

## ASCO 2006 Update of Recommendations for the Use of Tumor Markers in Gastrointestinal Cancer

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### A B S T R A C T

#### Purpose

To update the recommendations for the use of tumor marker tests in the prevention, screening, treatment, and surveillance of gastrointestinal cancers.

#### Methods

For the 2006 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.

#### Recommendations and Conclusion

For colorectal cancer, it is recommended that carcinoembryonic antigen (CEA) be ordered preoperatively, if it would assist in staging and surgical planning. Postoperative CEA levels should be performed every 3 months for stage II and III disease for at least 3 years if the patient is a potential candidate for surgery or chemotherapy of metastatic disease. CEA is the marker of choice for monitoring the response of metastatic disease to systemic therapy. Data are insufficient to recommend the routine use of p53, *ras*, thymidine synthase, dihydropyrimidine dehydrogenase, thymidine phosphorylase, microsatellite instability, 18q loss of heterozygosity, or deleted in colon cancer (DCC) protein in the management of patients with colorectal cancer. For pancreatic cancer, CA 19-9 can be measured every 1 to 3 months for patients with locally advanced or metastatic disease receiving active therapy. Elevations in serial CA 19-9 determinations suggest progressive disease but confirmation with other studies should be sought. New markers and new evidence to support the use of the currently reviewed markers will be evaluated in future updates of these guidelines.

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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 colorectal 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 2006 update, the Panel expanded the scope of the guideline to include a broader range of markers in colorectal cancer and, new to this guideline, pancreatic cancer markers (see Table 1).

### UPDATE METHODOLOGY

For the 2006 update, an Update Committee composed of members from the full Panel was formed to

complete the review and analysis of data published since 1999 (see Appendix). Computerized literature searches of Medline (National Institutes of Health, Bethesda, MD) and the Cochrane Collaboration Library (Oxford, United Kingdom) were performed. The searches of the English-language literature from 1999 to November 2005 (or from 1966 to November 2005 for the new markers) matched each of the markers with the corresponding disease site. 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. By and large, however, the literature is characterized by studies that included small patient numbers, studies that were retrospective, and studies that commonly performed multiple analyses until one revealed a statistically significant result ( $P < .05$ ). In

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**Table 1.** Summary of Guideline Recommendations

Specific Markers	2006 Recommendations for the Use of Tumor Markers in Gastrointestinal Cancer
1. CEA as a marker for colorectal cancer	<p>1a. Screening: CEA is not recommended as a screening test for colorectal cancer.</p> <p>1b. Staging/Treatment Planning: CEA may be ordered preoperatively in patients with colorectal carcinoma if it would assist in staging and surgical treatment planning. Although elevated preoperative CEA (&gt; 5 mg/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.</p> <p>1c. Postoperative: Postoperative serum CEA testing should be performed every 3 mo in patients with stage II or III disease for at least 3 yr after diagnosis, if the patient is a candidate for surgery or systemic therapy. An elevated CEA, if confirmed by retesting, warrants further evaluation for metastatic disease, but by itself does not justify systemic therapy for presumed metastatic disease.<sup>1</sup> Because chemotherapy may falsely elevate CEA levels, waiting until chemotherapy is finished to initiate surveillance is advised.</p> <p>1d. Monitoring Response to Therapy: CEA is the marker of choice for monitoring metastatic colorectal cancer during systemic therapy. CEA should be measured at the start of treatment for metastatic disease and every 1-3 mo during active treatment. Persistently rising values above baseline should prompt restaging but suggest progressive disease even in the absence of corroborating radiographs. Caution should be used when interpreting a rising CEA level during the first 4-6 wk of a new therapy, since spurious early rises may occur especially after oxaliplatin.<sup>2,3</sup></p>
2. CA 19-9 as a marker for colon cancer	2. Present data are insufficient to recommend CA 19-9 for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.
3. DNA ploidy or flow cytometric proliferation analysis as a marker for colon cancer	3. Neither flow cytometrically derived DNA ploidy (DNA index) nor DNA flow cytometric proliferation analysis (% S phase) should be used to determine prognosis of early-stage colorectal cancer.
4. p53 as a marker for colorectal cancer	4. 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.
5. <i>ras</i> as a marker for colorectal cancer	5. Present data are insufficient to recommend the use of the <i>ras</i> oncogene for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.
6. TS, DPD, and TP as markers in colorectal cancer (Note: This topic is new to the guideline.)	<p>6a. Screening: TS, DPD, and TP are tissue markers that have been used to predict response to treatment of established carcinomas and thus are not useful for screening.</p> <p>6b. Prognosis: None of the three markers—TS, DPD, or TP—are recommended for use to determine the prognosis of colorectal carcinoma.</p> <p>6c. Predicting Response to Therapy: There is insufficient evidence to recommend use of TS, DPD, or TP as predictors of response to therapy.</p> <p>6d. Monitoring Response to Therapy: There is insufficient evidence to recommend use of TS, DPD, or TP for monitoring response to therapy.</p>
7. MSI/hMSH2 or hMLH1 as markers in colorectal cancer (Note: This topic is new to the guideline.)	7. MSI ascertained by PCR is not recommended at this time to determine the prognosis of operable colorectal cancer nor to predict the effectiveness of FU adjuvant chemotherapy.
8. 18q <sup>-</sup> /DCC as markers for colorectal cancer (Note: This topic is new to the guideline.)	8. Assaying for LOH on the long arm of chromosome 18 (18q) or DCC protein determination by immunohistochemistry should not be used to determine the prognosis of operable colorectal cancer, nor to predict response to therapy.
9. CA 19-9 as a marker for pancreatic cancer (Note: This topic is new to the guideline.)	<p>9a. Screening: CA 19-9 is not recommended for use as a screening test for pancreatic cancer.</p> <p>9b. Operability: The use of CA 19-9 testing alone is not recommended for use in determining operability or the results of operability in pancreatic cancer.</p> <p>9c. Evidence of Recurrence: CA 19-9 determinations by themselves cannot provide definitive evidence of disease recurrence without seeking confirmation with imaging studies for clinical findings and/or biopsy.</p> <p>9d. Monitoring Response to Therapy: Present data are insufficient to recommend the routine use of serum CA 19-9 rules alone for monitoring response to treatment. However, CA 19-9 can be measured at the start of treatment for locally advanced metastatic disease and every 1-3 mo during active treatment. If there is an elevation in serial CA 19-9 determinations, this may be an indication of progressive disease and confirmation with other studies should be sought.</p>

Abbreviations: CEA, carcinoembryonic antigen; mo, months; yr, years; wk, weeks; TS, thymidine synthase; DPD, dihydropyrimidine dehydrogenase; TP, thymidine phosphorylase; MSI, microsatellite instability; PCR, polymerase chain reaction; FU, fluorouracil; LOH, loss of heterozygosity.

the scale developed by Hayes et al<sup>4</sup> for grading the clinical utility of tumor markers, these studies are designated as Level of Evidence III. The Level of Evidence in the Hayes et al system defines the quality of the data on a given marker. The Update Committee underscores here that the preferred way to assess tumor markers is within Level of Evidence II studies (prospective therapeutic trials in which marker util-

ity is a secondary study objective), or, ideally, within Level of Evidence I studies (single, high-powered, prospective, randomized controlled trials specifically designed to test the marker or a 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, per ASCO guideline policy. 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

### 1. Carcinoembryonic Antigen As a Marker for Colorectal Cancer

**2006 recommendation for carcinoembryonic antigen as a screening test.** Carcinoembryonic antigen (CEA) is not recommended for use as a screening test for colorectal cancer.

