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Colorectal Cancer Molecular Biology Moves Into Clinical Practice

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Abstract

The promise of personalized medicine is now a clinical reality, with colorectal cancer genetics at the forefront of this next major advance in clinical medicine. This is no more evident than in the recent advances in testing of colorectal cancers for specific molecular alterations in order to guide treatment with the monoclonal antibody therapies cetuximab and panitumumab, which target the epidermal growth factor receptor (EGFR). In this review, we examine genetic mechanisms of colorectal cancer and how these alterations relate to emerging biomarkers for early detection and risk stratification (diagnostic markers), prognosis (prognostic markers), and the prediction of treatment responses (predictive markers).

Keywords

Colon Cancer; Biomarkers; EGFR; KRAS; K-Ras; BRAF; Microsatellite Instability; MSI; Chromosome Instability; Cetuximab; Panitumumab; Personalized Medicine

INTRODUCTION

The promise of personalized medicine is now a clinical reality, with colorectal cancer genetics at the forefront of this next major advance in clinical medicine. This is no more evident than in the testing of colorectal cancers for specific molecular alterations in order to guide treatment with the monoclonal antibody therapies cetuximab and panitumumab, which target the epidermal growth factor receptor (EGFR).[1, 2, 3] Indeed, the discovery that acquired *KRAS* mutations are a robust predictive marker of resistance to cetuximab and panitumumab [4, 5] has led to clinically validated and cost-effective testing strategies to direct these drugs to appropriate patients. This discovery resulted from a detailed understanding of colorectal cancer genetics, including the role of *KRAS* mutations in colorectal carcinogenesis, as well as knowledge of the epidermal growth factor (EGFR) signaling pathways.[6] The success of *KRAS* mutation testing in predicting treatment response is just the

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beginning of the use of genetic markers for directing the care of colorectal cancer patients. Many other genetic markers in colorectal cancer show promise for their use in treatment selection, prognosis, and early cancer detection. In this context, knowledge of the underlying genetic mechanisms of colorectal tumorigenesis and the potential of specific genetic lesions for clinical decision making is expected to become part of the working knowledge of care providers managing colon cancer patients. However, despite the promising advances in the molecular pathology of colorectal cancer that are highlighted in this review, it is important to emphasize that clinicopathological staging of tumor tissue is still the cornerstone of prognostication and treatment selection. The modern tumor-node-metastasis (TNM) classification system is recommended, although the original Dukes staging system is still used by some clinicians and is taught to pathologists in training.[7] The pathologic features with greatest prognostic power are depth of tumor invasion, burden of lymphovascular invasion (estimated by the number of lymph nodes infiltrated by cancer), and presence of distant metastases. Efforts to correlate genetic alterations with histologic features have had limited success, although microsatellite instability is a molecular feature that shows modest correlation with certain histologic features such as cribriform architecture and medullary histology.8 Thus, molecular testing is usually required for accurate assessment of specific gene mutations or genomic instability that provide prognostic and predictive information beyond clinicopathologic features.

In this review, we examine genetic mechanisms of colorectal cancer and how these alterations relate to emerging biomarkers for early detection and risk stratification (diagnostic markers), prognosis (prognostic markers), and the prediction of treatment responses (predictive markers) (Table 1). The genetic features of colorectal cancer that are currently most clinically useful will be emphasized in this review, and a detailed description of the molecular genetics and molecular biology of the germane genetic and epigenetic alterations will be provided. We will conclude by reviewing the potential role for genetic markers in the selection of targeted colorectal cancer therapies that are in pre-clinical development or in Phase I and II trials.

MOLECULAR MECHANISMS OF COLORECTAL CARCINOGENESIS

The adenoma/carcinoma progression sequence

Colorectal cancer arises as the result of the accumulation of acquired genetic and epigenetic changes that transform normal glandular epithelial cells into invasive adenocarcinomas. Steps that transform normal epithelium to benign neoplasia (adenoma), followed by invasive carcinoma, and eventually metastatic cancer are described in the classic tumor progression model proposed by Fearon and Vogelstein (Figure 1).6 Since this model was originally proposed our understanding of the molecular pathogenesis has advanced considerably and led to numerous revisions of the Vogelstein and Fearon model. For instance, the original model proposed that only tubular and tubulovillous adenomas had the potential to progress to invasive adenocarcinoma. It is now recognized that serrated polyps including sessile serrated adenomas (SSA) and traditional serrated adenomas (TSA) also have the potential for malignant transformation.10, 11 These polyps are an alternative pathway to malignancy whereby a subset of hyperplastic polyps progress to serrated neoplasms (SSA or TSA) and a fraction of these serrated neoplasms progress to cancer. Premalignant serrated polyps more frequently arise in the proximal colon 12 and are associated with microsatellite instability and aberrant DNA methylation at CpG islands, whereas conventional tubular adenomas arise via biallelic inactivation of the *APC* tumor-suppressor gene and display chromosome instability.[13] Furthermore, other molecular lesions, such as *BRAF* V600E mutations, are characteristically found more often in tumors arising via the serrated neoplasia pathway.[13]

Genomic and Epigenomic Instability and Chromosomal Alterations

Genomic and epigenomic instability distinguishes neoplastic from normal colonic epithelium and is a hallmark feature of colorectal carcinogenesis.[14, 15] At least four kinds of genomic or epigenetic instability have been described in colorectal cancers: (1) chromosomal instability (CIN), (2) microsatellite instability (MSI), (3) CpG island methylator phenotype (CIMP), and (4) global DNA hypomethylation. Overlap between these categories and imprecise use of these terms has led to confusion and confounds interpretation of the literature.[16] Thus, in this section we will first define the different types of genomic and epigenetic instability in colorectal cancer and will delineate in general terms how these mechanisms are clinically relevant.

CIN—The most common form of genomic instability is chromosome instability, which is found in as many as 85% of colorectal cancers.[17] Chromosome instability, which can be recognized by the presence of aneuploidy, is defined as the presence of numerical chromosome changes or multiple structural aberrations and is typically assessed by DNA flow cytometry.[18] Despite the frequent occurrence of CIN in colorectal cancer, the mechanisms that give rise to this form of genomic stability and the role of aneuploidy in tumor progression remain poorly understood. However, there is some evidence that CIN promotes cancer progression by increasing clonal diversity [19, 20, 21]. Importantly, from a clinical perspective, large meta-analyses have demonstrated that CIN is a marker of poor prognosis in colorectal cancers.[18, 22]

