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# Colorectal Cancer Precision Medicine



William P.D. Hendricks



Daniel L. Edelstein

Clinicians have long endeavored to tailor treatment to individual patients. Today, a new wealth of clinically correlated genomic research is uncovering diagnostic and prognostic genetic markers in addition to driving development of new treatments capable of exploiting tumor-specific vulnerabilities. Clinical cancer management is thereby becoming increasingly precise. Colorectal cancer (CRC) patients have particularly benefited from this paradigm. Genetic tests are now available as standard of care for prognosis and prediction of treatment response while emerging technologies and new targeted treatments hold out hope for significant improvements in patient outcomes. Clinicians can now employ several patient-specific tests such as RAS, *BRAF*, and microsatellite instability testing to assess risk, enhance prognosis, and predict response to therapy. Meanwhile, new technological paradigms such as the liquid biopsy hold out promise for enabling more precise non-invasive, real-time analysis of patient tumor burden. Here, we review CRC's genetic basis, discuss diagnostic markers relevant to hereditary versus sporadic disease, summarize the recent data on CRC biomarkers, and point to new developments in CRC precision medicine.

## INTRODUCTION

**C**olorectal cancer (CRC) is a major worldwide public health problem with approximately 1.3 million new cases diagnosed per year.<sup>1</sup> It is the second leading cause of cancer mortality in the United States where 1.2 million people are estimated to be affected with the disease.<sup>2</sup> Disease stage at the time of diagnosis is the most significant prognostic factor<sup>3</sup>, but in spite of global screening efforts, 50% of patients will nonetheless either present with or later develop metastases.<sup>2,4</sup> In patients with metastatic disease, selection of the most effective treatment strategy remains challenging. The management of

patients with metastatic colorectal cancer (mCRC) has advanced significantly over the past decade with the pairing of advanced molecular diagnostic tools to targeted therapies. For example, therapies targeting the epidermal growth factor receptor (EGFR) have transformed mCRC treatment in many cases from non-specific cytotoxic regimens to precise and highly personalized treatments which target the unique genetic underpinnings of an individual patient's tumor.

Although the management of CRC has improved significantly over the past decade, with five-year survival for patients with localized CRC now at 90%, this number drops to a dismal 10% in patients with metastases at diagnosis. As discussed elsewhere in this issue, advances in screening have largely been responsible for recent progress in reducing CRC mortality. Similarly, the wide availability of risk

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William P.D. Hendricks, Ph.D.<sup>1</sup> Daniel L. Edelstein, M.S.<sup>2</sup>  
<sup>1</sup>Assistant Professor, Integrated Cancer Genomics Division, The Translational Genomics Research Institute (TGen), Phoenix, AZ <sup>2</sup>Clinical Research Associate, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD

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assessment tools such as genetic testing for hereditary CRC syndromes has increased the detection of familial CRC, thus allowing for early interventions and frequent surveillance to manage CRC risk. Screening has the potential to further dramatically reduce CRC mortality by facilitating detection of tumors at a curable stage. Yet, even with public education campaigns and the introduction of highly sensitive stool-DNA based diagnostics, adherence to screening guidelines is problematic. Regrettably nearly 50% of those at highest risk for developing CRC (individuals over the age of 50) are not screened and most patients are only diagnosed when symptoms present at advanced stage when the tumor is most difficult to treat.<sup>5</sup> This situation highlights the need for ongoing innovations not only in CRC treatment, but also in non-invasive screening for early detection.

## A Molecular Understanding of Colorectal Cancer

Cancer is fundamentally a genetic disease that arises as a consequence of the accumulation of mutations in genes that regulate cell growth and death. Progress in cancer medicine now depends on identification of these causative mutations alongside determination of their relationship to clinical phenotypes and development of drugs that are selectively toxic in defined mutational contexts. Explosive growth in the understanding of cancer's genetic basis over the past decade enabled by high-throughput genome sequencing technology has revealed that most cancers are far more complicated than anticipated. A great diversity of mutations exists both within specific tumor types as well as within individual patient tumors themselves.