**Literature update and discussion.** The specificity of CEA for identifying occult colorectal cancers is high but the sensitivity is very low across studies.<sup>5,6</sup> Accordingly, CEA should not be used for mass screening. This recommendation is in accordance with the 2003 recommendation for CEA by the European Group on Tumor Markers (EGTM).<sup>7</sup>

**2006 recommendation for preoperative CEA testing.** 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 mg/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.

**Literature update and discussion.** Studies published since the last update lend further support to the utility of preoperative CEA levels as prognostic factors.<sup>8-10</sup> Specifically, (1) a study of 2,230 patients demonstrated that preoperative CEA was an important independent prognostic variable in predicting outcome<sup>11</sup>; and (2) a study of 1,146 rectal patients using a multivariate analysis confirmed that preoperative CEA level was still a highly significant prognostic covariate even after stage and grade were included in the model.<sup>12</sup> These data, along with the data reviewed for the 2000 update, led the ASCO Tumor Marker Panel, as well as the EGTM,<sup>7</sup> to recommend that CEA should be used preoperatively to provide prognostic information. Furthermore, determination of CEA before resection aids in assessing its utility for postoperative surveillance. An elevated preoperative CEA suggests that the marker would be useful for surveillance. It is important to emphasize that measured levels of CEA may differ between laboratories and countries.

Preoperative CEA in patients undergoing resection of metastatic disease to the liver affects prognosis, and measurement in this circumstance is also indicated. Two very large case series<sup>13,14</sup> found preoperative CEA to be an important determinant of prognosis. Two smaller studies<sup>15,16</sup> corroborate the role of CEA in determining prognosis. For example, CEA of less than 30 ng/mL was associated with a median survival of 34.8 months whereas median survival was 22 months if CEA was more than 30 ng/mL.<sup>15</sup> CEA levels may also predict prognosis following cryosurgery for liver metastases.<sup>1,15</sup> Similarly, preoperative CEA may provide prognostic information for patients undergoing resection of pulmonary metastases.<sup>1</sup>

**2006 recommendation for postoperative CEA testing.** Postoperative serum CEA testing should be performed every 3 months in patients with stage II or III disease for at least 3 years after diagnosis if the patient is a candidate for surgery or systemic therapy. 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.<sup>1</sup> CEA elevations within a week or two following chemotherapy should be interpreted with caution.<sup>2</sup>

**Literature review and discussion.** In previous guidelines, the rationale for monitoring CEA has depended on the detection and treatment of isolated hepatic metastases. Since the 2000 guideline update, the importance of early detection of recurrent or metastatic disease has been further underscored by studies documenting the impact of systemic therapy on survival. Recent advances in systemic therapy and improved survival for patients with metastatic disease provide an additional rationale for monitoring CEA postoperatively. A recent meta-analysis<sup>17</sup> analyzed 13 randomized prospective trials of systemic chemotherapy in patients with advanced colorectal carcinoma; various chemotherapy regimens were given to asymptomatic patients with known metastatic disease who were compared with patients who received the same regimens when they became symptomatic. Three trials,<sup>18-20</sup> analyzed as part of the meta-analysis, randomized to either systemic chemotherapy administered to asymptomatic patients, or to best supportive care first and then chemotherapy when patients became symptomatic. In the systemic therapy subset of the meta-analysis, there was a significant improvement in rates of survival at 6 months in patients receiving chemotherapy first versus best supportive care. Scheithauer et al<sup>21</sup> also demonstrated a similar 5- to 6-month survival advantage in patients receiving a fluorouracil (FU)-containing regimen rather than just supportive care. Finally, quality of life studies suggest that, although early intervention in asymptomatic patients with advanced disease may have adverse effects, most data suggest that quality of life is improved.<sup>22</sup> Since the regimens for advanced colorectal cancer are more active with more and newer agents,<sup>23,24</sup> and because therapy may be given to older patients safely,<sup>25</sup> intervention is warranted. Thus, the use of CEA to identify recurrent or metastatic disease in asymptomatic patients is valuable because there is evidence to suggest that this enables identification of patients who are candidates for therapy that can prolong survival.

The EGTM<sup>7</sup> stated that CEA testing should be done in patients with Dukes' B and C who may be candidates for liver resection, every 2 to 3 months. CEA is considered a valuable component of postoperative follow-up, is the most frequent indicator of recurrence in asymptomatic patients,<sup>26,27</sup> is more cost-effective than radiology for the detection of potential curable recurrence,<sup>28</sup> and is the most sensitive detector for liver metastases.<sup>28</sup>

The detection of asymptomatic resectable metastatic disease remains another indication for routine measurement of CEA after

treatment of early-stage colorectal cancer. In a multicenter prospective randomized study<sup>29</sup> comparing the efficacy of two forms of chemotherapy in the adjuvant setting, 530 patients had CEA measurements every 3 months during the first year, every 6 months during the second year, and then annually. Computed tomography (CT) scans were done at 12 and 24 months. Relapses were detected by CT in 49 patients, by CEA in 45 patients, and by symptoms in 65 patients. Of the 49 patients whose relapses were detected by CT, 14 patients had a concomitant elevated CEA at relapse. By the time patients became symptomatic from their recurrent disease, only a small proportion of patients (3.1%) could undergo curative resection. In contrast, among asymptomatic patients in whom recurrence was detected by CEA or CT, resection could be performed in 17.8% and 26.5% of patients, respectively.<sup>29</sup> The authors concluded that in combination with CT examination, CEA was a valuable component of postoperative follow-up, especially if aggressive resection of metastatic disease could be performed.

Three meta-analyses confirm that such a combined intensive follow-up program results in a reduction in mortality.<sup>17,30,31</sup> One meta-analysis<sup>17</sup> noted more intensive follow-up was associated with a significant reduction in mortality ( $P = .007$ ). CT every 3 to 12 months and CEA every 3 to 6 months demonstrated the greatest reduction in mortality ( $P = .002$ ). Intensive follow-up was associated with earlier detection ( $P \leq .001$ ). A meta-analysis by Figuerado et al<sup>30</sup> concluded that the incidence of asymptomatic recurrence was significantly more common in patients with more intensive follow-up. This meta-analysis suggested that only trials using CEA and liver imaging demonstrated significant impact on overall survival (relative risks [RR], 0.71; 95% CI, 0.60 to 0.85;  $P = .0002$ ). In a meta-analysis, Rosen et al<sup>32</sup> compared outcomes in studies published between 1972 and 1996 (meta-analysis included 2,300 patients). In this analysis, the cumulative 5-year survival rate was 72.1% and 63.7% for the intensive and control groups, respectively ( $P \leq .0001$ ). Economic analyses suggest that intensive follow-up that incorporates CEA testing is cost-effective compared with conventional follow-up.<sup>33</sup> Recent ASCO guidelines have recommended that, in addition to CEA every 3 months, annual CT of the chest and abdomen should be performed for 3 years after primary therapy for patients who are at high risk of recurrence and who could be candidates for curative-intent surgery. Pelvic CT should be performed on the same schedule for rectal cancer surveillance, especially for patients with several poor prognostic factors, including those who have not been treated with radiation.<sup>4,34,35</sup>