MSI—Microsatellite unstable tumors, which account for approximately 15% of colorectal cancers, are generally regarded as being mutually exclusive of CIN tumors because they display a normal karyotype and exhibit unique genetic features, although there does appear to be a subset of tumors that show both CIN and MSI.16 MSI colorectal cancer has been defined by the presence of at least 30% unstable loci in a panel of 5–10 loci consisting of mono- and di-nucleotide tracts selected at a National Cancer Institute consensus conference. 23 Currently, many clinical laboratories assess MSI using a panel of 5 mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) that were selected for high sensitivity and specificity.[9] A subset of tumors with only 10–29% unstable loci has been designated as a form of microsatellite tumors designated “MSI-low”. Although there is evidence that MSI-low cancers have distinct features compared with MSI (also referred to as “MSI-High”, or “MSI-H”) and microsatellite stable tumors, there is considerable controversy regarding whether MSI-low is a unique molecular subclass of colorectal cancer. 16, 17, 24 Colorectal cancer patients with MSI tumors have been shown to have a better prognosis compared to patients with CIN tumors[18, 25], and probably respond differently to adjuvant chemotherapy compared to patients with microsatellite stable (MSS) cancer.[26, 27, 28]

In contrast to CIN, the mechanisms underlying MSI are relatively well understood and involve inactivation of genes in the DNA Mismatch Repair (MMR) family either by aberrant methylation or by somatic mutation.[21] Furthermore, individuals with Lynch syndrome (hereditary non-polyposis colorectal cancer, HNPCC) almost exclusively develop MSI colorectal cancers because they have germline mutations in the MMR genes, which include *MLH1, MSH2, MSH6*, and *PMS2*. In contrast, sporadic MSI colorectal cancer most often have loss of MMR activity as the result of silencing of *MLH1* by aberrant methylation.[21, 29] It is also now recognized that sporadic MSI tumors are associated with the serrated neoplasia pathway and frequently carry *BRAF* V600E mutations, while cancers resulting from germline mutations in MMR genes (Lynch syndrome) do not have mutated *BRAF*.[30, 31] Thus, the presence of a *BRAF* mutation in an MSI tumor effectively excludes the possibility that the tumor arose as the consequence of Lynch syndrome (Figure 2).

CIMP—Epigenetic instability in colorectal cancer is manifested as both hypermethylation of gene promoters that contain CpG islands (the CpG Island methylator phenotype, CIMP), and global DNA hypomethylation. Mechanisms that give rise to CIMP are not yet clear, although the strong association between *BRAF* V600E mutations and CIMP colorectal cancer suggests a role for activated BRAF in the pathogenesis of the methylator phenotype and a link between sporadic MSI and CIMP.[32, 33] However, in vitro studies of mutant *BRAF* in colorectal cancer cell lines have not demonstrated a direct cause and effect relationship between BRAF and CIMP.34 Furthermore, although CIMP tumors do appear to represent a distinct subset of colorectal cancer, the clinical utility of this designation is hindered by lack of a universally accepted definition of the methylator phenotype. CIMP is usually defined as methylation of at least three loci from a selected panel of five gene associated CpG islands. Because this panel is not always the same across studies, attempts are being made to facilitate standardization of CIMP markers for clinical use.[33, 35] Some authors have proposed two classes of CIMP, CIMP-low, and CIMP-high, depending on the number of methylated marker loci detected.[32] Another group suggested that CIMP colorectal cancers be divided into two distinct classes (called CIMP1 and CIMP2) based on the results of unsupervised cluster analysis of a large panel of methylation markers.[36] Finally, considerable overlap between CIMP and sporadic MSI tumors adds to the challenge of incorporating CIMP-status into clinical trials and clinical decision making.33 Retrospective studies suggest CIMP will ultimately be shown to be a predictive marker for colorectal cancer, but the data is not adequate at this time to recommend its clinical use.[36, 37] Thus, the discovery and classification of CIMP tumors has advanced our understanding of the molecular pathology of colorectal cancer but has not yet impacted clinical care.

In addition to aberrant gene methylation, a global decrease in methylation has also been identified in many colorectal cancers and is tightly associated with CIN tumors.[38, 39] Further research is necessary to determine if measurement of global DNA hypomethylation in colorectal cancer has any role in the clinical setting.

Role of Specific Genetic Alterations and Signal Pathway Deregulation

Just as important as genomic and epigenomic instability for the pathogenesis of colorectal cancer is the accumulation of mutations in specific genes and the resulting deregulation of specific signaling pathways that control the hallmark behaviors of cancer: cell proliferation, differentiation, apoptosis, immortalization, angiogenesis, and invasion. The best-studied pathways that are deregulated in colorectal cancer are the WNT-β-catenin signaling pathway, the transforming growth factor β (TGFβ) signaling pathway, the epidermal growth factor receptor (EGFR)-MAPK pathway, and the phosphatidylinositol 3-kinase (PI3K) pathway.[5, 16] Selected deregulated pathways in colorectal cancer and targeted therapies in clinical use or in clinical trials are summarized in Table 2.

Key tumor suppressor genes that do not necessarily mediate their effects through signal pathway deregulation, such as TP53, and recurrent cytogenetic aberrations such as 18q loss of heterozygosity (LOH) are also well-studied in colorectal cancer and affect the malignant transformation of colon epithelial cells through specific effects on the behavior of the cells (Figure 1). The use of these molecular alterations in the management of patients with colorectal cancer will also be discussed in more detail below.

WNT Pathway—Mutations in the adenomatous polyposis coli (*APC*) gene occur in up to 70% of sporadic colorectal cancers and are the cause of the familial adenomatous polyposis (FAP) cancer predisposition syndrome. *APC* mutations can be found at the earliest stages of neoplasia and are predominantly associated with the classic tubular adenoma pathway and CIN cancers (Figure 1).[6, 40, 41] The APC protein negatively regulates WNT signaling via

targeting β-catenin for ubiquitin-mediated proteasomal degradation. Disruption of the APC protein results in increased WNT signaling through stabilization of nuclear β-catenin. Activating mutations in the β-catenin gene (*CTNNB1*) that protect the protein from APC-mediated degradation are also observed in colorectal neoplasia, although they are found more frequently in adenomas (12.5%) than invasive cancer (1.4%), suggesting that *CTNNB1*-mutant tumors do not frequently progress to carcinoma.[42] Despite the critical and nearly universal role of WNT pathway activation in colorectal carcinogenesis, there is currently no clinical use for *APC* or *CTNNB1* mutations for treatment selection, prognosis, or early cancer detection. There has been intense effort to develop small molecule inhibitors of this pathway, but these efforts are still confined to the pre-clinical arena. If these agents eventually reach the clinic, the assessment of *APC* mutations or activated β-catenin (by the detection of nuclear localization of β-catenin by immunostaining) is likely to have a role in directing the selection of patients who will respond to these agents.

