Clinical Biomarkers in Colorectal Cancer

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Abstract: Colorectal cancer remains the second leading cause of cancer-related death in the United States. While chemotherapy remains the backbone upon which treatment for metastatic colorectal cancer is built, targeted therapies have been employed, albeit with mixed results, in the management of this disease. Nonetheless, increased understanding in recent years of the complexity and heterogeneity of cellular abnormalities driving these tumors has identified potential targets for future interventions. This article will review the seminal biomarkers of predictive and prognostic importance currently used in the treatment of patients with colorectal cancer, and will highlight additional promising biomarkers which may be incorporated into clinical practice in the future.

Introduction

Biomarkers in clinical oncology are detectable and characteristic alterations in DNA and proteins that provide insight into the mechanisms and phenotypic behavior driving a patient's cancer. The presence (or absence) of a selected biomarker offers the clinician the potential to plan therapies personalized to an individual patient's tumor. Over the past decades, research detailing critical molecular pathways implicated in the pathogenesis of colorectal cancer has identified various genetic and epigenetic alterations with critical predictive and prognostic utility in the management of this disease. This review discusses the role of validated biomarkers used in the clinical care of patients with colorectal cancer and will highlight newer markers that may be incorporated into standard practices in the future.

Microsatellite Instability

The presence of microsatellite instability (MSI) in a colorectal tumor is a biomarker of high clinical prognostic and predictive importance. It is most commonly used for the management of patients with stage II and III colorectal cancer. Microsatellites are short segments of repeating DNA nucleotides that are prone to developing

Keywords
Biomarker, chemotherapy, mutation, colorectal, metastasis

mutations. In microsatellite-rich regions, either nucleotides inserted aberrantly by DNA polymerases are not corrected because the microsatellite environment is not amenable to repair, or microsatellite repeats insert erroneously into exons to generate a frameshift mutation that alters the length and function of the resulting translated product. Normally, the mismatch repair (MMR) proteins—MLH1, MSH2, MSH6, and PMS2—correct these errors during DNA synthesis.[1] The absence of any of these enzymes (and the subsequent propensity for mutations) defines the existence of MSI. Either a germline mutation in any of the 4 aforementioned precursor genes or epigenetically regulated gene silencing through hypermethylation of the MLH1 promoter region can impair expression of normal MMR gene products.[2] Cells that harbor all 4 functional enzymes are deemed microsatellite stable (MSS). MSI status can be tested in colorectal tumors in one of 2 ways. The first is immunohistochemical staining to determine the presence and relative levels of expressed MMR proteins. The second is polymerase chain reaction (PCR) amplification of 5 gene loci (BAT25, BAT26, D5S346, D2S123, D17S250) susceptible to microsatellite insertion in order to determine whether the repeats are present.[3]

MSI-high tumors are found in approximately 15% of all colorectal cancers and are typically right-sided primary tumors with mucinous histopathology. MSI is associated with earlier disease stage at the time of diagnosis,[4,5] decreased rates of recurrence after resection of the primary tumor, lower incidence in distant metastases, and prolonged overall survival compared with patients with MSS tumors.[5-7] These findings suggest a unique biology associated with MSI and allow it to be considered a positive prognostic marker in patients with colorectal cancer.