Although surgery for isolated metastases and systemic therapy of patients with asymptomatic metastatic disease may improve the survival rate and therefore justify the use of CEA as a component of intensive monitoring, some controversy still surrounds the value of CEA-directed surgery. Lennon et al<sup>36</sup> reported on the Northover<sup>37</sup> study, which has been directly reported only in abstract form to date. In this study, patients were randomly assigned to symptom- or CEA-directed follow-up. Exploratory surgery was performed in patients with elevated CEA levels. Notably, the threshold for CEA elevation was defined as two measurements greater than 20 ng/mL, which is considerably higher than the upper limit of normal—5 ng/mL in most laboratories in the United States. Furthermore, the study was done before the widespread availability of sensitive imaging modalities. Survival at 5 years for the CEA monitoring group was 20.4% and was 22% for the control group. The trial was closed early with the recommendation that there was no survival advantage for second-look sur-

gery based on these relatively stringent criteria for CEA elevation. However, because the focus of this study was the value of second-look surgery, it did not definitively address the question of CEA monitoring for early detection of metastases.

**2006 recommendation for CEA testing to monitor metastatic colorectal cancer.** CEA is the marker of choice for monitoring metastatic colorectal cancer during systemic therapy. CEA should be measured at the start of treatment for metastatic disease and every 1 to 3 months during active treatment. Persistently rising values above baseline should prompt restaging, but suggest progressive disease even in the absence of corroborating radiographs. Caution should be used when interpreting a rising CEA level during the first 4 to 6 weeks of a new therapy, since spurious early rises may occur especially after oxaliplatin use.<sup>2,3</sup>

**Literature update and discussion.** In general, rising CEA during treatment indicates disease progression. Rising CEA should prompt re-evaluation and consideration of an alternative treatment strategy. There are some exceptions. Chemotherapy may transiently elevate CEA; a rising CEA by itself should not be considered evidence of disease progression,<sup>2,38</sup> particularly immediately after starting chemotherapy.<sup>2</sup> Chemotherapy-associated CEA increases may be related to treatment-induced changes in liver function.<sup>38</sup> Other non-cancer-related causes of elevated CEA include gastritis, peptic ulcer disease, diverticulitis, liver diseases, chronic obstructive pulmonary disease, diabetes, and any acute or chronic inflammatory state.<sup>39</sup>

## 2. CA 19-9 As a Marker for Colon Cancer

**2006 recommendation for use of CA 19-9 in colon cancer.** Present data are insufficient to recommend CA 19-9 for screening, diagnosis, staging, surveillance, or monitoring treatment of patients with colorectal cancer.

**Literature update: CA 19-9.** No support was identified in a review of the literature published since 1999 for CA 19-9 having a role in the management of colorectal cancer.

## 3. DNA Ploidy or Flow Cytometric Proliferation Analysis As a Marker for Colon Cancer

**2006 recommendation for DNA ploidy or DNA flow cytometric proliferation analysis to determine prognosis.** Neither flow-cytometrically derived DNA ploidy (DNA index) nor DNA flow cytometric proliferation analysis (% S phase) should be used to determine prognosis of early-stage colorectal cancer.

**Literature update and discussion: DNA Ploidy.** Fifteen articles (encompassing 14 independent series) that evaluated the prognostic role of DNA ploidy or index determined by flow cytometry in large colorectal adenocarcinomas were reviewed.<sup>40-53</sup> An additional study, inadvertently not considered in the previous analysis, is also included in this review.<sup>54</sup> Two recently published articles<sup>55,56</sup> that overlap with one previously reviewed<sup>57</sup> are not included. Studies using other techniques to assess DNA ploidy, such as image analysis, were not evaluated.

Nine of the 14 series included patients with both colon and rectal cancer; three studies addressed DNA ploidy only in colon cancer,<sup>40,52,54</sup> two considered only rectal cancer.<sup>42,47</sup> Six of the studies were performed on paraffin blocks<sup>40,42,44,47,50,51</sup> and the other eight were of fresh/frozen material. Two studies were of all (consecutive) patients from a defined time period<sup>41,48</sup>; two were of selected patients enrolled in randomized trials of adjuvant therapy.<sup>40,45</sup> The remainder were of selected cases from a given time period.

Of the 14 series, eight found that patients with a DNA aneuploid tumor or an elevated DNA index had a statistically significantly ( $P < .05$ ) worse survival after surgery than those patients with DNA diploid or low DNA index tumors.<sup>40-42,44,46,48,50,54</sup> These parameters had no significant effect on prognosis in the other six reports. In two of the eight positive studies, DNA ploidy or index remained prognostic in a multivariate analysis.<sup>41,50</sup> In five others, it was not a statistically significant independent predictor of survival<sup>42,44,46,48,54</sup>; in one, a multivariate analysis was not reported.<sup>40</sup>

*Literature update and discussion: Proliferation analysis (% S phase).* Ten series evaluating DNA flow cytometric proliferation analysis (% S phase) after surgical therapy of colorectal cancer were reviewed for the update<sup>41,42,44,46,50-52,58-60</sup>; an eleventh series did not contain statistical analysis by S phase alone and was not included.<sup>61</sup> One study was of patients with colon cancer,<sup>52</sup> one of patients with rectal cancer,<sup>42</sup> and the others of individuals with either colon or rectal adenocarcinomas. Two series were of selected specimens from randomized therapeutic trials.<sup>59,60</sup> One report was of consecutively treated patients,<sup>41</sup> and the remaining seven reports were of selected patients from a designated time period.

Five of the 10 series concluded that % S phase was a statistically significant predictor of survival for colorectal cancer patients in a univariate analysis<sup>41,42,50,51,60</sup>; five did not.<sup>44,46,52,58,59</sup> All five positive studies also found % S phase to be independently prognostic for survival in a multivariate analysis.<sup>44,46,52,58,59</sup>

The inconsistent results of the reviewed studies do not support the use of flow cytometrically derived DNA ploidy or proliferation analysis to determine prognosis of operable colorectal cancer.

*Areas for future research.* More than 50 series looking at the prognostic value of DNA ploidy and 20 series looking at DNA proliferation analysis (% S phase) in colorectal cancer have been reviewed in the last 10 years in the formulation of the ASCO Tumor Marker Guidelines.<sup>39,62</sup> The value of these DNA flow cytometrically derived parameters has still not been established. There may be more promising variants of DNA ploidy and proliferation analysis, such as correlating response to neoadjuvant chemoradiotherapy with serial determinations of tumor ploidy or S phase in patients with rectal cancer.<sup>47,63</sup> Nevertheless, for now, flow cytometric determination of DNA ploidy or proliferation should, at best, be considered an experimental tool.

#### 4. p53 As a Marker for Colorectal Cancer

*2006 recommendations for p53 testing.* 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.

*Literature update and discussion.* Loss of p53 function through inactivating mutation or deletion of the two alleles of the gene is one of the most common molecular events involved in tumorigenesis. As a consequence, p53 abnormalities have been studied extensively for more than two decades, including translational research into their role in prognosis and response to therapy in colorectal cancer. The results of the reported studies<sup>64-68</sup> are heterogeneous and often conflicting, in part because p53 abnormalities are usually detected through various methodologies that do not directly address the functional status of the two alleles of the gene.

