Biomarkers in Colorectal Cancer: Implications for Nursing Practice

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Abstract

Confirming the diagnosis of cancer has largely been the role of the pathologist, through examination and evaluation of tumor tissue. An adjunct to this process is the identification of a tumor marker, which can be found in tumor tissue or released from a tumor into the blood or other body fluids. The blood level of a tumor marker may indicate that a certain type of cancer is in the body. The use of tumor markers is increasing in the care of patients with colorectal cancer (CRC). One of the most commonly used tumor markers to monitor patients with stages II and III CRC for recurrence is carcinoembryonic antigen (CEA); however, the use of CEA has significant limitations. Biomarkers are biologic molecules found in the blood, other body fluids, or tissues that can represent a normal or abnormal process of a condition or disease. In CRC, *KRAS* has been the most notable biomarker advance, in terms of its predictive value for treatment with epidermal growth factor receptor inhibitors. The *BRAF* gene has more recently gained attention as a prognostic biomarker, although its predictive value for response to treatment is inconclusive. The recent upsurge of biomarkers in identifying disease prognosis, predictive response to treatment, or likelihood of treatment toxicities has contributed additional perspectives to the CRC disease management landscape.

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Over the past several years, new cases of colorectal cancer (CRC) have decreased in the United States from an estimated 150,000 in 2008 to 143,000 in 2010 (Jemal, Siegel, Xu, & Ward, 2010). In part, this decline can be directly attributed to early screening tests and the removal of colon polyps before they become cancerous (American Cancer Society [ACS], 2010; ACS, 2008a, 2008b; ACS, 2007; Sturgeon et al., 2008). However, even with a decline in the number of new cases projected for CRC, the disease remains ranked third in both incidence and cancer-related deaths for both genders, accounting for approximately 50,000 deaths within the United States annually (ACS, 2008b). Worldwide, CRC is the fourth most common cancer in men and the third in women, resulting in 1.2 million new cases annually (ACS, 2007).

Individuals with early CRC may have no symptoms, which makes

screening imperative. Unfortunately, for many, the diagnosis is not made until the tumor is large enough to cause symptoms such as bleeding or obstruction of the intestine (ACS, 2008).

The 5-year survival rate for patients with stage II CRC is 75%, but 40% of those who live beyond 5 years will experience a cancer recurrence during their lifetime. Adjuvant therapy improves disease-free survival in patients with stage III CRC. The role of adjuvant therapy for patients with stage II CRC is controversial, because the majority will be cured by surgery alone (Allen & Johnston, 2005). Therefore, there is a particular need for markers to ideally select patients with more aggressive disease who might benefit from adjuvant therapy (Deschoolmeester, Baay, Specenier, Lardon, & Vermorken, 2010).

Throughout the past 10 years, significant additions have been made to the treatment armamentarium for CRC. In addition to these modalities, prospective and retrospective research has been conducted to identify prognostic and predictive markers for CRC. Prognostic markers are characteristics that estimate the recovery (cure) or probability of disease recurrence. Predictive markers can help determine the likelihood of whether a person will respond to a specific treatment (NCI, 2010).

Function of Tumor Markers

The term "tumor marker" is used to describe any substance or process (for example, serum, tissue, DNA, apoptosis, or angiogenesis) that can be analyzed for specific aspects of cancer (Sturgeon, Lai, & Duffy, 2009; Yamamoto, Viale, Roesser, & Lin, 2005). Tumor markers are used as surrogate evidence for the behavior of a cancer and add to the clinician's understanding that a clinically important event has occurred, such as the development of a new cancer or a response to therapy (Diamandis, Hoffman, & Sturgeon, 2008). The field of molecular science has exploded, ushering in a new era of tumor marker use, which has outpaced all prior discoveries. A tumor marker can be used for more than one function, and endorsements of tumor markers for specific cancers are not uniformly embraced among all medical societies. The decision to use a tumor marker should be based on the effort to improve a patient's outcome in regard to survival, quality of life, and decreased medical costs (Duffy, 2004).

To best understand the utility of tumor markers, they should be categorized according to function, such as diagnostic, prognostic, predictive, staging, and monitoring. Diagnostic or screening tumor markers identify the presence of cancer, as in the case of prostate-specific antigen (PSA), which is used to screen for prostate cancer. Prognostic markers estimate the risk of cancer recurrence or death after removal of cancer without adjuvant therapies. Predictive markers forecast the likelihood of a response to a given therapy, and monitoring markers detect remission or recurrence after therapy has been completed.

Alpha-fetoprotein (AFP) and beta-human chorionic gonadotropin (β-hCG) are examples of established tumor markers with multiple functions. They are recommended for diagnosing, staging, determining prognosis, detecting recurrence, and monitoring therapy in patients with testicular cancer (Sturgeon et al., 2008). AFP and β-hCG concentrations so accurately reflect change that their measurement dominates over histology in making treatment decisions (Sturgeon, 2008). However, the biomarkers historically used in CRC have not been as selective, thus lessening the use of these tools in the decision-making process.

Carcinoembryonic Antigen

CEA is a well-established tumor marker used in patients with colorectal cancer. In 1981, a National Institutes of Health Consensus Conference concluded that CEA was the best available noninvasive test for detecting a recurrence of CRC (NIH, 1981). The sensitivity, or measurement of the proportion of actual positives that are correctly identified, and the specificity, or measurement of the proportion of correctly identified negatives, are factors to consider regarding the use of CEA as a tumor marker.

A CEA test has a sensitivity of approximately 80% and a specificity of approximately 70% in detecting a recurrence, but it lacks the sensitivity and specificity needed for use as a screening tool in asymptomatic patients (Duffy, 2004; Sturgeon et al., 2008). CEA levels can be elevated in benign conditions, such as liver cirrhosis, chronic active hepatitis, chronic renal failure, colitis, diverticulitis, irritable bowel syndrome, jaundice, and pneumonia, but also in neoplasms such as breast, gastric, lung, mesothelioma, esophageal, and pancreatic cancers (Sturgeon et al., 2009). Nevertheless, along with tissue staging, a CEA assay is uni-

versally used to determine prognosis in patients with CRC. A preoperative CEA level—along with other prognostic factors—is used in planning surgical treatment but should not be used to select patients for adjuvant chemotherapy (Sturgeon et al., 2008). An elevated preoperative CEA level has been shown to be a poor prognostic factor for CRC survival, especially for patients with stage II or III disease (Huh, Oh, Kim, & Kim, 2010; Sun et al., 2009).