TGF-β Pathway—Deregulation of TGF-β signaling, which is generally considered a tumor-suppressor pathway in the colon, occurs in the majority of colorectal cancers.[43] Inactivating mutations have been observed in receptor genes (*TGFBR2* and *TGFBR1*), post-receptor signaling pathway genes (*SMAD2*, *SMAD4*), and TGF-β superfamily members (*ACVR2*).[17, 44, 45, 46] Functionally significant mutations in *TGFBR2* are detected in as many as 30% of all colorectal cancer and are associated with the malignant transformation of late adenomas. *TGFBR2* mutations are most common in MSI tumors, but also occur in approximately 15% of microsatellite stable (MSS) tumors (Figure 1).[46, 47, 48] *SMAD4* is located on 18q in the region commonly deleted in colorectal cancer, and is associated with adenoma formation and adenoma-carcinoma progression in mouse models, supporting a role for *SMAD4* as a tumor suppressor gene.49 Furthermore, loss of SMAD4 expression as detected by immunostaining has been reported in >50% of colon cancers and is associated with lymph node metastases.[50] There is still not any definite clinical role for any genetic markers in the TGF-β signaling pathway, however there is some evidence that SMAD4 expression levels may be associated with prognosis and response to 5-Fluorouricial (5-FU) and there is ongoing investigation of 18qLOH as a predictive marker, which is discussed further in the next section.[51, 52]

18qLOH—Loss of the long arm of chromosome 18 (18q loss of heterozygosity; LOH) is the most frequent cytogenetic alteration in colorectal cancer and is observed in up to 70% of tumors.[6, 22] Two genes thought to have a role in the tumorigenic effects of this loss are deleted in colorectal carcinoma (*DCC*) and *SMAD4*. Additional mediators of the TGF-β pathway, including *SMAD2* and *SMAD7*, are also in the 18qLOH region, suggesting that 18qLOH promotes tumorigenesis at least in part through deregulation of TGF-β signaling. It appears that deletion at 18q is associated with a worse prognosis, however efforts to definitively link 18qLOH to prognosis are limited by a lack of consistent results across studies and heterogeneous detection methods.[22] Ongoing clinical trials (e.g. NCT00217737, also designated ECOG 5202) are assessing the utility of 18qLOH for treatment selection.

TP53—Mutations in the tumor-suppressor gene *TP53* occur in about half of all colorectal cancers and promote the malignant transformation of adenomas (Figure 1).[6] Like *APC*, *TP53* is a key tumor suppressor that has been extensively studied in colorectal cancer but currently has no predictive or prognostic role in the clinical setting.[16]

Mediators of EGF signaling: EGFR/RAS/RAF/RAF/MAPK—*KRAS*, a member of the *RAS* family of proto-oncogenes, is the most frequently mutated gene in all of human cancer and arguably the most clinically important oncogene in colorectal cancer. The KRAS protein is a downstream effector of EGFR that signals through BRAF to activate the mitogen

activated kinase (MAPK) pathway and promote cell growth and survival (Figure 3). Mutations in *KRAS* codons 12 or 13 occur in approximately 40% of colorectal cancers and lead to constitutive signaling by impairing the ability of GTPase activating proteins to hydrolyze KRAS-bound GTP.[53] *KRAS* mutations occur after *APC* mutations in the adenoma-to-carcinoma progression sequence, but are still a relatively early event in tumorigenesis (Figure 1).[6] Acquired *KRAS* mutations are maintained throughout carcinogenesis, as evidenced by the nearly perfect concordance of *KRAS*-mutation status in primary and metastatic colorectal cancer.[54, 55] This fact is critical to the utility of *KRAS* mutational analysis on archived primary tumor specimens in patients with metastatic disease and usually eliminates the need for additional biopsy tissue.

The *BRAF* gene, mutated in ~10–15% of colorectal cancers, encodes a protein kinase that is the direct downstream effector of KRAS in the Ras/Raf/MAPK signaling pathway. The majority of *BRAF* mutations are a single base change resulting in the substitution of glutamic acid for valine at codon 600 (V600E; sometimes referred to as "V599E").[5] *KRAS* and *BRAF* mutations are mutually exclusive, supporting the hypothesis that an activating mutation in either gene is sufficient to promote tumorigenesis via increased MAPK signaling.[56] As discussed above, *BRAF* mutations are much more frequent in MSI tumors (~35%) compared to MSS tumors (~5%) and are very tightly linked to CIMP cancers and the serrated neoplasia pathway.[56, 57] Emerging evidence supports a role for *BRAF* as a genetic marker for prediction, prognosis, and risk stratification.

Alterations in EGFR ligands and the *EGFR* gene itself are also observed in a subset of colorectal cancers. There is some data to support that upregulation of the EGFR ligands epigregulin and amphiregulin are associated with an anti-EGFR drug response.[58, 59]

Mediators of EGF signaling: The PI3K Pathway—Mutations in phosphatidylinositol 3-kinase (PI3K) pathway genes are observed in up to 40% of colorectal cancer and are nearly mutually exclusive of one another.[60] The most frequent mutations of the PI3K pathway occur in the p110α catalytic subunit *PIK3CA*, which are reported in up to 32% of colorectal cancers and may promote the transition from adenoma to carcinoma (Figure 1).[61] Mutations are also observed in *PTEN*, a tumor suppressor gene that negatively regulates PI3K signaling in as many as 30% of MSI tumors and 9% of CIN tumors.[62] The PI3K pathway is modulated by EGFR signaling in part via KRAS activation, and there is a plausible role for both *PIK3CA* and *PTEN* mutations as predictive markers of anti-EGFR therapy (Figure 3).[63, 64] Currently, there is not sufficient evidence from clinical studies to support the use of PI3K pathway mutations as predictive or prognostic biomarkers.

RISK STRATIFICATION AND EARLY DETECTION

One use of molecular markers in the management of colorectal cancer is in risk stratification for identifying individuals at high-risk for developing colorectal cancer and for the early detection of colon adenomas and early-stage colorectal cancers. With regard to risk stratification, the most robust molecular markers to date are germline mutations in genes that cause the hereditary colon cancer syndromes (e.g. *APC* mutations and Familial Adenomatous Polyposis, *BMPR1A* and Juvenile Polyposis, etc.) and MSI tumor status, which is an indicator of the possibility of Lynch Syndrome. The use of MSI tumor testing in the diagnosis of Lynch Syndrome will be discussed below in the context of MSI testing being a risk stratification marker because of its association with Lynch Syndrome.

