*B-2 testing at this time.

9. 2000 Recommendation: The use of c-*erb*B-2 data to decide whether to prescribe endocrine therapy either in the adjuvant or metastatic setting is not recommended.

Sensitivity or Resistance to Taxane Therapy

2000 Update: The available preclinical and clinical data regarding c-*erb*B-2 and taxanes are scant and contradictory. Peclinical evidence suggests that c-*erb*B-2 overexpression might be associated with resistance to taxanes.[72] Some clinical studies suggest an increase in response to taxanes for c-*erb*B-2–positive patients in the metastatic setting; others suggest the opposite.[73-76] These studies are plagued by small numbers, weak clinical trial design, and assay heterogeneity. Currently, we conclude that no decision regarding the use of taxanes should be made based on c-*erb*B-2 status.

10. 2000 Recommendation: The use of c-*erb*B-2 data to decide whether to prescribe taxane-based chemotherapy either in the adjuvant or metastatic setting is not recommended.

*Use of Measures of c-*erb*B-2 to Predict Patient Prognosis*

2000 Update: As previously described,[77] a prognostic factor is best evaluated in the absence of any therapies with which it may interact. It is also important that the test be prognostic in the patient population for whom treatment decisions will be affected (patients with stage I breast cancer). Further, prognostic factors that are to be used to determine whether to treat a patient after primary therapy is completed should be investigated in untreated patients from whom long-term follow-up data are available.

The prognostic significance of c-*erb*B-2 overexpression has been evaluated in several clinical trials, with some studies suggesting that c-*erb*B-2 has prognostic value[78-80] and others failing to find this association. This was particularly true for studies that concentrated on node-negative patients; some studies found c-*erb*B-2 overexpression to be predictive,[53,81-85] whereas several other studies did not find c-*erb*B-2 to be an independent prognostic variable.[53,86-94] Because of the multitude of IHCS assays and scoring systems used, and the rarity of cleanly designed truly prospective studies in the appropriate populations (node-negative untreated patients), there is insufficient evidence to endorse IHCS-based tests of c-*erb*B-2 overexpression for the prognostic assessment of patients.

The results of c-*erb*B-2 gene amplification as a prognostic factor are more consistent, with c-*erb*B-2 gene amplification often associated with poorer outcome in node-negative patients,[95-103] although this association was not always seen.[104-106] One prospective study concerned node-negative patients who did not receive adjuvant therapy. The results of this study were used to gain FDA approval for the Ventana (Tucson, AZ) c-*erb*B-2 FISH analysis kit.[99] In this study, c-*erb*B-2 amplification was a strong predictor of poor outcome in node-negative patients (conferred a relative risk of approximately 3) with T1a and T1b tumors. If this study can be replicated, Ventana FISH analysis and possibly other tests for c-*erb*B-2 amplification may permit selection of patients with stage I breast cancer who are at particular risk for disease recurrence if not treated with adjuvant therapy.

11. 2000 Recommendation: The data are insufficient to recommend the routine use of c-*erb*B-2 overexpression in patients with early breast cancer to identify patients with a higher risk of relapse.

*Utility of Measures of Circulating Extracellular Domain of c-*erb*B-2*

2000 Update: One might monitor circulating extracellular domain (ECD)/c-*erb*B-2 either to determine prognosis or to predict response to treatment, as described above for tissue evaluation of c-*erb*B-2. This serum marker might also be used to monitor a patient's clinical course in a manner similar to that discussed for CA15-3 and/or CEA above. Data to support either of these uses are level of evidence III at best. One study suggests that rising ECD/c-*erb*B-2 levels in patients who are known to have had previously c-*erb*B-2–positive tumors and who are free of detectable disease are strongly indicative of an impending clinically detectable relapse.[107] The ECD/c-*erb*B-2 detected relapse in patients who had normal levels of CA15-3 and CEA. However, as is the case for the other markers, the clinical utility of this knowledge is unclear, since it does not permit a decision that clearly improves outcomes for the patient.[23] Preoperative ECD/c-*erb*B-2 levels may be prognostic, but they are associated with tumor burden as well as c-*erb*B-2 expression and therefore are not usually independent prognostic factors.[108]

In the metastatic setting, ECD/c-*erb*B-2 levels may mirror the c-*erb*B-2 status at the tissue level. Moreover, investigators have suggested that circulating ECD/c-*erb*B-2 may predict resistance to hormone therapy or sensitivity and/or resistance to chemotherapy, in a manner similar to the way tissue c-*erb*B-2 does, as discussed above.[76,107-117] No data have been published regarding circulating ECD/c-*erb*B-2 and response to trastuzumab, although serial levels have been reported to reflect response to trastuzumab and cisplatin.[46] Other studies of serial circulating ECD/c-*erb*B-2 to monitor more standard therapies have been inconsistent.[118] In summary, the results are not sufficiently consistent or well validated to use ECD/c-*erb*B-2 to make clinical decisions.