In the postoperative setting, the use of serial CEA measurements is one component of a surveillance strategy for detecting early recurrence in stages II and III CRC, providing a median lead time of approximately 5 months (Duffy, 2004). The CEA test has an established role in detecting recurrences or metastases postoperatively. In metastatic CRC, the CEA serves as a monitor for response to treatment and should be measured every 1 to 3 months. In 2006, the American Society of Clinical Oncology (ASCO) stated that CEA should be the marker of choice for metastatic disease, and, in patients with stages II and III disease, a CEA should be followed every 3 months if the patient is a potential candidate for surgery or further treatment (Locker et al., 2006). The National Comprehensive Cancer Network (NCCN) guidelines call for CEA monitoring every 3 to 6 months for 2 years and every 6 months for a total of 5 years as part of standard CRC long-term follow-up care (NCCN, 2010).

The CEA test is fallible, because it will not be elevated in 30% of CRC recurrences. Moreover, it can be elevated as a result of other neoplasms and benign conditions or misleadingly elevated during the first 4 to 6 weeks of a new therapy, especially oxaliplatin (Locker et al., 2006; Yamamoto et al., 2005).

Biomarkers in Colorectal Cancer

EPIDERMAL GROWTH FACTOR RECEPTOR

Epidermal growth factor receptor (EGFR) is a protein and member of the ErbB family with tyrosine kinase activity, which is activated when growth factors or transforming growth factors bind to the receptor, causing the cells to grow and divide. The binding of these ligands to EGFR causes a cascade of intracellular signaling events that protect cells from apoptosis, facilitate invasion, inhibit DNA repair, and promote angiogenesis (Figure 1; Spano, Milano, Vignot, & Khayat, 2008). EGFR is found in high levels on the surface of many types of cancer cells, including CRC. Its identification has led to its use as a target in anticancer therapy. The U.S. Food and Drug Administration (FDA) has approved the use of cetuximab (Erbitux) and panitumumab (Vectibix)—both EGFR inhibitors—based on clinical trials of patients who are EGFR-positive.

Chung and colleagues (2005) conducted a retrospective review via the pharmacy computer database of all patients who had received cetuximab within the first 3 months of the drug's commercial availability. Of the 16 chemotherapy-refractory, EGFR-negative patients with CRC who received cetuximab, 14 had combination therapy with cetuximab plus irinotecan, and 2 received cetuximab monotherapy. Four major objective responses were found in this patient population (response rate, 25%; 95% confidence interval [CI], 4%–46%). The researchers concluded that patients with EGFR-negative tumors have the potential to respond to cetuximab-based therapies.

EGFR analysis by immunohistochemistry (IHC) does not appear to have predictive value in CRC. Thus, selection or exclusion of patients for cetuximab therapy should not be based on EGFR IHC.

EGFR AMPLIFICATION AND SKIN RASH

The anti-EGFR monoclonal antibodies panitumumab and cetuximab are important agents in the armamentarium of metastatic CRC (mCRC) therapies. Although the presence of *KRAS* mutation predicts for lack of response to these agents, studies are also exploring tumor *EGFR* expression and gene copy number (Deschoolmeester, et al., 2010). Patients with higher *EGFR* gene copy numbers have shown an improved response to anti-EGFR antibody therapy, but results have been inconsistent (Deschoolmeester et al., 2010).

The characteristic papulopustular rash seen in patients on anti-EGFR therapy is a common side effect, and studies have demonstrated that patients with more severe rash have improved clinical benefit compared with those who do not experience significant rash (Deschoolmeester et al., 2010). However, researchers are unclear as to the mechanism for this correlation, and more research is needed regarding the role of rash and EGFR amplification in patients receiving anti-EGFR therapy.

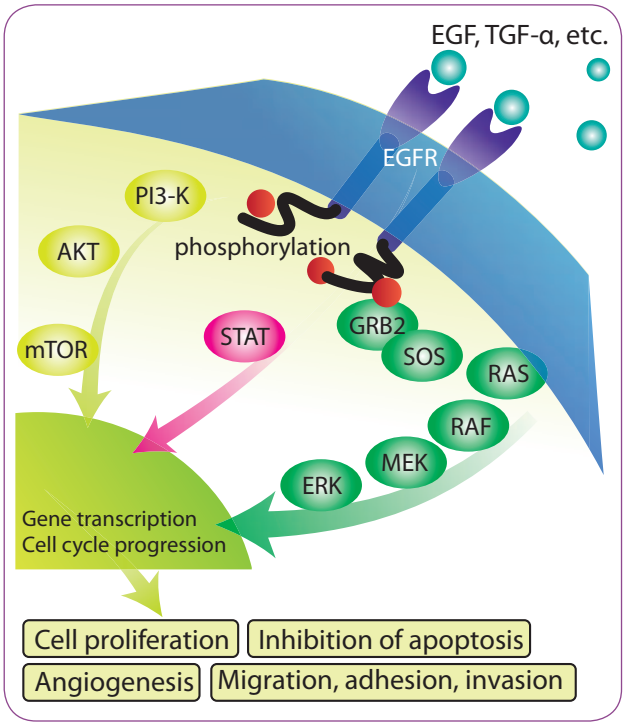

Figure 1. Illustration of the EGFR signaling pathway. The epidermal growth factor (EGF) ligand binds to the EGF receptor on the extracellular domain. Once bound, a cascade of downstream signaling occurs through multiple pathways including PI3-K and RAS, leading to cell proliferation, inhibition of apoptosis, angiogenesis, migration, adhesion, and invasion. EGFR = epidermal growth factor receptor; mTOR = mammalian target of rapamycin; PI3-K = phosphatidylinositol 3-kinase; TGF = transforming growth factor. Based on information from Adjei (2001); DiNicolantonio et al. (2008); NCCN (2010); Spano, Milano, Vignot, & Khayat (2008); and Richman et al. (2009).