CRC has long been one of the best understood cancers according to its genetic progression. It was additionally one of the first cancers to undergo comprehensive genomic analysis as early as 2006.<sup>6</sup> The CRC mutational landscape has since been exhaustively studied. Understanding of this landscape has radically transformed CRC classification, fueling treatment individualization that ultimately holds out the best hope for improvements in patient survival when screening and prevention fail. This approach is not new to CRC. Clinicians have historically relied on histopathology reports of tumor features and staging according to the TNM (Tumor, Node, Metastasis) system to inform design of treatment regimens. However, the current

depth of this individualization is driven by genomic knowledge and advanced diagnostics, both of which enable tumor characterization according to genetic features that may differ dramatically even within similar histological subtypes

The initiation and development of malignant CRC results from the stepwise accumulation of genetic events that transform normal epithelial tissue first into a benign polyp and then eventually into an invasive colorectal tumor.<sup>7</sup> This multistep process typically proceeds over decades as mutations accumulate, deactivating critical tumor suppressor genes that keep cell growth in check and activating tumor promoter oncogenes that drive growth and invasion into normal tissue, and spread to distant sites. In this traditional model, key events include inactivation of the tumor suppressor genes *APC*, *TP53* and/or *PTEN*, by which a loss of function transforms normal colonic mucosa into an adenoma. Further downstream, the activation of oncogenes such as *KRAS*, *NRAS*, *BRAF* and/or *PIK3CA* stimulates aberrant growth of the adenoma that, when coupled with genetic deletions of chromosome 18q, facilitates the final transition from adenoma to carcinoma.<sup>7,8</sup>

Comprehensive genomic sequencing of large cohorts of CRC tumors has now confirmed CRC is a heterogeneous genetic disease with substantial differences in tumors even of the same subtype.<sup>9-11</sup> Mutations in tumor suppressor genes and oncogenes as outlined above (in addition to other mutations) are found at variable frequencies in CRC tumors as are other types of molecular alteration such as methylation and gene expression. In the research setting, many such alterations are being assessed for their impact on tumor biology, potential correlations with clinical phenotypes, and their druggability. While many such novel findings are also currently under evaluation in clinical trials, a key set of foundational molecular markers have already been successfully translated to a CRC classification system to improve prognostic determinations and guide the administration of targeted therapy in routine clinical practice. This article will provide a review of those molecular markers in routine use for treating advanced disease with a look to the future implementation of advanced diagnostic technologies for managing the complex dynamics of tumor evolution in real-time.

## Hereditary and Sporadic Disease

The majority of CRC cases are due to sporadic genetic alterations that occur in somatic cells. However, up to

15-20% of all CRC cases have a hereditary component. In CRC, inherited or somatic mutations occur within the colonic epithelium to direct the transformation of a benign polyp into an invasive colorectal tumor. Two of the most common hereditary colorectal cancer syndromes, Familial adenomatous polyposis (FAP) and Lynch Syndrome (LS) have significantly different clinical phenotypes yet increase the lifetime risk of CRC to 80% in HNPCC<sup>12</sup> and 100% in FAP. Both syndromes are inherited in an autosomal dominant manner associated with specific germline mutations that significantly increase the risk of colorectal polyp and CRC development early in life. LS is typically caused by mutations in one of the mismatch repair (MMR) genes (*MLH1*, *MLH3*, *MSH2*, *MSH3*, *MSH6* or *PMS2*) and FAP results from a mutation in *APC*. These alterations are detectable with widely available germline genetic testing, results of which are evaluated in consultation with a physician and genetic counselor who consider an individual's family history and clinical symptoms. Germline genetic testing represents the first major advance in individualizing the management of CRC through the use of genomic information and is described in detail elsewhere in this series.

The individualized management of patients with sporadic CRC is achieved with the use of tumor-specific prognostic and predictive biomarkers. The distinction between prognostic and predictive markers is important. Prognostic markers provide an indication of the likely progression of disease whereas predictive markers predict response to treatment. An individual biomarker may have both prognostic and predictive clinical value. Such biomarkers are traditionally detected by the analysis of tumor tissue. However, recent advances in molecular diagnostics have led to wide clinical availability of blood-based and other minimally invasive methods of biomarker evaluation.<sup>13</sup> Although this technology is just now emerging for clinical use, blood-based diagnostics may soon simplify the process of detecting the presence of a tumor, monitoring response to therapy, and detecting recurrences, enabling new possibilities for monitoring disease status and modulating therapy in synchrony with ever-changing tumor dynamics.<sup>13</sup>