Munro, Lain, and Lane<sup>67</sup> conducted a comprehensive systematic review of data on p53 gene abnormalities in patients with colorectal cancer. They identified 168 reports in the literature that included 241

comparisons of relevant end points and survival data in a total of 18,766 patients. Data were available for the impact on response to chemotherapy in 1,514 patients, and for the relationship of abnormal p53 to the development of metastatic disease in 1,066 patients. They used funnel plots to illustrate the compensatory trim-and-fill approach to publication bias and found clear evidence of such bias that exaggerated estimates of the adverse effect of p53 on survival by a maximum of 0.20 for RR and of 10% for differences in absolute rate. They highlighted the variability in methodologies used for assessment of p53 status and for evaluation of clinical outcome. They reported subsets of papers that used similar methods and addressed clinical areas in which p53 could serve as a useful prognostic or predictive marker, including survival with advanced disease, survival after curative resection, development of metastatic disease in patients with apparently localized disease, response to therapy in patients with advanced disease, survival after curative resection and postoperative adjuvant chemotherapy, and response to radiotherapy with and without chemotherapy in patients with rectal cancer. The authors validated their data-pooling approach by comparing their extracted RR with the RR or hazard ratios reported in the articles they reviewed, and they found a strong correlation with Spearman's rank correlation coefficient 0.70 ( $P < .0001$ ), and  $y$ -axis intercept of their regression line of 0.01.

Review of studies of p53 as a potential prognostic marker showed that abnormal immunohistochemical expression of the p53 gene product and mutation of the p53 gene were each associated with increased risk of death (RR, 1.32; 95% CI, 1.23 to 1.42;  $P < .0001$ ; and RR, 1.31; 95% CI, 1.19 to 1.45;  $P < .0001$ , respectively). The adverse impact of mutated p53 was greater in patients with a good prognosis, as defined by expected baseline median survival rate of more than 65%, with RR 1.63 (95% CI, 1.40 to 1.90) versus RR 1.04 (95% CI, 0.91 to 1.19) in patients with poor baseline prognosis. For every 10% increase in baseline risk, the absolute rate difference associated with abnormal p53 decreased by 6% (95% CI, 4% to 8%;  $P < .0001$ ). Mutation was found to increase the risk of development of metastatic disease (RR, 1.67; 95% CI, 1.21 to 2.30;  $P < .002$ ), but immunohistochemistry had no effect (RR, 0.92; 95% CI, 0.61 to 1.39). Although p53 abnormalities were found more commonly in rectal cancers than colonic cancers, the adverse effects of p53 mutation were of similar magnitude for tumors in the two different primary locations.

Review of studies of p53 as a potential predictive marker showed that p53 mutation was associated with failure of response to radiotherapy or chemoradiotherapy in patients with rectal cancer (RR, 1.49; 95% CI, 1.25 to 1.77;  $P < .0001$ ), but immunohistochemistry was not predictive. There was no effect of abnormal p53 on outcome in patients treated with FU-based chemotherapy.

These authors emphasized the difference between biologically interesting observations and clinically useful tests. The calculated positive predictive values from their analyses were about 0.5, equivalent to a coin flip. The authors concluded that with current methods of assessment, p53 status is a poor guide to both prognosis and response or resistance to therapy in patients with colorectal cancer.

#### 5. ras As a Marker for Colorectal Cancer

*2006 recommendation for ras testing.* 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.

*Literature update and discussion.* *Ras* oncogene mutations in colorectal carcinomas and their precursors were identified early in research efforts directed at molecular pathogenesis. The possible roles of *ras* mutation as prognostic markers for natural history and as predictive markers for response or resistance to therapy have been studied extensively in colorectal cancer.<sup>64-66,68</sup> The results of the studies are heterogeneous and often conflicting.

The majority of reported studies show *ras* mutation is an adverse prognostic indicator, but the studies have wide variability in their specific results.<sup>64,68</sup> For example, some studies have shown that *ras* mutation is prognostic only in some stages of the disease (early or advanced), with particular mutation types (transitions or transversions, specific codons), with specific patterns of recurrence (lymph node or hematogenous), or with combinations of *ras* mutation with other molecular abnormalities (*p53* mutation). For example, a large study combined data from 42 centers in 21 countries for a total of 3,439 patients and used survival as the end point.<sup>69</sup> The results showed highly statistically significant impact on disease-free survival and on overall survival for patients with one mutation type (glycine to valine in codon 12 of *Ki-ras*) and stage III/Dukes' C disease, with hazard ratios of 1.5 and 1.45, respectively. Other *ras* mutation types were not prognostic, and the specific mutation was not prognostic in stage II/Dukes' B disease. In contrast, a substantial minority of reported studies has found no association of *ras* gene mutation with survival.<sup>64,68</sup>

Similar to the uncertain role of *ras* oncogene mutation in prognosis, its utility as a predictive marker is also unclear.<sup>65,66</sup> Interpretation of the literature is complicated by the variety of chemotherapeutic agents and regimens used. For example, studies that support *ras* as a useful predictive marker have evaluated patients treated with FU,<sup>70</sup> irinotecan/CPT-11 after FU,<sup>71</sup> and marimastat.<sup>72</sup>

## 6. Thymidine Synthase, Dihydropyrimidine Dehydrogenase, and Thymidine Phosphorylase As Markers in Colorectal Cancer

Note: These topics are new to the guideline.

*6a. 2006 recommendation for thymidine synthase, dihydropyrimidine dehydrogenase, and thymidine phosphorylase as screening tests.* thymidine synthase (TS), dihydropyrimidine dehydrogenase (DPD), and thymidine phosphorylase (TP) are tissue markers that have been used to predict response to treatment of established carcinomas and thus are not useful for screening.

*6b. 2006 recommendation for use of TS, DPD, or TP for prognosis.* None of the three markers—TS, DPD, or TP—are recommended for use to determine the prognosis of colorectal carcinoma.

*6c. 2006 recommendation for use of TS, DPD, or TP in predicting response to therapy.* There is insufficient evidence to recommend use of TS, DPD, or TP as predictors of response to therapy.

*6d. 2006 recommendation for use of TS, DPD, or TP in monitoring response to therapy.* There is insufficient evidence to recommend use of TS, DPD, or TP for monitoring response to therapy.

*TS: Marker definition.* TS is the rate-limiting step in the biosynthesis of thymidine, one of the four nucleotides required for DNA synthesis and cell proliferation. TS is the enzyme that produces de novo 2'-deoxythymidine-5'-monophosphate (dTMP) by methylation of 2'-monodeoxyuridine-5'-monophosphate (dUMP) in the presence of the methyl donor 5,10-methylene-tetrahydrofolate (CH<sub>2</sub>-THF). TS is inhibited by FdUMP, which is formed by thymidine

phosphorylase and thymidine kinase action on FU. FdUMP binds to TS in the presence of CH<sub>2</sub>-THF to prevent the formation of dTMP which ultimately leads to prevention of DNA synthesis.<sup>73,74</sup> Peters et al<sup>75</sup> have provided an excellent review of the interaction between FU and TS, as well as the other major participants in the analysis of FU effects. For instance, DPD converts FU to 5-fluorodihydrouracil, which is subsequently degraded while TP is essential for the conversion of FU to FudR, which is then converted to FdUMP.