Microsatellite status is also useful in the decision to administer adjuvant chemotherapy for early-stage disease. For example, a study of patients with stage II or III MSS colorectal cancer treated with adjuvant 5-fluorouracil showed that both overall survival and recurrence-free survival at 5 years were increased for patients if they received chemotherapy. However, patients with MSI-high stage II colorectal tumors, in the absence of other high-risk clinicopathologic features (eg, <12 lymph nodes examined, colonic obstruction or perforation, pathologic T4 tumors, lymphovascular invasion, or high tumor grade), fare worse if given adjuvant chemotherapy. In this study, patients with MSI-high colorectal tumors trended toward better outcomes if they received adjuvant chemotherapy than if they were not given additional treatment. The differences were not statistically significant, however: 70.7% vs 88.0% (P=.07) of patients were still alive and 69.3% vs 82.9% (P=.11) were without disease at 5 years.[8] A separate study of 5 clinical trials comparing adjuvant leucovorin (or levamisole) with surgery alone in patients with stages II or III colorectal cancer found no benefit for chemotherapy in those with MSI tumors; however, progression-free survival was prolonged (hazard ratio [HR], 0.67; *P*=.02) by chemotherapy for patients with MSS tumors.[9] We interpret these data as the rationale to offer 5-fluorouracil in the adjuvant setting to otherwise healthy patients with stage II, MSS colorectal tumors (in the absence of other high-risk features). Given these findings, we believe that MSI serves as a negative predictive marker for response to 5-fluorouracil and provides useful insight into the treatment planning for patients with stage II colorectal cancer. This marker is less influential in the decision-making process surrounding adjuvant treatment for patients with stage III disease, because the currently accepted standard of care is for all patients with lymph node metastases to receive chemotherapy following surgical resection of their primary tumor and lymph nodes.

Microsatellite testing is also employed to look for hereditary syndromes that may predispose to the development of colorectal cancer. In patients with colorectal cancer diagnosed before the age of 50 years or those with strong family histories of colorectal and/or uterine cancer, MSI testing is routinely used as a genetic biomarker to screen for Lynch syndrome, an autosomal dominant condition present in 2% to 4% of all colorectal cancers.[10-13] Patients with Lynch syndrome harbor germline mutations in the MMR proteins[1] and therefore lack expression of at least 1 MMR protein. If immunohistochemical testing indicates the absence of an MMR protein, then patients can be tested for specific germline mutations unique to Lynch syndrome. Recent data have suggested that testing for Lynch syndrome occurs more frequently in National Cancer Institute-designated Comprehensive Cancer Centers relative to community hospital cancer programs.[14] We hope that in the coming years, testing tumors for features found in the Lynch Syndrome will become routine practice universally in order to identify patients (and family members) at risk for developing other malignancies. If positive, patients and their family members should be referred to a genetic counselor for further recommendations regarding earlier cancer screening for tumors associated with this hereditary syndrome.

KRAS

For patients with metastatic colorectal cancer, the biomarker most commonly tested assesses for the presence of a mutation in the KRAS oncogene. The Ras family of signaling proteins consists of GTPases that alternate between an inactive guanine-diphosphate (GDP)-bound state (Ras-GDP) and an active guanine triphosphate (GTP)-bound (Ras-GTP) state.[15,16] When mutated, KRAS undergoes a

conformational change that impairs allosteric binding of the GTPase-activating proteins (GAPs), which hydrolyze GTP back to GDP and return KRAS-GDP to an inactive form.[17] With GAPs unable to access the mutated KRAS substrate, this kinase remains GTP-bound and constitutively activated.[18,19] Downstream effector pathways, such as Raf/Mek/Erk, phosphoinositide 3-kinase (PI3K)/Akt, and tumor invasion and metastasis induction protein 1 (TIAM1), are ensuingly triggered by activated KRAS to promote tumor cell proliferation, anti-apoptotic activity, and cytoskeletal reorganizations needed for the development of metastases.[20-23]

Mutations in the KRAS oncogene occur in approximately 35% to 40% of all colorectal cancers.[4,24,25] In the nonmetastatic setting, KRAS-mutated tumors tend to be of lower histologic grade and demonstrate microsatellite-low or MSS features.[4] Tissue samples collected prospectively from the PETACC-3, EORTC 40993, and SAKK 66-00 trials showed no difference in the frequency of a KRAS mutation according to stage at presentation. These findings suggest that these mutations occur early in tumor development. Additionally, these studies of patients with stage II or III colorectal cancer found no difference in recurrence-free or overall survival between patients with KRAS mutations and those with KRAS wild-type tumors following definitive resection.[4]