## CRC Biomarkers

In clinical practice, advanced colorectal tumors are treated according to their molecular classification. The extensive and growing list of mutations and mutational profiles associated with specific colorectal

tumor types, their clinical course, and their response to therapy has produced three predictive standard of care tests that are recommended by the *NCCN Clinical Practice Guidelines in Oncology for Colon Cancer* for use in clinical practice: RAS mutation analysis, *BRAF* mutation analysis, and Microsatellite Instability (MSI) or MMR testing.<sup>14</sup> These tests will be discussed in terms of their clinical context, their biology, and the evidence for their recommended use (Table 1).

## RAS Testing and Anti-EGFR Therapy

The rat sarcoma virus (RAS) gene signaling pathway plays an important role in the pathophysiology of CRC, with RAS mutations occurring in approximately 55% of mCRC patients. RAS comprises a family of genes including *KRAS*, *NRAS* and *HRAS*, which, under normal biological conditions mediate extracellular signaling from the epidermal growth factor receptor (EGFR) to deliver intracellular growth signals to the nucleus. In a tumor cell, the constitutive activation of RAS via mutations at specific hotspots such as one occurring in the gene's second exon (exon 2 RAS mutations) disrupts this normal signaling pathway to drive aberrant growth and metastases. Numerous Phase II and III randomized controlled trials have demonstrated the benefit of anti-EGFR monoclonal antibodies such as cetuximab and panitumumab as single agents or in combination with other chemotherapeutic regimens in mCRC patients.<sup>15-19</sup> However, in patients whose tumors harbor *KRAS* mutations, these drugs have been shown to be ineffective.<sup>1-5</sup> The selection of patients lacking *KRAS* exon 2 mutations (i.e. *KRAS* exon 2 wild-type patients) was shown to increase response rates to anti-EGFR therapy by as much 60%.<sup>20,21</sup> This finding reinforces the approach of incorporating highly specific molecular diagnostics into routine clinical practice.

With *KRAS* exon 2 mutation analysis widely incorporated into clinical practice for predicting lack of response to anti-EGFR therapy, it became clear that not all *KRAS* exon 2 wild-type patients responded to treatment. Further refinement in biomarker testing was pursued to improve patient outcomes and avoid unnecessary treatment-related side-effects and costs. Preclinical evidence showed that additional *KRAS* hotspot mutations such as those occurring in *KRAS* exons 3 and 4 and in the alternative RAS gene, *NRAS*, were similarly oncogenic to *KRAS* exon 2 mutations and conferred resistance to EGFR antibodies.<sup>22,23</sup> Thus, a series of retrospective evaluations were performed

Table 1. Available Somatic Tumor Mutation Tests with Clinical Utility

Test	Positive Result	Clinical Utility	Frequency/Incidence
RAS	KRAS/NRAS mutation in Exon 2 (codons 12, 13) Exon 3 (codons 59, 61) Exon 4 (codons 117, 146)	Tumors with RAS mutations exhibit limited to no response to anti-EGFR therapy. RAS testing should be performed on all patients considered for treatment with EGFR inhibitors.	50-55%
BRAF	V600E point mutation	Tumors with wild-type RAS should be tested for BRAF mutations. BRAF mutant tumors have poor prognosis. BRAF mutations may predict lack of response to anti-EGFR therapy, though conclusive evidence is lacking. Possible emerging data from clinical trials is encouraging for the use of dual blockade EGFR/BRAF inhibition.	10%
MSI	Loss of any one of MLH1, MSH2, MSH6 or PMS2 protein products via IHC or instability in <30% of microsatellite markers (MSH-H) via MSI analysis	MSI-H identifies Stage II patients with good prognosis who would not benefit from 5-FU based adjuvant therapy. MSH-H indicates dramatic and durable response to immunotherapy with PD-1 inhibition.	15%

on tumor specimens selected from clinical studies which had initially demonstrated the predictive value of *KRAS* exon 2 for response to anti-EGFR therapy. *KRAS* exon 2 wild-type tumors were evaluated for other RAS mutations and correlated to response to anti-EGFR therapy from numerous clinical trials including PRIME, OPUS, CRYSTAL, CALGB/SWOG-80405, FIRE-3, COIN, PEAK, PICCOLO, 20050181 and 20020408. In aggregate, data from all studies showed that a more comprehensive analysis of RAS mutations, so-called “expanded RAS” including *KRAS* and *NRAS* codons 12 and 13 (exon 2), 59 and 61 (exon 3), and 117 and 146 (exon 4), can identify an additional 11% of patients for which anti-EGFR therapy is contraindicated.<sup>24</sup>