KRAS

One of the most important downstream signaling pathways of the ErbB family is the Ras-Raf-MAP kinase pathway (Spano et al., 2008). Kristen rat sarcoma (*KRAS*) is a gene that has an early and important role in signal transduction downstream of EGFR in the signaling pathway (Adjei, 2001). *KRAS* is found in 70% of cancers (Bos, 1989). In mCRC, approximately 40% of tumors are found to have *KRAS* mutation (abnormal) on codon 12 or 13 of the *KRAS* gene, whereas 60% are *KRAS* wild-type (normal or nonmutated; Esteller et al., 2001). Even when monoclonal antibody EGFR inhibitors bind to the receptor, downstream signaling in *KRAS*-mutant tumors is dysregulated, thereby allowing activation to occur despite treatment with cetuximab or panitumumab (Baselga & Rosen, 2008).

Retrospective analysis across several randomized clinical trials suggests that EGFR inhibitors are not effective in treating metastatic CRC patients with *KRAS*-mutant tumors (Amado, 2008; DeRoock, et al., 2008). Karapetis and colleagues (2008) conducted a retrospective analysis of the phase III NCIC CO.17 randomized controlled trial, reporting on tumor samples from 394 patients with CRC who received either cetuximab plus best supportive care (BSC) or BSC alone. The investigators analyzed the effect of *KRAS*-mutation status in association with overall survival (OS) and progression-free survival (PFS) in both groups. *KRAS* mutations were found in 42.3% of tumors. Patients with *KRAS* wild-type tumors had significantly improved OS (median, 9.5 vs. 4.8 months) and PFS, with almost a doubling of the values (median, 3.7 vs. 1.9 months). No significant differences in OS or PFS were seen in the patients with *KRAS*-mutated tumors treated with cetuximab or BSC. The researchers concluded that colorectal tumors with mutated *KRAS* did not benefit from cetuximab, whereas patients with *KRAS* wild-type tumors did.

A change in drug labeling occurred in 2009 for both cetuximab and panitumumab, indicating that use of these agents was not recommended in the treatment of CRC associated with *KRAS* mutations. The NCCN identifies *KRAS* as a predictive biomarker and recommends the determination of *KRAS* gene status of either the primary tumor or a site of metastasis for patients with mCRC at diagnosis. *KRAS* mutations occur early in the formation of CRC, and there is strong concordance between mutation status of primary and metastatic tumors (NCCN, 2010). Additionally, the ASCO Provisional Clinical Opinion recommends *KRAS* testing for all patients with mCRC who are candidates for EGFR inhibitor therapy (Allegra, et al., 2009). Both guidelines recommend that cetuximab and panitumumab should only be given to patients with *KRAS* wild-type tumors.

BRAF

Although nearly 95% of mCRC patients with *KRAS* mutations will not respond to treatment with EGFR inhibitors—a highly specific predictive factor—the test is not sensitive in this set-

ting. This finding suggests that there are other confounding molecular determinants of response that have not yet been realized. *BRAF* is another component of the EGFR-MAPK pathway. The *BRAF* protein is downstream of *KRAS* and has activity in cellular growth and transformation. Mutations cause *BRAF* protein to be continuously active, relaying messages to the nucleus even in the absence of chemical signals, thereby contributing to the growth of cancers by allowing abnormal cells to grow and divide uncontrollably.

Oncogenic *V600E* mutations of *BRAF* in mCRC (Richman et al., 2009) may have negative predictive value in anti-EGFR therapy response. Recent small studies have begun to explore the impact of *BRAF* mutation and suggest that mCRC tumors with wild-type *KRAS* and mutated *BRAF* (DiNicolantonio et al., 2008; Richman et al., 2009) are unlikely to respond to EGFR inhibitors. The exact impact of this mutation to treatment decisions is uncertain, and therefore, testing remains optional in the clinical guidelines (NCCN, 2010).

Emerging Biomarkers in CRC: Prognostic Markers

Although the roles of *KRAS* (and to a lesser extent, *BRAF*) are firmly cemented as an important part of treatment decision-making in CRC today, other biomarkers of interest are currently under study. These markers may be prognostic or predictive and, in some cases, play both roles.

MICROSATELLITE INSTABILITY

The evolution of a tumor depends on many different factors, including mutations in specific genes and loss of tumor-suppressor function. As DNA undergoes replication, errors can occur, and correction of those errors is a function of the body repair systems. Mismatch repair (MMR) genes can undergo mutations leading to an inability of the MMR system to correct those errors (de la Chapelle, 2003; Soreide et al., 2009). Microsatellite instability (MSI) refers to an MMR deficiency where an accumulation of mutations occurs in short, repeating sequences called microsatellites. The germ-line microsatellite allele has either gained or lost repeat units and subsequently undergoes a change in length (de la Chapelle, 2003).

Approximately 15% to 20% of patients with CRC have high-frequency MSI (MSI-H; Soreide et al., 2009). Studies have demonstrated that MSI-H tumors are associated with more aggressive features, such as larger size, poor differentiation, and invasive tumor cell growth, as compared with their low-frequency MSI (MSI-L) or microsatellite stable (MSS) counterparts (Soreide, Janssen, Soiland, Korner, & Baak, 2006). However, studies of patients with MSI-H tumors that exhibit a decreased tendency toward metastasis have also demonstrated an improved survival. Interestingly, it appears that these patients may not derive benefit from adjuvant chemotherapy (Ribic et al., 2003).

The effect of MSI status on patients with stage II and III receiving 5-fluorouracil (5-FU) chemotherapy was examined in pooled data from randomized adjuvant trials (Sargent, Marsoni, & Thibodeau, 2008). The results showed that patients (n = 515) with MSI-H status or loss of an MMR protein not receiving chemotherapy had a 49% improvement in disease-free survival compared with patients with a non–MSI-H status (Sargent et al., 2008). However, variable results from previous studies demonstrate that the role of MSI status in the selection of appropriate patients for adjuvant therapy is not yet clear (Tejpar et al., 2010).