In the stage IV setting, differences in patterns of distant spread and survival outcomes based on KRAS status suggest that this mutation affects the clinical presentation and ultimate outcomes of patients with metastatic disease. For example, one retrospective review reported that patients who undergo hepatic resection for liver metastases have higher mortality rates if their tumors bear KRAS mutations (HR, 2.4; P=.004).[26] In a retrospective study at our institution of patients who underwent sequential hepatic resection of colorectal metastases, not only were KRAS mutations more common in the group of patients receiving adjuvant oxaliplatin, 5-fluorouracil, and leucovorin (FOLFOX) after the initial surgery (when compared with patients receiving 5-fluorouracil alone or no chemotherapy), but overall survival was also shortened in this group.[27,28] Perhaps then, the exposure to FOLFOX selects for a KRAS-mutated population present in recurring liver metastases that correlates to worse outcomes among patients with metastatic liver lesions harboring such mutations.

Another recent study reported that KRAS-mutated metastatic colorectal tumors have a predilection for distal spread to the lung, whereas KRAS wild-type tumors more commonly involve the liver and distant lymph nodes than KRAS-mutated tumors.[29,30] The authors also reported that colorectal tumors with lung involvement demonstrate a relatively high rate of discordance (32.4%) in KRAS status between the metastatic (mutated) site and the primary (wild-type) tumor compared with other sites of metastases, whereas other studies have shown a high rate of concordance between the primary tumor and associated liver metastases (3.6% discordance).[31] Whether or not lung-predominant KRAS-mutated colorectal cancers acquire additional de novo mutation after development of the primary tumor remains unclear at this time and supports the argument that KRAS-mutated tumors are a heterogeneous population; additional work to refine further substratifications within this group is needed.

Nonetheless, the most relevant aspect for KRAS mutation testing in clinical practice centers around the use of therapies with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies in patients with metastatic colorectal cancer. Cetuximab (Erbitux, Bristol-Myers Squibb) is a human-murine chimeric monoclonal antibody directed against the ligand-binding site of the EGFR receptor,[32] and panitumumab is a fully humanized monoclonal antibody also targeting EGFR. Initial early-phase studies using these antibodies as single-agent therapy in heavily pretreated patients with metastatic colorectal cancer demonstrated an overall response rate of approximately 10%.[33,34] However, no association was reported between immunohistochemical levels of EGFR expression and clinical outcomes with anti-EGFR treatment.[34,35]

In vitro work on activating mutations in KRAS first elucidated the negative relationship between the presence of a KRAS mutation and response to anti-EGFR therapy.[36] On the basis of these findings, a retrospective analysis of 572 patients with metastatic colorectal cancer treated with cetuximab and/or best supportive care showed significantly improved progression-free survival (3.7 vs 1.9 months; P<.001) and overall survival (9.5 vs 4.8 months; P<.001) in the subset of patients with KRAS wild-type tumors; however, these survival differences were not observed in patients with KRAS-mutated metastatic colorectal tumors.[37] This study was one of the first to suggest that the absence of a KRAS mutation in codon 12 may predict a benefit for patients receiving anti-EGFR therapy. Subsequently, multiple studies have confirmed this hypothesis with cetuximab or panitumumab, alone or in combination with cytotoxic chemotherapy. KRAS has been widely validated as a predictive marker for anti-EGFR therapy in metastatic colorectal cancer.[38-44] On the basis of these findings, the American Society of Clinical Oncology (ASCO) recommends that all such patients be tested for the presence of a KRAS mutation in codon 12 or 13 and be considered possible candidates for cetuximab and panitumumab, should KRAS wild-type tumors be present.[45]

Tumors with KRAS mutations are a heterogeneous population that differ biologically and clinically based on

the particular codon mutated. Codon 12 is the most common site for a KRAS mutation to occur, with these mutations present in approximately 25% to 35% of all colorectal cancers.[46,47] Within codon 12 mutations, various amino acids may be substituted for the physiologic glycine, with valine (G12V) thought to be most deleterious among the codon 12 mutations. Nonetheless, despite these interesting differences within a single codon, no codon 12 substitution has been shown prospectively to render these tumors sensitive to cetuximab or panitumumab. At this time, the particular amino acid substituted bears no significance in the decision to withhold anti-EGFR therapy in patients whose tumors bear these mutations.