*TS: Methodology.* Despite multiple reports, the best way to measure TS is unclear. Most US studies use immunohistochemistry (IHC) with cut-offs that are poorly defined, but revolve around focal versus diffuse expression of the marker. In contrast, European authors often use activity in fresh-frozen samples that provide a continuously distributed variable normalized to protein content. Reverse transcriptase polymerase chain reaction (RT-PCR) has also been used in several studies. However, levels of mRNA may not be surrogates for the activity of the enzyme or provide the most effective predictive marker. Since most studies have used IHC, it may be most appropriate to determine whether IHC can be better standardized and/or if enzyme activity is associated with IHC.

*TS: Literature review and analysis.* A recent meta-analysis of the data concerning expression of TS was performed by Popat et al.<sup>76</sup> The authors examined 32 published studies of TS expression that included reports of expression in primary and/or metastatic colorectal carcinomas assessed by IHC, RT-PCR, or enzyme assays. Twelve studies were considered unassessable. Thirteen assessable studies investigated outcome in advanced colorectal carcinoma, whereas seven investigated outcome in patients with stage I-III colorectal carcinoma. Although the majority of studies were based on IHC, the authors realized that different antibodies, methods of interpretation, and evaluation were performed. Even with that caveat, however, the authors were able to objectively determine that overall survival, but not disease-free survival, was poorer with high TS expression in both the advanced-disease and in the adjuvant-therapy settings. Interestingly, the authors found that there may have been a better inverse association between TS level and overall survival with RT-PCR analysis; but, since there were relatively few studies, the association was not as strong. Furthermore, it appeared that in the adjuvant surgery group, the association between expression of high intratumoral TS and survival was strongest in those patients treated by surgery alone and the association was markedly decreased in patients who received adjuvant therapy. This limits the usefulness of this marker because most patients with stage group II or III colorectal carcinoma receive adjuvant therapy. Finally, the authors still called for more prospective trials of TS expression, inclusion of standardized unbiased methods, and coded interpretation independent of knowledge of clinical outcome. This analysis suggests that TS expression still requires further evaluation as a prognostic or predictive marker in the adjuvant setting, although it may be useful in advanced disease.

Meta-analysis<sup>76</sup> indicates that there is an inverse relationship between the levels of TS protein expression and response to FU-containing regimens when IHC is used. However, controversy persists about the relative expression of TS and its relationship to resistance to FU-containing therapy. Similar concerns also exist for assessment of the relationship between TS expression and the response to adjuvant therapy in stage group II or III colorectal carcinoma. Thus, more research is necessary to determine the threshold for resistance to FU

therapy as measured by either immunohistochemistry or gene expression technology in advanced disease.

Recent studies suggest that polymorphisms in the promoter and the untranslated regions of the gene may be associated with different levels of TS protein in tumor,<sup>77</sup> and may also be associated with prognosis<sup>78-81</sup> and response to FU-based chemotherapy.<sup>78,79,81</sup> Although some of these studies contained more than 100 patients, there are still differences in the techniques used and the methods of evaluation across the studies. It is also not clear whether tumor or normal tissue should be studied.<sup>82</sup> In addition, at least one study suggests that another region of the TS gene may also contain polymorphisms that enhance the function of TS,<sup>83</sup> while polymorphisms may also be associated with toxicity from FU therapy.<sup>84</sup> Clearly, more research is needed to establish the value of these polymorphisms more precisely.

There is limited evidence regarding the utility of sequential values of TS in patients undergoing therapy. Thus, the role of TS in monitoring response to therapy or recurrence of disease is not clear.

**Dihydropyrimidine dehydrogenase (DPD): Marker definition.** DPD is the major enzyme that catabolizes FU. DPD converts FU to fluoro-5,6-dihydrouracil (FUH<sub>2</sub>) in a rate-limiting step, and then FUH<sub>2</sub> is rapidly converted to fluoro-ureidopropionate (FUPA) and subsequently to fluoro-b-alanine (FBAL) by dihydropyrimidinase and b-ureidopropionase, respectively.<sup>85</sup> More than 80% of the catabolism of FU occurs in the liver where the majority of DPD is concentrated.<sup>86</sup>

**DPD: Methodology.** Activity assays may be the most reliable method to measure DPD since immunohistochemistry does not appear to be reliably associated with activity. Evaluation of mRNA levels may be appropriate but requires more research to establish the methodology. Also the levels of DPD in circulating mononuclear cells have been used as a surrogate for the level of DPD within tumors. While this may be an important method to detect patients with germline mutations or polymorphisms that abolish DPD activity, the levels in mononuclear cells may not be related to those in the tumor.<sup>86</sup>

**DPD: Literature review and analysis.** Little empirical evidence supports DPD alone as a strong independent variable as a prognostic marker. DPD is important in predicting toxicity because the absence of DPD in surrogates such as circulating mononuclear cells portends possibly lethal complications in patients who receive standard FU therapy. In contrast, inhibiting the enzyme in patients with normal levels of DPD with either tegafur or eniluracil may be an important method to increase FU efficacy in patients with advanced colorectal carcinoma. To date, the data support the thesis that inhibiting DPD increases FU efficacy, so long as there is some expression of DPD to prevent the neutropenia and other complications that lead to lethal complications. In one adjuvant study, the complexity of tissue levels was confirmed when low tumor expression was associated with low levels of toxicity but was also associated with a poor prognosis.<sup>87</sup> Few data are available on the role of sequential values of DPD in patients undergoing therapy. Therefore, the role of DPD in monitoring response to therapy or recurrence of disease is not clear.

**TP: Marker definition.** TP is the enzyme that essentially activates the fluoropyrimidine by converting FU to FudR. Like TS, TP is an S phase protein that is essential for cell proliferation and passage through S phase because it is important for DNA synthesis.<sup>88</sup> High expression of TP may either prevent<sup>89</sup> or not affect FU activation.<sup>90</sup> However, TP also induces angiogenesis<sup>91,92</sup> and its expression complements that of vascular endothelial growth factor (VEGF) in colo-

rectal carcinoma.<sup>93</sup> The angiogenic function of TP is acknowledged by its other name of platelet-derived endothelial cell growth factor.<sup>92,94</sup>

**TP: Methodology.** Commonly detected by IHC or by enzyme-linked immunosorbent assay (ELISA), TP is often expressed in the stroma, either in infiltrating macrophages or other cells.<sup>95-97</sup>

**TP: Literature review and analysis.** TP expression may be important as a prognostic factor that can be independent of VEGF expression as well as stage and grade,<sup>98-100</sup> although data from well-designed studies are lacking. Other investigators have suggested that the level of immunoreactive TP and/or DPD<sup>95</sup> is not associated with stage, grade, or response to FU-based regimens. The role of TP is a complex one in colorectal carcinoma because it is essential for the activation of FU and its tumor inhibitory function; at the same time, it may enhance colorectal carcinoma survival through its angiogenic activity. Thus, TP may be an important molecule which has distinct and contradictory biologic functions that complicate analysis of its contributions to either prognosis or prediction of response to therapy. The role of TP in monitoring response to therapy is not apparent from present data.

## 7. Microsatellite Instability/hMSH2 or hMLH1 As Markers in Colorectal Cancer

Note: This topic is new to the guideline.