Soreide and colleagues (2009) reported on a study of MSI status and DNA ploidy to determine the influence of these factors on tumor recurrence in patients with CRC during surveillance after initial surgery. The study evaluated 186 consecutive, population-based patients with stages I–III CRC after surgery with curative intent. Patients were analyzed for MSI status by polymerase chain reaction, recurrence, recurrence-free survival, and disease-specific survival. Results demonstrated that patients with MSI (20%) were younger than patients without MSI (median age, 61 vs. 67 years; $p = .016$; Soreide et al., 2009). Proximal tumor location, larger tumor size, and poor tumor differentiation were associated with MSI. MSI was linked to an increased risk for locoregional tumor recurrence ($p = .016$) and shorter time to recurrence ($p = .60$). However, lymph node status predicted more accurately for the development of distant metastasis (Soreide et al., 2009).

Some clinicians believe that all patients with a diagnosis of CRC should undergo MSI testing to determine optimal candidates for systemic therapy. Confirmatory studies are needed to ful-

ly determine the role of MSI status in patients with CRC.

PIK3CA

Common somatic mutations in the *APC, TP53,* and *KRAS* genes, as well as the *PIK3CA* and *BRAF* mutations, contribute to the development of CRC (Markowitz & Bertagnolli, 2009; Souglakos et al., 2009; Wood et al., 2007). The cell-signaling pathways are integral to the development of treatments for CRC and represent targets for new therapies. The p53 pathway, once it has been inactivated by mutation of *TP53* (a tumor-suppressor gene), plays a critical role in the genetic development of CRC (Markowitz & Bertagnolli, 2009).

Activation of the *KRAS*, *BRAF*, and *PIK3CA* oncogenes stimulates tumor cell growth and enhances the process of metastasis (Souglakos et al., 2009). The phosphatidylinositol 3-kinase (PI3K)/AKT pathway is implicated in tumor cell growth. Current studies aim to define inhibitors of this pathway (Ogino et al., 2009). Phosphorylation of AKT by the interaction of PI3K with phosphatidylinositol-3-phosphate activates the downstream signaling pathway (Ogino et al., 2009). Mutated *PIK3CA* can subsequently stimulate the AKT pathway, leading to tumor cell growth of CRC (Ogino et al., 2009). These mutations occur in approximately 10% to 30% of colon cancers and are linked to mutations in *KRAS* and MSI.

Testing for mutations in *PIK3CA* is not standard of care at this time; however, available data are intriguing. Souglakos and colleagues (2009) aimed to determine the prognostic and predictive value of *KRAS*, *PIK3CA*, and *BRAF* mutations for patient response to active agents in the treatment of mCRC. The authors of the study reported that mutation status for all three markers was assessed in 168 patients treated for mCRC with 5-FU–based first-line chemotherapy at two institutions. Outcomes of the study were determined retrospectively (Souglakos et al., 2009). *PIK3CA* mutations were found in 26 of the cases (15%), with *KRAS* and *BRAF* mutations in 62 (37%) and 13 (8%) of the participants, respectively. The *KRAS* mutation was associated with a lack of response and shorter PFS (p = .002). The *PIK3CA* mutations predicted reduced PFS in response to salvage therapy with cetuximab (p = .01), but no differences in OS were seen. Reasons for this difference in OS could include the fact that 82% of patients received another regimen after disease progression on therapy with cetuximab or that cetuximab has a minimal response on the natural history of this common tumor type (Messersmith & Ahnen, 2008). Therefore, the authors of this study recommend further confirmatory studies to definitively confirm the role of *PIK3CA* mutations, as well as mutations in *KRAS* and *BRAF* (Souglakos et al., 2009).

PHOSPHATASE AND TENSIN HOMOLOG

Phosphatase and tensin homolog (PTEN) is a tumor suppressor. PTEN functions as a negative regulator of PI3K signaling, and mutations can cause subsequent loss of expression of PTEN (Jhawer et al., 2008). Approximately 20% to 30% of MSI colon cancer patients experience a loss of PTEN, and overactivation of the AKT cell-signaling pathway may result (Sartore-Bianchi et al., 2009; Tejpar & Odze, 2009; Jhawer et al., 2008). Previous studies have suggested that patients with PTEN-positive tumors demonstrate improved outcomes compared with patients with PTEN-negative tumors. Some studies have suggested that loss of PTEN and *PIK3CA* mutations can predict for lack of benefit from cetuximab therapy, although further research is needed to determine the definitive role of these markers (Jhawer et al., 2008; Frattini et al., 2007).

AMPHIREGULIN AND EPIREGULIN

There is great interest in discovering additional diagnostic features that may prove to be significant biomarkers. Material gathered from mRNA in stable formalin-fixed paraffin embedded (FFPE) tissues has allowed the measurement of potential new markers such as amphiregulin and epiregulin. Amphiregulin and epiregulin are ligands for EGFR that have been proposed as candidate molecular markers when evaluating disease control with cetuximab (Khambata-Ford et al., 2007).

A recent study identified a relationship between ligand expression and the level of sensitivity to EGFR inhibition. Gene expression and *KRAS*-mutation status were measured on FFPE primary tumors in 220 patients with chemorefractory mCRC (Jacobs et al., 2009). The authors demonstrated that a high level of ligand expression selects for a subgroup of *KRAS* wild-type patients who will most likely respond to EGFR

inhibition therapy, as opposed to wild-type patients with low ligand expression, whose disease behaves similarly to that of patients with *KRAS*-mutated tumors (Jacobs et al., 2009). They concluded that ligand expression had no predictive power in patients with *KRAS*-mutated tumors; however, expression of EGFR ligands did significantly predict outcome in *KRAS* wild-type chemorefractory mCRC in patients treated with cetuximab and irinotecan (Jacobs et al., 2009). Although further study is necessary to fully examine the role of amphiregulin and epiregulin in patients with CRC, current data suggest that these ligands may represent additional predictive markers for this tumor type.