In contrast to colorectal cancer cells with mutations in codon 13, KRAS 12 colorectal tumor cells demonstrate a more aggressive behavior in vitro with increased cellular proliferation, stronger resistance to apoptosis, and decreased cell-cell interaction.[48] When these findings are translated to clinical outcomes, retrospective analyses have suggested that patients with KRAS G13D substitutions have longer progression-free survival, increased overall survival, and an improved response to anti-EGFR antibody therapy compared with patients with metastatic colorectal tumors harboring codon 12 KRAS mutations.[49-52] Collectively, these laboratory and clinical results imply that codon 13 mutated tumors may behave more favorably than their codon 12 counterparts. However, these findings have thus far not been validated prospectively, and no benefit in survival with cetuximab or panitumumab has been demonstrated with patients bearing tumors with mutations in codon 13. Until further prospective studies can be completed, it is recommended that patients with KRAS 13 mutated tumors not be treated with anti-EGFR therapy.

KRAS mutations at non-exon 2 sites (eg, codons 61, 117, and 146) have been described as occurring in approximately 5% to 10% of the studied populations with colorectal cancer.[46,53-55] A recent analysis of the PRIME (Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy) trial showed that patients whose tumors have NRAS mutations or codon 61, 117, or 146 KRAS mutations demonstrate a worse progression-free survival and overall survival when treated with FOLFOX/panitumumab relative to FOLFOX alone.[56] Similar findings were seen in the analysis of the panitumumab vs best supportive care study.[57] Although different groups have reported differing survival outcomes and responses to anti-EGFR therapies for patients whose tumors bear these mutations,[46,58,59] most data suggest that tumors with "nontraditional" NRAS and KRAS non-codon 12 or 13 mutations do not respond to anti-EGFR therapy. We therefore recommend testing for NRAS mutations and KRAS 61 and 146 mutations in patients with metastatic colorectal cancer. Future prospective studies need to clarify the clinical significance of these "alternative" RAS mutations, given that these tumors would otherwise be considered "wild type" under current practices if untested, in order for patients to avoid treatment with ineffective and costly therapies should these tumors behave similarly to those with KRAS mutations at other codons.

Amphiregulin/Epiregulin

Despite the aforementioned successes with cetuximab and panitumumab, not all patients with KRAS wild-type metastatic colorectal cancer respond to these therapies. Two biomarkers that have surfaced in recent years that perpetuate activity of the EGFR, despite physiologically normal activity of the KRAS oncogene, are amphiregulin and epiregulin, ligands homologous to the epidermal growth factor. These ligands bind EGFR and activate its downstream signaling pathways. In colorectal tumor cells, amphiregulin and epiregulin can be secreted in an autocrine feedback loop[60] to perpetuate cell proliferation in a self-sustaining manner.[61] They also can promote oncogenic behavior through induction of anti-apoptotic behavior,[62] stimulation of angiogenesis,[63] and upregulation of genes involved in cell invasion and motility within the tumor microenvironment that promote metastatic activity.[64,65] In colorectal cancer patient specimens, these molecules have been associated with an increased depth of tumor invasion, pathologic detection of perineural invasion, and a higher rate of distant metastases.[66] Thus, the presence of these markers implies a more aggressive underlying tumor biology driven by the multiple aforementioned downstream effectors of these 2 ligands.