**2006 recommendation for use of microsatellite instability to determine prognosis.** Microsatellite instability (MSI) ascertained by polymerase chain reaction (PCR) is not recommended at this time to determine the prognosis of operable colorectal cancer nor to predict the effectiveness of FU adjuvant chemotherapy.

**MSI: Marker definition.** MSI is a measure of the inability of the DNA nucleotide mismatch repair system to correct errors that commonly occur during the replication of DNA. It is characterized by the accumulation of single nucleotide mutations and length alterations in repetitive microsatellite nucleotide sequences common throughout the genome.<sup>101,102</sup> It is an alternative pathway to chromosomal instability with loss of heterozygosity in the pathogenesis of colon cancer.<sup>102</sup> Initially, MSI was linked to hereditary nonpolyposis colorectal cancer (HNPCC) but it occurs in 8% to 18% of colon cancers in patients without a family history of the disease.<sup>78,103,104</sup> Microsatellite unstable colorectal cancers have distinctive phenotypic features,<sup>104</sup> with a predisposition to occur in the right colon and unusual histopathologic characteristics.<sup>102</sup> MSI has been suggested to be both prognostic for survival and predictive for response to therapy in patients with large bowel cancer.<sup>101,102</sup>

**MSI: Methodology.** In 1997, an international panel of experts met and developed consensus guidelines for the definition of MSI, the criteria for its measurement, and the choice of markers to be measured to facilitate uniformity across studies.<sup>101</sup> A recent National Institutes of Health (NIH; Bethesda, MD) conference suggested revised guidelines for HNPCC and MSI.<sup>105</sup> Microsatellite instability was defined as "a change of any length due to insertion or deletion of repeating (nucleotide) units in a microsatellite within a tumor when compared to normal tissue."<sup>101</sup> The tissue can be fresh-frozen or fixed-paraffin embedded and requires careful microdissection to optimize tumor yield and to obtain normal adjacent tissue for comparison. DNA is most often extracted from microdissected 10-micron-thick fixed-paraffin embedded tissue sections. For tumor tissue, those areas containing more than 70% tumor cells are typically used, and the corresponding normal control DNA is derived from adjacent normal mucosa. The PCR is used to amplify and radioactively or fluorescently

label a region of DNA containing a microsatellite sequence, followed by size-based separation of the PCR product. MSI can be observed by comparing the electrophoretic patterns of amplified DNA from both tumor and normal tissue and is scored as the presence of novel fragments in tumor DNA compared to normal DNA. The loci generally assayed are of normally occurring dinucleotide repeats, but several mononucleotide repeat loci are also widely employed in MSI testing. The original consensus group suggested a reference panel of five specific marker loci (three dinucleotide and two mononucleotide repeats) to be assayed but supplied a large number of alternative sites for testing.<sup>101</sup> It was urged that at least five sites be tested, with microsatellite–highly unstable (MSI-H) defined as  $\geq$  two loci abnormal if five are tested or  $\geq$  30% abnormal if more than five loci are tested. Low level of microsatellite instability (MSI-L) was defined as one of five loci abnormal or  $\leq$  30% abnormal if more than five sites are assayed. Microsatellite stable (MSS) tumors had no areas of abnormal length.<sup>101</sup> In most studies (and in this review), MSI-L and MSS tumors are considered as a single group in comparison with MSI-H tumors, though there is need for further study of the significance of MSI-L,<sup>105</sup> which may represent a biologically and clinically distinct entity.<sup>106,107</sup> Alternatively, investigators have assayed one to three mononucleotide repeat loci as a screen for MSI with some reports showing greater sensitivity and the ability to assay without comparison to normal tissue.<sup>105,108</sup> The recent NIH conference on revising guidelines for HNPCC and MSI advocated the use of mononucleotide loci testing to confirm MSI defined only by dinucleotide loci or the use of a panel of five monomorphic mononucleotide loci (with MSI-H defined as  $\geq$  three loci abnormal), but it did not endorse a more limited (one to three loci) mononucleotide repeat panel.<sup>105</sup>

HNPCC, the syndrome originally associated with MSI, is most often caused by germline mutations in the *hMSH2* or *hMLH1* genes. The protein products of those genes can be detected by IHC.<sup>109,110</sup> They are absent in the tumors of many cases of HNPCC-associated colon cancer,<sup>111</sup> but can also be absent in some sporadic large bowel carcinomas.<sup>112-114</sup> Multiple studies have suggested a high correlation between absent tumor staining for *hMSH2* or *hMLH1* and the presence of MSI-H tumors.<sup>109-114</sup> There are relatively few studies assessing the prognostic significance of absent IHC staining for *hMSH2* or *hMLH1* in early-stage sporadic colorectal cancer.<sup>55,113,115-117</sup> This technique will not be reviewed as a prognostic marker at this time.

*MSI: Literature review and analysis.* This review encompasses the literature on MSI testing as a prognostic test for operable colorectal cancer and as a predictive test for the response of colorectal cancer to chemotherapy. This use of MSI testing for HNPCC is beyond the scope of this review, which is limited to testing in nonfamilial/sporadic cases of colorectal cancer. Included in this analysis are all publications in English that determined MSI in accordance with the NIH consensus guidelines,<sup>101</sup> including at least 100 sporadic colorectal cancer patients (given the low rate of MSI positivity), looked at prognosis or predictive accuracy, and supplied survival data. A systematic review of MSI and colorectal cancer prognosis was recently published.<sup>77</sup> It used different criteria for inclusion in its analysis, such as methodology for determining MSI and definition of microsatellite instability (allowing for the inclusion of MSI-L). Its goal was to quantify the survival hazard ratio of patients with MSI tumors versus those with MSS cancers and was not geared toward making practice recommendations.

*MSI: Prognosis.* Seventeen series that looked at MSI in the prognosis of early-stage colorectal cancer were considered in this

review.<sup>27,40,56,68,77,78,103,109,118-126</sup> Three other series<sup>113,127,128</sup> overlapped two of the reviewed studies<sup>103,119</sup> and were not considered. Series that looked at only one or two mononucleotide repeat loci,<sup>53,129-133</sup> did not separate MSI-L from MSI-H in survival analysis,<sup>134</sup> did not compare MSI-H to MSI-L/MSS,<sup>106,107</sup> or had limited statistical analysis of overall survival data<sup>135,136</sup> were also excluded from the review. Six of the series included were analyses of patients on randomized therapeutic trials.<sup>40,56,68,122,123,125</sup> The other 11 were of selected patients treated in defined time periods. Eleven of the 17 series reviewed found that patients with colon or colorectal cancers that were MSI-high had a significantly better survival than those that were MSI-low or microsatellite-stable.<sup>27,56,68,78,103,109,119-122,126</sup> Two series that found no association of MSI-H with better survival were from randomized trials of adjuvant chemotherapy.<sup>40,125</sup> One of the studies did find a statistically significant association of MSI-H status with better disease-free survival.<sup>125</sup> Six of the 11 positive series also found MSI-H tumors to have a significantly better prognosis in a multivariate analysis.<sup>56,78,119,120,122,126</sup> Four did not find MSI-H to be independently prognostic.<sup>27,68,103,121</sup> Multivariate analysis was not done in one series.<sup>109</sup> Although there is suggestive evidence that MSI-H early-stage colon cancers have a more favorable prognosis than MSI-L or MSS tumors, the data are insufficient to recommend using MSI profile as an independent prognostic test for use in the clinic.