RAS genotyping of primary or metastatic tumors is strongly recommended in patients with mCRC and should be administered as a standard of care test since there is minimal rationale for patients with mutated RAS to endure the toxicity and expense of treatment with anti-EGFR therapy. Figure 1. illustrates the shift in our understanding of RAS. Incorporation of expanded RAS testing into routine practice is expected to increase the proportion of patients ineligible for anti-EGFR

therapy from approximately 40% to 55%.<sup>24</sup> Current clinical practice guidelines, including the 2016 NCCN CRC guidelines and the American Society of Clinical Oncology (ASCO), now recommend expanded RAS analysis be performed to more precisely identify patients for anti-EGFR therapy.<sup>14,25</sup> If RAS is wild type, *BRAF* testing should be considered as discussed below.

### **BRAF**

*BRAF* is an oncogene mutated in 5-10% of CRCs. It lies downstream of RAS in the same growth pathway that is activated upstream by EGFR signaling. *BRAF* codon 600 mutations are early events in CRC tumorigenesis and are typically mutually exclusive with RAS mutations. Currently *BRAF* is considered a prognostic marker. Patients with *BRAF* V600E mutations have shorter progression-free survival (PFS) and overall survival (OS), by approximately 15 months, as compared to wild-type patients<sup>26</sup>. *BRAF* also has diagnostic value in evaluating Lynch Syndrome because *BRAF* mutations have been shown not to occur in LS tumors.<sup>27</sup> Therefore in the clinical workup of a CRC tumor, the detection

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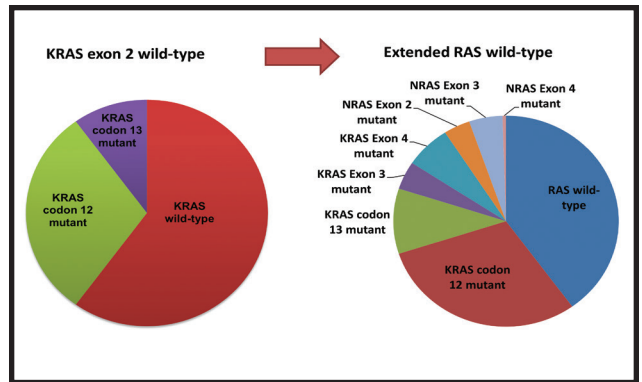


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of a *BRAF* mutation alongside microsatellite instability informs the clinician against the diagnosis of LS.<sup>28</sup> The workflow for diagnosing hereditary colorectal cancer is discussed elsewhere in this series.

As a predictive marker for anti-EGFR therapy, studies of patients with *BRAF* mutations have shown mixed results largely due to the small numbers of patients evaluated. Early studies pointed to a predictive role for *BRAF* mutation status in relation to first-line anti-EGFR therapy. However, recent data from CRYSTAL and OPUS clinical trials demonstrates lack of a relationship between *BRAF* and cetuximab in the first-line setting<sup>29,30</sup> In a retrospective analysis of 370 patients treated with anti-EGFR therapy, *BRAF* mutations were observed in 6.5% of patients with a treatment response of 8.3%, significantly less than in patients with wild-type *BRAF*.<sup>31</sup> While these results appear to substantiate the value of *BRAF* as a predictive marker for anti-EGFR therapy, the patient population was small and further clinical studies are required. The strong prognostic value of *BRAF* as a marker of rapid progression and inferior survival presents a chief challenge in validating the predictive effect of *BRAF* for anti-EGFR therapy. Any benefit from anti-EGFR seems minimal for patients with *BRAF* mutations as studies have consistently shown that the addition of anti-EGFR therapy to chemotherapy in first- and subsequent-line treatment does not increase OS or PFS.<sup>32</sup>