Multiple studies have retrospectively investigated the roles of amphiregulin and epiregulin as predictive biomarkers for anti-EGFR therapy. In one series of 110 patients with metastatic colorectal cancer receiving cetuximab monotherapy, these genes were highly expressed in 25% of all tumors. In patients treated with cetuximab, high levels of both amphiregulin and epiregulin were individually associated with prolonged median progression-free survival, which was doubled among those with higher expression of either ligand, relative to patients with low or undetectable levels (104 vs 57 days for amphiregulin, and 116 vs 57 days for epiregulin, respectively; $P<.001$ for each).[67] Similar findings were described for a group of 220 patients with metastatic colorectal cancer treated with irinotecan and cetuximab; here, both progression-free survival and overall survival were longer for those with tumors expressing higher levels of either amphiregulin or epiregulin.[68] It should be noted that these results were observed only in the setting of KRAS wild-type colorectal tumors; patients

with tumors harboring KRAS mutations, even if high ligand expression was present, showed no survival benefit with cetuximab. In addition, patients with low levels of both ligands do not appear to benefit from anti-EGFR therapy and fare similarly to those with KRAS-mutated tumors, having shorter response rates and worse survival outcomes when treated with cetuximab relative to their counterparts expressing higher levels of either ligand.[68,69]

These results imply that, in the absence of a constitutively activated KRAS-mutated protein, antibodies to the EGFR extracellular binding domain impair the amphiregulin:EGFR or epiregulin:EGFR interaction and, in doing so, prevent the colorectal tumor cells from activating the EGFR-mediated signaling pathways that propagate extension of disease in these patients. Additionally, these data are very encouraging in terms of further classifying patients with KRAS wild-type, amphiregulin/epiregulin highly expressed tumors as a more accurately defined group who may benefit from anti-EGFR therapies. One challenge in the development of these biomarkers has been using a continuous variable like the quantification of amphiregulin/epiregulin expression levels to categorize tumors, and then maintaining consistency of these results across different testing sites. Nonetheless, these findings need to be validated prospectively; should it occur, such validation would presumably be the basis for future routine testing of amphiregulin and epiregulin levels as biomarkers predictive for response to cetuximab.

BRAF

Another biomarker that has gained increased importance in subdefining populations of patients with colorectal cancer in recent years is the BRAF oncogene. BRAF is an isoform of the RAF kinase and serves as the downstream substrate to activated RAS. Mutations in the BRAF oncogene—most commonly a valine-to-glutamic acid substitution in codon 600 (V600E)—occur in approximately 8% to 10% of all colorectal cancers[70] and lead to constitutive activation of the MAPK pathway.[71] BRAF-mutated tumors are more commonly located in the proximal colon and are associated with female sex and older age[72]; histologically, they tend to have mucinous features, are extensively hypermethylated, and are highly correlated with an MSI-high phenotype.[73]

The presence of a BRAF mutation is widely accepted as a poor prognostic marker in patients with colorectal cancer. Multiple retrospective reviews have reported that, for patients with phase 2 and 3 BRAF-mutated tumors, recurrence-free survival and overall survival following surgical resection are historically worse than for their BRAF wild-type counterparts.[74,75] One retrospective study reported a median overall survival of 10 months for patients with stage IV disease with BRAF-mutated tumors, significantly worse than the 34.7 months for patients with metastatic, BRAF wild-type colorectal cancer.[76] Other studies have confirmed this finding that overall prognosis is worse in the metastatic setting for patients with BRAF mutations compared with wild-type BRAF.[46,77-80] Despite their KRAS wild-type status, patients with metastatic, BRAF-mutated colorectal tumors do not appear to respond to such therapies as cetuximab or panitumumab,[81] presumably owing to downstream activation of the MAPK pathway occurring independently of EGFR or KRAS activity.