ERCC1

Platinum agents are an important part of the armamentarium of chemotherapy agents utilized in the treatment of patients with cancer. Since its FDA approval in the metastatic setting, oxaliplatin has also been approved as adjuvant therapy for CRC and is considered standard of care. The effectiveness of oxaliplatin depends on its ability to cross-link complementary DNA strands, causing DNA damage and apoptosis (Stoehlmacher et al., 2004). Resistance to platinum compounds has been studied through the analysis of RNA and DNA, which demonstrates *ERCC1* as a possible cause for lack of drug effect.

ERCC1 stands for excision repair cross-complementing rodent repair deficiency complementation group 1, and it is an excision nuclease existing inside the nucleotide excision repair pathway (Reed, 2005). *ERCC1* plays a critical role in repair of DNA damage by platinum. Low gene-expression levels of *ERCC1* are associated with superior responses to chemotherapy agents such as oxaliplatin for CRC (Stoehlmacher et al., 2004). The findings of these studies suggest that *ERCC1* expression as measured by RNA or protein may be a useful marker of resistance to cisplatin and its analogs, such as oxaliplatin (Reed, 2006). These studies may help provide clinical information useful in planning platinum-based treatment plans. However, more data are needed to definitively confirm the role of *ERCC1* in therapy for CRC.

Predictive Markers of Toxicity

Although markers of toxicity exist, their use in clinical practice is not yet standard. The most relevant markers will be reviewed for their applicability in patients with CRC.

UGT1A1

Irinotecan is a critical component of the armamentarium of agents used for mCRC. It is a camptothecin analog that acts as a topoisomerase I inhibitor and is activated to SN-38, its active metabolite by carboxylesterase metabolism (Deeken, Slack, & Marshall, 2008). The inactivation of SN-38 is completed primarily by glucuronidation via uridine diphosphate glucuronyltransferases (UGTs), and the enzyme UGT1A1 is critical to this process (Schulz et al., 2009). For most patients receiving irinotecan, this process occurs without incident. However, some patients with a polymorphism in the *UGT1A1* gene have been reported to develop severe toxicity after receiving this chemotherapy agent. Irinotecan toxicity occurs as degradation of SN-38 is reduced, creating greater tissue exposure to the active metabolite. A patient with the *UGT1A1* polymorphism can suffer from diarrhea and neutropenia, and the reaction can be dose-limiting (Schulz et al., 2009; Deeken, Slack, & Marshall, 2008). In 2005, a change in the drug labeling warned clinicians of the possibility of irinotecan toxicity in selected patients with the polymorphism. A commercially available test can determine whether a potential candidate for irinotecan carries the polymorphism; however, it is not standard of care to test all patients.

A recently reported study addressed specific genotypes for the promoter region of the *UGT1A1* gene in blood samples from 105 patients who had received first-line therapy with irinotecan for mCRC (Schulz et al., 2009). The researchers examined the distribution of the genotypes: wild type (6/6), 39%; heterozygous genotype (6/7), 49.5%; and homozygous genotype (7/7), 9.5%—the last of these genotypes is thought to be the one most at risk for increased toxicity. Interestingly, the overall response rate was similar between patients carrying the heterozygous genotype 6/7 or homozygous genotype 7/7 and those carrying the wild-type genotype (6/6), and time to disease progression and OS were not significantly different (Schulz et al., 2009). The investigators found no significant difference in drug toxicity (grade 3 or 4 delayed diarrhea was 13% for the 6/7 heterozygous genotype and 7/7 ho-

mozygous genotype group vs. 6.2% for the 6/6 wild-type group). Treatment delays and dose reductions were statistically nonsignificant as well (Schulz et al., 2009). The authors of the study concluded that the *UGT1A1* gene polymorphism does not significantly influence efficacy and toxicity for patients receiving low-dose irinotecan therapy (Schulz et al., 2009).

Given the association between *UGT1A1* polymorphism and hyperbilirubinemia, irinotecan should be used cautiously in patients with Gilbert's syndrome (a genetic disorder in which the liver has difficulty breaking down bilirubin, occurring in approximately 2%–5% of the U.S. population) and in patients with elevated serum levels of bilirubin. Although no guidelines have been established for the testing of *UGT1A1* polymorphism in patients receiving irinotecan, clinicians should be aware of the potential for toxicity in select patients and should monitor accordingly.

DPD DEFICIENCY

Fluorouracil is integral to the treatment of patients with CRC, in both adjuvant and metastatic settings. A key treatment for 4 decades, 5-FU in conjunction with leucovorin is part of most regimens for CRC. This agent has a narrow therapeutic index; therefore, toxicity increases as the dose of drug is increased (van Kuilenburg et al., 2000). Previous studies have documented increased toxicity secondary to elevated plasma levels of the drug in patients with a complete or partial deficiency of dihydropyrimidine dehydrogenase (DPD). Some of these patients were found to be genotypically heterozygous for a mutant *DPD* allele, and the frequency of heterozygotes at risk for the mutation has been estimated to be as high as 3% (Etienne et al., 1994).

In a study of 37 patients with cancer, van Kuilenburg and colleagues (2000) examined the role of a partial DPD deficiency as related to unexpectedly severe 5-FU toxicity. The authors reported that 55% of patients with decreased DPD activity developed grade IV neutropenia, compared with 13% of patients with normal DPD activity ($p = .01$). Additionally, toxicity occurred twice as fast in patients with low DPD activity ($p = .05$).

A case report demonstrated a lethal outcome for a woman with a complete DPD deficiency after administration of palliative chemotherapy for recurrence of CRC. The patient developed stomatitis after receiving 5-FU (900 mg) and leucovorin (200 mg; van Kuilenburg et al., 2001). She received a second dose of chemotherapy and subsequently developed severe pancytopenia, dying from infectious complications 8 days post-therapy (van Kuilenburg et al., 2001). Although it is not standard practice to check all patients for DPD deficiency, clinicians should be aware of the possibility in patients receiving 5-FU. Investigation of DPD deficiency should be considered in patients experiencing unexpected toxicity with this chemotherapy.

Conclusion

Although testing for *KRAS* status—preferably at the time of diagnosis—has become routine in mCRC care, research on other predictive biomarkers in CRC continues to expand (Table 1). As these biomarkers move toward scientific validation with increasing levels of evidence, more of them may be incorporated into treatment decision-making.