*MSI: Utility of MSI for prediction of fluoropyrimidine efficacy.* Six series that addressed the value of MSI as a predictor for efficacy of FU given as an adjuvant to surgery for early-stage colon or colorectal cancer, and used methodology in keeping with that endorsed by the NIH Consensus Conference<sup>105</sup> are reviewed. Four studies were of patients enrolled onto randomized trials of adjuvant FU in colon cancer<sup>122,125</sup> or colorectal cancer.<sup>40,123</sup> One study was of patients receiving adjuvant FU from a prospective consecutive series of colorectal cancer.<sup>137</sup> One study was a retrospective series of unselected patients with stage II and III colorectal cancer.<sup>138</sup>

Four series evaluated MSI in both FU-treated and control patients; authors asked whether there was any difference in the benefit of chemotherapy versus no chemotherapy by MSI status.<sup>40,122,123,138</sup> Two reports<sup>122,138</sup> found that while intravenous FU was beneficial in improving survival in MSI-L or MSS patients, in MSI-H patients the chemotherapy did not improve survival. In one study,<sup>122</sup> there was actually a trend toward worse survival in MSI-H patients who received FU compared with those who did not.

In contrast, two series found no difference in the benefit of adjuvant portal vein infusion FU<sup>40</sup> or intravenous (IV) mitomycin/FU<sup>123</sup> in patients whose tumors were MSI-H versus those whose tumors were MSI-L or MSS. Two studies<sup>125,137</sup> only evaluated patients who received adjuvant FU. Both found a trend toward better survival (and significantly better disease-free survival) in MSI-H patients who received IV FU compared with non-MSI-H patients receiving the same therapy. This difference was not found in one of the aforementioned IV FU studies when only the chemotherapy patients were evaluated.<sup>122</sup> The contradictory conclusions may be an artifact of differences in the way FU was administered or the inclusion of rectal cancer in three series.<sup>40,137,138</sup> Nevertheless, the data reviewed do not support the use of MSI status in the prediction of benefit from FU chemotherapy as an adjunct to surgery for early-stage colorectal cancer at this time.

*MSI: Future studies.* The preliminary data that MSI status might predict efficacy of adjuvant FU chemotherapy<sup>122,138</sup> suggest incorporation of this marker into the biologic correlative studies to new

adjuvant chemotherapy trials in colorectal cancer to prospectively test this hypothesis.<sup>77</sup> Immunohistochemical determination of hMSH2 and hMLH1 protein in sporadic colorectal cancer is becoming a more widely used technique. It is simpler than determination of MSI by PCR and may correlate with different biologic and prognostic parameters.<sup>55,113,115-117</sup> These markers should be more extensively studied in retrospective and prospective series. If warranted by the results of these studies, they should be considered for inclusion in the correlative marker studies accompanying the large national adjuvant colorectal trials.

### 8. 18q-LOH/DCC As Markers for Colorectal Cancer

Note: This topic is new to the guideline.

*2006 recommendation for use of 18q-LOH/DCC to determine prognosis or to predict response to therapy.* Assaying for loss of heterozygosity (LOH) on the long arm of chromosome 18 (18q) or deleted in colon cancer (DCC) protein determination by IHC should not be used to determine the prognosis of operable colorectal cancer, nor to predict response to therapy.

*18q-LOH/DCC: Marker definition.* The long arm of chromosome 18 contains several genes with potential importance in colorectal cancer pathogenesis and progression. Deletion of portions of 18q has been implicated as an important step in the development of many colorectal cancers.<sup>139</sup> Among the genes located on 18q are the *DCC* gene that codes for a neutrin-1 receptor important in apoptosis, cell adhesion, and tumor suppression; the *SMAD-4* gene, which codes for a nuclear transcription factor in transforming growth factor- $\beta$  1 (TGF $\beta$ 1) signaling involved in tumor suppression; and the *SMAD22* gene involved in endodermal differentiation. For many years, reports have suggested that colorectal cancers with LOH on the long arm of chromosome 18 (18q-) or absent DCC protein<sup>143,144</sup> have a poorer prognosis compared with those without these abnormalities. A recent systematic review of 18q- and DCC assayed by various techniques also suggested prognostic significance but emphasized the need for studies using consistent methodology.<sup>77</sup>

*18q-LOH/DCC: Methodology.* There are several techniques employed to assess 18q/DCC status. The most commonly used assay for LOH at 18q examines polymorphisms at multiple microsatellites on 18q in patients whose normal cells are determined to be heterozygous at those loci ("informative"). The DNA of the microsatellites is amplified by PCR and revealed by electrophoresis. Loss of heterozygosity is detected by comparison of the results in normal cells to that in tumor. Two to 10 microsatellites are assayed to determine the status of 18q.<sup>40,56,125,127,140-142,145-149</sup> Polymorphisms in the area of the *DCC* gene (18q21) are most commonly assayed. Alternatively, DCC protein status in colorectal cancer cells is assessed by an IHC assay using a monoclonal antibody (clone C97-449; Pharmingen, BD Biosciences, San Jose, CA) directed to the DCC protein<sup>143,150,151</sup> or that monoclonal antibody in combination with polyclonal antibodies.<sup>144</sup> There are scattered reports of the use of other monoclonal antibody stains.<sup>152</sup> DCC protein is considered present in the tumor if there is any staining detected.

*18q-LOH/DCC: Literature review and analysis.* This review encompasses the literature on 18q LOH or DCC testing as a prognostic test for operable colorectal cancer. Included in the 18q LOH review were all publications in English that determined LOH by analysis of at least two microsatellites on 18q, had colorectal cancer patients informative at one or more loci, looked at prognostic accuracy, and

supplied survival data. Definition of 18q- varied from loss of heterozygosity at one locus to requiring that all tested loci be positive. Included in the DCC review were all publications in English that determined DCC status by IHC using the clone C97-449 monoclonal antibody, looked at prognostic accuracy, and supplied survival data.

*18q-LOH/DCC: Value of 18q-LOH in prognosis.* Sixteen series that looked at LOH at 18q in the prognosis of early-stage colorectal cancer were considered.<sup>27,40,56,97,125,127,141,142,145,146,148,149,153-156</sup> Two additional series<sup>140,147</sup> that reported subset analyses (with inconsistent results), but did not report on the effect of 18q LOH in the overall population of early-stage patients, were excluded from the review. Three of the series included were of patients on randomized therapeutic trials.<sup>40,56,125</sup> The others were of selected patients treated in defined time periods. Eight of the 16 studies found that patients with colorectal cancers who had loss of heterozygosity at 18q had a significantly worse survival than those who were heterozygous.<sup>27,125,127,142,148,154-156</sup> Four of the eight positive series also found 18q- tumors to have a significantly worse prognosis in a multivariate analysis with hazard ratios for death of 2.0, 2.75, and 7.30,<sup>125,142,148</sup> or recurrence of 9.60.<sup>27</sup> Three reports did not find 18q LOH to be independently prognostic<sup>127,154,156</sup>; one did not do a multivariate analysis.<sup>155</sup> In two studies where 18q- was not found to be prognostic in a univariate<sup>141</sup> or a multivariate analysis,<sup>127</sup> LOH at 18q did have a poor prognosis within stage II disease. Although there is suggestive evidence of an association of 18q loss with the natural history of colorectal cancer, the small number and retrospective nature of studies which found 18q status to be either an independent predictor of survival or of survival within stage II disease, makes it premature to use this marker to determine prognosis.