Vemurafenib is a tyrosine kinase inhibitor specific to the kinase domain of the V600E-mutated BRAF that blocks signaling of the MAPK pathway in vitro.[82] In a phase 3 trial for patients with metastatic melanoma, this drug improved overall survival compared with the standard of care cytotoxic agent dacarbazine.[83] However, a phase 1b trial in which vemurafenib was tested in patients with BRAF-mutated metastatic colorectal cancer reported a partial response in only 5% of patients treated with vemurafenib.[84] Several other patients in the same study did demonstrate a mixed response to vemurafenib, which suggests that this agent may serve as an effective backbone for combination therapy in the future. Recent in vitro studies in cell lines have implicated EGFR overexpression[85,86] and PI3K/Akt pathway activation[87] as mechanisms overcoming inhibition by vemurafenib; the combinations of vemurafenib/cetuximab and vemurafenib/PI3K inhibitors, respectively, may serve as the rationale for future clinical trials.

PI3KCA

Mutations in the PI3K/Akt signaling pathway generate oncogenic transformation in vitro by resisting apoptosis, stimulating cell proliferation, and promoting cell migration.[88-93] Approximately 10% to 30% of all patients with colorectal cancer have mutations in PI3K,[46,94-97] the vast majority of which localize to the helical domain (exon 9) and the kinase domain (exon 20).[98,99] Constitutive activation of the kinase occurs through 2 distinct mechanisms according to the specific exon mutated,[100] implying different underlying biological activity in oncogenesis based on the presence of a mutation in either exon 9 or exon 20.

The prognostic significance of PI3KCA mutations currently remains unclear, as discordant results have been reported regarding whether an isolated PI3KCA mutation is associated with survival outcomes in colorectal cancer.[46,80,94,95,97,101-107] Conclusions drawn from many studies must be interpreted with caution, owing to the relatively small populations examined and because many of the studies did not consider the influence of concomitant KRAS

or BRAF mutations in interpreting their analyses. Given the inconsistent findings to date, we do not recommend routine testing for PI3KCA mutations as a prognostic biomarker in patients with metastatic colorectal cancer.

Importantly, recent data have identified a role for PI3KCA mutations as a biomarker for benefit from aspirin therapy for stage II and III colon cancer. One recent retrospective series examined the use of aspirin in patients with colorectal cancer and found an improvement in both cancer-related mortality and overall survival for patients with PI3KCA mutations taking aspirin relative to those with wild-type PI3KCA oncogenes on aspirin therapy ([HR, 0.18; P<.001] and [HR, 0.54; P=.01], respectively).[108] An analysis of patients with stage II and III colorectal cancer adjuvantly treated with rofecoxib (a COX-2 inhibitor) or placebo showed no improvement either in recurrence-free survival or overall survival among patients with tumors bearing PI3KCA mutations. However, similar to the aforementioned study, adjuvant aspirin use (when compared to no aspirin use) was associated with an improvement in recurrence-free survival among patients with PI3KCA-mutated colorectal tumors (unpublished data). The strength of these effects is striking, as is the confirmed association with the outcome of recurrence and mortality. Most prior aspirin studies have focused on the role of aspirin in secondary prevention of new adenomas and adenocarcinomas through colorectal mucosal protection; however, these studies and others have confirmed a role for aspirin in preventing growth of subclinical micrometastatic disease.[109] Given the known bleeding risk of aspirin, a risk/benefit discussion with the patient is important, but in the presence of a PIK3CA mutation, the data suggest a net benefit from aspirin use for reduction in mortality after resection of stage II and III colon cancer.

Multigene Assays

Although adjuvant chemotherapy is the accepted standard of care for all otherwise capable patients who undergo resection for stage III colorectal cancer, chemotherapy is generally reserved for patients with resected stage II disease whose tumors exhibit particular high-risk clinical and pathologic features. To date, the use of single biomarkers as robust predictors for disease recurrence has been investigated but not validated in the adjuvant setting for patients with stage II colorectal cancer.[110-114] Because these tumors are complex, with multiple pathways implicated in the development of new disease following resection, multigene assays have been developed in recent years to identify these underlying elaborate molecular pathways implicated in disease recurrence, both for prognostication following curative surgery and for prediction of benefit of adjuvant chemotherapy.