Implications for Advanced Practice Clinicians

An understanding of biomarkers will assist advanced practitioners in appreciating more of the biology and aggressiveness of tumors. Knowledge of relevant biomarkers will continue to help clinicians refine individual treatment approaches for patients with CRC.

Advanced practitioners have a significant role in the education of patients who are being considered for treatment of CRC. Newly diagnosed patients have become savvy in obtaining their pathology reports and in deciphering the results. Advanced practitioners are uniquely positioned to elicit these discussions and assist patients in understanding the prognostic and predictive implications of these reports as they relate to disease and treatment outcomes. As the lexicon for biomarkers continues to emerge, oncology nurses can demystify the language through education and support of patients while underscoring the implications for the treatment trajectory.

DISCLOSURES

The authors have no potential conflicts of interest to disclose.

REFERENCES

Adjei, A. A. (2001). Blocking oncogenic ras signaling for can-

Table 1. Considerations about selected biomarkers used in the treatment of CRC

Marker	Use	Clinical significance	Agent
CEA	Along with tissue staging used to determine prognosis	Component of surveillance to detect early recurrence	Not agent-specific
EGFR	To determine use with EGFR inhibitors	Does not appear to have predictive value in patient selection for EGFR inhibitor therapy in mCRC	Cetuximab; panitumumab
KRAS	To determine response to EGFR inhibitors	Patients with mutated *KRAS* should not receive EGFR inhibitors; treatment is an option for patients with wild-type *KRAS*	Cetuximab; panitumumab
BRAF	To determine response to EGFR inhibitors	May have negative predictive value for response to EGFR inhibitors	Cetuximab; panitumumab
MSI	Measurement of MSI status could help determine appropriate patients for adjuvant chemotherapy	Patients with MSI-H status may not benefit from adjuvant treatment with chemotherapy	Fluoropyrimidine
ERCC1	Could determine potential resistance to platinum therapies	Patients with low *ERCC1* gene expression show greater responses to chemotherapy	Oxaliplatin
PIK3CA	Pathway is involved in tumor cell growth; *PIK3CA* mutations can stimulate AKT pathway	Mutations of *PIK3CA* could lead to CRC tumor cell growth; mutations have also predicted reduced PFS in patients treated with EGFR inhibitor therapy	Cetuximab
PTEN	Acts as a tumor suppressor	Patients with PTEN-positive tumors demonstrate improved outcomes compared with PTEN-negative tumors; some studies suggest PTEN can predict lack of benefit for EGFR inhibitor therapy	Cetuximab
Amphiregulin and epiregulin	Ligands for EGFR	Have been proposed as candidate markers for cetuximab disease control; ligand expression and the level of sensitivity to EGFR inhibition have been studied	Cetuximab
UGT1A1	Enzyme UGT1A1 is critical to the inactivation of SN-38, key to metabolism of irinotecan	*UGT1A1* polymorphism is associated with hyperbilirubinemia. Approximately 5% of the population have Gilbert's disease, which can cause problems with breakdown of bilirubin. Patients with the *UGT1A1*28* allele may have increased toxicity with chemotherapy agents in CRC	Irinotecan
DPD deficiency	DPD is important in the metabolism of 5-FU	Patients with partial or complete DPD deficiency have been noted to suffer unexpected profound toxicity with chemotherapy	5-FU

Note. CEA = carcinoembryonic antigen; CRC = colorectal cancer; DPD = Dihydropyrimidine dehydrogenase; EGFRI = epidermal growth factor receptor; 5-FU = 5-fluorouracil; mCRC = metastatic colorectal cancer; MSI = microsatellite instability; MSI-H = high-frequency microsatellite instability; PFS = progression-free survival; PTEN = phosphatase and tensin homolog. Based on information from Allegra et al. (2009); Chung et al. (2005); Deeken et al. (2008); Etienne et al. (1994); Frattini et al. (2007); Jhawer et al. (2008); Khambata-Ford et al. (2007); Locker et al. (2006); NCCN (2010); Sargent et al. (2008); Sturgeon et al. (2008); Tejpar & Odze (2009); van Kuilenburg et al. (2001).

cer therapy. *Journal of the National Cancer Institute, 93,* 1062–1074.

Allegra, C., Jessup, J. M., Somerfield, M. R., Hamilton, S. R., Hammond, E. H., Hayes, D. F.,...Schilsky, R. L. (2009). American Society of Clinical Oncology Provisional Clinical Opinion: Testing for *KRAS* gene mutations in patients with metastatic colorectal carcinoma to predict response to anti–epidermal growth factor receptor monoclonal antibody therapy. *Journal of Clinical Oncology, 27,* 2091–2096. doi:10.1200/JCO.2009.21.9170

Allen, W. L., & Johnston, P. G. (2005). Role of genomic markers in colorectal cancer treatment. *Journal of Clinical*