*18q-LOH/DCC: 18q status and prediction of response to therapy.* Three series looked at whether 18q- status was predictive for the effect of adjuvant FU chemotherapy. One study found greater survival<sup>141</sup> for 18q- patients receiving chemotherapy, a second reported worse survival,<sup>125</sup> and the third reported no difference in survival<sup>40</sup> compared with 18q+ patients receiving chemotherapy. The latter study did find greater benefit of chemotherapy (versus no chemotherapy) for patients whose tumors were 18q+ compared with those with loss of heterozygosity at 18q. Paradoxically, the study found untreated 18q- tumors to have a better prognosis than those that retained heterozygosity.<sup>40</sup> Based on these results there is insufficient information to recommend analysis of loss of heterozygosity at 18q as a predictive test to determine efficacy of FU-based adjuvant therapy in early-stage colorectal cancer.

*18q-LOH/DCC: DCC and prognosis.* Seven studies evaluating loss of DCC protein by IHC in early-stage colorectal<sup>125,144,151,157,158</sup> or rectal<sup>143,150</sup> cancer met the criteria to be considered in this review. Three of the seven found DCC-negative cancers to have a significantly worse survival than those that stain positive for the protein.<sup>143,144,157</sup> Two of the three positive studies found DCC to be independently prognostic in a multivariate analysis.<sup>144,157</sup> One study found that the loss of DCC predicts for lack of efficacy of adjuvant FU-based chemotherapy.<sup>157</sup> There is insufficient information to recommend evaluating DCC by IHC as a prognostic or predictive test in early-stage colorectal cancer.

*18q-LOH/DCC: Future studies.* There are suggestive data that the presence of LOH at 18q, in conjunction with other molecular changes, might predict response to adjuvant chemotherapy for operable colon cancer.<sup>125</sup> It would be appropriate to incorporate determination of LOH at 18q and polymorphisms in the genes located on 18q,

prospectively in the new generation of adjuvant trials in colorectal cancer. The currently accruing Eastern Cooperative Oncology Group 5202 Intergroup study is addressing the utility of LOH at 18q (and MSI) in the selection of patients with stage II colon cancer for adjuvant chemotherapy.

### 9. CA 19-9 As a Marker for Pancreatic Cancer

Note: This topic is new to the guideline.

**2006 recommendation for use of CA 19-9 as a screening test.** CA 19-9 is not recommended for use as a screening test for pancreatic cancer.

**2006 recommendation for use of CA 19-9 to determine operability.** The use of CA 19-9 testing alone is not recommended for use in determining operability or the results of operability in pancreatic cancer.

**2006 recommendation for use of CA 19-9 to provide evidence of recurrence.** CA 19-9 determinations by themselves cannot provide definitive evidence of disease recurrence without seeking confirmation with imaging studies for clinical findings and/or biopsy.

**2006 recommendation for use of CA 19-9 for monitoring response to therapy.** Present data are insufficient to recommend the routine use of serum CA 19-9 rules alone for monitoring response to treatment. However, CA 19-9 can be measured at the start of treatment for locally advanced metastatic disease and every 1 to 3 months during active treatment. If there is an elevation in serial CA 19-9 determinations, this may be an indication of progressive disease, and confirmation with other studies should be sought.

**CA 19-9: Marker definition.** CA 19-9 is a tumor-associated antigen, which was originally defined by a monoclonal antibody that has been produced by a hybridoma prepared from murine spleen cells immunized with a human colorectal cancer cell line.<sup>159</sup>

**CA 19-9: Methodology.** A radioimmunoassay is now available for the quantitation of CA 19-9.<sup>160</sup> CA 19-9 exists in tissue as an epitope of sialylated Lewis A blood group antigen. Those patients who are genotypically Lewis a-b (5% of the population) are unable to make CA 19-9 antigen and CA 19-9 testing should not be attempted in this patient population.<sup>161</sup>

**9a. CA 19-9: Screening.** CA 19-9 is not recommended as a screening test for pancreatic cancer.<sup>162</sup> Its specificity and sensitivity for this purpose are not adequate for accurate diagnosis. CA 19-9 is not specific for pancreatic cancer being elevated in many other tumors of the upper gastrointestinal tract, in ovarian cancer, hepatocellular cancer, and in colorectal cancer, in inflammatory conditions of the hepatobiliary system, and in many benign conditions (eg, thyroid disease).

It may be elevated as well in malignant and benign cases of biliary obstruction. CA 19-9 may not be elevated in small malignant tumors of the pancreas.

**9b. CA 19-9: Preoperative evaluation for resectability.** In the evaluation of patients for surgical intervention, preoperative CA 19-9 levels have been used to predict patient outcomes.<sup>163</sup> Some investigators have found that elevation of CA 19-9 above certain levels have correlated with unresectable disease, or disease that recurs early in the postoperative period. Such preoperative determinations alone have yet to be widely used as a means of establishing inoperability. Postoperatively, some studies have suggested that CA 19-9 do not fall to a normal range. Such studies are to some extent limited by lack of knowledge still of the CA 19-9 half-life. The use of CA 19-9 testing alone is not recommended for use in determining operability or the results of operability in pancreatic cancer.

**9c. CA 19-9: Indicator of asymptomatic recurrence.** Elevating levels of CA 19-9 postoperatively may predict for recurrent disease.<sup>164,165</sup> This may be helpful in the management of patients following attempted definitive surgery in patients who are receiving adjuvant therapy with either or both chemotherapy and radiation therapy, or who are being observed after surgery without adjuvant therapy. However, CA 19-9 determinations by themselves cannot provide definitive evidence of disease recurrence without seeking confirmation by imaging studies and/or biopsy.

**9d. CA 19-9: Monitoring of patients with locally advanced or metastatic disease receiving chemotherapy or radiotherapy.** CA 19-9 measurements have been used to monitor the clinical course of patients receiving cytotoxic chemotherapy, biologic response modifier therapy, or radiotherapy.<sup>166,167</sup> There have been a number of reports involving small numbers of patients showing a correlation between duration of patient survival and a fall in CA 19-9 levels. A fall in CA 19-9 levels has been used to help evaluate the effectiveness of a particular chemotherapy regimen and as a means to determine whether the regimen should be continued. Rising CA 19-9 levels have been taken as an indication to change a chemotherapy regimen and have been correlated with shorter survival times whenever they occur during an initial chemotherapy intervention. There is, however, no agreement about the frequency with which tests should be performed. There is no agreement about what magnitude of change or kinetics of change is likely to be significant and over what period of time such a change should be seen to be maintained for significance.

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### Appendix

For the 2006 update, methodology was used that was similar to that applied in the original ASCO practice guidelines for use of tumor markers. Pertinent information published from 1999 through November 2005 was reviewed for markers that were included in the last update of the guideline; information from 1966 to November 2005 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 corresponding disease site (colon, rectal, or pancreatic cancer). 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 using 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 section. 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).<sup>39</sup>

**Table A1.** Panel Members

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### Authors' Disclosures of Potential Conflicts of Interest

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Authors	Employment	Leadership	Consultant	Stock	Honoraria	Research Funds	Testimony	Other
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