Lynch Syndrome/Hereditary NonPolyposis Colon Cancer (HNPCC) syndrome

Identifying individuals with Lynch syndrome (also known as HNPCC) dramatically alters their clinical management, and can lead to effective colorectal cancer prevention programs

for these individuals and their family members. However, currently the definitive molecular diagnosis of Lynch Syndrome requires expensive germline DNA mutation analysis of multiple DNA Mismatch Repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). To facilitate the most cost-effective strategies for identifying patients at high risk for Lynch syndrome who are candidates for genetic testing, the evaluation of molecular features of colorectal cancers that have occurred in these individuals or other family members can be used to predict the likelihood of identifying a germline mutation in one of the MMR genes. It is now common practice for molecular diagnostics labs to offer a step-wise series of molecular tests that are used to identify colorectal cancers that likely arose in the setting of Lynch syndrome. These tests are based on the molecular pathology of colorectal cancer (Figure 2).[65] A common approach is to initially test the tumors for loss of MMR gene products (MLH1, MSH2, MSH6, PMS2) by immuohistochemistry (IHC) and for MSI by polymerase chain reaction (PCR) as the first-tier screening test (see for example http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/17073), although there is support for the use of IHC alone as a first-line test.[66] Tumors that display MSI and loss of MLH1 protein expression by IHC are then subjected to reflex testing for *BRAF V600E* mutation status and *MLH1* promoter hypermethylation to help distinguish sporadic MSI tumors (~35% *BRAF*-mutant and 99% *MLH1*-methylated) from Lynch syndrome MSI tumors (*BRAF*-WT, infrequent *MLH1*-methylation) (Figure 2, Table 3).[30, 67, 68] This strategy is most effective in excluding individuals who are unlikely to have a MMR gene mutation from undergoing germline mutation testing. It is notable that in those tumors that have MSI and loss of MSH2, MSH6, or PMS2 the likelihood of having a germline mutation is extremely high. Also of interest, it is now recognized that a strategy that relies on clinical criteria alone for the diagnosis of individuals at risk for Lynch Syndrome underdiagnose this syndrome [69]. In light of the substantial effect of a missed diagnosis on an individual's likelihood of developing cancer in the future, a strategy that employs universal testing of all colorectal cancer is being advocated by some experts in this area.[70] It remains to be determined if this strategy is cost-effective and if the benefits outweigh the risks.

Molecular markers and colorectal cancer early detection

Colonoscopy is the most accurate test currently for colorectal cancer screening, however, it is expensive and associated with procedure-related complications and poor patient compliance. In contrast, another commonly used colorectal cancer screening test , fecal occult blood testing (FOBT) is inexpensive and simple to perform, but has a relatively low sensitivity and specificity.[71] Advances in our understanding of the molecular pathology of colorectal cancer, has led to the identification of promising early detection molecular markers for use in non-invasive colorectal cancer screening assays.72, 73 Stool-based methylated *VIMENTIN* (mVim) is a clinically validated marker for early colorectal cancer detection that is now commercially available in the United States (Table 1).[74] The test relies on the fact that a majority of colorectal cancers (53–84%) carry an aberrantly methylated vimentin (*VIM*) gene. A PCR-based assay that simultaneously measured mVim and DNA integrity reported a sensitivity of 83% and a specificity of 82%, with approximately equal sensitivity in Stage I-III colorectal cancer patients .[75] At this time, methods are under development to enhance the performance of stool- and plasma-based methylation assays for clinical purposes.[76] The use of molecular assays, such as the fecal-methylated *VIM* assay, in the clinical care of patients is an area that is likely to undergo rapid advances in the near future.

GENETIC MARKERS AND PROGNOSIS

Genomic Instability and Prognosis: MSI vs. CIN

Meta-analyses across a diverse range of patients have firmly established that MSI colorectal cancer have a better prognosis and that CIN tumors have an unfavorable prognosis (Table 4).[18, 25] The combined hazard ratio for MSI colorectal cancers for overall survival was estimated to be 0.65 (0.59–0.71, 95% CI) with only one of thirty-two included studies reporting an HR >1.0.[25] Conversely, the overall HR associated with CIN colorectal cancer was determined to be 1.45 (1.27–1.45, 95% CI) based on 63 eligible studies and over 10,000 patients.18 Despite the clear association of MSI and CIN with prognosis, these markers have not yet been adopted into routine clinical decision making. It is most likely that MSI testing will be adopted into clinical practice before CIN testing because of the availability of a reliable assay for assessing MSI status.

18qLOH and Prognosis

Colorectal cancer patients with 18qLOH appear to have a worse prognosis compared to patients with tumors that do not carry 18qLOH. A meta-analysis of seventeen independent studies that was limited by evidence of publication bias found an overall HR of 2.00 (1.49–2.69 95% CI) for 18qLOH across all patients, and an HR of 1.69 (1.13–2.54 95% CI) in the adjuvant setting.[22] Candidate genes in the 18q region, including *DCC* and *SMAD4*, have been studied individually for prognostic roles, with inconsistent results.[77] The independent prognostic contribution of 18q deletion in colorectal cancer has been called into question due to the tight association between 18qLOH and CIN, and the inverse association of 18qLOH and MSI.[16] This assertion is supported by a recent study that found no difference in prognosis attributable to 18qLOH in a prospectively collected cohort of 555 non-MSI tumors Stage I-IV.[78] Thus, it is unclear at this time if 18qLOH represents an independent prognostic marker, or is merely a surrogate marker for CIN/MSS colorectal cancers.

Mediators of EGFR and Prognosis

Several recent studies have assessed the prognostic significance of *KRAS*, *BRAF*, and *PIK3CA* mutations in colorectal cancer.[24, 79, 80, 81, 82, 83] Mutant *KRAS* was not independently associated with differences in relapse-free or overall survival in Stage II or III colorectal cancer, but mutant *BRAF* was prognostic for overall survival (OS) in this group of patients.[24, 79] In contrast, mutant *KRAS* and *BRAF* have been reported as markers of poor prognosis in advanced colorectal cancer. In the largest study that has addressed the prognostic role of *KRAS* mutations in advanced colorectal cancer to date, patients with mutant *KRAS* cancers had a worse overall survival (HR= 1.40; 1.20–1.65 95%CI) but similar PFS compared to patients with tumors bearing wild-type *KRAS*.[81] The potential prognostic value of *KRAS* mutations is of particular interest in advanced colorectal cancers because the *KRAS* mutational status of tumors is now being routinely collected in this setting in order to assess for eligibility for treatment with cetuximab or panitumumab. At this time, the use of KRAS mutation status for prognosis in colorectal cancer is still premature but appears to have significant potential to be adopted into clinical use in the near future.

PREDICTIVE BIOMARKERS

Although the treatment of colorectal cancer still primarily relies on the surgical resection of the primary tumor to achieve a cure, considerable progress in the medical treatment of stage III and IV colorectal cancer has occurred over the last 15 years. The adjuvant therapy of stage III colorectal cancer has become more effective as the standard regimen has advanced from 5-fluorouracil (5FU) and leucovorin to 5FU and oxaliplatin or irinotecan [84]. Furthermore, the treatment of stage IV colorectal cancer patients has expanded to include

targeted therapies (cetuximab, panitumumab, bevacizumab; see Table 2) in addition to 5FU, oxaliplatin, and irinotecan. With the identification of multiple effective agents for the treatment of colorectal cancer has come a need for predictive markers for selecting optimal treatment regimens for patients. This is particularly applicable to colorectal cancer because of the heterogeneity in response among colon cancers and because of the toxicity and cost of the medical treatments. The potential of genetic and epigenetic alterations to be effective predictive molecular markers has received considerable attention lately and has led to the use of some of these markers in the routine care of patients with colorectal cancer (Table 5).