12. 2000 Recommendation: Measuring circulating extracellular domain of c-*erb*B-2 is not currently recommended for any clinical setting.

Areas for Future Research: First, the use of c-*erb*B-2 testing to select patients for trastuzumab needs to be further clarified, with response rates expected for patients with all levels of overexpression and gene amplification being defined.

Second, the use of c-*erb*B-2 testing for level of expression to select patients for specific adjuvant chemotherapy types needs to be improved by further studies and by publication of the information about the impact of gene amplification.

Third, studies evaluating the breadth of utility of FDA-approved c-*erb*B-2 methodologies and their interchangeability would be of value.

p53 *as a Marker for Breast Cancer*

13. 1997 Recommendation: Present data are insufficient to recommend use of *p53* measurements for management of patients with breast cancer.

2000 Update: None.

2000 Recommendation: No change.

Cathepsin-D as a Marker for Breast Cancer

14. 1997 Recommendation: Present data are insufficient to recommend use of cathepsin-D measurements for management of patients with breast cancer.

2000 Update: None.

2000 Recommendation: No change.

Future Directions

Several small retrospective studies have suggested that microvascular density is associated with a poor prognosis. However, present data are insufficient to recommend evaluation of markers for angiogenesis treatment decisions for patients with primary or metastatic breast cancer.

APPENDIX
Summary of Recommendations

Colorectal Cancer Guidelines

Carcinoembryonic Antigen as a Marker for Colorectal Cancer

1a. 1997 Recommendation: Carcinoembryonic antigen (CEA) is not recommended to be used as a screening test for colorectal cancer.
2000 Recommendation: No change.

1b. 1997 Recommendation: CEA may be ordered preoperatively in patients with colorectal carcinoma if it would assist in staging and surgical treatment planning. Although elevated preoperative CEA (> 5 ng/mL) may correlate with poorer prognosis, data are insufficient to support the use of CEA to determine whether to treat a patient with adjuvant therapy.
2000 Recommendation: No change.

1c. 1997 Recommendation: If resection of liver metastases would be clinically indicated, it is recommended that postoperative serum CEA testing may be performed every 2 to 3 months in patients with stage II or III disease for 2 or more years after diagnosis. An elevated CEA, if confirmed by retesting, warrants further evaluation for metastatic disease but does not justify the institution of adjuvant therapy or systemic therapy for presumed metastatic disease.
2000 Recommendation: No change.

1d. 1997 Recommendation: Present data are insufficient to recommend routine use of the serum CEA alone for monitoring response to treatment. If no other simple test is available to indicate a response, CEA should be measured at the start of treatment for metastatic disease and every 2 to 3 months during active treatment. Two values above baseline are adequate to document progressive disease even in the absence of corroborating radiographs. CEA is regarded as the marker of choice for monitoring colorectal cancer.
2000 Recommendation: No change.

Lipid-Associated Sialic Acid as a Marker for Colorectal Cancer

2. 1997 Recommendation: Present data are insufficient to recommend lipid-associated sialic acid (LASA) for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.
2000 Recommendation: No change.

CA 19–9 as a Marker for Colon Cancer

3. 1997 Recommendation: Present data are insufficient to recommend CA 19–9 for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.
2000 Recommendation: No change.

DNA Ploidy or Flow Cytometric Proliferation Analysis as a Marker for Colon Cancer

4. 1997 Recommendation: Present data are insufficient to recommend DNA flow cytometrically derived ploidy (DNA index) for the management of colorectal cancer.
2000 Recommendation: No change.

p53 as a Marker for Colorectal Cancer

5. 1997 Recommendation: Present data are insufficient to recommend the use of *p53* expression or mutation for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.
2000 Recommendation: No change.

ras as a Marker for Colorectal Cancer

6. 1997 Recommendation: Present data are insufficient to recommend the use of the *ras* oncogene for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.
2000 Recommendation: No change.

Breast Cancer Guidelines

CA 15–3 as a Marker for Breast Cancer

1. 1997 Recommendation: Present data are insufficient to recommend CA 15–3 or CA 27.29 for screening, diagnosis, staging, or surveillance after primary treatment. Although a rising CA 15–3 or CA 27.29 level can detect recurrence after primary treatment, the clinical benefit is

not established; therefore, it cannot be recommended. One well-designed study has shown that an increase in CA 27.29 can predict recurrence an average of 5.3 months before other symptoms or tests.[19] Options for therapy, however, remain unchanged, and there has been no demonstrated impact on the most significant outcomes (improved disease-free or overall survival, better quality of life, lesser toxicity, or improved cost-effectiveness).[20] The data used by the Food and Drug Administration (FDA) to approve CA 27.29 were available to the panel previously; although the assay was approved by the FDA, the FDA does not require tests to show clinical benefit. Given the small body of evidence and until there is evidence of clinical benefit, present data are insufficient to recommend routine use of CA 27.29.