While the predictive value of *BRAF* for anti-EGFR therapy remains controversial, the clinical utility of BRAF inhibitors in combination with anti-EGFR therapy is currently being explored in clinical trials with promising preliminary results. Hoping to replicate the exceptional efficacy of BRAF inhibitors in *BRAF*-mutant melanoma, early clinical trials of single-agent BRAF inhibitors in mCRC were disappointing. However, the administration of BRAF inhibitors in combination with anti-EGFR therapy in patients with *BRAF* mutant mCRC has shown responses in 35% of patients in one study<sup>33</sup> and 13% of patients in another.<sup>34</sup> These promising results for patients with otherwise dismal responses has led to the launch of a Phase II study of irinotecan and cetuximab +/- vemurafenib in patients with BRAF-mutant mCRC.<sup>33</sup> Current clinical practice guidelines recommend *BRAF* testing for its prognostic value, although it may very well have predictive value in identifying patients for clinical trials evaluating dual BRAF/EGFR inhibition.



**Figure 1.** Traditional understanding of KRAS exon 2 versus current understanding of expanded RAS analysis to select patients for anti-EGFR therapy. Data aggregated from meta-analysis of nine randomized controlled trials of anti-EGFR therapy and expanded RAS testing in mCRC.<sup>24</sup>

### Microsatellite Instability

Microsatellite instability (MSI) is a colorectal cancer phenotype and form of genetic instability caused by inactivating alterations in genes of the DNA mismatch repair (MMR) system. The MMR system normally functions to recognize and repair nucleotides mismatched by DNA polymerase. A defective MMR system leads to stretches of repetitive DNA sequences (microsatellites) and instability (MSI). All patients with hereditary Lynch Syndrome and approximately 15% of sporadic CRC patients exhibit MSI and a defective MMR system. In LS the loss of mismatch-repair function is due to an inherited germline mutation in one of the MMR genes (*MLH1*, *MSH2*, *MSH6* or *PMS2*). In sporadic CRC, MSI appears to be due to epigenetic modification of the *MLH1* gene,<sup>35</sup> MSI tumors present with distinct clinical and histologic features including the tendency to occur predominantly in the proximal colon, poor differentiation, lymphocytic infiltration and a signet-ring mucinous histology.<sup>36</sup> In both sporadic and familial tumors, the loss of MMR function is readily assessed by either molecular MSI testing with PCR or immunohistochemical analysis (IHC). Molecular MSI analysis is performed by comparing a panel of five microsatellite markers from both tumor and normal tissue. The size of the microsatellite fragments are evaluated and assigned a level of stability according to criteria established by the National Cancer Institute (NCI). Samples with instability in two or more markers, or <30% of the markers, are defined as MSI-H (high-frequency MSI). Samples with one unstable marker are designated MSI-L (low-frequency MSI) and

samples with no instability in any of the markers are microsatellite stable (MSS). IHC analysis can also be used to determine MMR function by confirming the presence or absence of each of the four MMR gene protein products from a tumor tissue sample. Molecular MSI testing is currently considered the gold standard, though IHC offers good sensitivity and specificity.<sup>37</sup> Both tests should be performed in a CLIA certified lab experienced with these methods.

MSI-H status has traditionally been used as a positive predictor to select patients for germline genetic testing for LS, however it has also recently been shown to correlate with patient survival and serve as a positive prognostic marker in sporadic CRC. Multiple studies have shown that patients with MSI-H tumors had better prognosis than those with MSS tumors.<sup>38</sup> MSI-H was shown to be an independent prognostic marker associated with tumors that were less prone to lymph node involvement and metastases, indicating a favorable outcome compared to tumors with MSS. Determination of MSI status is useful for stage II CRC patients, for whom the benefit of adjuvant therapy is often unclear, given their low risk of recurrence. Overall, MSI-H status has shown excellent clinical utility in identifying stage II patients with good prognosis who are unlikely to derive benefit from 5-fluorouracil adjuvant chemotherapy.<sup>39,40</sup>