The advent of cancer therapeutics that target specific molecules and pathways highlights the potential for underlying genetic and epigenetic lesions in colorectal cancer to guide personalized treatment decisions. A clear demonstration of the potential of mutant genes to direct therapy is that of mutant *KRAS* and treatment with cetuximab. Only ~15% of patients with metastatic colorectal cancer respond to monoclonal antibody (mAb) therapies targeting the epidermal growth factor receptor (EGFR), which prompted intense research into resistance mechanisms that could be secondary to alterations in the *EGFR* gene and/or mutations in downstream effectors. These studies have produced one well-validated and exceedingly robust predictive marker (mutant *KRAS*) and several more promising biomarkers that require further validation (mutant *BRAF*, *PIK3CA*, *PTEN*).85 Research efforts are also focused on identifying molecular features of colorectal cancer that predict response to adjuvant chemotherapy with cytoxic agents: 5-FU, irinotecan, and oxaliplatin.16 In this section we will discuss genetic features of colorectal cancer that have been evaluated for a role in guiding treatment selection. We have focused primarily on acquired tumor mutations as predictive markers, but it is important to note that inherited (germline) polymorphisms also influence the effects of chemotherapy on cancers and the risk for drug toxicity , particularly in the case of 5-FU and irinotecan (Reviewed in 16).

Predictors of Response to anti-EGFR mAb Therapies

EGFR-targeted monoclonal antibodies cetuximab (Erbitux®), and the fully humanized mAb panitumumab (Vectibix®), have proven to be effective in patients with metastatic colorectal cancer both as single agents and in combination with traditional chemotherapy.86, 87, 88 However, while these therapies improve both progression free survival (PFS) and overall survival (OS), they are effective in only a minority of metastatic colorectal cancer patients. 85 These drugs are generally well-tolerated, but are still associated with treatment-related morbidity, including skin rash, diarrhea, and nausea, and are also expensive. To better target anti-EGFR mAb therapy to patients most likely to benefit, *KRAS* mutation status and additional molecular markers of cetuximab and panitumumab resistance have been extensively evaluated.[5]

KRAS is an accurate predictive biomarker—Results of four large phase III randomized have established unequivocally that metastatic colorectal cancer patients with *KRAS* mutations in codon 12 or 13 do not benefit from cetuximab or panitumumab therapy.[4, 89, 90, 91] Prior to the publication of these pivotal trials, the link between *KRAS* mutation status and anti-EGFR mAb response was already firmly supported by several smaller studies[92, 93, 94], but the data was not sufficient to warrant routine clinical testing. The recently published randomized trials have established the use of *KRAS* mutational analysis as a predictive marker for anti-EGFR mAb resistance in patients with metastatic colorectal cancer in most of the relevant clinical settings. These settings include the use of cetuximab or pantumimab in combination with conventional cytotoxic chemotherapy (e.g. 5-FU, FOLFOX, FOLFIRI) as first line treatment of metastatic disease[90, 95, 96, and as monotherapy in relapsed/refractory patients.4, 89, 91]

A second relevant question related to anti-EGFR mAb therapy is whether mutant *KRAS* predicts an adverse outcome in the setting of these treatments. The reported hazard ratios (HR) were almost exactly 1.0 in a total of 348 *KRAS*-mutant chemotherapy-resistant or refractory cancers treated with either panitumumab89 or cetuximab4 as monotherapy, confirming lack of benefit, but also suggesting no harm from anti-EGFR mAb treatment related to PFS or OS in this population. In contrast, the reported HRs were usually greater than 1.0 in studies of cetuximab or panitumumab as first line treatment in combination with FOLFOX4 (fluorouracil, leucovorin, and oxaliplatin) or FOLFIRI (fluorouracil, leucovorin, and irinotecan) chemotherapy.[85] The results of the OPUS trial (Oxaliplatin and Cetuximab in First-Line Treatment of Metastatic Colorectal Cancer) in particular suggest it may be harmful to add anti-EGFR mAb treatments to 5FU, leukovorin, and oxaliplatin in patients with *KRAS*-mutant metastatic colorectal cancer.[90]

Based on the evidence from large trials, European and United States practice guidelines either recommend or require *KRAS* mutational analysis on colorectal cancer tumor tissue prior to the initiation of cetuximab or panitumumab treatment.[1, 2, 3] The European health authority confines use of panitumumab monotherapy, and cetuximab as mono- or combination therapy, to metastatic colorectal cancer patients who are found to carry non-mutated (wild-type, WT) *KRAS* in the primary tumors.[1] The American Society for Clinical Oncology recently published a provisional opinion stating that "All patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumors tested for *KRAS* [codon 12 and 13] mutations…and [*KRAS*-mutant] patients should not receive anti-EGFR antibody therapy".[3] Similarly, the National Comprehensive Cancer Network (NCCN) guidelines require evidence of wild-type *KRAS* prior to cetuximab or panitumumab therapy in all metastatic colorectal cancer settings.[2]

Despite the nearly perfect negative predictive value of mutant *KRAS*, it is still only a minority (~30%) of *KRAS* codon 12/13 wild-type patients who respond to anti-EGFR mAb therapy.[85] This has led to research into additional biomarkers that might predict lack of benefit in those individuals with tumors that have wild-type *KRAS*. There is evidence that rare *KRAS* mutations in codons 61 or 146 (~2% of colorectal cancer) behave similarly to codon 12/13 mutations[97], but incorporating these mutations into routine clinical practice will require analysis of a larger group of patients. Other promising markers of anti-EGFR mAb resistance are *BRAF V600E* mutations, *PIK3CA* mutations, and loss of PTEN protein expression.[5]

BRAF: another predictor of anti-EGFR mAb response?—The biological rationale for *BRAF V600E* mutations as an additional biomarker of anti-EGFR mAb resistance is strong: (1) BRAF is the immediate downstream effector of KRAS in the Ras/Raf/MAPK signaling pathway (Figure 3), and (2) *BRAF V600E* activating mutations are 100% mutually exclusive of *KRAS* mutations in colorectal cancer, implying that activation of either protein is sufficient for colon tumorigenesis. Existing limited data supports *BRAF V600E* mutations as a negative predictor of response to anti-EGFR mAb therapy, leading to the evolving use of *BRAF* mutation testing in *KRAS*-WT patients prior to treatment as a means to further stratify patients into responders and nonresponders. A retrospective analysis showed that 0/11 tumors with mutant *BRAF* responded to cetuximab or panitumumab compared to 22/68 (32%) of *BRAF*-WT/*KRAS*-WT patients.[98] Similar results were observed for patients treated with cetuximab plus irinotecan. None of the patients with tumors with mutant *BRAF* (N=13) responded compared to 24/74 (32%) patients with tumors with *BRAF*-WT/*KRAS*-WT.[97] These finding were supported by work presented at the 2009 American Association of Cancer Research and American Society of Clinical Oncology annual meetings[85], although not all studies have found as robust a relationship between *BRAF* V600E mutation status and anti-EGFR antibody response.[82, 99] *BRAF* mutations also appear to be associated with worse

prognosis independent of treatment, which can confound the assessment of its role as a predictive marker for response to EGFR directed therapies.[82, 99] Despite the currently limited data, and lack of complete consensus, it is likely that *BRAF* mutation status has a role in anti-EGFR mAb treatment decisions and soon will be adopted into the planning for treatment with cetuximab and panitumumab.