2000 Recommendation: No change.

CEA as a Marker for Breast Cancer

2a. 1997 Recommendation: CEA is not recommended for screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy.

2000 Recommendation: No change.

2b. 1997 Recommendation: Routine use of CEA for monitoring response of metastatic disease to treatment is not recommended. However, in the absence of readily measurable disease, a rising CEA may be used to suggest treatment failure.

2000 Recommendation: No change.

Estrogen Receptors and Progesterone Receptors as Markers for Breast Cancer

3. 1997 Recommendation: Estrogen and progesterone receptors are recommended to be measured on every primary 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 may be used to identify patients most likely to benefit from endocrine forms of adjuvant therapy and therapy for recurrent or metastatic disease.

2000 Recommendation: No change.

DNA Flow Cytometrically Derived Parameters as Markers for Breast Cancer

4a. 1997 Recommendation: Present data are insufficient to recommend obtaining DNA flow cytometry–derived estimates of DNA content or S phase in breast tissue.

2000 Recommendation: No change.

4b. 1997 Recommendation: DNA flow cytometry–derived ploidy are not recommended to be used to assign a patient to prognostic groupings. There is insufficient evidence to recommend the use of S phase determination for assigning patients to prognostic groupings.

2000 Recommendation: No change.

c-*erb*B-2 (*HER-2*/*neu*) as a Marker for Breast Cancer

5a. 1997 Recommendation: Present data are insufficient to recommend the use of c-*erb*B-2 (*HER-2*/*neu*) gene amplification or overexpression for management of patients with breast cancer.

2000 Recommendation: c-*erb*B-2 overexpression should be evaluated on every primary breast cancer either at the time of diagnosis or at the time of recurrence. Measures of c-*erb*B-2 amplification may also be of value.

Methods for Measuring c-*erb*B-2

5b. 2000 Recommendation: Because of the uncertain interchangeability, reproducibility, and clinical utility of different c-*erb*B-2 tests, it is important that clinical laboratories report not only an estimate c-*erb*B-2 but also a statement about the test's quality controls, the method, the specific kit or critical reagents, details of the scoring system, a statement regarding reproducibility, sensitivity, and specificity of the assay, and a reference to the clinical validation of the assay or its correlation with a clinically validated c-*erb*B-2 test.

Sensitivity to Trastuzumab

6. 2000 Recommendation: High levels of c-*erb*B-2 expression or c-*erb*B-2 amplification can be used to identify patients for whom trastuzumab may be of benefit for the treatment of metastatic, recurrent, and/or treatment-refractory unresectable locally advanced breast cancer.

Response to Cyclophosphamide/Methotrexate/Fluorouracil or Nonanthracycline-Based Adjuvant Chemotherapy

7. 2000 Recommendation: The question of whether c-*erb*B-2 overexpression affects the relative benefit of adjuvant cyclophosphamide methotrexate, and fluorouracil chemotherapy remains open, and the update committee cannot make a definitive practice recommendation at present.

Response to Anthracycline-Based Adjuvant Chemotherapy

8. 2000 Recommendation: High levels of c-*erb*B-2 expression, as determined by immunohistochemistry, may identify patients who particularly benefit from anthracycline-based adjuvant therapy, but levels of c-*erb*B-2 expression should not be used to exclude patients from anthracycline treatment.

Sensitivity to Endocrine Therapy

9. 2000 Recommendation: The use of c-*erb*B-2 data to decide whether to prescribe endocrine therapy either in the adjuvant or metastatic setting is not recommended.

Sensitivity or Resistance to Taxane Therapy

10. 2000 Recommendation: The use of c-*erb*B-2 data to decide whether to prescribe taxane-based chemotherapy either in the adjuvant or metastatic setting is not recommended.

Use of Measures of c-*erb*B-2 to Predict Patient Prognosis

11. 2000 Recommendation: The data are insufficient to recommend the routine use of c-*erb*B-2 overexpression in patients with early breast cancer to identify patients with a higher risk of relapse.

Utility of Measures of Circulating Extracellular Domain of c-*erb*B-2

12. 2000 Recommendation: Measuring circulating extracellular domain of c-*erb*B-2 is not currently recommended for any clinical setting.

p53 as a Marker for Breast Cancer

13. 1997 Recommendation: Present data are insufficient to recommend use of *p53* measurements for management of patients with breast cancer.
2000 Recommendation: No change.

Cathepsin-D as a Marker for Breast Cancer

14. 1997 Recommendation: Present data are insufficient to recommend use of cathepsin-D measurements for management of patients with breast cancer.
2000 Recommendation: No change.

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