Oncology, 23, 4545–4552.
Amado, R. G., Wolf, M., Peeters, M., VanCutsem, E., Siena, S., Freeman, T. J.,...Chang, D. D. (2008). Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colon cancer. *Journal of Clinical Oncology, 26,* 1626–1634. doi:10.1200/jco.2007.14.7116
American Cancer Society. (2007). *Global cancer facts & figures, 2007.* Retrieved December 7, 2008, from http://www.cancer.org/docroot/NWS/content/NWS_1_1x_ACS_Report_Puts_Global_Cancer_Death_Rate_at_76_Million.asp
American Cancer Society. (2008a). *Cancer facts & figures, 2008.* Retrieved October 15, 2010, from http://www.cancer.org/docroot/STT/content/STT_1x_cancer_facts_and_figures_2008.asp
American Cancer Society. (2008b). *Colorectal cancer facts & figures 2008-2010.* Retrieved June 2, 2010, from http://www.cancer.org/downloads/STT/F861708_finalforweb.pdf
American Cancer Society. (2010). *Cancer facts & figures 2010.* Retrieved June 2, 2010, from http://www.cancer.org/downloads/STT/Cancer_Facts_and_Figures_2010.pdf
Baselga, J., & Rosen, N. (2008). Determinents of RASistence to anti-epidermal growth factor receptor agents. *Journal of Clinical Oncology, 26,* 1582–1584. doi:10.1200/JCO.2007.15.3700
Bos, J. L. (1989). Ras oncogenes in human cancer: A review. *Cancer Research, 49,* 4682–4689.
Chung, K. Y., Shia, J., Kemeny N. E., Shah, M., Schwartz, G. K., Tse, A.,...Saltz, L. B. (2005). Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *Journal of Clinical Oncology, 23,* 1803–1810. doi:10.1200/JCO.2005.08.037
Deeken, J. F., Slack, R., & Marshall, J. L. (2008). Irinotecan and uridine diphosphate glucuronosyltransferase 1A1 pharmacogenetics. *Cancer, 113,* 1502–1510. doi:10.1002/cncr.23777
DeRoock, W., Piessevaux, H., DeSchutter, J., Janssens, M., DeHertogh, G., Personeni, N.,...Tejpar, S. (2008). K-ras wild-type state predicts survival and is associated with early radiological response in metastatic colorectal cancer treated with cetuximab. *Annals of Oncology, 19,* 508–515. doi:10.1093/annonc/mdm496
de la Chapelle, A. (2003). Microsatellite instability. *New England Journal of Medicine, 349,* 209–210.
Deschoolmeester, V., Baay, M., Specenier, P., Lardon, F., & Vermorken, J. B. (2010). A review of the most promising biomarkers in colorectal cancer: One step closer to targeted therapy. *The Oncologist, 15,* 699–731. doi:10.1634/theoncologist.2010-0025
Diamandis, E. P., Hoffman, B. R., Sturgeon, C. M. (2008). National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for the Use of Tumor Markers. *Clinical Chemistry, 54,* 1935–1939. doi: 10.1373/clinchem.2008.105494
DiNicolantonio, F., Martini, M., Molinari, F., Sartore-Bianchi, A., Arena, S., Saletti, P.,...Bardelli, A. (2008). Wild-type *BRAF* is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *Journal of Clinical Oncology, 26,* 5705–5712. doi:10.1200/JCO.2008.18.0786
Duffy, M. J. (2004). Evidence for the clinical use of tumour markers. *Annals of Clinical Biochemistry, 41,* 370.
Esteller, M., Gonzalez, S., Risques, R. A., Marcuello, E., Mangues, R., Germa, J. R.,...Peinado, M. A. (2001). K-ras and p16 abberations confer poor prognosis in human colorectal cancer. *Journal of Clinical Oncology, 19,* 299–304.
Etienne, M. C., Lagrange, J. L., Dassonville, O., Fleming, R., Thuss, A., Renee, N.,...Milano, G. (1994). Population study of dihydropyrimidine dehydrogenase in cancer patients. *Journal of Clinical Oncology, 12,* 2248–2253.
Frattini, M., Saletti, P., Romagnani, E., Martin, V., Molinari, F., Ghisletta, M.,...Mazzucchelli, L. (2007). PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *British Journal of Cancer, 97,* 1139–1145. doi:10.1038/sj.bjc6604009
Huh, J. W., Oh, B. R., Kim, H. R., & Kim, Y. J. (2010). Preoperative carcinoembryonic antigen level as an independent prognostic factor in potentially curative colon cancer. *Journal of Surgical Oncology, 101,* 396–400.
Jacobs, B., De Roock, W., Piessevaux, H., Van Oirbeck, B., Biesmans, B., Schutter, J. D.,...Tejpar, S. (2009). Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. *Journal of Clinical Oncology, 27,* 5068–5074. doi:10.1200/JCO.2008.21.3744
Jemal, A., Siegel, R., Xu, J., & Ward, E. (2010). Cancer statistics, 2010. *CA: A Cancer Journal for Clinicians.* Epub before print. Accessed from http://caonline.amcancersoc.org/cgi/content/full/caac.20073v1. doi:10.3322/caac.20073
Jhawer, M., Goel, S., Wilson, A. J., Montagna, C., Ling, Y-H., Byun, D.-S.,...Mariadason, J. M. (2008). PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. *Cancer Research, 68,* 1953–1961. doi:10.1158/0008-5472.CAN-07-5659
Karapetis, C. S., Khambata-Ford, S., Jonker, D. J., O'Callaghan, C. J., Tu, D., Tebutt, N.,...Zalcberg, J. R. (2008). K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *New England Journal of Medicine, 359,* 1757–1765.
Khambata-Ford, S., Garrett, C. R., Meropol, N. J., Basik, M., Harbison, C. T., Wu, S.,...Mauro, D. J. (2007). Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *Journal of Clinical Oncology, 25,* 3230–3237. doi:10.1200/JCO.2006.10.5437
Locker, G. Y., Hamilton, S., Harris, J., Jessup, J. M., Kemeny, N., Macdonald, J. S.,...Bast, R. C. (2006). ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *Journal of Clinical Oncology, 24,* 5313–5327.
Markowitz, S. D., & Bertagnolli, M. M. (2009). Molecular basis of colorectal cancer. *New England Journal of Medicine, 361,* 2449–2460.
Messersmith, W. A., & Ahnen, D. J. (2008). Targeting EGFR in colorectal cancer. *New England Journal of Medicine, 359,* 1834–1836.
NCCN. (2010). National Comprehensive Cancer Network Guidelines: Colon Cancer v.3.2010. Accessed from www.nccn.org
NCI. (2010). National Cancer Institute: Dictionary of Cancer Terms. Accessed from www.cancer.gov
NIH. (1981). National Institutes of Health Consensus Development Conference Statement: CEA (carcinoembryonic antigen): its role as a marker in the management of cancer. *Cancer Research, 41,* 2017.
Ogino, S., Nosho, K., Kirkner, G. J., Shima, K., Irahara, N., Kure, S.,...Fuchs, C. S. (2009). PIK3CA mutation is as-

sociated with poor prognosis among patients with curatively resected colon cancer. *Journal of Clinical Oncology, 27,* 1477–1484. doi:10.1200/JCO.2008.18.6544

Reed, E. (2005). ERCC1 and clinical resistance to platinum-based therapy. *Clinical Cancer Research, 11,* 6100–6102. doi:10.1158/1078-0432.CCR-05-1083

Reed, E. (2006). ERCC1 measurements in clinical oncology. *New England Journal of Medicine, 355,* 1054–1055.