The Oncotype DX assay (Genomic Healt.) for colorectal cancer was developed initially from 4 cohorts of patients with resected stage II or III colorectal cancer treated with either surgery alone or surgery plus 5-fluorouracil/leucovorin adjuvant chemotherapy (NSABP C-01/C-02, Cleveland Clinic study, NSABP C-04, and NSABP C-06).[115-118] RNA was extracted from formalin-fixed, paraffin-embedded tumor blocks, and reverse transcription PCR studies were then performed on the extracted RNA to identify 7 genes associated with recurrence and 6 genes associated with benefit from 5-fluorouracil/leucovorin chemotherapy.[119] Next, recurrence scores (based on the pattern of expression of the 7 aforementioned identified genes) were used to stratify patients into low-risk, intermediate-risk, and high-risk groups for recurrence. When these genes were examined in a validation cohort of tumor samples derived from patients who participated in the QUASAR (Quick and Simple and Reliable) study,[120] the previously described recurrence score was predictive of recurrence risk at 3 years (12%, 18%, and 22% for the low-, intermediate-, and high-risk groups, respectively; P<.01) independently of other clinicopathologic tumor features. Unfortunately, the 6-gene treatment score was unable to predict a population of patients with resected stage II colorectal cancer who could benefit from adjuvant chemotherapy. Given that finding, the Oncotype DX assay is not routinely used for treatment decisions regarding when to use 5-fluorouracil–based regimens postoperatively in the stage II setting.

ColoPrint (Agendia) is another multigene assay in which 18 genes were identified (using techniques similar to those used for the Oncotype DX assay) from tumors taken from 206 patients with stage I to III colorectal cancer to develop a gene signature with prognostic and predictive utility. Based on the pattern of expression of the 18 selected genes, patients were classified as either at low or high risk for recurrence. In one cohort studied, relapse-free survival rates were 87.6% and 67.2% in the low- and high-risk groups, respectively (HR, 2.5; P=.005).[121] The ability to predict recurrence was even stronger when stage III patients were excluded from the analysis, as the HR for recurrence for stage II patients was 3.34 (P=.017). A separate validation study independently corroborated the prognostic capabilities of ColoPrint in patients with stage II disease; here, 5-year distant metastasis-free survival rates were 94.9% and 80.6% for the low- and high-recurrence risk groups, respectively (HR, 4.28; P=.013).[122] Interestingly, these results predicted recurrence independently of the inclusion of the clinicopathologic considerations typically used to decide whom to treat with adjuvant chemotherapy. These findings further the notion that ColoPrint

Table. Recommended Biomarker Testing Based on Stage of Disease Presentation

	Stage II	Stage III	Stage IV
Microsatellite testing	+		
KRAS (codons 12, 13, 61, 146) + NRAS			+
Amphiregulin/epiregulin			?
BRAF			+
PI3KCA	+	+	?
Multigene assays	?		

+Authors recommend testing of the selected biomarker.

?Further prospective studies are needed to clarify the benefit of routine use of this biomarker in clinical practice.

may stand independently as a robust predictor for disease recurrence in the stage II setting. A prospective trial is currently under way comparing the ColoPrint assay with the clinical risk factors used in treatment decision-making for stage II disease; should the former be found superior, there may be a future role for this test in routine practice.

Conclusions

Although advances in our understanding of breast and lung cancers have led to the subclassifications of multiple populations of tumors according to the underlying molecular phenotypes, the diversity and heterogeneity of colorectal cancers are only now beginning to be better described and better appreciated. Even clinically relevant KRAS mutations demonstrate heterogeneity not only according to the particular codon mutated, but also according to the amino acid mutated within a specific codon. In addition, tumors now considered "KRAS wild-type" may harbor mutations (eg, BRAF) with added prognostic significance. Further research will continue to identify other effector molecules and pathways that dictate their driving biologic response (or lack thereof) to available therapies. Although our practices for routine biomarker testing are often dictated by the stage at presentation of the patient being treated (Table), we expect that the limited panel of testing markers will continue to expand in the near future as our understanding of the complex biology that affects treatment modalities in these patients evolves.

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