PI3K Pathway Activation and anti-EGFR mAb Resistance—Molecular lesions in the PI3K pathway, which in colorectal cancer are primarily mutations in *PIK3CA* and loss of PTEN protein expression, have been proposed as additional anti-EGFR mAb resistance markers because the PI3K pathway is also stimulated by EGFR.[85] However, the relationship of oncogenic alterations in PI3K signaling and cetuximab or panitumumab response is much less clear than that of *KRAS* and *BRAF* mutations. In several small studies published to date, *PIK3CA* mutations or PTEN loss have been associated with lack of response to cetuximab.[64, 100, 101, 102] Both *PIK3CA* mutations and PTEN loss may coexist with *KRAS* or *BRAF* mutations, which weakens the biological rationale of the activation of this pathway as an absolute predictor of anti-EGFR mAb therapeutic response. Nonetheless, the balance of evidence points towards a probable predictive role of molecular events that activate the PI3K pathway for being negative predictive markers for EGFR monoclonal antibody based therapy. In fact, there is modest data demonstrating that when *PIK3CA* mutations and PTEN loss of expression are combined with *KRAS* and *BRAF* mutational analysis, up to 70% of patients unlikely to respond to cetuximab or panitumumab may be identified.[85, 102] This observation has led to the idea that colon cancer may be able to be classified like breast cancers (e.g. triple negative breast cancers), and these cancers have been termed “quadruple-negative” for patients who do not have alterations in any of these four biomarkers.[85, 102] However, at this time, further studies are needed to determine if mutant *PIK3CA* or PTEN loss should be incorporated into clinical practice.

EGFR Mutations and Amplification—The most obvious candidate biomarker for resistance to antibodies which target EGFR is the *EGFR* gene itself. Early studies that focused on EGFR overexpression assessed by immunohistochemistry did not show a consistent relationship with treatment response, in part because of lack of standardization of the assay, which were based on either immunostaining, fluorescent in-situ hybridization (FISH) or quantitative RT-PCR, and inter-observer variability inherent in the technique.[103] *EGFR* gene amplification is more promising for being a predictive biomarker, but has also been fraught with technical challenges that limit the interpretation of existing data, such as dilution of tumor DNA with wild-type DNA in PCR-based assays, and lack of consistent tissue processing and scoring systems in FISH assays.[5] Activating mutations in the EGFR catalytic domain are seen frequently in lung cancer and are associated with sensitivity to anti-EGFR tyrosine kinase inhibitors, but these mutations are quite rare in colorectal cancer.[5] Thus, EGFR does not appear likely to be a clinically useful predictive marker for anti-EGFR monoclonal antibody therapy. Furthermore, although preliminary studies have shown that the EGFR ligands amphiregulin and epiregulin are overexpressed in colorectal cancer and may predict response to cetuximab, lack of standardization of the assays and studies that reproducibly demonstrate the same effect have prevented amphiregulin and epiregulin expression levels from being used as clinical biomarkers for directing therapy with EGFR monoclonal antibodies.[58]

Predictive molecular markers for response to 5-FU, irinotecan, and oxaliplatin

Currently, the tumor biomarkers that demonstrate the greatest promise for guiding adjuvant chemotherapy with conventional drugs in colorectal cancer patients include MSI and 18qLOH.

MSI—5-FU based regimens have been shown to be ineffective, or even detrimental to patients with MSI tumors.[28, 104] Evidence that a functioning MMR system is required for the cytotoxic effect of fluorouracil provides a plausible biological rationale for 5-FU resistance in MSI tumors.17, 27 However, the finding of 5-FU resistance in MSI colorectal cancer is not uniform, and may vary with tumor stage.105, [106] An ongoing phase III randomized trial of patients with completely resected stage II colorectal cancer (NCT00217737) will prospectively assess the role of MSI in predicting response to adjuvant chemotherapy in localized cancers.[16]

MSI tumors appear to be more responsive to irinotecan-based adjuvant chemotherapy.[26] Recently published results from a large randomized trial of Stage III colorectal cancer demonstrated improved outcomes (both PFS and OS) in MSI patients treated with an irinotecan-containing regimen that included 5-FU compared to 5-FU/luekovorin alone.[107] In light of the prior results of the CALGB 98303 study showing no benefit of adding irinotecan to 5FU as adjuvant therapy in unselected Stage III colorectal cancer patients, the finding that MSI is a predictive biomarker for irinotecan suggests MSI could be useful for adjusting adjuvant therapy for colorectal cancer patients.108 Replication of these results in independent studies is required to validate MSI-status as an inclusion criteria for irinotecan-based adjuvant chemotherapy. Currently, neither the European Group on Tumour Markers nor the American Society of Clinical Oncology have recommendations on the use of MSI for guiding therapy in stage II or stage III colorectal cancer patients.

An important issue to consider with regards to MSI is that the majority of colorectal cancers that have MSI are sporadic colorectal cancers that have inactivated the *MLH1* gene through aberrant promoter methylation. The majority of these sporadic MSI tumors also can be classified as CIMP cancers as well. It is not known whether the associations seen between 5FU and irinotecan effects in sporadic MSI tumors also apply to MSI tumors that arise in the setting of Lynch syndrome.

Loss of 18q—Loss of 18q has been associated with an adverse response to 5-FU based adjuvant chemotherapy.[52, 109] There is some evidence that this effect is due to loss of the *SMAD4* gene located in the 18q21 deleted region although this remains to be determined with more definitive studies.51, [52] A number of ongoing clinical trials are assessing the predictive value of 18qLOH and MSI status for the treatment of colon cancer. These include an ECOG trial of stage II colorectal cancer patients being treated with 5-FU, oxaliplatin and bevacizumab (NCT00217737), a trial in patients being treated with olaparib for metastatic disease (NCT00912743), as well as a retrospective analysis assessing MSI and 18qLOH in patients with colorectal cancer (stage II or III) treated with 5-FU or 5-FU and irinotecan (CLB-9581 or CLB-89803).