MSI status is now additionally guiding new developments in immunotherapy. Immune therapies which are able to activate the patient's own immune system against their tumor (e.g. "checkpoint blockade" with the anti-CTLA4 antibody ipilimumab or the anti-PD1 antibody nivolumab) have begun to show extraordinary activity in some cancers such as melanoma and lung cancer.<sup>41,42</sup> Significant correlations between high mutational burden and response rate to checkpoint inhibitors have been shown in lung cancer and melanoma.<sup>43</sup> Similarly, the overall better prognosis for MSI-H patients has been partially attributed to its correlation with increased tumor T-cell infiltration. This effect is thought to be driven by the increased mutational burden characteristic of tumors with defective MMR.<sup>44</sup> Somatic mutations in coding regions by definition create protein sequences that are foreign to the host and are immunogenic (or, immunologically speaking are "non-self" or neoantigens). They have the potential to be processed by intracellular antigen presentation machinery and presented to the immune system thereby triggering an immune response.<sup>45</sup> Indeed, studies

have shown that somatic mutation burden in tumors with MMR defects (1,782 mutations per tumor) is significantly higher than that in MMR-intact tumors (73 mutations per tumor).<sup>46</sup> Clinical trials are now testing these agents in MSI-H patients. A Phase II study of the anti-PD-1 antibody pembrolizumab in MMR-deficient versus MMR-intact patients with progressive disease found significantly better outcomes for MMR-deficient (immune-related overall response rate of 40%, PFS 78%) versus MMR-intact (immune-related overall response rate of 0%, PFS 11%) with data on PFS and OS still being collected for MMR-deficient patients. As in lung and melanoma studies, high mutational burden was correlated with response. Additional Phase II trials such as KEYNOTE-164 (pembrolizumab) and Checkmate 142; NCT02060188 (nivolumab) are also testing this approach in MMR-deficient CRC. Thus, molecular MSI analysis should now be used to identify candidates for checkpoint inhibitor treatment.

### Advanced Molecular Diagnostics and Targeted Therapy

New technologies are now shaping the next generation of tests that will improve clinical management of CRC patients. One such technological paradigm is the *liquid biopsy*, which exploits the fact that as tumor cells die, they release mutant gene fragments into circulation. The basis for this approach derives from the unique observation that patients with cancer have markedly higher concentrations of cell-free DNA (cfDNA) than healthy individuals.<sup>47</sup> As an alternative and complement to tumor tissue genotyping, analysis of tumor DNA derived from plasma of patients with mCRC has been shown to provide a rapid genotype result which accurately reflects the mutation status of tumor tissue.<sup>48,49</sup> Current evidence has shown that tumor cfDNA is more readily detected in the blood of patients with more invasive rather than earlier tumors, making it an excellent tumor marker in patients with metastatic disease.<sup>48,50</sup> This liquid biopsy approach to genotyping of a patient's systemic tumor burden can enable a real-time assessment of tumor evolution and response to therapy in contrast to reliance on single site tissue biopsies acquired at diagnosis.

Molecular heterogeneity may pose a diagnostic challenge for managing mCRC patients eligible for surgical resection. For example, patients whose primary tumors revealed RAS mutations may have metastases without RAS mutations, and vice-versa.

A unique feature of blood-based tumor genotyping is its ability to evaluate the extent of an individual patient's systemic tumor burden (e.g. identifying any mutant RAS even in the absence of such mutation in the primary tumor) thus eliminating tumor biopsy issues related to tissue molecular heterogeneity.<sup>48,49,51-56</sup> In the case of anti-EGFR therapy, blood-based RAS mutation testing is particularly useful for systemic assessment of tumor heterogeneity where RAS testing of a single tumor sample may not accurately reflect the RAS mutational status of the patient's overall disease burden.<sup>13</sup> Moreover, comprehensive assessment of RAS status by testing multiple tissue samples is neither practical nor feasible. Heterogeneity within individual tumors or tumor samples may also be substantial. Some reports have demonstrated that testing of DNA from a single colorectal tumor tissue block will wrongly assign *KRAS* wild-type status in 8-11.6% of patients.<sup>57-59</sup> Studies evaluating intertumor heterogeneity between primary tumors and metastases in the same patient have shown that the mutational discordance can be found in 3.6-32.4% of cases.<sup>60-66</sup> Both inter- and intratumor heterogeneity are inherent features of metastatic disease<sup>67-69</sup> and though outcome studies are just now being performed, preliminary results of select patients support the utility of a liquid biopsy to overcome sampling bias associated with single tumor site specific sampling.<sup>70</sup>