Ribic, C. M., Sargent, D. J., Moore, M. J., Thibodeau, S. N., French, A. J., Goldberg, R.,...Gallinger, S. (2003). Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *New England Journal of Medicine, 349,* 247–257.

Richman, S. D., Seymour, M. T., Chambers, P., Elliott, F., Daly, C. A., Meade, A. M.,...Quirke, P. (2009). KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: Results from the MRC FOCUS Trial. *Journal of Clinical Oncology, 27,* 5931–5937. doi:10.1200/JCO.2009.22.4295

Sargent, D. J., Marsoni, S., Thibodeau, S. N. (2008). Confirmation of deficient mismatch repair (dMMR) as a predictive marker for lack of benefit from 5-FU based chemotherapy in stage II or III colon cancer (CC): A pooled molecular reanalysis of randomized chemotherapy trials. *Journal of Clinical Oncology, 26* (suppl 15S):abstract 4008.

Sartore-Bianchi, A., Martini, M., Molinari, F., Veronese, S., Nichelatti, M., Artale, S.,...Bardelli, A. (2009). PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Research, 69*, 1851–1857. doi:10.1158/0008-5472.CAN-08-2466

Schulz, C., Heinemann, V., Schalhorn, A., Moosman, N., Zwingers, T., Boeck, S.,...Stemmler, H-J. (2009). UGT1A1 gene polymorphism: Impact on toxicity and efficacy of irinotecan-based regimens in metastatic colorectal cancer. *World Journal of Gastroenterology, 15,* 5058–5066. doi:10.3748/wjg.15.5058

Soreide, K., Janssen, E. A., Soiland, H., Korner, H., Baak, J. P. (2006). Microsatellite instability in colorectal cancer. *British Journal of Surgery, 93,* 395–406. doi:10.1002/bjs.5328

Soreide, K., Slewa, A., Stokkeland, P. J., van Diermen, B., Janssen, E. A. M., Soreide, J. A., . . . Korner, H. (2009). Microsatellite instability and DNA ploidy in colorectal cancer. *Cancer, 115,* 271–282. doi:10.1002/cncr.24024

Souglakos, J., Philips, J., Wang, R., Marwah, S., Silver, M., Tzardi, M.,....Shivdasani, R. A. (2009). Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. *British Journal of Cancer, 101,* 465–472. doi:10.1038/sj.bjc.6605164

Spano, J. P., Milano, P., Vignot, S., & Khayat, D. (2008). Potential predictive markers of response to EGFR-targeted therapies in colorectal cancer. *Critical Reviews in Oncology/Hematology, 66,* 21–30. doi 10.1016/j.critevonc.2007.11.005

Stoehlmacher, J., Park, D. J., Zhang, W., Yang, D., Groshen, S., Zahedy, S., & Lenz, H-J. (2004). A multivariate analysis of genomic polymorphisms: Prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. *British Journal of Cancer, 91,* 344-354. doi: 10.1038/sj.bjc.6601975

Sturgeon, C. M.(2002). Practice guidelines for tumor markers use in the clinic. *Clinical Chemistry, 48,* 1151–1159.

Sturgeon, C. M., Duff, M. J., Stenman, U., Lilfa, H., Brunner, N., Chan, D. W.,...Diamandis, E. P. (2008). National academy of clinical biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. *Clinical Chemistry, 54,* 11–79.

Sturgeon, C. M., Lai, L. C., & Duffy, M. J. (2009). Serum tumour markers: How to order and interpret them. *British Medical Journal, 339,* 852–858. doi:10.1136/bjm.b3527

Sun, L.C., Chu, K. S., Cheng, S.C., Lu, C. Y., Kuo, C. H., Hsieh, J. S.,...Wang, J. Y. (2009). Preoperative serum carcinoembryonic antigen, albumin and age are supplementary to UIICC staging systems in predicting survival for colorectal cancer patients undergoing surgical treatment. *British Medical Journal, 9.* Retrieved June 2, 2010 from http://www.biomedcentral.com/1471-2407/9/288 doi:10.1186/147-2407-9-288

Tejpar, S., & Odze, R. D. (2009). Accomplishments in 2008 in biologic markers for gastrointestinal cancers—focus on colorectal cancer. *Gastrointestinal Cancer Research, 3,* (suppl 2):S73–S78.

Tejpar, S., Bertagnolli, M., Bosman, F., Lenz, H-J., Garraway, L., Waldman, F.,...Roth, A. (2010). Prognostic and predictive biomarkers in resected colon cancer: Current status and future perspectives for integrating genomics into biomarker discovery. *The Oncologist, 15,* 390–404. doi: 10.1634/theoncologist.2009-0233

van Kuilenburg, A. B. P., Haasjes, J., Richel, D.J., Zoetekouw, L., Van Lenthe, H., De Abreu, R. A.,...van Gennip, A.H. (2000). Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: Identification of new mutations in the DPD gene. *Clinical Cancer Research, 6,* 4705–4712.

van Kuilenburg, A. B. P., Muller, E. W., Haasjes, J., Meinsma, R., Zoetekouw, L., Waterham, H. R.,...van Gennip, A. H. (2001). Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: Frequency of the common IVS14+1G>A mutation causing DPD deficiency. *Clinical Cancer Research, 7,* 1149–1153.

Wood, L. D., Parsons, D. W., Jones, S., Lin, J., Sjoblom, T., Leary, R. J.,...Vogelstein, B. (2007). The genomic landscapes of human breast and colorectal cancers. *Science,* 318: 1108–1113.

Yamamoto, D. S., Viale, P. H., Roesser, K., & Lin, A. (2005). The clinical use of tumor markers in select cancers: Are you confident enough to discuss them with your patients? *Oncology Nursing Forum, 32,* 1013–1022.