Topo1—In a large randomized trial that compared 5-FU alone to 5-FU with irinotecan and 5-FU with oxaliplatin in advanced colorectal cancer, higher expression of topoisomerase 1 (Topo1) measured by immunohistochemistry was significantly correlated with responsiveness to irinotecan.[110] Conversely, cancers with low Topo1 expression (602/1269; 47%) did not appear to benefit from the addition of irinotecan (HR 0.98; 95% CI, 0.78 to 1.22). Irinotecan is a Topo1 inhibitor, thus the level of Topo1 expression has a clear biological rationale as a biomarker for predicting irinotecan response. Replication of these initial results in multiple independent studies is required before Topo1 should be considered for use as a predictive marker.

Polymorphisms and their role as molecular markers for colorectal cancer

We emphasize again that germline polymorphisms that alter pharmacokinetics and pharmacodynamics of adjuvant chemotherapy are also potential biomarkers for guiding treatment selection. For example, alterations in thymidylate synthetase and dehydropyrimidine dehydrogenase have been extensively studied in relation to 5-FU response and look promising. However, very few of these polymorphisms have been thoroughly validated and so the majority are not ready to be used clinically.[111, 112] one exception to this generalization is a homozygous polymorphism that reduces the activity of UDP-glucuronosyltransferase (UGT1A1, an enzyme that detoxifies irinotecan), which is associated with a dose-related increased incidence of irinotecan toxicity.[113, 114] This has led to a commercial *UGT1A1* genotyping test that was approved by the Food and Drug Administration in 2005 to help guide irinotecan dosing.

CONCLUSIONS AND FUTURE DIRECTIONS

More than three decades of investigations into the molecular mechanisms of colorectal cancer carcinogenesis is finally culminating in biomarkers that are sufficiently validated for routine clinical use. *KRAS*-mutational analysis to guide anti-EGFR treatment stands as one of the first successes in the era of personalized medicine. MSI and *BRAF*-mutations already have a clear role in triaging molecular genetic testing in Lynch syndrome, and these markers are poised to take on a much greater role in prognostication and prediction of therapeutic responses for sporadic colorectal cancers. The use of assays for mutant *KRAS*, mutant *BRAF*, and MSI demonstrate how the molecular testing of colorectal cancer tissue can reduce medical costs and improve patient outcomes by targeting therapies to the appropriate patient population. Thus, it is anticipated that the use of molecular genetic markers in clinical decision making is likely to expand as more markers are identified and validated. For example, studies are in progress for assessing the efficacy of the multikinase/BRAF-inhibitor sorafinib, and specific inhibitors of PI3K signaling in the treatment of colorectal cancer.[5] There is evidence that sorafinib restores sensitivity to anti-EGFR mAb therapy in *BRAF*-mutant cell-lines, which has prompted an ongoing Phase II National Cancer Institute sponsored clinical trial of sorafinib plus cetuximab in metastatic colorectal cancer patients (NCT00343772).[98] If these initial findings are validated, the indications for mutational analysis of *BRAF* and *KRAS* would expand. Furthermore, colorectal cancer patients with tumors carrying mutant *BRAF* might also benefit from newer selective BRAF-inhibitors such as PLX-4032 combined with anti-EGFR mAb therapy. *PIK3CA* mutations or PTEN loss are likely to become clinically relevant for the treatment of colorectal cancer patients as specific PI3K pathway inhibitors (such as XL147, BGT226, GDC0941, XL765, and NVP-BEZ325) move into Phase II clinical trials.[115] The expanding repertoire of drugs designed to inhibit specific oncogenes and oncogenic signaling pathways again highlights that molecular mechanisms of colorectal cancer will increasingly play a role in the clinical care of patients with colorectal cancer. The use of molecular markers for risk stratification and early detection of colorectal cancer is also showing promise and will be part of the era of molecular medicine that is rapidly emerging.

Box 1: Summary Points

- Chromosome instability (CIN) and microsatellite instability (MSI) are distinct mechanisms by which colorectal cancers arise, associated with unique molecular features.
- Key pathways that drive colorectal cancer are WNT signaling, TGF-β signaling , and Epidermal growth factor receptor (EGFR) signaling; Ras/Raf/MAPK and phosphatidyl inositol 3-kinase (PI3K) pathways are both stimulated

by EGFR. Currently, only downstream mediators of EGFR have a clinical role as biomarkers.

- *KRAS* mutations in codon 12/13 are a highly validated predictive marker for resistance to monoclonal antibody drugs that target EGFR; *BRAF* V600E mutation is likely to be a second predictive marker. Additional resistance markers including *PIK3CA* mutations and PTEN protein loss are being evaluated.
- MSI+ cancers have a better prognosis and CIN+ cancers do worse; 18q LOH+ tumors also have a worse prognosis but are frequently with CIN. Downstream mediators of EGFR are under study for prognostication.
- The role of colorectal cancer molecular biomarkers in clinical decision making is likely to expand as more targeted drugs become available.

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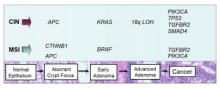

Figure 1. The adenoma-to-carcinoma progression sequence

Colorectal carcinogenesis progresses by at least two well-recognized pathways. The chromosome instability (CIN) pathway is characterized by classic tubular adenoma histology and the early acquisition of *APC* mutations that lead to deregulated WNT signaling, frequent activating mutations of the *KRAS* oncogene at the early adenoma stage, loss of heterozygosity at chromosome 18q (18qLOH) in late adenomas, and *TP53* mutations that facilitate the transition to frank malignancy. By contrast, tumors that harbor microsatellite instability (MSI) frequently acquire *BRAF* mutations and are not associated with 18qLOH or *TP53* mutations. Sporadic MSI cancers appear to commonly arise via the serrated neoplasia pathway, in which sessile serrated adenomas are the most frequently observed pre-cancerous lesions.

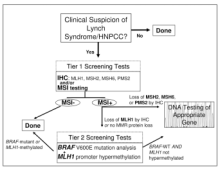

Figure 2. Testing strategies for Lynch Syndrome (HNPCC)

A multi-stage approach to facilitate the cost-effective diagnosis of Lynch Syndrome is outlined. Patients with a high clinical suspicion of Lynch Syndrome are first screened by immunohistochemistry (IHC) studies of the tumor tissue to assess for loss of Mismatch Repair proteins (MMR) expression and by MSI testing of the tumor DNA (Tier 1 Screening Tests). Patients with tumors that show microsatellite instability (MSI) with loss of MSH2, MSH6, or PMS2 by IHC undergo germline DNA mutation analysis of the gene corresponding to the missing protein. In contrast, patients with MSI tumors that lack MLH1 are further assessed with assessment of the tumor for *MLH1* promoter methylation and mutant *BRAF* V600E (Tier 2 Screening Test) because most sporadic MSI colon cancers have methylated *MLH1* and Lynch Syndrome MSI cancers rarely harbor *BRAF* mutations. When there is not evidence of *MLH1* promoter methylation or *BRAF* mutation, mutation analysis of the *MLH1* gene is performed to identify Lynch Syndrome patients with mutations in this gene.