In spite of the significant therapeutic advances made by targeting EGFR pathways, any clinical benefit is often short-lived due to the inevitable emergence of drug resistance. In RAS wild-type mCRC patients receiving anti-EGFR therapy, the detection of RAS mutations has been associated with disease progression.<sup>51,52,54</sup> In fact, using a liquid biopsy approach, independent research has demonstrated that RAS-mutant tumor DNA is detectable in plasma several months in advance of radiological progression,<sup>49,51</sup> a key insight that may lead to a refinement in the timing of subsequent treatments. As blood-based approaches allow for longitudinal monitoring of rising or falling levels of genetic mutations linked to resistance or susceptibility to targeted therapies, new clinical decision points will be illuminated.

Meanwhile, new innovative clinical trials aimed at assessing the efficacy of matching targeted therapeutics to genomically defined tumors are ongoing. These include the NCI-Molecular Analysis for Therapy Choice (MATCH) program (which focuses on multiple

tumor types including CRC<sup>71</sup>), the CRC-specific FOCUS 4,<sup>72(p4)</sup> and SPECTA studies.<sup>73</sup> Overall, the pairing of advanced diagnostics with new molecularly targeted therapeutics will ultimately deliver the promise of highly precise treatment individualization, leading to the improved management of and significantly better outcomes for mCRC patients.

### Additional Prognostic and Predictive Individualized Tests

There are several additional marketed tests based on genetic and molecular profiling that are intended to provide prognostic or predictive information for the individualization of CRC management, but whose utility in the clinic is still controversial. Such tests include Theraguide 5-FU, Oncotype Dx, ColoPrint, and circulating tumor cell (CTC) analysis. Theraguide 5-FU, recently discontinued by Myriad Laboratories, but still available from other reference laboratories, categorizes risk of 5-FU toxicity into "high," "moderate," "low," or "indeterminate" based on detection of variations in two genes involved in fluoropyrimidine metabolism. The test is indicated for patients experiencing severe toxicity after receiving 5-FU in order to adjust dosage or choose an alternative regimen. Overall, the use of Theraguide 5-FU is controversial as there are no data from prospective randomized trials to demonstrate its impact on improving toxicity without compromising efficacy, thus the test has not yet been widely adopted in the clinic. Though not intended for use in mCRC, Oncotype Dx and ColoPrint are prognostic tests that use gene expression profiling for stage II CRC. These tests evaluate expression of specific gene collections in CRCs that can be correlated to tumor behavior to evaluate risk of recurrence and possibly select low-risk patients who can be spared chemotherapy. Although an Oncotype Dx gene expression profile has been accepted by ASCO and the NCCN as standard of care in breast cancer for the prediction of recurrence in specific contexts, neither of these tests has experienced widespread clinical adoption for CRC since their absolute prognostic and predictive value is still unclear. Both require further validation in prospective trials. Finally, CTC analysis identifies and quantifies CRC cells in the plasma through the identification of tumor specific cell surface markers. Though the US Food and Drug Administration has approved certain methods for identifying CTCs, current guidelines do not recommend using CTCs to assess

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prognosis or to guide therapy selection due to their low sensitivity and specificity as well as a lack of clinical data. Ongoing evaluations may yet confirm some of these tests for standard of care.

## CONCLUSION

An individualized approach to managing colorectal cancer is gradually becoming an integral component of care. This approach will eventually result in more effective treatment with fewer side effects and will promote a more active role for patients in making decisions related to their health care. Future research promises to improve our understanding of the differences between those patients whose cancers respond favorably to certain therapeutics and those that do not. Just as pathology has been used to determine the stage of a colorectal cancer and subsequent prognosis for patients, molecular tools will be increasingly utilized to provide highly specific information to accurately diagnose and treat colorectal cancer. The advent of individualized medicine, while relatively new to CRC medicine, presents patients and physicians with a complex set of tools that when properly utilized, can lead to better treatment outcomes for those with cancer and provide important information for managing and largely eliminating the risk of cancer in hereditary conditions. ■

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