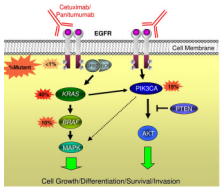

Figure 3. Mediators of EGFR signaling and anti-EGFR antibodies

EGFR forms a homodimer after ligand activation, which results in phosphorylation/activation of the intra-cellular kinase domain and a cascade of downstream signaling including activation of the Ras/Raf/MAPK and phosphoinositol-3-kinase (PI3K) pathways that are associated with cell growth, differentiation, survival, and invasion. Monoclonal antibodies used to treat patients with metastatic colorectal cancer including cetuximab and panitumumab bind to the extracellular portion of EGFR and inhibit signaling in some patients. Activating mutations in *KRAS* occur in ~40% of colorectal cancers and are thought to confer resistance to these drugs by bypassing the need for upstream EGFR signals. Activating mutations in *BRAF* – the direct downstream effector of KRAS – occur in ~10% of colorectal cancers and also probably confer resistance to anti-EGFR monoclonal antibodies. Emerging evidence supports an additional role of oncogenic aberrations in the PI3K pathway in cetuximab and panitumumab resistance.

Table 1

Selected Biomarkers That Have Been Evaluated in Colorectal Cancer

Biomarker	Molecular Lesion	Frequency in CRC	Prediction	Prognosis	Diagnosis
KRAS	Codon 12/13 activating mutations; rarely codon 61, 117,146	40%	Yes	Possible	-
BRAF	V600E activating mutation	10%	Probable	Probable	Lynch Syndrome
PIK3CA	Helical and kinase domain mutations	20%	Possible	Possible	-
PTEN	Loss of protein by IHC	30%	Possible	-	-
Microsatellite Instability (MSI)	Defined as >30% unstable loci in the NCI consensus panel or >40% unstable loci in a panel of mononucleotide microsatellite repeats[9]	15%	Probable	Yes	Lynch Syndrome
Chromosome Instability (CIN)	Aneuploidy	70%	Probable	Yes	-
18qLOH	Deletion of the long arm of chromosome 18	50%	Probable	Probable	-
CpG Island Methylator Phenotype (CIMP)	Methylation of at least three loci from a selected panel of five markers	15%	+/−	+/−	-
Vimentin (*VIM*)	Methylation	75%	-	-	Early Detection
TGFBR2	Inactivating Mutations	30%	-	-	-
TP53 Mutations	Inactivating Mutations	50%	-	-	-
APC Mutations	Inactivating Mutations	70%	-	-	FAP
CTNNB1 (β-Catenin)	Activating Mutations	2%	-	-	-
Mismatch Repair Genes	Loss of protein by IHC; methylation; inactivating mutations	1–15%	-	-	Lynch Syndrome

CRC- colorectal cancer; IHC- immunohistochemistry; FAP- Familial Adenomatous Polyposis

Table 2

Pathways Commonly Deregulated in Colorectal Cancer and Targeted Drugs in Clinical Use (**bold**) or in Clinical Trials

Pathway	Specific Target	Drugs
EGF/MAPK	EGFR (mAb)	**Cetuximab, Panitumumab**
	EGFR (TKI)	**Erlotinib, Gefitinib**
	KRAS	Tipifarnib, Lonafarnib
	BRAF	**Sorafenib**, PLX4032, XL281
	MEK	Selumetinib
PI3K	PI3K	BKM120, BGT226, XL147, GDC-0941
	mTOR	Everolimus, XL765
	AKT	Perifosine
WNT		Resveratrol
TGFβ	TGFβ2	AP 12009
VEGF	VEGF	**Bevacizumab**
	VEGFR	Vatalanib, AMG706, Pazopanib, Cediranib
HGF	HGF mAb	AMG102
IGF	IGF-1 mAb	AMG479, IMC-A12

EGF= epidermal growth factor; MAPK= mitogen activated protein kinase; mAb= monoclonal antibody; TKI= tyrosine kinase inhibitor; TGFβ= transforming growth factor beta; PI3K= phosphatidylinositol 3-kinase; VEGF= vascular endothelial growth factor; IGF= insulin-like growth factor; HGF= hepatocyte growth factor

Table 3

Biomarkers Used in The Diagnosis of Lynch Syndrome (HNPCC)

	Frequency	
Biomarker	**Sporadic**	**Lynch Syndrome**
Microsatellite Instability (MSI)	15%	>95%
BRAF V600E Mutations	35% of sporadic MSI 5% of MSS 10% overall	<1%
Mismatch Repair Protein Loss by IHC	10–15%, mostly MLH1	~90%
MLH1 Promoter Hypermethylation	~99% of sporadic MSI <1% MSS 15% overall	<1%

MSS= microsatellite stable; IHC= immunohistochemistry

Table 4

Prognostic Biomarkers in Colorectal Cancer

Biomarker	Mutation Frequency	Prognosis	Evidence	Status
Microsatellite Instability (MSI)	15%	Favorable	Strong	Testing available but not yet widely used
Chromosome Instability (CIN)	70%	Unfavorable	Strong	No readily available test, not in clinical use
18qLOH/*SMAD4* Loss	50%	Unfavorable	Moderate	No readily available test, not in clinical use
BRAF V600E Mutations	10%	Probably unfavorable	Moderate	Testing available but insufficient evidence to use for prognosis
KRAS Codon 12/13 Mutations	40%	Probably unfavorable in advanced disease	Limited	Testing widely available but insufficient evidence to use for prognosis
PIK3CA Mutations	20%	Possibly unfavorable	Limited	No readily available test, not in clinical use

Table 5

Colorectal Cancer Biomarkers as Predictors for Drug Selection

Biomarker	Mutation Frequency	Drug Selection	Evidence	Status
KRAS Codon 12/13 Mutations	40%	Predicts Resistance to anti-EGFR Therapy	Strong	Validated, In Routine Clinical Use
KRAS Codon 61/117/146 Mutations	1%	Probably Predicts Resistance to anti-EGFR Therapy	Moderate	In Clinical Use, Not Fully Validated
BRAF V600E Mutations	10%	Probably Predicts Resistance to anti-EGFR therapy, May Predict Response to BRAF-inhibitors	Moderate	In Clinical Use, Not Fully Validated
PIK3CA Mutations	20%	May Predict Resistance to anti-EGFR Therapy	Limited	No Readily Available Test, Not in Clinical Use
PTEN Loss	30%	May Predict Resistance to anti-EGFR Therapy	Limited	No Readily Available Test, Not in Clinical Use
Microsatellite Instability (MSI)	15%	May Predict adverse outcome with 5-FU and improved outcome with Irinotecan	Moderate	Not Yet in Routine Clinical Use As a Predictive Biomarker
18qLOH/*SMAD4* Loss	50%	May Predict Resistance to 5-FU	Moderate	No Readily Available Test, Not in Clinical Use
Topo1 Low	50%	May Predict Resistance to Irinotecan	Limited	No Readily Available Test, Not in Clinical Use

5-FU= 5-Fluorouricil